OPERATION, METHODS and QUALITY CONTROL of Technicon AutoAnalyzer II Systems for Nutrient Determinations in Seawater

by

Carl Zimmermann

Mary Price

John Montgomery
Supervisor

February, 1977

HARBOR BRANCH FOUNDATION, INC. TECHNICAL REPORT NO. 11

Acknowledgement

The authors would like to express their appreciation to the Harbor Branch Consortium for providing the ways and means for writing and publishing this report. We would particularly like to thank Ms. Pat Linley for her valuable editorial comments, Mrs. Jackie McKay and Mrs. Barbara Hermann for typing the manuscript, and Mr. Tom Smoyer for photographic aid.

Foreword

During 1975-1976, the first priority of this laboratory has been to examine the applicability of selected micronutrient methods for estuarine waters. Over 11,000 nutrients and standards have been analyzed to date. The second priority has been to check the techniques for accuracy and precision using statistical quality control techniques (Harbor Branch Consortium, 1976).

The following manual was written as a reference to the multiple nutrient determination procedures as conducted by this laboratory. The Technicon AutoAnalyzer II system employed allows rapid, simultaneous, and accurate determinations of nutrients in seawater when utilized with the outlined procedures.

This manual is divided into four main sections: components, operation, methods and quality control.

Descriptions of colorimeter, pump, recorder and manifold assemblies are found in the component section.

The operation section deals with general operating instructions, sample, glassware, standards preparation, data reduction computer input/output format.

The third section, methods, is a step-by-step description of the reagents, manifold assembly and standards for each determination.

Lastly, quality control of our system is discussed. Validity of method, comparison of slopes, minimum detectable concentration and interlaboratory comparisons are dealt with here.

Hopefully, this manual will inform the reader as to the methods and quality control routinely performed by this laboratory. For a more detailed discussion of the system, the reader is advised to refer to the Technicon Industrial Systems Manual.

List of Tables

List of Figures

Section 1. Components

The AutoAnalyzer II system operates on a continuous flow basis where samples and reagents are continuously added in a specific sequence along a path of glass tubing and mixing coils. Air bubbles are injected at precise intervals to sweep the walls of the tubing and to help prevent diffusion between successive samples. The reactions in the AutoAnalyzer do not develop to completion as in manual methods, but reach identical stages of development in each sample since every sample follows the same path, timing and exposure to specific reagents.

The basic function of each component of the AutoAnalyzer is briefly discussed in this section. This explanation is similar to that of Sanborn and Larrance (1972).

Sampler

A sampler probe alternately draws fluid from a tray of discrete samples and then from a wash-fluid receptacle. The probe dips into the sample to be extracted, and at a timed interval, moves to a wash solution while the tray of samples advances one position. A bubble of air, which acts as a diffusion barrier, is aspirated into the sample stream between sample and wash. The ratio of sample to wash time as well as the number of samples analyzed per hour are controlled by a cam, located in the top well of the sampler assembly. Cams are easily changed and are available for a varied range of sampling rates.

The wash solution separates successive samples as indicated on the graphical record by alternating minima (wash) and maxima (sample). The sample probe is connected to a stream divider which delivers identical samples simultaneously to each

manifold via the pump.

Proportioning Pump

The proportioning pump is a peristaltic type pump which continuously delivers air, reagents and samples to the manifold. Plastic pump tubes of various diameters are pressed between a series of moving rollers and a platen. The motion of the rollers along the tubes delivers a continuous flow. The delivery rate is determined by the inside diameter of the tube since the rollers move at a constant rate. These pump tubes are available in a large assortment of delivery rates. The pump will hold a maximum of 28 tubes and has an air bar which mechanically measures and injects identical air bubbles into the analytical stream. The pump tubes delivering reagents, air and samples are connected to appropriate manifolds.

Manifold

Each analysis requires a manifold specifically designed for the chemical method being used. The manifolds are composed of a series of horizontal glass coils, injection fittings and heating baths arranged for the proper sequence of reactions leading to color development. The sample and the reagents mix within the glass coils. As two solutions with different densities travel around each turn of the mixing coil, the denser solution falls through the less dense one causing mixing and resulting in a homogenous mixture of the two solutions. The length of the coil determines the amount of time allowed for chemical reaction between the addition of successive reagents. Injection fittings for each of the

reagents are placed between mixing coils. Thus, a sample enters one end of the manifold, a reagent is added, the solution is mixed and given time to react, and then another reagent is added and mixed. After the addition of all reagents, and an adequate reaction time, the solution flows into a colorimeter.

Colorimeter

The colorimeter measures the absorption of monochromatic light by the solution in the flow cell. Light from a single source passes through two separate but identical interference filters which emit light within a narrow spectral band, then through the appropriate flow cell, and finally projects onto a phototube which generates an electrical signal in response to the intensity of the impinging light. The output from each phototube is a measure of transmittance and is converted electronically by the colorimeter to a signal proportional to absorbance. The relationship between transmittance and absorbance is given by the equation $A = \log \frac{1}{T}$

where: A = absorbance

T = transmittance

The resulting signal is linear in absorbance and is directly proportional to concentration. As each sample passes through the cell, the signals are sent to a recorder.

Recorder

Results of the analyses are continuously recorded by two-pen strip chart recorders. Each recorder simultaneously monitors two separate analyses. The output of the colorimeter is

proportional to absorbance and standards of known concentration must be analyzed to relate absorbance to concentration on the chart (see Section 2). The analog signals can be converted to absorbance values by referring to the Technicon® reference curve and the standard calibration control in Section 2 of this report.

Section 2. General Operating Instructions

This section is a step-by-step outline of the operation of our system. It is believed that this type of presentation will be easier for the reader to follow.

Operating Procedures

1. Colorimeter - Turn power on - allow 10 minutes warm-up. Check standard calibration setting for desired determination.
2. Recorder - Model 69CU. Turn power on - allow 10 minutes warmup.
3. Recorder - Check paper supply.
4. Water Reservoirs - Check and fill with deionized water (DHOH).
5. Connect pump tubes. Attach platen to pump.
6. Pump - Start pump with deionized water running through the system. Check for leaks in tubes at connections and for a regular bubble pattern.
7. Recorder - Turn recorder on to '1 & 2' (chart paper should start moving).
8. Colorimeter - Check ZERO and FULL SCALE on the recorder.

   ZERO - Simulates a zero output so that ZERO adjustment (screwdriver) of the recorder can be made.

   FULL SCALE - Simulates a 5.0 volt output signal on the telemetry connector and a reference level so that FULL SCALE adjustment (screwdriver) or desired deflection of the recorder can be made.
9. Establish zero baseline with deionized water being

pumped through the system using the BASELINE CONTROL adjustment.

10. Allow reagents to pump through; note rise in baseline and readjust to zero. Refer to this rise as the REAGENT BLANK.
11. The first two samples on the tray should be synthetic seawater. Refer to the mean peak height as the SYNTHETIC SEAWATER BLANK, followed by the standards and samples to be analyzed.
12. At the end of the run, disconnect the reagents and place tubes in DHOH. After 15 minutes, establish zero baseline with the BASELINE CONTROL setting. Run approximately 10 seawater samples (mean peak height is called the TURBIDITY BLANK).

Shutdown Procedure

1. With completion of sample turbidity blanks, turn off recorder (turn the 3-position switch to the middle position).
2. Wash system with 1N HCl for 15 minutes, followed by 15 minute wash with deionized water, by placing pump tubes in deionized water.
3. Turn off pump, release proportioning platen, loosen pump tubes.
4. Turn off recorder and power supply to colorimeter.
5. If system is to be used for 2 or more days in a row, leave colorimeter lamp on.

Sample Preparation

1. Water samples should be collected in aged polypropylene bottles, prerinsed three times with the sample. Bottles are aged by repeated freezing, washing with 1N hydrochloric acid and rinsing with deionized water.
2. Mercuric chloride ($HgCl_2$) added to sample. Stock $HgCl_2$ - 1.0g $HgCl_2$ to 500ml deionized water. 10ml stock/500ml sample gives a 40mg $HgCl_2$/1000ml solution.
3. Chill and keep in the dark.
4. Upon return to laboratory samples are
   a. filtered through 0.4μ pore size Nuclepore® filters.
   b. placed in refrigerator or freezer.

Glassware and Sample Cups

Glassware and sample cups for all determinations are acid washed with 1N HCl followed by numerous deionized water rinses.

Standards

1. Stock solutions are prepared with deionized water. Silicates are prepared with cooled, boiled deionized water. As a general rule, stock solutions should be prepared every six months.
2. Secondary standards for all determinations are prepared using synthetic seawater.

add: 62.0g NaCl
20.0g $MgSO_4 \cdot 7H_2O$
0.08g $NaHCO_3$
with enough deionized water to make: 2000ml

Secondary standards should be prepared each day.

The standard curve must encompass the range of the samples.

Reading Charts

1. Subtract the synthetic seawater blanks from the standard peak height.
2. Subtract the turbidity blanks from the sample peak heights.
3. Be sure to record: REAGENT BASELINE
   SYNTHETIC SEAWATER BLANK
   TURBIDITY BLANK
   STANDARD CALIBRATION - adjusts the gain, the electrical output, to correspond to the concentration of a specific reference solution. This control should be set to include the maximum standard concentration.
   DRIFT - the upward or downward trend of the baseline after a number of samples have been run. Fluctuations may be due to chemical or mechanical problems.

DATA INPUT

All samples and standards are run in duplicate. The number of standard sets (minimum 4 concentrations) varies with the number of samples. After the peak heights of

standards and samples have been corrected for synthetic seawater blanks and turbidity blanks, the results are recorded on a data input sheet (Table 2-1). This information, along with oxygen, salinity, temperature and seston data, is sent to the computer section where a program is run to determine concentration of the samples based on the standard curve (see reduced data and quality control section). The results (Table 2-3) are placed in our files and copies are sent to the principal investigators.

Table 2-2. Raw physical data input format

INDIAN RIVER LAGOON PLANKTON STUDY

DATE: 29 Nov. 1976 CRUISE NUMBER 46

COLLECTION TIME		1344	1309	1129	1042	1004	0925	0840
STATION		1.140	1.172	1.172	1.184	1.908	1.188	1.197
TEMPERATURE (°C)	Surface	23.0	22.3	22.3	22.5	22.7	21.5	21.0
	Bottom	22.5	22.3	22.3	22.5	22.5	21.5	21.0
SALINITY (o/oo)	Surface	28.90	26.72	26.72	32.23	34.11	34.11	33.73
	Bottom	28.53	26.72	26.72	33.73	34.49	34.11	33.73
refractometer	Integrated	31.00	28.00	28.00	34.00	35.00	35.00	35.00
DISSOLVED OXYGEN (mg/ℓ)	Surface	7.46	7.41	7.41	6.75	7.17	7.17	6.78
	Bottom	7.21	7.28	7.28	6.36	7.14	7.14	6.72
SESTON DRY WEIGHT (mg/ℓ)		5.4	6.8	6.8	7.4	11.8	11.8	13.0
SESTON ASH WEIGHT (mg/ℓ)		NR	NR	NR	NR	NR	NR	NR

Table 2-3. Reduced chemical data

INDIAN RIVER LAGOON PLANKTON STUDY

REDUCED CHEMICAL DATA

DATE 112976 CRUISE 46

COLLECTION TIME	1344	1309	1129	1042	1004	925	840
STATION	1.140	1.146	1.172	1.184	1.908	1.188	1.197
SURFACE TEMPERATURE (°C)	23.0	22.5	22.3	22.5	22.7	21.5	21.0
BOTTOM TEMPERATURE (°C)	22.5	22.5	22.3	22.5	22.5	21.5	21.0
SURFACE SALINITY (o/oo)	28.90	28.53	26.72	32.23	34.11	34.11	33.73
BOTTOM SALINITY (o/oo)	28.53	28.17	26.72	33.73	34.49	34.11	33.73
INTEGRATED SALINITY (o/oo)	31.00	31.00	28.00	34.00	35.00	35.00	35.00
SURFACE D.O. (mg/l)	7.46	7.17	7.41	6.75	6.52	7.17	6.78
BOTTOM D.O. (mg/l)	7.21	6.86	7.28	6.36	6.56	7.14	6.72
SESTON DRY WEIGHT (mg/l)	5.40	7.00	6.80	6.60	7.40	11.80	13.00
SESTON ASH WEIGHT (mg/l)							
DISSOLVED SILICATE (ug at/l)	33.77	35.33	23.87	10.23	9.49	12.50	24.09
ORTHOPHOSPHATE (ug at/l)	1.35	1.49	.94	.46	.37	.27	.28
NITRITE (ug at/l)	.22	.57	.29	.27	.15	.11	.27
NO_3 + NO_2 (ug at /l)	.56	1.17	.65	.79	.42	.28	.89
AMMONIA (ug at/l)	2.68	4.45	2.06	2.13	1.95	2.00	2.47
UREA (ug at/l)							

REDUCED DATA

The reduced chemical data returned to us by the computer section includes a number of statistical tests, the standard curve values and tabulated physical-chemical results for each station.

The slope (B) and intercept (α) are calculated for each standard curve as well as for the combined curve. Sample concentrations are determined by the linear least squares methods (Sokal and Rohlf, 1969). Peak height is entered as the known value (y), the equation $y = \alpha + Bx$ is thus solved for concentration (x). Standard curves are analyzed for validity using analysis of variance (Table 2-4) (ANOVA, Sokal and Rohlf, 1969).

To determine if any significant difference exists ($P<0.05$) between the slopes of the standard curves, the test of $B_1 = B_2$ for all sets, is performed using Analysis of Covariance (Snedecor and Cochran, 1967). The F values (mean square between the two regression coefficients divided by the mean square within the two lines) for the results of the covariance significance test are listed under the column headed F and across the row for cov 1-2 (numbers 1-2,1-3,2-3, indicate which set of curves are being compared). Calculated values for F are compared against critical values for F at the appropriate degrees of freedom (DF). Critical values for F are found in statistical tables. When the calculated F value is greater than the critical F, there are significant differences between the curves. In Table 2-4, the calculated value of F between the 2nd and 3rd

standard curves equals 0.99 (DF=1,16), while the critical value of F at the 95% confidence limit equals 4.49 (DF=1,16). As the calculated F value is less than the critical F, there is no signficiant difference between the 2nd and 3rd standard curves.

Table 2-4. Computer printout of standard curve comparisons for silicate

	B	α	F	DF
1st standard set	2.016	-1.546	4814.195	9
2nd standard set	2.016	-1.546	4814.195	9
3rd standard set	1.977	3.787	1149.952	9
All std. sets	2.003	0.232	2765.976	29
For cov 2-3			0.99	16
For cov 1-3			0.00	16
For cov 1-2			0.99	16

For each station of a specific cruise, the mean concentration (Mean), standard deviation (Std. Dev.) and coefficient of variation (cv) are determined for the number (N) of replicates analyzed (Sokal and Rohlf, 1969). An example of a typical cruise is shown (Table 2-5).

Table 2-5. Sample printout of statistical data for one station

Station 1.146

	Mean	Std. Dev.	cv	N
SiO_3	35.32	0.00	0.00	4
PO_4	1.48	0.03	0.02	4
NO_2	0.57	0.00	0.00	4
NO_3	1.17	0.01	0.01	3
NH_3	4.45	0.17	0.04	4

The standard deviation is computed to give an estimate of the deviation of the sample points from the mean. The

coefficient of variation allows comparison of the amount of variation between replicate sets, which may have different means, for the same sample.

Section 3. Specific Determinations

ORTHO-PHOSPHATE

Method: Technicon Industrial Method - 155-71W-Modified

Manifold Assembly: See Figure 3-1

Range: 0-4.0µg at/l

Standard Calibration: 8.5

Damp: Normal

Sampling Rate: 30/hr.

2:1 wash-sample ratio

Filter: 880nm

Phototube: 199-BO21-04

General Description: The determination of ortho-phosphate in seawater depends upon the formation of a phosphomolybdenum blue complex.

A single reagent solution is used consisting of an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony.

Although arsenate produces a color similar to phosphate, seawater rarely contains arsenate in concentrations high enough to interfere (Technicon, 1973).

Reagents:

1. Sulfuric Acid (4.9N). Slowly add 136ml concentrated H_2SO_4 to 800ml deionized water, to prevent overheating. Cool solution, dilute to 1.0 liter with deionized water.
2. Ammonium molybdate - $(NH_4)_6\ MO_7\ O_{24}\cdot 4H_2O$. Dissolve 40.0g ammonium molybdate in 800ml deionized water. Dilute to 1.0 liter with deionized distilled water.

3. Ascorbic Acid - Dissolve 18.0g USP quality ascorbic acid in 800ml deionized water. Dilute to 1.0 liter. Place 100ml in labelled polypropylene bottles and freeze. Remove the day before analysis and thaw in refrigerator.

4. Antimony Potassium Tartrate (KAT) - $K(SbO)\ C_4H_4O_6 \cdot 1/2\ H_2O$. Dissolve 3.0g antimony potassium tartrate in 800ml deionized water. Dilute to 1.0 liter with deionized water.

5. Combined Working Reagent*

4.9N H_2SO_4	100ml
Ammonium Molybdate	30ml
Ascorbic Acid	60ml
Antimony Potassium Tartrate	10ml

*IMPORTANT

a. Mix after each addition.

b. With addition of ascorbic acid, a yellow color should develop.

c. A "cloud" should develop with addition of KAT.

d. The combined working reagent is stable for no longer than 8 hours.

e. A stable solution can be prepared by not including the ascorbic acid in the combined reagent. If this is done, the mixed reagent (acid, molybdate and tartrate) is pumped through the deionized water line and the ascorbic acid solution (30ml of 18g/l diluted to 100ml with deionized water)

through the original mixed reagent line.

f. The addition of Levor® as a wetting agent as recommended by Technicon, was found to give high blank readings and consequently is not being used.

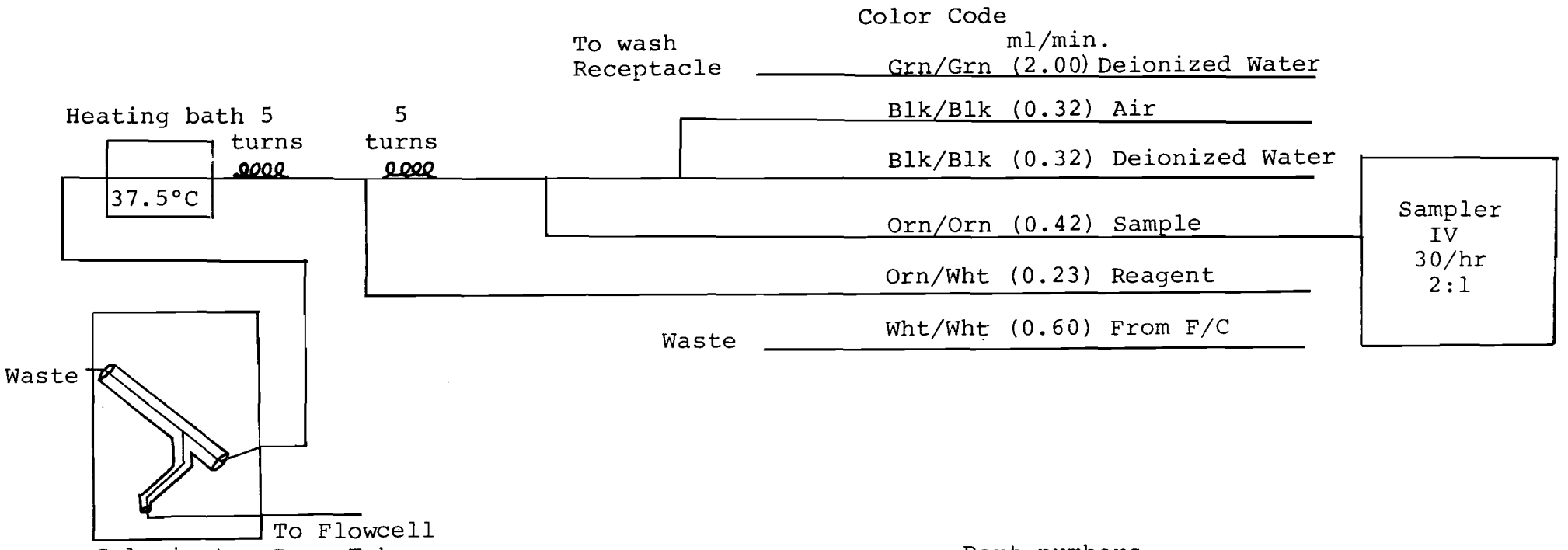
Standards:

1. Standards must bracket the sample range.
2. Stock Standard - 0.136g anhydrous potassium dihydrogen phosphate (KH_2PO_4) to 1000ml with deionized water (1000µg at P/l). Add 1.0ml chloroform to act as a preservative.
   a. 4.0ml of stock standard to 100ml with synthetic seawater (40µg at/l).
   b. Working Standards:

ml of "a"		final concentration µg at/l
0.5ml		0.2µg at/l
1.0ml		0.4µg at/l
2.0ml	Bring to 100ml with synthetic seawater	0.8µg at/l
4.0ml		1.6µg at/l
8.0ml		3.2µg at/l

RESULTS OF QUALITY CONTROL IN SEPARATE SECTION

Figure 3-1. Manifold assembly - Ortho-Phosphate



Colorimeter Pump Tube
Filter: 880nm
Phototube: 199-B021-04
Flowcell: 50mm x 1.5mm I.D.

Part numbers

5 turn coil: 170-0103

37.5° Heating Bath: 157-B273-03

SILICATE

Method: Technicon Industrial Method - 186-72W-Modified

Manifold Assembly: See Figure 3-2

Range: 0-40µg at/l

Standard Calibration: 4.0

Damp: 1

Sampling Rate: 40/hr.

4:1 sample-wash ratio

Filter: 660nm

Phototube: 199-B021-01

General Description: Determination based on reduction of silicomolybdate in acidic solution to "molybdenum blue" by ascorbic acid. Oxalic acid is introduced to the sample steam before the addition of ascorbic acid to eliminate interference from phosphate.

Reagents:

1. Ammonium Molybdate: $(NH_4)_6\ MO_7\ O_{24}\cdot H_2O$. Dissolve 10.0g ammonium molybdate in a liter of 0.1N sulfuric acid (2.8ml concentrated H_2SO_4/1000ml deionized water). Filter and store in an amber plastic container.
2. Ascorbic Acid: Dissolve 17.6g USP quality ascorbic acid to 800ml deionized water. Add one dropper Levor IV® per 100ml. Place 100ml in polypropylene bottles and freeze. Thaw in refrigerator prior to use.
3. Oxalic Acid: Add 50.0g oxalic acid to 900ml deionized water and bring up to 1 liter.

Standards:

1. Standards must bracket the sample range.
2. Stock Standard: 1.88g sodium fluosilicate ($Na_2Si\ F_6$) up to 1000ml with recently boiled and cooled deionized water (10,000µg at Si/l).

   Store in tightly stoppered plastic bottle.

   a. 1ml of stock - up to 10ml with synthetic seawater (1000µg at/l).

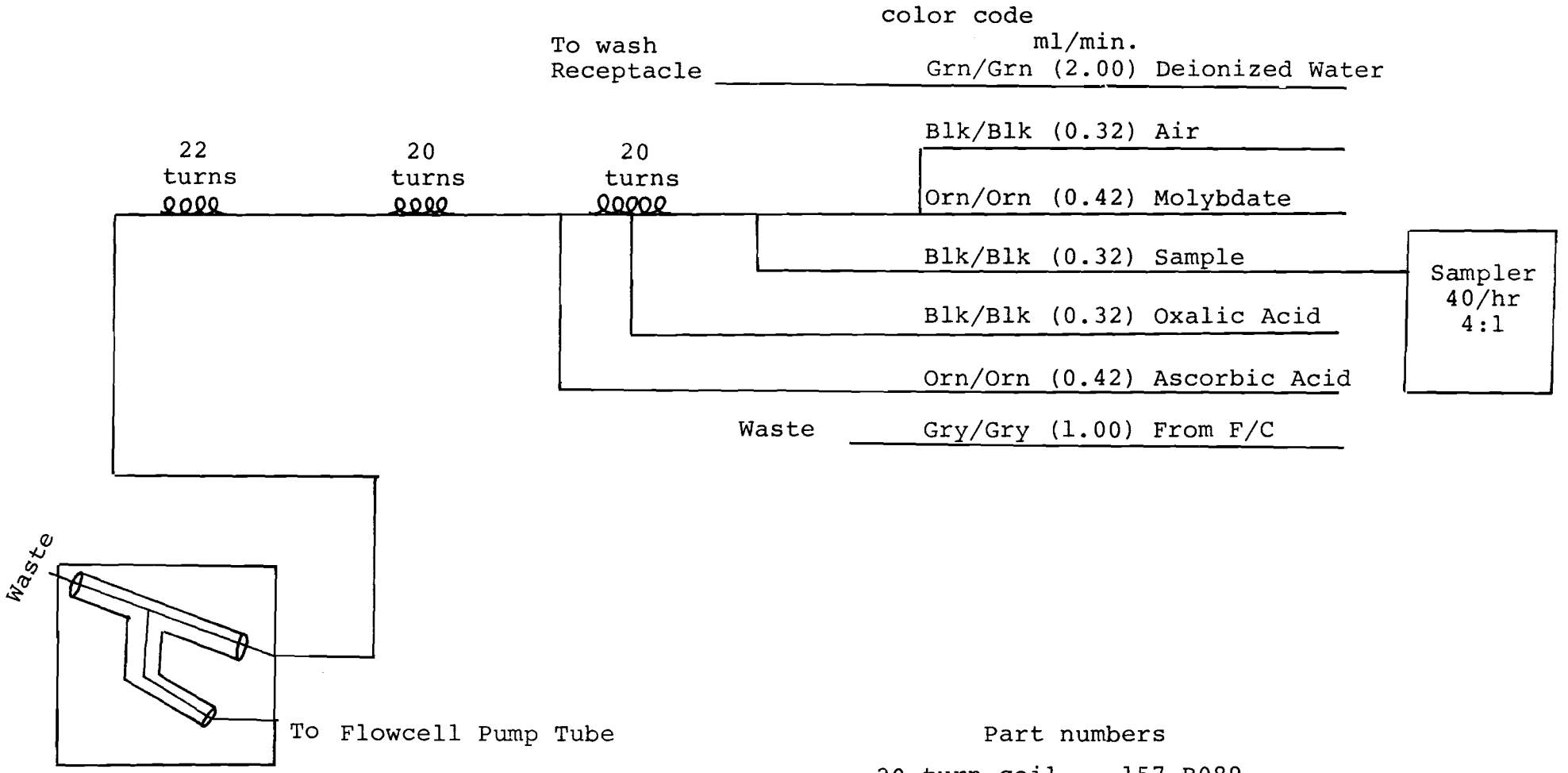
   Working Standards:

   b.

ml of "a"		final concentration µg at/l
0.5ml	Bring to 100ml with synthetic seawater	5µg at/l
1.0ml		10µg at/l
2.0ml		20µg at/l
3.0ml		30µg at/l
4.0ml		40µg at/l

RESULTS OF QUALITY CONTROL IN SEPARATE SECTION

Figures 3-2. Manifold assembly - Silicate



Colorimeter
Filter: 660nm
Phototube: #199-B021-01
Flowcell 50mm x 1.5mm I.D.

Part numbers

20 turn coil:	157-B089
20 turn coil:	157-B095
22 turn coil:	157-0370

NITRITE

Method: Technicon Industrial Method - 161-71W

Manifold Assembly: See Figure 3-3

Range: 0-2.0µg at/l

Standard Calibration: 7.5

Damp: Normal

Sampling Rate: 40/hr.

4:1 sample-wash ratio

Filter: 550nm

Phototube: 199-B021-01

General Description: Under acidic conditions, the nitrite ion reacts with sulfanilamide to yield a diazo compound which couples with N-1- naphthylenediamine dihydrochloride to form a soluble dye which is measured colorimetrically.

Interferences: High alkalinity (600 mg/l) will give low results due to a shift in pH of the color reaction.

Reagents:

Color Reagent:

a.	Sulfanilamide	20.0g
b.	Concentrated phosphoric acid	200.0ml
c.	N-1- naphthylethylendiamine dihydrochloride	1.0g
d.	Deionized water	2000.0ml

To ≈ 1500ml deionized water, add 200ml concentrated phosphoric acid and 20.0g sulfanilamide. Dissolve completely. Add 1.0g N-1- naphthylethylendiamine dihydrochloride and dissolve. Dilute to 2 liters.

Standards:

1. Standards must bracket the sample range.
2. Stock Standard - 0.345g sodium nitrite ($NaNO_2$) up to 1000ml with deionized water (5,000µg at N/l). Add 1.0ml chloroform to act as a preservative.
   a. 0.4ml stock standard to 100ml with synthetic seawater (20µg at/l).

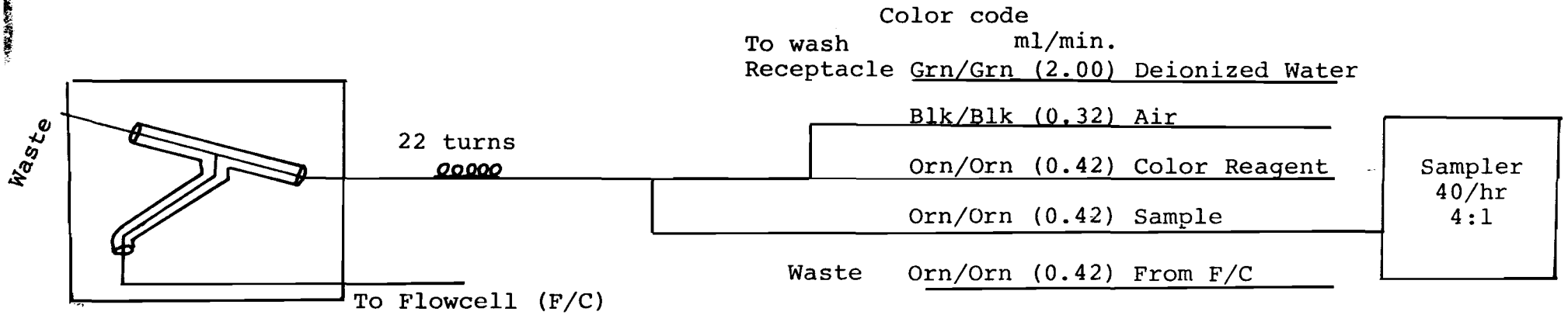
   Working Standards:

   b.

ml of "a"		final concentration µg at/l
0.25ml		0.05µg at/l
0.5ml		0.1µg at/l
1.0ml	Bring to 100ml with synthetic seawater	0.2µg at/l
1.5ml		0.3µg at/l
2.0ml		0.4µg at/l

RESULTS OF QUALITY CONTROL IN SEPARATE SECTION

Figure 3-3. Manifold assembly - Nitrite



Part Numbers

22 turn coil: 157-0370

AMMONIA

Method: Technicon Industrial Method - 154-71W-Modified

Manifold Assembly: See Figure 3-4

Range: 0-12 µg at/l

Standard Calibration: 7.5

Damp: 1

Sampling Rate: 30/hr.

2:1 sample-wash ratio

Filter: 630nm

Phototube: 199-B021-04

General Description: The formation of a blue colored compound believed to be closely related to indophenol, occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxide of calcium and magnesium.

Reagents:

1. Complexing Reagent:

a. Potassium Sodium Tartrate	33.0g
b. Sodium Citrate	24.0g
c. Deionized Water	1000.0ml

Dissolve 33.0g potassium sodium tartrate and 24.0g of sodium citrate in 950ml deionized water. Adjust the pH of this solution to 5.0 with concentrated sulfuric acid. Dilute to 1 liter with distilled water.

Add 1 dropper Brij-35® /250ml reagent before use.

2. Alkaline Phenol*: Phenol: 23.6ml 88% liquid phenol

NaOH: 45ml (20% w/v)

Deionized Water: Up to 250ml

To approximately 100ml deionized water add 23.6ml 88% liquid phenol. Add 45ml NaOH (20%) in small increments while cooling. Bring to 250ml with deionized water.

3. Sodium Hypochlorite: Dilute 50ml Hypochlorite (clorox) up to 250ml with deionized water.

4. Sodium Nitroferricyanide: Dissolve 0.5g sodium nitroferricyanide ($Na_2Fe(CN)_5(NO)\cdot 2H_2O$) in 900ml deionized water and dilute to 1.0 liter. This acts as a catalyst.

*Note: It has been found liquid phenol give better results than the crystalline form.

Standards:

1. Standards must bracket the sample range
2. Stock Standard: 0.4716 ammonium sulfate $(NH_4)_2SO_4$ bring to 1000ml with deionized water (7142µg at N/l). Add 1.0ml chloroform to act as a preservative.

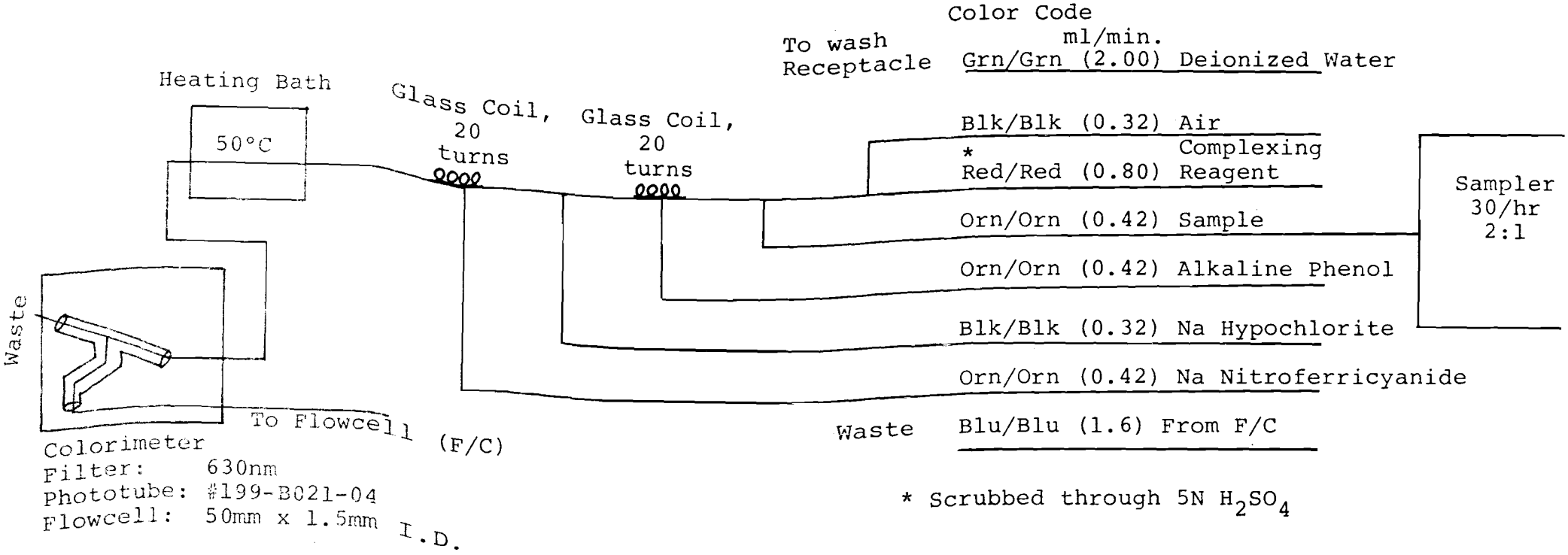
a. 2.0ml of stock standard up to 100ml with synthetic seawater (143µg at N/l).

Working Standards:

b.

ml of "a"		final concentration µg at/l
0.5ml		0.73µg at/l
1.0ml		1.45µg at/l
2.0ml	Bring to 100ml with synthetic seawater	2.9µg at/l
4.0ml		5.8µg at/l
8.0ml		11.6µg at/l

Figure 3-4. Manifold assembly - Ammonia

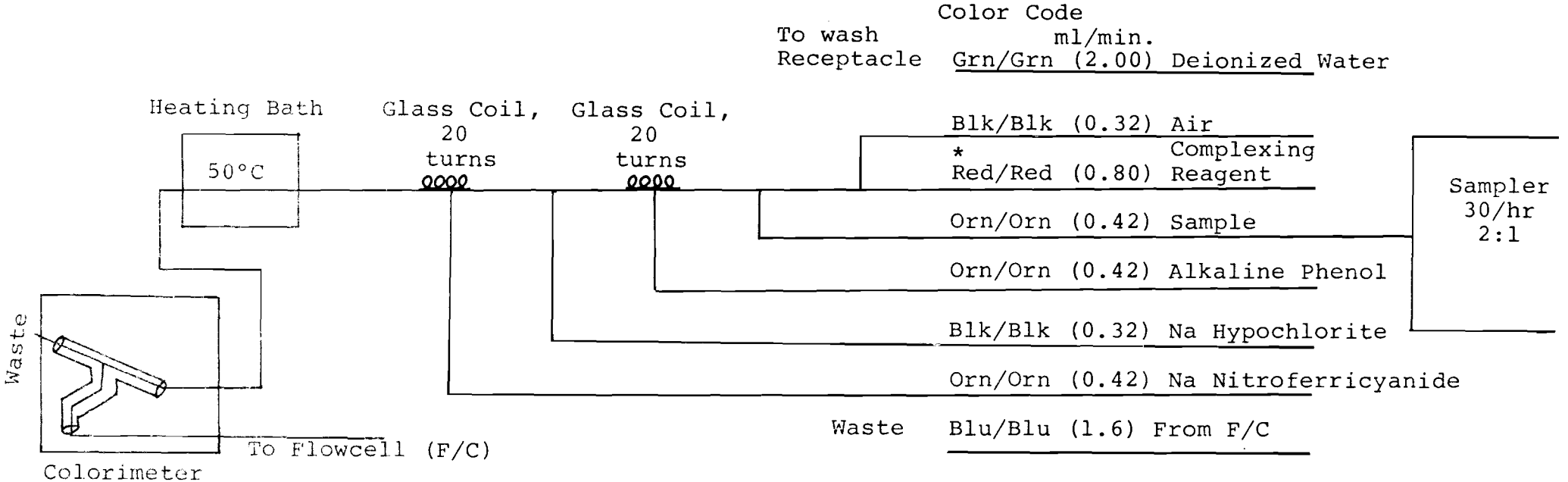


* Scrubbed through 5N H_2SO_4

Part Numbers

20 turn coil: 157-B089
20° turn coil: 157-B095
50° heating bath: 157-B273-09

Figure 3-4. Manifold assembly - Ammonia



Colorimeter
Filter: 630nm
Phototube: #199-B021-04
Flowcell: 50mm x 1.5mm I.D.

* Scrubbed through 5N H_2SO_4

Part Numbers

20	turn coil:	157-B089
20°	turn coil:	157-B095
50°	heating bath:	157-B273-09

NITRATE + NITRITE

Method: Technicon Industrial Method - 158-71W-Preliminary

Manifold Assembly: See Figure 3-5

Range: 0-5.0µg at/l

Standard Calibration: 8.5

Damp: 1

Sampling Rate: 40/hr.

4:1 sample-wash ratio

Filter: 550nm

Phototube: 199-B021-01

General Description: This method utilizes the procedure which reduces nitrate to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfamilamide under acidic conditions to form a diazo compound. This compound then couples with N-1 naphthyethylenediamine dihydrochloride, forming a reddish-purple azo dye.

Reagents:

1. Ammonium Chloride: Dissolve 10.0g of ammonium chloride in alkaline water and dilute to 1.0 liter. Brij-35® (1 dropper) is added immediately before use. Alkaline water is prepared by adding just enough ammonium hydroxide to deionized water to attain a pH of 8.5.
2. Color Reagent:
   a. Sulfanilamide 20.0g
   b. Concentrated phosphoric acid 200ml
   c. N-1- Naphthylethylenediamine Dihydrochloride 1.0g
   d. Deionized water 2000ml

To approximately 1500ml of deionized water, add 200ml concentrated phosphoric acid and 20g of sulfanilamide. Dissolve completely (heat if necessary). Add 1.0g N01-Naphthylethylenediamine dihydrochloride and dissolve. Dilute to 2 liters with deionized water. Refrigerate. Add 1 dropper Brij-35® before analysis. Stability: one month.

Preparation of Cadmium Column

Preweighed cadmium powder may be purchased from Technicon (T11-5063). Rinse the power twice with 1N HCl to remove dirt. Rinse several times with distilled water. Allow metal to air dry and store in stoppered bottle. The reductor column tube is a 35cm length of 0.081 ID standard Tygon tubing.

Wash 10g of previously cleaned cadmium with 50ml of 2% w/v copper sulfate until no blue color remains in solution and semi-colloidal copper particles begin to enter the supernatant liquid. It is extremely important that the cadmium not come into contact with air any time after having been treated with the copper-sulfate solution (Wood, 1967). Wash thoroughly with deionized water to remove colloidal copper. A minimum of 10 washings is usually required. Fill the reductor column tube with deionized water to prevent the entrapment of air bubbles during filling operation. Place glass wool plug at one end of the column. Transfer the prepared cadmium granules to the column avoiding contact with air (Figure 3-5). The use of a vibration source (Dremel Electric Engraver) along the outside walls of the column, helps pack the granules evenly. Approximately 8g is required to

pack the column but varies with particle size. Insert glass wool and connectors (G116-0010-01 N9).

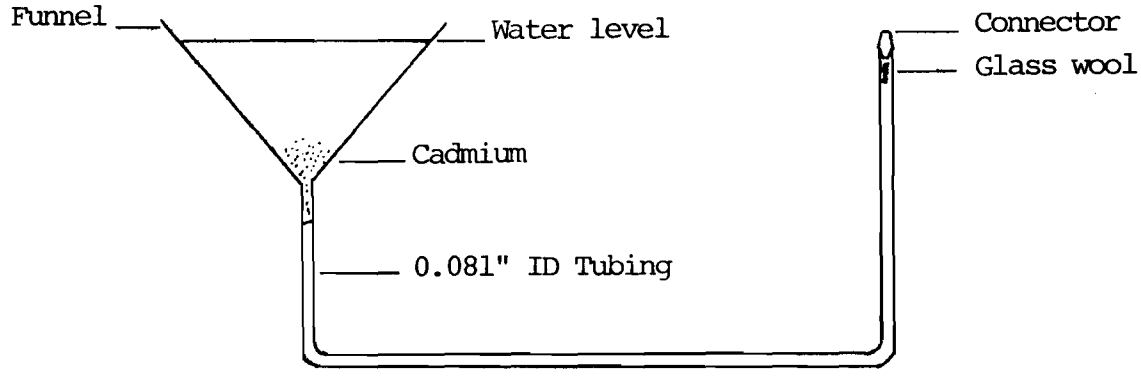


Figure 3-5. Filling the copper-cadmium column

When the pump tubes are filled with reagents and all air is removed from transmission lines, attached one side of the cadmium column to the resample line and the other end of the debubbler line. For initial activation, a midscale (3.0µg at N/1) standard should be pumped through the system for 1 hour.

When detaching column, reverse the procedure; store column by filling with 1% ammonium chloride.

To reactivate cadmium, remove cadmium, wash with 1N HCl, rinse thoroughly with deionized water, re-treat with copper sulfate and repack.

Standards

1. Standards must bracket the sample range.
2. Stock Standard: 0.101g potassium nitrate (KNO_3) bring to 1000ml with deionized water (1000µg at N/1). Add

1.0ml chloroform to act as a preservative. Store in a dark bottle.

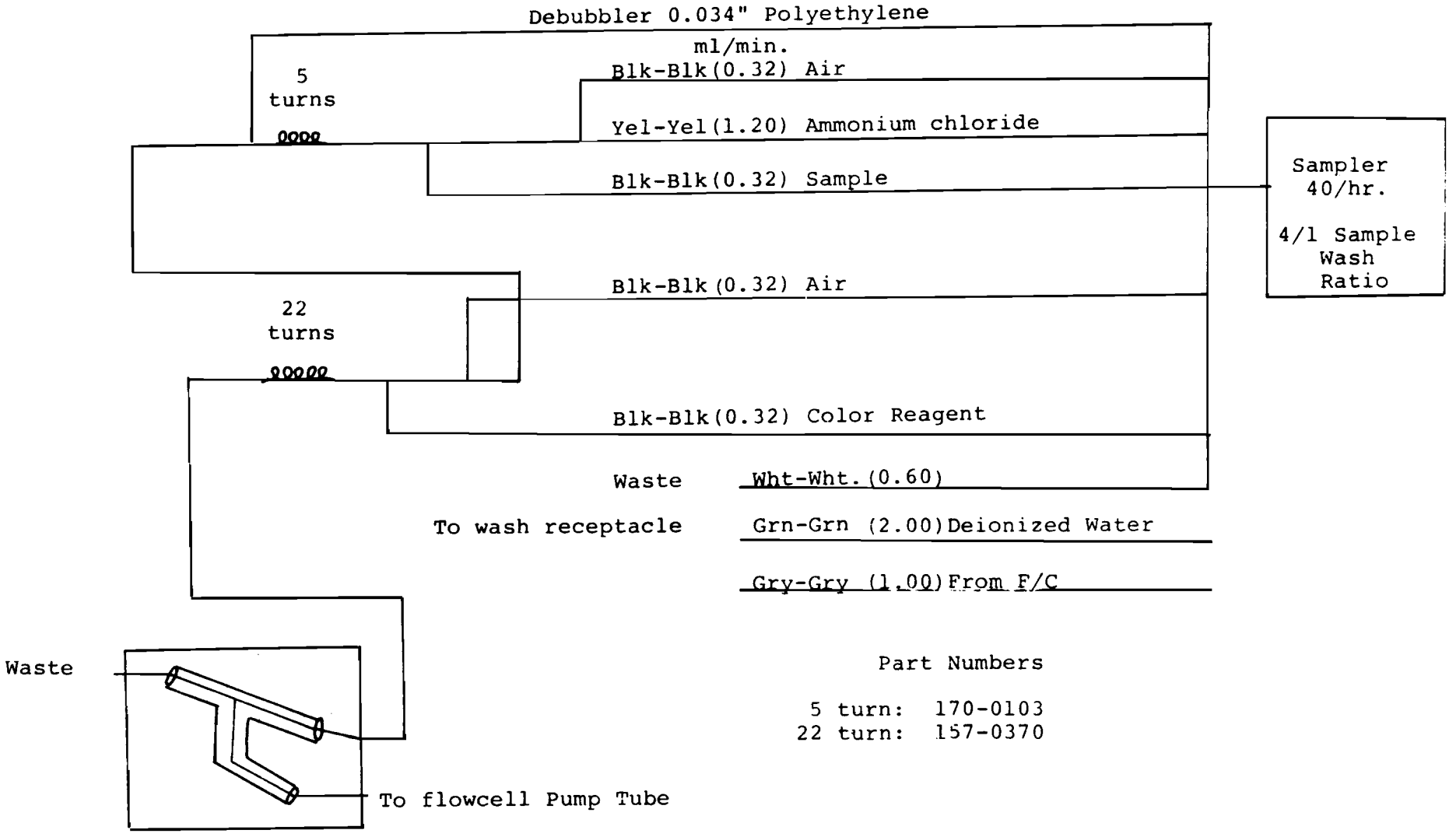
a. 5ml of stock standard up to 100ml with synthetic seawater (50µg at N/l).

Working Standards:

b.

ml of "a"		final concentration µg at/l
0.5ml		0.25µg at N/l
1.0ml		0.50µg at N/l
2.0ml	Bring to 100ml with synthetic seawater	1.00µg at N/l
4.0ml		2.00µg at N/l
6.0ml		3.00µg at N/l

Figure 3-6. Manifold assembly - Nitrate + Nitrite



Part Numbers

5 turn: 170-0103
22 turn: 157-0370

Colorimeter
Filter: 550nm
Flowcell: 50mmx 1.5mm Flowcell
Phototube: 199-B021-01

Section 4. Quality Control

The responsibilities of any analytical laboratory include insuring the validity of the methods and determining the quality of the obtained results. Thus, the two primary functions of the analytical chemist are monitoring the reliability of the reported results and controlling their quality in order to meet the program requirements for reliability (EPA, 1972). The following section discusses how this laboratory has developed its quality control program.

Validity of Method

As described in the previous section, all nutrient determination procedures are those of Technicon Industrial Systems with minor modifications (Technicon, 1973). These methods list the sensitivities in units of absorbance-concentration (μg at/ℓ).

A calibration curve has been devised (Technicon, 1973) plotting standard calibration control settings versus absorbance units (Figure 4-1).

All Technicon determinations list the absorbance per unit concentration (sensitivity) at a given concentration and what absorbance unit it should give (standard calibration setting). Table 4-1 is a comparison of our absorbance units compared to Technicon's.

Table 4-1. Comparison of absorbances Harbor Branch (HBF) vs. Technicon (Tech)

	PO_4		NO_2		$NO_2 + NO_3$		Si		NH_3	
	HBF	Tech.	HBF	Tech.	HBF	Tech.	HBF	Tech.	HBF	Tech.
Absorbance	0.18	0.15	0.20	0.18	0.14	0.17	0.38	0.38	0.20	0.15
µg at/l	3.2	4.0	1.6	2.0	4.0	5.0	40	50	11.6	10
Peak height	60	100	80	100	80	100	80	100	70	100
Sensitivity (Y)	0.06	0.04	0.12	0.09	0.04	0.03	0.01	0.01	0.02	0.02
Y x Peak Hgt.	3.36	3.70	10.0	9.0	2.80	3.00	0.73	0.71	1.26	1.50

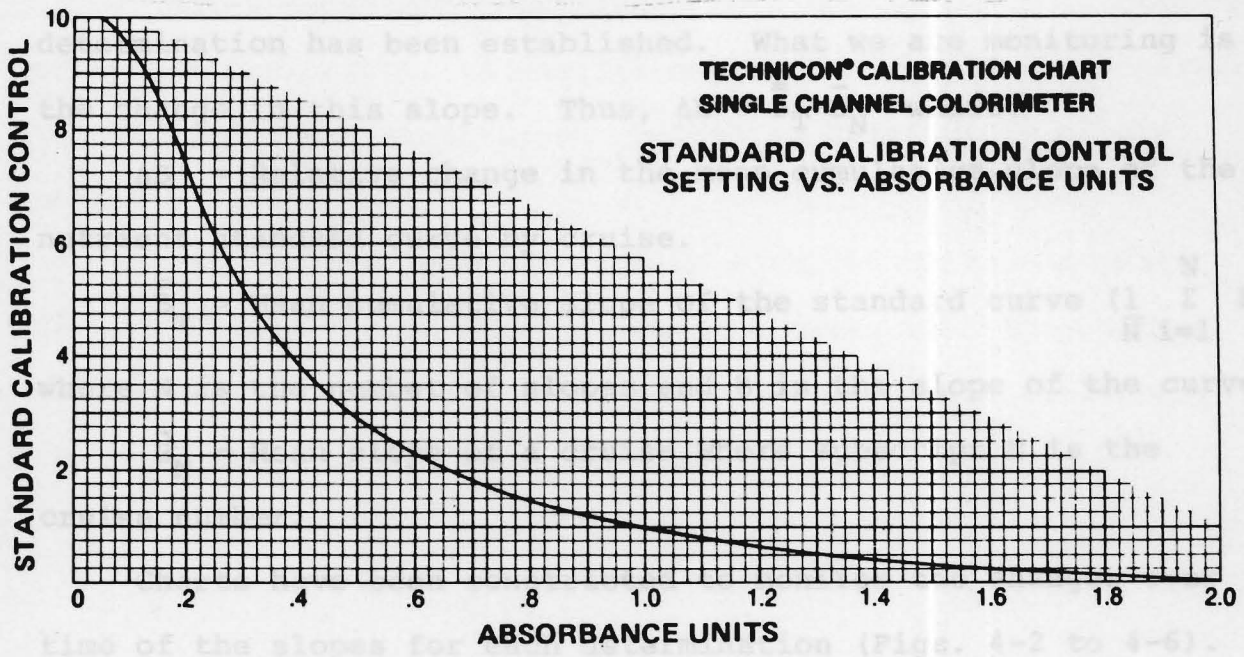


Figure 4-1. Standard calibration control setting versus absorbance units (Technicon Corporation, Tarrytown, New York)

All Technicon determinations list the absorbance per unit concentration (sensitivity) at a given concentration and what absorbance unit it should give (standard calibration setting). Table 4-1 is a comparison of our absorbance units compared to Technicon's.

Table 4-1. Comparison of absorbances Harbor Branch (HBF) vs. Technicon (Tech)

	PO_4		NO_2		$NO_2 + NO_3$		Si		NH_3	
	HBF	Tech.	HBF	Tech.	HBF	Tech.	HBF	Tech.	HBF	Tech.
Absorbance	0.18	0.15	0.20	0.18	0.14	0.17	0.38	0.38	0.20	0.15
µg at/l	3.2	4.0	1.6	2.0	4.0	5.0	40	50	11.6	10
Peak height	60	100	80	100	80	100	80	100	70	100
Sensitivity (Y)	0.06	0.04	0.12	0.09	0.04	0.03	0.01	0.01	0.02	0.02
Y x Peak Hgt.	3.36	3.70	10.0	9.0	2.80	3.00	0.73	0.71	1.26	1.50

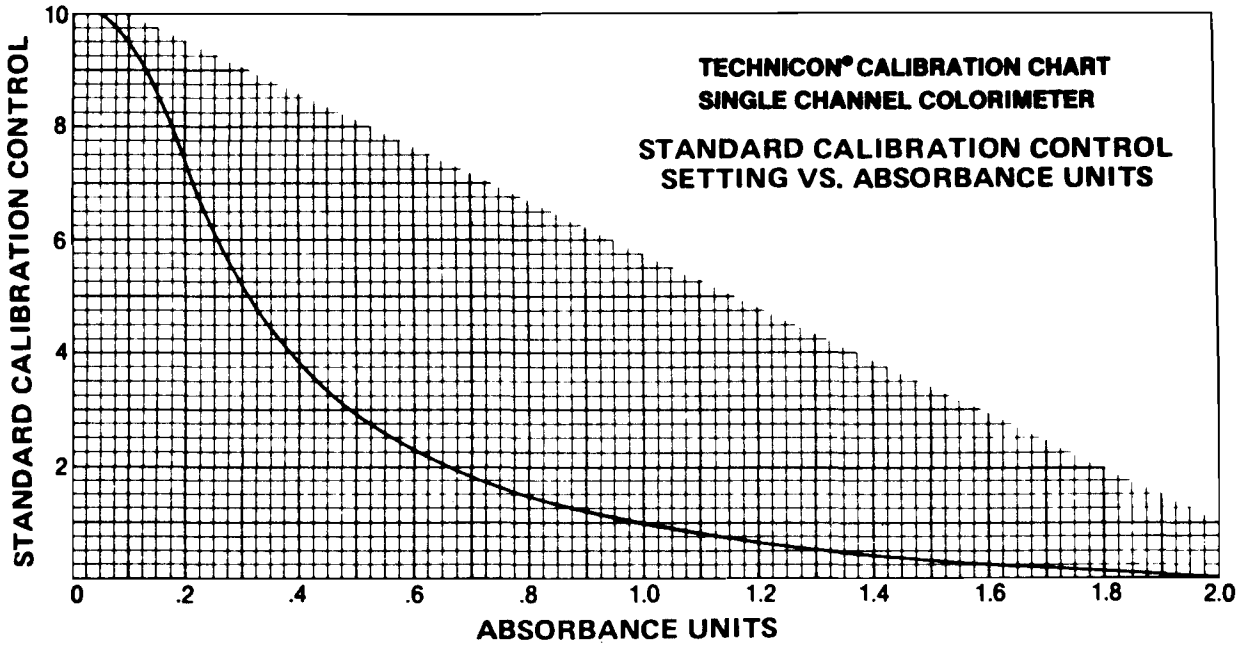


Figure 4-1. Standard calibration control setting versus absorbance units (Technicon Corporation, Tarrytown, New York)

As can be seen from Table 4-1, this laboratory's results are very close to the sensitivities determined by Technicon.

Comparison of Standard Curves

Once the validity and accuracy of the method has been assured, a program is then needed to monitor the slopes of the standard curves for each run.

These quality control charts allow us to determined if the system is operating within stated upper and lower limits (EPA, 1972).

A linear regression equation of the form $Y = \alpha + BX$ is utilized in this procedure, where:

α = intercept

B = slope

X = concentration of the sample in µg at/l

A mean cumulative slope for each nutrient standard curve determination has been established. What we are monitoring is the change in this slope. Thus, $\Delta B = \bar{\bar{B}}_T - \bar{B}_N$ where:

ΔB = Relative change in the mean cumulative slope of the nutrient standard curve by cruise.

$\bar{\bar{B}}_T$ = Mean cumulative slope of the standard curve $\left(\frac{1}{N} \sum_{i=1}^{N} B\right)$ where N is the number of slopes and B is the slope of the curve.

$\bar{B}_N$ = Mean slope of a cruise where subscript N is the cruise number.

Charts have been constructed to monitor the changes over time of the slopes for each determination (Figs. 4-2 to 4-6). This is accomplished by setting $\bar{\bar{B}}_T$ equal to zero and plotting ΔB by cruise. This graph should vary with a statistically normal

distribution around $\bar{\bar{B}}_T$. Trends toward one side or the other of zero are indicative of problems in the method, reagents, or sampling procedure. These upper and lower limits act only as a guide to the analyst.

As can be seen in Figures 4-3, 4-4, and 4-5 the relative change in slope is random with the majority of the points falling within the stated limits.

Nitrate (Fig. 4-6) indicates a random trend but the majority of points do not fall within the upper and lower control limits. This may be due to fluctuations in the efficiency of the copper-cadmium column. However, the narrow upper and lower limits (±1) indicate that the problem does not stem from variability in slopes within standard sets from one cruise, but rather wide slope variation between cruises. An alternative quality control method, cumulative sum with "V" mask (Montgomery, 1976), is currently under consideration. This method controls both types of statistical error, is easily computed and allows the operator to quickly detect significant changes in sensitivity of the method.

Figure 4-2 indicates a problem with the ortho-phosphate determination. After three successive dates with the ΔB much greater than normal, the colorimeter's optics were realigned. The next slope fell within the stated limits.

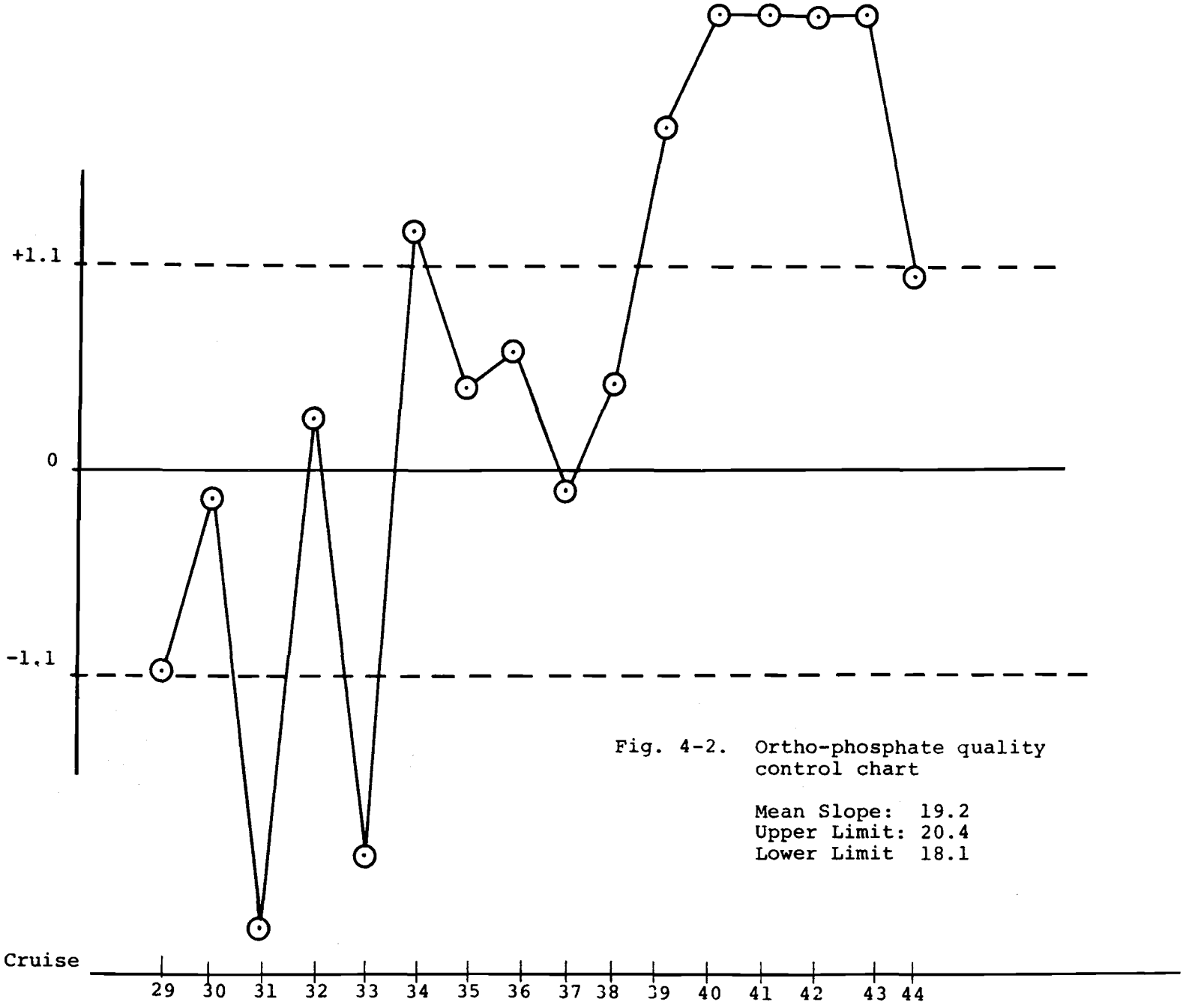


Fig. 4-2. Ortho-phosphate quality control chart

Mean Slope: 19.2
Upper Limit: 20.4
Lower Limit 18.1

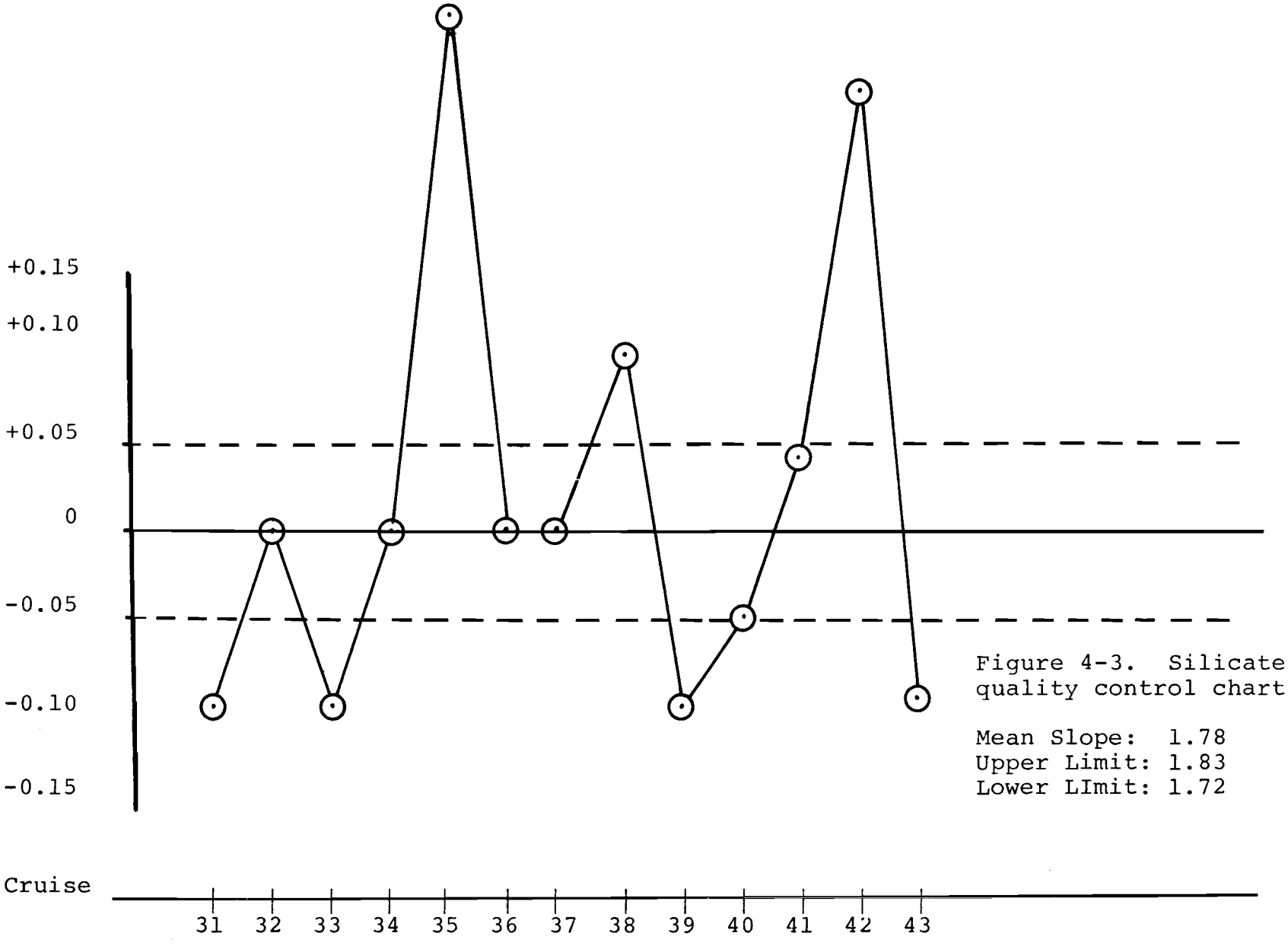


Figure 4-3. Silicate quality control chart

Mean Slope: 1.78
Upper Limit: 1.83
Lower LImit: 1.72

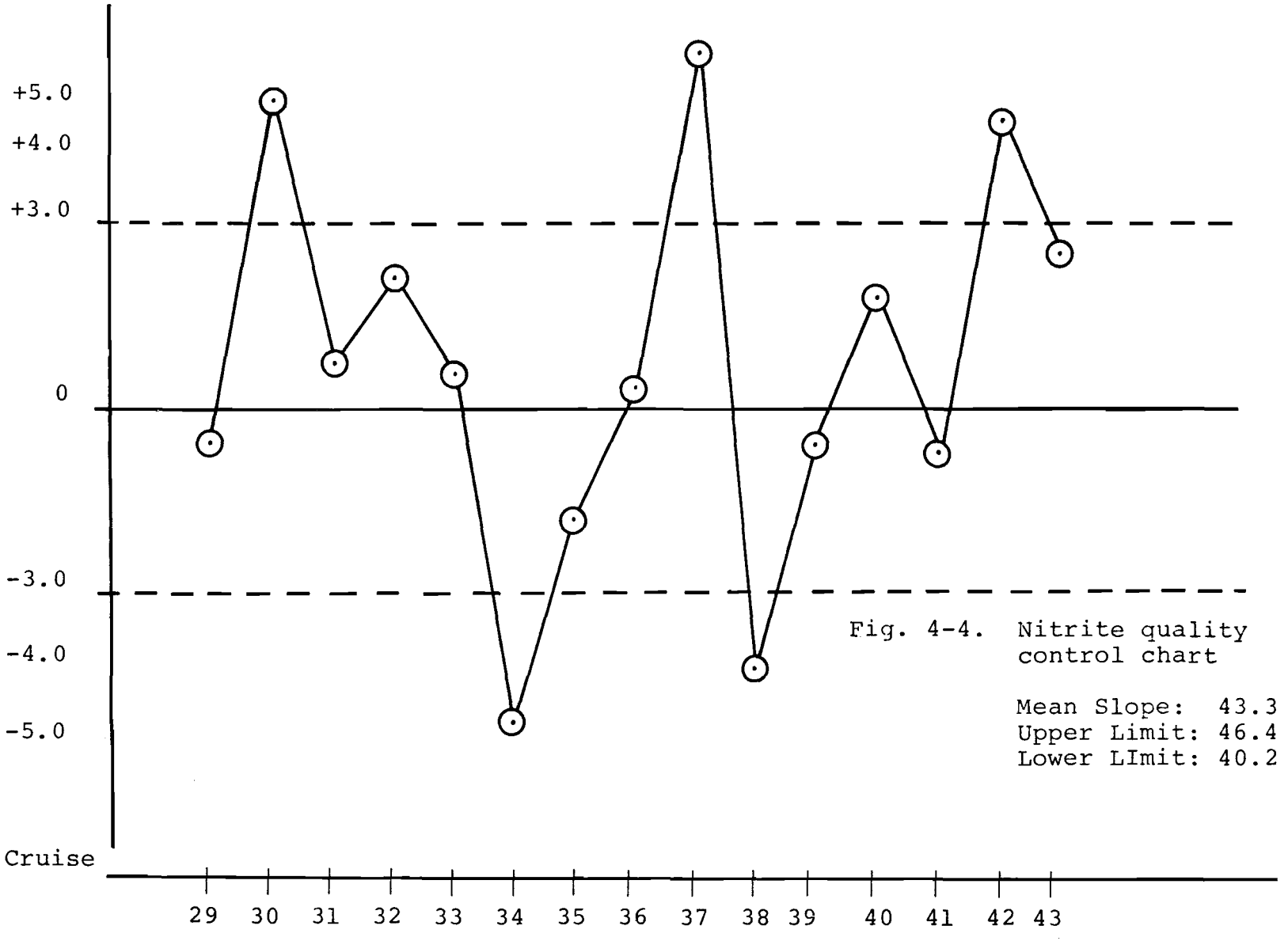


Fig. 4-4. Nitrite quality control chart

Mean Slope: 43.3
Upper Limit: 46.4
Lower LImit: 40.2

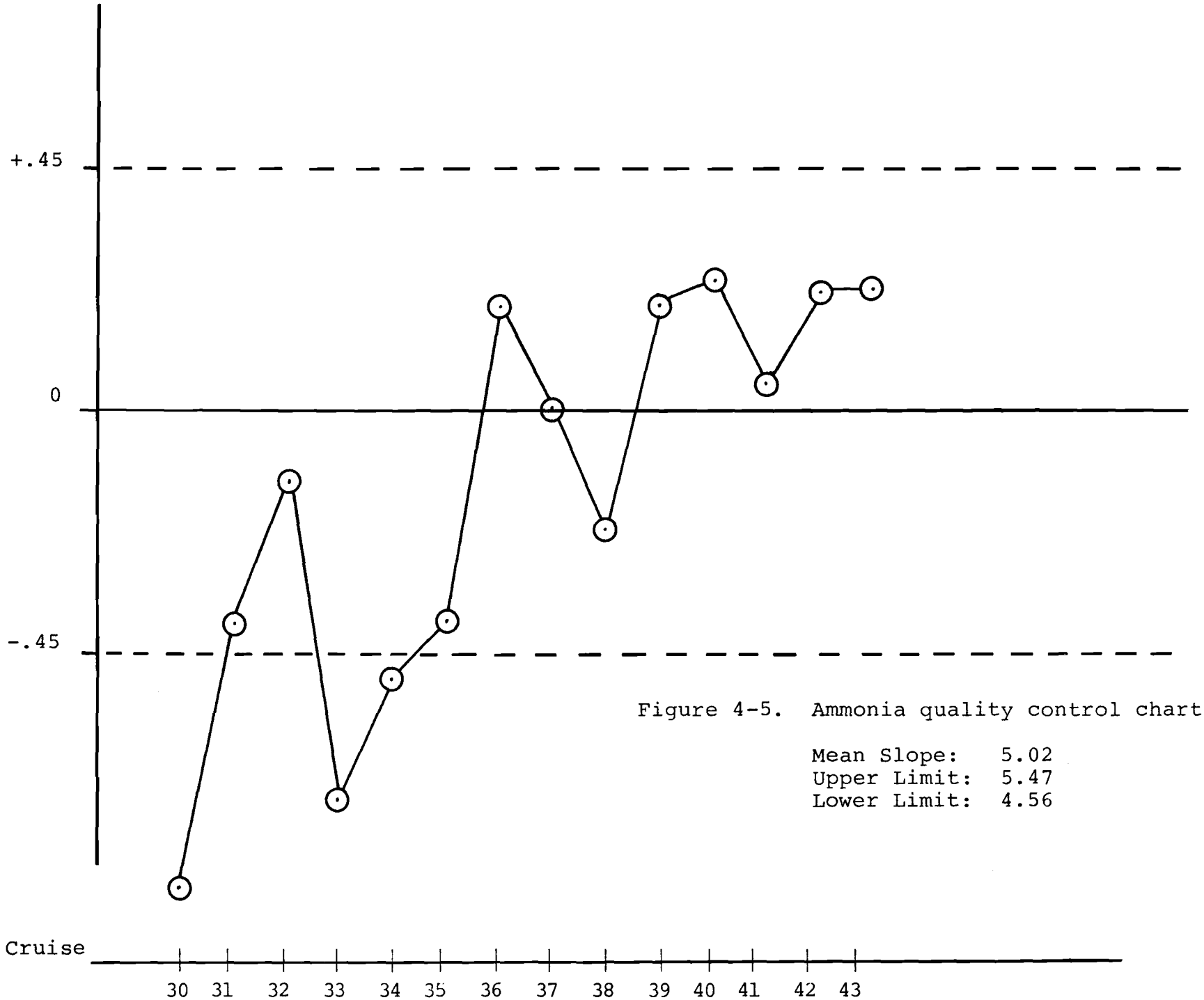


Figure 4-5. Ammonia quality control chart

Mean Slope: 5.02
Upper Limit: 5.47
Lower Limit: 4.56

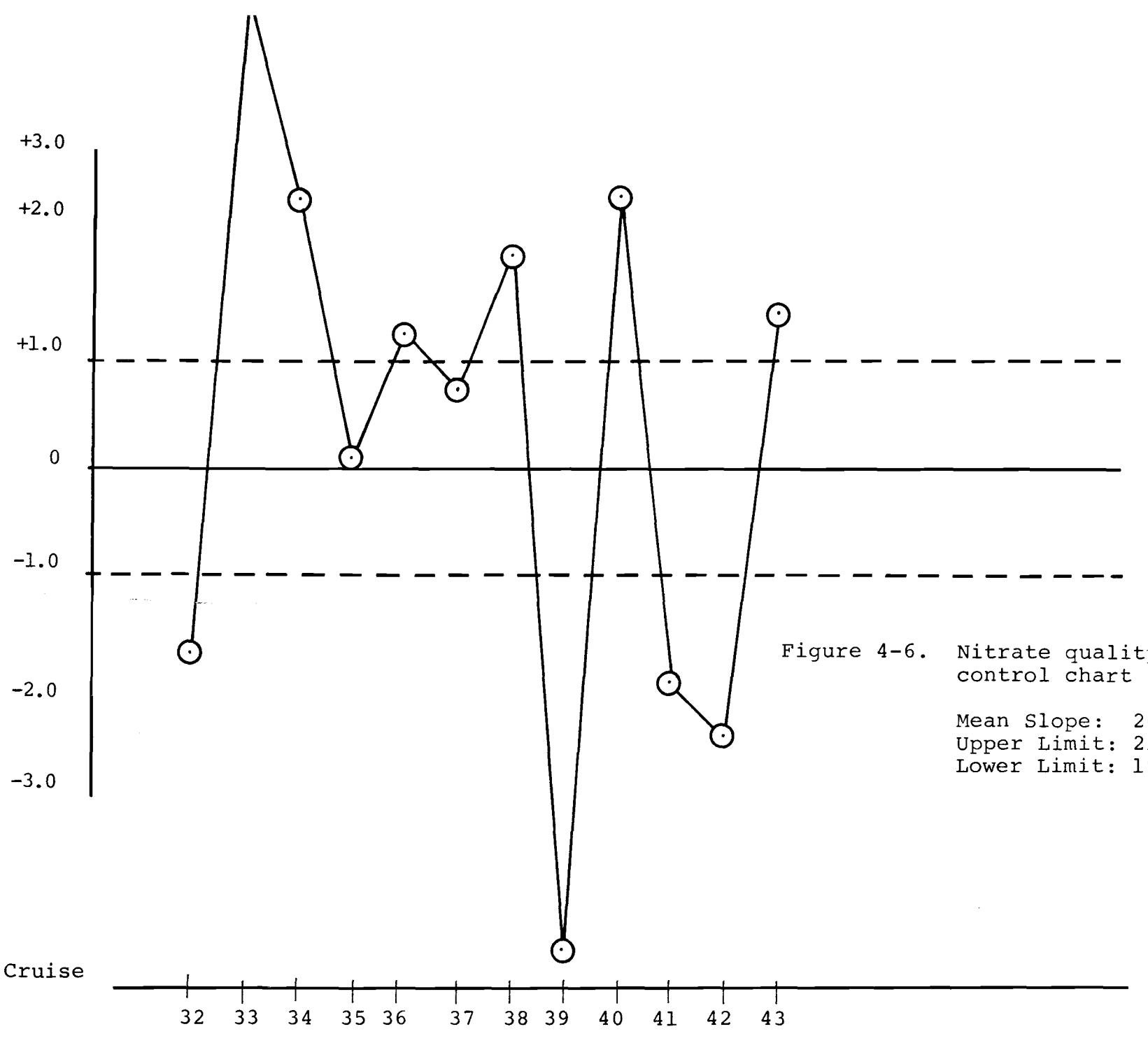


Figure 4-6. Nitrate quality control chart

Mean Slope: 20.73
Upper Limit: 21.73
Lower Limit: 19.73

Minimum Detectable Concentration

To determine the minimum detectable concentration, the sensitivity of the system must first be ascertained. Analytically, sensitivity should be described as the measure of the ability to determine a small change in concentration of analyte at any concentration level (Skogerboe and Grant, 1970). The factors which limit this ability are the slope of the analytical curve and the reproducibility (precision) with which the analytical signal can be measured (Skogerboe and Grant, 1970).

If two methods are equivalent in measurement precision, the one producing the curve with the greatest slope is most sensitive (Skogerboe and Grant, 1970).

Thus, to detect the minimum detectable concentration, the following formula is used: $MDC = S.D. \frac{[t(1-\alpha)\ (N-1)]}{m}$ where:

MDC = minimum detectable concentration.

SD = standard deviation of measurements. The mean square within groups from the ANOVA table was used (analysis of variance for the linear regression (Sokal and Rohlf, 1969)) or standard error (Syx).

$t(1-\alpha)(N-1)$ = students t value (with $\alpha = 0.05$, then $t_{.95}$ (N-1) gives the 95% confidence value) with N-1 degrees of freedom where N is the number of data points.

m = slope (regression coefficient) of the standard curve.

Table 4-2 indicates the minimum detectable concentrations for the nutrients this laboratory routinely analyzes, compared to Technicon's. These values are routinely re-evaluated.

Table 4-2. Comparison of minimum detectable concentration (in µg at/l)

Determ.	Harbor Branch	Technicon
NH_3	0.6 ± 0.03	0.2
OPO_4	0.1 ± .01	0.1
NO_2	0.04 ± 0.002	0.04
$NO_2 + NO_3$	0.2 ± 0.01	0.1
Si	1.4 ± 0.1	1.0

Interlaboratory Comparison

Interlaboratory comparisons have been conducted between this laboratory and the Quality Assurance Branch of the Environmental Protection Agency. Sealed ampoules of unknown concentrations of ammonia, nitrate, and ortho-phosphate are provided by the EPA. Answers are provided in a sealed envelope sent with the ampoules. Table 4-3 indicates the results of an initial set of unknown standards and the percent recoveries.

Table 4-3. Interlaboratory comparison of nutrients EPA vs. HBF in µg at/l, Test 1.

	HBF	EPA	% Recovery
PO_4	1.5	1.9	80
NO_3	16.2	14.3	113
NH_3	25.4	31.4	81

These differences may be due to a dilution error of the samples or the fact that our standard curves were prepared in artificial seawater then they should have been prepared in distilled water.

A second set of EPA samples were analyzed exactly according to their enclosed instructions. Results of these analyses are given in Table 4-4. The percent recovery is very good for ammonia and nitrate and average greater than 90% for orthophosphate. We will continue our quality control program using these EPA standards on a routine basis.

Table 4-4. Interlaboratory comparison of nutrients HBF vs. EPA in µg at/l, Test 2.

	EPA Standards	HBF ($N = 5$) $\bar{X}$ S.D.	Mean percent recovery (with 95% confidence level)
NH_3	16.4	16.6±0.0	101.2± 0.0
NO_3	7.8	8.0±0.0	102.6± 0.0
PO_4	1.7	1.6±0.0	95.2± 0.0
NH_3	113.6	115.4±1.7	101.5± 2.1
NO_3	27.1	28.8±0.0	106.2± 0.0
PO_4	6.1	5.4 0.1	88.5± 0.2

Literature Cited

EPA. 1972. Handbook for analytical quality control in water and wastewater laboratories. Environmental Protection Agency, Analytical Quality Control Laboratory, Cincinnati, Ohio. 103 pp.

Harbor Branch Consortium. 1976. Indian River coastal zone study, 1975-1976 annual report. Harbor Branch Consortium, Fort Pierce, Florida. 187 pp.

Montgomery, J.R. 1976. Monitoring of an atomic absorption spectrophotometer using cumulative sum statistical control charts. In National Bureau of Standards, Special Publication 422, 1023-1033.

Skogerboe, R.K. and C.L. Grant. 1970. Comments on the definitions of the terms sensitivity and detection limit. Spectroscopy Letters 3(8&9): 215-220.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. Iowa State Univ. Press, Ames, Iowa. 593 pp.

Sokol, R.R. and F.J. Rohlf. 1969. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., San Francisco, California. 776 pp.

Technicon. 1973. Technicon AutoAnalyzer II, Industrial methods. Technicon Industrial Systems, Tarrytown, N.Y.

Wood, E.D. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. of U.K. 47: 23-31.

Distributors of AutoAnalyzer Equipment

Fisher Scientific Co.
P.O. Box 13430
Orlando, Florida 32809
(305-857-3600)

Gamma Enterprises
561 South Fulton Avenue
Mt. Vernon, N.Y. 10550
(914-664-5533)

Technicon Industrial Systems
Tarrytown, New York
(914-631-8000)