# A Suggested Protocol for Continuous Flow Automated Analysis of Seawater Nutrients Using the Alpkem Flow Solution IV System

(Phosphate, Nitrate, Nitrite, Silicic Acid and Ammonia)

chemical protocols
used in the WOCE Hydrographic Program
and the Joint Global Ocean Fluxes Study

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# PREFACE

This protocol is the latest in a series designed to facilitate achieving uniform, high precision in nutrient analyses carried out in global programs such as the World Ocean Circulation Experiment Hydrographic Program (WHP) and the Joint Global Ocean Flux Study (JGOFS). The protocol has now been adapted to the Alpkem Flow Solution IV System. We have attempted to describe in detail the analytical considerations which we have found necessary in our own laboratory to obtain, over the years, a consistent set of nutrient data from a wide variety of oceanic locations. In writing this protocol we have tried to include sufficient detail that a competent analytical chemist can reproduce our techniques and achieve high precision. No specifications for accuracy are stipulated for but we hope that this protocol will also provide high accuracy. We have purposely included a large amount of repetition to provide emphasis where appropriate and to avoid forcing the reader to jump back and forth from place to place in the protocol to check details.

If you experience any problems please do not hesitate to contact us at the address given on the title page or, for email, below.

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## ABSTRACT

This Suggested Protocol, if executed by a careful, competent analytical chemist, will provide high quality nutrient concentration data using the Alpkem Flow Solution IV System, one realization of Segmented, Continuous Flow Analyzers. The nutrients covered are phosphate, nitrate plus nitrite, nitrite, silicic acid and ammonia. We present a very brief review of literature sources and principles of the analytical methods. The Protocol includes a description of the apparatus including continuous flow analyzers in general, volumetric ware, general notes on reagents, sampling and sample storage, sample containers and their maintenance, a calibration protocol, descriptions of the analytical methods, an outline of the processing of raw data to concentration units, and finally, a brief outline of quality assurance procedures. A brief bibliography and glossary appear at the end of the Protocol.

TABLE OF CONTENTS

## ACKNOWLEDGEMENTS

We are indebted beyond all description to a long list of treasured coworkers who have contributed the pioneering work that has resulted in this publication and the technology it attempts to document. Our earliest efforts were made possible by the foresight of P. Kilho Park who was among the first chemical oceanographers in this country to see the possibilities of continuous flow analyzers (CFA) in marine chemistry. He inspired his students to develop practical methods for use of the original Technicon™ AutoAnalyzer™ at sea. There followed a series of wonderfully patient friends and colleagues to whom we dedicate this effort.

The research on the analytical chemistry behind this presentation has been supported by a long series of research grants by the Marine Chemistry and Physical Oceanography Programs of the National Science Foundation (NSF). These grants began in the mid-1960's to Prof. P. Kilho Park, more recently to the first author. Additional support in came from the Office of Naval Research Marine Chemistry Program.

Support for this current effort, an instruction manual for the Alpkem Flow Solution IV System, has been provided by the Raytheon Corporation, Polar Services, under Purchase Orders RM77747-01 and RM77748-01.

To all, we gratefully say, Thank You!

## LIST OF TABLES



## LIST OF FIGURES

# 1. INTRODUCTION

## 1.1. Scope and Organization of this Protocol

This Suggested Protocol provides a description of procedures which, when implemented by a competent analytical chemist, can provide high quality measurements of the concentrations of the nutrients, silicic acid, phosphate, nitrate plus nitrite, and nitrite in seawater samples. These procedures are not necessarily the only procedures which will meet this claim. Nor are they necessarily the best procedures to use for all oceanographic studies. They have been optimized to provide data to be used in open ocean, deep water, descriptive and modeling studies. Careful adherence to the protocol and methods outlined can facilitate obtaining data which can meet JGPFS and U.S. WOCE specifications (U.S. WOCE Office, 1989). However, to accomplish this requires a great deal of attention to detail and to scrupulous monitoring of the performance of the FSS system.

For many oceanographic programs the nutrient concentration ranges are considerably lower than those experienced in the WOCE Hydrographic Program. Examples include the near surface experimental components of the JGOFS and studies in marginal, saline seas and lagoons. The protocols presented here can be adapted to lower concentration ranges by altering several experimental parameters.

## 1.2. Definitions of Terms for the Nutrients Addressed

Several conventions are used for denoting the nutrients discussed here: silicic acid, phosphate, nitrate plus nitrite, nitrite and ammonia. Although some of these conventions are more precise than the abbreviated terms used in this Suggested Protocol, the authors beg the readers' sympathy with the need to be concise. A glossary of terms including somewhat more detailed and precise nutrient definitions appears at the end of this document.

## 1.3. Principles of Continuous Flow Analysis of Seawater Nutrients

A Continuous Flow Analyzer (CFA) uses a multichannel peristaltic pump to mix samples and chemical reagents in a continuously flowing stream to automate colorimetric analysis. CFA's reduce technician error principally by treating samples and standards exactly alike and by precision in timing and proportioning of reagent addition. Segmenting the sample stream with air bubbles reduces mixing of adjacent samples and enhances mixing of the reagents within the sample stream. The segmented stream passes through a system of glass coils where mixing and time delays are accomplished. The sample-reagent mixture reacts chemically to produce a colored compound whose light absorbance is approximately proportional to the concentration of nutrient in the sample. Finally the absorbance is measured

by a flow-through colorimeter located at the end of the flow path. The colorimeter output is an analog voltage proportional to absorbance.

A fundamental difference between manual and CFA procedures is that complete color development is not required with CFA. Since all standards and samples are pumped through the system at the same rate and in constant proportion to the color developing reagents, all samples and standards achieve virtually identical degrees of color development. This saves considerable time and is one reason for the higher speeds attainable with CFA systems. However, this aspect can introduce errors from any factor affecting the kinetics of color development, eg. laboratory temperature. Laboratory temperature fluctuation historically has caused serious problems with the silicic acid analysis in particular. The modification described in this Suggested Protocol greatly reduces the effect of ambient laboratory temperature.

In the Oregon State University (OSU) and Scripps Institution of Oceanography - Oceanographic Data Facility (SIO-ODF) programs, the Technicon™ AutoAnalyzer™ II (AA-II) and Alpkem™ Rapid Flow Analyzer™ (RFA™) systems have been used to determine the seawater concentrations of silicic acid, phosphate, nitrate + nitrite and nitrite since the early 197O's. The principles of these methods are only briefly described here. Operational details for each method as adapted for the Flow Solution IV System are given in Section 6.

The phosphate analysis is a modification of the procedure of Bernhardt and Wilhelms (1967). Molybdic acid is added to the seawater sample to form phosphomolybdic acid, which is in turn reduced to phosphomolybdous acid using hydrazine as the reductant. Heating of the sample stream is used to speed the rate of color development.

Nitrate + nitrite and nitrite are analyzed according to the method of Armstrong et al. (1967). At a buffered, alkaline pH the sample nitrate is reduced to nitrite in a column of copperized cadmium. The sample stream with its equivalent nitrite is treated with an acidic, sulfanilamide reagent and the nitrite forms nitrous acid which reacts with the sulfanilamide to produce a diazonium ion. N-Naphthylethylene-diamine added to the sample stream then couples with the diazonium ion to produce a red, azo dye. With reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured; without reduction, only nitrite reacts. Thus, for the nitrite analysis no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by difference.

The silicic method is analogous to that described for phosphate. The method used is essentially that of Armstrong et al. (1967), wherein β-silicomolybdic acid is first formed from the silicic acid in the sample and added molybdic acid; then the silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using stannous chloride as the reductant. This method is quite sensitive to laboratory temperature. The method is also nonlinear at high silicate concentrations, necessitating on-line dilution of samples from deep and high latitude waters and/or correcting for the nonlinearity during data processing. The OSU choice has been to dilute high concentration samples on-line by using larger flow of a diluted molybdic acid reagent while the ODF choice has been to correct for the nonlinearity during data processing. An adaptation of the Armstrong et al. method by Gordon et al. (in

preparation) greatly reduces the effect of laboratory temperature and improves linearity. This adaptation is presented here.

## 1.4. Contents of this Document

Section 2 describes the analytical equipment for which this protocol has been developed. Section 3 contains general notes on the reagent and water quality required for this work. Sampling techniques, sample storage and some general considerations regarding sample storage appear in Section 4. The rigorous demands of the WOCE and JGOFS programs focussed considerable attention on calibration (or, "standardization") procedures, with respect to both methodology and laboratory techniques. Recognizing this focus, Section 5, entitled "Calibration Procedures," has been separated from the detailed discussion of the chemistry and mechanics of the CFA methods. Section 6 details the methods including reagent preparation and factors affecting accuracy and precision. Section 7 contains a discussion of the data processing from absorbance data (or their voltage analog) to nutrient concentrations. This section includes a brief discussion of concentration units and conversion from volumetric to gravimetric units. There is a brief overview of quality assurance procedures in Section 8, bibliographic references in Section 9, and a Glossary in Section. Appendix A is a reference chart for pump tube colors and Appendices B & C contain summary instructions for start up and shut down of the RFA System.

# 2. APPARATUS

## 2.1. Continuous Flow Analyzers.

This protocol covers use of the Alpkem Flow Solution IV system (FSS). In this Protocol, the abbreviation "CFA" refers to continuous flow analyzer systems including the FSS as well as both the Technicon AutoAnalyzer II and Alpkem systems. The Alpkem systems, using 1 mm ID flow systems, have the advantage of speed (ca. a factor of two), lower consumption rate of reagents and seawater samples (ca. a factor of four or more) and somewhat lower space requirements than for the AA-II.

Any CFA system includes an automated sampler that introduces the seawater samples into the analytical system at precise intervals. It separates the samples by introducing for short periods of time a "wash" consisting of low nutrient seawater or artificial seawater having low nutrient content. The effect of the wash is to provide a low-concentration marker (generally a negative-going "spike") between samples and between standards. It serves little useful purpose as an actual "wash" of the system. In fact, it could as well be a natural seawater of mid-range concentration. Use of deionized water as a wash or "base-line" sample between seawater samples is not advised because it introduces considerable noise in the system. In addition, slow washout of coatings on the flow-cell windows confuses the use of such base-line samples to provide correction for drift or as measures of reagent blank absorbances.

The next major component is a peristaltic pump that simultaneously pumps samples, reagents and air bubbles through the system. The pump is the analog of the chemist who pipets reagents into samples in manual methods. The analytical "cartridges" are systems of injection fittings, helical mixing coils and heating baths. Figure 2.1 schematically illustrates the general components of a CFA system.

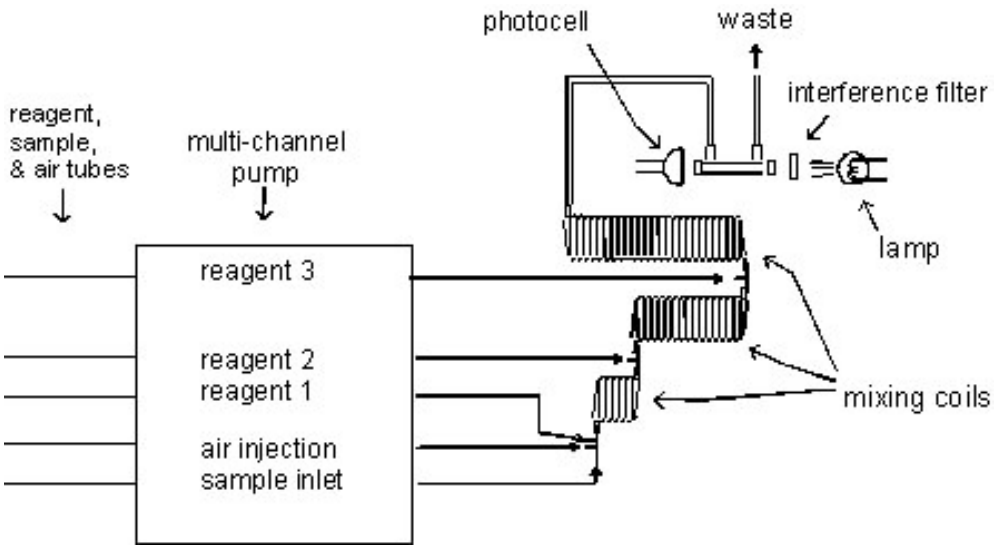


Figure 2.1. A generalized continuous flow analyzer, schematic picture

FOR SATISFACTORY RESULTS: The components must be arranged with several ideas in mind. First, the pathlengths between sampler and pump, pump and analytical "cartridges," etc. must be kept as short as possible. This is especially true of parts of the flow streams that are not segmented by air bubbles, eg. the lines between the sample "sipper" and the pump.[1] Otherwise excessive mixing between adjacent samples and between samples and wash water results. Second, all components should be arranged in a near horizontal plane. This is especially true of the relationships between the sample sipper tube, the flow stream "waste" outlets and the levels of reagents in the reagent reservoirs. Thus, it is not good practice to locate reagent reservoirs on shelves over the CFA, or drain waste tubes of small diameter into receptacles on the floor. The objective is to avoid large hydraulic pressure heads along the flow stream. Large hydraulic heads promote noisy output signals. A third point is to avoid "dead volumes" in the flow channels. These can be introduced by debubblers, voids in butt joints between ends of tubes, and unnecessarily large inside diameter tubing. The solutions are to avoid debubblers if not absolutely required, to use low-dead volume tube fittings or to cut the ends of connecting tubing square and make certain they are tightly butted together. Voids at joints between connecting tubing and glass fittings are notorious for disrupting bubble patterns.

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Regular bubble patterns are necessary for noise-free output signals. Achieving good bubble patterns primarily depends upon maintaining a clean system. Wetting agents specific to each analysis, at proper concentrations, are vitally important.

[1] The "sipper" is a ca. 1 mm I.D. stainless steel tube that dips into the successive sample containers on the sampler tray under control of the sampler timing circuit.

Heating baths set at excessively high temperatures can also seriously disrupt bubble patterns.

## 2.2. Volumetric Laboratory Ware

All volumetric glass- and plastic-ware used must be gravimetrically calibrated in order to achieve relative precision at the fractional-percent level. Plastic volumetric ware must be calibrated at the temperature of use, plus or minus 2-3°C. Temperature effects upon volumes contained by borosilicate glass volumetric ware are much less than those of plastic ware, are well documented, and volumes at normally encountered ship and shore laboratory temperatures can easily be computed from data at any usual calibration temperature (eg. Kolthoff et al., 1969; Weast, 1985). The weights obtained in the calibrations must be corrected for the density of water and air buoyancy. **The gravimetrically calibrated volumes must be used in computing concentrations of standard solutions, not nominal volumes!**

A note about the use of glass volumetric ware and contamination of standard solutions by dissolution of the glass is in order. Our group has collected data on dissolution rates of Pyrex™ volumetric flasks. Such flasks gave initial dissolution rates of 0.03 to 0.045 μM silicic acid per minute into LNSW and virtually no dissolution into DIW. Note that these data apply to the set of flasks tested and these flasks had had a varied history of prior use in our laboratories. Prior leaching by acid solutions, for example might profoundly influence the dissolution rate, probably but not necessarily, in the direction of slower dissolution.

Because of the marked superiority of borosilicate glass flasks to plastic with respect to thermal expansion and because of the very slow attack by DIW, borosilicate glass we recommend for preparation of the concentrated "A" and "B" standard solutions (the OSU "ABC" standard solution nomenclature is explained in Section 5). Exposure time to the glass is kept to minimum. The details of use of glass and plastic ware for standard preparation are given in Section 5.

### 2.2.1. Volumetric flasks

Volumetric flasks of NIST Class A quality, or the equivalent, should be used because their nominal tolerances are 0.05% or less over the size ranges likely to be used in this work. Class A flasks are made of borosilicate glass and, as just noted, solutions are transferred to plastic bottles as quickly as possible after they are made up to volume and well mixed.

Plastic volumetric flasks (polymethylpentene, PMP, or polypropylene) must be of ISO class 384 tolerance. **N.B. All volumetric flasks, including Class A, must be weight calibrated before use!** Occasional calibration errors are made by manufacturers.

Emphasizing once again, because of their larger temperature coefficients of cubical expansion and lack of tables constructed for these materials, the plastic volumetric flasks must be gravimetrically calibrated over the temperature range of intended use and used at the temperature of calibration within 2C. The volumes of plastic volumetric flasks calibrated in the OSU laboratory have been stable over several years' time. However, it is recommended that each volumetric flask be recalibrated once after an interval of ca. six months and annually after that in order to accumulate good replicate calibration data. Use of uncalibrated plastic volumetric ware and lack of attention to solution temperature at the time of making up standards can lead to aggregate errors on order of three percent or even more.

### 2.2.2. Pipets and pipettors

All pipets or pipettors should have precision and nominal calibration tolerances of 0.1% or better. These too must be gravimetrically calibrated in order to verify and improve upon this nominal tolerance.

Up to this time two commercial pipettors have proven to provide adequate precision for WOCE nutrient work in our experience. The first is the U.S.-made Lab Industries Standard REPIPET™ which dependably provides 0.1% precision. To achieve 0.1% accuracy the REPIPET must be gravimetrically calibrated; because its volume adjustment has been known to shift slightly it must be regularly recalibrated during and after a cruise. Considerable skill which can be attained with practice is required to achieve the 0.1% precision. Because REPIPETs employ a glass syringe they contaminate with silicic acid unless certain precautions are taken. A plastic reservoir minimizes contamination from that source. Flushing the syringe three or four times by dispensing to a waste receptacle immediately before use removes the most contaminated solution from the syringe.

The second, high precision pipettor readily available in the U.S.A. is the Eppendorf™ Maxipettor™. Its specifications claim 0.05 to 0.1% precision and accuracy in delivery volumes ranging from 10 to 1cc, respectively. These specifications apply to use with "positive displacement" tips. We individually calibrate matched pipettors and tips. The pipettors and tips must be serially numbered by the analyst and correct matching maintained during use. Gravimetric calibrations performed by five analysts and technicians of varying skill levels and with several different pipettors and dozens of tips have shown that these specifications are credible. These pipettors should nevertheless be gravimetrically calibrated by each analyst who will use them to verify accuracy for each new pipettor and set of tips and to ensure that each analyst's skill with the pipettor is adequate. Because the wetted parts of the Maxipettor are plastic, contamination with silicic acid is not a problem. In summary, Maxipettors appear to be remarkably insensitive to operator technique and are quite robust. They are much easier to use, particularly on a moving ship, that the Repipet.

There may be, by now, other commercially available pipettors that have sufficiently high precision and accuracy for this work. However we have not

certified any others as of this writing. Other nominations are welcome, particularly when accompanied by qualifying data.

Volumetric, borosilicate glass transfer pipets of the Mohr type are no longer recommended for preparation of reference or calibration standards in the WOCE Hydrographic Program (WHP). There are several reasons for this. Their accuracy and precision, with the most skillful use and gravimetric calibration, do not match those of the Eppendorf Maxipettor. Under marginal conditions of sea state it becomes difficult to maintain the attention to detail required for acceptable accuracy and precision. Their accuracy of delivery is extremely sensitive to their state of cleanliness. Being glass and of awkward dimensions they are susceptible to breakage. Their accuracy of delivery depends upon their state of cleanliness. Breakage at sea makes it impossible to recalibrate them should an error in their calibration be suspected.

### 2.2.3. The need for calibration of pipets at sea

Because their delivery volume settings can slip, REPIPETs must be calibrated once every week to ten days at sea to detect possible changes in delivery volume. At-sea "calibration" is done by dispensing replicate deliveries into glass ampoules and sealing the ampoules with a oxygen-gas torch. Care must be taken not to evaporate any of the water delivered, for instance from a drop deposited in the neck of the ampoule. The ampoules are returned to the shore lab where the volumes delivered are weighed and the delivery volumes calculated and checked. This is done as quickly as possible after the end of the cruise.

Note that during this step it is not important that glass drawn off from the ampoule neck be saved. It may be discarded. However, when the final opening, rinsing and drying of the ampoules is performed after obtaining their gross weights considerable care must be taken. One must not only not lose any fragments of glass when cracking off the necks but must keep each paired broken-off neck and parent ampoule together. This can be done by assigning each ampoule and broken-off neck to their own numbered and tared container such as a borosilicate glass Petri dish. The opened and rinsed (DIW) ampoules, necks and their Petri dishes are dried in an oven at 105-110 C overnight, cooled to room temperature and reweighed.

In our experience so far we have not found it necessary to check calibrations of Maxipettors while at sea. Pre- and post-cruise calibrations are sufficient.

## 2.3. Other Laboratory Ware

For the remaining laboratory ware the main requirements are convenience, scrupulous cleanliness, and guarding against exposure of either standard solutions or silicic acid reagents to contamination by glass dissolution. Unpublished results of work here at OSU and at the U.S. Geological Survey in Menlo Park, California, indicates that an effective method for cleaning and maintenance of standard and

sample bottles is by use of acetone (Gordon et al., unpublished results; S.W. Hager, personal communication) or 10% HCl (Gordon et al., unpublished results). The acetone procedure consists of rinsing once or twice with DIW to remove most dissolved salts, rinsing once with acetone to remove hydrophobic deposits (including cell-membrane lipids, rinsing with DIW two or more times and finally storage until next use, "shaken dry" and capped. For the HCl procedure simply rinsing with the HCl followed by thorough rinsing with DIW and storage as for acetone treatment suffices. The HCl procedure avoids the fire and toxicity hazard of acetone use.

Regular cleaning of storage containers reduces variance in the analytical results, i.e., samples degenerate more slowly in well maintained bottles than in dirty ones. Similar cleaning procedures using isopropyl alcohol or DIW instead of acetone or dilute acid did not maintain low variance as well as did the acetone or HCL.

# 3. REAGENTS AND CALIBRATION STANDARDS, GENERAL CONSIDERATIONS

## 3.1. General Specifications

In general all reagents must be of very high purity. Terms denoting adequate purity in the U.S.A. include "C.P. (Chemically Pure) Reagent Grade," "Analytical Grade," "Analyzed Reagent Grade" and others. It's important to remember that chemical manufacturers' specifications of "assay" on their labels are not to be taken as actual assays of the materials in the labeled bottles. There is more on this later.

**N.B.** When weighing and packaging "preweighed" reagents or "preweighs" for work at sea it is imperative that the label of each preweigh container contain the name of the manufacturer and lot number from the label of the original container. Further, when making up the actual reagent solutions, it is imperative that all of the information contained on the label of the preweigh package be copied into the laboratory notebook. The analyst must also note the time and date of reagent preparation and the time and date when its use is begun. Such information can be invaluable for tracing sources of problems arising from "bad batches" of reagents or improperly formulated or weighed reagents.

Special considerations apply for two of the reagents to be used for standard materials in this protocol, for nitrite and for silicic acid. Because some candidate materials are not available in sufficient or known purity or they may be unstable with time. For example, assays of nitrite salts given by reagent manufacturers are commonly in the range of 95-96%. The assays are often given to 0.1% but the figure is really a minimum guaranteed value and not necessarily precise or accurate; nitrites are unstable salts. Fortunately, nitrite concentrations in the oceans are generally low and the required analytical precision is usually only on order of 2-5% of water column maxima at best. When an assay is given on the reagent bottle one may use that value to adjust the weights taken. Reported nitrite concentrations using this procedure therefore might be biased by ca. five percent, a figure we regard as acceptable for nitrite. Note that no precision or accuracy specification has been adopted for nitrite concentration in the U.S. WOCE hydrographic program (U.S. WOCE Office, 1989, p. 30). An ultimate solution to this problem is to perform a chemical analysis for the nitrite salt in the material purchased. This is something we have not yet done.

The nitrite assay cannot be checked by passing the nitrite standard through the nitrate channel. Unfortunately, this is the very procedure we use to check the efficiency of the cadmium reductor, by comparing the responses of the nitrate channel to nitrite and to nitrate standards. This situation also impacts the accuracy of the procedure for checking reductor efficiency. Obviously, if the purity of the nitrite standard is only 95%, our reductor efficiency results will be biased by this amount. A reductor that appears to be 100% efficient might actually be only 95% efficient. We have not experienced apparent reductor efficiencies exceeding 100% leading us to believe that the nitrite "standard" materials we have been using are not substantially less than 100% pure.

In the WOCE Hydrographic Program the objective for silicic acid precision is much stricter. Although the specified objective is only 3% precision and accuracy, several laboratories routinely achieve short-term, within-laboratory precision of a few tenths percent (Weiss et al., 1983). Hence it would seem desirable to achieve accuracy in preparation of standards to this level. The goal of the protocols and methods set forth in this Suggested Protocol is on order of 0.1% for accuracy and precision of standard preparation. Even though sodium fluosilicate is a convenient and reproducible material for producing working standards to calibrate the CFA, it is not available in sufficient purity to function as a calibration standard on its own. Individual batches from the same or different manufacturers differ in equivalent silicic acid content by as much as 3% or more. Therefore, although fluorosilicate may be used as a routine calibration standard, its composition must be assayed by comparison with standards prepared by fusion of very pure silicon dioxide.

Sufficient replicate comparisons of pure silicon dioxide ($SiO_2$) with replicate standards prepared from sodium fluorosilicate must be made to assure adequate confidence in the assay. Extremely high purity $SiO_2$ is available from suppliers to the semi-conductor industry; more than 99.9% purity is readily available at modest cost. (It must be dried by ignition at high temperature following manufacture's' specifications in order to meet this purity criterion.)

A suitable procedure is given by Kolthoff et al. (1969, p. 651). This procedure is followed as far as the dissolution of the fusion cake. At that point the solution is diluted to a precise volume and a suitable aliquot is diluted to a working concentration. This concentration should be similar to that of a fluorosilicate working standard made from the fluorosilicate reagent to be assayed. Finally, the solutions are compared using the method given in this Protocol. Once a bottle of sodium silicofluoride has been so assayed it may be used for years if care is taken to prevent contamination. **N.B.** At the outset of the assay process the fluorosilicate should be mixed thoroughly using a scrupulously clean metal spatula to assure homogeneity.

Throughout the directions for reagent and standard preparation given later the amounts of reagents prepared at one time may be adjusted to suit the usage rates anticipated. Thus, the amounts for routine use at sea for the RFA are about one fifth the amounts for the AA-II. The only caveats are that the concentrations must be as specified here and attention must still be paid to reagent and standard storage conditions and useful lifetimes.

The chemist should note, in checking our molar concentrations of reagents prepared according to the directions in the next sections, that many of the reagents are quite concentrated. Therefore, molar concentrations (g-mole/liter) of the reagents cannot be simply calculated from the sums of, for instance, concentrated sulfuric acid and DIW taken in making up the 8.5 M sulfuric acid. In this example electrostriction greatly reduces the final volume from the sum of the volumes of acid and water originally taken. The molar concentrations of the final reagents are not simple values because the recipes were either taken from the literature or developed in terms of the simple volume/volume, volume:volume or weight/volume proportions given in the recipes here. The calculations of molar concentrations of

strong acid solutions given here were calculated using the tables given by Weast (1985, pp. D-232 and D-262).

### 3.2. Deionized Water

Dependable, pure water is an absolute necessity for the nutrient work. It may be double distilled water (DDW) or deionized water (DIW). In the case of DDW, the analyst must be careful to avoid contamination with silicic acid from dissolution of quartz or glass stills, connecting tubing or reservoirs. There are several high quality, commercially available systems that consistently deliver high purity DIW having 18.0 Megohm-cm specific resistance or better (American Society for Testing and Materials, or ASTM, Type I). These systems generally employ four steps including a prefilter, a high capacity resin cartridge and two tandem, ultrahigh purity, mixed-bed cartridges. This water suffices for preparation of reagents, higher concentration standards and for measurement of reagent and system blanks.

To be certain of an adequate supply of DIW or DDW at installation time in the shipboard laboratory it may be necessary to obtain reliable DIW or DDW supply from a local laboratory or vendor, perhaps 50 l or more. This supply may have to last through the first few days at sea while purer water from the ship's evaporator (distilling system) flushes shore water out of ship's storage tanks. In port water supplies are notoriously impure and can rapidly exhaust the very expensive cartridges in a demineralizer system. Furthermore, the high concentrations of silicic acid present in many coastal fresh waters cause some silicic acid to pass through many commercial water purification systems. Often it is best to obtain feed water for the laboratory deionizer system directly from the ship's evaporator if possible. The analyst must check the water immediately for possible contamination by phosphate and/or silicic acid. These are common ingredients in formulations for cleaning and eliminating boiler scale in evaporators.

### 3.3. Low-Nutrient Seawater (LNSW)

The final working, or calibration, standards are best prepared using natural seawater of low nutrient content as the matrix. Given the complex composition of seawater, there are many possibilities for interferences by exotic constituents. An inherently dependable way of compensating such errors is to make the working standards in a matrix as close in composition to the unknown samples as possible. Fortunately, low nutrient seawater is abundantly available in the central gyres of the open ocean in late spring and summer. Ideally, it should be collected and filtered through a filter having a pore size of 10 μm or smaller and then be stored in the dark for several months to stabilize. Filtration and storage are not absolutely necessary, but more consistent day-to-day results will result from use of filtered and aged seawater. The accuracy and precision of working standards will not suffer markedly using fresh, unfiltered seawater if the time between preparation and use of the standards is kept short, less than two or three hours, to avoid significant change.

It is possible to use coastal surface seawater as the LNSW but then considerable care and ingenuity must be applied to select the optimum season, to filter off the much higher biomass almost certain to be present and to age the seawater for several months. Subsequent refiltering might also be necessary. Finally, a commercial source of low nutrient natural seawater has recently become commercially available. It is sold in one liter bottles.

The nitrate concentration of the LNSW should be less than ca. 2 μM to avoid driving the total concentration of nitrate significantly out of the concentration range for which the nonlinearity has been measured.

# 4. SAMPLING AND SAMPLE STORAGE

Two factors dictate nutrient sampling procedures, a) the range of concentrations of nutrients present in the oceans, from extremely low to only moderate concentrations; and b) the biochemical and chemical reactivity of the nutrients present in seawater.

The extremely low concentrations present in oligotrophic surface waters of central gyres in spring and summer can be contaminated seriously during sampling and sample storage. Microbial films form on sampler and sample bottle walls in very short times, hours to a few days. Such films can take up or release nutrients significantly.

The nutrients vary widely in biochemical and *in vitro* reactivity. Nitrite and phosphate are the most labile while silicic acid appears to be the least reactive. Nitrite concentrations in seawater samples and standard solutions often change markedly in a few hours under common storage conditions. Yet silicic acid samples and standards can often be stored at room temperature (in the dark) for days with little detectable change.

The following sections outline procedures that have been found effective in producing high quality nutrient data. Close adherence to these or similarly effective alternates is necessary.

## 4.1. The Water Samplers

At the beginning of every cruise leg and at approximately weekly intervals or more often if indicated, the water samplers (usually 10L Niskin samplers in the WHP) must be inspected for evidence of biological or inorganic films on the interior walls, valves or end caps. A powerful flashlight or work light is necessary for this. Watch especially for iron rust staining on walls near the points where sampler handles are installed and on the end caps where coatings on springs may have worn through allowing the spring to corrode. If present the rust stains must be removed with 8M, or stronger, HCl. Springs whose coatings have worn through must be replaced and any other sources of rust must be eliminated or adequately protected from corrosion. Check with the hydrographic technicians for components and assistance. Accumulated microbial films should be removed using suitable brushes, scouring agents and detergent solutions. The scouring agents and/or detergents used must be checked to be certain they are nutrient-free.

## 4.2. Nutrient Sample Containers

These may be made of any of several plastics. Glass of any kind including "resistance glass" or "borosilicate glass" is not acceptable. Any glass contaminates the samples with silicic acid by easily measurable dissolution. High density polyethylene or polypropylene small mouth bottles ("Boston Rounds") of 30cc (1oz.) capacity and "Poly Seal " caps serve very well as do their wide mouth versions if

the samples are not stored in them too long.. These bottles, when filled ca. 2/3 full, contain ample water. Many laboratories have shown these bottle materials to be acceptable; they neither add nor remove nutrients from seawater samples. Before using them for the first time they are easily cleaned with warm detergent solutions but again, one must avoid nutrient-containing detergents. Some workers find 50cc screw-capped, plastic centrifuge tubes more useful. The particular plastics in these tubes should be checked for possible interferences such as adsorption of phosphate from the samples.

The nutrient sample bottles must be cleaned frequently to prevent nutrient uptake or release from microorganisms that colonize their inside walls. Our group has conducted experiments at sea to evaluate the variance in the data that arise from this source particularly if samples have to be held for a time before analysis, with or without refrigeration.

Cleaning at least once every four days with acetone or dilute acid following a procedure such as that in Section 2.3 significantly reduced variance in replicate samples. The experiments also showed that rinsing with DIW or isopropanol was not effective in stopping the activities of the microorganisms in these particular seawater samples (open ocean, central gyre).

Our work showed that the variance in all but the silicic acid analysis increased significantly (by factors of up to 3) within six hours even if the samples were refrigerated. In these experiments the accuracy did not deteriorate, an observation we do not understand.

After cleaning the bottles may be stored filled with DIW or shaken nearly dry and stored in that condition. They must not be stored filled or partially filled with seawater! At the very least the seawater remaining after analysis should be poured out and the bottles "shaken dry."

### 4.3. Sampling Order, Procedure and Precautions

In general, drawing the nutrient subsamples immediately after the samplers arrive on deck, although desirable, is not critically important. It is certainly less so than for some of the dissolved gases (eg. dissolved oxygen, CFC's and other trace gases such as nitrous oxide and carbon monoxide). The nutrients should be sampled before tritium samples if possible. This can save up to one hour of nutrient decomposition time. In any case, the analyst should not waste any more time at this stage than is necessary especially because perhaps an hour will have already been lost while the other preceding samples have been drawn. One should try to keep the interval between arrival on deck and start of analysis to less than an hour and a half if possible. When no other gas or tracer samples than dissolved oxygen are to be taken, the nutrients immediately follow oxygen sampling. When practical, preliminary startup of the CFA can be done before actually beginning the nutrient sampling in order to keep the delays to a minimum.

The sampling procedure is important. Sample containers must be rinsed three times with approximately 10-15cc of sample, shaking with the cap loosely in place

after drawing each rinse. Pour the rinse water into the cap to dissolve and rinse away any salt crusts remaining from earlier sampling and trapped in the threads of the cap. Finally, fill the sample container ca. 2/3 to 3/4 full (**no more,** see Section 4.4) and screw the cap on firmly.

During sampling care must be taken not to contaminate the nutrient samples with fingerprints. Fingerprints can contain measurable amounts of phosphate. One should not handle the end of the sample draw tube, touch the inside of the sample bottle cap or any place on the sample bottle neck. Another point to watch while sampling is not to let the nutrient samples be contaminated with seawater, rainwater or other spurious material dripping off the rosette or water samplers. This is somewhat more of a problem with wide mouth sample bottles than with narrow mouth Boston Rounds.

Immediately upon completion of the nutrient sampling take the samples to the analytical laboratory and begin the analyses as quickly as possible. Again, if possible, have the CFA running with reagents flowing before going to collect the samples. Often the preliminary blank and standard sequences can be programmed into the analyzer during waiting periods while sampling. In a series of observations, phosphate concentrations changed by 0.005 µM/hr for Antarctic waters while sitting in the sampler tubes on the analyzer sampler (Gordon and Dickinson, unpublished data).

## 4.4. Sample Storage

Nutrient samples must be analyzed immediately after sampling if at all possible! The only exception is if the CFA is not functioning correctly. Refrigeration of nutrient samples is not effective for more than two to four hours, depending upon precision and accuracy required. Refrigerator temperatures are not low enough to stop growth of many marine organisms, those which grow optimally at typical deep-sea temperatures of 1-4C. Obviously, samples from south of the Antarctic Convergence would not be preserved by the usual temperatures achieved by domestic refrigerators (typically, 4-5°C). There has not been a great deal of quantitative data published on this subject (but see Gilmartin, 1967; Grasshoff, et al., 1983; Macdonald et al., 1986; Chapman and Mostert, 1990). However most analysts agree that whenever possible natural seawater samples should be analyzed for nutrients as quickly as possible after collection. As a last resort, if the CFA is not operable and it appears that it can be repaired within less than eight or perhaps up to 12 hours, the samples can be refrigerated in the dark at 4 C or less. Should this happen, it must be noted in the laboratory notebook and/or on the sample log sheets. In general, the resulting variance and accuracy will suffer.

If longer storage is necessary samples should be frozen as soon after collection and as rapidly as possible. Before freezing ensure that no sample bottles are filled more than 3/4 full and all caps are firmly screwed on because loss of brine can cause extreme systematic errors. If a freezer is used, it should be a deep freezer ($t \leq$ -20C). Good air circulation around the bottles in the freezer is important. An open wire rack is preferable to wooden trays. Ensure that the

sample bottles remain upright while freezing and while in storage. Again, loss of unfrozen brine will be fatal to good results. Errors on order of several 100% can result! Often, when a low temperature freezer is not available, a better freezing method is to use an ice-salt bath and later to transfer the samples to the storage freezer. Another expedient is to use an anti-freeze solution in a bath in the ordinary freezer to improve heat transfer rates during the freezing step. Nutrient samples continuously degrade during frozen storage. Analyze them as soon as possible. Keep a maximum-minimum recording thermometer in the storage freezer to detect otherwise unnoticed, thawing temperatures that might occur before analysis. As a final note, samples should be frozen only as a last resort, when they cannot be analyzed within 8-10 hours of collection.

IMPORTANT! To thaw frozen samples for analysis use a tepid water bath (ca. 40C) and thaw the samples in less than 15 minutes; no more at a time than can be accommodated by the CFA, perhaps 5-10 at a time. A running (cold) water bath is also satisfactory if the samples can be thawed within 15 minutes. In either case take care not to contaminate the samples with the water used for thawing; make certain the caps are screwed on firmly and keep the bottles upright with the caps above the water line in the bath. ALSO IMPORTANT! Be certain to mix the samples thoroughly after thawing in order to mix the overlying, fresher water completely with the concentrated, underlying brine that was formed by the freezing. Do this by swirling not by shaking in order to avoid creating fine bubbles that will cause noisy traces. If the samples are not well mixed, errors can exceed 300% depending upon vagaries of geometry of the CFA sampler, ship motion and other conditions.

If silicic acid concentrations exceed ca. 40µM the samples will have to be saved after the first pass through the CFA and re-analyzed after standing for 24 hr. Silicic acid numbers will be biased low for the first pass. Store the samples in the dark at room temperature to allow polymerized silicic acid to depolymerize. Then, mix the samples thoroughly again before analysis.

## 4.5. Sampling Summary

To repeat briefly, because sample handling is so important, some of the factors affecting accuracy and precision:

a. Careful cleaning and maintenance of water samplers and nutrient sample bottles are essential for accurate and precise nutrient results.
b. Subsampling from the water samplers must be done carefully.
c. Nutrient samples should be analyzed as quickly as possible after sampling.
d. Accuracy and precision suffer with storage in a refrigerator or by prolonged frozen storage.
e. Gross silicic acid loss by polymerization can result if frozen samples are not thawed and analyzed properly.
f. Brine loss during freezing and frozen storage can cause dramatic loss or concentration of nutrients!
g. Incomplete or no mixing of brine and brackish supernatant water after thawing of frozen samples can cause enormous errors.
h. Carefully note in the field notebook any deviation from immediate analysis of the samples.

# 5. CALIBRATION PROCEDURES

## 5.1. Calibration Protocol

This protocol assumes that working standard solutions for calibration of the analyzers will be prepared by dissolution at sea of pure, crystalline standard materials, pre-weighed ashore, followed by dilution to appropriate, working concentrations (described in Sections 5.2-5.4). Efforts have been made in the OSU laboratory to prepare stable working calibration standards at oceanic concentrations that can be prepared ashore prior to an expedition, shipped to the expedition ports and stored with integrity for several months. These efforts have not been successful. Therefore this protocol continues the scheme of preweighing and packaging the dry, crystalline standard materials and making the working standard solutions at sea.

The procedure given here consists of first preparing a set of "A" standards using precisely weighed (to ±0.1 mg) primary standard materials (phosphate, nitrate, nitrite) dissolved in DIW and made up to accurately known volumes. The weights taken must be corrected to *in vacuo*. The nominal weights given here for standard preparation are **NOT** *in vacuo* weights. The correction is approximately 0.1%. The buoyancy correction should be calculated for the laboratory conditions of atmospheric pressure, temperature and humidity occuring at a given institution. It will be essentially constant and one value for the correction factor can probably be used at all times. However, this should be checked for each set of laboratory conditions. For all WOCE work and deep-water work in JGOFS, standard concentrations must be calculated for the exact weights taken, not the nominal weights.

Nitrite A standards are made separately but phosphate and nitrate may be made up as a single, mixed A standard. A "B" standard is next prepared by dissolving a preweighed silicic acid standard material in DIW, adding an aliquot of A standard(s) and making the solution up to an accurately known volume with DIW. Finally, an aliquot of the B standard and an aliquot of the nitrite A standard are made up to an accurately known volume using LNSW. This makes the working or calibration standard or "C" standard. This standard is in the range of typical, oceanic concentrations. The working standards are thus mixed standards containing all four nutrients. Note that when nitrite is present in the mixed standard appreciable systematic errors in the nitrate results can occur under certain conditions (Garside, 1993). These conditions are discussed in the section on nitrate analysis. Two tables, Table 5.1 and 5.2 give examples of concentrations of nutrients in the B standard and in the final working standards.

The proportions of the different nutrients in the standards may need to be adjusted to approximate ca. 80 ± 10% of their maximum concentrations in the ocean basin to be studied. This may be done by adjusting the weights of primary standard materials taken or the volumes of A standards pipetted into the B or working C standards, as appropriate. The proportions to be used should be decided before beginning a cruise leg and not changed during the leg if possible. The objective is to maintain constancy of analytical parameters and to avoid the

temptation to use less than adequate volumetric techniques to alter the working standard concentrations. Considerable latitude in working standard concentrations is shown in the examples in Table 5.2.

To summarize the standard solution nomenclature:

A standard ≡ stock standard solution containing primary standard nitrate, phosphate, or nitrite prepared in DIW. It may contain both nitrate and phosphate.

B standard ≡ stock standard solution containing aliquots of the phosphate and nitrate A standards plus the primary standard for silicic acid (also prepared in DIW).

C standard ≡ the calibration standard or working standard that is actually introduced into the CFA for calibration (prepared in low-nutrient seawater). It contains aliquots of the B standard and the nitrite A standard.

The timing and frequency of standard preparations, comparisons and analyzer calibrations given here represent minimum guidelines. Individual laboratories and analysts may have more stringent protocols. Other protocols are acceptable only insofar as they result in achieving or bettering the WOCE and JGOFS specifications of precision. Although no accuracy specification is given for WOCE, the analyst must do everything possible to also achieve high accuracy. The protocols given here, if carefully followed, will assure achievement of the WOCE and JGOFS specifications.

**N.B.** It is imperative the analyst keep a complete and detailed record in the laboratory notebook of all pipet, pipet tip and volumetric flask identities used for preparation of each standard. Further, the label information for each preweighed standard used must also be recorded in the notebook. Record the date and time of preparation and date and time placed in use.

5.1.1. Scheduling of preparation and specifications of A standards.

Prepare two sets of A standards at the beginning of a cruise or cruise leg. One will be used for preparation of working, calibration standards. The other will be used for preparation of reference standards to be used to check the integrity of the working A standard. Whenever possible, the first check should be carried out before the first station of the cruise or leg and certainly before the end of the first week. The absorbances of working standards prepared from the A standards must agree within 0.3 and 0.4% for nitrate and phosphate, respectively. Nitrite a standards must agree within an absorbance difference corresponding to 0.05 μM. If the standards do not agree within these specifications, a third and, if necessary, a third A standard is to be prepared and additional checks conducted immediately. Usually the first two standards will agree within specifications and either of them may be used to prepare the working standards. If repeated preparations do not

meet these specifications, fundamental studies of the gravimetric and volumetric techniques employed must be made and techniques improved until satisfactory results are obtained. Obviously, this should be accomplished before ever setting out to sea. In such cases something is likely to be seriously wrong with homogeneity of the standard reagent material, the weighings or the volumetric work. Any wildly discordant A standard preparations may be discarded after complete and appropriate notes have been entered in the field notebook. Thus, a sufficient number of dark, plastic storage bottles must be provided to save as many as four A standards.

Retain all concordant A standard preparations throughout a cruise leg, or until used up. Prepare a fresh A standard at least once a month and immediately check against the previously prepared standards. If possible, the working A standards should be compared with an A check standard once per week, the comparison data processed and examined that day and results of the comparison noted in the seagoing lab notebook.

5.1.2. Scheduling of preparation of B standards. At the beginning of a cruise leg duplicate B standards must be prepared and compared. Replicate B standards should agree within 0.2% for silicic acid concentrations. Similar considerations apply as for A standards if the initial duplicates do not agree. Prepare B standards at least once per week. This frequency must be monitored for the particular shipboard laboratory conditions by following this scheduling protocol. More frequent checking may be necessary under some conditions. Lack of agreement within the specifications indicates a similar procedure to that for the A standards: Preparation of additional replicates, identifying a possible bad preparation, etc. Note that each B standard preparation requires a new, preweighed silicic acid standard. Provision must be made for a sufficient number of B standard preparations to meet the worst-case number of preparations for the duration of the cruise.

5.1.3. Scheduling of preparation of C standards. These are, in general, stable for no longer than four to six hours. They must be prepared just before each station unless the stations are separated by no more than three hours. Lack of agreement between results from deep water samples from adjacent stations may indicate storage of working, calibration standards for too long.

5.1.4. Frequency of calibration of the nutrient analyzer. The drifts of the nutrient analyzer sensitivities for all the methods, colorimeters and laboratory conditions checked at OSU appear almost always to be monotonic and approximately linear with time. This seems valid for periods of about one to one and a half hours, approximately the time required to analyze one station's set of samples. It also assumes use of the low temperature drift modification of the silicic acid method described here (Gordon et al., in preparation).

Therefore the protocol presented here consists of running a complete set of reagent blank (DIW) samples, working standard matrix (MAT) and upscale concentration (STD) calibration standards only at the beginning and end of each

station's set of samples. If the time lapse between standard sets exceeds one and a half hours, sample degradation can become a problem. Possible remedies include dividing the samples into batches with standards and blanks at beginning and end of each, or the station sample sequence can be interrupted to allow a mid-batch standard and blank set. Of course, any data processing software being used must be modified if necessary to correctly process the data. The OSU software presently does not correctly process mid-batch standards and blanks.

5.1.5. Linearity ("Beer's Law") checks. Although all of the analytical methods described in this Suggested Protocol are sufficiently linear (when corrected as necessary), linearity must be checked at the beginning of the cruise or leg, before any samples are analyzed. The checks must be repeated once a week thereafter and again at the very end of the station work, just after or together with the last station's samples. There are several reasons for this. One is that performing a linearity check provides a good test of system performance. It helps assure that all of analytical parameters are correctly set up.

To perform a linearity check the analyst measures a set of working standard solutions, of increasing concentration (eg. as in Table 5.2). There should be at least five concentration steps including the standard matrix solution. Each concentration should be measured in duplicate or triplicate arranged in pairs or triplets of sequentially increasing or decreasing concentration. Our practice is to measure these working standards first in increasing and then immediately in decreasing order.

The data from the linearity test also can be used to evaluate the "carryover correction" for each channel, an excellent quality control check. To do this optimally, there should be in one or more points in the set of standards at least one full range step in concentration, i.e. from matrix to full scale standard or full scale standard to matrix.

If the data originating group chooses the approach of accepting significant non-linearity in their protocol, the linearity data are used to correct for nonlinearity. This approach won't be discussed here. Perhaps most important, if an operating parameter has inadvertently been changed, thereby making a method excessively nonlinear, the existence of the nonlinearity measurements permits immediate correction of the problem or post-cruise correction of the non-linear data.

All of the methods presented here are linear within experimental error on averaging of several linearity checks. These methods should provide a mid-scale offset from a straight line of less than 0.2%. Note that it is difficult to see this degree of non-linearity using a simple plot of absorbance (or output voltage) versus concentration of nutrient added. To visualize the non-linearity one should do a linear regression and plot the deviation from the linear fit versus concentration add. If this specification of the degree of non-linearity is not met using this protocol, something is wrong and troubleshooting must be started before any samples (or any more samples) are analyzed.

Many previous CFA methods for silicic acid from ca. 1973 or earlier to the present displayed a mid-scale non-linearity of ca. 0.4 to 0.7% or even much more. This is a sensitive function of the extent of dilution of the sample in the particular

method. The new silicic acid method described in this Suggested Protocol, optimized to reduce lab temperature sensitivity, also effectively eliminates the nonlinearity.

## 5.2. Materials for Preparation of Calibration Standards, General Considerations

We now give a detailed set of instructions for preparation of the working, or calibration standards. The reference A standards to be used for checking the working A standards are prepared according to the same instructions and using the same high-accuracy volumetric techniques as for the calibration standards. Again, the working, or calibration, standards are used for calibrating the CFA; the reference A standards are used for checking the integrity of the calibration standards.

**Important Note**: The technique used here for calibration of the sensitivity of the CFA system incorporates one of the valuable aspects of the "method of standard additions" widely employed by analytical chemists. In that method, known or "standard" additions of the analyte are added to aliquots of the samples being measured. The objective is to obtain a quantitative measure of the response of the method to the analyte when present in the actual sample with all of its possible interfering constituents. There is more to the method but this is the salient feature for our purpose. We know that so called "salt errors" are evident in many analyses, for instance, silicic acid and ammonia. These are sometimes the result simply of the ionic strength of seawater. The literature also contains references to specific interfering constituents, eg. sulfate ion in some silicic acid analyses. For this reason our protocol stipulates that if possible the working standards used to calibrate the CFA response be made up in a natural seawater matrix. For other reasons, it is desirable that the seawater used contain low concentrations of nutrients, hence the term, "**Low Nutrient Seawater**," or "**LNSW**." Its use and actual composition will be discussed in more detail later, in section 5.4.e.

5.2.1. Primary standard materials. These must be chemically pure, reagent grade or primary standard grade chemicals, crushed and dried at 105 C for $\geq$ 2 hours and stored in a desiccator over BaO or $MgSO_4$. **NB.** The chemicals are finely **crushed** using a carefully cleaned mortar and pestle; they must not be **ground**! There is a difference.[2] Again, weights must be corrected to *in vacuo* in order to achieve 0.1% accuracy which is desirable given the reproducibility attainable with

[2] Crushing is accomplished with use of minimum force, rocking the pestle back and forth over a small amount of the material to be crushed. Grinding is defined here as a vigorous circular movement of the pestle against the mortar, with maximum or strong force. Grinding can impart considerable energy to the material being ground, sufficient to cause chemical change in some cases. The need for crushing is to fracture coarsely crystalline material into a rather fine, fairly uniform powder so that water trapped in coarse crystals can evaporate during the drying process.

CFA. The weights given below are nominal. If, for efficiency, exact weights are not taken, careful track must be kept of the exact weights placed in each "preweigh" container, air buoyancy corrections made, and actual concentrations used in subsequent computations of concentrations.

5.2.2. Deionized water (DIW). This is prepared by passing fresh water through two or more research grade, mixed-bed, ion exchange columns. See Section 3.2 for more details on commercially available systems capable of producing acceptable deionized water.

5.2.3. Artificial seawater (ASW). ASW may be used for wash solution between seawater samples and in an emergency for making up the C standards, substituting for the LNSW. ASW of salinity ca. 34.7 is prepared by dissolving 128.5 g sodium chloride (NaCl); 28.5 g magnesium sulfate heptahydrate ($MgSO_4.7H_2O$); and 0.672 g sodium bicarbonate ($NaHCO_3$) in four liters of DIW. These reagents must be high quality, reagent grade to avoid excessive nutrient or trace metal contamination.

Some laboratories have been more or less successful in making "zero nutrient" artificial seawater for measuring reagent blanks. Usually the constituent salts are too contaminated with nutrients to make this feasible, particularly with respect to phosphate and silicic acid. With the advent of commercially available, ultra-high purity materials this might now be possible. If so it would be nice to have an artificial seawater of essentially zero nutrient concentration with which to measure reagent blanks without having to worry about refraction errors.

There appear to be two drawbacks to this approach but it should be pursued. First, it is likely to be quite expensive to make ASW in the necessary quantities. Second, it is possible that interfering substances in natural seawater but not present in the usual recipes for ASW might be quantitatively significant. This places a burden of responsibility upon a laboratory using that approach to check carefully for significant differences in response between artificial and natural seawater.

5.2.4. Low-nutrient seawater (LNSW). Natural seawater containing low concentrations of nutrients should be filtered upon collection and stored in the dark for three or four months to stabilize (see Section 3.3). This water is used for preparation of the C standards. It need not contain "zero" nutrient concentrations because it is **NOT** used for reagent blank measurements. Also, it is usually too precious to be used for "baseline checks." OSU requirements are usually ca. 100L for a typical one-month WOCE-type expedition leg.

5.2.5. Volumetric glassware. For reagent preparation it is not necessary to calibrate the volumetric ware used. For standard preparation it must be gravimetrically calibrated! (See Section 2.2)

## 5.3. Preparation of A Standards

5.3.1. Phosphate and nitrate A standards: 2,500 µM $HPO_4^{-2}$ and 37,500 µM $NO_3^-$. Quantitatively transfer 0.3402 g potassium di-hydrogen phosphate ($KH_2PO_4$) and 3.7912 g potassium nitrate ($KNO_3$) to a gravimetrically calibrated 1000 ml volumetric flask and dissolve in DIW, bring exactly to the mark with DIW. If using a plastic volumetric flask, the temperature of the DIW must be within 2C of its calibration temperature. This A standard may be made up as two individual phosphate and nitrate solutions with subsequent aliquots in Table 5.1 adjusted accordingly. Store in refrigerator.

5.3.2. Nitrite A standard: 2,000 µM $NO_2^-$. In a 1000 ml volumetric flask dissolve 0.1380 g sodium nitrite ($NaNO_2$) in DIW and dilute exactly to the mark with DIW. Pure $NaNO_2$ is difficult to obtain; one should check the manufacturer's assay (eg. Kolthoff et al., 1969, p. 821). The typical purities of 97-98% are usually adequate for oceanographic purposes (see Section 3.1).

5.3.3. Ammonium A standard: 2,000 µM $NH_4^+$. In a 1000 ml volumetric flask dissolve 0.1382 g Ammonium Sulfate $(NH_4)_2SO_4$ in DIW and dilute exactly to the mark with DIW.

## 5.4. B Standard: 2500 µM in silicic acid, 50 µM in phosphate, 750 µM in nitrate.

a. Quantitatively transfer 0.4701 g sodium silicofluoride ($Na_2SiF_6$) to a 1000 ml polypropylene or PMP Erlenmeyer flask containing ca. 800 ml of DIW, cover with plastic film and dissolve on an electric reciprocating shaker at moderate speed. Alternatively, the solution can be stirred with a shaft stirrer using a plastic stirrer. Complete dissolution usually requires 2-24 hours. Gentle warming can be used to speed dissolution of the fluorosilicate. Again, note that sodium fluorosilicate cannot easily be obtained in purities greater than 99%. Hence it must be assayed against pure $SiO_2$ (available in ultra-high purity grades, see Section 3.1).
b. Inspect the solution for undissolved material and record the observation in the notebook. Quantitatively transfer the solution to a 1000 ml Pyrex™ volumetric flask. Add: 20 ml nitrate plus phosphate mixed A standard or 20 ml each of the separate nitrate and phosphate A standards if so formulated. The actual 20 ml volumes dispensed must be known to ±0.02 ml and the volumetric flask volume to ±0.1 ml.
c. Add DIW exactly to the 1000 ml mark. Mix thoroughly.
d. Store refrigerated in the dark in a polyethylene bottle previously well-rinsed with acetone, DIW, then with three 15-20 ml portions of this B standard. Do not forget to rinse the bottle cap. Use within one week.

Possible changes in nutrient concentrations of the B standard over time must be monitored by comparing freshly prepared B standard with B standard that has been stored one day or more. In general, $HPO_4^{-2}$, $NO_3^-$ and $Si(OH)_4$

concentrations are stable for several days in the B standard (if $NO_2^-$ and/or $NH_4^+$ were also present in the B standard formulation their concentrations commonly would change appreciably after only 1 or 2 days). However, this is only a guideline. The B standards must be monitored and the guideline confirmed or adjusted for each expedition because the stability of the B standard may change as a function of the particular conditions prevailing during any given time.

e. DIW for making Matrix and C standard solutions

Save approximately 500 ml of the same DIW used for preparation of the B standard and store in the same way as for B standard. This "solution" is taken as the "DIW" in the third column of Table 5.2. Taking care at this point can eliminate contamination of the DIW used for making up standards and matrices as a source of systematic error.

Table 5.1 Concentrations of nutrients in the B standard

| | |
|---|---|
| $HPO_4^{-2}$ | 50 µM |
| $NO_3^-$ | 750 µM |
| $NO_2^-$ | 0 µM |
| $NH_4^+$ | 0 µM |
| $Si(OH)_4$ | 2,500 µM |

## 5.5. Working Calibration Standards

The protocol given here produces a series of solutions of the nutrients being measured in a matrix of composition close to that of natural seawater. The solutions are used to calibrate the CFA response to added quantities of nutrients added to the LNSW. The LNSW used to make up the working standards is diluted to a significant extent by the B standard. The B standard is essentially DIW having an ionic strength of only a very few millimolar (Table 5.1). Therefore it is important to dilute the LNSW to the same extent to hold the working standard and matrix solutions to nearly the same salinities and concentrations of possible interferents. When making up a series of working standards for linearity checks this provision is equally important.

### a. Zero-added-nutrient calibration standard, or "Matrix"

Pipet an appropriate volume of the DIW reserved from the preparation of the B Standard in section 5.4.e into a calibrated 500 ml volumetric flask. The volume to be taken will correspond to the highest volume of B Standard to be made up. For example, if making the entire set of C Standards listed in Table 5.2, pipet 30 ml of the DIW into the 500 ml volumetric flask. Make to volume with the same LNSW that will be used to make the accompanying C Standards. Store refrigerated in the dark in a plastic bottle well rinsed with portions of this matrix solution. Use within six hours if so stored, within less time if not. A final word: This matrix is not to be used as a "blank" or "reagent blank." It will in general contain more or less of all the analytes as present in the particular batch of LNSW at time of use. Further the concentrations of one or more of the analytes may change with times as short as a day depending upon conditions of storage.
When analyzing for $NH_4^+$, it is critical to acid wash (10% HCl) all preparation flasks and storage containers before EACH use. It is recommended that a fresh Matrix be made prior to each sample analysis (in a smaller volume if need be).

### b. Working standards containing added nutrients, or C Standards

Nominal concentrations, given in Table 5.2, are obtained by diluting the given volumes of B standard and reserved DIW to 500 ml with LNSW. These proportions between nutrient concentrations have been found convenient for Pacific and Antarctic work. As noted earlier, they may be, and should be, adjusted for other ocean basins. This best be done by adjusting weights of solid primary standard materials and/or the volumes of aliquots taken at suitable points in the preparations. In order to keep as many analytical parameters as constant as possible during a given cruise leg, we urge that the optimal concentrations for the full scale calibrations be kept constant during the leg and that they be determined before preparing for the leg.

All working standard concentrations are nominal and must be corrected according to the gravimetrically calibrated volumes contained by all the volumetric flasks and deliveries of all the pipets employed, corrected to the temperatures at which the flasks and pipets are used. For the best work, the calibrations must be checked before and after each cruise and no less often than every six months.

Table 5.2 Working calibration standard recipes and added concentrations produced when the exact volumes indicated are made up to exactly 500 ml with LNSW.

| | | Volume (cc) | | | Concentration added (µM) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| STD# | B | DIW | $NO_2^-$ A | $NH_4^+$ A | $HPO_4^{-2}$ | $NO_3^-$ | $NO_2^-$ | N+N | $SiO_2$ | $NH4^+$ |
| 0 | 0 | 30 | 0.00 | 0.00 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0.0 |
| 1 | 5 | 24.9 | 0.05 | 0.05 | 0.5 | 7.5 | 0.2 | 7.7 | 25 | 0.2 |
| 2 | 10 | 19.8 | 0.10 | 0.10 | 1.0 | 15.0 | 0.4 | 15.4 | 50 | 0.4 |
| 3 | 15 | 14.7 | 0.15 | 0.15 | 1.5 | 22.5 | 0.6 | 23.1 | 75 | 0.6 |
| 4 | 20 | 9.6 | 0.20 | 0.20 | 2.0 | 30.0 | 0.8 | 30.8 | 100 | 0.8 |
| 5 | 25 | 4.5 | 0.25 | 0.25 | 2.5 | 37.5 | 1.0 | 38.5 | 125 | 1.0 |
| 6 | 30 | 0 | 0.30 | 0.30 | 3.0 | 45.0 | 1.2 | 46.2 | 150 | 1.2 |

# 6. THE ANALYTICAL METHODS

This section presents the details of each of the analytical methods for use with the FSS. The wavelengths given here are all satisfactory if not always maximally optimum. To assure optimal wavelength selection, it is good analytical practice to measure the absorption spectrum of the colored species for each analysis as produced by the particular method used. This is done by collecting the effluent from the flowcell, preferably directly into a microflowcell, and measuring the spectrum using a high quality spectrophotometer as quickly as possible. Modern, linear diode array spectrophotometers help immensely in this regard. It is also good technique to regularly (at 6-12 month intervals) measure the band pass spectrum of each and every interference filter to be used in all of the analyses; this includes measurement of the spare filters as well. The interlayer metal films of interference filters are subject to corrosion with resultant loss of transmission and widening of bandwidth. Of course, if flow spectrophotometers are used this becomes irrelevant.

In order to maintain regular bubble patterns, necessary for noise-free signals, the flow channels must be cleaned frequently. This should be done using 1.2M HCl followed by thorough rinsing by flowing DIW through all reagent and sample tubes. Occasional washes with 2.5M NaOH are very helpful. Care must be taken to have thoroughly flushed reagents out of their tubes and out of the system before the acid or base wash. Some of the reagents will precipitate or decompose in strong acid or base solutions and cause minor to major havoc in the system tubing. Related to cleanliness and regular bubble patterns is the issue of wetting agents (surfactants). Consistency in use of particular wetting agents is an important consideration for long term consistency in results. Substitution of one surfactant for another without careful checking on many analytical factors should not be done without care. This is particularly true for the molybdenum blue methods where, in some cases, the colored compounds formed are in the colloidal state. In such cases surface chemistry and interfacial tensions can become important. If bubble patterns break up it is wiser to clean the system rather than trying to add more wetting agent or change to another, especially at sea.

**NB**. When preparing reagents is imperative that the analyst carefully record all of the label information for all preweighed reagents in the laboratory notebook. The analyst must also record the date and time of preparation, her or his initials as preparer and when each new batch of reagent is placed in use. At the beginning of the expedition leg the analyst should enter his or her full name and initials to be used to annotate each reagent preparation and the time of coming on watch.

## 6.1. Phosphate

The phosphate method is a modification of the molybdenum blue procedure of Bernhardt and Wilhelms (1967) employing hydrazine as the reductant. Hydrazine provides ca. 15% increased sensitivity over the ascorbic acid used in many methods and at the same time seems to reduce coating of the flowcell window. This may be particularly important when the reagents include antimony. Because of reduced flowcell coating hydrazine also causes less drift than does the stannous chloride method previously reported (Hager et al., 1968). Flowcell coating that develops or washes out with characteristic times on order of a minute to several minutes can make it extremely difficult to measure absorbances of successive samples or standards of widely differing concentrations. Slow coating of the flowcell windows can occur with hydrazine over a period of a few weeks. The coating can be removed by treatment with 5.4 M (30%) sulfuric acid approximately once a week.

The flow diagram for the analysis is shown in Figure 6.1. This phosphate method characteristically exhibits a linear response up to 5.0 μM $HPO_4^{2-}$ with a worst-case deviation from a linear regression through the Beers-Law check data of less than 0.1% of full scale. This was the highest concentration tested. At the wavelengths indicated the analytical sensitivity is 0.071 AU/μM phosphate in the seawater sample stream. Maximum absorbance for the highest open ocean concentrations is ca. 0.25 AU.

Note that the transmission line from the chemistry manifold to the detector has been jacketed with tygon tubing to insulate the sample stream. Drafty conditions in some shore based and shipboard labs might cause the sample stream entering the colorimeter to fluctuate in temperature and cause noisy colorimeter output.

### 6.1.1. Reagent Preparation

Molybdic acid reagent, 0.186 M in 6.3 M sulfuric acid.

a. Ammonium molybdate, 0.09 M; 109 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, diluted to 1000 ml with DIW. **NB**. Seven moles Mo/mole ammonium molybdate enters the calculation of the concentration of the final reagent.)
b. Sulfuric acid, 8.8 M; carefully add 1280 ml concentrated $H_2SO_4$ to 1620 ml DIW. Allow to cool between partial additions. Cool to room temperature.
c. Molybdic acid. Mix a. and b. and allow to cool. If the reagent has a bluish tinge or a precipitate develops, discard it and prepare a new solution. Store in a dark polyethylene bottle. This is usually stable for three to four months.
   Requirement: 144 ml/24 hours. Smaller amounts may be made for FSS use. Surfactant: 1ml dilute Witconate surfactant per 250ml may be added to this reagent.

NOTE: A molybdic acid reagent using 224 g ammonium molybdate instead of 109 g gave an increase in absorbance of approximately 15% at the level of 2.5 µMphosphate. However, this reagent caused very high reagent blanks and excessive baseline drift. All linearity and other tests were performed with the reagent concentration listed above.

HydrazineSulfate, 0.08-0.12 M (1% w/v). 2.5 g dihydrazine sulfate, $(N_2H_4)H_2SO_4(N_2H_4)$, are dissolved and diluted to 250 ml with DIW.
Alternatively, 2.5 g hydrazine sulfate, $(N_2H_4)H_2SO_4$ may be used, giving the lower concentration. The slightly higher concentration of hydrazine in the former preparation might provide somewhat higher reducing power and reagent lifetime. However, we haven't observed any significant difference in performance. This reagent is usually consumed before any sign of instability is noticed; no particular storage requirements.
Requirement: 144 ml / 24 hours. Surfactant: 1ml of dilute Witconate per 250ml may be added to this reagent.

DIW / SLS (wetting agent). 12ml 15% w/v SLS per 500 ml DIW.
Requirement: 144 ml / 24 hours.

Dilute Witconate. .25ml full strength Witconate diluted to 100 ml with DIW.

Wash Water. Artificial seawater should be used to wash between samples. This will greatly reduce noise in the recorder trace caused by refractive effects of switching between seawater and distilled water. Natural seawater having a very low concentration of nutrients also can be used if a plentiful and cheap source is available.

Wetting agents. Experience has shown that with this method, the use of wetting agents is critical for optimal performance.

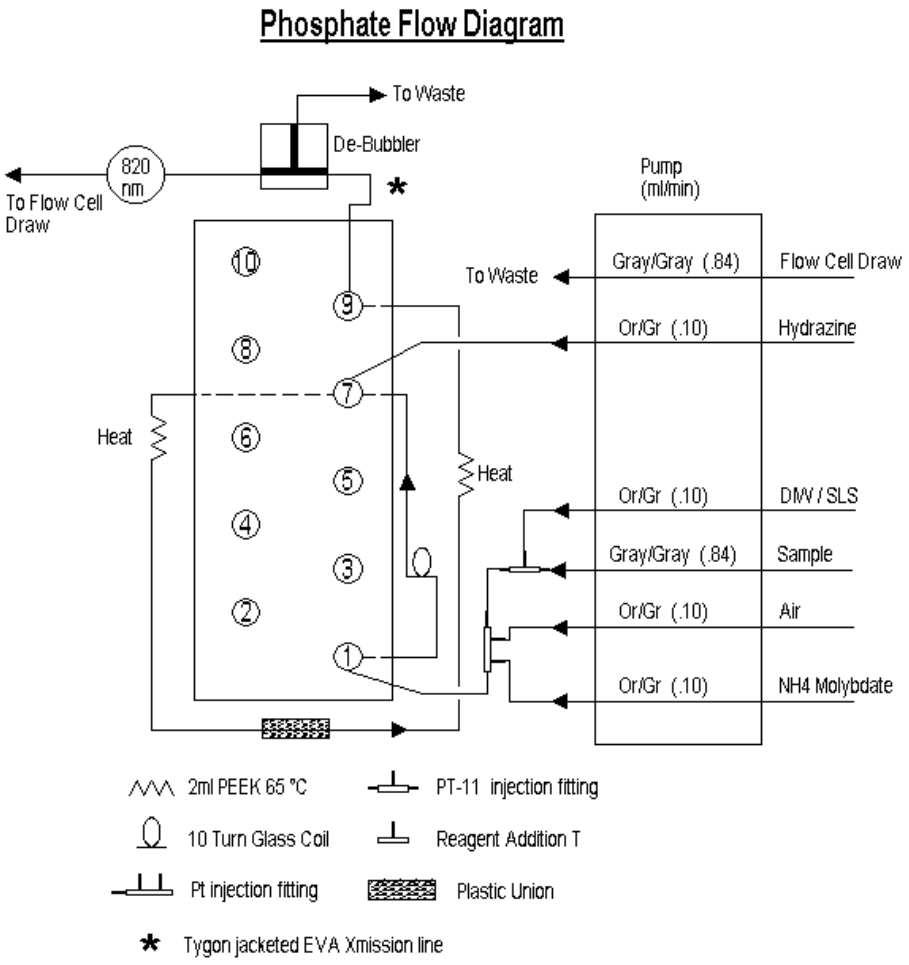


Figure 6.1. Flow diagram for the phosphate method. See Pump Tube Chart.

## 6.2. Nitrate

The nitrate + nitrite analysis uses the basic method of Armstrong et al. (1967) with modifications to improve the precision and ease of operation. The original method and many subsequent versions are unacceptably non-linear at concentrations above ca. 15 µM. Here, the buffer solution is diluted and its pump tube size increased to provide the necessary dilution while keeping the sample tube size constant in order to achieve satisfactory linearity. Conversely, at low concentrations, higher sensitivity can be had by concentrating the buffer solutions and using higher sample to buffer flow rate ratios. We prefer to use the imidazole buffer described by Patton (1983) because of its superior ability to complex cadmium ions. Nitrogen bubbles are introduced after the imidazole buffer.

The flow diagram for the analysis is shown in Figure 6.2. At the wavelength shown the analytical sensitivity is ca. 0.0048 AU/µM nitrate (and/or nitrite) in the sample stream. Maximum absorbance for the highest open ocean concentrations is ca. 0.25 AU.

Note the four-way valve used to isolate the Open Tube Cadmium Reductor (OTCR). Care must be used in selecting and using a suitable valve to minimize bubble breakup or introduction of unwanted dead volume into the sample stream. Care must be exercised to be sure the valve is fully open and aligned properly to avoid bubble breakup.

In some installations, only one three-way valve is used for bypassing the cadmium column, the downstream valve being replaced by a tee. This diverts the flow around the column but does not completely isolate it from the sample stream. The end left open can allow slow diffusion of unbuffered rinse water into the column, as the pressure in the system oscillates during the channel shutdown and startup periods. With attention paid to this possibility, the system can be operated without undesirable column degradation.

"Copperized" cadmium reduces nitrate to nitrite in either a packed column or an OTCR. (The method actually measure this nitrite.) The latter has the advantage of being more convenient to use, in causing less toxicity hazard in handling and in not requiring debubbling of the flow stream prior to its entry into the reductor. It can be purchased completely fabricated for conditioning and insertion into the system requiring only the internal cleaning, copperizing and conditioning steps. Its main disadvantage is its high cost. From time to time vendors have supplied defective columns which required (no-cost) replacement at some inconvenience to the user. For instructions on activating and maintaining the OTCR, see the Alpkem manual for the FS IV and the work of Patton (1983). We find their instructions complete and reliable. Although the OSU RFA method employs the OTCR, either reductor type can be used with good results. **N.B.** Take very seriously the Alpkem instructions for storage of the OTCR between measurement sessions. OTCR's can be irreparably damaged by improper storage!

If, for analytical efficiency, as is recommended in this Protocol, the nitrate and nitrite channels are calibrated using mixed nitrate and nitrite working standards, reductor efficiency must be carefully monitored (Garside, 1993). This is done by comparing the response of the nitrate channel alternately to nitrate and nitrite

standards at nearly full-scale nitrate concentrations. As an example one may place ten each, alternate 30 μM standard nitrate and nitrite solutions in the sampler. Note that nitrite salts are commonly less than 100% pure while nitrate reagent grade salts are typically 99.9% pure or better. This means that if the reductor were 100% efficient in reducing nitrate and also did not further reduce any nitrite it would be possible to observe 100% or greater efficiencies, that is, higher response to nitrate than to nitrite solutions of the same concentration. This rarely happens. Reductors usually gradually degrade yielding reduction efficiencies that can drop below 90%, 80% or less. Although the final degradation of the column can be rapid, the early stages of gradual degradation can be insidious. Garside (1993) has shown that for low column efficiencies (85%) and some combinations of nitrate and nitrite standard and sample concentration ranges systematic errors in observed nitrate concentration of more than 1 μM (up to 3% of deep water values) can occur.

To prevent this from happening, the analyst must regularly measure the reductor efficiency and monitor the magnitude of the nitrate sensitivity factor. The reductor efficiency should be checked at least once a week and the sensitivity factor should be checked as quickly as possible at or even before the end of every set of analyses. For the WOCE program the reductor should be reactivated if the efficiency drops below 95% and replaced if reactivation cannot bring the efficiency above 95%. To minimize the adverse impact of low reductor efficiency, the nitrite calibration standard concentration should be kept as low as possible for the oceanic region of study. For open ocean studies, away from intense upwelling systems (eg. northern Indian Ocean bays, Peruvian upwelling system) or open ocean locations like the Costa Rica Dome where high nitrite concentrations can be expected, nitrite calibration standards should be limited to at most ca. 1.0 μM.

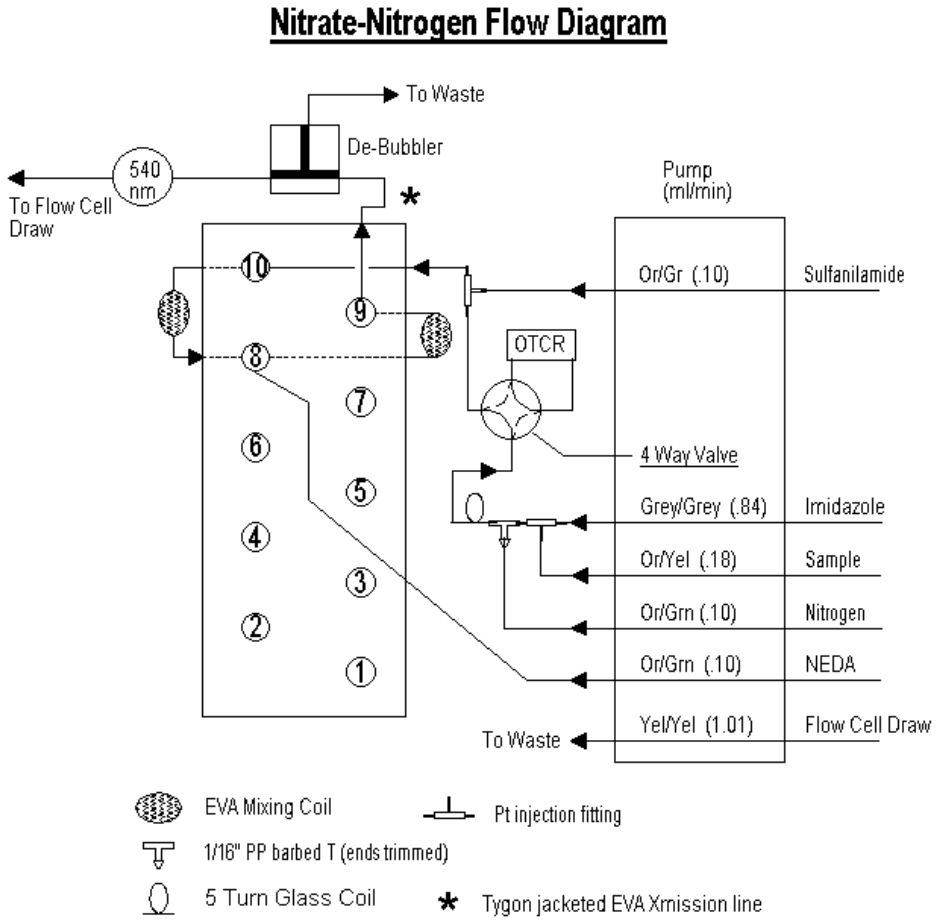


Figure 6.2. Flow diagram for the nitrate method. See text for discussion of wavelengths. See Pump Tube Chart.

### 6.2.1. Nitrate Reagents

Imidazole buffer/complexing agent, 0.05 M, containing copper (3 μM), for the RFA method. Dissolve 6.8 g imidazole, $C_3H_4N_2$, in ca. 1500 ml DIW; add 30 ml ammonium chloride-copper sulfate stock solution (described below) and 2ml BRIJ-35; make up to 2000 ml with DIW. Adjust the pH to 7.8-7.85 with concentrated HCl (ca. 2 ml). This reagent is usually consumed before showing any signs of instability; no particular storage requirement.
Requirement: 1210 ml/24 hours. Surfactant: Add 2-3 drops Brij-35 per 500ml.

Sulfanilamide, 0.06 M (1% w/v) in 1.2 M HCl. Dissolve 10g sulfanilamide, $4\text{-}NH_2C_6H_4SO_2NH_2$, in 1 L of 1.2 M (10%) HCl. Stable at room temperature.
Requirement: 144 ml/24 hours. Surfactant: Add 1ml Brij-35 per 500 ml.

N-1-Napthylethylene-diamine dihydrochloride, NEDA, 0.004 M. Dissolve 1 g NEDA, $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$, in 1 L of DIW. Refrigerate in an air-tight, dark bottle; discard if colored.
Requirement: 144 ml/24 hours.

Ammonium chloride-copper sulfate stock solution, 4.7 M $NH_4Cl$ - 0.2 mM $CuSO_4$. Dissolve 250 g ammonium chloride, $NH_4Cl$, in 1 L DIW, add 2.5 ml copper sulfate stock solution.
Requirement: One liter lasts for more than one month-long cruise.

Copper sulfate stock solution, 0.08 M. Dissolve 20 g cupric sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, in 1 L DIW. Stable at room temperature.
Requirement: One liter lasts for much more than a month-long cruise.

### 6.2.2. Cadmium Column Preparation and Maintenance

For the FS IV, an open tubular cadmium reductor (OTCR) is the preferred method for reducing nitrate to nitrite but a packed column could be used as a last resort. The OTCR has the advantage that the flow stream does not require debubbling before passage through the reductor. The presence of a debubbler in the system increases carryover as noted earlier in this Protocol. The useful lifetime of an OTCR seems to be comparable to that of a packed column reductor. Reduction efficiency is also comparable. If a packed column reductor is used for the FS IV its inside diameter should be approx. .1" and debubbling will be necessary. It may also be necessary to use a finer size fraction of Cd. The OSU group has no experience using packed column reductors with the FS IV system.

Preparation of the OTCR is similar to the preparation of the packed column reductor with obvious differences such as not having to transfer cadmium granules to the reductor. The OTCR is particularly convenient and easy to clean, copperize and condition. Most operations are easily performed using 5 or 10 ml plastic syringes to hold the successive reagents. The detailed instructions for preparing and maintaining the OTCR that come with the RFA systems are clearly written and should be followed carefully to assure proper operation and long life of the OTCR. Imidazole is the usual buffer/Cd complexing agent for the OTCR and may be used quite successfully with packed columns as well.

### 6.2.3. Factors Affecting the Success of the Methods

a. Colloidal copper formed during the "copperizing" step can cause serious problems if an OTCR is overcharged. It is recommended to run multiple high Nitrate standards to condition an OTCR after charging.
b. Whenever transmitting an unsegmented stream (eg. the output tubes from packed Cd-Cu columns and debubblers) use small bore (1 mm I.D.) tubing. This decreases transmission time and minimizes carryover of samples.
c. Both packed columns and OTCR's should be kept filled with buffered sample or buffered DIW stream at all times; Never with unbuffered DIW or sample. Before introducing the column into the flow stream, make certain that buffer has reached the reductor inlet point. When shutting down the system be sure to isolate the reductor before moving the buffer tube from the buffer reservoir to DIW. A microbore, four-way valve at this point in the system works very well for this as does a three-way valve (cf. section 6.2).
d. Linearity checks are important in the nitrate method.

## 6.3. Nitrite

Nitrite analysis is performed on a separate channel, omitting the OTCR and the buffer. The volume flow of the buffer is compensated by using a correspondingly larger sample pump tube; this also increases sensitivity. Nitrite concentrations never become high enough in the open oceans for the system response to become unacceptably nonlinear. Sensitivity may be increased by using the longest flow cell available. The resultant flow diagram is shown in Figure 6.4. All reagents required are described in Section 6.2.1. Under the conditions indicated the analytical sensitivity is 0.056 AU/μM nitrite in the sample stream. Maximum absorbance for the highest open ocean concentrations is ca. 0.25 AU.

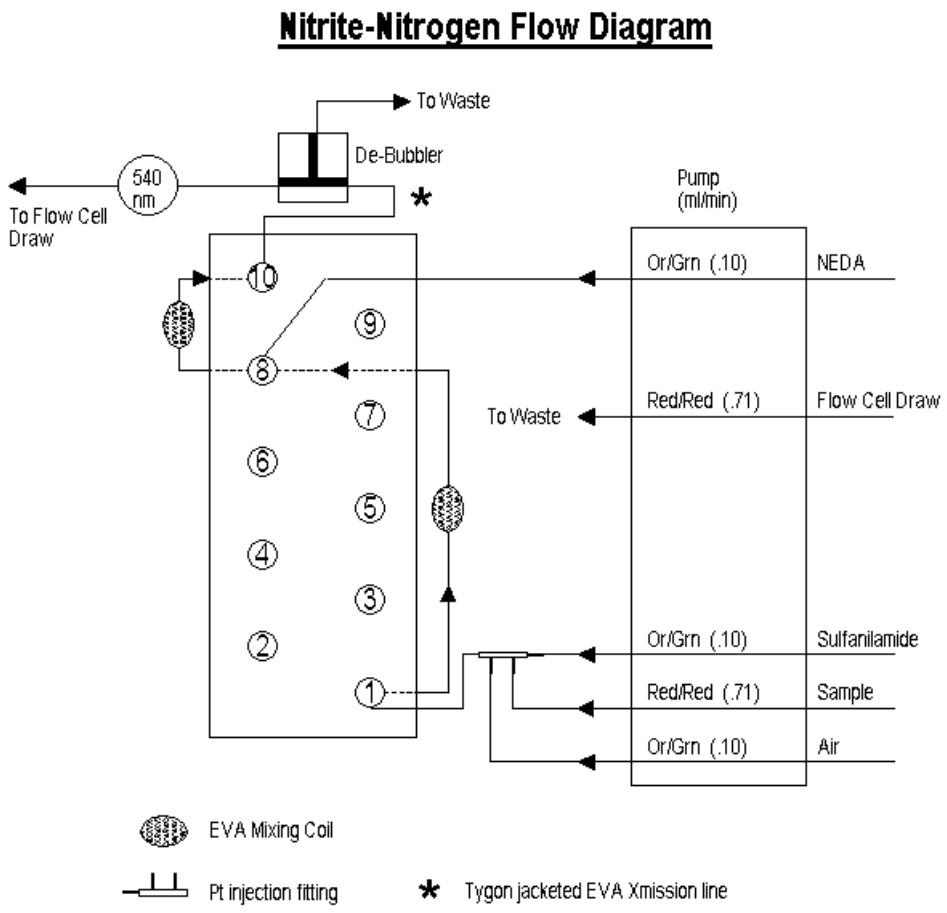


Figure 6.3. Flow diagram for the nitrite method. See text for discussion of wavelength. See Pump Tube Chart.

## 6.4. Silicic Acid

The method is based on that of Armstrong et al. (1967) as adapted by Atlas et al. (1971). The modifications presented here reduce its sensitivity to laboratory temperature (Gordon et al., in preparation). The rationale is explained in a later paragraph.

At the wavelengths indicated the sensitivity is ca. 0.006 AU/μM silicic acid in the sample stream. Maximum absorbance for the highest, open ocean concentrations is ca. 1.0 AU.

Figure 6.5 shows the flow diagram and operational parameters for the silicic acid analysis.

The marked temperature sensitivity of the Armstrong et al. method is caused by the very short time allowed for production of ß-silicomolybdic acid by reaction of the molybdic acid and the silicic acid in the sample. The kinetics of this reaction are, of course, temperature dependent. The initial rate of increase in ß-silicomolybdic acid, and hence the ultimate absorbance, is quite fast. By allowing the reaction to go closer to completion the temperature-dependent kinetics become less important. The laboratory temperature effect is ca. 20 times less than the Armstrong et al. method formerly used at OSU. A reviewer of an earlier draft of this manual stated that methods using ascorbic acid or metol as the reductant to ß-silicomolybdic acid are not dependent upon laboratory temperature. Because the effect appears to be caused by the formation of ß-silicomolybdic acid prior to reduction, it would be difficult to understand how this could be. This has not been checked at OSU. Some workers choose to heat the sample stream after addition of molybdic acid. This should also solve the temperature dependence problem but at the cost of more added complexity to the system.

Use of tin(II) as the reducing agent has worked well for us. However, considering its toxicity hazard and problems with waste disposal so as to avoid environmental pollution, we are testing the use of ascorbic acid. Many workers use this reductant. When we are assured that it meets the requirements of linearity, independence of laboratory temperature fluctuations and of interferences we will make the substitution.

### 6.4.1 Reagent Preparation

Molybdic acid reagent, 0.061M in 0.05M sulfuric acid. Dissolve 10.8 g ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 1000 ml DIW containing 2.8 ml concentrated $H_2SO_4$ and 2.0ml 15% SLS per liter.
Requirement: 806 ml/24 hours. Surfactant: 1ml SLS per 500 ml.

Tartaric Acid, 1.25M (20% w/v) in DIW. Dissolve 200 g tartaric acid, $HOCO(CHOH)_2COOH$, in 950 ml DIW. Refrigerate. Do not add chloroform; its solubility limit is ca. 0.6% in DIW; droplets of undissolved chloroform will cause noisy traces if they find their way to the flowcell.
Requirement: 259 ml/24 hours. Surfactant: 2ml SLS per 500 ml.

Stannous Chloride,

a. Hydrochloric acid, ca. 6M (50% v/v). Dilute 50 ml concentrated HCl to 100 ml with DIW. The resulting concentration is only approximately 6M but need not be more exact than this.
b. Stannous chloride stock solution, ca. 2.2M (50% w/v) in ca. 6M HCl. Dissolve 50 g $SnCl_2 \cdot 2H_2O$ in 6M HCl and make up to 100 ml with 6M HCl. STORE IN A PLASTIC BOTTLE IN A FREEZER at -10 C or below. IF NO FREEZER IS AVAILABLE, STORE UNDER MINERAL OIL WITH A PIECE OF MOSSY TIN ADDED. At freezer temperatures the solution is stable for one to two months.
c. Stannous chloride working solution, ca. 0.11M (ca. 1.1%) in 1.3M HCl. Dilute 10 ml of stannous chloride stock solution to 200 ml with 1.2M HCl (or 1 ml to 20 ml with 1.2M HCl). Make up fresh daily. Refrigerate whenever possible. A piece of mossy tin may be added. Requirement: 107 ml/24 hours.
d. SLS, 0.5M (15% w/v). Dissolve 15g sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$) in 87ml DIW.

6.4.2. Reagent Notes.

a. Deterioration of the stannous chloride reagent can be very rapid and may cause an unstable baselines, poor peak shapes and, in case of total deterioration, no response at all. When experiencing these problems with the silicate analysis, this is the first place to look for the remedy.
b. Stannous chloride as purchased, or sometimes after prolonged storage, does not always dissolve completely. An insoluble white residue remains and the reagent is unfit for use. Therefore, all new batches or batches that have been stored for some time since last being used should be tested!
c. Again, care must be taken to monitor the silicic acid concentration of the DIW used for measuring the reagent blank for several days after leaving port (see Section 3.2).

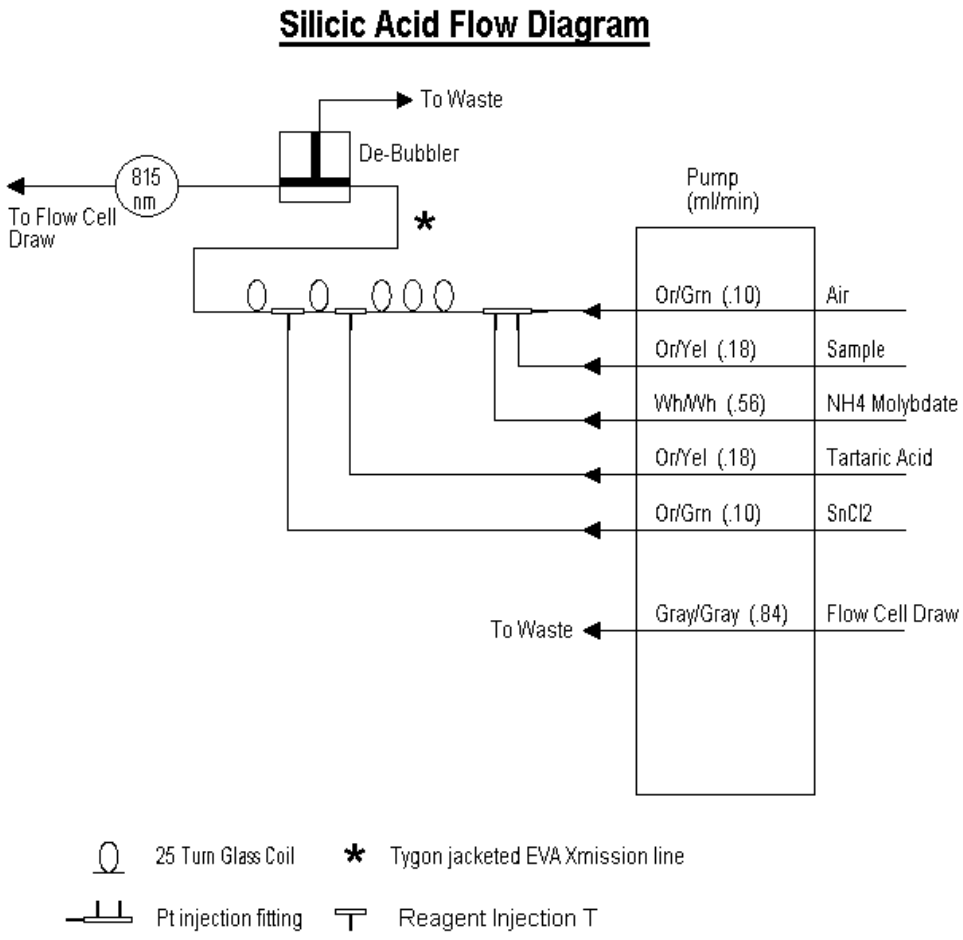


Figure 6.4. Flow diagram for the silicic acid method. See Pump Tube Chart.

## 6.5. Ammonia

This indophenol blue method is modified from ALPKEM RFA methodology which references Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen Ammonia", Method 350.1 (Colorimetric, Automated Phenate).

The flow diagram for the analysis is shown in Figure 6.5. The interference filters used are 640 nm and the recommended flowcell pathlength is 10 mm. This method has been determined to exhibit a linear response up to at least 6 µM. The full linearity range has not yet been tested. At the specified wavelength the analytical sensitivity is 0.022 AU/µM ammonia in the seawater as defined by twice the standard deviation. Maximum absorbance for the highest open ocean concentrations is ca. .13 AU.

### 6.5.1. Ammonia Nitrogen Reagents

Stock complexing agent: Sodium Citrate, Dihydrate, .476 M, $Na_3C_6H_5O_7 \bullet 2H_2O$.
Dissolve 140 g sodium citrate dihydrate in approximately 990cc DIW and adjust pH to 7.0 w/$H_2SO_4$. Dilute to 1000cc w/DIW. All brands of citrate do not perform the same in the analysis (high blanks, etc.).

Working Complexing Reagent: Add 1ml of Brij-35 to 500cc stock complex.
Requirement: 806 ml/24 hours.

Alkaline Phenol: Add 60cc of 10N NaOH to 700cc DIW. Add 12cc of liquified Phenol ($C_6H_5OH$ 88%). Dilute to 1000cc w/DIW. Store in dark plastic bottle and refrigerate.
Requirement: 144 ml/24 hours.

Sodium Hypochlorite: Add 2.5cc of 5.25% NaOCl (household bleach, eg. CLOROX) to 75cc of DIW and dilute to 100cc w/DIW. Make daily.
Requirement: 144 ml/24 hours.

Sodium Nitroprusside, .00168 M, $Na_2Fe(CN)_5NO \bullet 2H_2O$, (Sodium Nitroferricyanide): Dissolve .5 g in 800cc DIW. Dilute to 1000cc w/DIW. Store in dark container.
Requirement: 144 ml/24 hours.

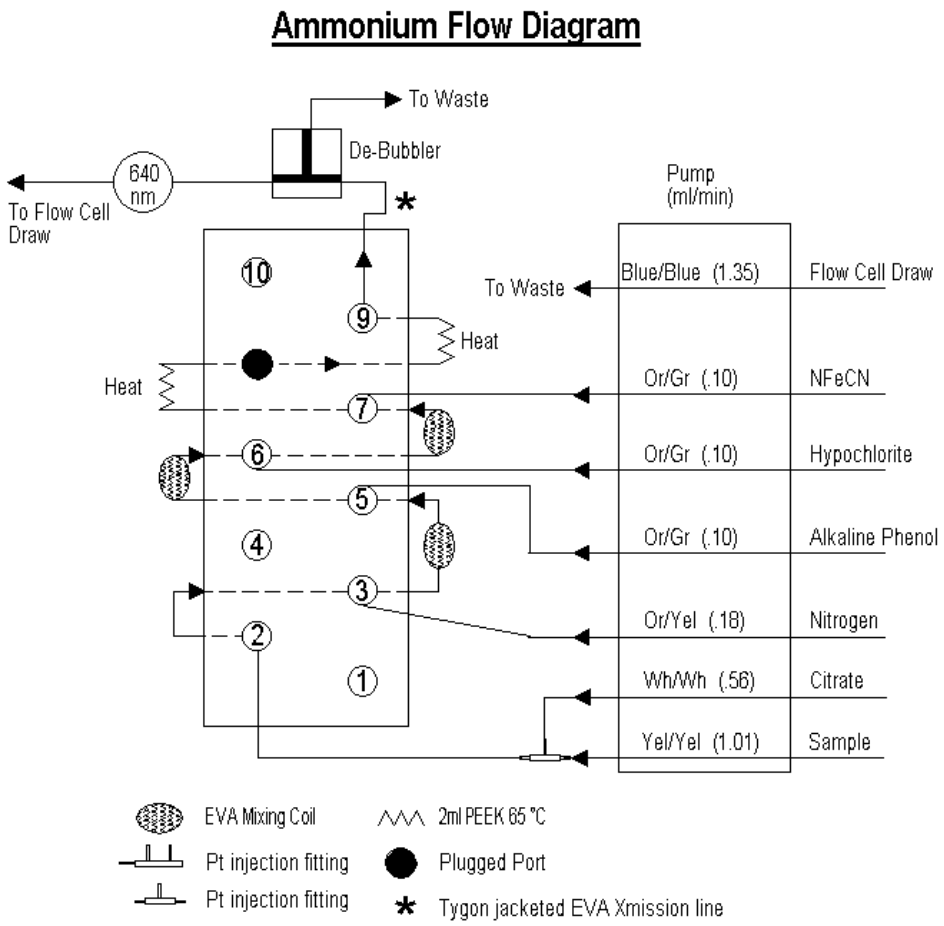


Figure 6.5. Flow diagram for the ammonia method. See Pump Tube Chart.

## 7. CALCULATIONS AND EXPRESSION OF RESULTS

Data processing using the FSS is carried out with a high degree of automation by the WinFlow software. It is well treated in the WinFlow documentation and nuances will be further explained in an appendix to this manual.

The data processing described in this section covers a manual backup procedure that can be used with the raw data saved by WinFlow. It consists of converting a set of absorbance analog readings to concentrations of nutrients in the samples analyzed.

The main steps are 1) to correct the observed absorbance data for zero-offset errors and, 2) to multiply the corrected absorbances by appropriate response factors, or "sensitivities," for the various analyzer channels.

The zero-offset corrections include:

a) Correction for nutrient impurities in the reagents and impurities in the reagents that behave like the nutrients in generating measurable color in the flow stream. This correction is termed the "reagent blank."

b) Optical errors arising from the difference in refractive index between deionized water and seawater. This correction is the "refraction correction," (Atlas et al., 1971).

c) The electronic and/or optical zero offset of the colorimeter. This correction is made automatically by the WinFlow software at the start of analysis.

d) The last zero-offset error for which we correct arises from the contamination of a sample in the flow stream by a residuum of the previous sample. This is commonly called the "washout" or "carryover" error. This affects all sample, standard and blank measurements, to a greater or lesser degree depending upon the differences in concentrations of successive samples entering the flow stream. Its magnitude is dependent upon the absolute and relative lengths of the sample and intersample wash periods. The shorter the sample time the larger the carryover effect. The effect of intersample wash time has a more complicated effect. The causes of the carryover effect are the presence of poorly flushed "dead volumes" in the flow stream and the non-uniform flow velocity profile across lumen of the flow paths in the system. Unfortunately this error can vary with time. In general, the longer and more complex the flow system, the larger is the carryover effect.

With some methods, for instance the antimony-catalyzed reduction to molybdenum blue by ascorbic acid in some phosphate methods, it typically has characteristic buildup and washout times on the order on the residence time of one or a few samples in the flow colorimeter. This makes it extremely difficult to obtain meaningful blank and standard calibration measurements.

We will discuss the measurement and correction of the zero-offset errors first, then the response factor and lastly the carryover correction.

## 7.1. Reagent Blank Estimation

Correction for the reagent blank depends upon a reliable source of a nutrient-free solution. Ideally, this would be nutrient-free natural seawater. However it is extremely difficult in practice to obtain, prepare or maintain nutrient-free seawater. Hence we use deionized water (DIW) instead. Sufficiently nutrient-free DIW is quite easy to prepare routinely and reliably at sea (see Section 2.2). **NB.** DIW prepared by ion exchange techniques can become contaminated by high levels of silicic acid in the fresh water supply. This can happen, and has too often happened, when ships take on fresh water in ports of call.

One measures the reagent blank by introducing two or more samples of DIW at the beginning and at the end of each batch of samples analyzed. In principle, the absorbance developed by these samples will result only from:

a) the presence of nutrient impurities in the reagents,

b) absorption by the reagents themselves or impurities in them at the analytical wavelengths,

c) contributions from the colorimeter's optics and electronics (instrumental zero) and,

and d) to the extent carried over, nutrients or other interferents present in the intersample wash.

Then one can subtract the reagent blank absorbance from all the remaining samples and standards and arrive at the absorbance arising just from the nutrients contained in those standards and samples. But the instrument zero can drift measurably with time; our experience has been that this drift is closely monotonic and linear. Therefore, in our software, the combined instrument zero and reagent blank absorbances (readings for DIW) are regressed upon position number in the batch being analyzed and interpolated values subtracted from all sample absorbances. They may also be subtracted from standard and standard matrix absorbances; in that case they cancel out upon taking differences between up-scale standards and matrices to calculate response (or "sensitivity") factors as will be explained later. Note that it is <u>not</u> necessary to bring the output signals down to the reagent blank level between each pair of samples by prolonged "wash times!" When operating properly a CFA should not drift enough to make this necessary for the nutrient methods described here. Operation with such long wash periods approximately doubles the analysis time; the result is more or less degeneration of the samples by bacterial activity and loss of operational efficiency. The only real purpose of the intersample wash in our protocol is to provide an easily detected mark between the output signals of adjacent samples!

## 7.2. Refraction Error Estimation

The use of DIW to measure the reagent blank introduces a new source of error, the refraction error (Atlas et al., 1971). It derives from the difference in refractive indices of pure water and seawater and the imperfect optics of the AutoAnalyzer or RFA flow cell. (Were the end windows of the flow cell planar and parallel to each other, the light beam perfectly collimated and the flow cell's inside diameter sufficiently larger than the diameter of the light beam there would be no error from this cause.) The measured "reagent blank" therefore includes both the true reagent blank and this refractive error.

To measure the refractive error itself one first removes a critical reagent from each analyzer flow stream, replacing the reagent with DIW. The critical reagent selected is the one contributing least to the total ionic strength of the stream and its total flow rate and whose absence assures complete elimination of color development at the analytical wavelength. Then, one passes a series of alternating DIW and natural seawater samples through the system, records the absorbances and computes the refraction error, _, as the average difference with regard to sign. At least ten differences should be obtained.

$$\bar{d} = \frac{\text{Sum } ( A_{sw} - A_{dw})}{n} \qquad (7.1)$$

where: $\bar{d}$ = Refraction error
A = Absorbance
n = Number of differences between seawater and DIW peaks
dw = DIW
sw = Seawater

This procedure is followed for all analytical channels and the resulting average refractive corrections are subtracted from the signals of all samples, including in our software, working calibration standards and matrices. The refractive correction is sensitive to reagent and sea salt concentrations in the flow cell, colorimeter "Standard Cal." or range settings, and recorder gain settings. Therefore it must be remeasured after any change in pump tubes, even if no pump tube sizes have been changed, and any change in any of these colorimeter or recorder settings! Note that the sign of the refraction error may be negative. Given CFA system optics, this is a possible and acceptable case and attention must be paid to the sign of the correction.

Typical refraction errors range from ca. zero for silicic acid to one to three percent of full scale concentration for phosphate. The error, with the AA-II optics especially, can be as much as three percent of deep-water phosphate concentrations. Fortunately these errors are quite constant and measurable with good precision. Thus, the uncertainty from this cause is less than 0.1% for silicic acid, ranging to ca. 0.3% at most for phosphate, with respect to deep water concentrations.

## 7.3. Computation of Carryover Correction

Carryover results from the finite and more or less incomplete flushing of the flow system between samples. Thus an error is present in any given absorbance reading. Angelova and Holy (1983) have shown that the carryover signal can be approximated as linearly dependent upon the difference between the absorbance of a given sample and that of the preceding sample:

$$o = k( A_i - A_{i-1} ) \qquad (7.2)$$

where $o$ = Carryover correction
k = Carryover coefficient
i = Sample position number

To correct a given absorbance reading, $A_i$, one then adds the carryover correction:

$$A_{i,c} = A_i + o \qquad (7.3)$$

where: $A_{i,c}$ = Corrected absorbance

The carryover coefficient, **k**, is obtained for each channel by measuring the difference between the absorbances of the second and first full-scale standards following a near-zero standard or sample, all having the same, natural seawater matrix composition. It can equally well be calculated from the difference between the first two near-zero standards following a full-scale standard or sample. Measurement of the carryover should be done in triplicate at the beginning of a cruise in order to obtain a statistically significant number. Then it can be re-measured from the beginning or ending standard and matrix peaks for a given batch of samples and its magnitude compared with the earlier value. Keeping a running average of the carryover coefficient and having the data processing software monitor its magnitude and signal the analyst in case of sudden departures from the average would be an ideal quality control procedure. However we have not yet implemented this in our software to be described later. Note that the

carryover correction must be carefully re-measured every time any change in plumbing of a channel is done, including simple pump tube or coil replacement.

The formula for **k** is:

$$k = \frac{A_{i+1} - A_i}{A_i - A_{i-1}} \qquad (7.4)$$

where: $A_i$ = Absorbance of the first full-scale standard
$A_{i+1}$ = Absorbance of the second full-scale standard
$A_{i-1}$ = Absorbance of the matrix sample preceding the first full-scale standard

Note that **k** is also valuable for monitoring system performance. Its value depends strongly upon several operational conditions such as constant timing of the pump and minimal dead volume in the flow system. Mechanical wear in the pump or pump tubes or dead volume accidentally introduced when maintaining the flow system can often be detected very quickly by monitoring **k**. To monitor for these effects, one should carefully record values of **k** and, if possible, accumulate them in a data quality control file and frequently and regularly plot **k** against time.

Carryover corrections for well designed and maintained channels are usually less than 0.3%. The worst cases are for systems with large volumes such as those containing heating baths (phosphate) or debubblers (AA-II channels) or packed-bed cadmium columns. In general, the shorter the sample and the longer the wash periods the larger the carryover effect. Lengthening the sample period and reducing the wash period, in principle, reduces the carryover magnitude.

## 7.4. Calibration of analyzer response

The response of each analytical channel per unit nutrient concentration is obtained by addition of known nutrient concentrations to natural seawater and measuring the resultant increases in absorbances. Using natural seawater assures that systematic effects (possible, unknown interferences) derived from natural seawater constituents will be present in both the calibration standards and seawater samples. However the natural seawater used for this purpose will, in general, contain finite concentrations of nutrients. It is not necessary that these concentrations be zero, only low, thus, "low-nutrient seawater" (LNSW). If the concentration were high to begin with, adding sufficient additional nutrients to obtain a usefully large signal might increase the total nutrient concentration enough that the analyzer response becomes nonlinear. In particular, this must be avoided if linear formulae for data processing are used. Even when nonlinear responses are corrected using nonlinear data processing techniques application of the corrections can become complicated if the matrix seawater contains appreciable nutrient concentrations. (OSU protocols strive for a mid-range nonlinearity of no more than 0.4% in all analyses and use a linear algorithm for data processing. The SIO-ODF employs a nonlinear algorithm.) In general, LNSW is acceptable if it contains less than ca. five percent of full-scale concentrations of all the nutrients. Given this condition the calibration procedure then consists of measuring both the LNSW and the LNSW with known additions of nutrients. The system response to nutrient addition is computed from the slope of the "Beer's Law" plot of measured absorbance versus standard additions to the matrix LNSW. Again, a nonlinear fit to this plot may be used.

Other than to correct the responses to the working standards for the nutrients or interferents present in the matrix LNSW the signals from the LNSW are of no intrinsic value. They can be valuable for monitoring the DIW used for reagent blank measurement. For example a sudden increase in absorbance of the matrix relative to the reagent blank might indicate contamination of the DIW supply.

Calibration standards (at least in duplicate, preferably triplicate) must be placed at the beginning and end of each and every set of samples analyzed. Batches of samples should be kept small enough so that the elapsed time between beginning and ending blank and standard sets does not exceed one and a half hours. We selected this based on observed instrument drift rates for all of our systems. Drifts in our CFA responses are, if something is not failing catastrophically, linear and monotonic with time, similar to the situation with the zero offsets. The OSU data processing protocol linearly regresses the observed beginning and ending response or "sensitivity factors" on sample number (counting blanks, matrices and standards as samples in this instance) and applies linearly interpolated "response factors" when computing concentrations. (Strictly speaking, the response factor as defined in Equation 7.5 is the reciprocal of sensitivity, hence the quotation marks.)

The response factors are computed from:

$$f = \frac{C_a}{(A_s - A_m)} \qquad (7.5)$$

where: $f$ = response factor (or "sensitivity")
$C_a$ = Added concentration of nutrient in the calibration standard
$A_s$ = Absorbance of calibration standard
$A_m$ = Absorbance of standard matrix seawater (LNSW)

## 7.5. Summary of Steps for Computing Concentration

To summarize, the data processing involves both additive corrections to the absorbances and multiplication of the fully corrected absorbances by the response factor to obtain the sample concentrations. The additive corrections can be made in the following sequence:

a) Correct all absorbances for carryover.
b) Regress the reagent blank absorbances against position number in the sample set and subtract the interpolated reagent blank from all absorbances. Strictly speaking, there is no need to do this for the calibration standard absorbances and their associated LNSW absorbances but there is no harm in doing so. It is simply easier to do it this way in most computer programs.
c) Subtract the refraction correction from all seawater sample absorbances. Again, there is no need to do this for the calibration standard and LNSW absorbances but it does no harm if done. This step produces fully corrected absorbances for all seawater samples.
d) Calculate the beginning and ending response factors, regress them against position number in the set and multiply sample absorbances by the interpolated values. The results are the desired seawater concentrations.

Some of these computations can be done using different sequences than given here. Three important points to note are, a) that this procedure gives correct results, b) that the analyst must thoroughly understand the concepts involved before making any changes in the procedure and c) that the analyst must compare the results obtained by the changed procedure with those resulting from this one and be certain they agree over a variety of conditions and concentration levels before accepting the new procedure. The last point assures that, even though the new algorithm or software is correct, an error in reprogramming has not occurred.

## 7.7. Units for Expression of the Final Results and Conversion Factors

The concentrations resulting from the preceding calculations are micromolar, that is, micromoles per liter ($\mu\underline{M}$ or $\mu mol \cdot dm^{-3}$) of the nutrient ion. Expressing nutrient concentrations in these volumetric units makes them pressure and temperature dependent. In order to be free of this dependence many workers choose the pressure and temperature independent units, $\mu mol \cdot kg^{-1}$. To accomplish the numerical conversion it is necessary to know the density of the seawater samples at the time they are volumetrically drawn into the CFA pump and compared with the working standards whose concentrations are known in volumetric units. To do this one requires knowledge of the salinity of the samples and their temperature at analysis time (the pressure is, of course, one atmosphere). The salinities are generally known from the concomitant hydrographic observations. The sample temperatures closely enough approximate the laboratory temperature at the time the samples are analyzed. Fofonoff and Millard (1983) give a convenient algorithm for computing the density. The volumetric units are simply divided by the density to convert to pressure-independent gravimetric units.

The procedures presented thus far produce "nitrate" concentrations that are actually the sums of the concentrations of both nitrate and nitrite present in the samples. Stating the obvious, it's necessary to subtract the nitrite concentrations from the nitrate plus nitrite concentrations to obtain concentrations of nitrate alone. A last caveat relates to the potentially large sources of error possible for certain combinations of nitrate and nitrite standard and sample concentrations, particularly if the cadmium reductor's efficiency is allowed to deteriorate (Garside, 1993).

## 7.8. Computer Software

Our group has developed a series of programs for nutrient data acquisition and processing. The first of these, "DATABEEP," is a QuickBASIC program for control of a Keithley Instruments System500 data acquisition system in an IBM-PC type environment. It controls acquisition and digitization of the voltage-analog data from the flow colorimeters. It does this in "background" allowing the analyst to interact with DATABEEP's operational parameters in "foreground" to accomplish tasks like adjusting peak window delays and widths. DATABEEP's output is a raw data file that can be edited and processed by the second program, "NUTCALC."

NUTCALC is also a QuickBASIC program. It carries out the computations described in this section in a menu-driven environment, operating upon an array of blank, standard and sample absorbances or voltage analogs. These can have been constructed by any digital data acquisition system including DATABEEP or by manually digitizing the data. It takes the raw data file through editing and processing steps to a new data file in concentration units. NUTCALC applies baseline and sensitivity drift corrections (assumed linear and monotonic), applies carryover corrections, computes sensitivities (or calibration factors) and computes concentrations in micromolar units. Its simple spreadsheet module facilitates entry of hydrographic and other bottle data into the nutrient data file. Replicate samples

can then be averaged, sample depths entered, etc. Output from the program is in ASCII format.

DATABEEP, NUTCALC and some smaller, companion programs including a multivariable plotting program are available on request from the authors via ftp at no cost.

# 8. QUALITY ASSURANCE

Quality assurance in nutrient analyses as with any analytical procedure begins with well designed and meticulously executed sampling methods. These have already been described. The same must be said for the execution of the actual analyses themselves. The analyst must carefully monitor the performance of the CFA at all times, correcting and noting any deviations from normal and acceptable performance.

It is imperative that the analyst not continue operation of the CFA should its performance not guarantee acceptably high quality data. In such a case operation must be halted and the problem corrected. It's obvious that a CFA can generate a distressingly large amount of bad data in a short time if not properly maintained and operated. A gap in a data set is far less objectionable than a set of bad data!

We'll go through a plan of quality assurance steps that can facilitate producing a good data set. This will include a program of replicate sampling to provide a measure of short-term, within laboratory precision, both for sampling from the water column and for analysis of homogeneous water samples by the CFA. Somewhat longer term precision can be evaluated by examining consecutive station agreement of deep samples and more rigorously by examination of variance along isopycnal surfaces over not-too-long horizontal distances.

Every laboratory must develop a rigorous QA program, either along these lines or with equal or greater rigor, one of its own.

## 8.1. Replicate sampling from the same water sampler

Draw duplicate samples from two water samplers at each station. One pair is to be drawn from one of the deepest depths or from mid-depth, for instance at the nitrate/phosphate maximum if present. Another can be a duplicate from the mixed to obtain a low nutrient concentration sample. The duplicates should be well separated in the sample tray and not placed in consecutive positions. As the cruise proceeds, maintain a cumulative log file of these replicate measurements.

Another plan, giving more information on CFA stability is to draw the deep or mid-depth sample in triplicate. Two of these samples are analyzed with the first samples analyzed and the third is analyzed with the last analyzed, perhaps just before the ending working standards. This arrangement works well with the common situation where the deepest samples are analyzed first, working up the cast to the samples from the shallowest depths.

## 8.2. Replicate sampling from different samplers tripped at the same or similar depths

If possible, casts should be designed such that either every cast or periodic casts entail tripping samplers at the same depth, or nearly the same depth. This is of most value when the selected depths are in a range where the vertical gradients are minimal. This allows an estimate of the effects of different samplers and slightly differing sampling times on sample variance. Because of the obvious loss of sampling detail this option is not often available. It should be encouraged, even if only at relatively infrequent intervals.

## 8.3. Quality checks during operation

There are two general areas where the analyst must be diligent in maintaining quality assurance while operating the CFA and in the first steps of processing the data. First, the analyst must be conscientious to almost an extreme in constantly watching the flow characteristics of all channels of the CFA and monitoring the quality of the strip chart recorder traces. Second, if the data logging software implements an on-line computation and printout or display of root-mean-square noise on the sample peaks, the analyst must pay particular attention to abnormal variance and to correction of the cause.

## 8.4. Multivariate plotting of vertical profiles

As soon as possible following analysis of each station the analyst should construct a composite vertical profile plot of the nutrient data. Abnormal performance of water samplers and/or the CFA often show up as "flyers" in one or more of the nutrients. The nutrient analyst can often be the first person to discover a particular water sampler that habitually or often leaks. Sudden jumps in deep water concentrations observed upon overlaying subsequent vertical profiles can alert the analyst to a problem with preparation of a working or earlier stage calibration standard or with an unstable standard.

## 8.5. Use of the Q1EDIT program

The Q1EDIT program developed by the WOCE Hydrographic Program (WHP) Office serves as a highly sensitive device for the detection of flyers and offsets in nutrient and other data. We heartily endorse its use where possible.

## 8.6. Comparison with historical data

If the analysts have time at sea and if adequate historical data are available, overlaying plots of the current data with the historical data is an excellent quality assurance technique. Care must be taken that the historical data are, in fact, of quality adequate for the purpose! The analyst and chief scientist must also keep in mind that differences between historical and contemporary data may in many cases be real and not analytical artifacts.

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# 10. GLOSSARY

Aerosol-22 ≡ a proprietary surfactant, widely sold under this name

AU ≡ absorbance unit {$\log_{10}(I_0/I_t)$, where $I_0$ is the light intensity incident upon the colorimeter flowcell, $I_t$ is the light intensity transmitted through the flow cell}

ASW ≡ artificial seawater (cf. Section 2.2 for recipe)

BPM ≡ Bubbles per minute

Brij-35 ≡ a proprietary surfactant, widely sold under this name

CFA ≡ Continuous flow analysis (or analyzer)

DIW ≡ deionized water

F/C, f/c ≡ flowcell

I.D. ≡ inside diameter (in reference to pump tubing)

I/F ≡ interference filter

IPH ≡ inches per hour (1 IPH = 7.06 x $10^{-4}$ cm·$sec^{-1}$)

LNSW ≡ Low-nutrient natural seawater

M ≡ Molar (1 gram mole of solute / liter of solution, 1 g-mol·$dm^{-3}$)
**M** or <u>M</u>

Nitrate ≡ Dissolved reactive nitrate ion, $NO_3^-$

Nitrite ≡ Dissolved reactive nitrite ion, $NO_2^-$

O.D. ≡ Outside diameter (refers to glass or plastic tubing)

OSU ≡ Oregon State University

OTCR ≡ Open tube cadmium reductor

Phosphate ≡ Dissolved, reactive, inorganic ortho-phosphate ion, $HPO_4^{-2}$

psi ≡ pounds $in^{-2}$ (1 psi = 6.895 x $10^3$ Pa)

Silicic acid ≡ Dissolved reactive ortho-silicic acid, $Si(OH)_4$. This acid and its dissociation products are usually the most abundant species of silicon present in seawater. Theoretically, it accounts for approximately 80-90% of the silicic acid present in seawater with its first dissociation product constituting most of the remainder. A very small fraction might be present in low molecular weight polymers; however dimers, and to some extent, trimers are recovered by the method given.

Silicate, dissolved silica, or sometimes "silica" (Used in this sense, "silica" is not correct chemical nomenclature. Strictly speaking, "silica" denotes solid, amorphous $SiO_2$!)

SIO-ODF ≡ Scripps Institution of Oceanography, Oceanographic Data Facility

SLS ≡ sodium lauryl sulfate, $C_{12}H_{25}NaO_4S$; sodium dodecyl sulfate

Witconate ≡ a proprietary surfactant

μM ≡ micromolar ($10^{-6}$ moles of solute/liter of solution)
"u**M**"

## 11. APPENDIX A Pump Tube Chart

| Flow rate: (mL/min) | Ismatec Pump (Speed @ 35) | Color |
|---|---|---|
| | .10 | orange/green |
| | .18 | orange/yellow |
| | .25 | orange/white |
| | .32 | Black |
| | .41 | Orange |
| | .56 | White |
| | .71 | Red |
| | .84 | Gray |
| | 1.01 | Yellow |
| | 1.12 | Yellow/Blue |
| | 1.35 | Blue |
| | 1.57 | Green |

# 12. APPENDIX B HOW TO RUN A STATION
## on the Flow Solution IV System

### 12.1 Start-up and Baseline

Check and refill as necessary the sampler wash reservoir and all reagent wash solutions, adding surfactant as necessary. Connect reagent lines to appropriate wash bottle positions.

Clamp down the pump cartridges and turn on the pumps. Turn on heat baths. Completely flush the system; it takes at least 10 minutes. Longer flushing and washing helps promote better performance. The two way valve connected to the sample probe should be positioned to draw from the DIW reservoir.

While the system is flushing and being cleaned, check the levels of all reagents in their bottles, topping up if needed and preparing fresh working solutions ($SnCl_2$, $OCl^-$) if necessary. Check to be sure that there is ample nitrogen gas in the “Pillow,” and that the on/off valve is open.

While the system is flushing, start the WINFLOW software and edit the template sample table file (FS_table.tbl) to contain information on the samples to be to be analyzed. When completed and "saved as" the appropriate station filename, start the data acquisition by clicking on “collect data”. You will be prompted for which sample table (*.tbl) and methods (fs_5chan.mth) file to use, followed by your initials and filename for the results (the filename used for the sample table is the default). Once the system has flushed and you have removed air bubbles from in the flowcells (see below) click on the pre-run start button to begin collecting baseline data. Occasionally the software does not initially display properly. To correct, double click on one of the channels and then minimize back to viewing all channels.

Now add the reagents by moving the reagent tubes from their wash bottles to the individual reagent bottles following the tags and labels carefully. NOTE: The $SnCl_2$ should not be added to the silicic acid channel for at least 5 minutes after the tartaric acid and molybdate solutions have been added. Premature addition of the $SnCl_2$ will result in an ugly black precipitate being deposited on the walls of the system tubing and coils. Do not turn the valve to put the OTCR (Open Tube Cadmium Reductor) into the nitrate plus nitrite (N+N) channel until you're sure the imidazole buffer has reached that point in the flow system, usually after about three minutes of pumping buffer.
Once the N+N reagents have been pumping for a few minutes, turn the valve to switch the OTCR into the N+N channel.

You will notice deflections of the baselines as the reagents reach the colorimeters. Once all of the reagents have had time to reach the colorimeters, note the differences between the original DIW baselines and the new reagent baselines. This should be quite consistent from day to day and is a good initial check that none of the reagents have deteriorated. After recording the signal shifts due to reagents, check the DIW + reagent baseline for stability.

If the DIW + reagent baseline is drifting or abnormally noisy at this point, momentarily pinch the flow cell draw line closed for several seconds and then release. This is often a good way to clear flow cells of small bubbles and even particles. If you suspect something is lodged in the flow cell and this quick fix doesn't work, the next thing to try is to disconnect the flowcell draw connection and reverse flush the flowcell with DIW using a syringe fitted with appropriate connecting tubing. Also, check to make sure that reagent lines are connected properly and that reagent is indeed flowing. Any extra air bubbles in the flow pattern usually mean loss of reagent either due to an empty reagent bottle or that a reagent line came disconnected.

Next, valve in the LNSW "wash" and wait for a shift in the baseline response. The delays you observe are a measure of the transit times of the sample streams from probe to colorimeter for the various channels. Note the size of shift in baseline as well. At this point, it is usually a good idea to try a few test standards while in the “pre-run” mode to check the chemistries. At this point, if the baselines are smooth and not drifting, you are ready to set up the sample run.

12.2 The Sample Run

We usually run a "default" sample arrangement (The following resembles what is found within the “FS_table.tbl” template table file.) It’s intended to minimize carryover effects from one sample to the next. The sequence we've found useful is:

One “sync peak;” (required for auto-peak detection by the WINFOW software.)
3 LNSW bottles (the "CO" samples required by the software for carry-over deter.)
3 DIW bottles;
3 LNSW (low nutrient seawater) matrix bottles;
3 higher concentration standard;
1 “old” higher concentration standard (QC check);

- the actual rosette bottle samples run in sequence and/or "special/experimental" samples;

3 standards (repeat of the ones used at the start.)
3 LNSW matrix bottles; and finally

3 DIW bottles.

While waiting for all reagents to be on-line, you can begin preparing the sample run by filling the DIW and LNSW matrix bottles with the appropriate solutions and making up a working standard if a new one is needed. We normally label sets of 30 ml polyethylene bottles at the beginning of each cruise, each with its own color. The number of bottles in each set corresponds to the number of nutrient samples being taken on each cast. If using four cc cups, label or number them but only use them once. Typical use of 4 cc cups is for special (non-CTD/Rosette samples).

DIW, LNSW and working standard bottles have distinctly colored labels. This helps prevent mixing up bottles used for DIW and matrix with bottles used for working standards or high nutrient seawater samples on a previous sample run. We also label one or two extra sample bottles with duplicate numbers to sample some of the bottles in duplicate from each cast for monitoring precision.

The ideal situation now is to have the tested the chemistries in WINFLOW's "pre-run" mode, prepared the DIW, matrix and working standard bottles, and ready to start the sample run just after the CTD comes up on deck. The sampler is normally set for 90-95 seconds per sample, including wash. This means that the beginning series of DIW, LNSW, and STDs (standards) will take approximately 21 minutes for the FS to run before a "real" sample is required to be placed in the system's sampler tray.

Once the first set of samples (sinc, 3x lnsw, etc.) have been placed in the tray and the total sample count adjusted on the sampler, zero the baselines if necessary on the WINFLOW software by clicking on the ∅ button and click on the >> button to start the sample analysis run. After approximately 75 seconds start the sampler by pressing the start button located on the front of the sampler to begin the run.

During the sample run, the analyst's main tasks are; re-filling the sample tray with samples, checking the chemistry flow patterns, and keeping a sharp lookout for baseline jumps or drift in the colorimeter output on the data acquisition screen. It is also important to watch the sampler carefully because its sample probe can miss a sample bottle neck and suck air. This, and most other, problems which could develop during a run will signal themselves in the form of wildly oscillating or erratic traces. Other, less dramatic, causes are a bubble in a flow cell or a reagent reservoir running dry. It is a good idea to regularly check all of the channels for even bubble spacing, or spurious, small bubbles in the manifold or reagent lines and reagent /sampler wash reservoirs.

## 12.3 Shutting the System Down After Completing a Sample Run

After a set amount of time has elapsed after the last DIW "peak" has been recorded, the data acquisition software will stop automatically and save the run data. It is now time to begin flushing the system with DIW preparatory to shutting down until the next run. THIS MUST BE DONE CAREFULLY!

Remove all* tubes from their corresponding reagent bottles (*except for the tartaric acid (Si), imidazole Buffer (N+N) and citrate complexing reagent ($NH_4^+$)) and attach them to their own wash bottle locations. Switch the two-way valve on the sampler to the DIW draw position. Turn off heat baths.

Before removing the imidazole buffer tube from its reservoir, remove the OTCR from the N+N channel by isolating it using the three- or four-way valve. After the OTCR is off of the system, flushing of the N+N channel may begin. The life of the reductor will be drastically reduced if it is not filled with buffer while being stored between runs.

In order to prevent deposition of the tin oxide film in the silicic acid channel it is essential to move the $SnCl_2$ tube briefly to a 10% HCl reservoir and then to DIW. Then allow sufficient time for complete flushing of the $SnCl_2$ out of the silicic acid channel before moving the tartaric acid sipper to the DIW wash for flushing. This usually takes about four or five minutes. We have not yet evaluated the necessity for careful flushing when using ascorbic acid instead of $SnCl_2$ .

Finally, the citrate complexing reagent line is moved to the 10% HCl wash solution once the other $NH_4^+$ reagents have been flushed out.

After these steps have been completed, the system is completely flushed for about ten to fifteen more minutes and the pumps shut off after the pump cartridges are dis-engaged.

## 12.4 Miscellaneous Items.

Shutdown, startup, and general operation of the data acquisition system is a separate topic and will not be treated here.

## 13. BRIEF FS STARTUP CHECK OFF LIST

1. REPLACE ALL RINSE SOLUTIONS (mostly deionized water, some special ones) WITH FRESH ONES. Check all reagent bottles, top up as necessary.

2. CHECK TO SEE THAT PHOTOMETER POWER, AND HEATING BATH(s) ARE ON.

3. TURN ON NITROGEN. CHECK SAMPLER WASH LEVEL.

4. LOCK DOWN ALL CARTRIDGES AND TURN ON PUMPS.
   Again, check to see if reagent levels are adequate, and If necessary, prepare new working $OCl^-$ , and $SnCl_2$ (or ascorbic acid).

5. START WINFLOW DATA ACQUISITION SOFTWARE.
   Prepare sample table and initiate "Pre-Run" mode.

6. After a good bubble pattern is achieved using rinse solutions;
   BEGIN ADDING REAGENTS. HOLD OFF ON ASCORBIC ACID OR $SnCl_2$ UNTIL TARTARIC ACID IS IN THE SYSTEM. DON'T VALVE OTCR IN UNTIL ITS BUFFER REACHES IT.

7. AFTER A FEW MINUTES, ADD THE ASCORBIC ACID OR $SnCl_2$ AND THE OTCR.
   Check to see if you need a new working standard, more matrix, etc.
   If you've timed it right, the CTD is coming aboard about now.

8. VALVE IN THE SAMPLER LNSW "WASH". CHECK FOR CONSISTENT BUBBLE PATTERNS WHEN ALL REAGENTS ARE IN.

9. OBSERVE BASELINES FOR 5 - 10 MINUTES. Zero ADJUST IF OR AS NECESSARY. PINCH FlowCell draw lines to clear bubbles!

10. RUN ONE TO THREE STANDARDS OR HIGH NUTRIENT SAMPLES TO BE SURE ALL OF THE CHANNELS ARE BEHAVING CORRECTLY.

11. LOAD THE SAMPLER TRAY WITH ONE SYNC-PEAK STANDARD, BLANKS, MATRIX SAMPLES, STANDARDS AND SAMPLES. AFTER THE TEST STANDARDS HAVE COME THROUGH AND LOOK OK, START THE DATA ACQUISITION. AFTER APPROX. 75 SEC., START THE SAMPLER.

    **** CHECK THE SAMPLER STOP COUNT ****

12. HAVE A GOOD RUN!