CLEAR TECHNICAL REPORT NO. 205

CLEAR ANALYTICAL METHODS MANUAL

Compiled by

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COLUMBUS, OHIO

December 1982

PREFACE

This manual constitutes the methods used by the Center for Lake Erie Area Research in the water quality laboratory and the field. Analytical methods have been drawn from standard references, current publications, communication with other water quality laboratories and experience of personnel and adapted to meet specific conditions.

The biological section presented is minimal and deals mainly with collection rather than identification. More detailed procedures for handling microbiological, phytoplankton, zooplankton and benthos samples will be discussed in a separate manual. The CLEAR Analytical Methods Manual is designed for in house use only.

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PART I - INTRODUCTION

DESCRIPTION OF SPECIFIED PURE WATERS USED IN THIS MANUAL

Distilled Water

Distilled water is prepared from the copper still located at the Put-in-Bay laboratory. Hard well water is used as the source and it is necessary to clean the still at least yearly to prevent excessive build-up of scale. Distilled water is used when preparing acid wash water, Lugol's preservative for phytoplankton, and in washing glassware.

Double Distilled Demineralized Water

DDD water is produced by OSU lab stores and comes in 2.5 gal., sealed plastic containers. It is used to prepare the silica reagents, conductivity standards and is used as source water for making CO_2-free water and deionized water (super water) at Put-in-Bay.

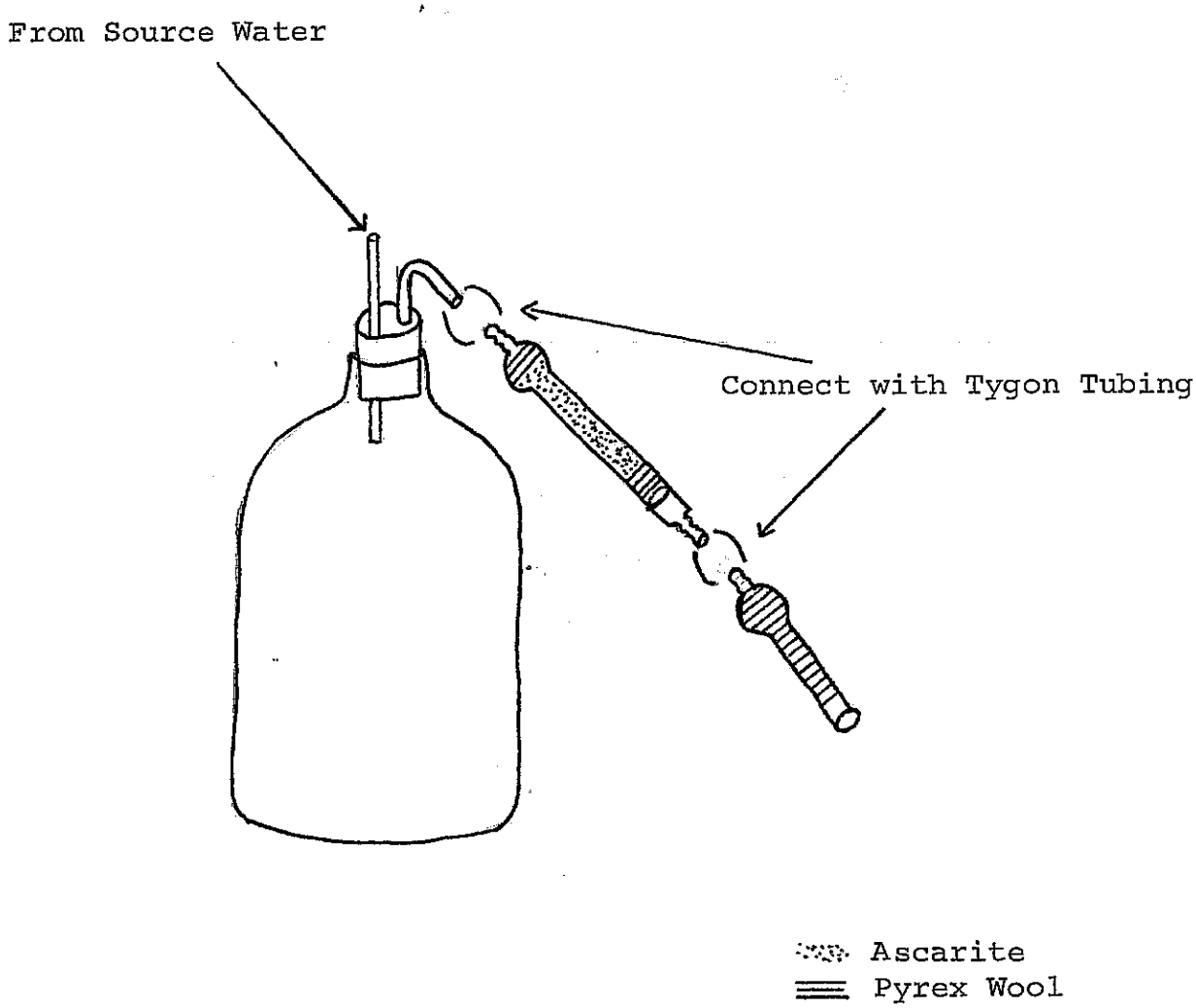
Deionized Water

Deionized water is obtained in two ways:

A. At the Columbus lab, deionized water is made by passing tap water through a Continental Water Purification system. This includes an organics removal filter and two columns of mixed bed resin. This water is used in preparing all reagents and standards used for analyses performed in Columbus. It is also used for any procedure requiring the use of distilled water (in Columbus).

B. At the Put-in-Bay lab and in the field (i.e. onboard R/V Hydra) an acceptable grade of source water for the Continental System is not available. Therefore, the double distilled demineralized water is passed through a mixed bed deionizer column to obtain deionized water. This water (also referred to as super H_2O) is used for all reagents, standards and analyses not specifically mentioned elsewhere. A trap is attached to the air vent to prevent contamination from free ammonia and other gases present in the ambient air. This trap is set up as described below.

1. Fit collecting container with a tight-fitting 2-holed stopper.
2. One hole is connected to the water source; the other hole is for venting.
3. Fill half a bowl section of a plastic drying tube with pyrex wool.
4. Fill the rest of the tube with Ascarite and a 1/2" plug of pyrex wool. Attach this tube to the vent hole with bowl end closest to the hole.
5. Fill a second drying tube with pyrex wool and add 1 ml. concentrated H_2SO_4.
6. Connect the second tube to the first tube at the bowl end.



CO_2 Free Water

Boil double distilled demineralized water for 15 minutes, cover loosely with a beaker and cool prior to use.

WASHING GLASSWARE

General Glass Washing

1. All glassware is washed with 1:1 HCl*.
2. Rinse 3 or 4 times with distilled water.
3. Allow to air dry. Dry thoroughly before storing.

*In a well-ventilated area or under the hood add 2 l concentrated HCl to 2 l of distilled water and mix.

Washing Chloride Glassware and Sample Containers

1. Wash glassware with 1:1 HNO_3*.
2. Rinse 3 or 4 times with distilled water.
3. Allow to air dry.

*In a well-ventilated area or under the hood add 2 l concentrated HNO_3 to 2 l of distilled water and mix.

Washing Total Phosphorus Sample Bottles

Prepare Acid Bath.

1. Fill acid bath to water mark with distilled water.
2. Add concentrated HCl to the mark.
3. Cover with heavy plastic.

Washing Bottles

1. Remove tape and caps from bottles.
2. Submerge bottles in acid bath and allow to soak for 1 hour or more.
3. Rinse bottles 3 times with distilled water and a final rinse with deionized water.
4. Invert bottles in paper towel lined tray and allow to dry completely before storing.
5. Wash caps in a beaker with 1:1 HCl and rinse well. Don't let caps soak.

QUALITY CONTROL

Quality control is an important part of all sample analyses. The precision and accuracy of a method, instrument and sampling procedure can be consistently monitored and maintained by comparing the results of replicate analysis and spikes. The quality control program constitutes about 10 percent of the total sampling effort and from this data standard deviations for individual methods can be estimated using the procedure outlined by the IJC as described in this section.

In addition to establishing guidelines for calculating standard deviations, quality control is essential in the sampling procedure itself. When collecting and processing samples it is imperative to be consistent from station to station and person to person. Measurements for most physical parameters in the field can often be taken by more than one instrument. In this case, the data obtained from the instrument routinely used are periodically compared with the values obtained using the alternate instrument. This serves as a check to correct for any bias and also establishes a reliable backup unit. It also serves as quality control where it is not applicable or possible to analyze replicates and splits.

General Water Sampling Procedure

1. All water samples are obtained by a submersible pump or Niskin bottle. When collecting replicate samples, two separate casts are made and labeled as Ol_1, Ol_2 and/or B_1 and B_2. Sufficient pumping time is allowed to clear the line of any previous sample before collecting water. (Ol - surface, B - bottom)

2. A split, as referred to in the following procedures, indicates a sample run twice from the same cast (i.e., Split Ol_1 Split Split Ol_2 Split

Auto Analyzer QC

This program is more extensive than for other parameters because there are more variables involved in the determination of concentrations and the analytical procedure is more conducive to running replicates and splits.

1. A replicate sample is taken at every QC station (Ol_1, Ol_2 etc.)
2. Every replicate sample is analyzed twice as separate runs. This is a split making a total of four values for each QC sampling depth.
3. Replicates and splits are recorded in the QC logbook and the difference between splits (within cast replicate) is calculated as the range.
4. Replicate samples at each QC station (Ol_1, Ol_2, B_1, B_2) are spiked with a known standard concentration in a 1:1 ratio (usually 4 ml sample + 4 ml standard mixed in a test tube). These results are used to calculate percent recovery which gives an indication of the accuracy of the method.

$$\% \text{ recovery} = \frac{\text{actual spiked value}}{\text{theoretical value}} \times 100$$

$$\text{Theoretical Value} = \frac{\text{Spiking standard + Original Value of Sample}}{2}$$

Actual Spike = Read off chart

All of these values are recorded in the AA QC logbook.

Estimating Standard Deviations (IJC Procedure)

1. Add ranges derived from differences in the splits and calculate the mean. Discard any ranges that differ significantly from the majority.
2. Divide the mean by 1.128 to obtain standard deviations.
3. Control limits are determined by multiplying the estimated standard deviation by 3.686. Whenever a range falls out of the boundaries of this limit, the system is said to be out of control.
4. To pool estimates of standard deviations use the following equation.

$$s^2 = \frac{(n_1 - 1)(s_1^2) + (n_2 - 1)(s_2^2)}{(n_1 - 1) + (n_2 - 1)}$$

n_1 = number of values in first set

S_1 = standard deviation of first set

n_2 = number of values in second set

S_2 = standard deviation of second set

QC Program for Particulates (Chlorophylls, Suspended Solids, POC, PON and Turbidity)

1. An identical aliquot is filtered from each replicate cast ($O1_1$, $O1_2$ and B_1, B_2).

2. A split is filtered from only one of the replicate casts at each QC station and labeled as a split (i.e., $O1_1$ split). There will be 3 QC samples from the O1 depth ($O1_1$, $O1_2$ and $O1_1$ split).

QC Program for All Remaining Parameters

1. Replicate samples are taken wherever possible (DO, alkalinity, conductivity and pH).
2. Splits are done for conductivity and pH when using YSI and Orion meters.
3. Replicate EBT traces are taken from surface to bottom at QC stations. When applicable, replicate readings are taken for conductivity and pH.
4. Secchi and extinction depth replicate values are recorded by two individuals.

Selection of QC Stations

1. Selection is random and one station is picked each morning prior to arriving at the first station.
2. If stratification exists, quality control sampling is done at surface and bottom depths. When the water column is unstratified QC is done only at one depth.

Interlaboratory Quality Control

1. IJC Round Robin samples are periodically analyzed for selected parameters and compared with results of other laboratories.
2. Known sample concentrations provided by the EPA are analyzed periodically.

Transfer of QC data into the DEMON File

1. Stations are entered in order sampled, cruise by cruise.
2. QC sampling procedures at individual stations will follow set pattern as closely as possible (i.e., QC will be done at the same stations both on the Hydra and in Columbus).
3. The following codes will be used when transferring data.

QC Code	Value
11	replicate 1, split 1 ($O1_1$ value 1)
12	replicate 1, split 2 ($O1_1$ value 2)
21	replicate 2, split 1 ($O1_2$ value 1)
22	replicate 2, split 2 ($O1_2$ value 2)
81	original value ($O1_1$) before spiking
82	observed value of spike
83	spike standard
84	volume of sample } if ratio is different than 1:1
85	volume of spike } if ratio is different than 1:1
91	original value ($O1_2$) before spiking
92	observed value of spike
93	spike standard
94	volume of sample } if ratio is different than 1:1
95	volume of spike } if ratio is different than 1:1

4. Data should always be entered in the above order (not including 84, 85, 94 and 95), entering N if no data exists.
5. Two DEMON data sheets are available, one for parameters run in Columbus and one for parameters run on the boat. The order of entries should be identical.
6. Any QC data run which is above and beyond that required, or is just different from the set pattern, should be entered at the end of each cruise interval.

TABLE 1. SYNOPSIS OF METHODS

Parameter	Method	Range	Detection Limit
Specific Conductance	In situ probe (InterOcean, Martek) Electrode (Beckman, YSI)	0-1000 μmhos/cm	.02 μmhos 1%
Extinction Depth	Licor Quantum/Radiometer/Photometer Protomatic submarine photometer	NA	0.1 m 0.1 m
Suspended Solids	Gravimetric, using Whatman GF/C glass fiber filters	1-10,000 mg/l	.01 mg
Temperature	Thermistor (InterOcean, Martek) Mechanical Bathythermograph NBS Calibrated Thermometer Reversing Thermometer	0-35°C	0.2°C
Transmission Transparency Turbidity	Martek, Hydro Products Transmissometer Secchi disk Hach Turbidimeters (Model 2100A and Ratio)	NA NA 0-1000 NTU (3 lower ranges are available on each meter)	 .01 m .02 NTU
Alkalinity	Titrimetric (.02N HCl)	0-250 mg/l $CaCO_3$	.5 mg/l
Chloride	Ferricyanide, AAII	.5-50 mg Cl/l	.5 mg/l
Ammonia	Phenate Method, AAII	0.5-100 μg N/l 1.0-200 μg N/l 2.0-400 μg N/l	.5 μg/l 1.0 μg/l 2.0 μg/l

TABLE 1 CONTINUED

Parameter	Method	Range	Detection Limit
Nitrate + Nitrite	Cadmium Reduction, AAII	.005-1 mg N/l	.005 mg/l
pH	Electrode (InterOcean, Martek, Orion)	0-14	0.1
Dissolved Oxygen	Electrode (InterOcean, Martek) Titrimetric (Winkler azide modification)	0-20 mg O_2/l	0.05 mg O_2/l
Soluble Reactive Phosphorus	Stannous Chloride, AAII	.5-50 µg P/l	.5 µg/l
Total Phosphorus	Persulfate Digestion, Stannous Chloride	.5-50 µg/l 1.0-100 µg	.5 µg/l 1.0 µg/l
Total Filtered Phosphorus	Persulfate Digestion, Stannous Chloride, AAII	Expanded further by Machine or Manual Dilutions	
Particulate Phosphorus	Persulfate Digestion, Stannous Chloride, AAII NaOH Extraction, Stannous Chloride, AAII CDB Extraction, Stannous Chloride, AAII H_2SO_4 Extraction, Stannous Chloride, AAII		
Dissolved Reactive Silicate	Molibdosilicate-Ascorbic Acid-Oxalic Acid, AAII	.03-5.00 mg SiO_2/l	.03 mg/l
Sulfate	Methylthymol Blue, AAII	.5-50 mg SO_4/l	.5 mg/l
Dissolved Organic Carbon	UV Digestion - Phenolphthalein, AAII	.2-10 mg C/l	.2 mg/l

TABLE 1 CONTINUED

Parameter	Method	Range	Detection Limit
Chlorophyll *a*	Acetone extinction Varian Spectrophotometer	0-50 µg/l	.02 µg/l
Pheopigment	Acetone extinction Varian Spectrophotometer	0-50 µg/l	.04 µg/l
Kjeldahl Nitrogen	Continuous Helix Digestion with H_2SO_4 and Hydrogen Peroxide	10-1000 µg N/l	10 µg/l
Particulate Organic Nitrogen	Perkin-Elmer Model 240 Elemental Analyzer	0-5000 mg N	.002 mg
Particulate Organic Carbon	Perkin-Elmer Model 240 Elemental Analyzer	0-5000 mg C	.005 mg
Benthos	Optical examination (Ponar Grab)		species
Phytoplankton	Optical examination (Collected w/ Niskin Bottle, Preserve w/Lugols)	macro + nanoplankton	species
Zooplankton	Optical examination (64 µ net, $CaCO_3$ formalin + sucrose)	.075 mm	species

TABLE 2. SAMPLE CONTAINERS AND PRESERVATION OF CONSTITUENTS

Parameter	Container	Preservative	Storage Time
Alkalinity	poly	cool, 4°C	24 hrs.
Carbon, Organic	glass	cool, 4°C	1 month
Carbon, Particulate Org.	plastic petri dish	filter thru GF/F glass fiber or Quartz glass pad; desiccate	6 months
Chloride	poly	cool, 4°C	1 month
Chlorophyll	plastic petri dish	filter on GF/C; add $MgCO_3$; freeze	6 months
Dissolved Oxygen	glass 300 ml BOD	fix on site	no hold
Nitrogen			
Ammonia	poly	cool, 4°C	24 hrs.
Kjeldahl	glass	freeze	6 months
NO_3+NO_2	poly	cool, 4°C	24 hrs.
Organic, particulate	plastic petri dish	filter thru GF/F glass fiber or Quartz glass pad; desiccate	6 months
pH	poly	none	6 hrs.
Phosphorus			
Soluble Reactive	poly	cool, 4°C	24 hrs.
Total Filtered	glass	cool	1 month
Total	glass	cool	1 month
Particulate	glass	filter on .45 µ membrane filter	1 month
NAIP	poly	concentrate	2 weeks
Apatite	poly	freeze	2 weeks

TABLE 2 CONT.

Parameter	Container	Preservative	Storage Time
Silica	poly	cool, 4°C	1 month
Specific Conductance	poly	cool, 4°C	24 hrs.
Sulfate	poly	cool, 4°C	1 month
Turbidity	poly	cool, 4°C	7 days
Suspended Solids	plastic petri dish	filter thru GF/C; desiccate	1 month
Phytoplankton	poly	Lugols + $MgCO_3$ buffered formalin	
Zooplankton	poly	Carbonated H_2O, $MgCO_3$ buffered formalin + sucrose	
Benthos	poly	10% formalin	

PART 2 - PHYSICAL PARAMETERS IN WATER

CONDUCTIVITY

Scope and Application

All methods used here to measure conductivity can accommodate a variety of ranges, but generally a range of .2-2500 µmhos/cm is sufficient to cover values encountered within the Great Lakes.

Principle and Theory

Conductivity is a measure of the ability of a substance to conduct an electric current. This property is related to the total concentration of ionized substances in the water and the temperature at which the measurement is made. The conductance cell contains a positive and negative electrode and the positive ions in solution migrate toward the negative electrode while the negative ions migrate toward the positive electrode. Since the measure of an electric current relys on the movement of ions, it follows that the greater the number of dissociated molecules in the solution the higher will be the conductivity. Conductivity is useful in measuring the purity of distilled water (fresh has conductance of .5 to 2 µmhos, older from 2 to 4 µmhos), and in monitoring daily fluctuations in polluted waters, particularly near sources of pollution. Comparisons of seasonal variations in water quality can also be made using conductivity. Several different instruments are used to measure conductance: the YSI Model 31 and Beckman conductivity meters as described below and in situ probes (Martek, InterOcean). All conductivities are corrected to 25^{o}C using factors in Table 3.

Procedure

A. YSI Model 31.

1. Set function switch to LINE and warm up for 5 minutes.

2. Place conductivity cell in sample, submerging electrodes and eliminating any trapped air bubbles.

3. Set SENSITIVITY control to minimum.

4. Rotate the RANGE switch to the range which gives maximum shadow length on indictor tube.

5. Adjust DRIVE control for longest shadow.

6. If dial indication is above 20 or below 2.0 set RANGE to next higher or lower scale.

7. Set SENSITIVITY control to maximum and readjust DRIVE for maximum shadow.

8. The conductivity of the sample is the dial reading times the multiplier which is in line with the pointer.

9. Correct conductivity to $25^{o}C$ using conversion given in Table 3.

10. Follow instructions in instrument manual for cleaning and replatinizing cells.

B. Beckman Conductivity Meter.

1. Check battery power.

2. Adjust temperature control knob to sample temperature.

3. Place electrodes in sample, being careful not to trap any air bubbles in the cell.

4. Press black button on top and adjust knob to conductivity reading producing an even charge.

C. Calibration Standards.

for KCl 74.55

C = milliformula weight per liter

M = Molarity

C = .1 (.0001M)	.0075 g. KCl/l.	14.9 µmhos
C = .5 (.0005M)	.0373 g.	73.9
C = 1 (.001 M)	.0746 g.	147
C = 2 (.002M)	.1491 g.	294
C = 3 (.003M)	.2237 g.	441
C = 5 (.005M)	.3728 g.	717.8

These standards are used for setting and checking both meters and in situ probes. Probe descriptions and calibration procedures are found in the section on in situ probes.

Precision and Accuracy

This meter is accurate to ± 1%.

References

1. American Public Health Association. 1971. Standard methods for the examination of water and wastewater. 13th Edition. p. 323-327.

2. Yellow Springs Instrument Co. 1977. Instruction manual YSI Model 31 Conductivity Bridge. Yellow Springs Instrument Company, Scientific Division, Yellow Springs, Ohio. 16 p.

TABLE 3

FACTORS FOR COMPUTATION OF SPECIFIC CONDUCTANCE
AT 25.0°C FROM MEASURED SPECIFIC CONDUCTANCE FOR GREAT LAKES WATERS

TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)
0.0	1.888	2.6	1.764	5.2	1.646	7.8	1.534	10.4	1.432	13.0	1.337
0.1	1.884	2.7	1.759	5.3	1.641	7.9	1.529	10.5	1.428	13.1	1.333
0.2	1.879	2.8	1.754	5.4	1.637	8.0	1.526	10.6	1.425	13.2	1.329
0.3	1.874	2.9	1.749	5.5	1.632	8.1	1.522	10.7	1.421	13.3	1.327
0.4	1.869	3.0	1.745	5.6	1.627	8.2	1.518	10.8	1.418	13.4	1.324
0.5	1.864	3.1	1.740	5.7	1.624	8.3	1.514	10.9	1.414	13.5	1.320
0.6	1.859	3.2	1.735	5.8	1.619	8.4	1.511	11.0	1.409	13.6	1.317
0.7	1.854	3.3	1.731	5.9	1.614	8.5	1.506	11.1	1.406	13.7	1.313
0.8	1.849	3.4	1.726	6.0	1.611	8.6	1.502	11.2	1.402	13.8	1.309
0.9	1.845	3.5	1.721	6.1	1.607	8.7	1.499	11.3	1.398	13.9	1.306
1.0	1.840	3.6	1.717	6.2	1.602	8.8	1.494	11.4	1.394	14.0	1.302
1.1	1.835	3.7	1.713	6.3	1.598	8.9	1.489	11.5	1.391	14.1	1.299
1.2	1.830	3.8	1.708	6.4	1.594	9.0	1.486	11.6	1.387	14.2	1.295
1.3	1.826	3.9	1.704	6.5	1.591	9.1	1.482	11.7	1.384	14.3	1.292
1.4	1.821	4.0	1.699	6.6	1.586	9.2	1.478	11.8	1.380	14.4	1.289
1.5	1.816	4.1	1.694	6.7	1.581	9.3	1.474	11.9	1.377	14.5	1.287
1.6	1.810	4.2	1.691	6.8	1.577	9.4	1.471	12.0	1.373	14.6	1.284
1.7	1.806	4.3	1.686	6.9	1.572	9.5	1.466	12.1	1.369	14.7	1.280
1.8	1.801	4.4	1.681	7.0	1.568	9.6	1.462	12.2	1.366	14.8	1.277
1.9	1.798	4.5	1.677	7.1	1.565	9.7	1.459	12.3	1.362	14.9	1.273
2.0	1.793	4.6	1.672	7.2	1.560	9.8	1.454	12.4	1.359	15.0	1.269
2.1	1.788	4.7	1.666	7.3	1.555	9.9	1.451	12.5	1.355	15.1	1.266
2.2	1.782	4.8	1.662	7.4	1.551	10.0	1.447	12.6	1.352	15.2	1.262
2.3	1.778	4.9	1.659	7.5	1.546	10.1	1.444	12.7	1.348	15.3	1.259
2.4	1.773	5.0	1.654	7.6	1.542	10.2	1.440	12.8	1.345	15.4	1.257
2.5	1.768	5.1	1.649	7.7	1.539	10.3	1.436	12.9	1.341	15.5	1.254

TABLE 3 CONT.

TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)	TEMP	FACTOR (X)
15.6	1.251	18.2	1.171	20.8	1.100	23.4	1.035	26.0	.980	28.6	.933
15.7	1.247	18.3	1.167	20.9	1.096	23.5	1.033	26.1	.979	28.7	.932
15.8	1.244	18.4	1.165	21.0	1.094	23.6	1.031	26.2	.976	28.8	.929
15.9	1.240	18.5	1.162	21.1	1.092	23.7	1.028	26.3	.974	28.9	.927
16.0	1.238	18.6	1.159	21.2	1.089	23.8	1.027	26.4	.972	29.0	.926
16.1	1.235	18.7	1.155	21.3	1.086	23.9	1.025	26.5	.969	29.1	.925
16.2	1.231	18.8	1.153	21.4	1.085	24.0	1.022	26.6	.968	29.2	.922
16.3	1.228	18.9	1.151	21.5	1.082	24.1	1.020	26.7	.967	29.3	.921
16.4	1.225	19.0	1.148	21.6	1.079	24.2	1.018	26.8	.965	29.4	.920
16.5	1.221	19.1	1.146	21.7	1.076	24.3	1.015	26.9	.962	29.5	.918
16.6	1.219	19.2	1.142	21.8	1.074	24.4	1.013	27.0	.961	29.6	.916
16.7	1.216	19.3	1.139	21.9	1.072	24.5	1.012	27.1	.960	29.7	.915
16.8	1.213	19.4	1.136	22.0	1.069	24.6	1.009	27.2	.958	29.8	.913
16.9	1.209	19.5	1.134	22.1	1.067	24.7	1.007	27.3	.955	29.9	.911
17.0	1.207	19.6	1.132	22.2	1.065	24.8	1.005	27.4	.954	30.0	.909
17.1	1.204	19.7	1.129	22.3	1.061	24.9	1.002	27.5	.953	30.1	.908
17.2	1.200	19.8	1.126	22.4	1.059	25.0	1.000	27.6	.951	30.2	.907
17.3	1.198	19.9	1.122	22.5	1.056	25.1	.998	27.7	.948	30.3	.906
17.4	1.194	20.0	1.120	22.6	1.054	25.2	.996	27.8	.947	30.4	.905
17.5	1.191	20.1	1.118	22.7	1.052	25.3	.995	27.9	.946	30.5	.904
17.6	1.188	20.2	1.115	22.8	1.049	25.4	.993	28.0	.944	30.6	.901
17.7	1.186	20.3	1.113	22.9	1.047	25.5	.991	28.1	.941	30.7	.899
17.8	1.182	20.4	1.109	23.0	1.045	25.6	.988	28.2	.940	30.8	.898
17.9	1.179	20.5	1.107	23.1	1.042	25.7	.986	28.3	.939	30.9	.896
18.0	1.176	20.6	1.105	23.2	1.040	25.8	.985	28.4	.936	31.0	.895
18.1	1.174	20.7	1.102	23.3	1.038	25.9	.981	28.5	.934	31.1	.894

X = Multiply

EXTINCTION DEPTH

Scope and Application

Extinction depth is measured at all stations with a Li-Cor Quantum/Radiometer/Photometer (Lambda meter) or a Protomatic photometer.

Principle and Theory

The extinction depth is that point in the water column where downwelling light is equal to 1% of the amount of light measured at the surface. This indicates the point below which no significant photosynthetic activity occurs. The Lambda meter measures light as irradiance or photosynthetically active radiation using the units μ Einsteins/m^2/sec which includes dimensions of area and time. The Protomatic measures light as illumination in units of footcandles.

Procedure

A. Protomatic.

1. Check battery power.
2. Set scale at highest range.
3. Set console switch to up position.
4. Lower sensor to just below water surface and adjust scale to lowest range where reading does not go off scale.
5. Record surface reading and lower sensor until meter reading is 1% of surface reading.
6. Measure length of cable to the nearest centimeter.

B. Licor Lambda Meter.

1. Check battery power.
2. Set range scale to highest range (10,000).
3. Set control switch to "quantum" and take reading for deck cell.
4. Lower sensor to just below water surface and record reading for downwelling light.
5. Lower sensor meter by meter, recording readings until illumination is equal to 1% of surface downwelling reading.
6. Note length of cable to determine extinction depth. Measure to nearest centimeter.

References

1. Li-Cor, Inc. 1981. Radiation Measurement. LI-COR Instrumentation for biological and environmental sciences. LI-COR, Inc., Lincoln, Nebraska. p. 42-46.

Conversions

10.74 footcandles = 1 lux

1000 lux = 1 klux

1 klux = 19.53 $\mu Em^{-2}sec^{-1}$

The conversion from lux to $\mu Em^{-2}sec^{-1}$ is dependent upon the light source. The above conversion is based on daylight. For conversions using other light sources check reference listed.

SUSPENDED SOLIDS (GRAVIMETRIC)

Scope and Application

This method can be used for a wide range of water samples measured on a scale of 1-10,000 mg/l.

Principle and Theory

Several components of nonfilterabe suspended matter are being determined here. Total nonfilterable suspended matter is determined by filtering a known volume of water through a prepared filter pad, desiccating it and drying at 107^{o}C. The pad is cooled, weighed and the original weight is subtracted to obtain total nonfilterable suspended matter. Volatile solids present are measured by igniting the pad to 550^{o}C, cooling and reweighing. This value is subtracted from the dried weight (weight after drying at 107^{o}C) to obtain volatile solids. Nonvolatile solid content can be obtained by subtracting the value for volatile solids from the total solids.

Suspended solid concentrations are an indication of the trophic status of an aquatic area. High suspended solids can be interpreted as a high concentration of sediment and/or plankton matter. Sediment generally contains a higher concentration of pollutants (i.e. phosphorus, heavy metals, pesticides, etc.) than does the overlying water, so a higher suspended solids content indicates poor quality water. Suspended solids are also directly related to turbidity, conductivity, transparency (secchi), transmission and extinction depth.

Procedure

A. Preparation of filter pads.

1. Use GF/C glass fiber filters.
2. With wrinkled side up, filter 100 ml deionized water through each pad.
3. Ignite pads at 550^{0} C in muffle furnace for 20 minutes. Cool in desiccator.
4. Weigh each pad on the Sartorious balance and record weight in log book as mg to two decimal places.
5. Transfer to a petri dish which has been labelled SS and numbered on the bottom.
6. Bundle pads consecutively in groups of ten, secure with masking tape and label each bundle with range of pad numbers.

B. Filtering Samples.

1. Label bottom of petri dish as follows: station number - Julian date -depth.
2. Transfer pad to fritted glass filter holder using tweezers, wrinkled surface up, and filter appropriate volumes of well-mixed sample. Record volume filtered on bottom of dish and on field sheet.
3. Rinse down filter funnel with deionized water and allow pad to suck dry.
4. Return pad to petri dish, being careful to include all pieces which may have torn off.

5. At day's end place pads in desiccator with lids off and evacuate to 25 psi.
6. After 48 hours, bundle pads consecutively, label with Julian date, cruise and range, and store in desiccator until analysis.

C. Analyzing Samples.

1. Dry pads at 107°C for 1 hour. Cool in desiccator.
2. Weigh each pad and record weight along with station, date, depth and amount filtered next to corresponding pad number in log book.
3. Ignite pads for 20 minutes at 550°C in muffle furnace. Cool in desiccator.
4. Reweigh pads and record weight in log book.

D. Calculations.

$$\text{Total Solids (mg/l)} = \frac{\text{Dried wt (107°C) - Initial pad wt.}}{\text{liters filtered}}$$

$$\text{Volatile Solids (mg/l)} = \frac{\text{Dried wt (107°C) - After combustion wt (550°C)}}{\text{liters filtered}}$$

Nonvolatile Solids (mg/l) = Total Solids - Volatile Solids

References.

1. American Public Health Association. 1974. Standard Methods for the Examination of Water and Wastes. 14th Edition. p. 537-538.

TEMPERATURE

Scope and Application

Temperatures are measured at all depths with in situ probes (i.e. InterOcean, Martek), mechanical bathythermograph, reversing thermometers or regular thermometer corrected to an NBS thermometer. Temperature is measured in ^{o}C to at least one decimal place.

Principle and Theory

Temperature is one of the most important parameters measured as it indicates the presence and extent of stratification. The concentrations of a number of parameters (i.e. dissolved oxygen, conductivity, etc.) are temperature-dependent.

Procedure

1. Methods for in situ measurement by InterOcean and Martek probes are found in the section on in situ probes.

2. Surface temperatures are measured by collecting a sample in a bucket and measuring with an NBS corrected thermometer.

3. Mechanical bathythermograph (BT) readings are obtained by lowering the BT to the bottom and raising at a fairly constant rate. A trace of the thermal structure is etched on a gold-plated slide by a pressure sensitive needle. The slide is then read on a BT slide reader calibrated to the BT being used.

4. An electrical bathythermograph (EBT) trace can be obtained using the Hewlett Packard X-Y recorder hooked up to the InterOcean as described in the in situ probe section. Here a direct trace of temperature vs. depth is drawn on a large sheet of graph paper.

5. The reversing thermometer is used mostly to ascertain a change in temperature. One thermometer will measure the original temperature, while the other will measure the temperature at the greatest extreme from the original.

TRANSMISSION

Scope and Application

Transmission measurements can be taken at all stations we sample and are recorded as percent of light reflected back to the sensor.

Principle and Theory

Transmission gives an indication of density at a particular depth. Taking a meter by meter profile can reveal any layering or where patches of particulates may occur. The instrument contains its own light source which shoots a beam of light across a set path length. The light is reflected back to a photocell and the percent of light reflected is read as transmission. Two transmissometers are used: one with a light path of 1 meter and one with a light path of 10 cm. The 1 meter light path transmissometer is by Martek and is used in the central basin and less eutrophic waters. The water in the western basin is too turbid to use such a long light path, so a Hydro Products transmissometer having a 10 cm light path is used instead.

Procedure

1. Turn Power Switch on Power Supply Box to "on" position.
2. Mechanically zero meter using existing mirror scale behind needle to minimize parallax error.
3. Turn Range Switch to 10% position. Adjust zero potentiometer for zero reading on meter.

TRANSPARENCY (SECCHI DISC)

Scope and Application

This method can be used in any type of water and can be measured to the nearest centimeter if desired.

Theory and Principle

The standard secchi disc used is a 30 cm Whipple disc with black and white quadrants. The line attached to the secchi disc is calibrated in meters. The secchi disc has long been used as a simple, direct and common method of measuring transparency and models have been developed for determining trophic levels by comparing secchi depth, chlorophyll a and total phosphorus (Gregor and Rast 1979). For our purposes, when filtering in the field, the secchi depth gives an indication of how much water can be easily filtered and is a quick check of when to anticipate high nutrient, turbidity and conductivity readings.

Procedure

1. Take secchi measurement on shady side of boat to avoid any interference from glare at the water surface.
2. Lower secchi until disc is no longer visible.
3. Raise disc until black and white quadrants are distinguishable.
4. Measure the length of line using meter markings and meter stick.

References

1. Gregor, D.J. and W. Rast. 1979. Trophic characterization of the U.S. and Canadian nearshore zones of the Great Lakes. Report to Pollution from Land Use Activities Reference Group, IJC, Windsor, Ontario. 38 p.

TURBIDITY (TURBIDIMETRIC)

Scope and Application

This method is applicable to all samples with turbidities in the range of 0-1000 ntu's. Higher samples can be measured by dilution.

Principle and Theory

Hach turbidimeters (Models 2100A or Ratio Turbidimeter) are used. A light beam is shone upward through a glass tube containing the sample and the light reflected by suspended particles is received by photocells whose electrical response is proportional to the sample turbidity. The turbidimeters are calibrated against formazin standards.

Interferences

1. Any fingerprints or smudges on the glass sample tube can interfere with the reading so handle tubes as little as possible and wipe with a tissue before placing in turbidimeter.
2. Avoid getting air bubbles in sample.
3. Highly colored or extremely turbid samples can cause a lower reading than is actually present. Samples reading over 40 should be diluted and run again.

Calibration Procedure for Model 2100A

1. Using 10 ntu standard set 10 on 100 ntu scale.
2. Switch scale to 10 and readjust if necessary.

Calibration Procedure for Ratio Turbidimeter

1. With instrument set on 20 scale, and using the 18 ntu standard provided, adjust the readout to 18 1 ntu.

Procedure

1. Allow machine to warm up for at least an hour.
2. Mix sample well before taking aliquot.
3. Fill sample cuevettes nearly full.
4. Place cuevette in turbidimeter, cover with light shield and adjust range to lowest range on which sample can be read.

InterOcean

pH. Sensor incorporates the reference electrode and the measuring electrode into a single combination sensor. The voltage output is proportional to the pH value. This voltage is amplified through a temperature-compensated amplifier to correct for temperature differences from sample to sample. For convenience in the readout an offset voltage of 0.7 volts is added to produce the conventional pH readout scale of 1 to 14.

Dissolved oxygen. Monitor utilizes a voltaic polarographic membrane sensor. The sensor consists of a platinum cathode and a silver anode in a solution of potassium chloride. A potential difference of 0.55 volts is established between the cathode and anode. The cell is separated from the sample by a membrane which is permeable to dissolved oxygen. The special membrane assembly used by InterOcean provides for extreme dimensional stability which results in excellent long-term reliability without recalibration. Dimensional stability is achieved by using stainless steel construction in the sensor and by a special process of depositing the membranes on a fine mesh stainless steel screen. The screen is held securely in place by a stainless steel screw on assembly. The oxygen which enters through the membrane is reduced at the cathode and a very small current is produced. By designing the sensor to consume oxygen at a slow rate compared to the

net mass transport of oxygen through the water, the sensor output is not affected by changes in water flow rate past the membrane. No pump or stirrer mechanisms, which are subject to fouling and clogging, are required. Even in stagnant water the sensor will read 97 percent of the equilibrium value.

The output current is proportional to the diffusion rate of oxygen across the membrane. The oxygen diffusion rate for a given membrane configuration is proportional to the partial pressure of oxygen in the sample.

Most researchers prefer their data in terms of concentration of dissolved oxygen in the water. To relate the partial pressure value to an actual concentration it is necessary to measure and correct for the sample temperature and salinity. Figures 1 and 2 show the relationships between oxygen solubility and temperature and salinity respectively.

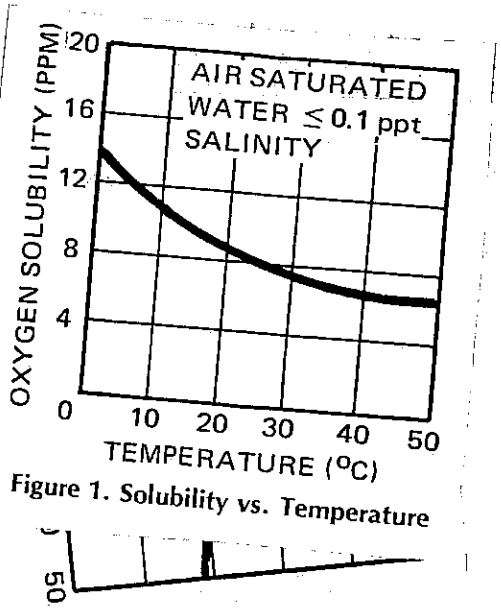


Figure 1. Solubility vs. Temperature

Figure 2. Solubility vs. Salinity

The standard InterOcean Dissolved Oxygen Monitor provides for automatic temperature compensation. A thermistor is used to measure the temperature of the water and its output is used to adjust the gain of the signal processing amplifier.

Conductivity. is measured by the inductive technique. InterOcean's conductivity sensor is completely encapsulated in dense epoxy making it extremely rugged and insensitive to fouling. Square waves are the standard alternating current source for inducing a known voltage by transformer action in the water sample. A current transformer detects the current flowing in the water loop. Rectification converts the current square waves back into a direct current voltage which is proportional to conductivity. All circuitry and voltage regulation is contained in the probe housing. After calibration, the conductivity sensor head constant is determined and a table of characteristic values for known resistances placed through the sensor is provided. The user may therefore easily check the calibration in the field without using elaborate test equipment. See Model 500CS Conductivity Sensor Stimulator. Conductivity measurements must be corrected to temperature using Table 3 in manual conductivity method.

Temperature. is measured by use of a linearized thermistor. The special designed probe insures rapid response and accurate temperature measurements. The thermistors are assembled in thin walled stainless steel tubes which isolate them from contact with the water. This provides protection and an almost unlimited lifetime even when continuously submerged.

Depth. measurement is made using a bonded strain gauge pressure transducer. An oil-filled compliance chamber isolates the strain gauge from the water environment but does not alter or affect the sensitivity of the measurement. Sensitivity of the 0-100 meter depth gauge is about 4 cm. Pre-calibrated pressure sensors with different ranges may be easily installed in the field by the user. A depth calibration circuit is incorporated in the depth electronics circuit board. By shunting the depth "calibration posts" a factory determined characteristic value is displayed on the system's readout. The depth parameter may be recalibrated, in the field, by shunting the "calibration posts" and adjusting the signal amplifier on the depth circuit board.

Calibration

1. Remove pressure case from probe circuitry.

2. On the current regulator board there should be only 2 millivolts difference between the 2 white test points.

   Use the white test point next to the red test point as a circuit common for all further steps.

3. Check that the red and black test points are the + and - voltages being supplied to the Probe (15 ± 4 volts).

4. On the voltage regulator board there are red and black test points (+8, -8). Adjust the trimpots adjacent to each test point to obtain 8.000 ± .002 volts.

5. Check chopper, amplifier and demodulator boards at their test points prior to calibrating for conductivity or salinity. No adjustments can be made.

6. For conductivity board zero adjustment be sure that the conductivity head is clean and dry. Move the positive voltmeter lead to the blue test point. Adjust trimpot (located on the left side of the conductivity board) to obtain 0.000 ± .002 volts.

   To make gain adjustment, place the 50 ohm wire resistor through conductivity probe head while it is in air and attach the alligator clips. The readout should be 4800. Make adjustments to the trimpot on the right side of the board.

7. The auto range board has no adjustments. The following voltages can be read at the test points if it is working properly.

	20 salinity	20 salinity
gray	2.005 + .005 volts	+1.99 + .01 volts
green	6.50 + .75 volts	-6.50 + .75 volts

8. To calibrate temperature, place voltmeter on the temperature output test point (blue) and remove the shroud from the temperature sensor.

Place the temperature sensor so that it is completely immersed in an ice and water mixture. When thermal equilibrium is reached at about $0^{o}C$ adjust the zero trimpot so that the output of the circuit reflects the temperature at the ratio of .1 $V/^{o}C$.

Substitute a room temperature bath for the ice bath. Adjust the gain trimpot until the output reflects the temperature at the ratio of .1 $V/^{o}C$.

Cycle between ice water and room temperature a couple of times and check the zero adjustment as it may have been affected by the gain adjustment. Repeat until no more adjusting is necessary.

9. For calibration of depth attach the positive meter lead to the blue test point on the depth board. Adjust the zero trimpot (next to the blue test point) until a reading of less than ± 2MV is read on the meter. Short together the two turret terminals on the left hand side of the depth card. Record the number generated by the short on the Digital Volt meter (approximately .8 or .9). Multiply this number by the factor 1.0256 and reset this new value by adjusting the gain on the right hand side of the depth card.

10. If the disposable cartridge has been removed or replaced, it is recommended that the dissolved oxygen sensor be soaked overnight with the probe powered.

Connect the voltmeter test lead to the green test point on the DO board. Remove the bottom cap and disposable cartridge from the sensor, wash off all charging fluid with distilled water and allow the sensor to completely dry. Adjust zero.

Soak DO sensor overnight with probe powered prior to adjusting the gain.

Immerse the probe up to the base plate in circulating water whose O_2 content and temperature are stable. Run a winkler titration. Adjust the gain trimpot located furthest from the green test point so that the output represents the O_2 concentration at the ratio of .1v/ppm O_2.

11. Clean pH sensor, by wiping the bulb with dilute alcohol and soak it in saturated potassium chloride solution overnight if it has dried out. Always have sensor soaking in potassium chloride solution when not in use.

    Wash the sensor with distilled water, wipe it dry, and immerse the sensor in a pH 7 buffer solution. Measure the voltage at the yellow test point on the pH board. Adjust the trimpot next to the yellow test point until its voltage equals .700 v $\pm$ .005.

An EBT can be obtained by hooking the InterOcean up with a Hewlett Packard X-Y recorder. For the intial hook-up, a connection is made at the recorder plug on the back of the InterOcean as follows:

Temperature (x-axis)	filter +	temp pin
	-	common*
	G	ground
Depth (y-axis)	filter +	depth pin
	-	common*
	G	ground

*The small wire attached to the common must be connected to the hole under the ground pin and screwed in.

Specifications for Recorder

Axis	Parameter	Scale	Chart Scale	Polarity	Chart Response	Full Scale On Chart
x	Temp	0-25^{o}C	.05 v/cm	+ up	fast	40 div.
y[1]	Depth	0-25m	5 mv/cm	RT +	fast	25 div.

Procedure

1. Connect modulating filters.
2. Turn on line switch at beginning of day and leave on. Recorder should be left in standby position when not in use.
3. Turn on Servo and Chart Hold when arriving on station.
4. Place the InterOcean probe in the water. Raise it so that all the probes are in the air.
5. Place recorder pen in down position and begin trace. Lower the probe system using speed 3 on the winch control.
6. Lift pen when probe reaches bottom and switch x-y recorder to the standby position.

All units should be plugged into the voltage stabilizer outlet.

Martek

A. pH

1. Remove the two set-screws securing the protective shield, remove reference electrode from plastic bottle used for shipping and long-term storage. Rinse in tap water to remove KCl from surfaces. Dry connector and remove greased cork.
2. If pH electrode has been stored in a dry state, immerse in water for 24 hours.
3. Turn function switch to "EXP" and expanded range switch to "6+." Reading of approximately 1.0 indicates proper readout operation.

4. Turn function switch to "OFF" position and connect plug on deck end of cable to input receptacle (on rear of case).
5. Connect sensor plug to the waterproof female connector on the underwater end of cable and securely tighten.
6. "Slope" and "Zero" calibration adjustments should be checked every time the electrode tips are acid cleaned. Otherwise they should remain stable for several months or longer.
7. Turn the function switch to "OFF" and adjust the mechanical "zero" control on the face of the panel meter. Rinse electrodes in distilled water and shake to remove excess.
8. Pour pH 7 buffer into a 250 ml beaker and immerse electrodes. Level of the buffer solution should be 2 inches above base of shield. Turn function switch to "exp" and the expanded range switch to "7". Allow 30 minutes equilibration.
9. Remove calibration access place and adjust zero potentiometer to indicate zero.
10. Change expanded range switch to "6+" position and adjust calibration potentiometer to obtain 1.00 on the meter.
11. Turn function switch to "OFF" and rinse with distilled water and shake off excess liquid.
12. Squirt probe with pH 10 solution and immerse electrodes in pH 10 solution. Turn the function switch to "exp" and the expanded range to the 9+ position.
13. Adjust the slope potentiometer so that the meter reads 1.00 (pH = 10.00)

NOTE: The exact values given by the buffer manufacturer for the temperature of measurement should be used for the most precise calibration.

B. Conductivity

1. Clean conductivity cell by flushing it with tap or distilled water, and then connect it to the instrument by matching brown color-coded leads. Tighten waterproof connectors.
2. Turn conductivity range switch to "CAL" position.
3. Adjust conductivity CAL potentiometer, as necessary, to position the dial-pointer of the conductivity meter at exactly "10" (full scale point).
4. Place conductivity probe in each of 4 different standards. Allow a period of at least 6 seconds stabilization time. Turn conductivity range switch clockwise until the highest reading on the conductivity panel meter is reached without the dial pointer exceeding the full-scale point. Read scale as shown below.
5. Reading should be within 1.5% of known value. Use a mercury thermometer to determine temperature.
6. If cell reading is low, check for physical bore obstructions. If there are no obstructions, set K = 2 and 0.2 cells, dip the cells for 20 seconds in 5% (wt.) hydrochloric acid (17 ml conc HCl diluted to 100 mls) followed by a fresh-water rinse. This should restore the cells.

Switch Position	Full-scale Measuring Range Selected Using micromhos/cm
100	0-1000 µmhos/cm
50	0-500 µmhos/cm
25	0-250 µmhos/cm
10	0-100 µmhos/cm
5	0-50 µmhos/cm
2.5	0-25 µmhos/cm

PART 3 - INORGANIC CONSTITUENTS IN WATER

ALKALINITY (TITRATION)

Scope and Application

This manual method can be used to determine alkalinities up to 500 mg $CaCO_3$/l. It is more than sufficient to cover the range of values we might encounter.

Principle and Theory

Alkalinity is a function of carbonate, bicarbonate and hydroxide content. It is determined by titration with a strong acid to a designated pH. Both phenolphthalein and total alkalinity can be measured with this method, the phenolphthalein alkalinity read at a pH of 8.3 and total alkalinity at a pH of 4.8.

Method

A. Sample Preparation

1. Samples are not filtered and should be run as soon as possible after sampling. Hold at $4^{o}C$.

B. Reagents

1. .02 N Hydrochloric acid

   200 ml .1 N HCl (8.3 ml HCl/l distilled water*)

   Dilute to 1 l with distilled water*

*Water used for titrant should be freshly boiled and cooled CO_2 free distilled water.

2. TRIS

   2.422 g. TRIS

   Dilute to 1 liter with distilled water.

3. Standardization of .02N HCl

   20 ml TRIS

   100 ml distilled water

   Titrate against .02N HCl to a pH of 4.8.

   Do three times and take an average. Average should be close to 20 ml.

C. Calculations

1. Determine normality using the following formula.

   $N_1 x V_1 = N_2 x V_2$

   N_1 = theoretical normality (.02)

   V_1 = amount that should be titrated (20 ml)

   N_2 = actual normality

   V_2 = actual amount titrated

2. Factor equals $\frac{N_2}{N_1}$

3. To determine the actual concentration of alkalinity in a sample as mg $CaCO_3$/l, multiply the volume titrated by 10 and then by the factor determined in Step 2 above.

D. Procedure

1. Measure 100 ml of sample into a beaker.

2. Place calibrated pH electrode in sample and titrate to designated pH (4.8 for total alkalinity).

E. References

1. American Public Health Association. 1974. Standard Methods for the Examination of Water and Wastewater. 14th Edition. p. 48-52.

CHLORIDE (FERRICYANIDE - AAII)

Scope and Application

This automated method is used to analyze natural water samples for chloride. It is usually run on a scale of 0-50 ppm but can be run on a lower or higher scale, depending on the concentration of chlorides from the area being studied. A larger range can be accommodated by using machine dilutions.

Principle and Theory

This procedure depends on the liberation of the thiocyanate ion from mercuric thiocyanate by the formation of unionized but soluble mercuric chloride. In the presence of ferric ion, the liberated thiocyanate forms a highly colored ferric thiocyanate proportional to the original chloride concentration. This method is not linear, particularly at very low concentrations, so some chloride stock standard is added to the wash water to compensate for correct baseline setting.

Interferences

No significant interferences have been encountered. All glassware used in preparing samples, standards and reagents should be acid washed with 1:1 HNO_3 rather than the usual 1:1 HCl wash to avoid possible Cl^- contamination.

Method

A. Sample Preparation

1. Filter sample through .45 μ membrane filter.
2. Hold at $4^{o}C$ if samples cannot be analyzed immediately.

B. Reagents

1. Mercuric Thiocyanate (2 l)

   10 g mercuric thiocyanate

   2 l deionized water

   Stir overnight. Filter 1 liter just before use and add 1 ml Brij per liter. The original stock can be recycled by replacing the volume used with additional deionized water. Water can be added until mercuric thiocyanate no longer precipitates when allowed to settle for a few minutes.

2. Ferric Nitrate (1 l)

   202 g ferric nitrate (Fe_2NO_2)

   Dissolve in 500 ml of deionized water

   50 ml nitric acid

   Top off to 1 l with deionized water.

   Let settle overnight and filter before using.

3. Wash Water

   3.5 ml Stock A per 1 l deionized water

   Add 1 ml of Brij for each liter of water.

3. During this time mercuric sulfide will precipitate and H_2S gas will be released.
4. After 24 hours, siphon liquid through a Buchner funnel and store residue in glass jar. The clear filtrate may be flushed down the hood sink.

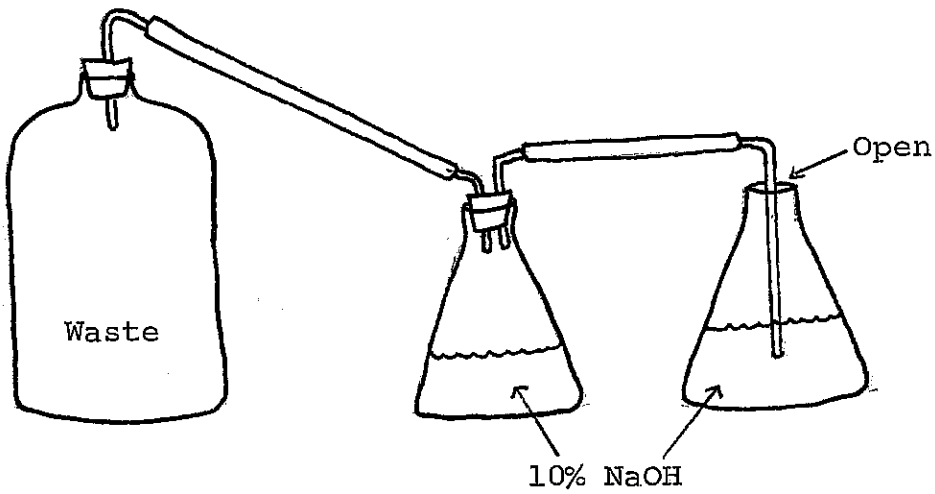
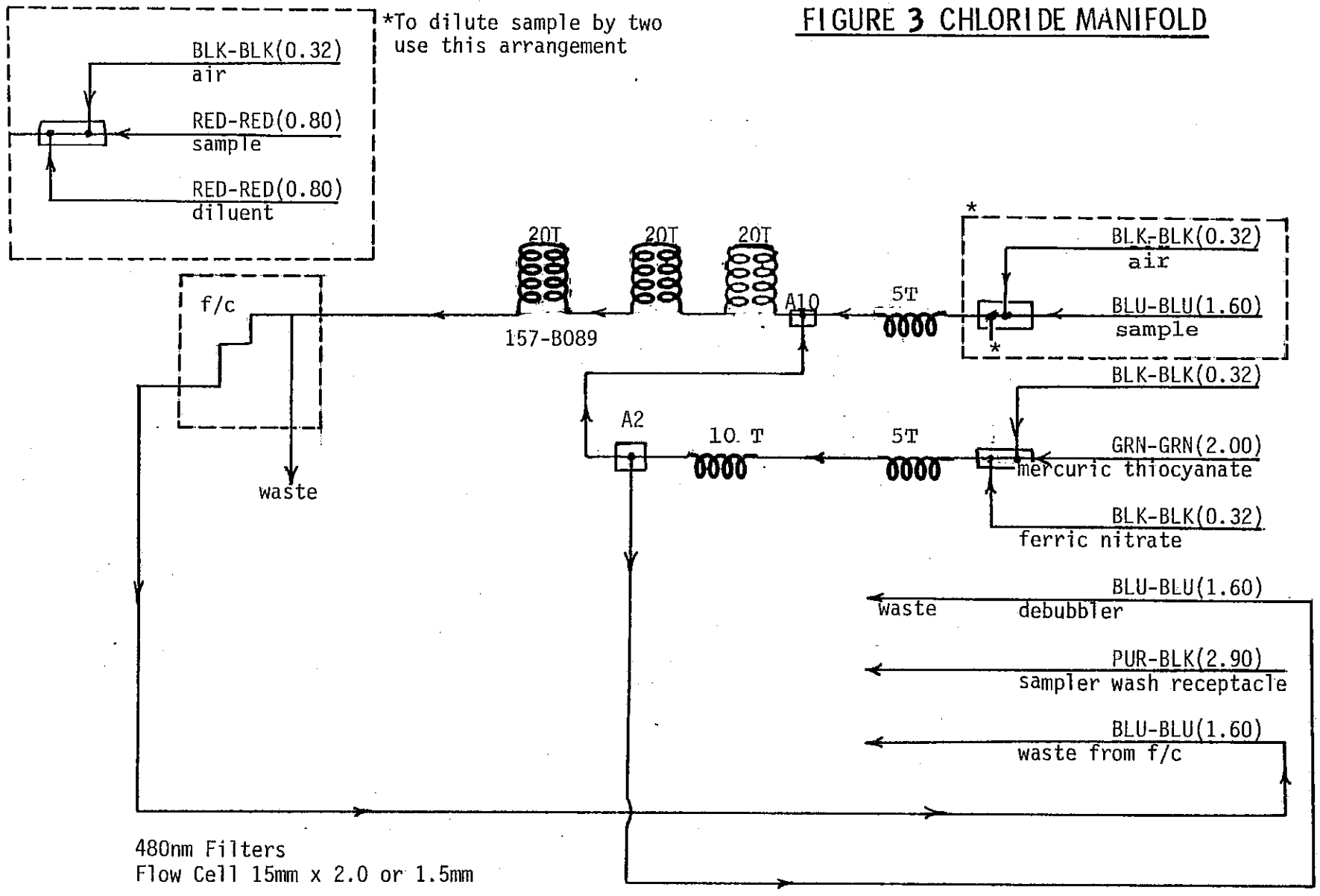


FIGURE 3 CHLORIDE MANIFOLD



AMMONIA (PHENATE METHOD -- AAII)

Scope and Application

The automated phenate method is used in determining ammonia concentrations in all our samples. Samples can be run in a range from 0 to 400 ppb. For samples with higher concentrations, samples are individually diluted or the manifold is modified to accomplish automatic dilutions.

Principle and Theory

The automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue-colored compound closely related to indophenol occurs when ammonia is added to sodium phenol followed by the addition of sodium hypochlorite. Potassium sodium tartrate and sodium citrate are added to the sample stream to eliminate the precipitation of hydroxides of calcium and magnesium. Sodium nitroprusside is added as a catalyst to intensify the color reaction.

Interferences

Calcium and magnesium ions may be present in sufficient quantity to cause precipitation problems. For the amounts we may encounter in concentrated areas, such as Maumee Bay, a complexing reagent containing potassium sodium tartrate and sodium citrate is added to eliminate any precipitation. It is very easy to contaminate samples and reagents with ammonia present in the surrounding lab area. To lessen this possibility all reagents are covered, the air is scrubbed with 5 N H_2SO_4 before entering the system and samples are not set up until immediately before analysis.

Method

A. Sample Preparation

1. Samples are filtered through .45 u membrane filters and run immediately.
2. Store at $4^{o}C$ if samples cannot be run right away.

B. Reagents

1. Complexing Agent (1 liter)

   33 g potassium sodium tartrate.

   24 g sodium citrate.

   Dilute to 1 l with deionized water.

   Adjust to pH 5.0 with H_2SO_4.

   Add 0.5 ml Brij.

   Store in poly container at $4^{o}C$. Make sure all crystals are dissolved before using.

2. Alkaline Phenol (1 liter)

   83 g phenol (This chemical can cause painful burns. Take care when using it.)

   Dissolve in 500 ml deionized water.

   Slowly add 180 ml 20% NaOH (20 g/100 ml).

   Dilute to 1 l with deionized water.

   Store in glass container at $4^{o}C$. Reagent is no longer good when it turns dark brown. Should undissolved crystals remain after reagent is made, filter through .45 u filter paper (GF/F or membrane filter).

3. Sodium Nitroprusside (1 liter)

   0.5 g sodium nitroprusside.

   Dilute to 1 l with deionized water.

   Store in glass container at $4^{o}C$.

4. Sodium Hypochlorite (250 ml)

   50 ml 5.25% chlorox

   Dilute to 250 ml with deionized water.

   Make daily.

C. Standards

1. Ammonia Standard Stock A (100 ppm)

   0.3819 g ammonium chloride (NH_4Cl)

   Dilute to 1 l with deionized water.

   1 ml chloroform

   Store in glass container at $4^{o}C$.

2. Stock B (1 ppm)

   10 ml Stock A

   Dilute to 1 l with deionized water.

3. Working Standards (make daily)

ml Stock A in 100 ml deionized water	Concentration
20 ml	200 ppb
10 ml	100 ppb
5 ml	50 ppb
3 ml	30 ppb
2 ml	20 ppb
1 ml	10 ppb

G. Re

1.

2.

D. Procedure

1. Set up system as described in Figure 4.
2. Attach air tubing to acid scrubber of 5 N H_2SO_4 (14 ml/100 ml).
3. Allow system to warm up for at least 20 minutes with reagents pumping.
4. Zero and full-scale recorder.
5. Run a full series of standards and blanks to establish linearity, setting the standard closest to anticipated concentration of samples.
6. Run samples in the following pattern: set standard, 6-10 samples, second standard within range of samples.

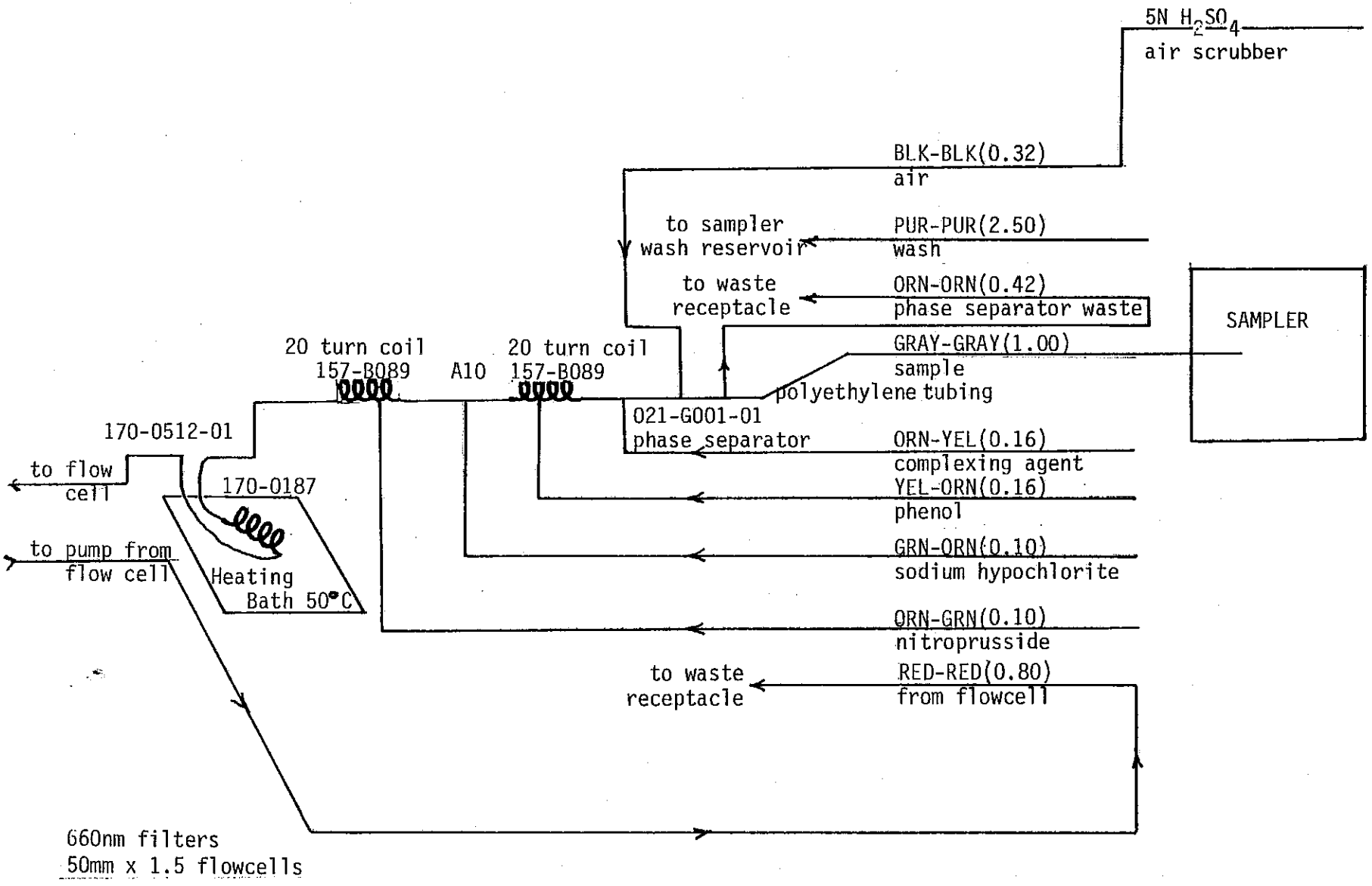
E. Calculations

1. Prepare a calibration curve with peak heights obtained from standards.
2. Compare peak heights of samples with calibration curve.

F. Precision and Accuracy

1. Samples should be run at a standard calibration setting of approximately 6.0 to 8.0 for a 0-100 ppb scale; 5.00-6.00 for a 0-200 ppb scale and 2.00-3.00 for a 0-400 ppb scale. Detection limits are 1.0 ppb, 2 ppb and 4 ppb, respectively.

FIGURE 4 AMMONIA MANIFOLD



NITRATE PLUS NITRITE (AAII - Cadmium Reduction)

Scope and Application

The cadmium column reduction method is used to determine a combined concentration of nitrate plus nitrite. The range used is 0-1 ppm with dilution of individual samples used when occasional values go off scale.

Principle and Theory

Nitrate in the sample is reduced to nitrite by passing it through a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. The compound then couples with N-1-napthylethylenediamine dihydrochloride to form a purple dye the intensity of which is then measured colorimetrically.

Interferences

No interfering substances have been encountered. However, air bubbles allowed to enter the cadmium column will greatly shorten the life of the column and result in a loss of sensitivity.

Method

A. Sample Preparation

1. Samples are filtered through a .45 μ Sartorius membrane filter and run immediately.

2. If samples cannot be run within several hours of collection, store at 4°C and run as soon as possible.

B. Reagents

1. Ammonium Chloride (2 liters)

   20 g ammonium chloride

   Dilute to 2 l with deionized water.

   Adjust to pH 8.5 with NH_4OH (If pH is overshot, use HCl to bring it down).

   Store in poly container at 4°C.

2. Color Reagent (2 liters)

   1500 ml deionized water

   200 ml concentrated phosphoric acid

   20 g sulfanilamide

   1.0 g. N-1-napthylethylenediamine dihydrochloride

   Dilute to 2 l with deionized water.

   Add 1 ml Brij.

   Store in glass container at 4°C.

C. Preparation of Cadmium Column

1. Weigh 8-10 g cadmium on weighing paper.
2. Sort out large chunks and discard.
3. Place cadmium in beaker.
4. Rinse twice with 1 N HCl (83 ml HCl/1 l H_2O).
5. Rinse 5 times with distilled water.
6. Rinse 3 times with 2% $CuSO_4$ (2 g/100 ml). A reddish-brown precipitate should appear.

7. Rinse cadmium at least 20 times with deionized water.
8. Fill column under water to avoid trapping any air in column. Pack cadmium by tapping column as it is filled.
9. Loosely pack pyrex wool 1/4-inch from each end.
10. Store under water.

D. Standards

1. Nitrate + Nitrite Standard Stock A (1000 ppm)

   7.2469 g potassium nitrate (KNO_3)

   Dilute to 1 l with deionized water.

   1 ml chloroform

   Store in glass container at $4^{o}C$.

2. Stock B (10 ppm)

   10 ml Stock A

   Dilute to 1 l with deionized water.

3. Working Standards (make daily)

ml Stock B to 100 ml deionized water	Concentration
10 ml	1 ppm
5 ml	.5 ppm
3 ml	.3 ppm
2 ml	.2 ppm
1 ml	.1 ppm

H. References

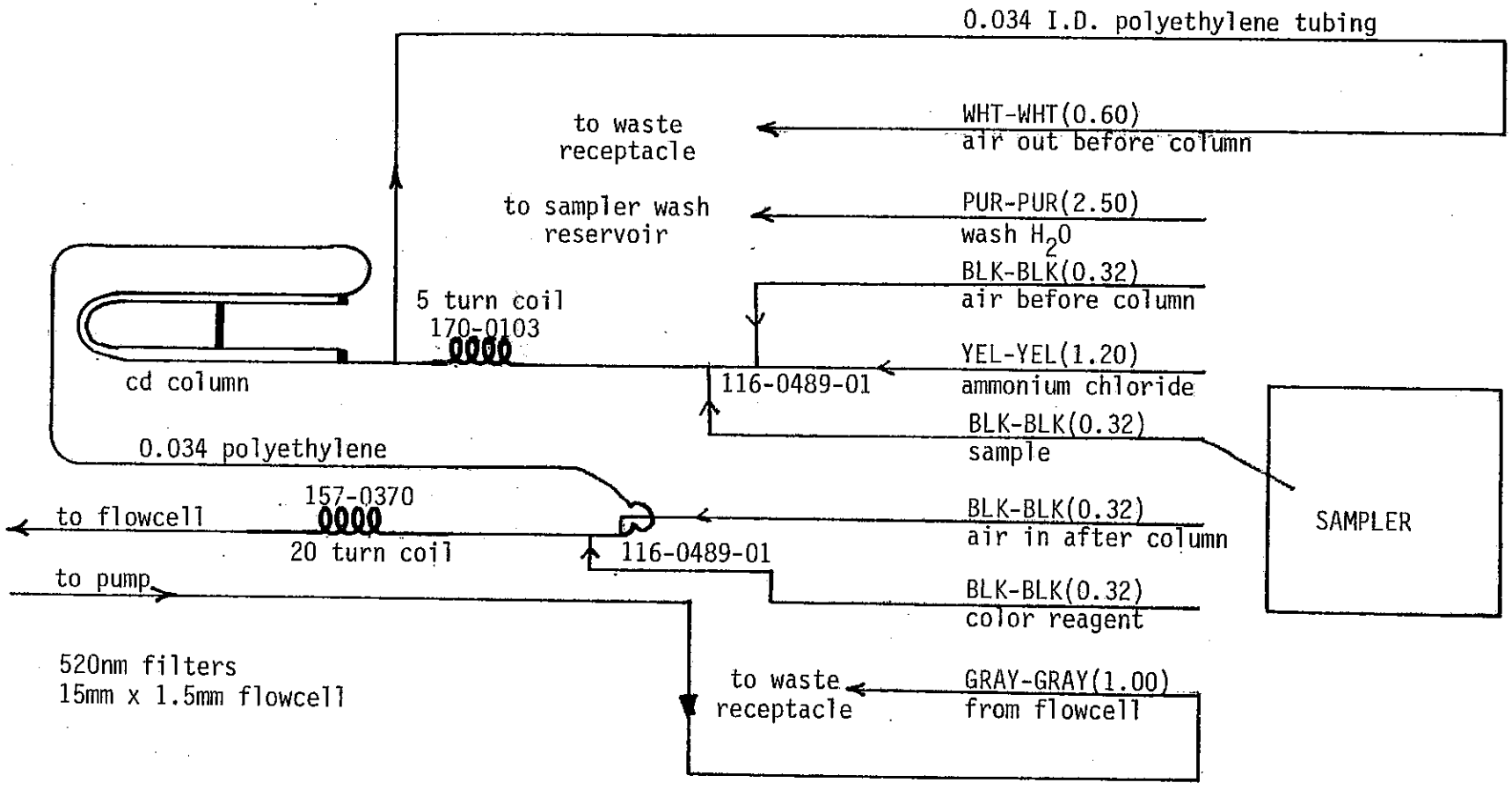
1. Technicon Industrial Method No. 100-70W.

2. Standard Methods for the Examination of Water and Wastewater. 1976. Fourteenth Edition. American Public Health Association, Washington, D.C.

Note

In the event that concentrations of nitrite alone are desired rather than the combination of nitrate + nitrite, the same manifold is used minus the Cadmium Column. Standards prepared with a nitrite stock are used for calibration.

FIGURE 5 NITRATE + NITRITE MANIFOLD



pH (ELECTROMETRIC)

Scope and Application

This method is applicable to all waters over the whole pH scale. Lake Erie water generally runs around 8 so the pH meter is calibrated to 7 and sloped to 10.

Principle and Theory

The glass electrode in combination with a reference electrode is used to measure pH. The glass electrode is a membrane of special glass. The membrane forms a partition between two liquids of differing hydrogen ion concentration and a potential is produced between the two sides that is proportional to the difference in pH between the liquids. A change in pH produces an electrical charge allowing the pH value to be read on the pH meter. Temperature has a significant effect on the pH measurement in two ways, both of which can be compensated for. The first involves differences in potential of the electrodes which can be corrected by adjusting the temperature control on the meter. The second is inherent in the sample and is handled by recording both temperature and pH of the sample. pH is also measured in situ as described in the section on in situ probes.

Method

Sample Preparation

1. Take a whole water sample and analyze as soon as possible after collection. Hold at $4^{o}C$.

Procedure*

1. Calibrate pH meter
   a. Set meter to 7.00 with electrodes in Buffer 7.
   b. Adjust slope to pH 10 with electrodes in Buffer 10.
      If samples are anticipated to fall below 7.00 adjust slope to pH 4 with Buffer 4.
   c. Set temperature calibration knob to temperature of buffer.
2. Pour sample into beaker and place on stir plate.
3. Place electrodes in sample, stir and allow readout to stabilize.
4. To insure accurate measurements, regularly fill and clean reference electrode according to instrument specifications.
5. Store electrodes in water or pH buffer.

*Leave meter on pH whenever transferring electrodes from one solution to another. Only use Standby mode when instrument is not in continual use. Rinse electrodes with distilled water between samples and blot dry.

Precision and Accuracy

1. pH is accurate to ± .05 units.

References

1. American Public Health Association. 1974. Standard Methods for the examination of Water and Wastewater. 14th Edition.

DISSOLVED OXYGEN (WINKLER TITRATION-AZIDE MODIFICATION)

Scope and Application

This method is recommended for determining oxygen concentration in water containing more than .05 ppm of nitrite and less than 1 ppm of ferrous iron. Most of the waters we analyze are included in this category. Oxygen is measured as mg O_2/l.

Principle and Theory

The water sample being tested for dissolved oxygen is treated with a manganese sulfate solution and a strongly alkaline iodide reagent. The manganese hydroxide formed reacts with the dissolved oxygen present to form a brown precipitate. Upon the addition of acid, in the presence of iodide, iodine is liberated in an amount equal to the amount of dissolved oxygen originally present. The iodine is then titrated with standard sodium thiosulfate, using starch as an indicator to visually intensify the end point.

Interferences

Samples with high ferrous iron content or high in suspended solids cannot be accurately analyzed by this method.

Method

A. Sample Preparation

1. Collect sample in 300 ml BOD bottle being careful not to introduce any air bubbles.

B. Reagents

1. Manganese Sulfate (1l)

   364 g. $MnSO_4 \cdot H_2O$*

   Dilute to 1 l. with distilled water.

   Filter and store in poly bottle at 4^oC.

   *If $MnSO_4 \cdot H_2O$ is not available use 480 g. $MnSO_4 \cdot 4H_2O$, or 400 g. $MnSO_4 \cdot 2H_2O$.

2. Sodium Azide (1 l)

   500 g. sodium hydroxide (NaOH) or 700 g. potassium hydroxide (KOH)

   150 g. potassium iodide (KI) or 135 g sodium iodide (NaI).

   Dissolve in distilled water and dilute to 1 l. To this solution add 10 g. sodium azide (NaN_3) dissolved in 40 ml. distilled water. Store in poly container at 4^oC.

3. Sulfuric Acid, concentrated

4. Starch (1 l.)

   5-6 g. starch

   Prepare an emulsion with starch and pour into 1 l. of boiling distilled water. Boil for a few minutes and let settle until cool. Add a few drops of toluene as a preservative. Store in poly container at 4^oC.

5. Sodium Thiosulfate, .025N (2 l)

   12.41 g. sodium thiosulfate

   Dissolve in and dilute to 2 l. with freshly boiled and cooled distilled water (CO_2 free).

   Add .8 g. NaOH as a preservative.

6. Potassium Dichromate

   1.226 g potassium dichromate ($K_2Cr_2O_2$) dried at 103°C for 2 hr. Dissolve in 1 l. distilled water.

7. Standardization of Thiosulfate

   2 g. potassium iodide (KI)

   100 to 150 ml distilled water

   10 ml. H_2SO_4 (1 ml. concentrated H_2SO_4 + 9 ml. distilled water)

   20 ml. potassium dichromate

   Combine in wide mouth erlenmeyer flask in above order. Place in dark for 5 minutes, dilute to approximately 400 ml. and titrate with thiosulfate. Amount of thiosulfate used in titration should equal amount of potassium dichromate.

   Repeat procedure three times and take the average as V_2.

C. Calculations

1. To determine normality of sodium thiosulfate use the following formula:

   $$N_1 x V_1 = N_2 x V_2$$

   N_1 = theoretical normality (.025)

   V_1 = volume that should be titrated (20 ml)

   N_2 = actual normality

   V_2 = actual volume titrated (average of three titrations)

2. Factor = $\frac{N_2}{N_1}$

D. Procedure

1. Collect sample in 300 ml BOD bottle.
2. Add 2 ml. $MnSO_4$ followed immediately by 2 ml. sodium azide solution, adding below the surface of the liquid and taking care not to introduce any air bubbles.
3. Mix well by inverting and allow to settle. Shake and settle twice.
4. Add 2 ml. conc. H_2SO_4 and mix to produce a clear liquid.
5. Measure 203 ml. of sample into erlenmeyer flask and titrate with standardized thiosulfate to a light straw color.
6. Add a squirt of starch and continue titrating until clear.
7. Multiply ml. titrated by correction factor for thiosulfate to obtain DO in mg O_2/l.

E. References

1. American Public Health Association. 1974. Standard Methods for the examination of water and wastewater. 14th Edition, p. 406-410.

ORTHOPHOSPHATE (STANNOUS CHLORIDE - AAII)

Scope and Application

The stannous chloride method is used for analysis of all phosphorus forms which we measure including total, total filtered, soluble reactive, particulate, non-apatite and apatite. All forms are first converted to soluble orthophosphate which is then measured colorimetrically on a Technicon Auto Analyzer II. Each form requires a different preparation method, has a different range, detection limit and standard deviation; so each form is discussed separately. Ranges vary from 0-50 ppb to 0-1,000 ppb.

Interferences

A positive interference is caused by high iron concentrations. High peaks which collapse approximately halfway through the reaction are indicative of this problem. The only solution we have discovered is dilution. Care also must be taken to have all samples, reagents and standards at the same temperature, preferably between 20^{o} and $30^{o}C$. Samples which are colder than the system will produce a falling peak or no reaction at all. Mercuric chloride used as a preservative presented problems as a precipitate of mercurous chloride was formed within the system and interfered with the colorimetric reading causing noisy peaks. The deionized water used in preparing reagents and standards must be free from phosphorus. This is particularly important when running SRP as concentrations often run very close to the detection limit.

After several days of continual running a stannous chloride coating will build up in the flow cell and a loss of sensitivity will result. The system can be flushed out by pumping 1 N NaOH for 15 minutes, water for 5 minutes, and 20% H_2SO_4 for 15 minutes.

SOLUBLE REACTIVE PHOSPHORUS (AAII)

Principle and Theory

Soluble reactive phosphorus is already in the form of soluble orthophosphate. The orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid which is reduced to intensely-colored molybdenum blue by stannous chloride. Hydrazine sulfate is added to the stannous chloride reagent to prevent oxidation of stannous chloride and rapid deterioration of the reagent. It also aids as a reducing agent.

Method

A. Sample Preparation

1. Filter sample through .45 μ Sartorious membrane filter.
2. If samples cannot be run within several hours of collection, hold at 4°C.

B. Reagents

1. Ammonium Molybdate (1 liter)

   11.9 g ammonium molybdate

   Dissolve in 450 ml deionized water

   73.8 ml concentrated H_2SO_4

   Added to 450 ml deionized water

   Cool solutions, mix and dilute to 1 l.

   Store in poly container at 4°C. This is good until it begins to turn cloudy.

2. Stannous Chloride Stock Solution (100 ml)

   1.2 g stannous chloride

   100 ml concentrated HCl

   Store in glass container at $4^{o}C$. Good for about two weeks.

3. Hydrazine Sulfate (200 ml)

   4.0 g hydrazine sulfate

   200 ml deionized water

   Store in poly container at room temperature. Take care all crystals are dissolved before pipetting. This will last indefinitely.

4. Stannous Chloride Working Solution

   80 ml deionized water

   10 ml hydrazine sulfate stock solution

   0.7 ml Levor IV

   4.0 ml stannous chloride stock solution added in 0.5 ml aliquots and mixed well after each addition. Make daily.

C. Standards

1. Phosphorus Standard Stock A (100 ppm)

   0.4394 g potassium phosphate (KH_2PO_4)

   Dilute to 1 l with deionized water

   1 ml chloroform

   Store in glass container at $4^{o}C$.

2. Stock B (1 ppm)

   10 ml Stock A

   Dilute to 1 l with deionized water

3. Working Standards

ml Stock B per 100 ml deionized water	Concentration
5 ml	50 ppb
3 ml	30 ppb
2 ml	20 ppb
1 ml	10 ppb
0.5 ml	5 ppb

D. Procedure

1. Set up system using manifold as seen in Figure 6.
2. Zero and full-scale recorder.
3. Let system warm up for at least 20 minutes with reagents pumping.
4. Run a full series of standards and a blank to establish linearity, setting the standard running closest to anticipated sample concentrations.
5. Run samples between the set standard and a different standard at the end of the run.

E. Calculations

1. Prepare a calibration curve derived from the peak heights obtained with the standard solutions.
2. Determine the concentration of SRP in the samples by comparing peak heights with the calibration curve.

F. Precision and Accuracy

1. Run on a scale of 0-50 ppb with a detection limit of 0.5 ppb.
2. Standard calibration setting is between 7 and 8 on the colorimeter.

G. References

1. Canada Centre for Inland Waters. 1979. Analytical Methods Manual. NAQUADAT No. 15363, 15313 and 15254.
2. Standard Methods for the Examination of Water and Wastewater. 1976. Fourteenth Edition. American Public Health Association, Washington, D.C.
3. Unpublished information. Modifications developed by the Center for Lake Erie Area Research.
4. Unpublished information. Canada Centre for Inland Waters.

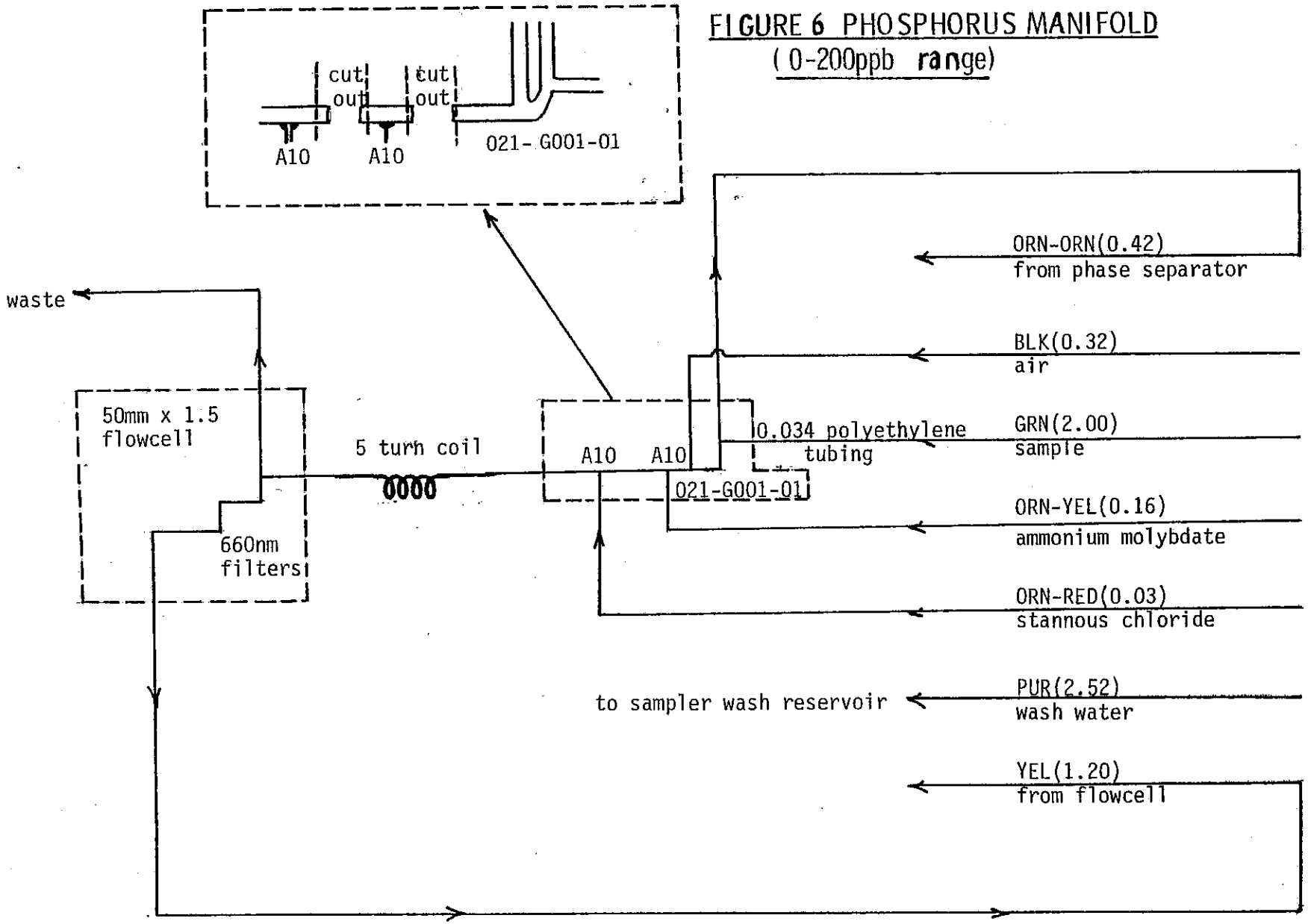


FIGURE 6 PHOSPHORUS MANIFOLD
(0-200ppb range)

TOTAL PHOSPHORUS, TOTAL FILTERED PHOSPHORUS AND PARTICULATE PHOSPHORUS (AAII)

Principle and Theory

Total phosphorus includes all phosphates potentially available. Total filtered phosphorus and particulate phosphorus are separated by filtration through a .45 µ membrane filter with the filtrate being analyzed for TFP and the pad being analyzed for PP. After being subjected to acid hydrolysis and oxidative digestion by sulfuric acid and potassium persulfate, the resulting soluble orthophosphate is combined with ammonium molybdate to form molybdophosphoric acid. This is reduced by stannous chloride to form molybdenum blue which is then measured colorimetrically.

Method

A. Sample Preparation

1. For TP, measure 100 ml of well-mixed sample into a 125-ml pyrex bottle (acid washed).
2. For TFP, measure 100 ml of sample water which has been filtered through a .45 µ membrane filter into a 125-ml pyrex bottle (acid washed).
3. For PP, filter 100 ml of sample through a .45 µ membrane filter, fold filter pad and place in 125-ml pyrex bottle (acid washed). When ready to analyze sample, add 100 ml of deionized water.

4. Digestion procedure for TP, TFP and PP:

   Add 2 ml of 11 N H_2SO_4 to each 100-ml sample.

   Add 0.8 g potassium persulfate (2 scoops) to each 100-ml sample.

   Cover bottles loosely with small beakers and autoclave at 121°C for 1 hour.

   Cool samples slowly in autoclave to prevent ebullition.

B. Reagents

1. Ammonium Molybdate (1 liter)

   11.9 g ammonium molybdate

   Dissolve in 450 ml deionized water.

   128 ml 11N H_2SO_4

   Add to 450 ml deionized water.

   Cool and mix 2 solutions and dilute to 1 l. Store in poly container at 4°C. This is good as long as it remains clear.

2. Stannous Chloride Stock Solution

   1.2 g stannous chloride

   100 ml of HCl(conc)

   Store in glass container at 4°C. Make up new for every cruise (1-2 weeks).

3. Hydrazine Sulfate (200 ml)

   4.0 g hydrazine sulfate

   200 ml deionized water

   Store in poly container at room temperature. Take care all crystals are dissolved before pipetting. This will last indefinitely.

4. Stannous Chloride Working Solution

   80 ml deionized water

   10 ml hydrazine sulfate

   0.7 ml Levor IV

   4.0 ml stannous chloride stock solution added in 0.5-ml aliquots and mixed well after each addition. Make daily.

5. 11N H_2SO_4

   Slowly add 620 ml concentrated H_2SO_4 to 1200 ml deionized water. Cool and dilute to 2 l with deionized water.

6. Wash Water

   Add 40 ml 11N H_2SO_4 to 2 l of deionized water.

   Add 2 ml Levor IV and mix well.

C. Standards

1. Phosphorus Standard Stock A (100 ppm)

   0.4394 g potassium phosphate (KH_2PO_4)

   Dilute to 1 l with deionized water.

   1 ml chloroform

   Store in glass container at 4^oC.

2. Stock B (1 ppm)

   10 ml Stock A

   Dilute to 1 l with deionized water.

3. Working Standards

ml Stock B to 500 ml deionized water	Concentration
100 ml	200 ppb
75 ml	150 ppb
50 ml	100 ppb
25 ml	50 ppb
15 ml	30 ppb
10 ml	20 ppb
0.5 ml	10 ppb

Add 11N H_2SO_4 to each standard in the ratio of 2 ml acid per 100 ml standard to maintain the same pH as the samples.

D. Procedure

1. Set up system using manifold as seen in Figure 6. If a range greater than 0-100 ppb is required, refer to Figure 7.
2. Zero and full-scale recorder.
3. Let system warm up for at least 20 minutes with reagents pumping.
4. Select a series of standards for the range desired.
5. Run a series of standards and blanks covering the entire scale to establish linearity, setting the standard running closest to anticipated sample concentrations.
6. Run samples between the set standard and an additional standard within the sample range.

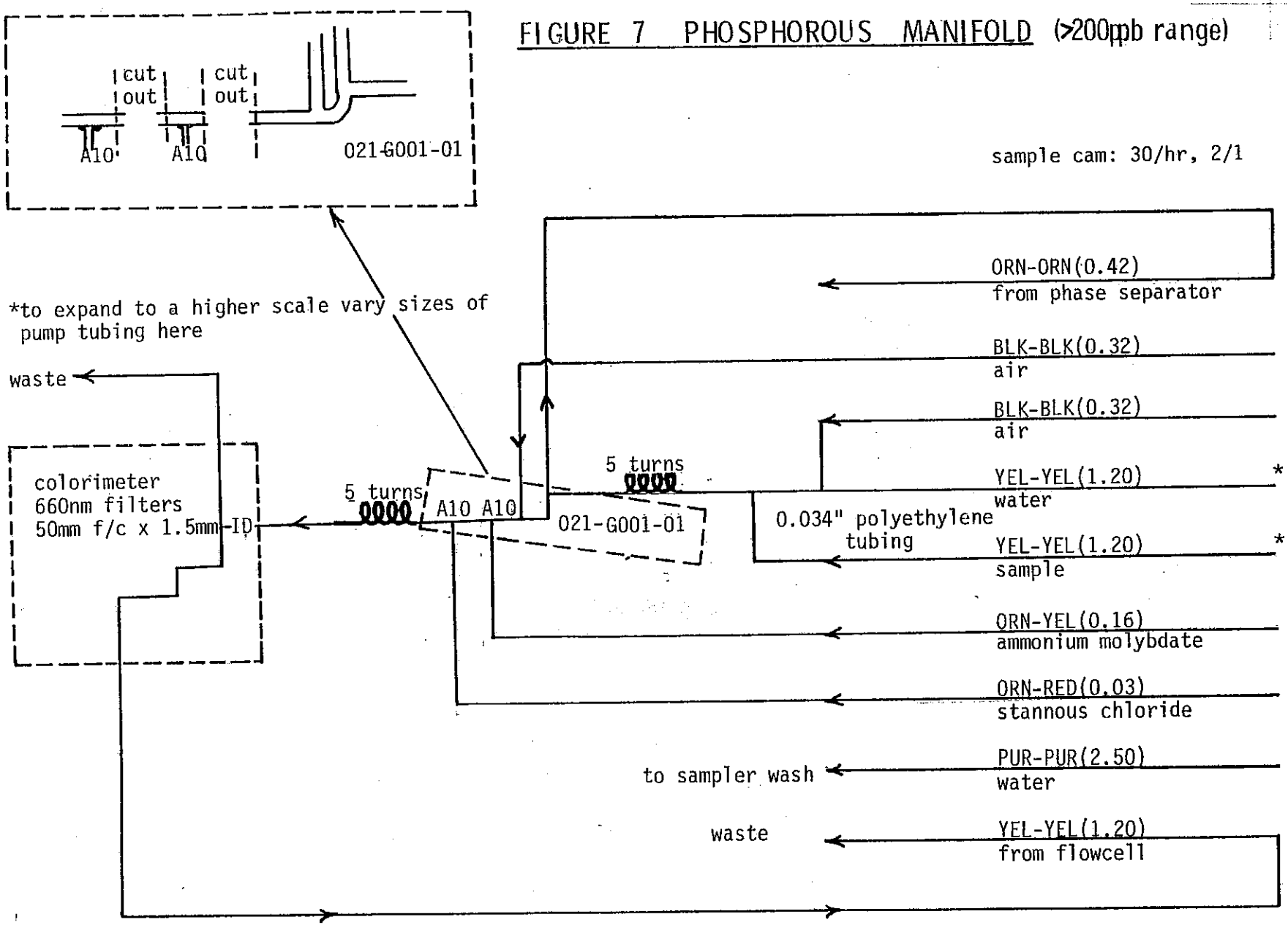


FIGURE 7 PHOSPHOROUS MANIFOLD (>200ppb range)
cut out
cut out
A10
A10
021-G001-01
sample cam: 30/hr, 2/1
*to expand to a higher scale vary sizes of pump tubing here
waste
colorimeter
660nm filters
50mm f/c x 1.5mm-ID
5 turns
5 turns
A10 A10
021-G001-01
0.034" polyethylene tubing
ORN-ORN(0.42)
from phase separator
BLK-BLK(0.32)
air
BLK-BLK(0.32)
air
YEL-YEL(1.20)
water
*
YEL-YEL(1.20)
sample
*
ORN-YEL(0.16)
ammonium molybdate
ORN-RED(0.03)
stannous chloride
PUR-PUR(2.50)
water
to sampler wash
waste
YEL-YEL(1.20)
from flowcell

TP SUPPLEMENT I.

(Phosphorus Correction When Running Non-Autoclaved Standards)

As of 1980 the Columbus lab began to run non-autoclaved standards in the determination of phosphorus. Because of this an autoclave correction factor was needed to obtain correct values. (This was discovered when IJC test lab samples were run.)

Through experimentation a blank value of 2.0 ppb was found, and phosphorus values were, on the average, 6.5% high after autoclaving. So the correction procedure will be to subtract blank value (2.0) and multiply that number by .935 to get correct phosphorus concentration. This was determined by the following procedure:

Standards were made up: 0, 10, 20, 30, 50, 80, 100, 200, 300 ppb. Half of each standard was transferred to TP bottles, persulfate and acid added and then autoclaved. These autoclaved standards and non-autoclaved standards were run on the AA and percent difference calculated. Also a blank value was determined. Data from this test is in the following table.

Non-Autoclaved Standards µg/l	AA Value µg/l	Autoclaved Standards µg/l	AA Value µg/l	Standard µg/l	Percent* Difference
0	.2	0	2	0	0
10	10.0	10	12.7	10	7.0
20	20.0	20	23.3	20	6.5
30	30.0	30	34.6	30	8.7
50	48.5	50	53.1	50	5.4
80	80.0	80	86.3	80	5.4
100	100	100	108.0	100	6.0
200	200	200	216.0	200	7.0
300	298	300	316.0	300	5.4

Average Percent Difference = 6.43

*After several blanks it has been determined that 2.0 ppb will be subtracted from every autoclaved sample when running non-autoclaved standards.

TP SUPPLEMENT II.

DILUTION PROCEDURES FOR THE AUTO ANALYZER IN RUNNING HIGH CONCENTRATION PHOSPHORUS SAMPLES

The basic straight (no dilution) system is shown in Figure 6. This system can be run up to a 0-200 ppb scale. A scale of 0-400 can also be reached but such a low setting is needed on the colorimeter (less than 1) that a lesser degree of accuracy may be entailed.

Figure 7 represents a 1:1 dilution with sample and dilution (wash) water. Before the phase separator a five turn mixing coil is added along with a connector (part No. 116-0489-01 E) to allow for addition of mixing bubble. This creates a faster flow and so a larger tubing from phase separator might be needed to compensate for the bubble and additional flow. This system is good for a scale of 0-200 ppb (setting about 5) and also 0-400 ppb (setting about 3).

Since the dilution of samples depends upon the pump tubing size it is conceivable that any dilution may be obtained with proper pump tubing size. A dilution of 1:10 was used to run samples on the Auto Analyzer with great success. The only difference in set-up from Figure 7 is a 10 turn mixing coil needed to properly mix the sample and dilution (wash) water, along with a sample pump tubing of orange-white (.23 ml/min) and dilution water of purple-purple (2.50 ml/min). A red-red (.80 ml/min) phase separator waste tubing was

needed to compensate for the faster flow. This system allowed a 0-1000 ppb scale to be run (setting about 5).

The same set-up was also used for a dilution of approximately 1:20 with a sample tubing of orange-yellow (.16 ml/min) and a dilution water pump tubing of purple-orange (3.40 ml/min). The phase separator waste tubing was green-green (2.00 ml/min). This allowed for a scale of 0-2000 ppb or 0-3000 ppb (setting about 6).

Additional higher scales and dilutions may be used but are not advised since the accuracy will be decreased due to increased increments of chart reading.

PARTICULATE PHOSPHORUS FRACTIONS

NONAPATITE AND APATITE (AAII)

Scope and Application

This method can be applied to water samples containing suspended material. A range of 0-400 µg/l is used for extractions 1 and 3, while a range of 0-100 µg/l is needed for extraction 2.

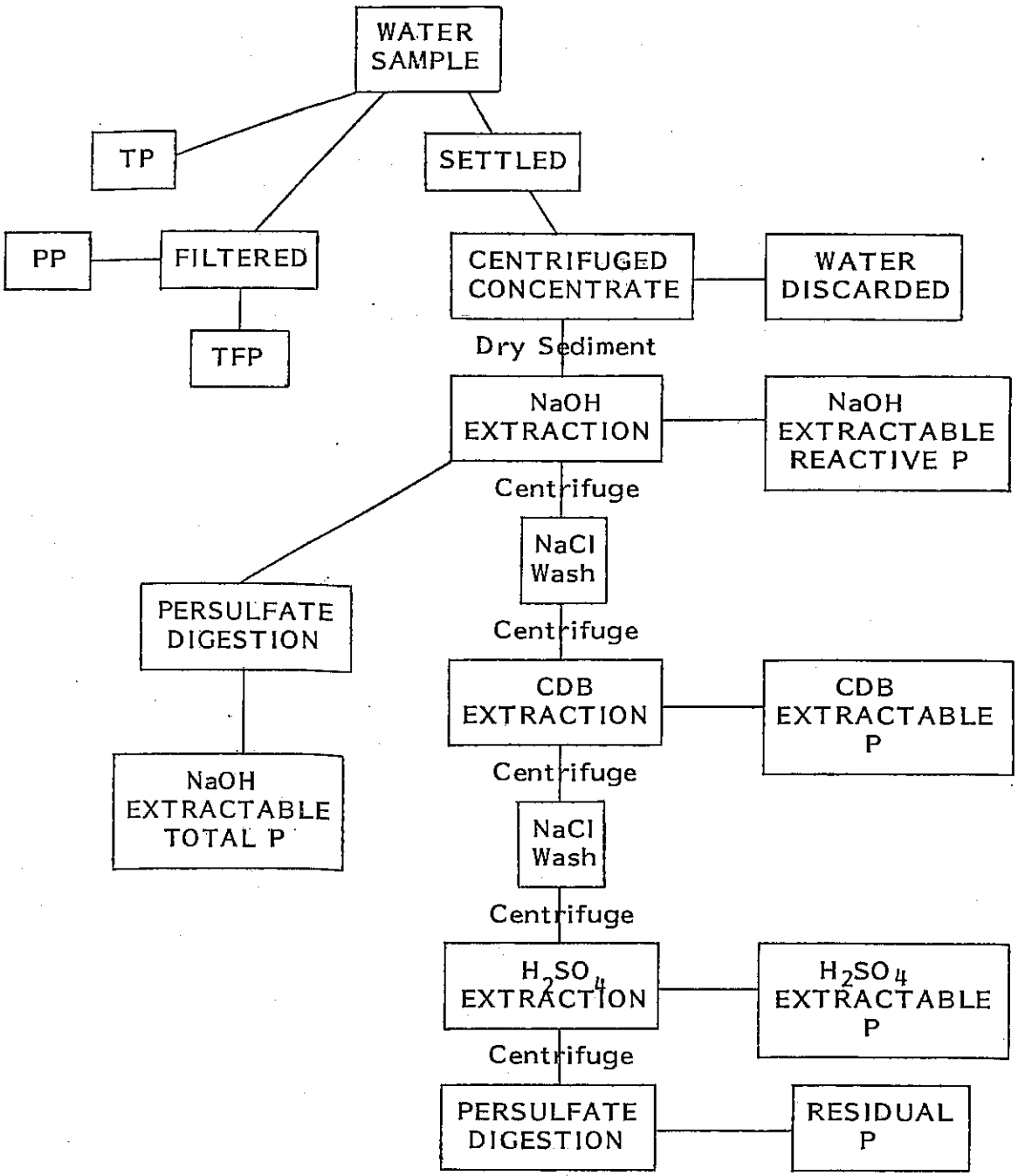
Principle and Theory

Particulate phosphorus refers to that fraction of total phosphorus in a sample that is bound to suspended material. It can be further defined as nonapatite and apatite, or bioavailable and non-bioavailable, respectively. A sample volume greater than 10 liters is concentrated, the particulate matter harvested and subjected to sequential chemical extractions (see flow chart). A sediment sample weighing 30 mg is used whenever possible. After each extraction the sample is analyzed on the AAII as described for SRP or TP. The first two extractions, NaOH and CDB, estimate the amount of available phosphorus present, while sulfuric acid extraction and the residue phosphorus estimate the amount of nonavailable phosphorus in the sample.

Method

A. Sample Preparation

1. Collect a known volume of water greater than 10 liters.
2. Concentrate sample using a Millipore Pellicon cassette system or by centrifuging.



PARTICULATE PHOSPHORUS FRACTIONATION PROCEDURE

3. Dry the resultant sediment by desiccation or freeze drying.
4. Record the weight of dried sample before beginning the series of extractions. If the sample weighs more than 35 mg, a 30 mg sample is used. If the sample weighs less than 35 mg, the entire sample is used.

Extraction 1 - NaOH Extraction

A. Reagents

1. 1.0N Sodium Chloride - 0.1N Sodium Hydroxide (2 l) (enough to run 60 samples)

   117.0 g. NaCl

   1500 ml deionized water

   8.0 g. NaOH

   Dilute to 2 l. with deionized water after solution has cooled and is clear.

2. 1.0N Sulfuric Acid (4 l)

   112 ml concentrated H_2SO_4

   Add to 3 liters of deionized water, cool and dilute to 4 liters.

3. 1.0N Sodium Chloride (2 l)

   117.0 g NaCl

   Dilute to 2 l with deionized water

4. 2N Sodium Chloride/.2N sodium hydroxide (2 l)

   234 g NaCl

   1500 ml deionized H_2O

   16 g NaOH

   Dilute to 2 liters with deionized H_2O

5. 2 N Sulfuric Acid (1 l)

   56 ml concentrated H_2SO_4

   Add to 500 ml deionized water.

   Cool and dilute to 1 liter with deionized water.

C. Standards

1. Phosphorus Standard Stock A (100 ppm)

   .4394 g potassium phosphate (KH_2PO_4)

   Dilute to 1 liter with deionized water

   1 ml chloroform

   Store in glass container at 4^oC

2. Stock B (1000 ppb)

   10 ml Stock A

   Dilute to 1 l with deionized H_2O.

3. Standard Stock H_2O (2 l)

   As determined by titration, mix correct ratio of 2N NaCl/.2N NaOH and 2N sulfuric acid to achieve 2 liters of a .44N acid solution.

4. Working Standards (250 ml flasks)

ml Stock B	ml Stock H_2O	Concentration
0	125	Blank
12.5	125	50
25	125	100
50	125	200
75	125	300
100	125	400

D. Procedure

1. Place dried weighed sample in a 50 ml polypropylene centrifuge tube and add 30 ml 1.0N NaCl/0.1N NaOH.
2. Shake tube for 18 hours, preferably with a wrist-action shaker.
3. Centrifuge for 30 minutes at 4000 rpm.
4. Decant all the liquid from the centrifuge tube into a standard TP bottle, being careful not to dislodge any sediment. Only a drop or two of liquid should remain.
5. Add 20 ml of 1.0N NaCl to the sediment, shake for 5 minutes, centrifuge as above and discard solution.

   Save the sediment for the next extraction.
6. Add a predetermined amount of 1.0 N H_2So_4 to the TP bottle to achieve a .22N acid solution.*
7. Run the sample on AAII using procedure outlined for TP, but without digesting.
8. Digest sample as described in TP procedure and run on AAII again.

*To determine how much 1.0N H_2SO_4 to add to each sample, the following procedure can be used.

1. Pipet 30 ml of 1.0 N NaCl/0.1 N NaOH into an appropriate vessel.
2. Add 3 drops of Methyl Orange indicator and titrate with 1.0 N H_2SO_4 to endpoint (yellow).
3. Assuming the 1.0N NaCl/0.1 N NaOH is 0.1000 N, calculate the normality of the H_2SO_4 by the following formula:

$N_B \times V_B = N_A \times V_A$

Where

N_B = normality of base

V_B = volume of base

N_A = normality of acid

V_A = volume of acid

i.e. Assume 2.7 ml H_2SO_4 was needed to reach endpoint then

N

x .030 l = N_A x .0027 l

N_A = 1.1111

4. The normality of the H_2SO_4 (N_A) is now used to estimate the amount of acid needed to reach 0.22 N.

$0.22N \times Y = X \times N_A$

Where

.22N = normality desired

Y = an estimate of final volume after titration (around 40)

X = additional amount of 1.0 N H_2SO_4 needed to reach .22 N

N_A = normality of H_2SO_4, as calculated above.

i.e. 0.22N x .040 l = X x 1.1111N

x = .0079 l

So Y = 30 ml + 2.7 ml + 7.9 ml = 40.6 ml.

Substituting .0406 in the above equation for .040 X = 8.1.

Since 2.7 ml + 8.1 ml = 10.8 ml, in this case 10.8 ml of 1.0N H_2SO_4 must be added to each sample to achieve .22N acidity.

Place above chemicals in each of three polyethylene test tubes. Heat tubes in water bath for 15 minutes at 80-85°C. Cool and bubble moist air through solution overnight.

4. Working Standards

Flask Size (ml)	ml of Stock B	ml CDB	ml 5% Am Moly	Concentration (ppb)
50	0	10	10	Blank
50	.5	10	10	10
50	1.0	10	10	20
50	1.5	10	10	30
50	2.5	10	10	50
25	2.0	5	5	80
25	2.5	5	5	100
25	5.0	5	5	200

Add 0.5 ml of 30% H_2SO_4 to each 25 ml of standard.

C. Procedure

1. To the centrifuge tube containing the sediment left from the first extraction add 30 ml 0.3M sodium citrate, 3.75 ml 1.0 M sodium bicarbonate and 0.75 g sodium dithionite.
2. Place tube in a beaker of water on a magnetic stirrer/hot plate and heat to 80-85°C while stirring. Remove cap while heating and take care no exchange of liquid occurs between water bath and sample.
3. Cool and centrifuge 30 minutes at 4000 rpm.
4. Decant all solution into a test tube, being careful not to dislodge sediment at the bottom.
5. Bubble moist air through the sample for 18 hours.

6. Pipet 5.0 ml of sample from the test tube to a 25 ml volumetric flask.
7. Add 5 ml. 5% ammonium molybdate, dilute to 25 ml with deionized water and add .5 ml 30% H_2SO_4.
8. Run on AAII as before.
9. Wash sediment remaining in centrifuge tube with 20 ml 1.0N NaCl, shake for 5 minutes, centrifuge and discard solution.

Extraction 3 - Acid Extraction

A. Reagents

1. 1.0 N Sulfuric Acid

   Same as in NaOH extraction.

2. 1.0N Sodium Hydroxide (1 l)

   40 g NaOH

   Dilute to 1 liter with deionized water.

3. Standards are prepared as for first extraction.

B. Procedure

1. Add 30 ml of 1.0N H_2SO_4 to centrifuge tube containing sediment.
2. Shake for 1 hour.
3. Centrifuge for 30 minutes at 4000 rpm and decant into standard TP bottle.
4. Since the sample is over the .22N acidity required to run on the AA, the normality must be stepped down from 1.0 N to .22N by adding an amount of 1.0N NaOH determined as follows.

Example: Pipet 30 ml of 1.111N H_2SO_4 (determined in NaOH extraction) into an appropriate container. Add 3 drops Methyl Orange indicator and titrate to end point (yellow) with 1.0N NaOH. Assume it took 29.9 ml to reach neutrality. Then:

N_A = .22N V_A = 49 ml (estimate)

N_B = 1.0 N (assume) V_B = X

So .22N x .049 l = 1.0N x X

X = 10.8 ml

Since it took 29.9 ml of 1.0N NaOH to neutralize 30 ml of the H_2SO_4, the difference of volume needed to reach neutrality and to reach .22N will be the amount of 1.0N NaOH needed to be added to each sample. 29.9 - 10.8 = 19.1 ml, so 19.1 ml of 1N NaOH must be added to each 30 ml 1.0N H_2SO_4 sample to achieve a .22N acidity.

5. Run sample on AAII following TP procedure.

Residual Digestion

A. Procedure

1. Wash out sediment remaining in the centrifuge tube into a standard TP bottle to a volume of 100 ml.
2. Digest sample as a TP and run on AAII.

SOLUBLE REACTIVE SILICA (AAII)

Scope and Application

This method is used to measure silicate in the form of SiO_2 for all samples tested. The range is .03 to 5.00 ppm and any samples running higher than 5 ppm are diluted and run again on the same scale.

Principle and Theory

Ammonium molybdate at a pH of about 1.2 reacts with both silica and phosphorus to form acids. The addition of oxalic acid eliminates the molybdophosphoric acid leaving only the molybdosilicic acid which produces a yellow color proportional in intensity to the concentration of silica. Ascorbic acid is then introduced to reduce the molybdosilicic acid to molybdenum blue which is a more intense color and increases the sensitivity of the method.

Interferences

There are no significant interferences; however, to avoid any contamination from glassware, use poly containers for samples and reagents as much as possible.

Method

A. Sample Preparation

1. Filter sample through .45 μ Sartorious membrane filter.
2. If samples cannot be run immediately, store in plastic containers at $4^{o}C$.

B. Reagents

1. Ammonium Molybdate (1 l)

   10 g ammonium molybdate

   2.8 ml concentrated H_2SO_4

   Add 2.8 ml H_2SO_4 to approximately 800 ml double distilled demineralized water. Dissolve ammonium molybdate in this and dilute to 1 liter. Store in poly container at $4^{o}C$.

2. Oxalic Acid (1 l)

   50 g oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)

   Dissolve in 900 ml double distilled deminearlized water. It may be necessary to heat slightly to get all oxalic acid in solution.

   Cool and top off to 1 l.

   Store in poly container at room temperature.

3. Ascorbic Acid (1 l)

   17.6 g ascorbic acid

   50 ml acetone

   1000 ml double distilled demineralized water

   Add acetone to 500 ml double distilled deionized water.

   Dissolve ascorbic acid in this and top off to 1 liter.

   Add 0.5 ml Levor IV.

   Store in poly container at $4^{o}C$.

C. Standards

1. Silica Standard Stock A (1000 ppm)

   3.13 g sodium silicofluoride (Na_2CO_3)

   Dilute to 1 liter with double distilled demineralized water.

   Add 1 ml chloroform

   Store in poly container at 4^oC.

2. Stock B (100 ppm) (prepare daily)

   10 ml. Stock A

   Dilute to 100 ml with deionized water

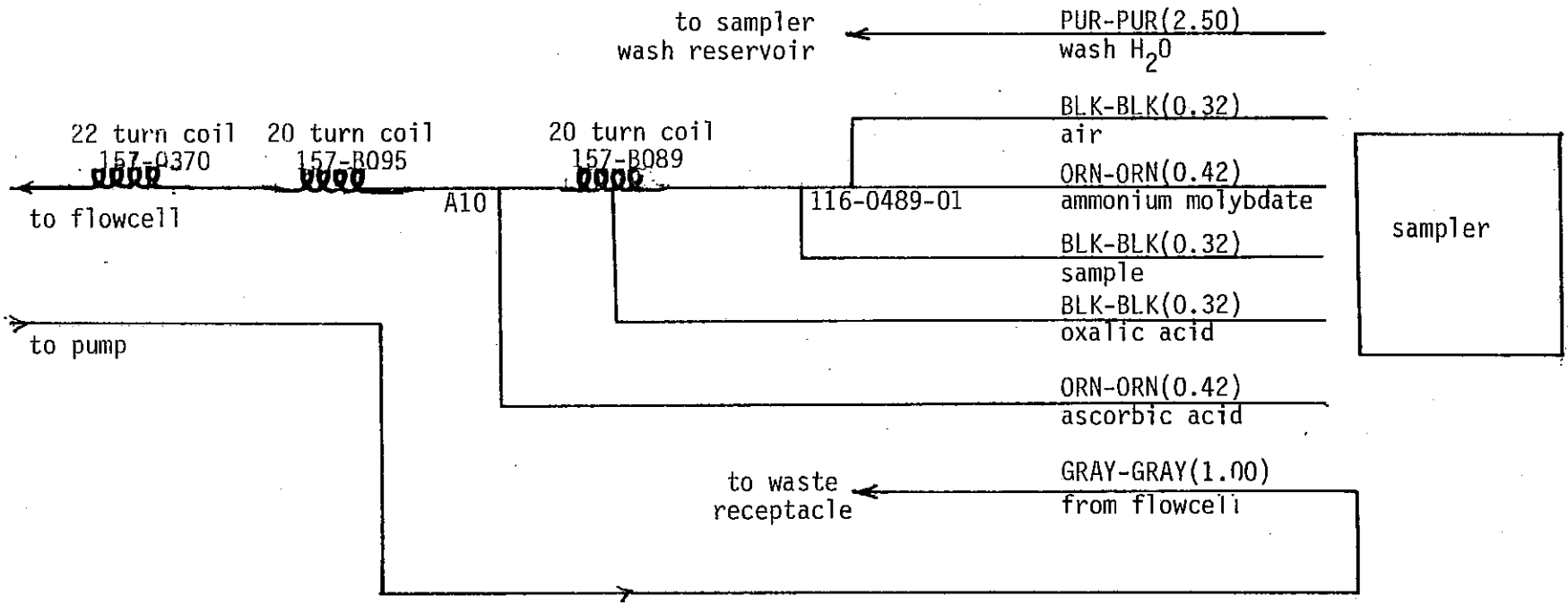
3. Working standards (prepare daily)

ml Stock B per 100 ml deionized water	Concentration
5 ml	5 ppm
3 ml	3 ppm
2 ml	2 ppm
1 ml	1 ppm
.5 ml	.5 ppm

D. Procedure

1. Set up system using manifold in Figure 8.
2. Zero and full-scale recorder.
3. Let system warm up for at least 20 minutes with reagents pumping.
4. Run a full series of standards and blanks to establish linearity, setting a mid scale standard closest to anticipated sample concentrations.
5. Set up sample tray as follows: set standard, 6 to 10 samples, another standard similar to sample range.

FIGURE 8 SILICA MANIFOLD



660nm filters
50mm x 2.0mm flowcell (199-B023-01)

E. Calculations

1. Prepare a calibration curve derived from peak heights obtained with standard solutions.
2. Determine concentration of silica in sample by comparing peak heights with calibration curve.
3. To determine silica as Si mg/l instead of SiO_2 mg/l, multiply by 0.46.

F. Precision and Accuracy

1. Run samples on a scale of 0-5 ppm with a detection limit of .03 ppm.
2. Standard calibration setting on colorimeter should be between 2.0 and 3.0.

G. References

1. Technicon industrial method 186-72W.
2. Standard Methods for the Examination of Water and Wastewater. 1976. Fourteenth Edition. American Public Health Association, Washington, D.C.
3. Lind, O.T. 1979. Handbook of Common Methods in Limnology. C.V. Mosby Company, St. Louis, Missouri. 199 p.

SULFATE (METHYLTHYMOL BLUE - AAII)

Scope and Application

This method is utilized for surface and groundwaters in the range of 0-50 ppm. Samples with higher concentrations of sulfate are analyzed by manual or machine dilutions.

Principle and Theory

The sulfate in the sample is reacted with an equimolar solution of barium chloride and methylthymol blue at a pH of 2.5 to 3.0 to form barium sulfate. After sufficient time for production of barium sulfate the pH is readjusted to between 12.5 and 13.0 and the excess barium ions complex with methylthymol blue to produce a blue color. This leaves an amount of gray uncomplexed methylthymol blue in solution which is equivalent to the quantity of sulfate removed as barium sulfate and equal to the concentration of sulfate in the sample.

Interference

Cations such as calcium, aluminum and iron can interfere by complexing with the methylthymol blue. Therefore, the sample is passed through an ion exchange column before the reagents are introduced.

Method

A. Sample Preparation

1. Filter sample through .45 μ membrane filter.
2. Store samples in a plastic container at 4°C if they are not run immediately.

B. Reagents

1. Barium Chloride Stock (1 l)

   1.526 g barium chloride

   Dilute to 1 l with deionized water.

   Store in dark poly bottle.

2. Sodium Hydroxide .18 N (1 l)

   7.2 g sodium hydroxide

   1000 ml deionized water

3. Buffer, pH 10.5 (1 l)

   6.75 g ammonium chloride

   57 ml ammonium hydroxide

   1000 ml deionized water

   Store in dark poly bottle

4. Buffered EDTA (1 l)

   40 g tetrasodium EDTA (ethylenediaminetetraacetic acid)

   1000 ml buffer, pH 10.5 (see 3 above)

   Store in dark poly bottle.

5. Methylthymol blue/Barium Chloride (500 ml)

25 ml barium chloride stock (see 1 above)

.1056 g MTB (methylthymol blue)

4 ml 1 N HCl

71 ml deionized water

Add EtOH to 500 ml

Mix MTB with barium chloride solution. Add 1 N HCl. Add water using it to rinse weighing paper used for MTB. Top off to 500 ml with EtOH.

Keep in a dark bottle and stir contents while in use.

Prepare daily.

6. Preparation of Ion Exchange Column

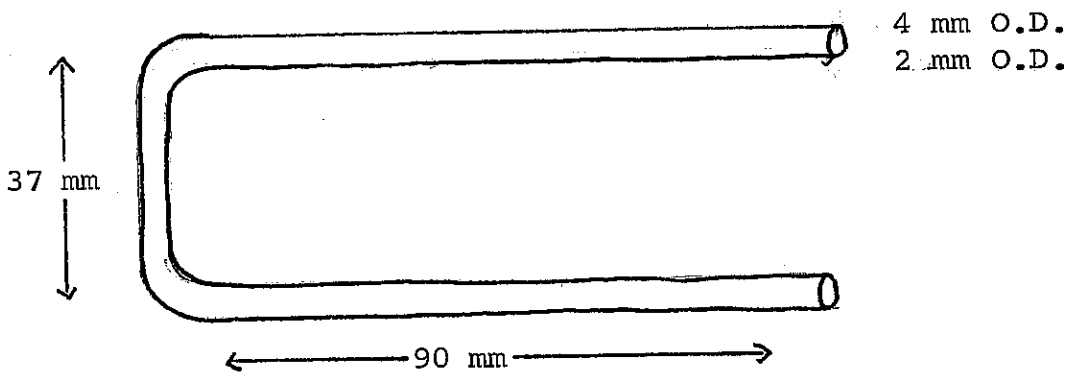
Prepare Bio Rex 70 beads by stirring in a small beaker of water and quickly pouring off small beads before settling. Repeat 3 or 4 times.

Pack glass U-shaped column underwater to avoid introducing air into the column.

Pack ends loosely with pyrex wool.

Prepare new column daily.

Specifications for column:



7. Wash Water - add 1 ml Brij and 3.5 ml Stock standard to each liter of wash water. The stock is added to compensate for the fact that this method is not linear at low levels.

C. Standards

1. Sulfate Standard Stock A (1000 ppm)

   1.4790 g sodium sulfate (Na_2SO_4)

   Dilute to 1 l with deionized water

2. Working Standards

ml Stock A to 100 ml deionized water	Concentration
1 ml	10 ppm
2 ml	20
3 ml	30
4 ml	40
5 ml	50

D. Procedure

1. Set up manifold as seen in Figure 9.
2. Zero and full scale recorder.
3. Allow system to warm up for at least 20 minutes with reagents pumping.
4. Run a full series of standards and blanks to establish linearity, setting a mid scale standard close to the anticipated sample concentrations.

5. Set up sample tray as follows: set standard, samples, a different standard close to average concentration of sulfates in the sample.
6. When shutting down the system or in the middle of a long day place NaOH and MTB/$BaCl_2$ lines in water for about 5 minutes. Then place the line in EDTA until the purple residue has been removed from glass coils. Rinse with water for 5 minutes and place lines back in reagents or rinse 15 minutes and shut down.

E. Calculations

1. Prepare calibration curve derived from peak heights obtained with the standards.
2. Determine sulfate concentrations by comparing peak heights with calibration curve.

F. Precision and Accuracy

1. This method is run on a scale of .2 to 50 ppm with a detection limit of 0.2 ppm.

References

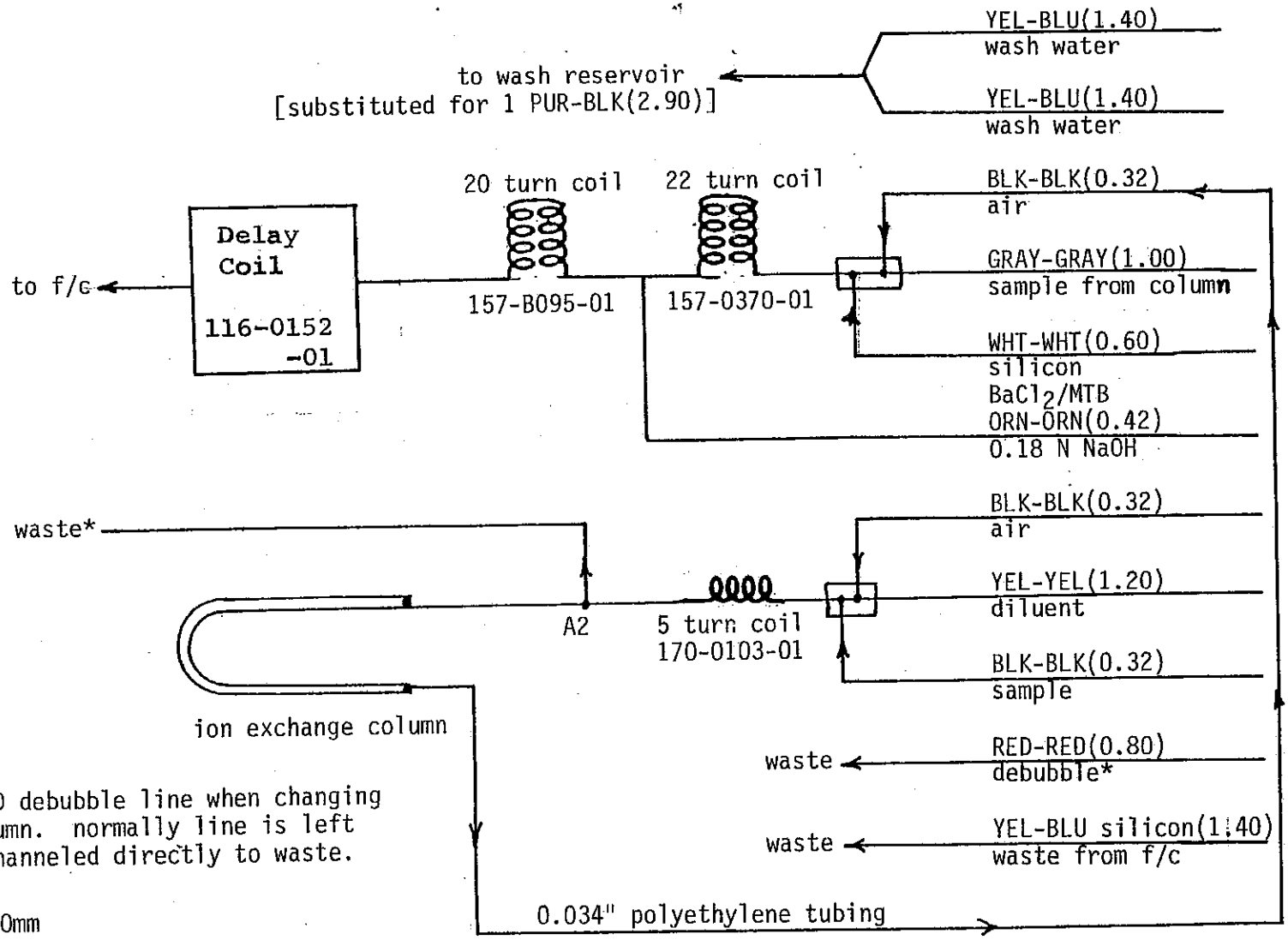
1. Technicon Instruments Corporation. 1971. Technicon AutoAnalyzer II Industrial Method No. 118-71W. Tarrytown, New York.
2. Canada Centre for Inland Waters. 1979. Analytical Methods Manual. NAQUADAT No. 16306.
3. American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. p. 628-630.

4. EPA Methods Manual. Method 375.2 Storet No. 00945.

5. Adamski, J.M. and S.P. Villard. 1975. Application of the methylthymol blue sulfate method to water and wastewater analysis. Analytical Chemistry 47(7):1191-1194.

6. Colovas, G., M.R. Panesar and E.P. Parry. 1976. Linearizing the calibration curve in determination of sulfate by the methylthymol blue method. Analytical Chemistry 48(12):1693-1696.

FIGURE 9 SULFATE MANIFOLD



*attach to RED-RED debubble line when changing ion exchange column. normally line is left unattached and channeled directly to waste.

460nm filters
flowcell 15mm x 2.0mm

PART 4 - ORGANIC CONSTITUENTS IN WATER

DISSOLVED ORGANIC CARBON (AAII)

Scope and Applications

This automated method can be used for determining dissolved organic carbon in the range of .2 to 10 mg/l C.

Principle and Theory

This method closely follows Technicon Industrial Method No. 451-76W. The determination of organic carbon requires the removal of inorganic carbon present in the sample as carbonate. The aspirated sample is mixed with an acid diluent and segmented with CO_2-free air. A portion of the CO_2 generated is removed at the first C3 debubbler. The acidified stream is entrained with a high velocity stream of nitrogen air (750 cc/min.). The sample is transformed into a thin turbulent liquid film which is transported rapidly through a large bore coil, providing the necessary surface area for complete CO_2 removal. An aliquot of the carbonate-free sample is then segmented, mixed with acid and potassium persulfate and subjected to UV radiation. The organic compounds present are completely digested and converted to CO_2. A wetting agent is then added to improve flow hydraulics and insure subsequent smooth flow through the gas dialyzer. The sample stream then passes through a 6-inch dialyzer containing a gas-permeable silicone rubber membrane. The CO_2 in the sample stream diffuses into a weakly buffered phenolphthalein indicator solution. The decrease in absorbence of the phenolphthalein solution is a measure of the carbon originally present in the sample.

Method

A. Sample Preparation

1. After the sample is collected, it is filtered through a .45 u membrane filter and stored at $4^{o}C$ until analysis.

B. Reagents

1. Acid Diluent (1 l)

   28 ml sulfuric acid (H_2SO_4)

   Add to 800 ml deionized water and dilute to 1 liter.

2. Triton (1:1 in isopropyl alcohol)

   50 ml Triton X100

   50 ml isopropyl alcohol

3. Triton X-100 solution (wetting agent)

   10 ml Triton (1:1 in isopropyl alcohol)

   Dilute to 1 l with deionized water

4. Sodium Carbonate (.1 M)

   10.6 g sodium carbonate

   Dissolve and dilute to 1 l with deionized water

5. Sodium Bicarbonate (.1 M)

   8.4 g sodium bicarbonate

   Dissolve and dilute to 1 l with deionized water.

6. Carbonate - Bicarbonate buffer

   50 ml sodium carbonate .1 M (#3)

   100 ml sodium bicarbonate .1M (#4)

Method

A. Sample Preparation

1. After the sample is collected, it is filtered through a .45 u membrane filter and stored at $4^{o}C$ until analysis.

B. Reagents

1. Acid Diluent (1 l)

   28 ml sulfuric acid (H_2SO_4)

   Add to 800 ml deionized water and dilute to 1 liter.

2. Triton (1:1 in isopropyl alcohol)

   50 ml Triton X100

   50 ml isopropyl alcohol

3. Triton X-100 solution (wetting agent)

   10 ml Triton (1:1 in isopropyl alcohol)

   Dilute to 1 l with deionized water

4. Sodium Carbonate (.1 M)

   10.6 g sodium carbonate

   Dissolve and dilute to 1 l with deionized water

5. Sodium Bicarbonate (.1 M)

   8.4 g sodium bicarbonate

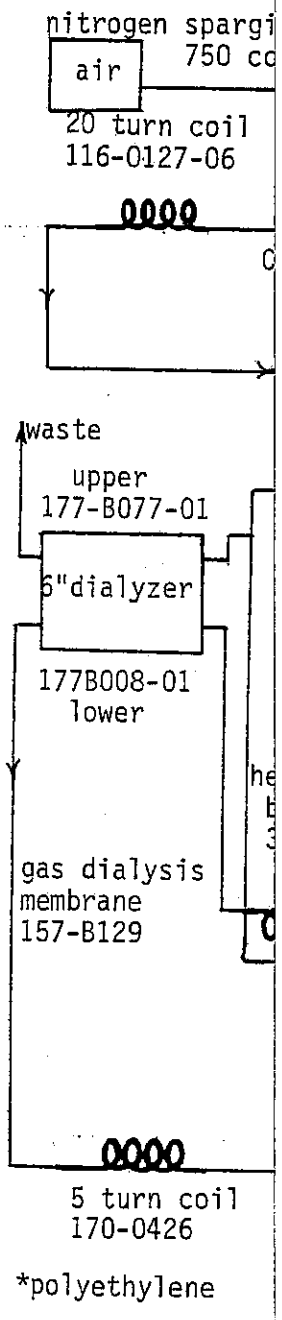
   Dissolve and dilute to 1 l with deionized water.

6. Carbonate - Bicarbonate buffer

   50 ml sodium carbonate .1 M (#3)

   100 ml sodium bicarbonate .1M (#4)

FIGURE



2. Ehrh
diss
3. Goul
diss
47(1

7. Phenolphthalein, 1%

   1.0 g. phenolphthalein

   Dissolve in 80 ml methanol and dilute to 100 ml with methanol.

8. Persulfate 4%

   40 g potassium persulfate

   Dissolve and dilute to 1 l with deionized water.

9. Color Reagent

   .6 ml phenolphthalein (1%)

   10.0 ml carbonate-bicarbonate buffer

   1000 ml deionized water

   .5 ml Triton X-100 (1:1 in isopropyl alcohol)

   Pipet .6 ml phenolphthalein into a 1 l volumetric flask. Add 800 ml. deionized water and mix. Pipet 10 ml carbonate-bicarbonate buffer and mix. Dilute to 1 l with deionized water and add .5 ml Triton X-100 (1:1 in isopropyl alcohol).

C. Standards

1. Potassium Biphthalate Stock A Standard (100 ppm)

   .2125 g potassium biphthalate

   Dissolve in 800 ml deionized water

   5 ml 1 N H_2SO_4

   Dilute to 1 l with deionized water

CHLOROPHYLL

Scope and Application

This method is used for analyzing water samples containing the various forms of chlorophyll.

Principle and Theory

Chlorophyll is measured as an estimate of the phytoplankton standing crop and primary productivity, thus providing an indication of the level of eutrophication. Chlorophyll a, b, c, pheophytin and chlorophyll a, corrected for pheophytin are measured by spectrophotometric analysis. Although all forms are measured, only corrected chlorophyll a (active chlorophyll) and pheophytin (inactive chlorophyll) are significant for our purposes.

Samples are concentrated by filtering through a 1.0 μ glass fiber filter pad. The pad is ground in 90% acetone which extracts the chlorophyll present in the algal cells. The detritus and pad fibers are centrifuged out. The concentrations of chlorophyll present are measured on the spectrophotometer by comparing the light absorbance between a blank (90% acetone) and the sample solution (in 90% acetone). These absorbances are sent to the computer which calculates the concentrations of chlorophyll present. Pheophytin is determined by acidifying the sample and rerunning as before.

Method

A. Field Sampling

1. Filter enough water through a Whatman GF/C glass fiber filter to obtain a dense color on the pad.
2. When only 50 ml of sample remains to be filtered, add 3 drops of $MgCO_3$ (magnesium carbonate suspension). This serves as a buffer to help prevent acid degradation of the sample.
3. Fold pad in quarters with Millipore tweezers, place in labeled petri dish and freeze immediately. At the end of the day, wrap pack in aluminum foil, label and return to freezer.

B. Reagents

1. Magnesium carbonate (used as preservative in the field)

   1 g $MgCo_3$

   100 ml distilled water
2. 90% Acetone

   100 ml distilled water

   900 ml acetone
3. 1 N HCl

   8.3 ml concentrated HCl

   Add to 75 ml distilled water and dilute to 100 ml

Preparation of Sample for Spec Analysis

A. Grinding

1. Fold pad and place in grinding vessel. If pad is coated with ice, thaw, fold and blot on a paper towel.
2. Transfer station number, date, depth and amount filtered to chlorophyll data sheets.
3. Add 90% acetone to a depth of one to two centimeters (approximately 4 mls).
4. Grind pad for a maximum of one minute, avoiding heating up the liquid. Sample should look milky when grinding is complete.
5. Pour sample into graduated centrifuge tube, rinsing vessel and pestle with 90% acetone and adding each rinse to centrifuge tube. After the final rinse, fill tube to 12 ml with 90% acetone. Record number of centrifuge tube next to corresponding station number on data sheet. Record amount of extraction solution (should be 12 ml).
6. Cap centrifuge tube and place in freezer for 4 to 24 hours. Samples should be kept in the dark as much as possible.

B. Centrifuging

1. Replace glass stoppers with yellow caps (or appropriate lids).
2. Place rubber pads on bottom of centrifuge tubes and load centrifuge, making sure it's balanced.
3. Centrifuge for 10 minutes at 3000 rpms (setting 6½ on MSE).
4. Remove each tube, shake to dislodge fibers along walls and centrifuge for 10 more minutes. Hold in a dark place until analysis.

C. Transferring to sample cuvette

1. Rinse cuvette with distilled water and then with 100% acetone. Let air dry.
2. Fill cuvette almost completely full and avoid getting bubbles in the light path. Wipe the ends with lens paper.
3. Reference cuvette should be filled with 90% acetone.
4. Place cuvettes in spec with numbers facing forward and left ends against the holder. It is important that the cuvettes are consistently used as either the reference or the sampling cell.

D. Operating Spectrophotometer and Wavelength Programmer

1. Allow wavelength programmer and spec to warm up for 24 hours.
2. Set sample time at a minimum of 12 seconds.
3. Set wavelengths at 750, 663, 645 and 630 nm.
4. Zero spec and find line loss (calculated by computer).
5. Turn on chart recorder
6. Start computer and run through program as outlined in Table 4. Select Spec 1.
7. After Spec 1 exits, acidify sample with 2 drops of 1 N HCl, agitate gently, let set for 3 minutes and start Spec 2.
8. The computer program compares Spec 1 and Spec 2 values to determine absorbances at the set wavelengths and calculates chlorophyll a, b and c concentrations using SCOR/UNESCO (1966), and corrected chlorophyll a and pheophytin after Lorenzen (1967).

References

1. Fay, L.A. 1976. An investigation of corrected chlorophyll a and pheophytin a in Lake Erie's western and central basins. MS Thesis, The Ohio State University, Columbus. 177 p.

2. Lorenzen, C.J. 1967. Determination of chlorophyll and pheopigments spectrophotometric equations. Limnol. Oceanogr. 12:343-346.

3. SCOR/UNESCO. 1966. Monograph on oceanographic methodology. I. Determination of photosynthetic pigments in sea water. Paris. 69 p.

TABLE 4

INSTRUCTIONS FOR RUNNING THE CHLOROPHYLL ANALYSIS PROGRAM

If nobody is logged on:

1. Type HELLO 100,1 on terminal
2. Type the password CHL
3. Type CHL. The program should now be running.
4. When the program stops, type BYE

To start the program (if someone else is logged in and the program is not running)

1. Type CHL. The program should now be running.

To log off terminal

1. Type correct code from program on terminal and program will automatically stop.

KJELDAHL NITROGEN (AAII)

Scope and Application

This automated method for the determination of total Kjeldahl nitrogen (TKN) was developed for use in analyzing low levels found in the waters of the Great Lakes. It is used for the range of 0-500 or 0-1000 µg N/l.

Principle and Theory

Total Kjeldahl nitrogen includes ammonia and organic nitrogen but not nitrite or nitrate. The organic nitrogen is digested in a continuous helix digester with sulfuric acid and hydrogen peroxide through a temperature gradient of 300°C to 180°C. The sample is cooled and mixed with sodium salicylate and dichloroisocyanurate to effect a color reaction at an alkaline pH. The optimum pH for greatest color intensity is 12.5 which is achieved by the addition of NaOH.

Method

A. Sample Preparation

1. A whole water, unfiltered sample is used. Samples are preserved by adding 2 ml 11 N H_2SO_4 per 100 ml sample.

B. Reagents

1. Sodium Salicylate (500 ml)

   100 g sodium salicylate

   Dissolve in 300 ml deionized water

   Add 0.3 g sodium nitroferricyanide (nitroprusside)

   Dilute to 500 ml with deionized water

2. Sodium Dichloroisocyanurate (500 ml)

   25 g sodium hydroxide

   Dissolve in 300 ml deionized water and cool

   Add 3.5 g cyanurate

   Dilute to 500 ml with deionized water.

   Make fresh daily.

3. 30% Sodium Hydroxide (1 l)

   300 g sodium hydroxide

   Mix with 500 ml deionized water, cool and dilute to 1 l.

4. 10% Hydrogen Peroxide (100 ml)

   10 ml hydrogen peroxide

   Dilute to 100 ml with deionized water.

5. Concentrated Sulfuric Acid

6. 11 N Sulfuric Acid (1 l)

   Slowly add 310 ml sulfuric acid to 500 ml deionized water.

   Cool and dilute to 1 l.

7. Dilution/Wash Water

   Add 40 ml 11 N H_2SO_4 to 2 l deionized water.

C. Standards

1. Stock A Standard (1000 ppm)

   4.7619 g ammonium sulfate ($(NH_4)_2\ SO_4$)

   Dilute to 1 l with deionized water.

2. Stock B (10 ppm)

   Dilute 10 ml of Stock A to 1 l with deionized water.

3. Working Standards

ml of Stock B per 500 ml deionized water	Concentration ppb
5 ml	100 ppb
10	200
20	400
25	500
40	800
50	1000

Add 2 ml 11 N H_2SO_4 to each 100 ml of standard to match the 2 ml 11 N H_2SO_4 added to the samples as a preservative.

D. Procedure

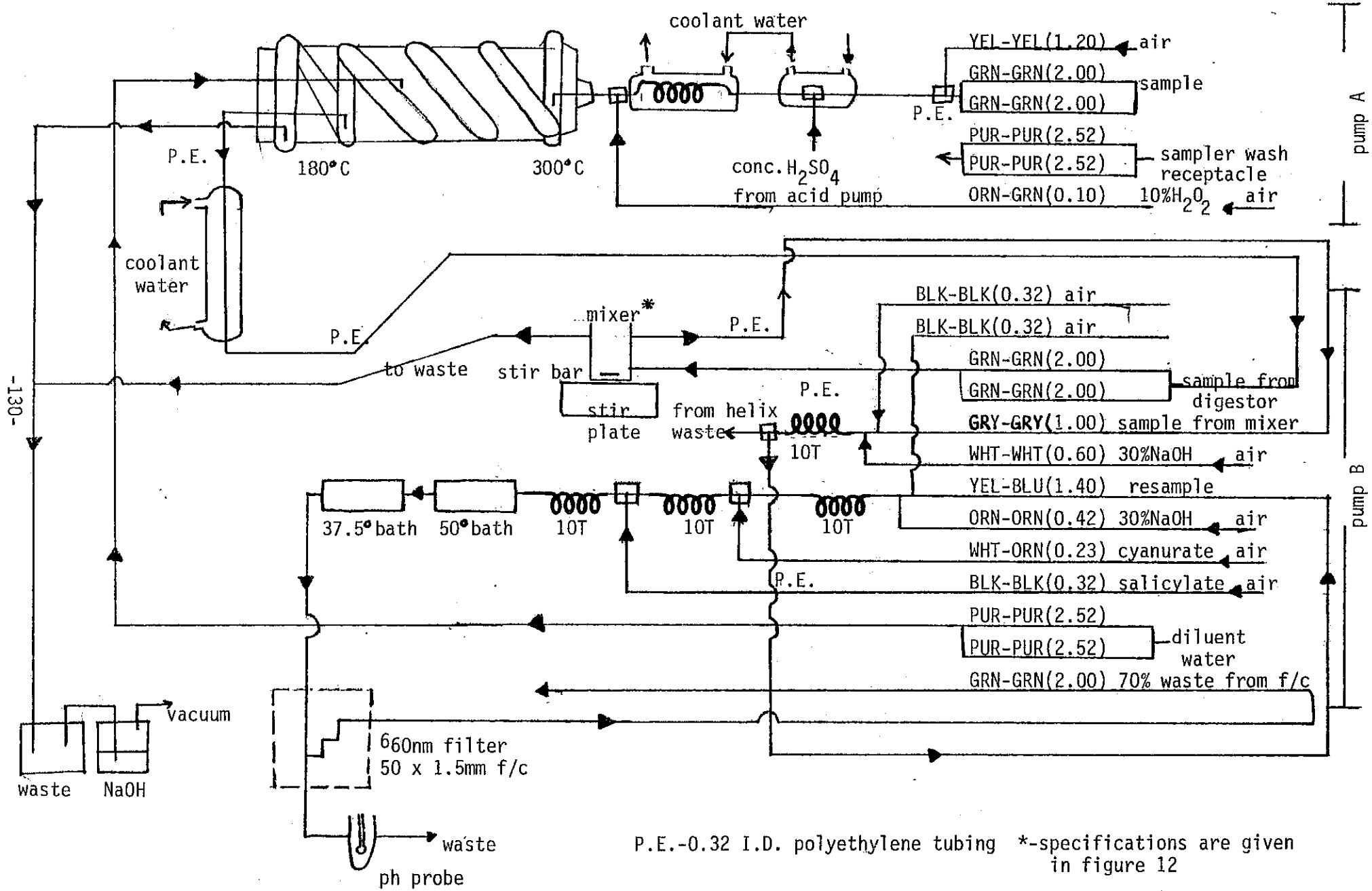
1. Set up system as shown in Figure 11, being careful to follow all instructions as closely as possible.

2. Start components in the following order
   a. vacuum pump
   b. continuous digestor motor
   c. proportioning pumps
   d. FMI fluid metering pump connected to H_2SO_4
   e. helix heating elements
   f. stir plate under mixing chamber
3. When helix temperatures have stabilized place sodium hydroxide, cyanurate and hydrogen peroxide reagents on line.
4. After the pH of the system has stabilized at 12.5, put salicylate on. If salicylate is introduced under non-alkaline conditions salicylic acid will precipitate, clogging the lines.
5. Run standards to establish linearity and run samples.
6. Shutdown system in the following order
   a. Remove salicylate and turn off heat
   b. When salicylate is washed out (indicated by a drop in the baseline) remove the rest of the reagents
   c. Disconnect acid line and place in water when helix temperature falls below $100^{o}C$.
   d. Wash out system until pH drops and then wash for an additional 15 minutes.
   e. Turn off acid pump and proportioning pumps. Remove plunger from acid pump.
   f. When most of water has drained from helix, turn off helix, vacuum pump, stirrer, cooling water and the remainder of the system.

References

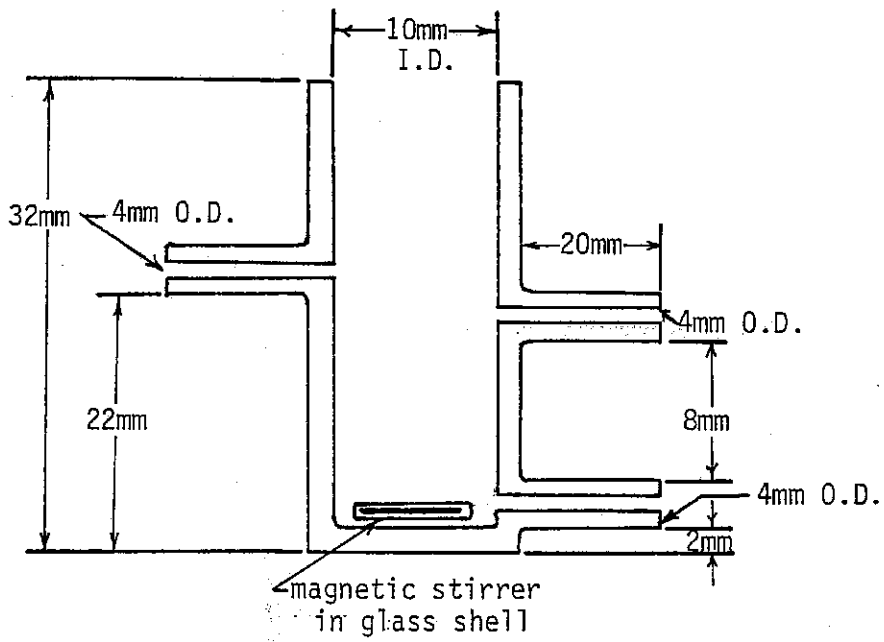
1. El Kei, O. 19 . An automated method for the determination of low level Kjeldahl nitrogen. Analytica chimica acta.

FIGURE II TOTAL KJELDAHL NITROGEN MANIFOLD



P.E.-0.32 I.D. polyethylene tubing *-specifications are given in figure 12

FIGURE 12 SPECIFICATIONS FOR MIXING CHAMBER



PARTICULATE ORGANIC CARBON AND NITROGEN

Scope and Application

This automated method is used for natural water samples containing organic carbon and nitrogen in the range of 1 to 1000 mg/l.

Principle and Theory

The Perkin Elmer Model 240 Elemental Analyzer determines the particulate organic carbon and nitrogen of samples by detecting and measuring their combustion products (CO_2 and N). Hydrogen content is also measured as H_2O. The sample is combusted in an atmosphere of pure oxygen and a reduction furnace reduces any oxides of nitrogen present to nitrogen. The combustion products are then mixed with inert helium which carries the gasses to the point of detection.

Field Sampling

A. Preparation of filter pads

1. Heat Whatman GF/F* glass fiber filters (.45 µ) for 20 minutes at 550°C in a muffle furnace.
2. Place in tight sealing petri dishes.

*Use of Gelman Micro-Quartz glass fiber filters (1 µ) have been found to be more advantageous and will be used after 1982.

B. Field sampling

1. Filter as much water as possible through prepared GF/F pad, using specially designed CN filter head.
2. Allow filter to suck dry and rinse with 10 ml of 1 N HCl* followed by several small portions of deionized water.
   *(83 ml HCl to 1 l distilled water)
3. Place in petri dish labelled on the bottom with station number, Julian date, depth and amount filtered.
4. Desiccate at 25 psi for at least 24 hours in open petri dishes.
5. Sort and ship to Columbus for analysis.
6. Store in desiccator.

Analysis of Samples on Perkin-Elmer 240 Elemental Analyzer

A. Set up and condition machine according to procedures outlined in instrument manual. Any troubleshooting hints are also in manual.

B. Daily Operation

1. Check temperatures
   a. Combustion temperature should be 830°C.
   b. Reduction temperature should be 650°C.
2. Zero chart recorder
   a. Set input switch on recorder to short.
   b. Adjust zero to 0 chart divisions on scale.
   c. Return input switch to source.

3. Condition system by running 1-2 mg of acetanilide in platinum boat and standard ladle through cycle. When conditioning, set each element zero to ten using fine adjustment. If necessary, readjust coarse adjustment located inside the machine.

4. Calibration

   a. Weigh between 1000 and 2000 ug acetanilide out in platinum boat on autobalance. Run through cycle in standard ladle. Record weight, signals and zeroes on calibration data sheet. When sample read comes through, set attenuation switch to the lowest point where concentration can be read.

   b. Run a blank (Standard ladle and boat without acetanilide)

   c. Run a second standard using a weight of acetanilide close to that of the first standard

   d. Run a second blank

   e. Run a third standard

   f. Run a third blank

   g. Average three blanks to obtain value used for blank on calibration sheets

   h. Average sensitivities for each element and calculate standard deviations. Deviations must fall within the following limits:

      N ± 0.30

      C ± 0.10

      H ± 3.58

   i. Run three blanks using sample ladle, average and use this value as blank on sample sheet

5. Running samples

   a. Trim filter pads so only portion containing sample remains.

   b. Fold pad and place in sample ladle.

   c. Run through cycle.

   d. Record signals on sample data sheet and perform calculations indicated to obtain amounts of C and N present in µg/l.

Guidelines for changing traps and columns.*

A. Combustion Column - change after ∿ 350 samples

B. Reduction Column - change after ∿ 90 samples

C. Traps and Scrubbers - change when colorcarb turns from white to blue

*Follow instructions in instrument manual for filling and changing.

Use of Autobalance

1. Allow balance to warm up for at least 1 hour.
2. Place a 200 mg weight on right-hand pan. This weight will most often be left on the pan so this step can be skipped.
3. Set calibration to 1.
4. Place clean platinum boat on left hand pan and set to zero with coarse and fine adjustment.
5. Remove platinum boat, place acetanilide in boat, and replace boat in balance. Record weight in ug.
6. Rezero before each use.

PART 5 - BIOLOGICAL COMPONENTS

BENTHOS

Field Sampling Procedure

1. Obtain sample using a Ponar grab sampler.

2. Sieve sample through 30 USGS standard sieve screen (.91 mm opening).

3. Wash retained material into plastic jar and preserve with 10% formalin.

Sorting Procedure

A solution of Rose Bengal and ethyl alcohol is added to each sample to facilitate separation of benthos from sediment. Stain for at least 24 hours.

Benthos Removal

The benthos is removed from each sample in its entirety. This insures that all species and organisms are accounted for, and does not skew the results in favor of dominant species. Each sample is rinsed in a sieve and then poured into a white enamel pan. The substrate is flushed with water several times and the water poured into a fine mesh sieve (40 mesh). This process is repeated several times until visual observation indicates the absence of organisms in the substrate. A careful check of the substrate randomly has insured a recovery of 95-100% of benthos in each sample. This method has proved efficient for the smaller benthos which constitutes the major proportion of the

organisms. The sieve is rinsed into a clean enamel pan and the benthos placed in numbered vials of 70% ethanol for later identification.

Identification

The contents of each vial are placed into a plastic petri dish for gross "identification" under 15-30X using a dissecting microscope. This stage of identification is used to separate immature from mature oligochaetes and for identification of larger benthos (i.e., amphipods, oligochaetes, leeches, etc.). Representative specimens of chironomid genera are wet-mounted for examination of the head capsule. This is the recommended method where large numbers of midges are to be identified. Clearing of head capsules is not usually necessary for identification to the general level because of morphological features specific to each of the existing genera. At this stage any unidentified species are retained for further examination and the rest are stored.

Adult oligochaetes are placed in an ascending alcohol series to prepare them for permanent slides. The procedure is 70% ETOH (24 hours), 95% ETOH (2 hours), 100% ETOH (2 hours), Xylene (12 hours) to fix the specimens. Oligochaetes are finally mounted in Permount. Each slide is labeled with station number and allowed to dry 48 hours. Identification is made with a compound microscope with special attention given to the setae size and shape along with size and shape of penis sheath and head.

References

1. Brinkhurst, R.O. and B.G.M. Jamieson. 1971. Aquatic Oligochaeta of the World. Toronto, University of Toronto Press. 860 p.

2. Pennack, R.W. 1953. Fresh Water Invertebrates of the United States. The Ronald Press Company, New York, New York. 769 p.

3. Ward, H.B. and G.C. Whipple. 1959. Freshwater Biology. Second Edition. John Wiley and Sons, Inc.

PHYTOPLANKTON METHODS

Field Sampling

Use flat 300 ml Monsanto bottles. Label bottles with station, Julian date and depth. Obtain whole water samples with Niskin bottle. Fill Monsanto bottle with sample water, add 3 ml Lugol's solution.*

Sample Transfer

Label 40 ml glass vials with station, Julian date and depth. Transfer contents of Monsanto bottle into a beaker, add 10 ml $MgCO_3$ buffered formalin and 3 drops of Lugol's solution. Pour water back and forth between two beakers 10 times until well-mixed. Pour sample into phytoplankton vial until it is completely filled, discard remainder of sample. Cap vial tightly with poly-seal closure-type cap.

Counting

Settle water 24 hours. Count using inverted microscope until between 15 and 20 mm^2 of the chamber have been covered or until at least 300 individuals have been observed.

*Lugol's Solution:

300 g potassium iodide (KI)

600 ml distilled water

150 g iodide (I_2)

1500 ml distilled water

150 g sodium acetate

ZOOPLANKTON METHODS

Field Sampling

From R.V. Hydra: a half meter, 64 u mesh net is used. Meters inside and outside the net are set to zero. Net is lowered to within one meter of the bottom, then raised at a constant speed with the winch. Rinse plankton into collecting jar with hose. Record meter readings. Add approximately 4 oz. (40 ml) carbonated water. After 5 minutes add 10 ml $MgCO_3$ buffered formalin to which 40 g/liter of sucrose has been added.

From small vessel: a half meter, 64 u mesh net is used. Lower top frame of net to just above the bottom, taking care not to disturb the sediments, (reset meters before lowering net). Raise net completely out of the water, let plankton run down into the collecting jar. It may be necessary to immerse the net two or three times to get most of the plankton down. Rinse the last of the plankton into the jar with lake water in a large squirt bottle. Add carbonated water, buffered formalin as on large vessel. Note: Label all bottles carefully, including station, Julian date and meter readings.

Sample transfer:

Allow plankton to settle to the bottom of the collecting jars. Use suction flask to remove most of the water above the settled plankton. Transfer the settled plankton into labeled RX bottles, add 5 ml $MgCO_3$ buffered formalin, bring up to 100 ml with distilled water. Retain for further analysis.

Counting

Shake sample well and sample with a large-bore pipette. Place sub-sample into plankton counting wheel and count at 50X under Wild M5 dissecting microscope.

Count at least 200 adult cladocerans and copepods, ignoring all rotifers and immature crustaceans. Using a second (usually more dilute) sub-sample, count at least 200 rotifers and immature crustaceans. Return all sub-sampled material to the original container after all counts have been made.

APPENDIX A

STORET CODES

PARAMETER	STORET CODE
Alkalinity	
Total	00410
Phenolphthalein	00415
Ammonia	00608
Carbon, Particulate Organic	80102
Carbon, Dissolved Organic	00684
Chloride	82295
Chlorophyll a, corrected	32211
Extinction Depth	00204
Kjeldahl Nitrogen	00625
Nitrate + Nitrite	00631
Nitrite	00613
Nitrogen, Particulate Organic	
Oxygen	
Electrode	00299
Winkler	00300
% Saturation	00301
pH	00400
Pheopigment	32218
Phosphorus	
Soluble Reactive	
Total Filtered	00666
Total	00665
Particulate	
Plankton, Biomass	00570
Silica, Soluble Reactive	00955
Specific Conductance, corrected	00095
Sulfate	00946
Suspended Solids	
Total	00530
Volatile	00535
Temperature	00010
Transmission	00074
Transparency	00078
Turbidity	00076

3. For digestion, add 0.8 g of ammonium persulfate and heat for 30 minutes in an autoclave at 121 C (15-20 psi). Cool and dilute samples to 30 ml. Adjust pH to 7.0 + 0.2 with 1 N NaOH using a pH meter. Dilute to 50 mls.

B. Reagents

1. Sulfuric Acid solution - 5 N.

   70 ml of concentrated H_2SO_4

   Add 430 ml of distilled water

2. Antimony potassium tartrate solution

   1.3715 g $K(SbO)C_4H_4O_6$ $1/2H_2O$

   Dissolve in 400 ml distilled water

   Dilute to 500 ml

   Store at 4 C in a dark, glass stoppered bottle

3. Ammonium molybdate solution

   20 g $(NH_4)_6Mo_7O_{24}$ $4H_2O$

   Dissolve in 500 ml of distilled water

   Store in plastic bottle at 4 C

4. Ascorbic Acid

   1.76 g Ascorbic Acid

   Dissolve in 100 ml of distilled water

   Stable only for 1 week at 4 C

5. Combined reagent

   50 ml of 5 N H_2SO_4

   5 ml of antimony potassium tartrate

   15 ml of ammonium molybdate

   30 ml of ascorbic acid

   Add each of the above reagents in the order given

   Mixing after each reagent

   Prepare daily

6. Sulfuric acid solution - 11 N

   Add 310 ml concentrated H_2SO_4

   To 600 ml distilled water

   Dilute to 1 l when cool

7. Ammonium persulfate

C. Standards

1. Phosphorus Standard Stock (100 ppm)

   0.4394 g potassium phosphate (KH_2PO_4)

   Dilute to 1 l with deionized water

   1 ml chloroform

   Store in glass container at 4 C

2. Stock B (1 ppm)

   10 ml Stock A

   Dilute to 1 l with deionized water

3. Working Standards

ml Stock B to 500 ml deionized water	Concentration
100 ml	200 ppb
75 ml	150 ppb
50 ml	100 ppb
25 ml	50 ppb
15 ml	30 ppb
10 ml	20 ppb
5 ml	10 ppb

Add 11 N H_2SO_4 to each standard in the ratio of 2 ml acid per 100 ml standard to maintain the same pH as the samples.

D. Procedure

1. Set up system using manifold as seen in Figure 13.
2. Zero and full-scale recorder.
3. Let system warm up for at least 20 minutes with reagents pumping.
4. Select a series of standards for the range desired.
5. Run a series of standards and blanks covering the entire scale to establish linearity, setting the standard running the closest to the anticipated sample concentrations.
6. Run samples between the set standard and an additional standard within the sample range.

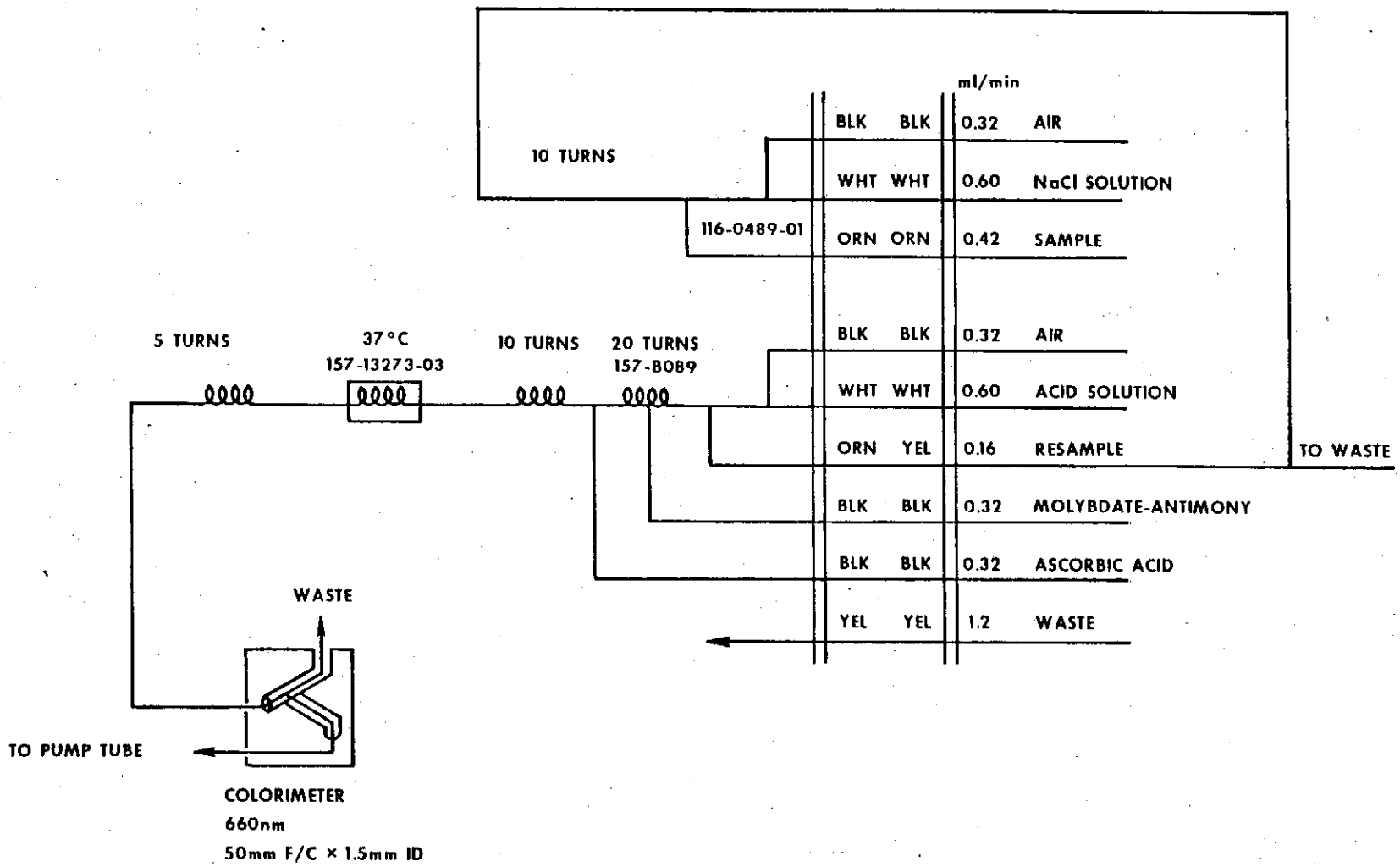


Figure 13. Phosphorus Manifold (Ascorbic Acid)