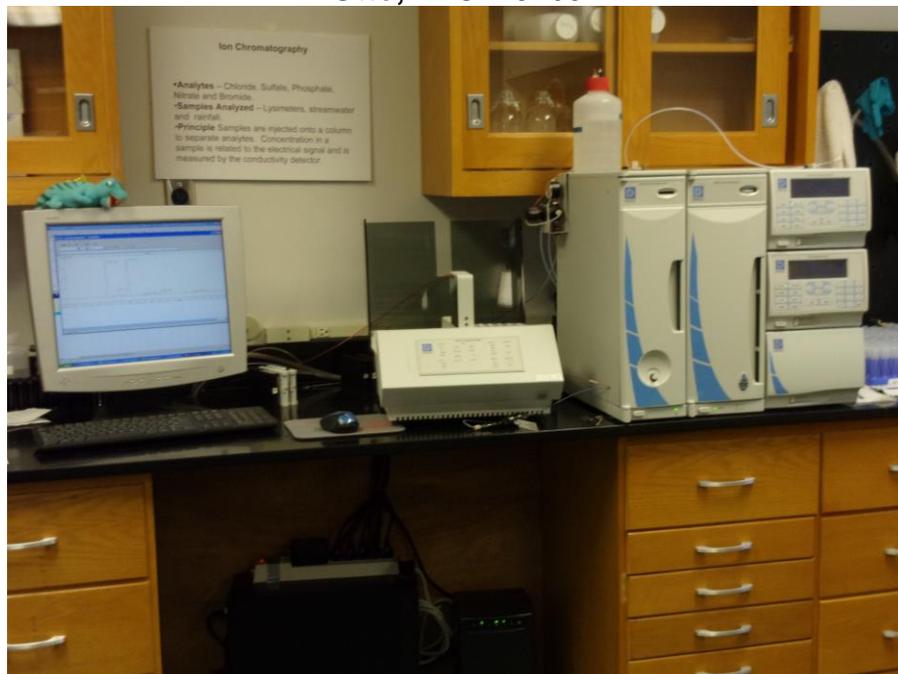


PROCEDURES FOR CHEMICAL ANALYSIS

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FOREWORD

This manual represents the combined efforts of many persons, past and present. The Coweeta chemistry laboratory has evolved since the early 1970's into a first class analytical facility designed to support a wide range of ecological research. I think it is important to mention some people who have contributed to the methods development and long term data sets.

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This manual does not include the Coweeta Chemical Hygiene and Safety Plan. Prior to working in the chemistry lab all personnel will receive safety training and will get approval from the lab manager before starting any procedure.

Cindi Brown, Laboratory Manager and Chemist
Coweeta Hydrologic Laboratory
November 18, 2013

INTRODUCTION

Coweeta Hydrologic Laboratory was established in 1934 to study watershed ecosystem responses to natural, management, and other human disturbances of southeastern forest. Samples from the Coweeta 5,400 acre experimental forest consist of weekly streamflow grab samples, bulk and dryfall precipitation, soil solutions, soil and plant samples, and throughfall. Analyses are performed primarily with seven analytical instruments: an Astoria 2 autoanalyzer, a Perkin-Elmer AAnalyst 300 atomic absorption spectrophotometer (cations), a Thermo Scientific iCAP9300 inductively couple plasma spectrometer, a Dionex ICS 4000 ion chromatograph (anions), a Flash EA 1112 series NC Soil Analyzer, an Orion pH meter, and a Shimadzu TOC-Vcph/TNM-1 DOC/TN analyzer. The following sections discuss laboratory techniques used to process field samples and to determine mineral and nutrient concentrations. Details of field methods will be omitted, except when necessary to identify samples or to clarify a laboratory process. For field collection protocols see 'Field Technician Manual' (<http://coweeta.ecology.uga.edu/webdocs/1/pdf/FieldTechnicianManual.pdf>) or study plan.

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SAMPLE COLLECTION

Samples collected for analysis at Coweeta include a wide variety of water, soil, and plant tissue. Sample collection protocols are specified in the experimental study plan. All samples must be clearly labeled with study name, identifying sample number, and date of collection. All samples entering the lab should be added to the Analytical sample log file, with all sample label information as well as sample type, processing and analyses required.

Water samples are normally collected in clean 50ml vials or 250 ml polypropylene bottles. Samples are labeled and refrigerated at 4°C for immediate analysis or frozen at a temperature of -18 °C for subsequent analysis. Water samples for DOC analysis are collected in 40ml muffled glass vials. Precipitation samples are collected in 2 liter polypropylene bottles that have been treated with 1ml of a 1000 mg/l phenol mercuric acetate solution. This preservative has proven to be effective in retarding biological activity which could change the concentration of certain nutrients. Wetfall/Dryfall samplers use a 14 liter polyethylene bucket. The samples collected in buckets are emptied into clean labeled 500ml polypropylene bottles and refrigerated prior to analysis or frozen for subsequent analysis. Soil solutions collected using tension Lysimeters are composited monthly by freezing a subsample on collection day; composites remain frozen until ready to analyze.

Soil samples are generally collected in plastic ziplock bags. Bags are labeled and placed into a cooler with blue ice prior to return to lab. Gloves should be worn when collecting soils from hazardous sites. Soil samples may be extracted in the field, in the lab, or following air or oven drying, as directed by the study plan.

Plant tissue samples vary greatly as to the method of collection and include fresh green leaves and stems, litter fall, and forest floor. All plant tissue is oven dried at no more than 65 degrees C. Samples are then ground to <2mm and stored in 20ml glass scintillations vials.

QUALITY ASSURANCE/QUALITY CONTROL

For the full Coweeta Analytical Lab Quality Assurance/Quality Control Protocol go to the Coweeta website <http://www.srs.fs.usda.gov/coweeta/index.html>

Quality Control Samples

Blanks

1. Deionized water blanks are analyzed weekly with the DWS samples. These blanks serve as a check on the DI water system. The conductivity of 5% of all sample bottles and sample vials washed is tested to ensure clean sample containers. If any bottles are found to have conductivity greater than 1.0 micromhos/cm then that set will be rinsed again and checked before being used.

Calibration and Standards

1. Certified stock solutions used to make calibrants and certified reference standards are purchased yearly.
2. Calibration of the Flash CN Analyzer is done with Aspartic Acid. Acceptable range is given by the manufacturer as: Carbon (35.79-36.39), and Nitrogen (10.22%-10.82%). After calibration, a certified reference soil or certified plant tissue standard is analyzed to verify the calibration. The reference is run after every ten samples to check the stability of the instrument.
3. A standard curve is determined before every analysis with the Ion Chromatograph, colorimetric analyzer, spectrometers, and the DOC/TN analyzer. An R squared value must equal 0.99 or greater before samples are analyzed. Certified QC solutions are analyzed after the calibration of the instrument to verify the calibration curve. Check calibrants and/or certified standards are analyzed after every tenth sample to check the stability of the instrument.

QA/QC

1. QC concentrates from NSI and ERA are sent to the lab quarterly. They are analyzed for SO₄, Br, Cl, NO₃-N, NH₄-N, PO₄, K, Na, Ca, Mg, DOC, TN, Al, P and TP. A value within the manufactures range is considered acceptable. Samples are run in triplicate. The percent error versus the true value are plotted yearly for each analyte to show trends.
2. NIST Pine Needles or Apple Leaves are digested and analyzed in triplicate with each set of plant samples analyzed on the ICP. Any dilutions needed for soil or plant analysis are done in duplicate.
3. NADP samples analyzed here for pH and conductivity are also analyzed by the USGS Central Analytical Lab. The USGS conducts semi-annual blind audits of our pH and conductivity measurements.
4. Method limits of detection are determined quarterly for all analytes for all instruments.
5. All balances are checked for calibration each year by a certified technician.
6. The calibration of oven temperatures are checked yearly and posted on the oven.
7. Long term chemistry data is verified by comparison of past data to current. Values that fall out of range are analyzed again.
8. All precipitation samples collected in the same week should have similar ion concentrations. A large discrepancy, particularly in NH₄-N or PO₄, indicates contamination and the sample's data are not used. Contamination usually results from insects or birds.
9. The internal Coweeta soil reference sample is run with each soil cation analysis.

LABORATORY PROTOCOL

I. Laboratory Water (DI)

A Millipore Milli-Q Integral system is used to give two types of water, Type II and Type I (ultra-pure). The system has a 200 liter storage tank that uses UV to eliminate biological activity in the tank. Conductivity is checked weekly and should never exceed 1.0 uS/cm for the Type II water. The Ultra-pure water produced by the Milli-Q has a built in check for the resistivity which is maintained at 18.2 mega ohm cm.

II. Washing Procedures for Glassware and Sample Containers

Samples at Coweeta are characterized by very low concentrations of nutrients. The mean concentration for NO₃-N in a control watershed in 1992 was 6 µg/l. The need for careful washing of collection bottles cannot be overstated.

A. General Washing Procedures for Glassware and Plastic Bottles

USE THE SINK IN THE AUTOANALYZER LAB OR THE SOIL LAB

1. Rinse out any non-hazardous liquids left in the glassware or plastic bottle into the sink. Hazardous liquids should only be disposed of by qualified technicians.
2. Wash bottles and glassware in hot soapy water, using Liquinox soap and the proper brush for the size bottle.
3. Rinse bottles and glassware in tap water 5 times.
4. Rinse bottles and glassware in Type II deionized water 5 times.
5. Hang bottles and glassware on drying racks.
6. When bottles are dry, cap and put a piece of white label tape on each one. Cover glassware with a piece of plastic wrap.

B. ICP and AA

USE THE SINK IN THE AUTOANALYZER LAB

Washing procedure with 5% Nitric acid (HNO₃) rinse

1. Rinse out any non-hazardous liquids left in the glassware or tubes into the sink. Hazardous liquids should only be disposed of by qualified technicians.
2. Wash glassware and tubes in hot soapy water, using Liquinox soap and the proper brush for the size bottle, or vial.
3. Rinse glassware and tubes in tap water 5 times.
4. Fill volumetric 1/3 full with 5% HNO₃, cover opening and sit overnight. Rinse 5 times using type II DI.
5. Soak tubes overnight in 5% HNO₃ and then rinse in type II DI 5 times.
6. Hang to dry on designated rack.

C. Soil Nitrogen Bottles and Centrifuge Tubes

USE THE SINK IN THE SOIL LAB

Washing procedure with 10% hydrochloric acid (HCl) rinse

1. Rinse out any non-hazardous liquids left in the plastic bottle or tube into the sink. Hazardous liquids should only be disposed of by qualified technicians.
2. Wash plastic bottle or tube in hot soapy water, using Liquinox soap and the proper brush for the size bottle.
3. Rinse plastic bottle or tube in tap water 5 times.
4. Fill plastic bottles and tubes 1/4 full with 10% HCl acid. Invert, swirl and shake briefly (20 sec.). Allow the acid to sit overnight.
5. Rinse with deionized water 2 times.
6. Fill plastic bottles and tubes with deionized water and allow to soak at least 2 hours.
7. Empty and rinse with deionized water 2 - 3 times. Put on racks to dry.

D. IC

START WITH SINK IN THE SOILS LAB

Remove vial caps from vial using a hemostat. Empty any liquid left in vial into the sink.

1. Vials
 - a. In the soils lab soak vials in 10% HCl for 2 hours (can be overnight) in the acid only tub.

- b. Remove vials from acid and place in acid only tub for DI soak. Fill with DI. Soak in DI overnight.
 - c. Drain DI out of tub. Bring tub to IC lab and fill tub with DI.
 - d. While vials in DI, grab a handful of vials out of the tub and rinse 4 times in DI and then place on racks.
2. Vial caps
 - a. In the soils lab soak vial caps in 10% HCl for 2 hours (can be overnight) in the acid only tub.
 - b. Remove the caps from acid and place in acid only tub for DI soak. Fill with DI. Soak in DI overnight.
 - c. Drain DI out of tub. Bring tub to IC lab and fill tub with DI.
 - d. Drain the tub and fill with DI again. Repeat for a total of 4 times.
 - e. Place vial caps in colander to dry.

Note: Always start with empty carboy. Do not use DI left in carboy overnight.

E. DOC

USE THE SINK IN THE SOIL LAB

Washing procedure with 10% hydrochloric acid (HCl) rinse

1. Rinse out any non-hazardous liquids left in the glassware into the sink.
 2. Wash all glassware, vials and caps in hot soapy water using Liquinox.
 3. Rinse in tap water 5 times.
 4. Rinse with 10% HCl acid. Invert, swirl and shake briefly.
 5. Rinse with type II DI 5 times.
 6. Put on racks to dry.
 7. Once dry, cover all glassware in aluminum foil and muffle for 4 hours at 450°C.
- III. Acid washing for soil lysimeters using dilute Hydrochloric acid (HCl)

This step is for new lysimeters only

Step 1

 - a. Remove the nalgene tubing; it does not need to be acid washed.
 - b. Rinse lysimeter with DI water and place into a 5gal bucket with enough DI to cover the porous cup.
 - c. Pressurize lysimeter and check for leaks by looking for air bubbles.

Step 2

 - a. Depressurize and remove DI.
 - b. Pour 100 – 250ml of 10% HCl acid inside the lysimeter. Place a stopper on the lysimeter and invert several times. Remove stopper and pour the acid down a drain.
 - c. Put the lysimeter into the empty bucket. Carefully add enough 10% HCl acid to bucket to cover the cup.
 - d. Pull a tension on the lysimeter. Place a warning sign on the bucket indicating acid. Allow to sit overnight.
 - e. Remove the acid from the lysimeter and the bucket and rinse both thoroughly with DI.
 - f. Add DI to bucket.
 - g. Pull DI thru the cup until the conductivity is below 5 uS/cm.

For used lysimeters:

 - a. Using a dry brush, clean off soil. If needed add DI and scrub.
 - b. Rinse with DI 5 times.
 - c. Place lysimeter into a 5gal bucket with enough DI to cover the porous cup.
 - d. Pull DI thru the cup until the conductivity is below 5 uS/cm.
- IV. Preparation of PMA Preservative Solution
 1. Read the MSDS sheets for phenyl mercuric acetate and 1,4 dioxane. Wear safety glasses, lab coat, and gloves. Always work under a fume hood.
 2. Dissolve 0.1g phenyl mercuric acetate (PMA) $C_8H_8HgO_2$ in 15.0 ml of 1,4 dioxane. Use small glass beaker with magnetic stir bar under fume hood.
 3. Add 85 ml of DI water and stir for 10 - 15 min. Solution will appear slightly cloudy.

4. Pour into 125 ml polypropylene bottle and label with date and a poison sticker.
5. PMA should be handled with care and should be stored in the flammables cabinet.
6. Bulk precipitation collection bottles receive 1.0 ml of the PMA solution. The amount of Hg in 1.0 ml of this PMA solution is 0.6 mg. The amount of bulk precipitation varies from 500 ml to 2000 ml so the concentration of Hg in a sample will vary from approximately 1.0 mg/l to 0.3 mg/l. This concentration range has been found sufficient to retard most biological activity.

V. Preparation of Soils for Chemical Analysis

Soil samples for Exchangeable Cations, Total Cations and CN are air dried prior to being sieved through a 2 mm sieve. A small subsample is powdered with a mortar and pestle prior to analysis on the Flash EA NC analyzer. Fresh soil samples for NH₄-N and NO₃-N extraction are sieved through a 6mm sieve and processed the same day.

VI. Sediment Protocol

Sediment samples are collected from Watersheds 2 and 7 when the ponding basins are cleaned out, approximately once a year.

VII. Preparation of Plant Tissue for Chemical Analysis

Plant tissue samples should be dried in the forced air ovens at 65° C to a constant weight prior to weighing. Dried material is then ground in the Wiley mill to pass through a 1mm sieve. Subsamples are collected and stored in 20 ml glass scintillation vials.

PROCEDURES FOR THE ANALYSIS OF WATER

I Procedure for Determining pH

A. Materials and Equipment needed:

pH meter
4.0 and 7.0 buffer for calibrating meter
stop watch

B. Procedure

Calibrate the pH meter using the 4 and 7 buffer.
Place pH probe in sample and time for two minutes.
Record the pH

II. Procedure for Filtering Sample for DOC

A. Materials and Equipment needed:

Millipore glass fibre prefilters Cat#APFF04700
Muffle Furnace
Filtration flask
Filtration funnel
Glass vacuum filter holder
Vacuum pump
40ml sample vials with screw top lid containing septa
Organic free deionized water (Type I)

B. Procedure

1. All glassware, sample vials, and filters are muffled at 450°C for 4 hours.
2. Set up filtering device with filter paper and attach to the vacuum pump.
3. Filter 50-100ml of sample.
4. Pour filtrate into 40ml sample vial. Cap sample.
Rinse filter flask, funnel and glass filter holder in organic free deionized water three times.
Shake out excess water, prepare to filter next sample. The same filtration equipment can be used for like samples.

III. Compositing Water Samples

Samples are composited on a volume weighted basis or a set volume basis.

For Volume Weighted Basis:

Each week, the collection volume of each sample is noted. The total volume for the month for each sample is then used to calculate what percentage of each sample is used for the total sample volume. A composite of 250 ml is made using these representative percentage calculations.

Example:

| | Lysimeter 118-1s | | |
|---------|------------------|---------------------|-----------------------------|
| | Volume ml | Percentage of total | Amount needed for composite |
| Week 1 | 150 | 21 | 53.6 |
| Week 2 | 200 | 29 | 71.4 |
| Week 3 | 100 | 14 | 35.7 |
| Week 4 | <u>250</u> | <u>36</u> | <u>89.3</u> |
| Total = | 700 | 100 | 250 |

For Set Volume: Some samples are composited using a set volume. Example: Linville Gorge Lysimeters are sampled twice a month, monthly composites contain 20ml from each sample date .

IV. Total Acidity in Water Samples

Total Acidity measurements must be done immediately.

Equipment:

1. Microburet

Reagents:

2. 0.0100N potassium biphthalate: dissolve 2.0425 g anhydrous $\text{KHC}_8\text{H}_4\text{O}_4$ and dilute to 1 liter with CO_2 -free DI water.
3. 1N NaOH: dissolve 40 g NaOH and dilute to 1 liter with DI water.
4. 0.01N NaOH: dilute 10.0 ml 1N NaOH with CO_2 -free DI water to 1 liter. Make up and standardize weekly.
5. Phenolphthalein: dissolve 2.5 g phenolphthalein disodium salt in 250 ml DI water and 250 ml ETOH.

Procedure:

6. Standardize the 0.01N sodium hydroxide
 - a. use 25 ml of $\text{KHC}_8\text{H}_4\text{O}_4$ in flask
 - b. add 1 drop 0.1N sodium thiosulfate to the $\text{KHC}_8\text{H}_4\text{O}_4$
 - c. add 3 drops phenolphthalein
 - d. titrate with NaOH until get faint pink (pH of 8.3)
 - e. repeat two more times
 - f. normality of NaOH = $\frac{\text{ml KHC}_8\text{H}_4\text{O}_4 \times \text{Normality of KHC}_8\text{H}_4\text{O}_4}{\text{ml NaOH}}$

7. Determine acidity of samples

- a. use 100 ml of sample
- b. add 1 drop of 0.1N sodium thiosulfate
- c. add 3 drops phenolphthalein
- d. titrate with 0.01N NaOH until see faint pink
- e. calculation of acidity as:
$$\text{mg/l CaCO}_3 = \frac{\text{ml NaOH} \times \text{N of NaOH} \times 50,000}{\text{ml sample}}$$

V. Total Alkalinity in Water Samples

Total Acidity measurements must be done immediately.

Equipment:

1. Microburet
2. ph Meter

Reagents:

3. Stock sulfuric acid, 0.1N: 2.8 ml of concentrated H_2SO_4 diluted to 1 liter DI water.
4. CO_2 -free DI water: prepare fresh as needed by boiling DI water for 15 minutes and cooling rapidly to room temperature. Cap the flask with an inverted beaker while cooling.
5. Sulfuric acid, 0.01N: dilute 100 ml of 0.1N stock to 1 liter with CO_2 -free DI water. Make up weekly.
6. NaOH, 0.01N: see Total Acidity.
7. 0.1N sodium thiosulfate: dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 1 liter DI water.
8. Mixed bromcresol green-methyl red: dissolve 20 mg methyl red sodium salt and 100 mg bromcresol green sodium salt in 100 ml DI water.

Procedure:

9. Standardize the 0.01N sulfuric acid against NaOH of known concentration (approximately 0.01N, see Total Acidity).
 - a. use 25 ml of 0.01N NaOH
 - b. add 1 drop 0.1N sodium thiosulfate
 - c. add 3 drops mixed bromocresol green-methyl red indicator
 - d. titrate with ~ 0.01N sulfuric acid until solution turns pale orange. Solution will go from blue to gray to pale orange.
 - e. repeat two more times
 - f. normality of $H_2SO_4 = \frac{\text{ml NaOH} \times \text{N of NaOH}}{\text{ml } H_2SO_4}$

10. Determine alkalinity of samples: use pH meter
 - a. use 100 ml of sample
 - b. titrate with 0.01N H_2SO_4 and record volume (C) required to reach pH 4.5
 - c. continue to titrate and record total volume (D) needed to reach pH 4.2
 - d. total alkalinity as: $\text{mg/l } CaCO_3 = \frac{(2C-D) \times \text{N of } H_2SO_4 \times 50,000}{\text{ml of sample}}$

VI. Determination of Bicarbonate as $CaCO_3$

1. Measure out 25ml of sample into beaker.
 2. Note pH of sample.
 3. Using 0.01N H_2SO_4 titrate sample to point where last drop gives a pH just below 4.5.
 4. Record the ml used.
- $\text{Bicarbonate as } CaCO_3 = \frac{50000(.00000043)(\text{normality of } H_2SO_4)(\text{ml of acid used})}{25} / ((.00000043) + 10^{-\text{pH}})$

VII. Method for Nitrogen Analysis of Dry Deposition

A monthly composite of the weekly collection for the dry deposition (DWS) and the Blank are made. Each composite is filtered, using the liquid filtrate to measure total dissolved Nitrogen on the Shimadzu; the particulates remaining on the filter paper are used to measure total dry deposition N on the Flash EA1112.

Procedure

1. Collect sample weekly from DWS-1 and DWS-2 plus 2 Blanks which contain lab DI water.
2. Composite the above monthly combining DWS-1 and DWS-2 into one sample and the Blanks into a second sample.
3. Measure the volume of DWS composite and the volume of Blank composite.
4. Filter each composite as follows:
 - a. Rinse several disks of Whatman 934-AH 25mm filter paper with DI water.
 - b. Dry the filter paper and then muffle at 450°C for 4 hours.
 - c. Weigh filter separately.
 - d. Filter each composite.
 - e. Set aside a sample of each liquid filtrate for analysis on the Shimadzu.
 - f. Dry paper at 65°C (until dry). Weigh paper again. The weights are used for turbidity data. Use this sample for N analysis on the Flash.

Calculations:

Measure filtered contents for total dissolved Nitrogen on Shimadzu (see instrument section).

Measure particulates on filter for %N using the Flash (see instrument section).

Calculate mg/L N from %N (obtained from Flash) as follows: $\frac{(\%N/100) \times (\text{amt weighed out for Flash})}{((\text{Total Volume used for filtrate})/1000\text{ml/L})}$

Calculate the weighted amount of NO₃ as follows:

Use the value obtained from the IC for NO₃-N in mg/L minus the value of the blank for each week collection included in the monthly composite times weighted volume.

NO₃ -N wtd = ((weekly value NO₃-N mg/L- blk value NO₃-N mg/L) x (volume for week NO₃ measured/monthly volume)

TKN = TNmg/L+N_{flash}-NO₃

TDN= TNmg/L-NO₃

VIII. Turbidity – Total Suspended Solids (TSS)

Equipment: Vacuum pump, Millipore filtering apparatus, Whatman GF/C glass 1.5 micro fiber filter paper, 5.5 cm or 2.5 cm. Do not handle filters with your fingers or drop on the floor. If you do, throw it away.

Filters used for total carbon and nitrogen analysis (2.5 cm) must be muffled at 480°C prior to use.

A. Procedure for Turbidity only:

1. Holding filter with flat bladed tweezers, rinse both sides of the filter with a squeeze bottle filled with DI. Using the tweezers, place the filter on vacuum filtration and filter 500 ml of deionized water through each filter. Wash at least four (4) more filters than there are samples. These will serve as blanks.
2. Place on aluminum foil which has been numbered (or number the filter). Cover with a towel.
3. Dry in oven for 1 1/2 hours at 125°C.
4. Weigh each filter, recording the weight. Do not remove more than 5 filters from the oven at a time.
5. Filter samples, recording the mls of sample filtered. Use all the sample if the sample is very clear. Hopefully, this will be at least 700 mls. DI can be used to obtain sediment left in graduated cylinder if needed. Do not include as volume. **SHAKE SAMPLE THOROUGHLY BEFORE FILTERING!**
6. Wash extra filters second time with average volume used for samples.
7. Rinse filter holders between samples.
8. Dry filters in oven for 2 1/2 hours at 105 °C .
9. Weigh each filter, recording the weight in grams to five decimal places.
10. Calculations: $(\text{final wt mg} - \text{initial wt mg}) \times \frac{1,000 \text{ ml}}{\text{vol. filtered ml}} = \text{mg/l suspended sediment}$

B. Procedure for Turbidity, total carbon and total nitrogen on filters:

1. Use pre-muffled 2.5 cm Whatman GF/C glass 1.5 microfibre filters.
2. Assemble the small filter holder (2.5 cm) apparatus and filter 15 ml of DI water.
3. Carefully remove filter with forceps and place on pre-numbered aluminum foil.
4. Dry in oven for 1 1/2 hours at 125°C.
5. Weigh each filter, recording the weight to five decimal places.
6. Filter samples, recording the mls of sample filtered. **SHAKE SAMPLE THOROUGHLY BEFORE FILTERING!** Clear samples will require 100 to 200 mls of water in order to collect enough particulates to analyze for total carbon and total nitrogen. Muddy samples may only require 5 to 10 mls of water.
7. Dry in oven for 2 1/2 hours at 65°C. **DO NOT DRY AT 125°C!**
8. Weigh each filter, recording the weight in grams to five decimal places.
9. Calculations: $(\text{final wt mg} - \text{initial wt mg}) \times \frac{1,000 \text{ ml}}{\text{vol. filtered ml}} = \text{mg/l suspended sediment}$
10. Refer to instrumental section on Combustion Analysis of Total Carbon and Nitrogen in Soil and Plant Tissue Samples for instructions for analysis of total carbon and total nitrogen on the filters.

C. Procedure for Volatile Solids

1. Follow procedure for TSS, muffling the filters at 550 °C in a premuffled crucible for 4 hours after each drying step. Record the weight of the filter + crucible before and after muffling.
2. Calculation: $\text{mg Volatile Solids/L} = (\text{final wt mg} - \text{initial wt mg})/\text{sample volume L}$

PROCEDURES FOR THE ANALYSIS OF SOILS

I. Preparation of Soils for Chemical Analysis

Soil samples for Exchangeable Cations, Total Cations and CN are air dried prior to being sieved through a 2 mm sieve. A small subsample is powdered with a mortar and pestle prior to analysis on the Flash EA CHN analyzer. Fresh soil samples for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ extraction should be sieved through a 6mm sieve and processed the same day.

II. Exchangeable Soil Cations

A. NH_4Cl extraction for cation concentration and Effective Cation Exchange Capacity

Exchangeable cations (Ca, Mg, K, Na, Al,) can be extracted using a mechanical vacuum extractor.

Equipment:

1. Centurion mechanical vacuum extractor
2. Extraction tubes and syringes, see figure below
3. Connector tubing - 24 pieces/ 13cm long
4. Filters
5. Balance

Reagents:

6. 1M NH_4Cl - dissolve 53.5 grams NH_4Cl in 1 liter deionized water.
7. 1M KCl - dissolve 74.55 grams KCl in 1 liter deionized water.
8. Ethanol

Procedure Day 1

9. Weigh 10g of soil (record weight), and put into labeled extraction tubes.
10. Label both upper and lower tubes.
11. Place extraction tubes in the upper tray of the extractor. Include a blank (no soil), and a reference standard for every 10 to 12 samples. See Figure below.
12. Place vacuum extractor tube in bottom tray. Attach tips of the upper and lower tubes together with rubber tubing. Be sure tubing is not too long or the syringes may fall out.
13. Fill extraction tubes (upper reservoir) with 50 ml 1M NH_4Cl solution.
14. Set control wheel and extract for 12 hours. This will extract exchangeable Ca, Mg, K, Na, and Al.

Procedure Day 2

15. Empty the contents of vacuum extractor (lower syringes) into clean labeled sample bottles.
16. Repeat steps 11 and 12, making sure that the vacuum extractors remain with its respected original extraction tube to prevent contamination.
17. Fill extraction tubes with 50 ml ethanol.
18. Set control wheel for 1 hour and extract. This will flush out interstitial $\text{NH}_4\text{-N}$.
19. Discard contents of vacuum extractor, no analysis needed.
20. Repeat steps 11 and 12, making sure that the vacuum extractors remain with its respected original extraction tube to prevent contamination.
21. Fill extraction tubes with 50 ml 1M KCl solution. This will extract $\text{NH}_4\text{-N}$ on exchange sites.
22. Set control wheel and extract for 12 hours.

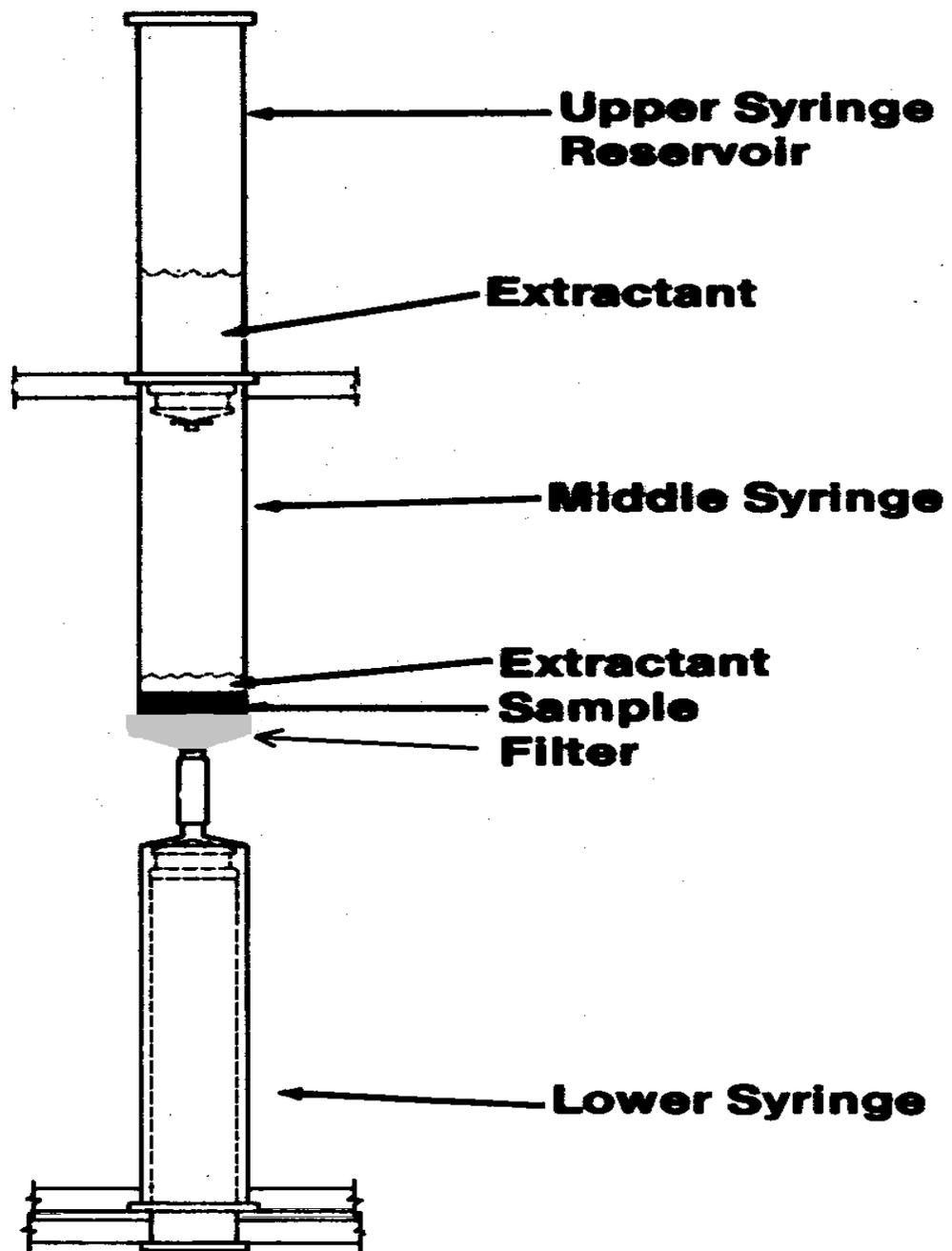
Procedure Day 3

23. Empty the contents of vacuum extractor (lower syringes) into labeled sample bottles.

Analysis:

24. Analyze KCl as soon as possible on the autoanalyzer system for $\text{NH}_4\text{-N}$.

25. Analyze NH_4Cl as soon as possible on the ICP for Ca, Mg, K, Na and Al. Freeze the sample if cation analysis is delayed.



B. Double Acid Method - Exchangeable Soil Cations Extraction

Equipment:

1. 50 ml capped centrifuge tubes, acid-washed
2. Shaker box and shaker table

Reagents:

3. Double-acid extraction solution. Add 12.15 ml HCl and 2.1 ml H₂SO₄ to 2 liters of DI water. Dilute to 3 liters with DI water.

Procedure:

4. Sieve dry soil through #10 mesh sieve (2 mm).
5. Weigh approximately 5.0 g \pm .05g dried soil into tube; record exact weight.
6. Add 20 ml extraction solution to each vial. Prepare 4 or 5 reagent blank vials and 3 standard reference samples.
7. Place tubes horizontally in wooden shaker box and shake for about 15 minutes.
8. Centrifuge samples at 8000 rpm for 10 min or until clear. Pipette into acid washed plastic bottles.
9. Store in refrigerator and analyze within 1 week.

Analysis:

Analyze on the ICP. Make up standards and blanks in extraction solution.

C. Cation Exchange Capacity (NH₄Ac)

Reagents:

1. 1N NH₄Ac
2. In a 1 liter flask add 600 ml DI water.
3. Add 58.0 ml of Acetic Acid
4. Add 70.0 ml of NH₄OH
5. Dilute to 1 liter with DI water.
6. Adjust pH to 7 with Acetic Acid or NH₄OH

Procedure:

1. Weigh 5.0 grams of sieved (2mm) air dried soil into labeled centrifuge tube.
2. Add 25 ml of 1N NH₄Ac
3. Cap and shake sideways for 30 minutes.
4. Centrifuge for 10 minutes at 8000 rpm.
5. Pour off supernatant into weighted 60 ml plastic bottle.
6. Repeat steps 2 – 5 and bring final weight to 50 grams with 1N NH₄Ac.
7. Shake bottle by hand to insure mixing.
8. Run 3 blanks and 3 standard reference samples through the procedure.
9. Analyze on the ICP.

III. NH₄ - NO₃ Extraction Procedure (Soil and Forest Floor)

Equipment:

1. Centrifuge tubes, 50 ml, 15ml
2. Shaker box
3. Shaker table

Reagents:

4. Potassium chloride extraction solution (2M KCl).
5. Dissolve 147 gm KCl (Note amount of NO₃ contamination in the KCl) in 800 ml DI water and swirl solution. Dilute to 1 liter.

Procedure:

6. Weigh 5.0 g (\pm 0.05 g) of fresh sieved soil (6mm sieve) or weigh 2-3 g of O₂ litter into 50 ml centrifuge tube. Record exact weight. Extract duplicates every 10th sample.
7. Add 20 ml KCl extraction solution to each tube (use repipet, 20 ml capacity). Include 4-6 blanks.
8. Place tubes into wooden shaker box.
9. Turn wooden box upside down approximately 10 times.
10. Place wooden box on shaker table for approximately 1 hour at medium speed - 200-300 rpm; remove from shaker.
11. Centrifuge at 8000 rpm for 10 min. Pipette the solution into labeled clean 15 ml plastic centrifuge tubes. Store in the refrigerator at 4C.
12. Analyze as soon as possible on the Astoria system for NH₄-N and NO₃-N. All standards, blanks, and QC standards are made up in 2M KCl using KCl with same or similar NO₃ contamination as that used in step 5.

IV. Mehlich I PO₄ Extraction

Equipment:

13. 50 ml capped centrifuge tubes, acid-washed
14. Shaker box and shaker table

Reagents:

15. Double-acid extraction solution. Add 12.15 ml HCl and 2.1 ml H₂SO₄ to 2 liters of DI water. Dilute to 3 liters with DI water.

Procedure:

16. Sieve dry soil through #10 mesh sieve (2 mm).
17. Weigh approximately 5.0 g \pm .05g dried soil into tube; record exact weight.
18. Add 20 ml extraction solution to each vial. Prepare 4 or 5 reagent blank vials and 3 standard reference samples.
19. Place tubes horizontally in wooden shaker box and shake for about 15 minutes.
20. Centrifuge samples at 8000 rpm for 10 min or until clear. Pipette into acid washed plastic bottles.
21. Store in refrigerator and analyze within 1 week.

Analysis:

Analyze on the ICP. Make up standards and blanks in extraction solution.

V. Nitrification: 30-Day Soil Laboratory Incubation

Equipment:

1. 2 mm sieve
2. 250 ml polyethylene bottles
3. Incubator

Reagents:

4. Potassium chloride extraction solution (2M KCl): Dissolve 147 gm KCl (Use KCl purchased from Sigma. This vendor appears to have lower NO₃ contamination) in 800 ml DI water and swirl solution. Dilute to 1 liter. Mix well.

Procedure:

5. Sieve field-moist soil through 6 mm openings.
6. Initial NO₃ and NH₄ concentrations were determined in triplicate for each composited soil sample by

extracting 5 g of fresh soil with 20 ml of 2 M KCl.

7. Soil moisture content was determined by drying a 10g sample overnight at 105°C. Dry one subsample at 105°C until constant weight is achieved.

8. Determine % moisture (90% of dry wt.) = $\frac{\text{wet-dry}}{\text{dry}}$

9. Place 10 grams of soil in 0.94L bottle and adjust moisture content to 33% of dry weight. Jars were covered with plastic wrap and soil moisture was adjusted weekly as necessary.

10. Incubate for 30 days at 25°C.

11. After 30 days, 40 ml of 2 M KCl was added to each jar plus soil to extract NO₃ and NH₄-N.

Nitrification rates equal NO₃ concentration at 33 days minus NO₃ at time zero. Nitrogen mineralization rates equal as NH₄+NO₃ at 33 days minus NH₄+NO₃ at time zero.

VI. Resin Membrane Anion and Cation Sheets

Methods Sheet SPWQ-001, Wesley M. Jarrell, September 1996

Type AR204-SZRA (anion exchanger), or CR67-HMR (cation exchanger), Ionics, Inc.; available from Soil Plant, Water Quality, Inc., 12505 NW Cornell Road, Portland, OR 97229, (503) 641-0560 or (503) 671-0855; e-mail wjarrell@esc.ogi.edu

Equipment:

1. 10 cm Petri dishes
2. Resin sheets cut into 5 cm by 5 cm squares
3. Shaker table and box
4. Plastic zip lock bags

Reagents:

1. 0.5 M NaHCO₃ – Dissolve 42.0g of NaHCO₃ in 900 mL of DI water and dilute to 1L.
2. 0.5 M HCl – Into 900 mL of DI water, slowly add 41.5 mL of concentrated HCl acid and dilute to 1L.
3. 2.0 M HCl - Into 900 mL of DI water, slowly add 166 mL of concentrated HCl acid and dilute to 1L.
4. 500ppm KH₂PO₄ – 0.71639 KH₂PO₄ to 500ml distilled water in a 1L volumetric flask. Fill to the mark with DI.

Procedure:

A variety of resin-impregnated membranes are available. Those from Ionics, Inc. have been found to be most robust and reliable in the field.

Membrane size:

Membranes are normally prepared by first cutting into 5 cm by 5-cm squares or 5 cm by 2.5 rectangles, although other sizes are acceptable.

Preparation of resin-impregnated membranes:

Anion exchange resin:

These typically arrive from the supplier saturated with Cl. However, in most cases both Cl and OH have higher affinities for the resin than does P, so saturation with a weakly held anion, bicarbonate, effectively increases the membrane's affinity for P. Cover anion membranes with 2 M HCl in a beaker and shake for 10 minutes. Rinse with DI water. Membranes are converted to the bicarbonate form by covering them with 0.5 M NaHCO₃ in a beaker and shaking for 10 minutes.

This procedure is repeated again twice (a total of three saturations), rinsing with DI water between each step. Make sure that both sides of the membrane are exposed to the solution, and that they not be stored more than 10 days after saturation in most cases. Put six buffered sheets in 100ml of 500ppm KH_2PO_4 contained in a 500ml beaker. Cover with plastic wrap and store in the refrigerator. These will be desorbed and analyzed at the same time the sample sheets are brought in and desorbed. This will allow a history of absorption to be documented.

Cation exchange resin:

Follow the same procedure for anion sheets, rinsing first with 2M HCl then using the 0.5 M NaHCO_3 solution. Put six buffered sheets in 100ml of 500ppm KH_2PO_4 contained in a 500ml beaker. Cover with plastic wrap and store in the refrigerator. These will be desorbed and analyzed at the same time the sample sheets are brought in and desorbed. This will allow a history of absorption to be documented.

Installation:

Ion sinks Membranes are placed in the soil beneath a soil core removed with a bulb planter, soil is replaced carefully to minimize disturbance. They can be placed at any depth; in most cases, the primary root zone is the region of greatest interest. One cation sheet and one anion sheet can be placed at each location. They can then be treated as a unit through desorption and analysis phases.

Time of exposure:

The membranes are left in place for up to 2 weeks. Beyond this time the membrane may no longer maintain a near-zero concentration of P or nitrate at its surface; if the concentration near the surface becomes significant and interpretation is complicated. They can be left in longer, but they may load up with ions and no longer maintain a zero concentration boundary condition.

Collection:

The ion sink should be gently removed from the soil, although slight scraping causes little change in amount extracted. Clinging soil particles should be removed gently with a rubber spatula. A small amount of soil on the membrane will not cause problems in the extraction. When returned to the lab the membrane is rinsed with deionized water to remove any additional soil prior to cation extraction. The membranes are relatively rugged and mild abrasion does not affect results. The membranes should be kept moist, e.g., in a ziploc bag with a few drops of deionized water, prior to desorption. However, in most cases, drying does not appear to adversely affect sorption properties.

Desorption:

The ion sink is dabbed dry with a clean cloth, placed in 25 ml of 0.5 M HCl in a Petri plate, and gently shaken for 20 to 30 hours. The desorption sample solution can be stored in polyethylene bottles for analysis using appropriate laboratory techniques. Include the resin sheets in KH_2PO_4 stored in the refrigerator for desorption.

Analysis:

Analyze K, Ca, Mg on the Atomic Absorption Spectrophotometer.
Analyze $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and P on the Alpkem autoanalyzer.

Data analysis:

The time factor t (seconds) is recorded when the sample is collected. To determine Mt, divide

the total amount of ion extracted from the resin, μ moles, by the surface area of the resin. For a 5 cm X 5 cm sheet, with one side in contact with soil, the area is 25 cm². If both sides of the ion sink are exposed to soil, then both sides are counted in the area term (50cm² for the above example). To best compare basic fertility among similar sites, ion sinks should be inserted after a soaking rain, irrigation, or thoroughly wetted just prior to installation, to make water content more comparable among treatments.

VII. Phosphate Extractable Sulfate

Equipment:

12. 50 ml capped centrifuge tubes, acid-washed
13. Shaker box and shaker table

Reagents:

3. Sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) 0.016 M (500 mg P/L) - Dissolve 4.46 g NaH_2PO_4 in DI and dilute to 2.000 L. or Potassium phosphate (KH_2PO_4) 500mg P/L= 2.197g/L or 4.394g/2L.

Procedure:

1. Weigh out 2.000 g of air-dried soil into 50 ml centrifuge tube.
2. Add 15 ml of 500 mg P/L solution.
3. Shake tube and contents for 30 minutes on shaker table.
4. Centrifuge for 10 minutes at 1500 rpm. Decant supernatant into a clean 50 ml disposable centrifuge tube.
5. Repeat extraction and centrifugation (Steps 2, 3, and 4) two times for a total of three extractions. Combine all 3 supernatants.
6. Filter the solution through a 0.45 μm membrane filter and place in a LPE bottle.
7. Store the solution at 3°C and analyze for sulfate by ion chromatography within 24 hours if possible. Immediate analysis is desirable because biological activity in this nutrient-rich extract may reduce the concentration of sulfate in solution.

Quality Control:

1. 2 blanks and 2 NIST Estuarine soil standards are run with each set of 24.

Analysis:

Make all standards in phosphate used for extraction.

Ion Chromatograph:

1. Use method so4soil052903.met.
2. Switch to the 90 μl sample loop.
3. October 2012 – Samples analyzed for Sulfur on ICP.

VIII. Langmuir sulfate adsorption isotherms were generated for soil samples using a modified method as described by Harrison et. al. (1989) and Strahm and Harrison (2007)

1. Set up mechanical extractor and syringes for extraction process. See page 18 of this document for example of syringe set up.
2. Weigh 5.0 g soil in syringe. (5 g Sample #1)

Step 1—Native SO₄ determination

- a. Native SO₄ desorption; Add 25 ml of DI to each syringe. Allow mechanical extractor to run for one hour. After an hour collect sample in 50ml vial and record volume. Repeat until SO₄ concentration is <0.005meq/L, approximately 4 extractions.
- b. Extract finally with 0.01 M Ca(H₂PO₄)₂ –3 times. Note – will have to add .3ml H₃PO₄ per liter to get the Ca(PO₄)₂ to dissolve.

Notes: 1. Extract soil with solution for 1 hr. Analyze supernatant for SO₄. Record final solution volume for the first extraction to estimate retention by soil following extraction to allow correction of solution concentration.

Step 2. Soil SO₄ adsorption. (5 g Sample #2)

Extract 5 g of soil on mechanical extractor with 25 ml of solution in the following sequence with K₂SO₄ solution in the following concentrations: 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 mmol SO₄-S L⁻¹. Add 2-3 drops toluene per sample (first extraction only) to inhibit any biological activity. Use the same 5 g sample for all SO₄ concentrations.

Extract soil with solution for 1 hr. Analyze supernatant for SO₄. Record final solution volume for the first extraction to estimate retention by soil following extraction to allow correction of solution concentration.

Make the following in a volumetric flask and bring to the mark with DI:
For 500ppm KH₂PO₄(5.26meq/L K₂PO₄) = 0.716g KH₂PO₄ per liter

IX. Soil Bulk Density Determination

- a. Collect 'intact' soil core, as much as possible.
- b. Place entire soil core sample in jar of known weight.
- c. Oven dry at 105°C. Weigh entire jar for total weight.
- d. Sieve through 6 mm sieve, Weigh < 6 mm fraction.
- e. Sieve through 2mm sieve. Remove organic materials, roots, etc. and weigh.
- f. Weigh < 2mm fraction.
- g. Record following weights:
 - Total core.
 - < 2mm fraction.
 - Organic fraction

X. Soil pH Procedure - H₂O and 0.01M CaCl₂

Equipment:

- a. pH meter
- b. paper cups
- c. glass rod

Reagents:

- d. pH buffers 4 and 7
- e. 1M CaCl₂ - dissolve 14.7g CaCl₂ in 100 mL DI water.
- f. .01M CaCl₂ – dilute 1M CaCl₂ – 10mL in 1 liter DI water.

Procedure:

- g. Weigh 5 g of air-dried soil into 4oz paper cup.
- h. Add 10 ml of .01M CaCl₂ to each cup.
- i. Mix thoroughly for 5 seconds with glass rod.
- j. Let stand 30 minutes – stirring several times during the 30 minutes.
- k. Insert electrodes into container and stir soil suspension by swirling around electrodes slightly
- l. Read pH immediately on standardized pH meter. Record as soil pH in .01M CaCl₂.

Note: some studies may call for pH measured in DI H₂O—procedure is the same, except use H₂O.

XI. Exchangeable Acidity in Soils (Adams Buffer Method)

A. Exchangeable acidity (cmol H⁺) is determined by pH change in a 1:2 soil: Adams and Evans buffer slurry. Cmol H⁺ was calculated from the change in buffer pH (8.0), 0.8 cmol H⁺ kg⁻¹ soil for every 1.0 unit pH decrease.

Equipment:

1. ph meter
2. paper cups
3. glass rod

Reagents:

4. Buffer Solution: dilute to 1 liter with DI water
 - a. p-Nitrophenol 20.0g
 - b. Boric Acid (H₃BO₃) 15.0g
 - c. Potassium Chloride (KCl) 74.0g
 - d. Potassium Hydroxide (KOH) 10.5g
5. Standard pH meter buffers: 4.0 and 7.0

Procedure:

6. Weigh 20g air-dry soil into small cup
7. Add 20ml DI H₂O and stir intermittently for 1 hour
8. Read pH
9. Adjust pH meter to 8.0 with diluted (20ml H₂O +20ml buffer) solution
10. Add 20ml buffer solution to soil-water
11. Stir every 10 minutes and immediately before measuring pH
12. Measure pH

Calculations:

13. Cmol H⁺ kg⁻¹ = change in buffer pH (8.0) * 0.8
0.8 cmol H⁺ kg⁻¹ soil for every 1.0 unit pH decrease.

For 0.25mM CaSO₄ = 0.043g CaSO₄ per liter

XII. Total Carbon and Nitrogen

1. Use mortar and pestle to grind up one spoonful of each sample.
2. Dry in oven overnight at 50 – 60 C.
3. Work from desiccator when weighing out samples, use 40 – 60 mg of soil per sample.
4. Analyze sample on the Flash EA 1112
5. Include reference standard after calibration and after every tenth sample.

XIII. Total Organic Phosphorous – Ignition Method

Organic phosphorous is determined by the difference between the amounts of extractable phosphorous determined from ignited and unignited H_2SO_4 extractable P.

P ignited:

1. Weigh 1.0g of soil into a porcelain crucible.
2. Muffle at $550^\circ C$ for 1 hour.
3. After the crucible has cooled, transfer the contents to a centrifuge tube.
4. Add 25ml of 0.5M H_2SO_4 to the centrifuge tube.
5. Shake for 16 hours.
6. Centrifuge the sample to obtain a clear solution.
7. Transfer to a 50ml vial.

P unignited:

1. Weigh 1.0g of soil into a centrifuge tube.
2. Add 25ml of 0.5M H_2SO_4 to the centrifuge tube.
3. Shake for 16 hours.
4. Centrifuge the sample to obtain a clear solution.
5. Transfer to a 50ml vial.

QC:

Include 3 blanks and 3 soil reference to each set.

Calculation:

Total extractable P = $(P_{ug}/mL) \times (50/v) \times (50/g \text{ of soil})$

V=sample volume

$P_{organic} = P_{ignited} - P_{unignited}$

PROCEDURES FOR ANALYSIS OF SEDIMENT

I. Sediments Protocol

A. Collection

Sediment samples are collected from Watersheds 2 and 7 when the ponding basins are cleaned out, approximately once a year. Each sample should receive the following treatment:

1. Dry each sample to constant weight at 50°C. Record dry weight.
2. Separate samples by sieving through standard soil mesh sieves, resulting in 3 size fractions: < 1 mm, 1-2 mm, and > 2 mm. Weigh each fraction and record.
3. Save the > 2 mm fraction for carbon determination. Toss out rocks and other minerals; weigh what remains after oven drying again at 50°C. Grind.
4. Watershed 2: Combine the < 1 mm and 1-2 mm fractions. Mix well. Remove 5 subsamples approximately 30 g each. Three to four samples are taken from each ponding basin.
5. Watershed 7: Samples are collected along 6 sample lines, which are numbered either 1-6 or A-F. Combine all samples from a given line (< 1mm and 1-2 mm), thus making 6 composite samples. Remove duplicate subsamples from each composite, resulting in 12 samples for chemical and mechanical analysis, need about 300 g.
6. Analyze for total cations (Ca, Mg, Na, K, and P), exchangeable cations (Ca, Mg, Na, K, and P), mechanical analysis, CHN composited samples and for > 2 mm fraction.

B. Separation of Organic Material from > 2 Sediment Fraction

Equipment:

1. Deionized water
2. Small white enamel pans
3. Forceps
4. Large beaker covered with plastic screen
5. Aluminum weighing pans or #2 paper bag

Procedure:

6. Pour > 2 sample into enamel pan.
7. With forceps, pick out larger leaf fragments and other organic material.
8. Add water to pan, slosh around till small organic fragments float to surface.
9. Stir with forceps so small pieces of quartz and dirt sink.
10. Pour off into screened beaker.
11. With forceps, remove organic matter to numbered pan or bag.
12. Dry and weigh organic and inorganic fractions.
13. Save organic fraction CHN analysis.

C. Mechanical Analysis for Coweeta Sediments (mostly sand)

Materials:

1. Hamilton Beach mixer and dispersion cups.
2. Bouyoucos hydrometer graduated in grams per liter of water.
3. Soil sedimentation cylinders, 1,205 ml.
4. Thermometer, graduated in °F.
5. Plastic 250 ml beakers.
6. Wash bottles.
7. Stop watch.
8. Sodium hexametaphosphate solution, 10 g/l deionized water.

Procedure:

9. Dispersion

- a. Weigh to the nearest 0.1 g, 100 g air dried soil into 250 ml beaker.
- b. Add 40 ml sodium hexametaphosphate solution, and then 150 ml distilled water. Let stand for at least 18 hours, but do not permit to dry out.
- c. Use distilled water to wash soil into dispersion cup, then add water to within 2 inches of the rim. Disperse the sample with Hamilton Beach stirrer for 8 minutes.

10. Hydrometer Measurements

- a. Wash contents of dispersion cup into soil sediment cylinder. Place hydrometer in cylinder and then add distilled water to upper mark, 1,250 ml
- b. Remove hydrometer from cylinder.
- c. Blank: add 40 ml sodium hexametaphosphate solution to an empty soil cylinder and dilute to upper mark with distilled water. Take hydrometer readings as for samples.
- d. Use rubber ball to close top of cylinder and invert cylinder at least 10 times. Allow time after each inversion for sand to fall from bottom of cylinder but not so much that it begins to accumulate at opposite end. When suspension is uniform, place cylinder on bench and note exact time to the second. This is time zero.
- e. Insert hydrometer quickly but carefully, steadying it with finger tip at first. Record hydrometer reading (top of meniscus) exactly 40 seconds after time
- f. Move hydrometer up and down in the suspension to displace soil particles which have settled on it and then remove it from cylinder. Re-suspend soil and obtain second reading. If it agrees within 1.0 of scale unit, proceed to next determination; otherwise, repeat the reading. Then take temperature.
- g. After two satisfactory 40-second readings have been made, re-suspend the soil and let sit for 3 hours, + or - 15 minutes; take one reading then and record, along with temperature.

D. Calculations

- a. Since hydrometer was calibrated at 68°F, data obtained at other temperatures must be corrected. Less error is introduced if the temperature is above 68°F than below. Avoid extreme temperatures.
For degrees above 68°F: add 0.2 units per degree above 68°F to hydrometer reading. For degrees below 68°F: subtract 0.2 units per degree below 68°F from hydrometer reading.

- b. For the following calculations, subtract % O.M. from weight of soil:

- c. 40-second hydrometer reading:

$$\frac{\text{Hydrometer reading (sample-blank) + temp. correction}}{\text{weight of soil, grams}} = \% \text{ clay + silt}$$

- d. $100 - (\% \text{clay} + \text{silt}) = \% \text{sand}$

- e. 3-hour reading:

$$\frac{\text{Hydrometer reading (sample-blank) + temp. correction}}{\text{weight of soil, grams}} = \% \text{ clay}$$

- f. Determination of silt:

$$100 - \% \text{ sand} - \% \text{ clay} = \% \text{ silt}$$

II. Digestion for Total Cations in Sediments

Wear safety glasses, lab coat, and gloves when performing this procedure.

Equipment:

1. Flume Hood
2. Hot Plate
3. 600 ml Glass Beakers
4. Watch Glass Covers 11cm
5. Muffle Furnace
6. Porcelain Crucibles

Reagents:

7. Hydrogen Peroxide 30%
8. Acid Digestion Solution: To 500 ml DI water slowly add 250 ml of HNO_3 and 30 ml of H_2SO_4 . Mix well.
9. HNO_3 - 2%

Procedure:

10. Weigh approximately 1 gm sediment material into tared and pre-muffled crucible, record exact weight.
11. Muffle at 500°C for 4 hours.
12. Transfer each sample to an acid-washed 600 ml beaker. (Rinse crucible with DI water if necessary).
13. Add enough H_2O_2 to cover sample (approx. 10 ml) and cover with watch glass. Place on hot plate on high temperature and allow to react. When reaction stops fuming, remove watch glass and evaporate to dryness. (At this stage lid can explode if left on).
14. Add acid mixture to cover sample and allow to react as above. Evaporate to dryness. Sample at this point should be a yellow-white sediment. Start blanks here.
15. Add 20 ml 2% HNO_3 to each sample. Wash down beaker sides with warm deionized water.
16. Filter through Whatman # 42 Ashless filter paper into 50 ml volumetric. Bring to volume with DI water.
17. Store samples in acid-washed plastic bottles in refrigerator at 4°C .

Analysis:

18. Analyze for K, Na, Ca, and Mg on atomic absorption and PO_4 on Autoanalyzer.

PROCEDURES FOR ANALYSIS OF PLANT TISSUE

I. Preparation of Plant Tissue for Chemical Analysis

Plant tissue samples should be dried in the forced air ovens at 65° C. Dried material is the ground in the Wiley mill to pass through a 1mm sieve. Subsamples are collected and stored in 20 ml glass scintillation vials.

II. Digestion for Total Cations in Plant Tissue

Wear safety glasses, lab coat, and gloves when performing this procedure.

Equipment:

1. Muffle Furnace
2. Porcelain Crucibles, acid washed in 5% HNO_3
3. 50 ml graduated tubes
4. Guth Wash Flask
5. NIST Pine Needles or suitable reference standard

Reagents:

6. HNO_3 20%

To 600 ml of deionized water add 200 ml of HNO_3 and dilute to 1 liter with DI water.

Procedure:

7. Dry the samples for 2 hours at 70°C.
8. For ash free dry weight, muffle crucibles prior to use (OR DRY IN OVEN AT 105 FOR 2 HOURS). Allow cooling in oven and store in desiccator. Record the weight for each crucible.
9. Weigh approximately 0.5g sample into a tarred crucible. Record the weight to the nearest mg. Include 3 reference standards.
10. Muffle 3 extra crucibles and carry them through the procedure for blanks.
11. Muffle at 500°C for at least 4 hours. Wood material will take longer to ash. Material that has been completely ashed will not have any black material present.
12. Allow cooling in oven. For ash free dry weight; store in desiccator and record weight of crucible and ash.
13. Working under a hood, dissolve the ash in 5 ml of 20% HNO_3 . The ash may not dissolve completely.
14. Use a Guth Wash Flask filled with hot deionized water to rinse the crucible contents into a 50ml graduated tube that has been acid washed in 5% HNO_3 .
15. Fill tubes to the 50ml mark with DI water.

Analysis:

Analyze for K, Na, Ca, Mg, P and Al (if needed) on ICP.

Calculations:

$$\text{Percent cation} = \frac{50 \text{ ml} \times \text{conc. mg/l in solution}}{\text{wt. sample g} \times 10000}$$

III. Ash Free Weight

Equipment:

1. Muffle Furnace
2. Porcelain Crucibles
3. NIST Pine Needles or suitable reference standard

Procedure:

4. Dry the samples for 2 hours at 60°C. Keep in a desiccator until ready to use.
5. Dry the crucibles for 2 hours at 105°C. Keep in a desiccator until ready to use. Record the weight of the crucible.
6. Weigh approximately 0.5g sample into a tarred crucible. Record the weight to the nearest mg. Include 3 reference standards.
7. Muffle 3 extra crucibles and carry them through the procedure for blanks.
8. Muffle at 500°C for 6 hours. Material that has been completely ashed will not have any black material present.
9. Allow cooling in oven. Store in a desiccator and record the weight of crucible and ash.

IV. Total Carbon and Nitrogen

1. Dry in oven overnight, temp 50 – 60 C. Dry in original container if glass, otherwise pour into a tin pan.
2. Work from desiccator when weighing out samples, 13 – 17 mg of plant per sample.
3. Analyze sample on the Flash EA 1112
4. Include reference standard after calibration and after every tenth sample.

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2. Walsh, L.M., "Instrumental Methods for Analysis of Soils and Plant Tissue", 1971, Soil Science Society of America, Madison, Wisconsin 53711
3. Greenberg, A.E., Trussell, R.R., Clesceri, L.S., "Standard Methods for the Examination of Water and Wastewater", 16th Edition, 1985, American Public Health Association
4. "Methods for Chemical Analysis of Water and Wastes", 1983, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio, 45268
5. Cronan, C.S., Goldstein, R.A., "ALBIOS: A Comparison of Aluminum Biogeochemistry in Forested Watersheds Exposed to Acidic Deposition", 1989, Acidic Precipitation, Volume 1, Case Studies, Springer-Verlag, New York
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INSTRUMENTAL METHODS

This section will deal with analytical methods for analysis of Anions, Cations, pH, Carbon, and Nitrogen. Coweeta methods are similar to the EPA and Standard Methods. Each instrumental section will be a stand-alone instruction manual for an element, ion, or group of ions.

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|--|--------------------|
| Combustion Analysis of Total Carbon, Hydrogen, and Nitrogen in Soil and Plant Tissue Samples | 1 |
| Micro-membrane Suppressed Ion Chromatography Chloride, Nitrate, Orthophosphate, Bromide, and Sulfate | 2 |
| Automated Wet Chemistry Ammonium | 3 |
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| Electrometric Determination of pH and Titration for Bicarbonate | 7 |
| Atomic Absorption Spectrophotometer Potassium, Sodium, Calcium, and Magnesium | 8 |
| Inductively Coupled Plasma Spectrophotometer Potassium, Sodium, Calcium, Magnesium, Aluminum and Phosphorus | 9 |
| Combustion Analysis of Water Samples..... Dissolved Organic Carbon and Total Nitrogen | 10 |

Combustion Analysis of Total
Carbon, and Nitrogen
In Soil and Plant Tissue samples

Coweeta Hydrologic Laboratory
3160 Coweeta Lab Road
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October 2009

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TABLES

1. Suggested Reference Materials for Samples at Coweeta.
2. Acceptable Ranges for Aspartic Calibration Standard.
3. Suggested Weights for Various Sample Types.
4. Method Detection Limits.

FIGURES

1. System diagram for Flash EA 1112 Analyzer.
2. Filling Diagram for NC Soils

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Total Carbon, and Nitrogen in soil and plant tissue samples. Water samples can be analyzed by filtration and combustion of filter or by use of evaporative pan.
- 1.2 Method detection limits are summarized in Table 5.

2. SUMMARY OF METHOD

- 2.1 The Flash EA series 1112 NC Elemental Analyzer is based on the Dynamic Flash Combustion technique. Samples are converted to simple gases (CO₂, H₂O, and N₂) by combustion in a pure Oxygen environment. The gases are separated chromatographically and eluded to a Thermal Conductivity Detector for quantification. Prior to analysis, sieved or ground samples are weighed into tin capsules on a micro balance. The sample weights are entered and analytical results are reported in elemental percent.

3. DEFINITIONS

- 3.1 Dynamic Flash Combustion Technique - The sample enters the reactor, inserted in the special furnace heated at 900 – 1000°C, a pulse of pure oxygen is added to the system containing a catalyst, the temperature goes up and combustion occurs, converting the sample into elemental (simple) gases.
- 3.2 GAS CHROMATOGRAPHY -- A separation technique based on molecules having a distinct affinity for a particular material.

4. INTERFERENCES

- 4.1 Use only ultra-high purity Helium and Oxygen gases.
- 4.2 Heterogeneous materials can cause poor reproducibility.

5. SAFETY

- 5.1 When preparing reactor tubes, wear safety glasses, gloves, and lab coat. Work under hood to prevent inhalation of dust from reagents.
- 5.2 Do not touch furnace surface when in use. Furnace is at a very high temperature.
- 5.3 When removing a hot reactor tube, wear high temperature gloves and safety glasses.
- 5.4 Make sure to use proper gas regulators on the Helium, Air and Oxygen tanks.

6. APPARATUS AND EQUIPMENT

- 6.1 Flash EA series 1112 CN Analyzer
- 6.2 Analytical balance – Denver Instruments PI-225D

7. REAGENTS AND CONSUMABLE MATERIALS

- 7.1 Purchase from CE Elantech, Inc.:

| | |
|---|------------|
| Tin Capsules 9x5mm(set of 250) | 240-064-25 |
| Tin Capsules 10x12mm(set of 100) | 252-080-00 |
| Small Adsorption Filter W/O Fast Connectors | 281-131-04 |
| Flash Fast Connectors(set) | 190-502-08 |
| Seal for Adsorption Traps SM/Med W/FC(set) | 290-036-35 |
| Copper Oxide(flask of 100G) | 338-217-15 |
| High Quality Copper for Flash EA 1112(50G) | 338-353-12 |
| Oxidation Catalyst (40G) | 338-400-00 |
| Soil NC REF Material (5G) | 338-400-25 |

8. QUALITY CONTROL

- 8.1 Use NIST Apple leaves or Peach leaves for a Carbon and Nitrogen reference standard when running plant tissue.
- 8.2 Use Coweeta 1A, Coweeta 1B, or Thermo Soil reference standard for Carbon, Hydrogen and Nitrogen when running soil samples.
- 8.3 Run a reference standard after every tenth sample..
- 8.4 Check calibration of Flash EA after every 50 samples by running an Acetanilide as an unknown. Refer to table 2 for acceptable values for Carbon, Hydrogen and Nitrogen.

9. PROCEDURE

Soil Sample Prep

- 1 Use mortar and pestle to grind up one spoonful of each sample.
2. Fill out data sheet to correspond samples with tin they are in.
3. Dry in oven overnight at 50 – 60 C.
4. Work from desiccator when weighing out samples, use 40 – 60 mg of soil per sample.

Plant Sample Prep

1. Dry in oven overnight, temp 50 – 60 C. Dry in original container if its glass, otherwise pour into a tin and mark on data sheet.
2. Work from desiccator when weighing out samples, 13 – 17 mg of plant per sample.

Weighing samples / preparing QC's

1. Ensure weight is in mg's .
2. Tare tin before putting sample in.
3. Standards (Aspartic) and QC's (plant or Thermo soil) are in glass desiccator.
4. Seal tin before taking final weight, record on data sheet.

Machine Startup

1. Turn on computer.
2. Turn on EA1112, oxygen, and helium tanks.

Machine pre-sample checks

1. Left Tank 1 is the drying cylinder, has to be cleaned after every sample run:
 - 1.1. Take out using tongs and place in rack to cool.
 - 1.2. Use backup stored underneath the machine, place quartz wool in bottom.
2. Left Tank 2 is Reactor 1, to refill:
 - 2.1. Remove sample dispenser.
 - 2.2. Use special tongs to remove cylinder – left tank 1.
 - 2.3. Scrape out waste, use tools on shelf under the machine, clean as much as possible.
 - 2.4. Check glass wool in bottom, replace if it's not intact.
 - 2.5. To remove left tank 2 use special tongs.
 - 2.6. Take off rubber o-ring, inspect for cracks or dirt.
 - 2.7. Tube is filled from bottle labeled oxidation catalyst, ensure mixture is homogenous.
 - 2.8. Use Figure 2 and markings on the metal rod to determine how to fill.
 - 2.9. 50mm glass wool, 130mm oxidation catalyst, 10mm glass wool.
 - 2.10. Use hood when filling tube.
 - 2.11. Use a beaker to mix oxidation catalyst, fill and plug w/10mm glass wool.
 - 2.12. After placing cylinder and tube back and reassembling perform leak test.
 - 2.13. In software go to – maintenance program – edit – reset maintenance for left 1 & left 2.
 - 2.14. Used material can be disposed of in regular trash.

3. Right Tank 1 is Reactor 2
 - 3.1. Remove using oven mitts if the tube is hot.
 - 3.2. Remove quartz wool using tweezers, save for reuse if in good condition.
 - 3.3. Clean out used copper using the tools on the shelf underneath the machine.
 - 3.4. Replace copper according to Figure 2.
4. The Adsorption FilterTube is in the other compartment
 - 4.1. Clean out after a full sample run.
 - 4.2. Pull out wool in hood.
 - 4.3. Rinse out magnesium in sink.
 - 4.4. Dry with the air hose on the hood.
 - 4.5. Replace wool in one end and pour magnesium perchlorate in , loose packing of mg is ideal.
 - 4.6. Ensure no wool is hanging out to provide proper seal.

* In software go to – maintenance program – edit – reset maintenance for any column cleaned and refilled

Sample Run

1. Computer and machine need to be on.
2. Check maintenance status to ensure no tubes need replaced, visually inspect adsorption tube.
3. Perform a leak test. The carrier flow result needs to be below 5 to operate.
4. Check machine status to ensure combustion tubes have reached proper temperature and you have a green light.
5. Ensure there is a folder for data: go to flash data folder, data folders are grouped by month, create folder for your data based on the date you run the sample.
6. Create a new spreadsheet by bringing up previous, emptying, then importing (remember to change start number from 2 to 1).
7. Run QC's first: bypass is aspartic, blank is an empty tin, 3 aspartic, 3 Peach Leave for plant or 3 Thermo's for soil.
8. View calibration curve to ensure that QC's were all close enough together to use – if there's an outlier do not highlight it.
9. If all the QC's are within parameters load sample tray, note that 31 samples take app. 3hrs 15min.
10. Click on green arrow to run samples, view sample being run and previous sample results.
11. When complete run another plant or soil standard to assure machine is still calibrated.

10. PRECISION AND BIAS

Single operator precision and bias were obtained from the analysis of NIST Pine Needles, NIST Apple Leaves and NIST Peach Leaves. Table 4 summarizes the current data.

11. REFERENCES

1. http://weather.nmsu.edu/Teaching_Material/SOIL698/flash/New%20WebSite.htm
2. <http://www.ceelantech.com/>

Table 1. Suggested Reference Materials for Samples at Coweeta.

| Sample Type | Reference Material |
|-------------------|--------------------|
| Soil -- A horizon | Coweeta Hi OM (1A) |
| Soil -- B horizon | Coweeta Lo OM (1B) |
| Plant -- Foliage | NIST Pine Needles |
| Plant -- Foliage | NIST Peach Leaves |

Table 2. Acceptable Ranges for Aspartic Calibration Standard

| Analyte | Range % |
|----------|---------------|
| Carbon | 35.79-36.39 % |
| Hydrogen | 5.00-5.60 % |
| Nitrogen | 10.22-10.82 % |

Table 3. Suggested Weights for Various Sample Types.

| Sample Type | Weight mg. |
|-------------------|------------|
| Soil -- A horizon | 40-60 |
| Soil -- B horizon | 40-60 |
| Plant -- Foliage | 12-17 |
| Plant -- Other | 12-17 |

Table 4

Method Detection Limit using Coweeta Soil Reference 1b

| Analyte | wt. in mg | %N | %C |
|-------------------|--------------|--------------|--------------|
| Coweeta 1B | 51.06 | 0.053 | 1.377 |
| Coweeta 1B | 47.08 | 0.047 | 1.382 |
| Coweeta 1B | 50.43 | 0.051 | 1.384 |
| Coweeta 1B | 50.95 | 0.052 | 1.383 |
| Coweeta 1B | 45.98 | 0.053 | 1.406 |
| Coweeta 1B | 50.56 | 0.050 | 1.378 |
| Coweeta 1B | 51.56 | 0.054 | 1.370 |
| Coweeta 1B | 48.22 | 0.046 | 1.360 |
| Coweeta 1B | 51.43 | 0.050 | 1.381 |
| Coweeta 1B | 48.89 | 0.047 | 1.374 |
| | avg | 0.050 | 1.376 |
| | std | 0.004 | 0.002 |
| | mdl | 0.011 | 0.006 |

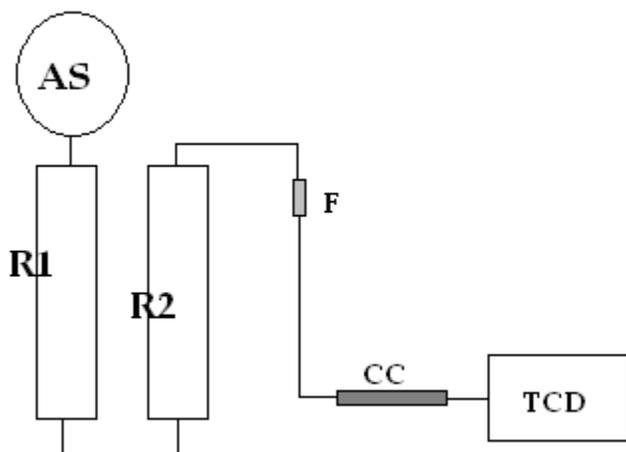
Method Detection Limits Using NIST Peach Leaves

| Analyte | | %N | %C |
|--------------|------------|--------------|---------------|
| Peach Leaves | | | |
| Peach Leaves | | 2.984 | 47.074 |
| Peach Leaves | | 2.970 | 47.137 |
| Peach Leaves | | 2.826 | 47.005 |
| Peach Leaves | | 3.010 | 47.002 |
| Peach Leaves | | 2.977 | 47.116 |
| Peach Leaves | | 2.981 | 46.891 |
| Peach Leaves | | 2.997 | 46.980 |
| Peach Leaves | | 2.997 | 46.876 |
| Peach Leaves | | 2.967 | 46.945 |
| | avg | 2.968 | 47.003 |
| | std | 0.055 | 0.092 |
| | mdl | 0.155 | 0.261 |

Figure 1. CHN System Diagram

7.1.1 Description of Method for NC-Soils configuration¹:

Diagram of instrument parts:



Where:

AS- autosampler

R1-left furnace (combustion reactor)

R2- right furnace (oxidation reactor)

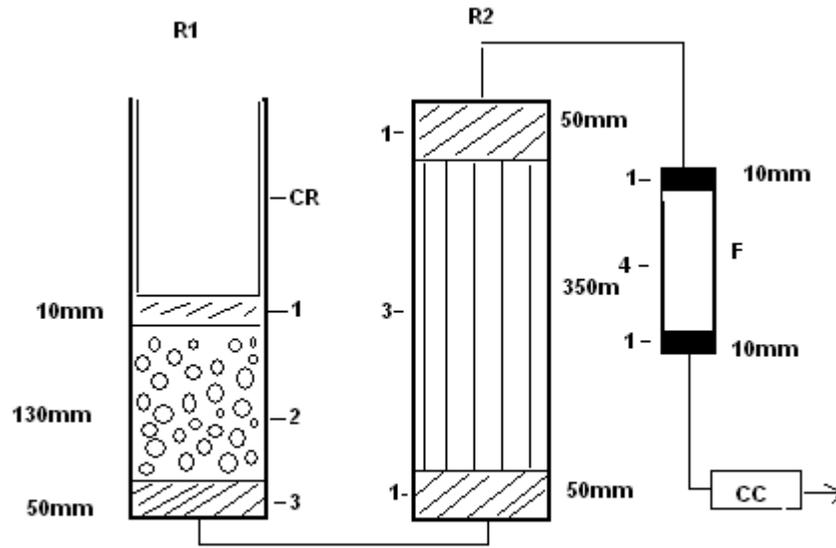
F- Adsorption filter

CC- chromatographic column

TCD-thermal conductivity detector

Autosampler AS is connected to the steel combustion reactor R1. Combustion reactor is placed in a furnace at a set temperature of 900°C. The combustion reactor is connected to the oxidation reactor R2 which is set in a furnace at 680°C. The oxidation reactor is in turn connected to the adsorption filter F and the filter outlet is connected to the analytical column CC. The CC is connected to the thermal conductivity detector TCD.

Figure 2 Filling Diagram for NC- Soils



Legend

Reactor R1

- 1. Quartz Wool
- 2. Oxidation Catalyst

Reactor R2

- 1. Quartz Wool
- 3 Reduced Copper

Adsorption filter F

- 1. Quartz Wool
- 4. Magnesium Perchlorate

Note: The R1 reactor requires use of a crucible CR

Micro-membrane Suppressed
Ion Chromatography
for Chloride, Sulfate, Bromide, Nitrate, and Orthophosphate

October 2009

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TABLES

1. Method Detection Limits.
2. Suggested Calibration Standards for Stream, Precipitation, Thrufall, and Lysimeter Samples at Coweeta.

FIGURES

1. Drawing of pump showing transducer.
2. Plumbing diagram for Dionex ICS2500
3. Chromatogram for a Coweeta Sample containing Chloride, Bromide, Nitrate, Orthophosphate and Sulfate.

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Chloride, Bromide, Nitrate, Orthophosphate, and Sulfate in Stream, Precipitation, Thrufall, and Lysimeter samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that may contain high concentrations of salts or acids.

2. SUMMARY OF METHOD

- 2.1 Ion Chromatography utilizes the separation capacity of an ion exchange column and the detection signal from a conductivity detector. Aliquots of samples are poured into sample vials with caps. The automatic sampler loads the sample into the sample loop. The pump controls a micro-injection valve which introduces the sample into a high pressure stream of eluent. As the sample passes through the guard column and the separator column each analyte will be retained to a certain degree by the stationary phase material in the column. After separation the analytes are pushed through the micro-membrane suppressor. The eluent ions are neutralized and the sample ions are converted to their corresponding strong acids. The conductivity detector responds to each ion as it eludes off the column. Peak area data is acquired through computer interface. Calibration curves are constructed from standards with known concentrations of each analyte. Concentrations of the unknown samples are determined based on the calibration curve.

3. DEFINITIONS

- 3.1 ION EXCHANGE -- a reversible process by which ions are interchanged between an insoluble material (stationary phase) and a liquid (mobile phase) with no substantial structural changes of the material.
- 3.2 SUPPRESSOR -- a semipermeable membrane containing cation exchange sites to suppress the eluent background conductivity.
- 3.3 ELUENT -- the ionic liquid mobile phase used to transport the sample through the exchange columns.
- 3.4 RESOLUTION -- the ability of a column to separate constituents under specified test conditions. Peak resolution is a function of column efficiency, selectivity, and capacity. Separation of peaks can also be a function of eluent strength.
- 3.5 RETENTION TIME -- the interval measured from the point of sample injection to the point of maximum peak area for a given analyte.

4. INTERFERENCES

- 4.1 Shifting retention times can cause peaks to be misidentified or unidentified. Retention times will shorten over the life of the column due to contamination of the stationary phase. It is extremely important that the eluent be made up in the same molar concentration each time. This will minimize changes in retention times.
- 4.2 Peaks that elude close together may not be properly integrated if one peak is disproportional to the other peaks.
- 4.3 Noisy baselines can interfere with peak sensitivity. See section 10.7.
- 4.4 New samples should be checked for late eluding peaks by running the chromatogram for 22 minutes. Also, check for co-eluding peaks by spiking samples with pure standards.

5. SAFETY

- 5.1 Most of the reagents used in this method are not hazardous. Follow the American Chemical Society guidelines when using all chemicals.
- 5.2 The eluent cartridge can be thrown in the garbage after emptying the solution remaining in the cartridge (KOH) under the hood. Wear protective gloves, lab coat, and safety glasses when working with caustic.
- 5.3 High pressures in excess of 3000PSI are generated by the pump. Column compartment should be shielded and operator should wear safety glasses when working on high pressure lines.

6. APPARATUS AND EQUIPMENT

- 6.1 ION CHROMATOGRAPH:
The Dionex ICS2500 ion chromatograph is equipped with an Autosampler, Micro Injection Valve, Gradient pump, Slider Valve, Conductivity Detector, Guard Column, Separator Column, Anion Self Regenerating Suppressor, and Computer software that runs under Windows operating system. See Figure 2.
 - 6.1.1 AS40 Autosampler:
Samples are loaded into 5ml disposable plastic vials. Autosampler can be operated in loop or concentrator mode. In loop mode the autosampler fills a sample loop which attached is to the Micro Injection valve. In the concentrator mode the autosampler will slowly fill a concentrator column which is used in place of the sample loop. Autosampler can hold 11 cassettes of 6 vials each.
 - 6.1.2 Rheodyne Micro Injection Valve:
Injection valve is electric operated. It receives load and inject instructions from the gradient pump program. The use of a Micro Injection Valve and sample loop will give reproducible chromatograms.
 - 6.1.3 GP50 Gradient Pump:
Steady pressure is the single most important parameter for good chromatography. The Dionex GPM is a programmable dual head gradient pump capable of delivering pressure up to 3000 psi. The GPM receives programming from the computer. GPM controls the timing of the injection, system pressure, and regen solution to the micro

membrane suppressor. See figure 2 for a plumbing diagram.

- 6.1.4 CD25 Conductivity Detector:
The Conductivity Detector responds to each ion as they elude off the column. Communications with the computer allow for automatic range, zero offset, and conductivity cell on and off.
- 6.1.5 AS18 Guard Column:
Guard Columns provide protection for the more expensive Separator Column. Samples are filtered as they are injected but some very small particulates will get through and collect on the Guard Column. The Guard Column is usually a short version of the longer Separator Column. We are presently using a Dionex AG18a Guard Column.
- 6.1.6 AS 18 Separator Column:
The 4 x 200 mm Dionex AS18a Separator Column has an ion exchange capacity of approximately 285 $\mu\text{eq}/\text{column}$. This resin is composed of a highly cross-linked (55%) 9 μm macroporous (2,000 A pore size) polyethylvinylbenzene/divinylbenzene substrate agglomerated with anion exchange latex that has been completely aminated. The latex has a polyvinylbenzyl backbone and carries the actual ion exchange sites which have a nominal efficiency for sulfate using standard operating conditions of at least 20,000 plates/meter. The highly cross-linked (55%) substrate core permits the use of organic solvents in the eluent without loss of bed stability. This column usually operates at a back pressure of 2,000psi at 1.0 mL/min. However, the column is capable of operating at back pressures up to 4,000 PSI.
- 6.1.7 Anion Self Regenerating Suppressor:
Suppression of the eluent or background conductivity allows the detector to see lower concentrations of ions. The Dionex ASRS-II Anion Self Regenerating Suppressor utilizes a semi-permeable membrane in combination with an electrical current to suppress eluent conductance.
- 6.1.8 LC25 Chromatography Oven – Maintains constant temperature.
- 6.1.9 Computer Software:
The Dionex ICS2500 system uses a windows based software package. The Chromel software affords the user flexibility during operation. Operator has control over all modules by setting up a Methods file. Sequence files are set up and run. Data is plotted in real time and results are integrated. Chromatograms are stored on disk and can be reprocessed if need be.

7. REAGENTS AND CONSUMABLE MATERIALS

- 7.1 Eluent:
The recommended eluent for running Cl, Br, SO₄, NO₃-N, O-PO₄, on the AS18a column must be purchased from Dionex. The cartridge is supplied with a serial number required to operate the system. As the system runs the eluent is depleted until 0% is reported by the system and a new cartridge is required.
- 7.2 ASRS:
The Dionex utilizes the Self-Regenerating Suppressor (SRS). AutoSuppression technology allows the SRS to continuously produce the ions required to neutralize the eluent by the electrolysis of water. The water supply is drawn from recycled eluent.

- 7.3 Sample Vials:
The Autosampler uses Dionex .5 mL or 5.0 mL plastic vials with 20 μ filter caps. Each 5mL cassette holds 6 vials and the .5mL cassettes hold 8 vials.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion Stock Solutions are 1000ppm Ricca© single analyte calibrants.
- 8.2 Working Standards:
Tare 1000 mL flask on balance and make the following standards on w/w basis.
Chloride: .050 mg/L, .200 mg/L, 1.000 mg/L, 2.000 mg/L
Bromide : .010mg/L, .050mg/L, .100mg/L, .25mg/L
Nitrate - Nitrogen: .010 mg/L, .050 mg/L, .100 mg/L, 0.500mg/L
Phosphate: .010 mg/L, .050 mg/L, .100 mg/L, .500 mg/L
Sulfate: .250 mg/L, .500 mg/L, 2.000 mg/L, 4.000 mg/L

9. QUALITY CONTROL

- 9.1 Stock solutions are purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.2 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO₄, Cl, NO₃-N, PO₄ and Br. The certified samples have a tolerance range associated with each value. Samples are run in triplicate.
- 9.3 A standard curve is determined before every analysis with the Ion Chromatograph. R squared must equal 0.99 or greater before samples are analyzed.
- 9.4 Daily QC checks are performed using solutions made from Spex IC Instrument Check Standard. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.5 Check limits of detection annually for all instruments.

10. PROCEDURE AND DATA PROCESSING

10.1 I.C. Startup:

1. Turn on computer.
2. Open up Helium tank and set pressure to 110 psi (preset), side regulator to water reservoir should be at 0psi.
3. Empty DI and put in fresh DI.
4. Set aside regulator to approximately 70psi.
5. Turn on instrument power strip.
6. Open IC software by clicking on the Chromeleon icon on desktop. If needed open browser and bring up the IC control panel by clicking on the panel file found near the bottom of the file, Deal_local.
7. Prime the pump. In the IC control panel window (detector, conductivity and pump), uncheck the box for pump connected (signifies local). Open the compartment door under the gradient pump module. Turn the transducer, (see Figure 1A and 1B) ½ turn counter clockwise. Push the prime button on the pump module and allow to run 1 minute. Turn off prime by pressing the prime button. Close the transducer by turning clockwise ½ turn. **DO NOT OVERTIGHTEN.**
8. In the IC panel, set the computer to remote by clicking on connected for the pump, detector and conductivity.
9. In the IC panel, click on startup. The pressure should be about 2000psi.
10. The instrument is stable once the background conductivity is stable. This can take 3-6 hours.
11. Make up calibrants and QC.
12. To run samples a sequence is created as follows:
 - a. Open a previous run and copy first seven lines (test thru Anion QC).
 - b. Open sequence wizard (file → new → using sequence wizard).
 - c. **Time base** = Coweeta. Click next, paste via clip board, click paste, click apply, click next.
 - d. **Method files** Program – browse for programs → anion2. **Quantification files** Browse for Method → anion2. **Sequence name** = file name = run date. **Data Source** = My Documents → Coweeta → year. Click Finish.
13. After new sequence is created copy and then paste .pgm, .qnt and shutdown program from last run sequence.
14. Pressure should rise to about 2000 psi. If not then pump may have lost it's prime. Refer to trouble shooting guide. When stable, pump pressure should level out near 2000 psi, conductivity ≤1us.

10.2 Sampler startup:

1. Load first sample cassette as follows: #1 test standard, #2 lowest standard, #3 next highest, #4 next highest, #5 highest standard, #6 QC Anions check standard.
2. Sample vials come in two sizes, .5mL and 5mL. Normally we use the 5mL size. Fill each vial to within ½ inch of the top. Caps and vials should be handled with gloves, taking precautions against possible contamination. Using the black insertion tool, caps should be pushed down into the vial such that the top of the cap is flush with the top.
3. Open top of sampler and find cassette holder on left side. Push cassette holder back and insert cassette with black dot at top right (closest to sample probe).
4. Press run on sampler and first vial will move into position.

10.3 To start the run:

1. On computer, click on **Batch** toolbar, click on add, click ready check then start.
2. Sampler must be in run mode.

Note: Data Acquisition must be off to begin.

- 10.3 Calibration:
1. Each chromatogram will take about 10 minutes. After the standards have run, look for any abnormalities such as: peak areas not close to what you would expect, peaks not identified correctly, or noisy baseline. If needed refer to trouble shooting guide.
 2. After test sample, all four standards and QC sample have run (about 60 min.) the system will automatically update the method. You may view the calibration of standards using the Qnt (Anions2.qnt) program.
 3. To look at the calibration curve, open the qnt file and click on the calibration tab. You should see a straight line intersecting each of the four points. The correlation coefficient should yield r^2 values of .98 or better. If not, then the standards are not made correctly or peaks were not integrated correctly. Make sure you use the standards specified in section 8.2. The anions2.met expects to see these concentrations.
 4. Check QC chromatograms and confirm that all ions are within acceptable limits.
- 10.4 Shutdown:
1. After last sample has finished pump will automatically stop if shutdown.pgm was loaded as the last sample in the sequence. System can be left in this mode until the next day.
 2. Release the pressure off the water reservoir (use side arm regulator).
 3. Turn gas off at helium tank.
 4. Turn off power to I.C. unit.
 5. Shut down computer.
- 10.5 Data Processing:
1. The Batch program is used to process the data file. After the Sequence has finished, double click on a sample in the sequence. The chromatogram of the sample will come up. First make sure the retention times are correct and each peak has been integrated correctly. Refer to section 10.6.2. If changes are made to the retention times of any components then the Method needs to be saved before running the Batch program.
 2. Reports are generated by the Batch program. The entire sequence must be highlighted by clicking on the square No. (to left of Name) located in the sequence header. Then right click to bring up selection and choose Batch Report. Choose report type (ie anions) under Use Report Definition. Selected Channel should be ECD_1. Do not print out. Select Export Option by checking Export. Location = MyDocument/year/Coweeta/Data Year/ Month Year. Directory Formula is left blank. File Name Formula = Sequence ID = ddmmy. For Export Format choose Excel file format, click next. Summary – Amount Only should be checked. File Format Excel version 5 or higher should be selected.
 3. Text files can be viewed using Excel spreadsheet program. Open the text files and look for any abnormalities before coping and pasting into the Workbook.
- 10.6 Trouble shooting:
1. Pump- loss of pressure or erratic pressure: Sometimes the pump will lose it's prime because of outgasing of eluent. In order to restore pump to normal operation you will need to force the gas out of the pump. Locate priming block, see Figure 1B. Insert 10ml syringe into luerloc and open valve on top of priming block. Slowly withdraw 8-10ml of eluent. Start the pump. Locate needle valve on pressure transducer (Figure 1A) and open counterclockwise while at the same time forcing eluent into the priming block with the syringe. The pump will make a sound like it is speeding up, this is

normal. Close valve on pressure transducer and stop the pump. Close valve on priming block and turn pump back on. Pressure should return to normal.

2. If peaks are not identified correctly you may need to alter retention times in the component table. Located in the sequence at the top are files to tell the instrument how to run the sequence and how to integrate the chromatogram. Double click on the .qnt file to open. **Only experienced operators should modify a file.** Click on the peak table tab and enter in the current retention times. Save the file.
3. If peaks areas are not integrated correctly you can modify integration parameters under the qnt file located in the sequence. **Only experienced operators should modify a file.**

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from Environmental Resource Associates and were diluted according to manufacturer's directions.

12. REFERENCES

- 12.1 Dionex Reference Library, P/N 053891-35, 2009, Dionex Corporation, 1228 Titan Way, Sunnyvale, California 94088-3603

Table 1. Method Detection Limits and Concentration Ranges for the Determination of Anions in Streamflow and Precipitation samples.

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|----------------|-----------------------------|--------------------------|
| Chloride | .009 | 0.05 - 1.00 |
| Bromide | .005 | 0.01 - 1.00 |
| Nitrate | .001 | 0.01 - 1.00 |
| Orthophosphate | .009 | 0.01 - 1.00 |
| Sulfate | .002 | 0.25 - 4.00 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 7

Table 2. Suggested Calibration Standards for Streamflow, Precipitation, Thrufall, and Lysimeter Samples at Coweeta

| Analyte | Calibration Standards mg/L |
|----------------|----------------------------|
| Chloride | 0.05, 0.20, 1.00, 2.00 |
| Bromide | 0.01, 0.05, 0.10, 0.25 |
| Nitrate | 0.01, 0.05, 0.10, 0.50 |
| Orthophosphate | 0.01, 0.05, 0.10, 0.50 |
| Sulfate | 0.25, 0.50, 2.00, 4.00 |

Figure 1A. Drawing of pump showing transducer.

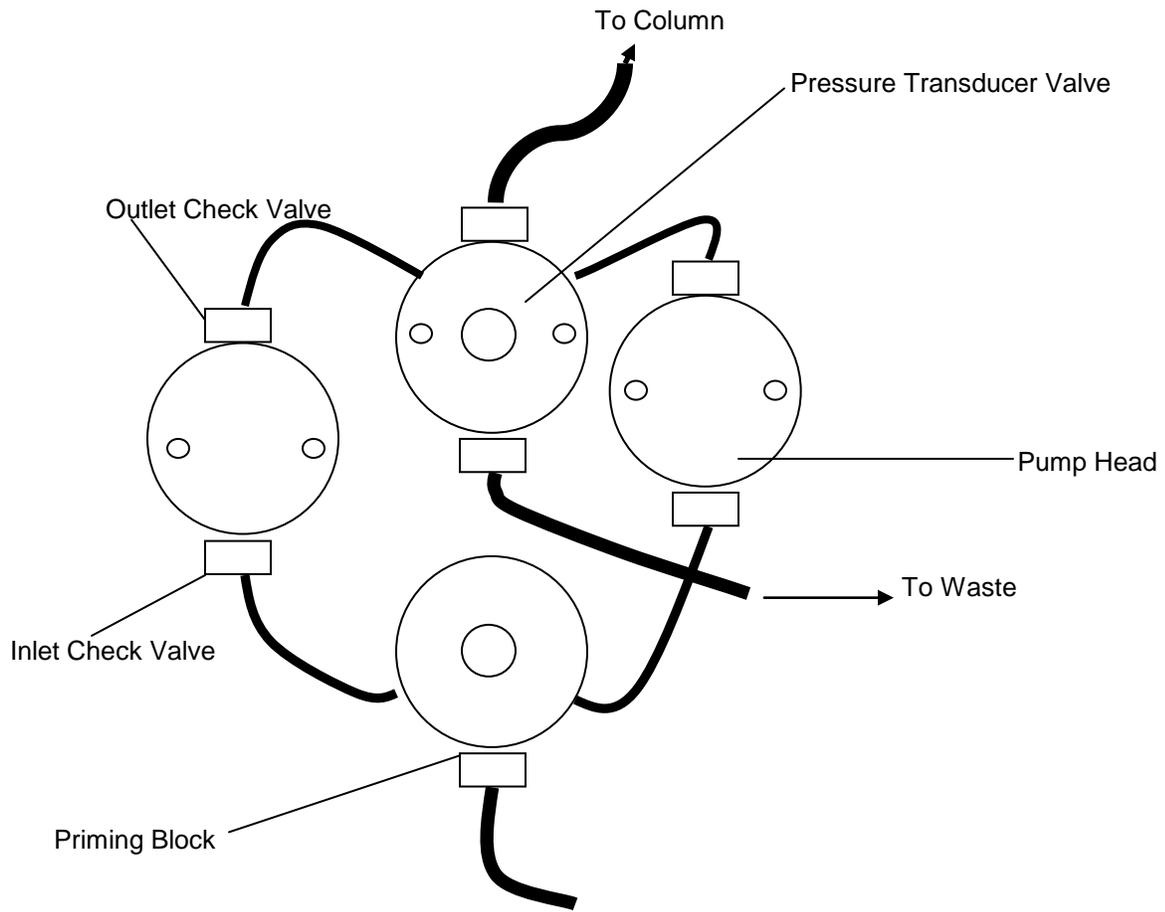


Figure 1B

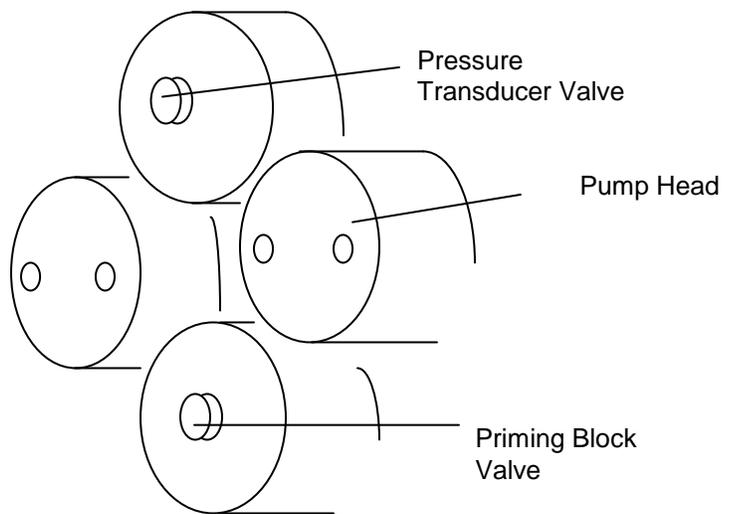


Figure 2 - Flow Diagram for ICS2500

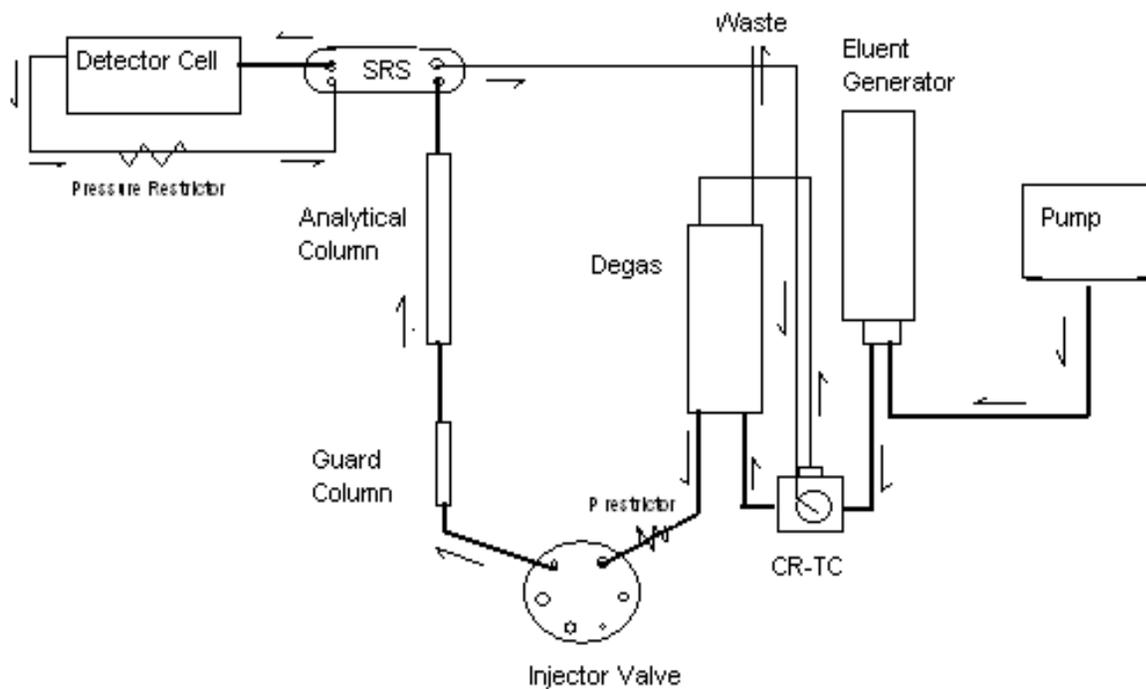
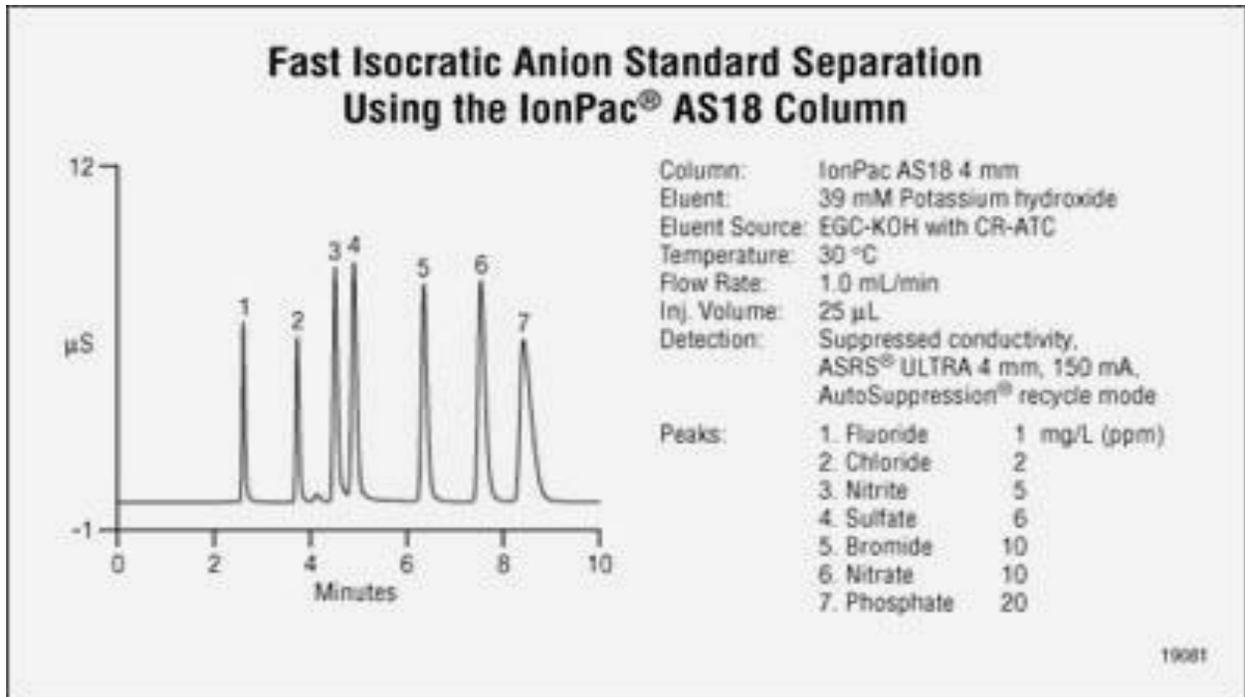


Figure 3. Chromatogram containing Chloride, Sulfate, Bromide, Nitrate, Orthophosphate



Automated Wet Chemistry
for Ammonium

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

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Laboratory Manager:

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FIGURES

1. System diagram for Astoria.
2. Manifold setup for Ammonium.

Astoria

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Ammonium in Stream, Precipitation, Thrufall, Lysimeter, and in extracted soil samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that are highly colored.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Ammonium reacts with alkaline phenol and hypochlorite to form indophenol blue. Sodium Nitroprusside is added to intensify the blue color. The reaction is speeded up by running the solution through a heating bath coil at 50⁰C. After the reaction has developed a color the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance
a = absorptivity
b = length of light path
c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentrations of the samples are calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 620 - 640 nm can cause

false positive readings. Samples at Coweeta are usually clear so this is no problem.

4.2 Avoid using Ammonia based cleaning products in the lab.

5. SAFETY

5.1 Extra precaution should be used when handling the liquid Phenol. Wear lab coat, gloves, and eye protection when using Phenol. Always work under a hood when making up the reagent, vapors can also be harmful.

5.2 Wear protective clothing when using Sodium Hypochlorite.

5.3 Wear protective clothing when using Sodium Nitroferricyanide.

5.4 Turn on the exhaust vent over the Astoria system. Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.

5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. Apparatus and Equipment

6.1 Base Module:

The Astoria 2 Analyzer base module incorporates the Micropump, Cartridge Base, and Detector into one compact unit. The unit also contains digital heating bath controls and power supply.

6.2 Autosampler:

The Astoria 311 XYZ sampler module, with traveling washpot, holds up to three 60 position sample racks and a 20 position calibrants rack. Sample and wash times, automatic shut off and sampling mode are all microprocessor controlled from the FASPac software package.

6.3 Auxiliary Pump:

The 322 Auxiliary Pump provides an external supply of solution to the 311 sampler.

6.4 Analytical Cartridge:

The analytical cartridge is a tray with the necessary fittings and components to bring together reagents, sample, and air to effect a chemical reaction, and is specifically configured for a particular analysis. Components include one or more of the following: glass mixing coils and fittings, heat baths, dialyzers, phase separators, and flowcell.

6.5 Detector

The detector measures the optical density (absorbance) of the product produced and converts this absorbance to a digital signal. The detector uses changeable wavelength filters and flowcells

6.6 Data Acquisition System

FASPac II data acquisition system provides hardware control and data reduction. It provides graphical presentation of data, which can be customized and/or exported. Analysis parameters are entered via on screen windows.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in ammonia-free deionized water.

- 7.1 Stock Complexing Reagent (1L):
Dissolve 33 g of potassium sodium tartrate and 24 g of sodium citrate in 800 ml of DI water. Adjust the pH of the solution to pH 5.0 with sulfuric acid (2.0 to 2.2 ml). Dilute to 1 L and filter to 0.45 μm .
- 7.2 Working Complex Reagent(100mL):
Add 4 drops of Brij-35 for each 100 ml of complexing reagent required for the day's run.
- 7.3 Stock 10 N Sodium Hydroxide (1L):
Dissolve 50 g of NaOH in 100 ml of DI water and dilute to 125 ml. Cool and store in a tightly capped, plastic container.
- 7.4 Alkaline Phenol (125 mL):
Place stir bar in 125 ml flask with 100 ml DI water. While stirring, add 11 ml of 10 N NaOH. Slow add 1.5 mL of Liquid Phenol (88%) and dilute to 125 ml. Filter to .45 μm . Store in dark polyethylene bottle at 4°C. Stability is approximately 1 month. Discard the reagent if it becomes dark amber in color.
- 7.5 Sodium Hypochlorite (100 mL):
Add 2.5 ml of sodium hypochlorite solution to 75 ml DI water. Dilute to 100 ml. Prepare daily.
- 7.6 Sodium Nitroferricyanide (250 ml):
Dissolve .125 g sodium nitroferricyanide in 200 ml DI water and dilute to 250 ml. Filter to 0.45 μm . Store in an amber bottle at room temperature, where it is stable approximately 1 month.
- 7.7 Diluent and Startup/Shutdown Solution (1L)
Add 1 to 2 ml of Brij-35 to 1000 ml DI water.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Store certified stock (1000 mg/L) in original container at 4°C. Replace yearly.
- 8.2 Working Standards:
0.01mg/L, 0.05mg/L, 0.10mg/L, 0.20mg/L, 0 .60 mg/L, 1.00 mg/L and 3.00 (for some samples).

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989.

- 9.4 A standard curve is determined before every analysis. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. Astoria 2 Operation

10.1 Preparation

- 1. Make sure all reagents are freshly prepared or are within allowable holding times and are free of particulates.
- 2. Verify that the correct filters and flowcell are installed in the detector.
- 3. Make sure there are no loose fittings or connections, and inspect pump tubes for excessive wear.

10.2 Operation

- 1. Turn on all instrument modules and computer.
- 2. Place all reagent lines in startup solution (DI water with appropriate surfactant). Place sampler wash lines in wash solution. Latch pump platens.
- 3. After flow has stabilized, verify a uniform bubble pattern throughout the manifold.
- 4. Turn on heating bath controls if required and adjust temperature properly for analysis.
- 5. Run the FASpac II software, enter user name and password.
- 6. Select the appropriate configuration from the list box in the tool bar at the top of the main menu. If one doesn't exist, see Configuration Setup in the operations manual. Enter a name for the run.
- 7. Connect the program to the system, select System – Connect from the main menu or click on the Connection (joined buckle, located in tool bar) or Connection Status (unjoined buckle) icon.
- 8. Select Channels button to bring channel windows to top. Select Options – Display Signal All and Options – Zero Signal and observe baseline on startup solution. When baseline is stable, place all lines in their appropriate reagents and allow to stabilize. Observe baseline, check for noise or drift, and aspirate a high standard to verify response. Consult troubleshooting guidelines if needed. Zero signal again.
- 9. Select the Sample Table button in the toolbar to access the sample table. Sample table should start with a Synchronization (SYNC), or high standard, followed by blanks to readjust baseline (w, W), calibrants (C1, C2, C3 ...), QC standard, blanks, unknowns, Check Calibrant (CC1 or QC1), blanks, unknowns, etc. Check Calibrants should be run periodically to verify run is still in calibrations, and blanks should be run periodically to readjust baseline.
- 10. To start the run select Run – Begin, or select the green “start” icon.
- 11. When the calibration peaks have been displayed, select View – Calibration (or calibration icon) to view the calibration curve
- 12. When the run is complete, select the Sample Run or Sample Report icon to view results. Select File – Print to print a report; select File – Export to export to an Excel file. Select the red “abort” icon to end run.

10.3 Shutdown

1. Short term shutdown (1-4 days): Place reagent lines in startup solution for 15 – 20 minutes. Use high pump speed for only short periods, as extended periods will cause premature pump tube wear. Unlatch platens. Caution: Serious pump tube damage will occur if platens remain latched with the pump power off. Cover and store reagents and calibrants as appropriate to prevent deterioration.
2. Long term shutdown (4 or more days): Place reagent lines in deionized water for 15 – 20 minutes. Remove reagent lines from water and pump air until the cartridge is dry. Unlatch platens.

10.4 General Guidelines for Troubleshooting

1. Isolate and define the problem.
Categorize the problem or symptom as chemistry, hydraulic, or electronic.
2. Do not overlook the obvious.
3. Rule out operator induced errors.
4. Eliminate one variable at a time.
5. Document the solution.
6. Refer to the operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions.

12. References

Astoria 2 Analyzer: Operation Manual, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II: Flow Analyzer Software Package, Version 2.12, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II Quick Start, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

Table 1 - Method Detection Limits

| Name | NH4-N |
|-------------|-------|
| NH4 | 0.045 |
| NH4 | 0.046 |
| NH4 | 0.050 |
| NH4 | 0.050 |
| NH4 | 0.047 |
| NH4 | 0.043 |
| NH4 | 0.042 |
| NH4 | 0.044 |
| NH4 | 0.045 |
| NH4 | 0.047 |
| avg | 0.046 |
| std | 0.003 |
| mdl | 0.008 |

Table 2 - Recommended Sample Tray/Table Layout

| Sample # Rack Position | Contents | Function | FASpac ID |
|-----------------------------------|---------------------|---------------------|------------------|
| 1 SR:1 | 80 – 100 % Standard | Synchronization | SYNC |
| 2 SR:2 | Blank | Calculate Carryover | CO |
| 3 SR:3 | Blank | Wash | w |
| 4 SR:4 | Blank | Readjust baseline | W |
| 5 SR:5 | Standard 1 | Calibration | C1 |
| 6 SR:6 | Standard 2 | Calibration | C2 |
| 7 SR:7 | Standard 3 | Calibration | C3 |
| 8 SR:8 | Standard 4 | Calibration | C4 |
| 9 SR:9 | Standard 5 | Calibration | C5 |
| 10 SR:10 | Standard 6 | Calibration | C6 |
| 11 SR:11 | QC Standard | Verify calibration | QC1 |
| 12 SR:12 | Blank | Wash | w |
| 13 SR:13 | Blank | Readjust baseline | W |
| 14-24 1:1 – 1:10 | Samples (unknowns) | | |
| 25 1:11 | Check Calibrant | Verify calibration | CCV |
| 26 1:12 | Blank | Readjust baseline | W |
| 27-37 1:13 – 1:22 | Samples (unknowns) | | |
| 38 1:23 | Check Calibrant | Verify calibration | CCV |
| 39 1:24 | Blank | Readjust baseline | W |
| 40 1:25 | Blank | Pause sampler | PAUSE |

Table 3: Example of Sampler Setup Window (Astoria *FASPac*)

| | | | |
|-------------------|-----------|---|-----------|
| Timing | | <input checked="" type="checkbox"/> Enable Autowash | |
| Sample Time | <u>25</u> | Autowash Interval | <u>10</u> |
| Wash Time | <u>35</u> | | |
| Repetition Counts | | | |
| Unknowns | <u>1</u> | | |
| Autowash | <u>2</u> | | |
| Calibrants | <u>2</u> | | |

Table 4: Example of Channel Properties – Calibration Window (Astoria *FASPac*)

| | | |
|---|----------------|-------|
| Calculate Concentrations From | Concentrations | |
| <input checked="" type="checkbox"/> Curves before | C1 | 0.000 |
| Curves around | C2 | 0.010 |
| Average all curves | C3 | 0.050 |
| | C4 | 0.100 |
| Curve Type | C5 | 0.200 |
| 1 st Order Polynomial ▼ (drop down) | C6 | 0.600 |
| Curve 1 Curve 2 | C7 | 1.000 |

Figure 1

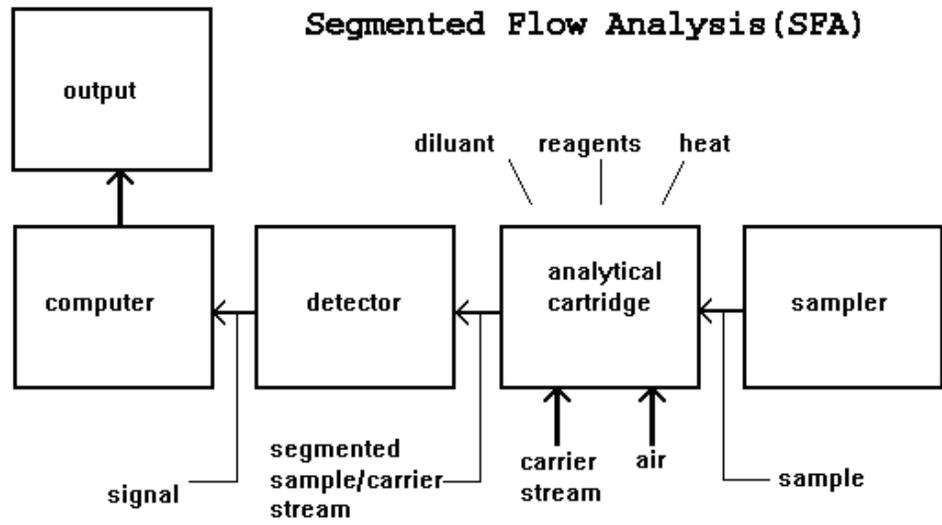
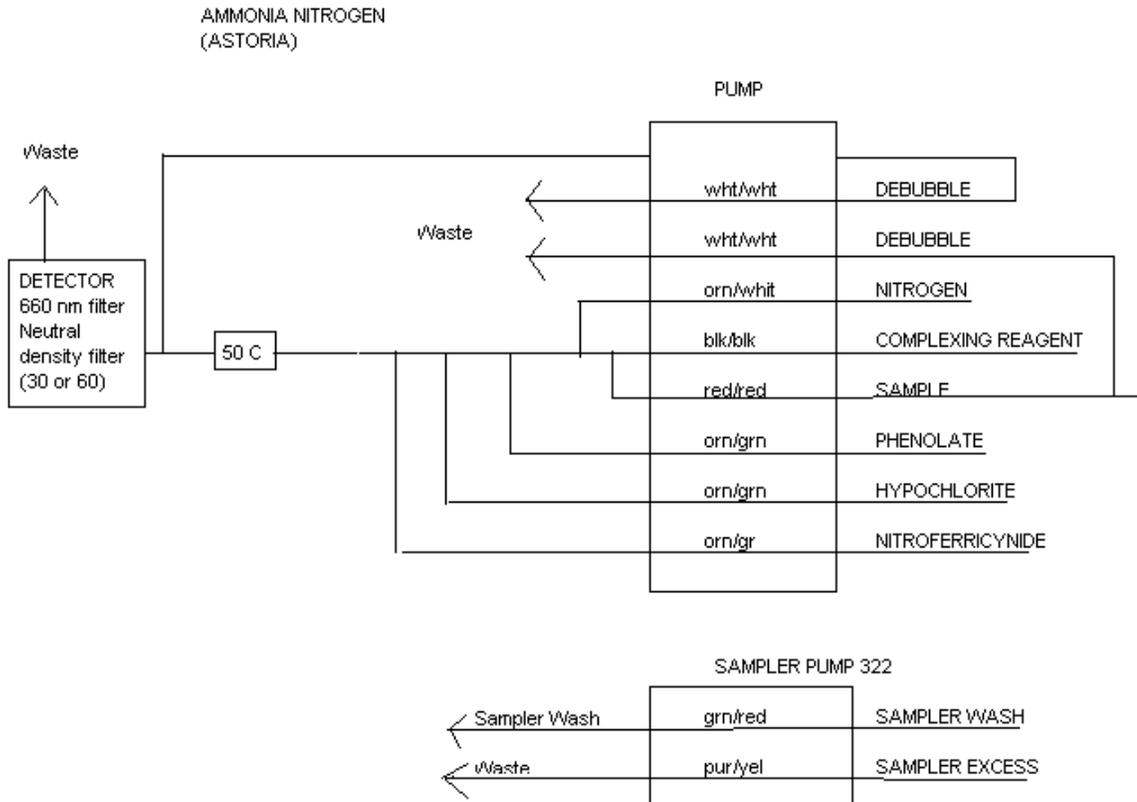


Figure 2



Automated Wet Chemistry
Nitrate by Cadmium Reduction

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

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4. Example of Channel Properties – Calibration Window.

FIGURES

1. System diagram for Astoria.
2. Manifold setup for Nitrate.

Astoria

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Nitrate in Stream, Precipitation, Thrufall, Lysimeter, and extracted soil samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that are highly colored.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Nitrate in the sample is reduced to Nitrite by passing through a cadmium coil reactor. The Nitrite then reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye. The reaction is measured at 520nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance

a = absorptivity

b = length of light path

c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 520 - 540 nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.
- 4.2 Samples high in turbidity must be filtered prior to analysis.
- 4.3 Samples high in iron or copper will need EDTA to form a complex.
- 4.4 Keep glassware that has been rinsed in HNO₃ separated for cations only.

5. SAFETY

- 5.1 Wear lab coat, gloves, and eye protection when using Hydrochloric Acid, Phosphoric Acid and Ammonium Hydroxide. Always work under a hood, vapors can be harmful.
- 5.2 Wear protective clothing when handling Cadmium coil.
- 5.3 When Cadmium coil has expired, store in tightly sealed container for later disposal at a hazardous waste treatment storage facility.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. Apparatus and Equipment

- 6.1 **Base Module:**
The Astoria 2 Analyzer base module incorporates the Micropump, Cartridge Base, and Detector into one compact unit. The unit also contains digital heating bath controls and power supply.
- 6.2 **Autosampler:**
The Astoria 311 XYZ sampler module, with traveling washpot, holds up to three 60 position sample racks and a 20 position calibrants rack. Sample and wash times, automatic shut off and sampling mode are all microprocessor controlled from the FASPac software package.
- 6.3 **Auxiliary Pump:**
The 322 Auxiliary Pump provides an external supply of solution to the 311 sampler.
- 6.4 **Analytical Cartridge:**
The analytical cartridge is a tray with the necessary fittings and components to bring together reagents, sample, and air to effect a chemical reaction, and is specifically configured for a particular analysis. Components include one or more of the following: glass mixing coils and fittings, heat baths, dialyzers, phase separators, and flowcell.
- 6.5 **Detector**
The detector measures the optical density (absorbance) of the product produced and converts this absorbance to a digital signal. The detector uses changeable wavelength filters and flowcells
- 6.6 **Data Acquisition System**
FASPac II data acquisition system provides hardware control and data reduction. It provides graphical presentation of data, which can be customized and/or exported. Analysis parameters are entered via on screen windows.

7. REAGENTS AND CONSUMABLE MATERIALS

- 7.1 Working Imidazole (0.05) M Buffer
Add 0.5 ml stock copper sulfate 'B', 0.001 M to 50 ml of stock imidazole buffer, 0.1 M. Dilute to 100 ml with DI water, add 0.1 ml (3 drops) 50 % Triton X-100 and mix thoroughly. Prepare fresh daily.
- 7.2 1.0 N Hydrochloric Acid
Add 8.3 ml of concentrated hydrochloric acid to about 80 ml deionized water contained in 100 ml volumetric flask. Dilute to the mark with deionized water and mix well.
- 7.3 Cupric Sulfate Solution
Dissolve 20 g of cupric sulfate in approximately 900 ml deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix well.
- 7.4 Stock Copper Sulfate Solution 'A', 0.01 M
Dissolve 2.5 g of copper sulfate in about 600 ml of DI water. Dilute to one liter with DI water.
- 7.5 Stock Copper Sulfate Solution 'B', 0.001 M
Dilute 10.0 ml of stock copper sulfate, 'A' 0.01 M to 100 ml with DI water
- 7.6 50 % Triton X-100
Add 50 ml Triton X 100 to 50 ml isopropanol. Mix thoroughly.
- 7.7 Start-Up Solution
Add .1 ml of 50 % Triton X-100 solution to every 100 ml DI water.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Store certified stock (1000 mg/L) in original container at 4°C. Replace annually.
- 8.2 Working Standards:
0.01, 0.05, 0.10, 0.20, 0.600 mg/L, 1.000 mg/L

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989.
- 9.4 A standard curve is determined before every analysis.
R squared must equal 0.98 or greater before samples are analyzed.

- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable range for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.
10. Astoria 2 Operation
- 10.1 Preparation
1. Make sure all reagents are freshly prepared or are within allowable holding times and are free of particulates.
 2. Verify that the correct filters and flowcell are installed in the detector.
 3. Make sure there are no loose fittings or connections, and inspect pump tubes for excessive wear.
- 10.2 Operation
1. Turn on all instrument modules and computer.
 2. Place all reagent lines in startup solution (DI water with appropriate surfactant). Place sampler wash lines in wash solution. Latch pump platens.
 3. After flow has stabilized, verify a uniform bubble pattern throughout the manifold.
 4. Turn on heating bath controls if required and adjust temperature properly for analysis.
 5. Run the FASPac II software, enter user name and password.
 6. Select the appropriate configuration from the list box in the tool bar at the top of the main menu. If one doesn't exist, see Configuration Setup in the operations manual. Enter a name for the run.
 7. Connect the program to the system, select System – Connect from the main menu or click on the Connection (joined buckle, located in tool bar) or Connection Status (unjointed buckle) icon.
 8. Select Channels button to bring channel windows to top. Select Options – Display Signal All and Options – Zero Signal and observe baseline on startup solution. When baseline is stable, place all lines in their appropriate reagents and allow to stabilize. Observe baseline, check for noise or drift, and aspirate a high standard to verify response. Consult troubleshooting guidelines if needed. Zero signal again.
 9. Select the Sample Table button in the toolbar to access the sample table. Sample table should start with a Synchronization (SYNC), or high standard, followed by blanks to readjust baseline (w, W), calibrants (C1, C2, C3 ...), QC standard, blanks, unknowns, Check Calibrant (CC1 or QC1), blanks, unknowns, etc. Check Calibrants should be run periodically to verify run is still in calibrations, and blanks should be run periodically to readjust baseline.
 10. To start the run select Run – Begin, or select the green “start” icon.
 11. When the calibration peaks have been displayed, select View – Calibration (or calibration icon) to view the calibration curve
 12. When the run is complete, select the Sample Run or Sample Report icon to view results. Select File – Print to print a report; select File – Export to export to an Excel file. Select the red “abort” icon to end run.

10.3 Shutdown

1. Short term shutdown (1-4 days): Place reagent lines in startup solution for 15 – 20 minutes. Use high pump speed for only short periods, as extended periods will cause premature pump tube wear. Unlatch platens. Caution: Serious pump tube damage will occur if platens remain latched with the pump power off. Cover and store reagents and calibrants as appropriate to prevent deterioration.
2. Long term shutdown (4 or more days): Place reagent lines in deionized water for 15 – 20 minutes. Remove reagent lines from water and pump air until the cartridge is dry. Unlatch platens.

10.4 General Guidelines for Troubleshooting

1. Isolate and define the problem.
Categorize the problem or symptom as chemistry, hydraulic, or electronic.
2. Do not overlook the obvious.
3. Rule out operator induced errors.
4. Eliminate one variable at a time.
5. Document the solution.
6. Refer to the operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions.

12. References

Astoria 2 Analyzer: Operation Manual, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II: Flow Analyzer Software Package, Version 2.12, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II Quick Start, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

Table 1 - Method Detection Limits

| Name .050ppm | NO3-N |
|-------------------------------|--------------|
| NO3 | 0.051 |
| NO3 | 0.049 |
| NO3 | 0.047 |
| NO3 | 0.049 |
| NO3 | 0.052 |
| NO3 | 0.052 |
| NO3 | 0.048 |
| NO3 | 0.046 |
| NO3 | 0.047 |
| NO3 | 0.047 |
| avg | 0.049 |
| std | 0.002 |
| mdl | 0.006 |

Table 2. Recommended Sample Tray/Table Layout

| Sample # Rack Position | Contents | Function | FASPac ID |
|-----------------------------------|---------------------|---------------------|------------------|
| 1 SR:1 | 80 – 100 % Standard | Synchronization | SYNC |
| 2 SR:2 | Blank | Calculate Carryover | CO |
| 3 SR:3 | Blank | Wash | w |
| 4 SR:4 | Blank | Readjust baseline | W |
| 5 SR:5 | Standard 1 | Calibration | C1 |
| 6 SR:6 | Standard 2 | Calibration | C2 |
| 7 SR:7 | Standard 3 | Calibration | C3 |
| 8 SR:8 | Standard 4 | Calibration | C4 |
| 9 SR:9 | Standard 5 | Calibration | C5 |
| 10 SR:10 | Standard 6 | Calibration | C6 |
| 11 SR:11 | QC Standard | Verify calibration | QC1 |
| 12 SR:12 | Blank | Wash | w |
| 13 SR:13 | Blank | Readjust baseline | W |
| 14-24 1:1 – 1:10 | Samples (unknowns) | | |
| 25 1:11 | Check Calibrant | Verify calibration | CCV |
| 26 1:12 | Blank | Readjust baseline | W |
| 27-37 1:13 – 1:22 | Samples (unknowns) | | |
| 38 1:23 | Check Calibrant | Verify calibration | CCV |
| 39 1:24 | Blank | Readjust baseline | W |
| 40 1:25 | Blank | Pause sampler | PAUSE |

Table 3: Example of Sampler Setup Window (Astoria *FASPac*)

| | | | |
|-------------------|-----------|---|-----------|
| Timing | | <input checked="" type="checkbox"/> Enable Autowash | |
| Sample Time | <u>25</u> | Autowash Interval | <u>10</u> |
| Wash Time | <u>35</u> | | |
| Repetition Counts | | | |
| Unknowns | <u>1</u> | | |
| Autowash | <u>2</u> | | |
| Calibrants | <u>2</u> | | |

Table 4: Example of Channel Properties – Calibration Window (Astoria *FASPac*)

| | | |
|---|----------------|-------|
| Calculate Concentrations From | Concentrations | |
| <input checked="" type="checkbox"/> Curves before | C1 | 0.000 |
| Curves around | C2 | 0.010 |
| Average all curves | C3 | 0.050 |
| | C4 | 0.100 |
| Curve Type | C5 | 0.200 |
| 1 st Order Polynomial ▼ (drop down) | C6 | 0.600 |
| Curve 1 Curve 2 | C7 | 1.000 |

Figure 1

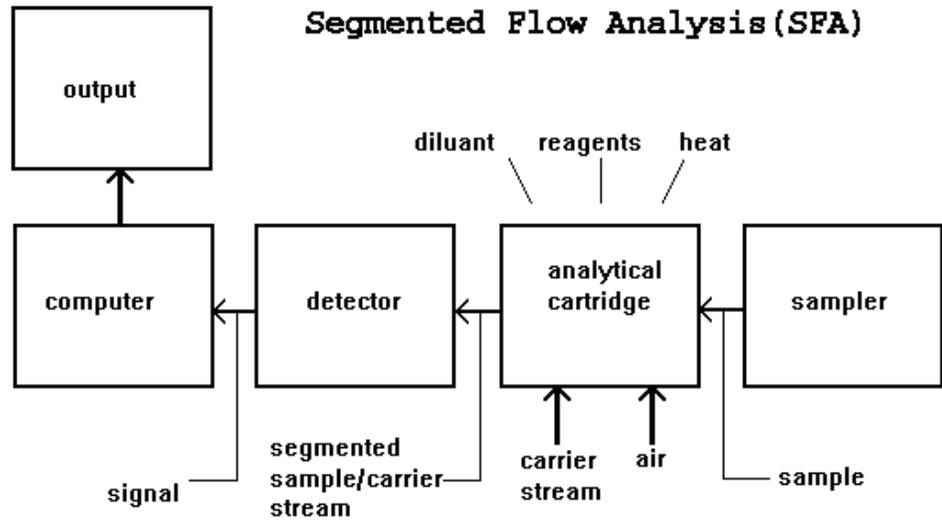
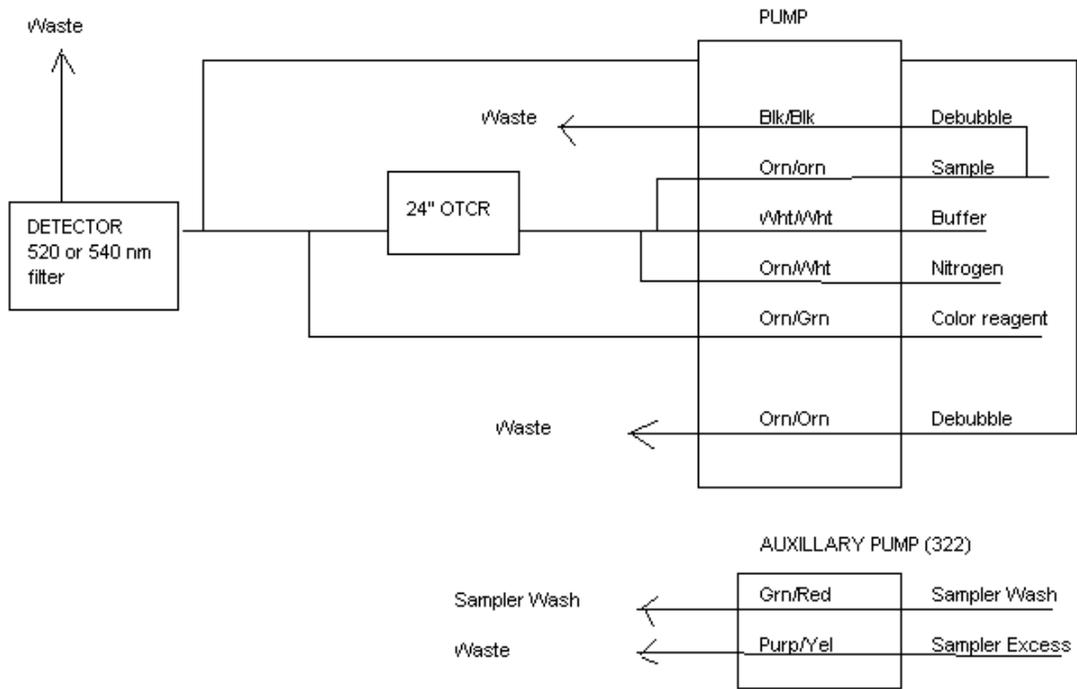


Figure 2

NITRATE/NITRITE NITROGEN
(ASTORIA)



Automated Wet Chemistry
For Orthophosphate

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
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Sponsoring Agency:

James M. Vose, Project Leader

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INDEX

Astoria

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1. Method Detection Limits.
2. Recommended Sample Tray/Table Layout
3. Example of Sampler Set up Window
4. Example of Channel Properties – Calibration Window.

FIGURES

1. System diagram for Astoria.
2. Manifold setup for Orthophosphate.

Astoria

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Orthophosphate in Stream, Precipitation, Thrufall, Lysimeter, and extracted soil samples.
- 1.2 Orthophosphate measured by this method is Total Reactive Phosphorus. Refer to section 424 in Standard Methods for the Examination of Water and Wastewater for further information on fractions of Phosphorus.
- 1.3 Method detection limits are summarized in Table 1.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Orthophosphate in the sample reacts with Ammonium Molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ and Antimony Potassium Tartrate $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot \frac{1}{2}\text{H}_2\text{O}$ in acid media to form an Antimony-phosphomolybdate complex. The complex is reduced with Ascorbic Acid $\text{C}_6\text{H}_8\text{O}_6$ to form a blue color that is measured at 660nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance

a = absorptivity

b = length of light path

c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 640 - 660nm can cause false positive readings. Samples from coastal waters can present problems.
- 4.2 Samples high in turbidity must be filtered prior to analysis.
- 4.3 Silica concentrations greater than 10mg/L can cause positive interference. SiO₂ concentrations of 20mg/L would cause .005mg/L positive readings. Samples at Coweeta run below 20mg/L.

5. SAFETY

- 5.1 Wear lab coat, gloves, and eye protection when using Sulfuric Acid, Antimony Potassium Tartrate, and Ammonium Molybdate. Always work under a hood, vapors can be harmful.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. Apparatus and Equipment

- 6.1 Base Module:
The Astoria 2 Analyzer base module incorporates the Micropump, Cartridge Base, and Detector into one compact unit. The unit also contains digital heating bath controls and power supply.
- 6.2 Autosampler:
The Astoria 311 XYZ sampler module, with traveling washpot, holds up to three 60 position sample racks and a 20 position calibrants rack. Sample and wash times, automatic shut off and sampling mode are all microprocessor controlled from the FASPac software package.
- 6.3 Auxiliary Pump:
The 322 Auxiliary Pump provides an external supply of solution to the 311 sampler.
- 6.4 Analytical Cartridge:
The analytical cartridge is a tray with the necessary fittings and components to bring together reagents, sample, and air to effect a chemical reaction, and is specifically configured for a particular analysis. Components include one or more of the following: glass mixing coils and fittings, heat baths, dialyzers, phase separators, and flowcell.
- 6.5 Detector
The detector measures the optical density (absorbance) of the product produced and converts this absorbance to a digital signal. The detector uses changeable wavelength filters and flowcells
- 6.6 Data Acquisition System
FASPac II data acquisition system provides hardware control and data reduction. It provides graphical presentation of data, which can be customized and/or exported. Analysis parameters are entered via on screen windows.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Phosphate free DI water. Filter the all reagents prior to use.

7.1 Sulfuric Acid, .5N (250 mL)

Add 35 mL of concentrated Sulfuric Acid H_2SO_4 to 200 mL of DI water. Mix well and dilute to final volume of 250 mL.

7.2 Stock Ammonium Molybdate Reagent (250 mL)

Dissolve 10g of Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 200 mL of DI water. Dilute to 250 mL with DI water. Store reagent in dark polyethylene bottle at 4° C. Reagent is not stable for more than two days. If reagent turns a faint blue, then remake.

7.3 Stock Antimony Potassium Tartrate (250mL)

Mix well Dissolve .75g of Antimony Potassium Tartrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}2H_2O$ in 200 mL of DI water. and dilute to 250 mL with DI water. Store in a plastic bottle at 4°C for no more than one week.

7.4 Ascorbic Acid (250 mL)

Dissolve 4.4g of Ascorbic Acid $C_6H_8O_6$ in 200mL DI water with 12.5 mL of Acetone. Dilute to 250 mL with DI water. Store in a plastic bottle at 4°C for no more than one week.

7.5 Color Reagent (200 mL)

Stock Sulfuric acid, 5N-----100mL
Stock Antimony Potassium Tartrate Solution-----10mL
Stock Ammonium Molybdate Solution-----30mL
Stock Ascorbic Acid Solution-----60mL
Dowfax 2A1-----0.5mL

Add reagents in the order stated and mix after each addition. This will prevent the ascorbic acid from turning a darker color when the solution is first made. Prepare reagent daily.

8. CALIBRATION AND STANDARDIZATION

8.1 Anion calibrants, 1000mg/L are purchased from Ricca yearly.

8.2 Working Standards:

For Coweeta samples: .010 mg/L, .050 mg/L, .100 mg/L, .200 mg/L
For Double Acid Extraction samples: 5.0mg/L, 10.0mg/L, 20.0mg/L, 30.0mg/L

9. QUALITY CONTROL

9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μ mho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μ mho indicates a dirty cartridge and the cation exchange cartridges are changed.

9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.

- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO₄, Cl, NO₃, NH₄, PO₄, and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.
- 9.4 A standard curve is determined before every analysis with the Astoria. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.
- 10.1 Preparation
1. Make sure all reagents are freshly prepared or are within allowable holding times and are free of particulates.
 2. Verify that the correct filters and flowcell are installed in the detector.
 3. Make sure there are no loose fittings or connections, and inspect pump tubes for excessive wear.
- 10.2 Operation
1. Turn on all instrument modules and computer.
 2. Place all reagent lines in startup solution (DI water with appropriate surfactant). Place sampler wash lines in wash solution. Latch pump platens.
 3. After flow has stabilized, verify a uniform bubble pattern throughout the manifold.
 4. Turn on heating bath controls if required and adjust temperature properly for analysis.
 5. Run the FASPac II software, enter user name and password.
 6. Select the appropriate configuration from the list box in the tool bar at the top of the main menu. If one doesn't exist, see Configuration Setup in the operations manual. Enter a name for the run.
 7. Connect the program to the system, select System – Connect from the main menu or click on the Connection (joined buckle, located in toolbar) or Connection Status (unjoined buckle) icon.
 8. Select Channels button to bring channel windows to top. Select Options – Display Signal All and Options – Zero Signal and observe baseline on startup solution. When baseline is stable, place all lines in their appropriate reagents and allow stabilizing. Observe baseline, check for noise or drift, and aspirate a high standard to verify response. Consult troubleshooting guidelines if needed. Zero signal again.
 9. Select the Sample Table button in the toolbar to access the sample table. Sample table should start with a Synchronization (SYNC), or high standard, followed by blanks to readjust baseline (w, W), calibrants (C1, C2, C3 ...), QC standard, blanks, unknowns, Check calibrant (CC1 or QC1), blanks, unknowns, etc. Check Calibrants should be run periodically to verify run is still in calibrations, and blanks should be run periodically to readjust baseline.
 10. To start the run select Run – Begin, or select the green “start” icon.
 11. When the calibration peaks have been displayed, select View – Calibration (or calibration icon) to view the calibration curve

12. When the run is complete, select the Sample Run or Sample Report icon to view results. Select File – Print to print a report; select File – Export to export to an Excel file. Select the red “abort” icon to end run.
- 10.3 Shutdown
1. Short term shutdown (1-4 days): Place reagent lines in startup solution for 15 – 20 minutes. Use high pump speed for only short periods, as extended periods will cause premature pump tube wear. Unlatch platens. Caution: Serious pump tube damage will occur if platens remain latched with the pump power off. Cover and store reagents and calibrants as appropriate to prevent deterioration.
 2. Long term shutdown (4 or more days): Place reagent lines in deionized water for 15 – 20 minutes. Remove reagent lines from water and pump air until the cartridge is dry. Unlatch platens.
- 10.4 General Guidelines for Troubleshooting
1. Isolate and define the problem. Categorize the problem or symptom as chemistry, hydraulic, or electronic.
 2. Do not overlook the obvious.
 3. Rule out operator induced errors.
 4. Eliminate one variable at a time.
 5. Document the solution.
 6. Refer to the operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions.

12. References

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FASPac II: Flow Analyzer Software Package, Version 2.12, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II Quick Start, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

Table 1 - Method Detection Limits

| Name | O-PO4 |
|---------------------|-------|
| .050pp m | |
| O-PO4 | 0.044 |
| O-PO4 | 0.045 |
| O-PO4 | 0.051 |
| O-PO4 | 0.052 |
| O-PO4 | 0.048 |
| O-PO4 | 0.056 |
| O-PO4 | 0.051 |
| O-PO4 | 0.055 |
| O-PO4 | 0.041 |
| O-PO4 | 0.052 |
| avg | 0.050 |
| std | 0.005 |
| mdl | 0.014 |

Table 2: Recommended Sample Tray/Table Layout

| Sample # Rack Position | Contents | Function | FASPac ID |
|-----------------------------------|---------------------|---------------------|------------------|
| 1 SR:1 | 80 – 100 % Standard | Synchronization | SYNC |
| 2 SR:2 | Blank | Calculate Carryover | CO |
| 3 SR:3 | Blank | Wash | w |
| 4 SR:4 | Blank | Readjust baseline | W |
| 5 SR:5 | Standard 1 | Calibration | C1 |
| 6 SR:6 | Standard 2 | Calibration | C2 |
| 7 SR:7 | Standard 3 | Calibration | C3 |
| 8 SR:8 | Standard 4 | Calibration | C4 |
| 9 SR:9 | Standard 5 | Calibration | C5 |
| 10 SR:10 | Standard 6 | Calibration | C6 |
| 11 SR:11 | QC Standard | Verify calibration | QC1 |
| 12 SR:12 | Blank | Wash | w |
| 13 SR:13 | Blank | Readjust baseline | W |
| 14-24 1:1 – 1:10 | Samples (unknowns) | | |
| 25 1:11 | Check Calibrant | Verify calibration | CCV |
| 26 1:12 | Blank | Readjust baseline | W |
| 27-37 1:13 – 1:22 | Samples (unknowns) | | |
| 38 1:23 | Check Calibrant | Verify calibration | CCV |
| 39 1:24 | Blank | Readjust baseline | W |
| 40 1:25 | Blank | Pause sampler | PAUSE |

Table 3: Example of Sampler Setup Window (Astoria *FASPac*)

| | | | |
|-------------------|-----------|---|-----------|
| Timing | | <input checked="" type="checkbox"/> Enable Autowash | |
| Sample Time | <u>25</u> | Autowash Interval | <u>10</u> |
| Wash Time | <u>35</u> | | |
| Repetition Counts | | | |
| Unknowns | <u>1</u> | | |
| Autowash | <u>2</u> | | |
| Calibrants | <u>2</u> | | |

Table 4: Example of Channel Properties – Calibration Window (Astoria *FASPac*)

| | | |
|---|----------------|-------|
| Calculate Concentrations From | Concentrations | |
| <input checked="" type="checkbox"/> Curves before | C1 | 0.000 |
| Curves around | C2 | 0.010 |
| Average all curves | C3 | 0.050 |
| | C4 | 0.100 |
| Curve Type | C5 | 0.200 |
| 1 st Order Polynomial ▼ (drop down) | C6 | 0.600 |
| Curve 1 Curve 2 | C7 | 1.000 |

Figure 1

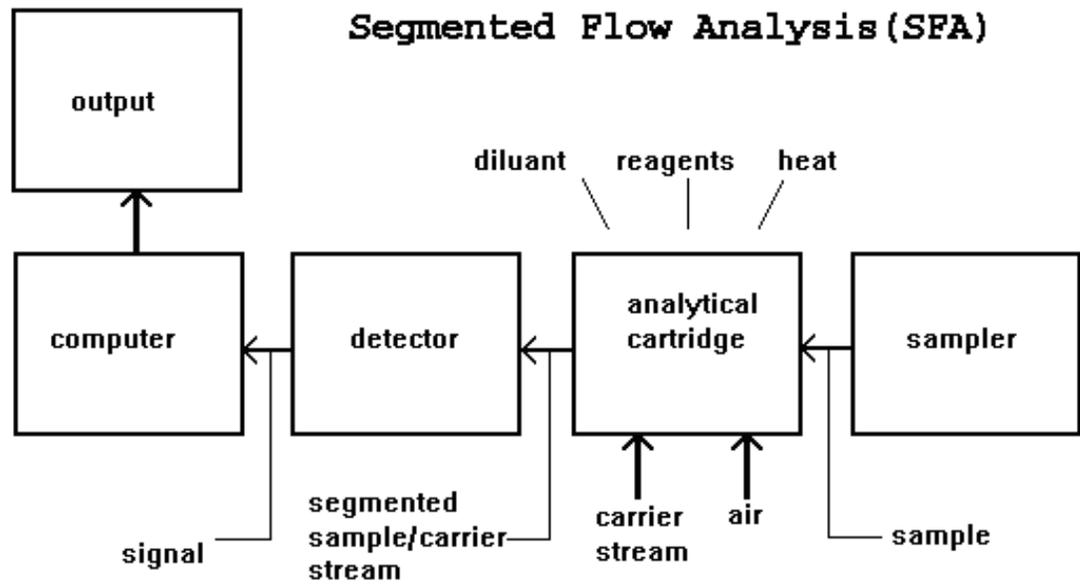
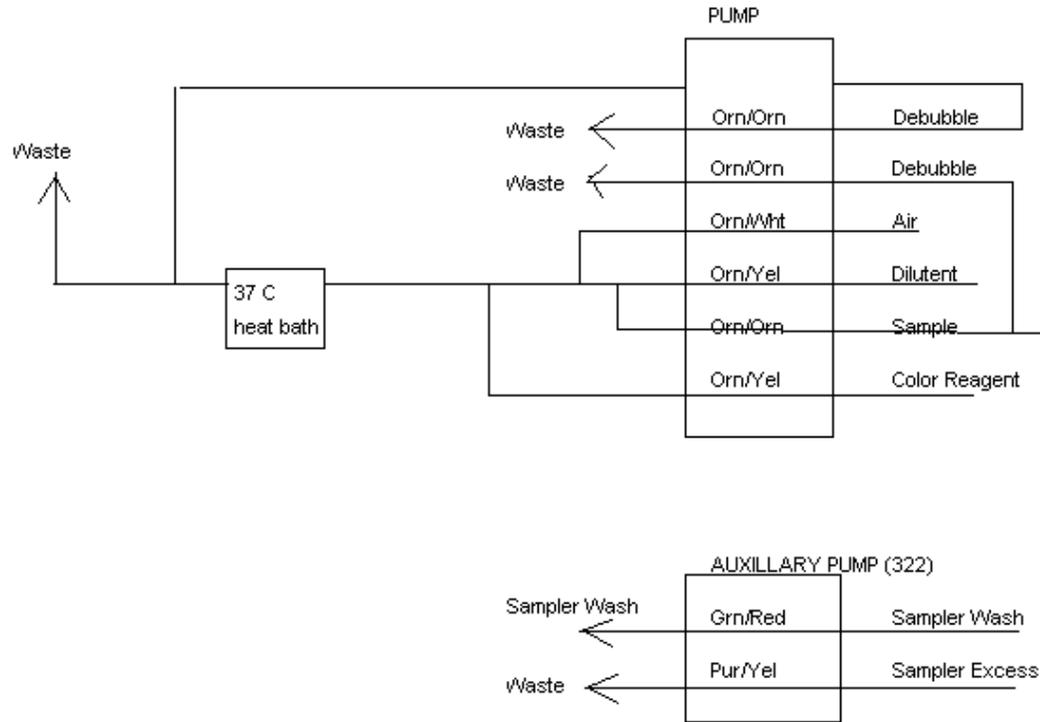


Figure 2

ORTHO-PHOSPHATE
flow-diagram ASTORIA2



Automated Wet Chemistry
For Silicate

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
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Sponsoring Agency:

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U.S. Forest Service
University of Georgia

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Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

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Astoria

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1. Method Detection Limits.
2. Recommended Sample Tray/Table Layout
3. Example of Sampler Set up Window
4. Example of Channel Properties – Calibration Window.

FIGURES

1. System diagram for Astoria.
2. Manifold setup for Silicate.

Astoria

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Silica in Stream, Precipitation, Thrufall, and Lysimeter samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that have been frozen.

2. SUMMARY OF METHOD

2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discrete. The Silicate then reacts with Ammonium The complex is reduced by Ascorbic Acid $C_6H_8O_6$ to form Molybdenum Blue. The reaction is measured at 660nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance
a = absorptivity
b = length of light path
c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 640 - 660nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.
- 4.2 Samples high in turbidity must be filtered prior to analysis.
- 4.3 Phosphates interfere but are suppressed by Oxalic Acid.
- 4.4 Do not freeze samples, silica will precipitate out of solution and cause false negative readings.

5. SAFETY

- 5.1 Wear lab coat, gloves, and eye protection when using Sulfuric Acid, Oxalic Acid Acetone, and Ammonium Molydate. Always work under a hood, vapors can be harmful.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. Apparatus and Equipment

- 6.1 **Base Module:**
The Astoria 2 Analyzer base module incorporates the Micropump, Cartridge Base, and Detector into one compact unit. The unit also contains digital heating bath controls and power supply.
- 6.2 **Autosampler:**
The Astoria 311 XYZ sampler module, with traveling washpot, holds up to three 60 position sample racks and a 20 position calibrants rack. Sample and wash times, automatic shut off and sampling mode are all microprocessor controlled from the FASPac software package.
- 6.3 **Auxiliary Pump:**
The 322 Auxiliary Pump provides an external supply of solution to the 311 sampler.
- 6.4 **Analytical Cartridge:**
The analytical cartridge is a tray with the necessary fittings and components to bring together reagents, sample, and air to effect a chemical reaction, and is specifically configured for a particular analysis. Components include one or more of the following: glass mixing coils and fittings, heat baths, dialyzers, phase separators, and flowcell.
- 6.5 **Detector**
The detector measures the optical density (absorbance) of the product produced and converts this absorbance to a digital signal. The detector uses changeable wavelength filters and flowcells
- 6.6 **Data Acquisition System**
FASPac II data acquisition system provides hardware control and data reduction. It provides graphical presentation of data, which can be customized and/or exported. Analysis parameters are entered via on screen windows.

7. REAGENTS AND CONSUMABLE MATERIALS

- 7.1 Add 2.8 ml concentrated sulfuric acid to 800 ml DI water. Mix well and dilute to 1L.
- 7.2 **Stock Stannous Chloride**
While stirring, add 10 ml of hydrochloric acid to 10 ml DI water. Dissolve 10 g of stannous chloride in the acidic solution. Heating may be necessary to obtain complete dissolution. Store the stock solution in a tightly closed plastic container and refrigerate at 2 – 8 °C.
- 7.3 **Hydrochloric Acid 1.2 N**
Add 100 ml of hydrochloric acid to 800 ml DI water. Dilute to 1 L. Filter to 45 µm and store in a plastic container.
- 7.4 **Working Stannous Chloride Reagent**
Mix 1.0 ml of stock stannous chloride solution with 50 ml of 1.2 N hydrochloric solution in a plastic container. Prepare fresh daily. Add to sample stream last (at least 5 minutes after adding molybdate solution).
- 7.5 **Startup Solution/Shutdown Solution**
Add 2-3 ml Dowfax 2A1 per 1 L DI water.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Use certified anion stock solution (1000 mg/L). Store in original plastic container at 4 °C. Replace after 1 year.
- 8.2 **Working Standards:**
For stream samples: 2.0 mg/L, 4.0 mg/L, 8.0 mg/L, 12.0 mg/L, 20.0 mg/L.

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 µmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 µmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO₄, Cl, NO₃, NH₄, PO₄, and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.

- 9.4 A standard curve is determined before every analysis with the Astoria. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable range for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.
- 10.1 Preparation
1. Make sure all reagents are freshly prepared or are within allowable holding times and are free of particulates.
 2. Verify that the correct filters and flowcell are installed in the detector.
 3. Make sure there are no loose fittings or connections, and inspect pump tubes for excessive wear.
- 10.2 Operation
1. Turn on all instrument modules and computer.
 2. Place all reagent lines in startup solution (DI water with appropriate surfactant). Place sampler wash lines in wash solution. Latch pump platens.
 3. After flow has stabilized, verify a uniform bubble pattern throughout the manifold.
 4. Turn on heating bath controls if required and adjust temperature properly for analysis.
 5. Run the FASPac II software, enter user name and password.
 6. Select the appropriate configuration from the list box in the tool bar at the top of the main menu. If one doesn't exist, see Configuration Setup in the operations manual. Enter a name for the run.
 7. Connect the program to the system, select System – Connect from the main menu or click on the Connection (joined buckle, located in toolbar) or Connection Status (unjoined buckle) icon.
 8. Select Channels button to bring channel windows to top. Select Options – Display Signal All and Options – Zero Signal and observe baseline on startup solution. When baseline is stable, place all lines in their appropriate reagents and allow to stabilize. Observe baseline, check for noise or drift, and aspirate a high standard to verify response. Consult troubleshooting guidelines if needed. Zero signal again.
 9. Select the Sample Table button in the toolbar to access the sample table. Sample table should start with a Synchronization (SYNC), or high standard, followed by blanks to readjust baseline (w, W), calibrants (C1, C2, C3 ...), QC standard, blanks, unknowns, Check calibrant (CC1 or QC1), blanks, unknowns, etc. Check calibrants should be run periodically to verify run is still in calibrations, and blanks should be run periodically to readjust baseline.
 10. To start the run select Run – Begin, or select the green “start” icon.
 11. When the calibration peaks have been displayed, select View – Calibration (or calibration icon) to view the calibration curve
 12. When the run is complete, select the Sample Run or Sample Report icon to view results. Select File – Print to print a report; select File – Export to export to an Excel file. Select the red “abort” icon to end run.

10.3 Shutdown

1. Short term shutdown (1-4 days): Place reagent lines in startup solution for 15 – 20 minutes. Use high pump speed for only short periods, as extended periods will cause premature pump tube wear. Unlatch platens. Caution: Serious pump tube damage will occur if platens remain latched with the pump power off. Cover and store reagents and calibrants as appropriate to prevent deterioration.
2. Long term shutdown (4 or more days): Place reagent lines in deionized water for 15 – 20 minutes. Remove reagent lines from water and pump air until the cartridge is dry. Unlatch platens.

10.4 General Guidelines for Troubleshooting

1. Isolate and define the problem.
Categorize the problem or symptom as chemistry, hydraulic, or electronic.
2. Do not overlook the obvious.
3. Rule out operator induced errors.
4. Eliminate one variable at a time.
5. Document the solution.
6. Refer to the operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions.

12. References

Astoria 2 Analyzer: Operation Manual, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II: Flow Analyzer Software Package, Version 2.12, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

FASPac II Quick Start, Astoria-Pacific International, Clackamus, Oregon, 97015-0830, USA.

Table 1 - Method Detection Limits

| Name | SIO2 |
|----------------|-------|
| .050ppm | |
| SIO2 | 0.054 |
| SIO2 | 0.056 |
| SIO2 | 0.057 |
| SIO2 | 0.054 |
| SIO2 | 0.051 |
| SIO2 | 0.049 |
| SIO2 | 0.052 |
| SIO2 | 0.057 |
| SIO2 | 0.052 |
| SIO2 | 0.054 |
| avg | 0.054 |
| std | 0.003 |
| mdl | 0.007 |

Table 2: Recommended Sample Tray/Table Layout

| Sample # Rack Position | Contents | Function | FASpac ID |
|-----------------------------------|---------------------|---------------------|------------------|
| 1 SR:1 | 80 – 100 % Standard | Synchronization | SYNC |
| 2 SR:2 | Blank | Calculate Carryover | CO |
| 3 SR:3 | Blank | Wash | w |
| 4 SR:4 | Blank | Readjust baseline | W |
| 5 SR:5 | Standard 1 | Calibration | C1 |
| 6 SR:6 | Standard 2 | Calibration | C2 |
| 7 SR:7 | Standard 3 | Calibration | C3 |
| 8 SR:8 | Standard 4 | Calibration | C4 |
| 9 SR:9 | Standard 5 | Calibration | C5 |
| 10 SR:10 | Standard 6 | Calibration | C6 |
| 11 SR:11 | QC Standard | Verify calibration | QC1 |
| 12 SR:12 | Blank | Wash | w |
| 13 SR:13 | Blank | Readjust baseline | W |
| 14-24 1:1 – 1:10 | Samples (unknowns) | | |
| 25 1:11 | Check Calibrant | Verify calibration | CCV |
| 26 1:12 | Blank | Readjust baseline | W |
| 27-37 1:13 – 1:22 | Samples (unknowns) | | |
| 38 1:23 | Check Calibrant | Verify calibration | CCV |
| 39 1:24 | Blank | Readjust baseline | W |
| 40 1:25 | Blank | Pause sampler | PAUSE |

Table 3: Example of Sampler Setup Window (Astoria *FASPac*)

| | | | |
|-------------------|-----------|---|-----------|
| Timing | | <input checked="" type="checkbox"/> Enable Autowash | |
| Sample Time | <u>25</u> | Autowash Interval | <u>10</u> |
| Wash Time | <u>35</u> | | |
| Repetition Counts | | | |
| Unknowns | <u>1</u> | | |
| Autowash | <u>2</u> | | |
| Calibrants | <u>2</u> | | |

Table 4: Example of Channel Properties – Calibration Window (Astoria *FASPac*)

| | | |
|---|----------------|-------|
| Calculate Concentrations From | Concentrations | |
| <input checked="" type="checkbox"/> Curves before | C1 | 0.000 |
| Curves around | C2 | 0.010 |
| Average all curves | C3 | 0.050 |
| | C4 | 0.100 |
| Curve Type | C5 | 0.200 |
| 1 st Order Polynomial ▼ (drop down) | C6 | 0.600 |
| Curve 1 Curve 2 | C7 | 1.000 |

Figure 1

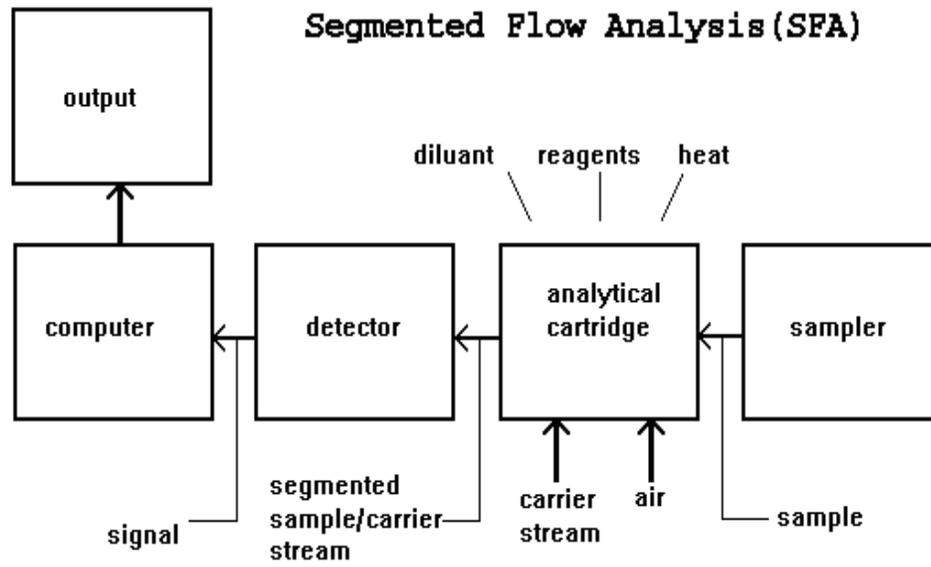
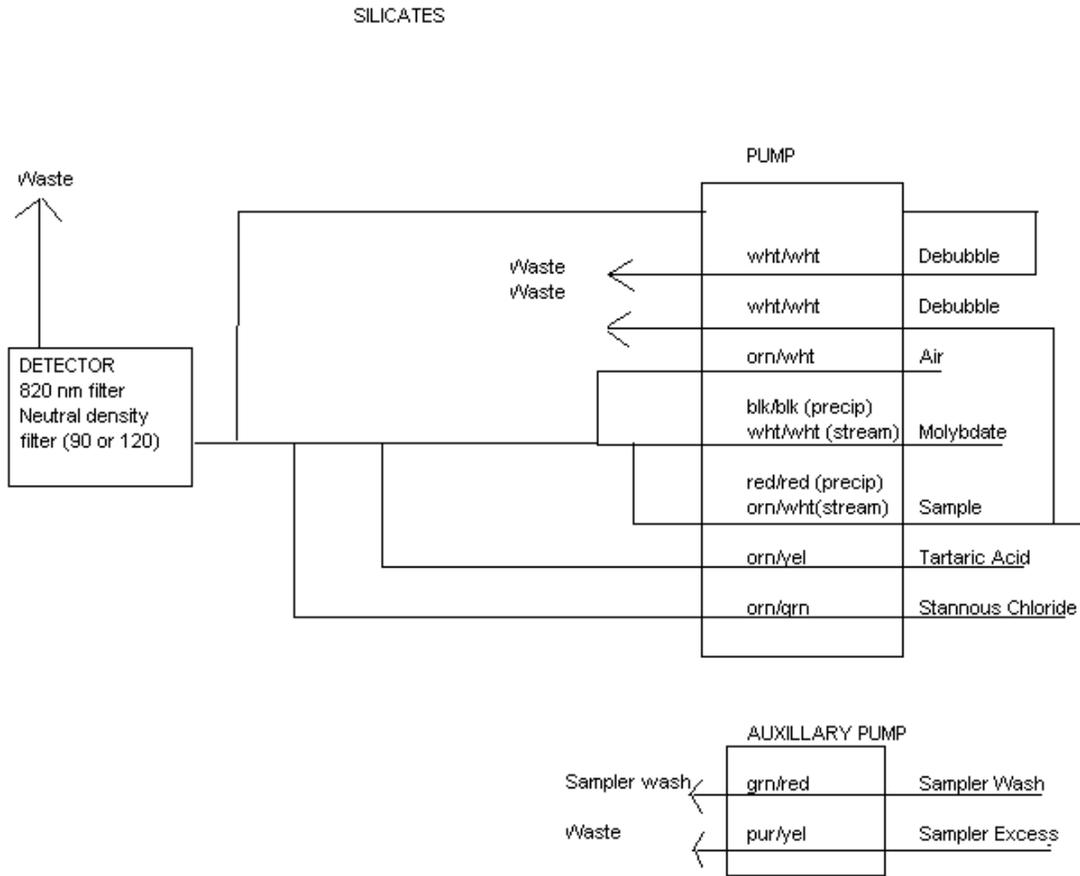


Figure 2



Electrometric Determination of pH
and Titration for Bicarbonate

October 2009

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1. Suggested Calibration Standards for Samples at Coweeta.
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FIGURES

1. System diagram for pH meter and Brinkman Dosimat dispenser.

1. SCOPE AND APPLICATION

1.1 This method is applicable to the determination of Hydrogen ions and Bicarbonates in Stream, Precipitation, Thrufall, and Lysimeter samples.

1.2 Method detection limits are summarized in Table 1.

1.3 The Bicarbonate titration method is not recommended for samples with pH values lower than 4.50.

2. SUMMARY OF METHOD

2.1 The pH value is determined by the measurement of the activity of the Hydrogen ion in an aqueous solution. The electromotive force produced by the combination electrode varies linearly with pH. The linear relationship is described by calibration with pH 4 and pH 7 buffers.

3. DEFINITIONS

- 3.1 pH -- negative log of the activity of the Hydrogen ion.
- 3.2 ELECTRODE -- a probe when immersed into a liquid produces an electromotive force.
- 3.3 BUFFER -- solutions of a known pH used to calibrate pH meter.
- 3.4 BICARBONATE -- HCO_3 represents the buffering capacity of the sample.

4. INTERFERENCES

- 4.1 Temperature can influence the electrode performance. Avoid excessive drift by warming samples to room temperature prior to analysis.
- 4.2 Stirring the sample can speed electrode response but avoid taking the reading until the sample returns to a quiescent state.

5. SAFETY

- 5.1 Prepare .01N H_2SO_4 under fume hood. Wear lab coat, gloves, and safety glasses.

6. APPARATUS AND EQUIPMENT

- 6.1 pH Meter: Orion model 611
Meter should be readable to .01 pH units. Meter should have controls for calibration and slope adjustments. Temperature compensation is also a desirable feature.
- 6.2 Combination Electrode: Broadley - James #E-1229-EC2-A03SC
Probe should respond rapidly 1-3 minutes.
- 6.3 Automatic buret: Brinkmann Dosimat model 645
Dispenser should be accurate to .01mL.
- 6.4 Stopwatch

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in CO_2 free DI water.

- 7.1 pH buffer 4 and 7: Labcraft
Purchase buffers from commercial sources that are traceable to NIST standards.
- 7.2 Standardized 0.01N sulfuric acid. See section below.
- 7.3 Saturated KCl solution. Electrode requires periodic refill with KCl.
- 7.4 Storage solution for Orion series A meter electrode: Dissolve 1g of KCl into 200ml pH7 buffer.

8. STANDARDIZATION OF SULFURIC ACID

8.1 Reagents:

- a. 0.0100N potassium biphthalate: dissolve 2.0425 g anhydrous $\text{KHC}_8\text{H}_4\text{O}_4$ and dilute to 1 liter with CO_2 -free DI water.
- b. 1N NaOH: dissolve 40 g NaOH and dilute to 1 liter with DI water.
- c. 0.01N NaOH: dilute 10.0 ml 1N NaOH with CO_2 -free DI water to 1 liter. Make up and standardize weekly.
- d. Phenolphthalein: dissolve 2.5 g phenolphthalein disodium salt in 250 ml DI water and 250 ml ETOH.
- e. Stock sulfuric acid, 0.1N: 2.8 ml of concentrated H_2SO_4 diluted to 1 liter DI water.
- f. CO_2 -free DI water: prepare fresh as needed by boiling DI water for 15 minutes and cooling rapidly to room temperature. Cap the flask with an inverted beaker while cooling.
- g. Sulfuric acid, 0.01N: dilute 100 ml of 0.1N stock to 1 liter with CO_2 -free DI water. Make up weekly.
- h. 0.1N sodium thiosulfate: dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 1 liter DI water.
- i. Mixed bromcresol green-methyl red: dissolve 20 mg methyl red sodium salt and 100 mg bromcresol green sodium salt in 100 ml DI water.

8.2 Standardize the 0.01N sodium hydroxide

- a. use 25 ml of .01N $\text{KHC}_8\text{H}_4\text{O}_4$ in flask
- b. add 1 drop 0.1N sodium thiosulfate to the $\text{KHC}_8\text{H}_4\text{O}_4$
- c. add 3 drops phenolphthalein
- d. titrate with \sim .01N NaOH until get faint pink (pH of 8.3)
- e. repeat two more times
- f. normality of NaOH = $\frac{\text{ml KHC}_8\text{H}_4\text{O}_4 \times \text{Normality of KHC}_8\text{H}_4\text{O}_4}{\text{ml NaOH}}$

8.3 Standardize the 0.01N sulfuric acid against NaOH of known concentration (approximately 0.01N, see above).

- g. use 25 ml of 0.01N NaOH
- h. add 1 drop 0.1N sodium thiosulfate
- i. add 3 drops mixed bromcresol green-methyl red indicator
- j. titrate with \sim 0.01N sulfuric acid until solution turns pale orange. Solution will go from blue to gray to pale orange.
- k. repeat two more times
- l. normality of H_2SO_4 = $\frac{\text{ml NaOH} \times \text{N of NaOH}}{\text{ml H}_2\text{SO}_4}$

9. QUALITY CONTROL

- 9.1 Calibration buffers should be traceable to NIST standards.
- 9.2 Recalibrate ph meter each day.
- 9.3 Check ERA quality control mineral sample quarterly.
- 9.4 Check NADP quality control sample each Tuesday.
- 9.5 Remake and standardize the .01N H_2SO_4 acid every two weeks.

10. PROCEDURE AND CALCULATIONS

- 10.1 Samples and buffers should be at room temperature. (20° - 25° C)
- 10.2 Raise electrode and remove the storage bottle from tip.
- 10.3 Calibration:
1. Rinse electrode with DI water using wash bottle. Shake or blot off excess water.
 2. Pour pH 7 buffer into a small 4 ml sample cup.
 3. Lower electrode into pH 7 buffer.
 4. Adjust slope control knob to 100.
 5. After 2 minutes, adjust calibration control knob so that meter reads 7.00.
 6. Rinse electrode with DI water using wash bottle. Shake or blot off excess water.
 7. Pour pH 4 buffer into a small 4 ml sample cup.
 8. Lower electrode into pH 4 buffer.
 9. After 2 minutes, adjust slope control knob so that meter reads 4.00.
- 10.4 Sample run:
1. Turn power on to Brinkman Dosimat and fill the syringe supply.
 2. Dispense 2 - 3 ml of acid to clear any air that may be trapped.
 3. Rinse electrode with DI water using wash bottle. Shake or blot off excess water.
 4. Measure 25 ml of sample and pour into small beaker.
 5. Lower electrode into sample and allow to stabilize for 2 minutes.
 6. Record pH.
 7. Record beginning number on the Brinkman Dosimat.
 8. Slowly dispense acid into sample stopping to swirl occasionally.
 9. Observe pH and stop adding acid when pH reads 4.50.
 10. Record ending number on the Brinkman Dosimat.
- 10.5 Calculations: Bicarbonate mg/l
1. Bicarbonate is determined by electrometric titration with 0.01N H₂SO₄ to pH 4.5.

Calculations for HCO₃⁻ are as follows:

$$\text{mg/L Alk. (as CaCO}_3) = \frac{\text{ml acid to pH4.5} \times 0.01\text{N} \times 50,000}{\text{Vol. Sample (ml)}}$$

$$\text{mg/L HCO}_3^- = \frac{(4.6 \times 10^{-7}) \text{ mg/L CaCO}_3}{([\text{H}^+] + 4.6 \times 10^{-7})}$$

$$4.6 \times 10^{-7} = K_a \text{ H}_2\text{CO}_3$$

The hydrogen ion concentration is calculated from the original sample pH.
Samples are analyzed the day of collection.

- 10.6 Shutdown:
1. Rinse electrode with DI water using wash bottle. Shake or blot off excess water.
 2. Place storage bottle containing KCl on the tip of electrode.
 3. Turn pH meter to Standby.
 4. Turn power off to Brinkmann Dosimat.

- 10.7 Trouble shooting:
1. If the electrode responds erratically it may need filling with KCl.
 2. If the electrode has a sluggish response then the junction may be clogged.
 3. The Dosimat often gets air in the lines. Allow it to dispense 2 - 3 ml of acid before starting the first titration.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. Table 3 summarizes the data.

12. REFERENCES

- 12.1 Methods for Chemical Analysis of Water and Waste, Method 150.1, EPA, 1983.
- 12.2 Methods for Chemical Analysis of Water and Waste, Method 310.1, EPA, 1983
- 12.3 Standard Methods for the Examination of Water and Wastewater, Method 423, "Determination of pH", 16th edition, 1985.
- 12.4 Standard Methods for the Examination of Water and Wastewater, Method 403, "Determination of Alkalinity", 16th edition, 1985.

Table 1. Suggested Calibration Standards for Samples at Coweeta

| Analyte | Calibration Standards |
|---------|-----------------------|
| pH | 4.00 and 7.00 |

Table 2. Single Operator Precision and Bias for pH determined from Quality Control Samples

| Analyte | True Value | Number of Samples | Mean Measured | Mean Bias | Standard Deviation | Relative Standard Deviation, % |
|---------|------------|-------------------|---------------|-----------|--------------------|--------------------------------|
| pH | 9.08 | 10 | 9.12 | .04 | .01 | .11 |

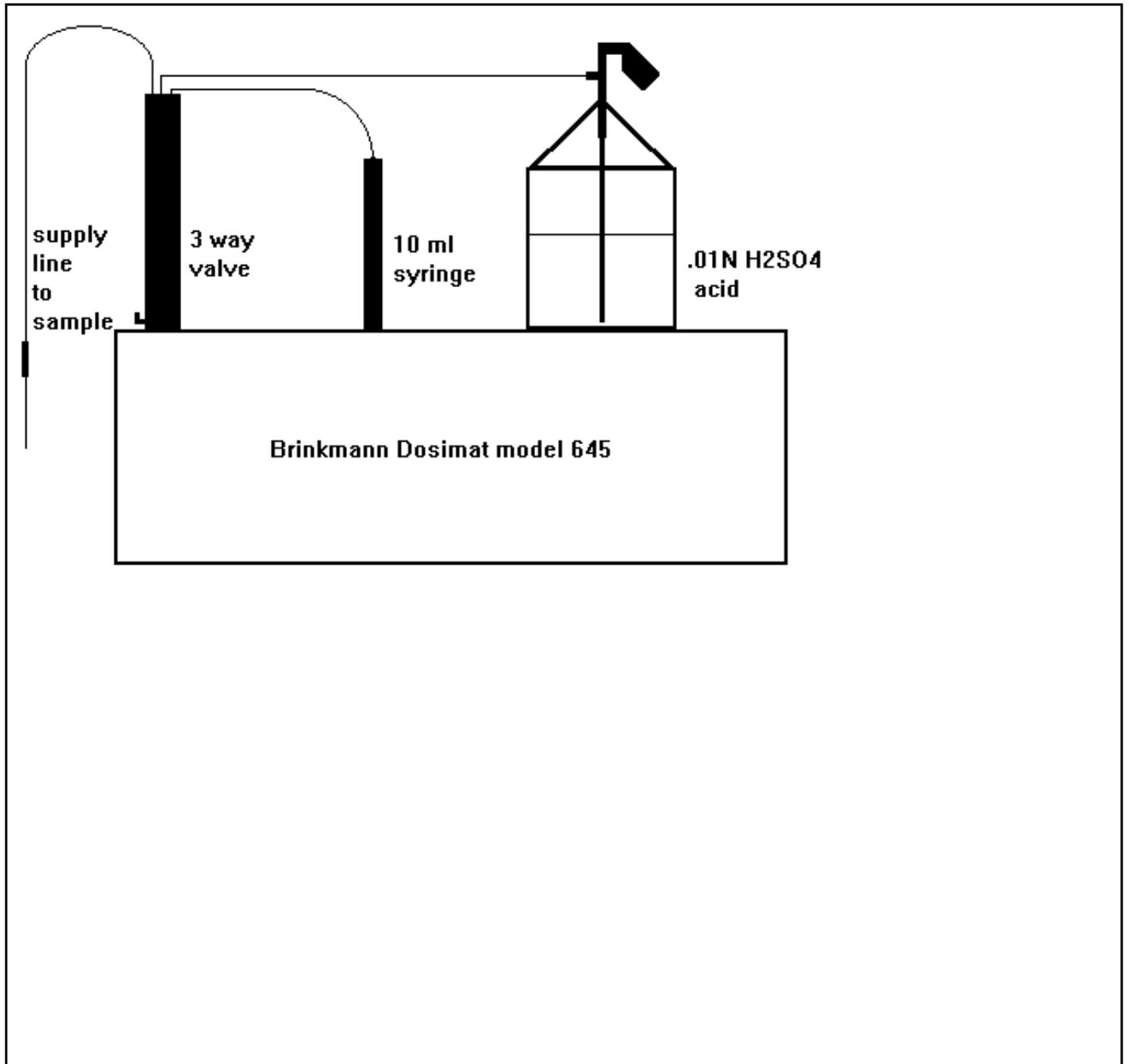
Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Table 3. Single Operator Precision and Bias for HCO₃

| Analyte | True Value | Number of Samples | Mean Measured mg/l | Mean Bias mg/l | Standard Deviation | Relative Standard Deviation, % |
|------------------|------------|-------------------|--------------------|----------------|--------------------|--------------------------------|
| HCO ₃ | 152 | 10 | 159 | 7 | 1.5 | .99 |

Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Figure 1. Brinkmann Dosimat model 645



Atomic Absorption Spectroscopy
Determination of
Potassium, Sodium, Calcium,
And Magnesium by

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

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Laboratory Technician:

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ATOMIC ABSORPTION SPECTROSCOPY

1. SCOPE AND APPLICATION

- 1.1 This method is applicable for the determination of K, Na, Mg, and Ca in sample matrices of water, 2% nitric acid, 0.5M HCl, and soil extract (See cation soil procedure).

2. SUMMARY OF METHOD

- 2.1 Atomic absorption utilizes the principle that each atom absorbs light at a specific wavelength. Therefore, at a specific wavelength the quantity of the absorbing element can be measured and is proportional to its concentration.

- 2.2 A sample is aspirated into an air-acetylene (C_2H_2) or nitrous oxide (N_2O)- C_2H_2 flame. The molecules are atomized in the flame having a specific wavelength of light diverted through it. The atoms absorb light. The amount of light absorbed quantifies the amount of element present by use of Beer's Law $A=abc$.

Where, A=absorbance

a=absorption coefficient for the absorbing species

b=length of light path

c=concentration

- 2.3 A three point calibration curve is developed for each element plotting absorbance versus concentration.

3. INTERFERENCES

- 3.1 A chemical interference can arise when calcium is being measured and phosphate is present. The calcium and phosphate will combine to form calcium phosphate ($Ca_3(PO_4)_2$). Calcium phosphate does not completely atomize in an air-acetylene flame. Therefore, an excess of lanthanum chloride ($LaCl_3$) is added. $LaCl_3$ will react with the $Ca_3(PO_4)_2$ to form calcium chloride ($CaCl_2$) and lanthanum phosphate ($LaPO_4$). Calcium chloride is easily atomized in the air-acetylene flame.

- 3.2 Ionization interference can arise when using the hot $N_2O-C_2H_2$ flame. Because Ca is easily ionized, the hotter $N_2O-C_2H_2$ flame supplies the excess energy to ionize Ca. Therefore, there are fewer ground state atoms available for absorption. To control the ionization, an excess of an element easily ionized is added. Potassium chloride (KCl) is easily ionized and therefore used as an ionization suppressant.

4. RANGE

- | | | |
|-----|----|---|
| 4.1 | K | 0.05 - 12.0ppm, using 1.0 and 3.0ppm standards or 1.0 and 6.0ppm standards. |
| | Na | 0.1 - 6.0ppm, using 1.0, 2.0, and 3.0ppm standards. |
| | Ca | 0.1 - 14.0ppm, using 1.0 and 3.0ppm standards or 1.0 and 6.0ppm standards. |
| | Mg | 0.02 - 5.0ppm, using 0.5, and 1.0ppm standards or 1.0 and 2.0ppm standards. |

- 4.2 Note: The working range can be doubled by increasing the standard concentrations and changing the burner head from 10cm to 5cm. This is possible due to the relationship $A=abc$ (see section 2.2 for definition). Since the burner head has been decreased in half, it will now take two times the concentration for the same absorbance.

5. APPARATUS AND EQUIPMENT

- 5.1 Perkin Elmer model AAnalyst 300 Atomic Absorption Spectrophotometer
- 5.2 Perkin Elmer model 90plus Autosampler
- 5.3 Perkin Elmer Auto-Prep 50 dilutor
- 5.4 Dell GX1 Computer – Pentium II 450 – CD rom
- 5.5 Hewlett Packard desk jet 695C printer
- 5.6 Interfacing software - Perkin Elmer AA WinLab version 3.2
- 5.7 Corning 15ml polypropylene centrifuge tube

6. SAFETY

- 6.1 All gas cylinder connections must be leak free.
- 6.2 Before igniting the flame ensure that:
 - a. The vent is on and drawing properly.
 - b. The burner door is closed.
 - c. The siphon interlock is filled with water.
- 6.3 When the flame is burning:
 - a. Never change the pressure at the gas regulator.
 - b. Never close a valve at either a regulator or a gas cylinder.
 - c. Never leave the flame unattended for long periods of time.
 - d. Never open the cover on the siphon interlock.
- 6.4 Make certain air bubbles do not remain trapped in the siphon U-loop underneath the interlock.
- 6.5 Do not adjust the nebulizer when using the N₂O-acetylene flame.
- 6.6 Drain System
 - a. Do not kink or fold the drain tube.
 - b. Do not modify the float in the siphon interlock.
 - c. Do not store the waste vessel in a confined area.
 - d. Never use a glass container as the waste vessel.
 - e. Never close the neck of the waste vessel.
 - f. Never place the drain tube directly into a laboratory sink.
- 6.7 See Safety Practices for flame analysis located in the Perkin Elmer AAnalyst 300 Hardware guide.

7. REQUIREMENTS

- 7.1 Gases
Acetylene - AA Grade, 99.6% pure
Compressed Air - Breathing quality
Nitrous Oxide - CP Grade
- 7.2 Regulator Setting
Acetylene - 15 psig
Compressed Air - 58 psig
Nitrous Oxide - 58 psig

8. REAGENTS

- 8.1 1% LaCl_3
Weigh 5.9g La_2O_3 into a 500ml erlenmeyer flask. Rinse with a small amount (20ml) of deionized water. CAUTION - THIS NEXT STEP WILL PRODUCE A VIGOROUS REACTION!!! Slowly add 35ml concentrated HCl (trace metal grade). Swirl until dissolved. Using deionized water, transfer to a 500ml volumetric flask and bring to volume.
- 8.2 10% LaCl_3
Weigh 117.28g La_2O_3 into a 1000ml erlenmeyer flask. Rinse with a small amount (50ml) of deionized water. CAUTION - THIS NEXT STEP WILL PRODUCE A VIGOROUS REACTION!!! Slowly add 260ml concentrated HCl (trace metal grade). Swirl until dissolved. Using deionized water, transfer to a 500ml volumetric flask (containing 100ml of water) and bring to volume.
- 8.3 2% Nitric Acid
Pipette out 2ml of concentrated nitric acid (trace metal grade) into an acid washed (see section 12.1 for procedure) 100ml volumetric flask containing 50ml of deionized water. Bring to volume with deionized water.
- 8.4 1N NH_4 Acetate
Add 600ml DI to a one liter volumetric. Add 58ml acetic acid and 70ml NH_4OH . Fill to the mark with DI. Adjust as necessary to obtain a pH of 7.

9. STANDARDS

- 9.1 Reference Standard
Perkin Elmer AS STD, ALTERNATE WATER POLLUTION, 4 METALS, PE # N930-0215, containing 100ug/ml Mg, K and 500ug/ml Ca, Na in 2% nitric acid.
- a. Pour out a 0.5ml aliquot of the reference standard into a 5ml disposable beaker. Allow to come to room temperature.
 - b.1. In an acid washed (see section 12.1 for procedure) 100ml volumetric flask, pipette out 0.1ml. Bring to volume with deionized water. Cover and then invert 20 times to mix. The concentrations of the analytes are as follows: K,Mg - 0.1ppm, Ca,Na - 0.5ppm
 - b.2. In an acid washed (see section 12.1 for procedure) 100ml volumetric flask, pipette out 0.5ml. Bring to volume with deionized water. Cover and then invert 20 times to mix. The concentrations of the analytes are as follows: K,Mg - 0.5ppm, Ca,Na - 2.5ppm

- 9.2 Stock Standard Solution
Perkin Elmer AS STD, INSTRUMENT CALIBRATION-1, PE # N930-0218, containing 5,000ug/ml Ca, Mg, K, Na.
- Pour out a 15ml aliquot of the standard solution into a disposable weigh boat. Cover and allow to come to room temperature.
 - Pipette 10ml of the standard into an acid washed (see section 12.1 for procedure) 100ml volumetric flask. Bring to volume with 2% nitric acid (see section 8.3). Cover and then invert 20 times.
- 9.3 Calibration Standards
- Pour out a 2ml aliquot of the stock standard solution into a 5ml disposable beaker. Allow to come to room temperature.
 - Using an acid washed (see section 12.1 for procedure) 100ml volumetric flask, pipette out the following:
 - For 0.5ppm - 0.1ml; used for Mg
 - For 1.0ppm - 0.2ml; used for K, Na, Ca, Mg
 - For 2.0ppm - 0.4ml; used for K, Na, Ca, Mg
 - For 3.0ppm - 0.6ml; used for K, Na, Ca
 - For 6.0ppm - 1.2ml; used for K & Ca
 - Bring to volume with deionized water and invert 20 times to mix.

10. PROCEDURE

- 10.1 Pour 10ml±0.5ml of sample into a centrifuge tube. Place in sample tray. Repeat for all samples listed on the Sample ID Sheet.
- 10.2 Make up the reference standard and calibration standards (see section 9).
- 10.3 Spectrometer Start Up
- Turn on computer.
 - Turn on the spectrophotometer.
 - Double click on the AA WinLab Analyst icon.
 - The instrument will now go through several system checks.
 - After the checks are completed, click on the workspace icon. Select the appropriate workspace.
- In the Automated Analysis window under Set Up:
- Click on Sample Info File. Make sure the appropriate file is open.
 - Click on Results Data Set and enter the day's date using the established format.
- Flame Ignite
 - Turn on hood.
 - Open compressed air valve.
 - Open acetylene tank.
 - Click open the flame control and switch flame on.
 - Maximize Absorbance
 - Put sample probe into deionized water.
 - Under the Tools Menu go to continuous graph and ensure maximum absorbance is obtained (consult table 1). If not, adjust the nebulizer and burner.
 - Begin Analysis by clicking on analyze all in the automated analysis window.

- 10.4 For a N₂O-C₂H₂ Flame using a 5cm burner head:
- a. Optimize the burner using an air-C₂H₂ flame.
 - b. Follow analysis procedures in 10.3.
- 10.5 Shut down
- a. Go to the flame icon on the menu bar.
 - b. Turn flame off.
 - c. Close off acetylene.
 - d. Depress check gases.
 - e. Close off air and N₂O (if applicable) and press check gases.
 - f. Continue depressing check gases until all are clear.
 - g. Exit program.
 - h. Turn off spectrophotometer.
 - i. Turn off computer.
 - j. Turn off hood.
- 10.6 Special Considerations
- a. Place red lens in the path of the light for K analysis.
For Ca analysis of water samples add 0.05% La to the samples and standards.
For the La addition to samples other than water, consult soil cation procedure.
11. QUALITY CONTROL
- 11.1 A three point calibration curve is generated at the start of the run.
- 11.2 The calibration curve is checked using a reference standard.
An accuracy of ±5% and a precision of 2% or less is maintained.
- 11.3 During the run the instrument recalibrates as dictated in the method.
- 11.4 Quarterly checks on the instrument and standards are made using National Standards Institute. At this time the reference standard is also checked using calibration standards.
12. WASHING PROCEDURE FOR GLASSWARE AND CENTRIFUGE TUBES
- 12.1
- a. Wash in Joy dishwashing liquid.
 - b. Rinse with tap water.
 - c. Rinse with 5% HNO₃.
 - d. Rinse five times in deionized water.
13. REFERENCES
- 13.1 Analytical 100/300 Atomic Absorption Spectrophotometer Hardware Guide, Perkin-Elmer, Revision 0993-6088 Rev. E, June 1998.

Table 1. Setup Values for AAnalyst 300

| Lamp# | Element | Wave length | Energy | Current | Slit | Max. Abs. with std()ppm |
|-------|---------|-------------|--------|---------|------|-------------------------|
| 1 | K | 766.4 | 74 | 8 | .7H | .12(1) |
| 3 | Na | 588.9 | 74 | 6 | .2H | .20(1) |
| 2 | Ca | 422.7 | 74 | 10 | .7H | .05(1) |
| 2 | Mg | 285.2 | 74 | 10 | .7H | .25(.5) |

Table 2 - PRECISION AND BIAS

| Element(Actual Conc) | K(0.049) | Na(0.050) | Ca(0.050) | Mg(0.010) |
|----------------------|----------|-----------|-----------|-----------|
| | 0.051 | 0.057 | 0.049 | 0.012 |
| | 0.051 | 0.057 | 0.053 | 0.011 |
| | 0.052 | 0.057 | 0.051 | 0.013 |
| | 0.054 | 0.055 | 0.053 | 0.012 |
| | 0.053 | 0.056 | 0.049 | 0.012 |
| Average | 0.052 | 0.056 | 0.051 | 0.012 |
| ΣBias | 0.0002 | 0.0006 | 0.0001 | 0.0002 |
| std. dev. | 0.002 | 0.002 | 0.003 | 0.001 |
| MDL | 0.003 | 0.003 | 0.008 | 0.002 |

Figure 1 - ID/Wt Sheet

CATION DATA SHEET

SAMPLE ID _____
DATE ANALYZED _____

- 9. _____
- 10. _____
- 11. _____
- 12. _____
- 13. _____
- 14. _____
- 15. _____
- 16. _____
- 17. _____
- 18. _____
- 19. _____
- 20. _____
- 21. _____
- 22. _____
- 23. _____
- 24. _____
- 25. _____
- ⋈ _____
- 49. _____
- 50. _____
- 51. _____
- 52. _____
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- 54. _____
- 55. _____
- 56. _____
- 57. _____

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- 65. _____
- 66. _____
- 67. _____
- 68. _____
- 69. _____
- 70. _____
- 71. _____
- 72. _____
- 73. _____
- 74. _____
- ⋈ _____
- 98. _____
- 99. _____
- 100. _____
- 101. _____
- 102. _____
- 103. _____
- 104. _____
- 105. _____
- 106. _____

Figure 4 Methods Page

Inst.

Date: 03/06/30

Identification Potassium low stds Element K

Spectrometer

Timing

Wavelength

Read Time (sec)

Slit width

Read delay time (sec)

Modified settings No Yes

Signal

Flame

Type.....

Type

Measurement.....

Oxid flow L/min

Fuel flow L/min

Calib.

Autoprep – Dilutor

Dilution
No

Calibration Equation

Equation

Max Decimal Places

Max Significant Figs

Units

Calibration

Sample

Replicates

Number

Standard Conc

Standard Concentrations

| | ID | Conc | A/S Loc. |
|--------------|-------|-------|----------|
| Calib. Blank | Calib | ----- | 1 |
| Reslope Std. | | | |

Inductively Coupled Plasma Spectroscopy
Determination of
Potassium, Sodium, Calcium,
Magnesium, Aluminum, Sulfur and Phosphorous
Thermo Scientific

November 2012

Coweeta Hydrologic Lab
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| 1. | Method Detection Limits |
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Inductively Coupled Plasma Spectroscopy

1. SCOPE AND APPLICATION

1.1 This method is applicable for the determination of K, Na, Mg, Ca, Al and P in sample matrices of water, 2% nitric acid, 0.5M HCl, and soil extract (See cation soil procedure).

2. SUMMARY OF METHOD

Inductively Coupled Plasma (ICP) Spectroscopy utilizes a high energy plasma not only to dissociate the sample but excite and ionize the atoms for atomic and ionic emission. The light emitted is focused onto a diffraction grating via a slit and a mirror. As the grating rotates a different wavelength is focused onto a second mirror and the exit slit. The light is then directed to a Charge Injection Device where the signal is amplified to a measurable amount. A calibration curve is developed for each element plotting intensity versus concentration.

3. INTERFERENCES

ICP was developed to eliminate interferences encountered using Atomic Absorption. However there are usually more than one wavelength associated with each element and some wavelengths from different elements can overlap and interfere. Therefore a profile must be developed for each line and background correction applied if necessary.

4. RANGE

K, Na, Ca, Mg, Al and P all have a working range up to 1000ppm.

5. APPARATUS AND EQUIPMENT

5.1 Thermo Scientific iCAP 6300 Inductivity Coupled Plasma – Optical Emission Spectrometer

5.2 CETAC ASX-520 autosampler

5.3 Thermo Scientific ThermoFlex 900 water recirculating chiller

6. SAFETY

6.1 Never look directly at the plasma.

6.2 The exhaust must be on.

6.3 Follow all safety practices provided by the manufacture. Do not disable the safety interlocks!

7. REQUIREMENTS

- 7.1 Gases
Liquid Argon
- 7.2 Water recirculating chiller

8. REAGENTS

- 8.1 All calibrants and QC's are made in the same matrix of the samples being analyzed. Trace metal acids are used when needed.
- 8.2 1000ppm Yttrium for setting the nebulizer pressure and as an internal standard is purchased from Fisher Scientific.
- 8.3 ICP Instrument Check 1, cat# JYICP-QC1 purchased from Horiba, used to determine stability and LOD.
- 8.4 Aqua regia – Using trace metal acids make up a 3 to 1 HCl HNO₃ solution under the hood using caution. Wear safety goggles, gloves and lab coat.

9. STANDARDS AND CALIBRANTS

- 9.1 Calibrant – ICP custom mix #Q-5067 purchased from NSI Solutions Inc
- 9.2 QC calibration check -ICP custom mix #Q-5068 purchased from NSI Solutions Inc
- 9.3 Quarterly QC – Certified samples purchased from Environmental Resource Associates cat#ERA530
- 9.4 ICP Instrument Check 1, cat# JYICP-QC1 purchased from Horiba, used to determine stability and LOD

10. PROCEDURE

- 10.1 Before turning on the instrument, open argon to purge the optical components.
- 10.2 After purging two hours, turn the instrument on.
- 10.3 Spectrometer Start Up – The following directions are brief. Before using the instrument read the manufactures instructions
 - a. Turn on computer.
 - b. Open iTEVA software.
 - c. For user name select admin.
 - d. Instrument will now connect to the PC.
 - e. In the iTEVA control center under applications, click on Analyst.
 - f. Select a Method should come up. Highlight the appropriate method and click OK.
 - g. Turn on chiller and exhaust.
 - h. Allow chiller to circulate for 3 minutes. Turn on plasma
 - i. Go to sequence tab, at top click on Auto-Session, select new auto sampler.

- j. New Automation Session will come up. Select the appropriate rack configuration, then click on new. Fill out as required and click OK.
- k. On left side of page, a new icon will come up (looks like an auto sampler). Right click on the icon and choose Auto-locate all. This fills in sample information.
- l. On the top bar of the page click on auto sampler with lightning bolt icon. This will initialize the auto sampler.
- m. Depress the green arrow to begin the analysis.
- n. To view the progress of the analysis return to the analysis tab.
- o. After the analysis right click on the sequence (method name located in the analysis tab) and choose export all samples. This will save the data in comma delimited format.
- p. Turn off plasma, release platens, turn off chiller and exhaust.
- q. Put the argon at a trickle using the plasma control page.

10.4 Maintenance

Consult the manufactures instructions in the manual for cleaning instruction for the torch, spray chamber and nebulizer.

11. QUALITY CONTROL

- 11.1 A three point (or more) calibration curve is generated at the start of the run.
- 11.2 The calibration curve is checked using a certified standard.
An accuracy of $\pm 10\%$ and a precision of 2% or less is maintained.
- 11.3 During the run the instrument recalibrates as dictated in the method.
- 11.4 Quarterly checks on the instrument are made using Environmental Resource Associates and NSI Solutions Inc certified QCs.

12. REFERENCES

iCAP 6000 Series ICP-OES Spectrometer Customer Training/Maintenance Manual, 2005 Thermo Electron Corporation Stafford House, Boundry Way, Hernel Hampstead, HP27GE, United Kingdom.

Table 1 - PRECISION AND BIAS

| | Matrix= | 2%HNO3 | | | | |
|------------|--------------|--------------|--------------|--------------|--------------|--|
| | K | Na | Ca | Mg | Al | |
| 1 | 0.474 | 0.4957 | 0.5865 | 0.5676 | 0.4802 | |
| 2 | 0.4403 | 0.4924 | 0.5887 | 0.569 | 0.4714 | |
| 3 | 0.4476 | 0.4981 | 0.594 | 0.5701 | 0.4732 | |
| 4 | 0.465 | 0.4893 | 0.5921 | 0.5666 | 0.4904 | |
| 5 | 0.443 | 0.4969 | 0.5935 | 0.5651 | 0.4757 | |
| 6 | 0.4566 | 0.4737 | 0.5781 | 0.5581 | 0.4797 | |
| 7 | 0.4092 | 0.4813 | 0.5704 | 0.5543 | 0.4395 | |
| 8 | 0.4415 | 0.4776 | 0.5714 | 0.5507 | 0.4247 | |
| 9 | 0.4826 | 0.4929 | 0.5819 | 0.5646 | 0.4814 | |
| 10 | 0.455 | 0.5286 | 0.5875 | 0.6069 | 0.5071 | |
| avg | 0.451 | 0.493 | 0.584 | 0.567 | 0.472 | |
| std | 0.021 | 0.015 | 0.009 | 0.015 | 0.024 | |
| mdl | 0.058 | 0.043 | 0.024 | 0.043 | 0.067 | |

Matrix = DI

| | K | Na | Ca | Mg | P | Al |
|------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1 | 0.1004 | 0.099 | 0.1058 | 0.1022 | 0.1017 | 0.1075 |
| 2 | 0.1092 | 0.0976 | 0.1087 | 0.0992 | 0.1018 | 0.105 |
| 3 | 0.1016 | 0.1007 | 0.1059 | 0.102 | 0.1026 | 0.1073 |
| 4 | 0.0821 | 0.1012 | 0.1059 | 0.1017 | 0.1022 | 0.1072 |
| 5 | 0.0866 | 0.0995 | 0.1043 | 0.1019 | 0.1024 | 0.1074 |
| 6 | 0.0904 | 0.1021 | 0.1065 | 0.1016 | 0.1027 | 0.1078 |
| 7 | 0.0861 | 0.1023 | 0.108 | 0.102 | 0.1031 | 0.1071 |
| 8 | 0.0999 | 0.103 | 0.1091 | 0.1016 | 0.1032 | 0.1074 |
| 9 | 0.0801 | 0.1017 | 0.1074 | 0.1025 | 0.1024 | 0.1068 |
| 10 | 0.0889 | 0.0995 | 0.11 | 0.1021 | 0.1026 | 0.1075 |
| avg | 0.093 | 0.101 | 0.107 | 0.102 | 0.102 | 0.107 |
| std | 0.010 | 0.002 | 0.002 | 0.001 | 0.000 | 0.001 |
| mdl | 0.027 | 0.005 | 0.005 | 0.003 | 0.001 | 0.002 |

student t with nine degrees of freedom and a 99% confidence level = 2.821

Combustion Analysis of Water Samples
for
Dissolved Organic Carbon
and Total Nitrogen

Coweeta Hydrologic Laboratory
3160 Coweeta Lab Road
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October 2009

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U.S. Forest Service
University of Georgia

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Chemist:

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Laboratory Technicians:

Carol Harper
Neal Muldoon
Sheila Gregory

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TABLES

1. Method Detection Limits.

FIGURES

1. System flow diagram for Shimadzu TOC-V_{CPH} TNM-1 Analyzer.

1. SCOPE AND APPLICATION

1.1 This method is applicable for the determination of Dissolved Organic Carbon and Total Nitrogen in filtered water samples.

1.2 Method detection limits are summarized in Table 1.

2. SUMMARY OF METHOD

2.1 Dissolved organic carbon is measured by combustion technique and detection using a non-dispersal infrared detector. Inorganic carbon is removed by acidifying the sample and purging with carbon-free air. The remaining carbon is converted to carbon dioxide through oxidation by high temperature combustion. Carbon dioxide is measured by an infrared detector, using the region of infrared light specific to carbon dioxide.

Total nitrogen is measured by chemiluminescence, or emission of light as the result of a chemical reaction. The sample is combusted to nitrogen monoxide and nitrogen dioxide, which react with ozone to create an excited state in nitrogen dioxide. When electrons return to ground state, light energy is emitted; this energy is measured with a chemiluminescence detector

3. DEFINITIONS

3.1 Nondispersive Infrared (NDIR) sensors are simple spectroscopic devices often used for gas analysis.

The key components are an infrared source (lamp), a sample chamber or light tube, a wavelength filter, and an infrared detector. The gas is pumped or diffuses into the sample chamber, and gas concentration is measured electro-optically by its absorption of a specific wavelength in the infrared (IR).

3.2 Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction

4. INTERFERENCES

None

5. SAFETY

5.1 Do not touch furnace surface when in use. Furnace is at a very high temperature.

5.3 Use hood to make up acids. Wear safety glasses, gloves and a lab coat.

5.4 Make sure to use proper gas regulators on the Helium, Air and Oxygen tanks.

6. APPARATUS AND EQUIPMENT

6.1 Shimadzu TOC-V_{CPH} TNM-1 analyzer

6.2 Shimadzu ASI-V autosampler

7. CONSUMABLE MATERIALS

| | |
|---------------------------------|---------------|
| 7.1 Purchase from Shimadzu: | |
| halogen scrubber | 630-00992 |
| CO ₂ absorber | 630-00999 |
| o-ring teflon | 036-11408-84 |
| o-ring black | 0936-11209-84 |
| Combustion tube | 638-41323 |
| Viton coupling | 631-40316 |
| o-ring, ozone treatment unit | 036-19004-19 |
| Gasket for TN detector entrance | 631-43818-00 |
| Plunger tip | 638-59296-01 |
| syringe | 638-59296-00 |
| Membrane filter | 046-00044-11 |
| union | 631-40315-00 |
| Pt catalyst | 638-60116 |

8. REAGENTS

2N HCl - Add 300ml ultra pure DI to a 500ml volumetric flask. Working under the hood measure 83 ml of trace concentrated HCL (fisher cat# A508-500) in a graduated cylinder. Pour into volumetric containing DI. Swirl under the hood, pointing the open end away from you. Allow to cool. Fill to the mark with DI.

9. CALIBRANTS

1. To make up calibrants

10ppm TC+TN calibrant – Weigh 2.5g of 1000ppm TOC from ERA plus 2.5g of 1000ppm NO₃-N from NSI into a 300ml muffled DOC bottle. Add organic free DI to a mass of 243.75g. Add 6.25 ml of 2N HCl.

50ppm TC+TN calibrant – Weigh 12.5g of 1000ppm TOC from ERA plus 12.5g of 1000ppm NO₃-N from NSI into a 300ml muffled DOC bottle. Add organic free DI to a mass of 243.75g. Add 6.25 ml of 2N HCl.

10. QUALITY CONTROL

10.1 Use NSI Solutions QCI-013 for DOC and QCI 138 for TN.

10.2 Run QC after calibration, every 10th sample, and at the end of the run.

11. PROCEDURE

11.1 To start Analysis

1. Open zero air
2. Open compressed air
3. Turn on instrument by pressing power switch on TOC_V_{CPH} analyzer.
4. Open program by double clicking TOC-Control V icon on desktop of computer. Double click sample table editor. Click OK. On the top tool bar open a new analysis by clicking on the icon of white paper. Enter the days date, format ddmmyy.
5. Connect the instrument to the computer by clicking on the lightning bolt located on the upper tool bar.

11.2 Prepare for Analysis

1. Make sure there is organic free DI in the rinse reservoir (located in back of the instrument), the dilution bottle and the bottle of zero DI w/acid (both located between the instrument and sampler).
2. Ensure there are enough calibrant and QC's for the analysis.

11.3 Set up Analysis Sheet

1. Insert InjectionCkNPOC.cal calibration curve. This is to check the injection.
2. Insert Autogenerate
 - a. For WS analysis use ws_10ppm_analysis_NPOC_TN.met
 1. calibration curve
Use 10NPOC_wTN_0_2.5.cal plus
10TN_0_1_wNPOC.cal
 2. Controls
Use 10NPOC_0_2.5_wTN.tpl plus
10TN_0_1_wNPOC.tpl

- b. For RB use
 - RB_analysis_50ppm_NPOC_TN.met
 - 1. calibration curve
 - Use 50NPOC_wTN_0_10.cal plus 50TN_0_5w_NPOC.cal
 - 2. 50NPOC_0_10_wTN.tpl
 - 50TN_0_5_wNPOC.tpl

11.4 Begin analysis by clicking the stop light icon, click on standby, click OK, and click start.

11.5 To shut down the instrument click on the clock icon, and click on standby. The instrument will shut down in 30 minutes.

Note: The stop sign icon will stop analysis after finishing current sample being analyzed. The stop sign with bulb flash will stop analyzing and abort the current sample.

12. PRECISION AND BIAS

Single operator precision and bias were obtained from the analysis of NSI QC in DI. Table 1 summarizes the current data.

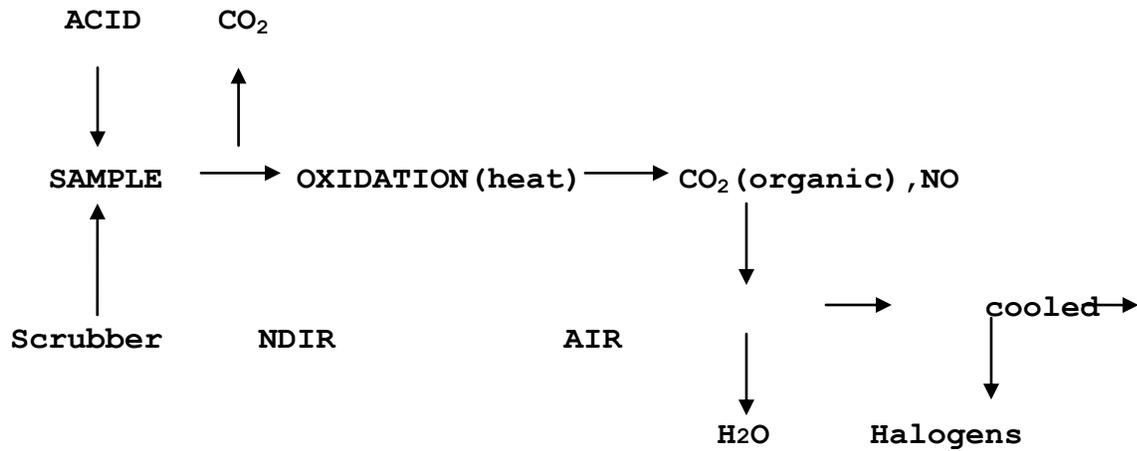
13. REFERENCES

Shimadzu TOC-VCPH/CP Users Manual, Shimadzu Corporation, 2003.
www.intl-lighttech.com/applications/light-source.../ndir.../ndir.../file

Table 1 Method Detection Limit for DOC and TN

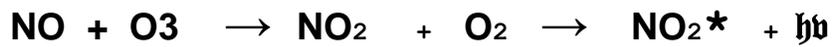
| Matrix= DI | DOC=.553(mg/L) | TN=.100 (mg/L) |
|------------|----------------|----------------|
| 1 | 0.597 | 0.118 |
| 2 | 0.607 | 0.113 |
| 3 | 0.610 | 0.108 |
| 4 | 0.606 | 0.110 |
| 5 | 0.597 | 0.115 |
| 6 | 0.614 | 0.113 |
| 7 | 0.610 | 0.105 |
| 8 | 0.607 | 0.112 |
| 9 | 0.618 | 0.111 |
| 10 | 0.607 | 0.109 |
| avg | 0.607 | 0.111 |
| std | 0.006 | 0.004 |
| mdl | 0.018 | 0.010 |

Figure 1- Flow Diagram for DOC Analysis



Flow Diagram for TN Analysis

NDIR (NO) → Chemiluminescence



Appendix 1. – Methods Archive

I. Digestion for Total Cations in Water

Equipment:

1. Scientific Block Digester Model AD-40
2. Digestion Tubes
3. Flume Hood
4. Oxford Pipet
5. Repipet Dispenser
6. Vortex Mixer
7. Boiling Chips - Teflon and Hengar
8. Parafilm

Reagents:

9. HNO₃, concentrated
10. H₂SO₄ concentrated
11. H₂O₂ 30%

Procedure:

12. Measure 40 ml of sample into acid-washed TKN tube. Fill to mark on tube.
13. Add 2.5 ml H₂SO₄ and 2.5 ml HNO₃. Vortex.
14. Place on block digester at 105°C.
15. After 2-4 hours, remove and cool slightly. Then add 3 ml of H₂O₂ with plastic syringe, Vortex, Put back on block.
16. After 20 hours total time on block remove and refill tubes to mark with deionized water.
17. Follow same procedure for blanks (3) and standards.

Analysis:

Analyze K and Ca on AA using digested standards. Zero instrument with digested blanks. For Ca, add at least 0.2 ml of 5% LaO₂ solution to 5 ml of sample. The addition of the same amount of 5% NaCl may be helpful in suppressing ionization.

II. Total Phosphorus in Soil and Sediment Samples

Equipment:

1. 70 ml Pyrex screw capped culture tubes with teflon lined caps (Corning 9826-25x).
2. Pressure cooker (sterilizer).
3. Pipette.
4. Vortex mixer.

Reagents:

5. Potassium Persulfate K₂S₂O₈
6. Sulfuric Acid H₂SO₄
7. Potassium Sulfate K₂SO₄
8. Phosphorus standard 2.0 mg/l

Procedures:

9. Acid wash tubes with 10% HCl - rinse with DI water.
10. Prepare soil - dry and sieve (2mm)
11. Prepare 5.5 M H₂SO₄ solution. (29.5ml/100ml)
12. Prepare 8.0 M H₂SO₄ solution. (42.8ml/100ml)
13. Prepare K₂SO₄-H₂SO₄ solution. (7.36g K₂SO₄ + 25.0ml 8.0M H₂SO₄ per liter).
14. Weigh 10mg-50mg soil into each tube.
15. Be sure to include a QC check sample. (NIST estuary)
16. Dispense 1.0 ml DI water and 1.0 ml 5.5 M H₂SO₄ into the sample and 3 blanks.
17. Weigh .400 g K₂S₂O₈ into the tubes.
18. Cap tightly and pressure cook (8-9psi) for 1 hour at 130°C.
19. Allow to cool then add 33.0 ml DI water to samples and two blanks.
20. Add 28.0 ml of DI water and 5.0 ml of the 2.0 mg/l phosphorus standard to two tubes.
21. Add 23.0 ml of DI water and 10.0 ml of the 2.0 mg/l phosphorus standard to two tubes.
22. Mix on vortex mixer and allow particulate material to settle, preferably overnight.
23. Dilute 5.0 ml of the supernatant solution in each tube to 50.0 ml using K₂SO₄-H₂SO₄ solution.
24. The diluted standards in the last 6 tubes will yield concentrations of 0.0 mg/l, 0.029 mg/l, and 0.057 mg/l of phosphorus.

Analysis:

Analyze on Perstorp system as Phosphorus using digested standards and blanks for calibration.

III. Centrifuging Whole and Filtrate Samples for TKN Analysis

The purpose of centrifuging these samples is to obtain a sub-sample free of suspended material. The TKN value of this sample will be subtracted from the TKN value of its non-centrifuged component to obtain the TKN value for the suspended material.

- A. Shake the monthly composite thoroughly.
- B. Immediately pour up 60 ml into a labeled 125 ml bottle. This will be the whole (W) sample. Shake again and pour composite into 3 or 4 centrifuge tubes; keep a list of which tubes represent which sample.
- C. Centrifuge for 10 minutes at 15,000 rpm.
- D. Pour supernatant (clear liquid at top) into another labeled bottle. Do not pour over the material which has collected on the outer side of the tube. Do not pour out any liquid from the bottom of the tube which may have particles in it. This is the filtered (F) sample.
- E. Save the rest of the composite for turbidity.

IV. Kjeldahl Digestion for Water Samples

Wear safety glasses, lab coat, and gloves when performing this procedure.

Equipment:

25. Scientific Block Digester Model AD-40
26. Digestion Tubes
27. Flume Hood
28. Oxford Pipette
29. Repipet Dispenser
30. Vortex Mixer

31. Boiling Chips - Teflon and Hengar
32. Parafilm

Reagents:

33. Mercuric Sulfate (1)
 - a. Dissolve 8g of HgO₂ in 50 ml of warm 1:4 H₂SO₄
 - b. Dilute to 100 ml with DI water
34. Potassium Sulfate (2)
 - a. Dissolve 133g K₂SO₄ in 700 ml DI water
 - b. Add 200 ml of H₂SO₄ acid
35. Working Digestion Solution
 - a. Add 25 ml of solution 1 to solution 2
 - b. Dilute to 1 liter

Procedure:

36. Turn on fume hood and block digester on and set to 160°C, allow 1 hour to warm up.
37. Prepare tubes - number and place 3 teflon and 1 hangar chips in each tube
38. Pipette 30 ml of sample into tubes - allow 8 tubes for standards and 3 tubes for blanks, 3 tubes for QCs and 26 for unknowns. Total = 40.
39. Pipette 6 ml of Digestion solution into each tube and vortex
40. Place tubes into block using the digester stand and side plates
41. Set block controller to run 1 hour at 160°C and 2 1/2 hours at 380°C and press start
42. When digestion cycle is complete, remove digester stand and tubes from block and allow to cool inside the hood for 15 to 20 min.
43. Dilute each tube with 30 ml of H₂O and vortex
44. Cover each tube with parafilm and refrigerate until analysis

Calculation of Percent TKN in Particulates

$$\%TKN = \frac{(w - f)}{(\text{turb})} \times 100$$

w= whole concentration mg/l
 f= filtrate concentration mg/l
 turb= turbidity mg/l

V. Compositing Lysimeters and Well Samples

The following samples will be composited monthly for chemical analysis:

- Riparian Lysimeters
- Gradient Lysimeters
- Restoration Study Lysimeters
- Riparian Well Samples
- Slagle Restoration Lysimeter

Samples will be composited on a volume weighted basis. Each week's collection volume is calculated as a percentage of the total volume. A composite of 250 ml is made using these representative percentage calculations. Example:

| Lysimeter 118-1s | | |
|------------------|---------------------|-----------------------------|
| Volume ml | Percentage of total | Amount needed for composite |
| 150 | 21 | 53.6 |
| 200 | 29 | 71.4 |
| 100 | 14 | 35.7 |
| <u>250</u> | <u>36</u> | <u>89.3</u> |
| Total = | 700 | 100 |
| | | 250 |

VI. Compositing Restoration Overland Flow Samples

Overland Flow samples are also composited on a volume weighted basis. Volumes are calculated using a depth measurement from each collector. When sampling the collection carboys in the field, be sure to stir well to ensure a homogeneous subsample. Composite samples will be analyzed for total solids, total carbon, and total nitrogen prior to any other analysis. Refer to section VIII for instructions on total solids. Refer to instrumental section on Elemental Analysis of Total Carbon, Hydrogen, and Nitrogen in Soil and Plant Tissue Samples for instructions for analysis of total carbon and total nitrogen on the filters.

VII. Speciation of Stream and Soil Solution Aluminum

This procedure is for the fractionation of dissolved Al into the organically-complexed and the ionic plus inorganically-complexed fraction of monomeric Al.

Equipment:

1. Pipettes (eppendorf and glass pasteur)
2. Peristaltic pump (Masterflex)
3. Powder free gloves
4. 250 ml plastic bottles
5. 30 ml plastic bottles
6. 15 ml plastic centrifuge tubes (polyethylene)
7. 3 ml plastic centrifuge tubes with caps
8. 1.5 ml plastic centrifuge tubes with caps
9. Custom make reaction column (see below)

Stock Reagents: (ACS grade)

10. MIBK (methyl isobutyl ketone)
11. Glacial Acetic Acid (CH_3COOH)
12. Nitric Acid (HNO_3) - trace metal grade
13. 8-Hydroxyquinoline ($\text{HOC}_6\text{H}_3\text{N}:\text{CHCH}:\text{CH}$)
14. Ammonium Hydroxide (NH_4OH)
15. Sodium Chloride (NaCl)
16. Rexyn-101 cation exchange resin beads (Fisher Scientific)

Working Reagents:

17. 1% 8-Hydroxyquinoline in 10% glacial acetic acid (100 ml)
 - a. Dissolve 1g 8-Hydroxyquinoline in 10 ml glacial acetic acid then dilute to 100 ml with DI water.
18. 10M metal free Ammonium Hydroxide (200 ml)
 - a. Add 135.2 ml of NH_4OH to 64.8 ml of DI water.
19. NH_4AC Buffer (500 ml)
 - a. Add 111.5 ml of 10M NH_4OH and 57.5 ml of glacial acetic acid to 250 ml of DI water, Adjust pH to 8.3 and dilute to 500 ml with DI water. CAUTION! - will give off fumes and heat. Check pH before using. Usually takes 3-5 ml of acid to bring pH down to 8.3.
20. 0.1M NaCl (1000 ml)
 - a. Dissolve 5.844g NaCl in 900 ml of DI water and dilute to 1 liter.
21. 0.001M NaCl (1000 ml)
 - a. Dilute 10 ml of 0.1M NaCl to 1 liter with DI water.
22. 1N HNO_3 (1000 ml)
 - a. Add 64.0 ml of HNO_3 acid to 800 ml of DI water and dilute to 1 liter.

Procedure:

23. MIBK extraction

- a. Make ready MIBK, 1% 8-Hydroxyquinoline and NH_4AC buffer (pH 8.3).
- b. Make ready 1 ml and 4 ml pipettes with acid washed tips.
- c. Label 15 ml centrifuge tubes with sample IDs, standards, and blanks.
- d. Pipette 1 ml NH_4AC into a tube.
- e. Pipette 4 ml of sample into the tube.
- f. Immediately add 1 ml of 1% 8-Hydroxyquinoline to tube.
- g. Immediately forcibly pipette 4 ml of sample into tube.
- h. Immediately add 2 ml MIBK, cap and shake tube for 10 sec.
- i. Allow to stand for 2 to 3 hours.
- j. Proceed with HNO_3 extraction.

24. CEC extraction

- a. Make ready peristaltic pump set at 13 ml per min.
- b. Make ready custom CEC extraction column.
 - (1) Cut a 17 cm piece of 6 mm ID hard polypropylene tubing.
 - (2) Attach 4 cm length of 9 mm OD flexible tubing to each end.
 - (3) Attach pipette tip to bottom end of column.
 - (4) Place 3 cm of glass wool in one end of tube.
 - (5) Fill column with DI water and add Rexyn-101 cation exchange resin beads. Get as much trapped air out of the column as possible.
 - (6) Connect an empty 60cc syringe to the top of the tube. This will act as a reservoir as the pump draws liquid through the column.
 - (7) Support column and reservoir with ring stand and connect tubing to pump. Make sure pump is set at correct speed and direction.
 - (8) Pretreat column by pumping through 200-300 ml of 0.1M NaCl. Check stability of column pH by pumping 0.001M NaCl through column. Column should be in the pH range of samples.
- c. Rinse column with 50 ml of 0.001M NaCl.
- d. Pour in 50 ml of sample. Let first 20 ml go to waste. Save last 30 ml.
- e. Rinse column with 50 ml of 0.001M NaCl.
- f. Use 8 ml of the saved sample for MIBK extraction. The rest may be analyzed later.

25. HNO_3 extraction

- a. Set eppendorf pipet to .666 ml and use acid-washed tips.
- b. Pipet 2 aliquots of .666 ml each into labeled 3 ml centrifuge tubes.
- c. Add .666 ml of 1N HNO_3 to the tube.
- d. Cap tightly and put on shaker for 20 minutes.
- e. Stand tubes upright for a few minutes, then using small pasteur pipet withdraw ~ .30 ml of yellow liquid from the very bottom of each tube.
- f. Place extractant into a labeled 1.5 ml centrifuge tube.

Analysis:

26. Samples can be stored in freezer until analyzed on graphite furnace.
27. Refer to section on Atomic Absorption Spectrophotometer - Part II

Appendix II – Instrumentation Methods No Longer in Use

Methane and Carbon Dioxide Gas Analysis by
Gas Chromatography

December 1995

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Sponsoring Agency:

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FIGURES

1. Diagram for 10 Port Injection Valve

GAS CHROMATOGRAPHY

1. SCOPE AND APPLICATION

- 1.1 This method is applicable for the determination of CH₄ and CO₂ in atmospheric samples.

2. SUMMARY OF METHOD

- 2.1 Gas Chromatography utilizes the attractive forces between the sample effluent and the stationary phase of the column to separate the components in the sample. The more the sample is like the column the longer the species will be retained. The components are detected by a selected detector. The components can then be quantified using a standardization curve.

A sample is injected into a heated port and vaporized. The sample is carried onto the column and to the detector by the carrier gas. The carrier gas is an inert gas such as Helium or Nitrogen. The sample and carrier gas make up the mobile phase. Detector selection is dependent on the species of interest and the concentration of the species.

- 2.2 The Thermal Conductivity Detector (TCD) is a universal detector capable of detecting all compounds. Detection is limited by concentration with a range in the upper ppm. The TCD consist of four filaments which make up the arms of a Wheatstone bridge. The column effluent passes through one pair of filaments, the second pair is a reference with just helium passing through. The conductivity of helium is greater than most other compounds. Therefore, the presence of the sample causes the filament temperature of the sample side to rise creating an imbalance between the pair. This imbalance is measured and quantified.

- 2.3 The Flame Ionization Detector (FID) is used for detection of organic molecules. The FID has a detection limit in the upper ppb and low ppm range.

The FID measures changes in an applied voltage to detect ions formed. Ions are formed when the effluent stream moves up and into a jet with a H₂/air flame. The organic molecules present in the sample are then ionized. The ions accumulate in a collection cup located above the flame. This results in a change in voltage which is amplified and sent to the integrator.

3. EQUIPMENT

- 3.1 Varian Model 3700 Gas Chromatograph
The Varian is equipped with two detectors. A Thermal Conductivity Detector and a Flame Ionization Detector. Column capability is packed.
- 3.2 Spectra Physics Model SP4270 Integrator
The spectra Physics is connected to the Varian.
- 3.3 Syringe - BD 60cc
Needle - BD 25 gauge

4. SAFETY

4.1 All gas cylinder connections must be leak free.

5. STANDARDS

5.1 All gas standards are certified and in nitrogen.

6. REQUIREMENTS

6.1 Gases

- a. Compressed Air - breathing grade, tank pressure = 60psi
- b. H₂ - zero grade, tank pressure = 40psi
- c. He - ultra high purity, tank pressure = 60psi

6.2 Column

- a. FID - 80/100 mesh Hayesep Q $\frac{1}{8}$ "x10ft
- b. TCD - 80/100 mesh Hayesep Q $\frac{1}{8}$ "x10ft

6.3 Sample valve

Valco 10 port sampling valve for FID/TCD. Schematic shown in Figure 1.

6.4 Sample Loop Volumes - Each = 1.2ml

6.5 Septa

- a. Varian Injector
 - 7/16" Blue Septa
 - Supplier - ALLTECH
 - CAT # 6518
- b. Gas Sampling Valves
 - 3/8" Grey Septa
 - Manufacturer - Unknown

6.6 Special Requirements

Because atmospheric samples contain moisture, which can damage the TCD (corrodes the filaments), a 4½"x¼" copper tube packed with silica gel (mesh size 6-16) using glass wool to plug is placed before the sample loops and after the injection port.

7. INSTRUMENT CONDITIONS

- 7.1
 - a. Injector - off
 - b. Oven - 40°C
 - c. Detector
 - 1. FID - 100°C
 - 2. TCD - 160°C, Filament=250°C
 - d. Flow
 - 1. FID - 22 ml/min
 - 2. TCD - 30 ml/min
 - e. Electrometer
 - 1. FID - ATTENUATOR - ∞
RANGE - 10⁻¹²amps/mV
 - 2. TCD - ATTENUATOR - ∞
RANGE - 0.5mV

8. PROCEDURE

- 8.1 Start Up
 - 1. Set instrument conditions.
 - 2. Open helium tank.
 - 3. Turn on instrument.
 - 4. Allow TCD to stabilize for 6 hours. While waiting for stabilization, set up integrator (section 8.3a).
 - 5. Open air and H₂ tanks. After 30 seconds ignite flame. Do not allow toggle switch to stay in ignite position for more than 4 seconds.
 - 6. While waiting for warm up (5 minutes), change all septa.
 - 7. Start injecting.
- 8.2 Standardization

To calibrate the instrument three standardized gases of appropriate concentration are injected in triplicate. For each concentration, the peak areas are compared and must be within 10% of each other. If not, additional injections are made until a 10% agreement is achieved.
- 8.3 Integrator Set up: FID and TCD
 - 1. Turn power on.
 - 2. Enter date and time.
 - 3. With edit mode in A, press DIALOG.
 - 4. Enter the following;
 - FILE NAME= CO2
 - TT=.01 TF=AZ TV=1
 - TT=.02 TF=II TV=1
 - TT=2 TF=II TV=0
 - TT=2.01 TF=PM TV=1
 - TT=3.2 TF=ER TV=1
 - Press Enter, Press enter
 - 5. With edit mode in B, press DIALOG.

6. Enter the following;
FILE NAME= CH4
TT=.01 TF=AZ TV=1
TT=.02 TF=II TV=1
TT=1 TF=II TV=0
TT=1.01 TF=PM TV=1
TT=2 TF=ER TV=1
Press enter, press enter

8.4 Shut Down

1. Close H₂ and air tanks.
2. Turn off power.
3. After the TCD is cool (12 hours), close He tank.

9. QUALITY CONTROL

- 9.1 After every tenth sample a reference standard is injected to check the calibration of the instrument. The reference must be within 5% of the mean value calculated from the initial standardization of the instrument. If value is out of range, the instrument must be recalibrated.

10. MAINTENANCE

- 10.1 To remove water from the silica gel trap and recondition the column, the Varian is baked out the night before a day's run.
- a. To bake out set temperature programming to the following:
 1. Initial temperature - 40°C Time - 0 minutes
 2. Program rate - 1°C/minute
 3. Final temperature - 160°C Time - ∞
 - b. Press start to begin.

Figure 1 - Valco 10 Port valve

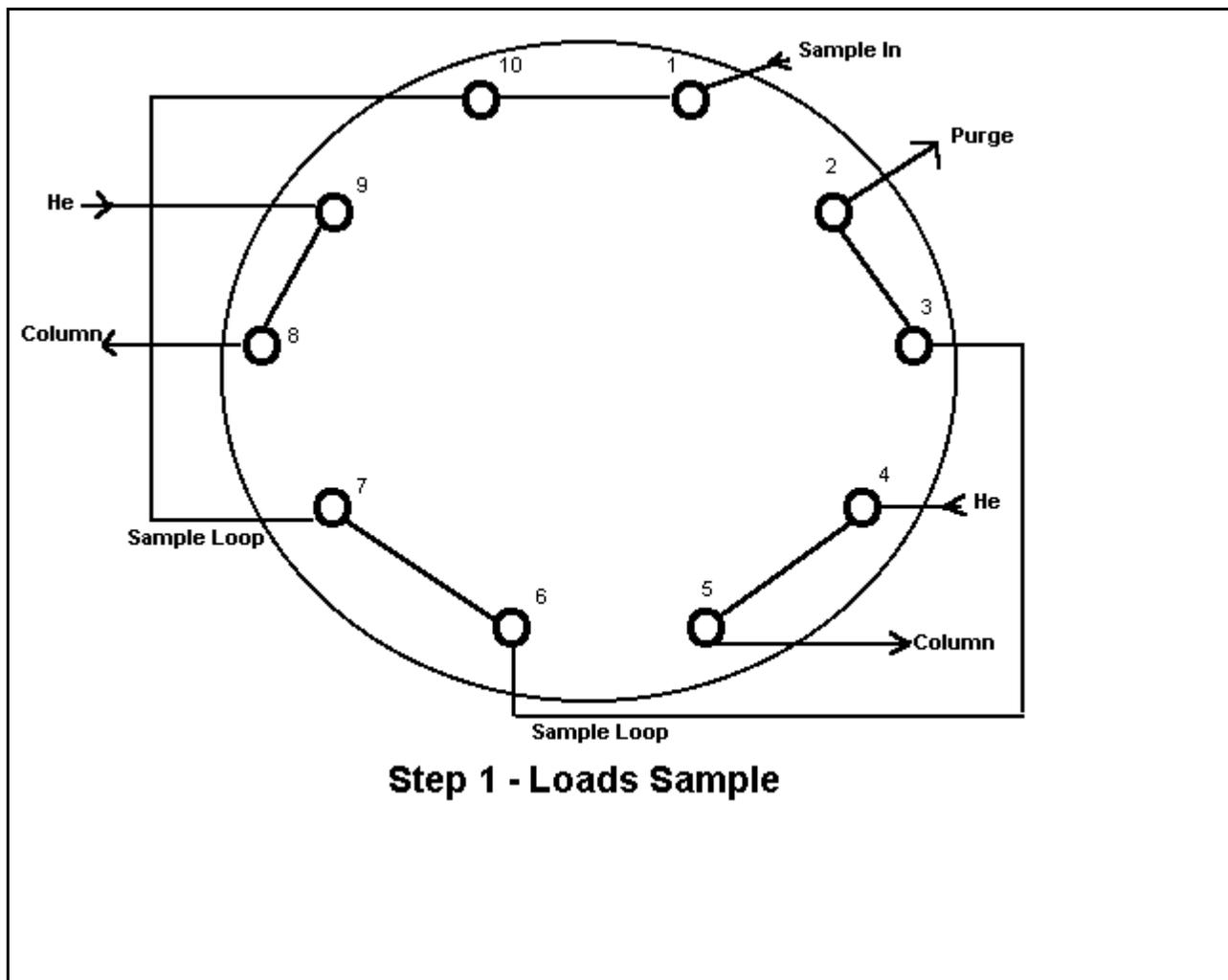


Figure 2 - Valco 10 Port valve

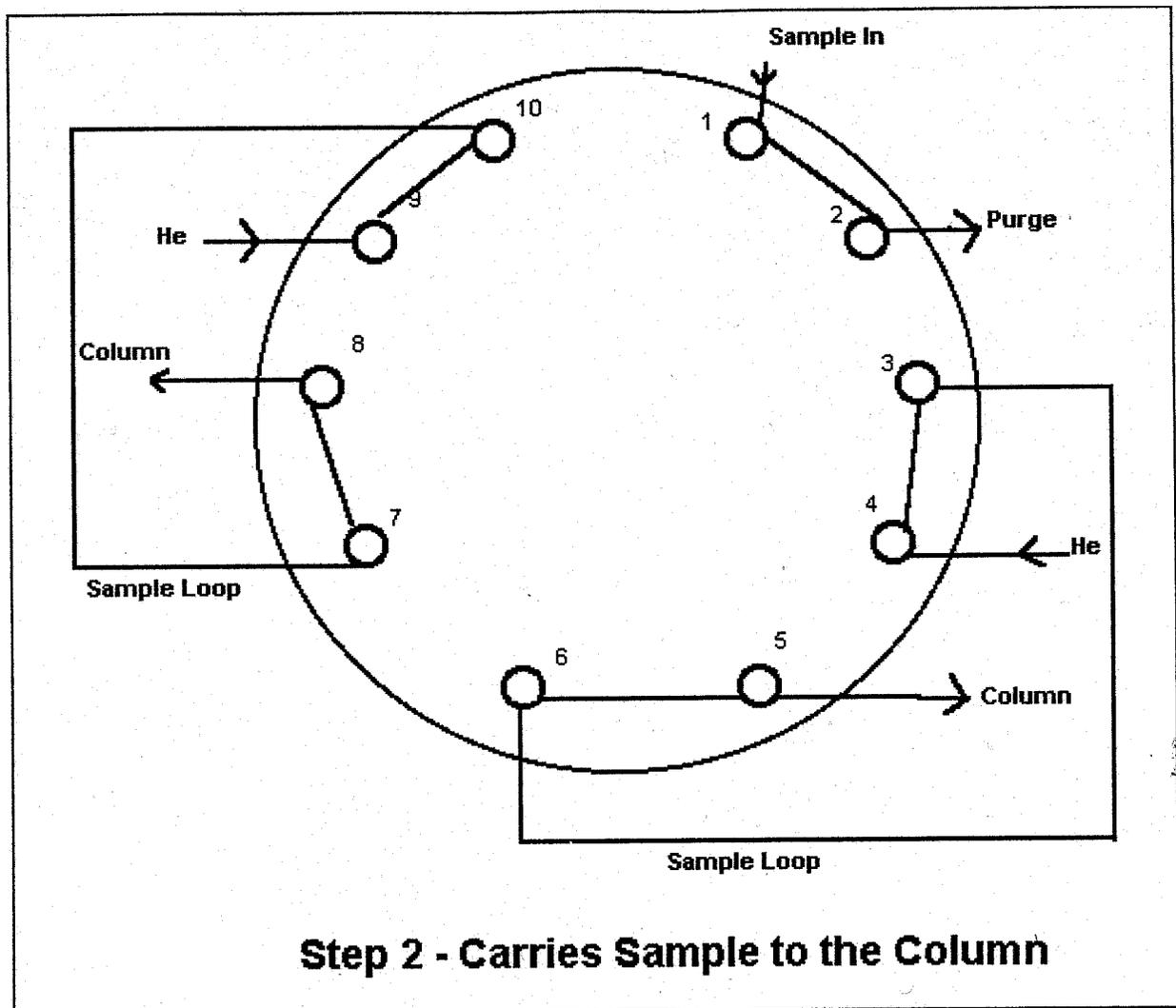


Table 1 - PRECISION AND BIAS

| act()ppm | CH ₄ (1.039) | CO ₂ (1023) |
|-----------|-------------------------|------------------------|
| | 1.18 | 1153.3 |
| | 1.07 | 1050.6 |
| | 1.03 | 1216.2 |
| | 1.05 | 1128.4 |
| | 1.00 | 1096.6 |
| | 1.00 | 1017.3 |
| | 1.02 | 1027.0 |
| | 1.07 | 1088.4 |
| | 1.03 | 1147.2 |
| | 1.02 | 1056.3 |
| Average | 1.047 | 1098.13 |
| ΣBias | 0.0008 | 7.51 |
| Std. Dev. | 0.053 | 63.25 |

Nitrous Oxide Gas Analysis by
Gas Chromatography

December 1995

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
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GAS CHROMATOGRAPHY

1. SCOPE AND APPLICATION

- 1.1 This method is applicable for the determination of N₂O in atmospheric samples.

2. SUMMARY OF METHOD

- 2.1 Gas Chromatography utilizes the attractive forces between the sample effluent and the stationary phase of the column to separate the components in the sample. The more the sample is like the column the longer the species will be retained. The components are detected by a selected detector. The components can then be quantified using a standardization curve.

A sample is injected into a heated port and vaporized. The sample is carried onto the column and to the detector by the carrier gas. The carrier gas is an inert gas such as Helium or Nitrogen. The sample and carrier gas make up the mobile phase. Detector selection is dependent on the species of interest and the concentration of the species.

- 2.2 The Electron Capture Detector (ECD) detects electrophilic molecules (electron seeking). The ECD has a detection limit in the high ppt and the very low ppb range.

The radioisotope Ni⁶³ emits a high energy electron (beta) which bombards the carrier gas causing a plasma of positive ions, radicals and thermal electrons to form. An electron capture cell collects the thermal electrons yielding the baseline signal when only the carrier gas is going through. When electrophilic species are present they react with the thermal electrons to produce negative ions of larger mass. The decrease in detector current due to the loss of thermal electrons by recombination is quantified.

3. EQUIPMENT

- 3.1 Hewlett Packard Model 5890 Gas Chromatograph
The Hewlett Packard is equipped with an Electron Capture Detector. Column capability is packed.
- 3.3 Hewlett Packard Model 3392A Integrator
The Hewlett Packard integrator is connected to the Hewlett Packard Gas Chromatograph.
- 3.4 Syringe - 500ul SGE gas tight
Needle - beveled 25 gauge

4. SAFETY

- 4.1 All gas cylinder connections must be leak free.
- 4.2 The operator must be familiar with the ECD safety warnings (section 11-24 and 11-25, in the HP 5890A gas chromatograph reference manual).

5. STANDARDS

5.1 All gas standards are certified and in nitrogen.

6. REQUIREMENTS

6.1 Gases

N₂ - grade 5.0, tank pressure = 60psi
note - N₂ must go through oxygen scrubber.

6.2 Column - 80/100 mesh Hayesep Q _"x10ft

6.3 Septa

- a. Hewlett Packard
3/8" Thermogreen LB-2
Supplier - SUPELCO
CAT # 2-0677
- b. Gas Sampling Valves
3/8" Grey Septa
Manufacturer - Unknown

7. INSTRUMENT CONDITIONS

- 7.1
 - a. Injector - off
 - b. Oven - 60°C
 - c. Detector - 325°C
 - d. Flow - 30ml/min
 - e. Electrometer
ATTENUATOR - 2⁰
RANGE - 2⁰

8. PROCEDURE

8.1 Start Up

- 1. Set instrument requirements.
- 2. Open Nitrogen tank.
- 3. Turn on instrument.
- 4. Allow to stabilize 24 hours.
- 5. While waiting for stabilization, change all septum and set up integrator (section 8.3b).
- 6. Start injecting.

8.2 Standardization

To calibrate the instrument three standardized gases of appropriate concentration are injected in triplicate. For each concentration, the peak areas are compared and must be within 10% of each other. If not, additional injections are made until a 10% agreement is achieved.

8.3 Integrator Set up

- 1. Turn power on.
- 2. Enter Date and Time by pressing OP(1).
- 3. Set Chart Speed to 0.5.
- 4. Set Attenuation to 2².
- 5. Control integration by:

- pressing INTG(9)TIME 0.01.
- pressing INTG(-9)TIME 2.0.
- 6. STOP run TIME 5.0.

8.4 Shut Down

1. Turn off ECD.
2. After the ECD is cool, close off N₂.
3. Turn off power.

9. QUALITY CONTROL

- 9.1 After every tenth sample a reference standard is injected to check the calibration of the instrument. The reference must be within 5% of the mean value calculated from the initial standardization of the instrument. If value is out of range, the instrument must be recalibrated.

10. MAINTENANCE

- 10.1 The Hewlett Packard is baked out when chromatograms are consistently poor and a 15 minute injection break does not improve the quality.
- a. To bake out set temperature programming to the following:
 1. Initial temperature - 60°C Time - 0 minutes
 2. Program rate - 1°C/minute
 3. Final temperature - 160°C Time - 700 minutes
 - b. Press start to begin.

Note: The integrator for the Hewlett Packard will begin when the start button is pressed. Therefore, disconnect the integrator.

11. REFERENCES

- 11.1 HP 5890A Gas Chromatograph Operators Manual, Hewlett Packard, 1988

Table 1 - PRECISION AND BIAS

| | NIST Value act(.495ppm) |
|---------------|----------------------------|
| | .49 |
| | .55 |
| | .57 |
| | .49 |
| | .48 |
| | .50 |
| | .40 |
| | .40 |
| | .40 |
| | .39 |
| Average | .467 |
| Σ Bias | .0028 |
| Std. Dev. | .066 |

Aluminum by
Graphite Furnace Atomic Absorption Spectroscopy

December 1999

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3160 Coweeta Lab Road
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GRAPHITE FURNACE

1. SCOPE AND APPLICATION

- 1.1 This method is applicable for the determination of Aluminum (Al) in sample matrices of water, 2% nitric acid and soil extracts.

2. SUMMARY OF METHOD

- 2.1 Atomic absorption utilizes the principle that each atom absorbs light at a specific wavelength. Therefore, at a specific wavelength, the quantity of the absorbing element can be quantified and is proportional to its concentration. A sample is placed in a graphite tube which is in the path of light with a wavelength of 309.3nm(Al absorption band). The sample then undergoes temperature programming which dries, ashes and atomizes the sample. The absorption of light by the atoms quantifies the amount of Al present through the use of Beer's Law $A=abc$.

Where, A=absorbance
a=absorption coefficient for the absorbing species
b=length of light path
c=concentration

A three point calibration curve is developed by plotting absorbance versus concentration.

3. INTERFERENCES

- 3.1 Because the matrix does not completely volatilize off during the ashing step, spectral interference can arise through background absorption of the matrix. This is controlled by the addition of $Mg(NO_3)_2$ (Magnesium Nitrate).

4. RANGE

- 4.1 0.01ppb - 0.12ppb

5. APPARATUS AND EQUIPMENT

- 5.1 Perkin Elmer model 2100 Atomic Absorption Spectrophotometer
5.2 Perkin Elmer model AS 70 Autosampler
5.3 Perkin Elmer HGA 700 Graphite Furnace
5.4 Magitronic 486DX2-50
5.5 Epson model EX-800 printer
5.6 Interfacing software - Perkin Elmer m2100 - V9.5 Feb. 17, 92

6. SAFETY

- 6.1 Because temperatures reach 2700°C a white light is produced. Therefore avoid looking at the furnace during a run.

7. REQUIREMENTS

7.1 Gases - argon, zero grade

7.2 Regulator Setting - 40 psi

7.3 Water - 2.4 liters/minute

8. REAGENTS

8.1 1% Mg(NO₃)₂ - Weigh out 1.0g of ultra-pure Mg(NO₃)₂ . Transfer to a 100ml volumetric flask. Bring to volume with deionized water.

8.2 0.2% HNO₃ sampler wash - Add 700ml deionized water to a 1L volumetric flask. Pipette in 2ml trace metal grade concentrated HNO₃. Fill to the mark with deionized water.

9. STANDARDS

9.1 2mg/L Stock Standard Solution
Perkin Elmer AS STD, INSTRUMENT CALIBRATION-1, PE # N930-0100, containing 1,000ug/ml Aluminum. Pour out a small quantity of standard into a disposable beaker. Allow to come to room temperature. Pipette out 2ml into an acid washed (see procedure 11.1) 1L volumetric flask. Bring to volume with deionized water.

9.2 Calibration Standards

a. Using acid washed (see section 11.1 for procedure) 1L volumetric flask, weigh out the following:

For 20ppb - 10.0g

For 40ppb - 20.0g

For 60ppb - 30.0g

b. Bring to volume with deionized water and invert 20 times To mix.

9.3 Reference Standard

Perkin Elmer AS STD, ALTERNATE WATER POLLUTION, 11 METALS, PE # N930-0214, containing 20ug/ml Al in 2% nitric acid

a. Pour out a 4ml aliquot of the reference standard into a 5ml disposable beaker. Allow to come to room temperature.

b. In an acid washed (see section 12.1 for procedure) 1L volumetric flask, pipette out 1ml. Bring to volume with deionized water. Cover and then invert 20 times to mix. This will give a concentration of 20.0ppb Al.

10. PROCEDURE

- 10.1 Turn on computer, spectrophotometer, and graphite furnace.
- 10.2 At the C> prompt, type AAS.
- 10.3 Element Selection
 - a. Type in lamp #4 for Al and depress lamp soft key.
 - b. Depress Current.
 - c. Select Furnace Analysis.
- 10.4 Set-Up and Alignment
 - a. Swing furnace out of light path.
 - b. Depress set up.
 - c. Turn off background correction by depressing BG CORR soft key.
 - d. Depress Gain.
- 10.5 Data Management
 - a. Go to data management by depressing Data Manag.
 - b. Fill in ID/WT Spreadsheet.
 - b. Save file.
- 10.6 Program Element
 - a. Press Prog Elem key. Enter element, ID weight file, and storage file (see figure 1).
 - b. Press Recall File. Check Instru Page (see figure 2). Check auto sampler page (AS Page) and enter in sample location number "from" and "to" (see figure 3).
 - c. Check HGA Page for conditions (see Figure 4).
- 10.7 Furnace Alignment
 - a. Turn on Argon and open up water valve.
 - b. Remove windows and clean with ethyl alcohol.
 - c. Depress Cont Graph and move furnace back into the path of light.
 - d. Depress Auto zero. Replace windows.
 - e. Absorbance should be <0.80. If not, clean windows again.
- 10.8 Autosampler Alignment
 - a. Select Samp Contr and depress F6. Rotate sampler arm and determine if tip enters tube without touching the sides. The tip should not quite touch the platform of the tube.
- 10.9 Run
 - a. Go back to Set Up and turn on background.
 - b. In Samp Contr press reset until all bubbles are gone in sample line.
 - c. Depress Atom Cont and heat out furnace for 5 seconds. Enter 190 for manual temperature.
 - d. Depress Run Elem, turn on Printer, and depress Sampl Contr.
 - e. Type 2 and depress Sampler Man. Run until a reading of 0.03 absorbance or less is obtained (0.3 for extracted std).
 - f. Reset Sampler and start program.
- 10.10 Shut Down
 - a. Go to Elem Select and exit to DOS.
 - b. Turn off spectrophotometer, Graphite Furnace and the Computer.
 - c. Close off water and Argon.

11. QUALITY CONTROL

11.1 A three point calibration curve is generated at the start of the run.

11.2 The calibration curve is checked using a reference standard.
An accuracy of $\pm 15\%$ and a precision of 3% or less is maintained.

11.3 During the run the instrument recalibrates every tenth sample.

12. WASHING PROCEDURE FOR GLASSWARE

- 12.1
- a. Wash in Joy dish washing liquid.
 - b. Rinse with tap water.
 - c. Rinse with 5% HNO₃.
 - d. Rinse five times in deionized water.

13. REFERENCES

13.1 Analytical Methods For Atomic Absorption Spectrophotometry, Perkin-Elmer, Revision 0303-0152, January 1982.

Table 1. Setup Values for PE 2100

| Lamp# | Element | Wave length | Energy | Current | Slit |
|-------|---------|-------------|--------|---------|------|
| 4 | Al | 309.3 | 62 | 18mA | 0.7L |

Table 2 - PRECISION AND BIAS

| | Al Total 20ppb | Al Extract 20ppb |
|---------------|-------------------|---------------------|
| | 24 | 22 |
| | 24 | 20 |
| | 24 | 18 |
| | 23 | 25 |
| | 26 | 22 |
| | 25 | 19 |
| | 24 | 21 |
| | 22 | 20 |
| | 22 | 20 |
| | 19 | 17 |
| Average | 23.3 | 20.4 |
| Σ Bias | 0.33 | 0.04 |
| std. dev. | 1.95 | 2.27 |
| MDL | 5.49 | 6.40 |

Figure 1. Sample ID/Wt File

Program ID/Weight

Date: 95/10/23

ID/Weight File Name: totalal

| Page 1 | AS/Location | Sample ID | Weight | Volume |
|--------|-------------|------------|--------|--------|
| | 0 | | 1.0 | 1.0 |
| | 1 | Mg(NO3)2 | 1.0 | 1.0 |
| | 2 | blk | 1.0 | 1.0 |
| | 3 | 60 | 1.0 | 1.0 |
| | 4 | 40 | 1.0 | 1.0 |
| | 5 | 20 | 1.0 | 1.0 |
| | 6 | PE Ref 20 | 1.0 | 1.0 |
| | 7 | ws 18 5/9 | 1.0 | 1.0 |
| | 8 | 27 | 1.0 | 1.0 |
| | 9 | blk | 1.0 | 1.0 |
| | 10 | blk2 | 1.0 | 1.0 |
| | 11 | ws 18 5/16 | 1.0 | 1.0 |

Figure 2. Instrument Page

Program Element - Instrument Page - FURNACE

Date: 95/09/12

Element: Al Wavelength (nm): 309.3 Slit (nm): 0.7
Pre-Temp.: 1700 At-Temp.: 2500 M0: 10.0 Modif.: 0.05mg Mg(NO3)2

Wavelength (nm): 309.3 Slit (nm): 0.7 L Lamp Number: 4
Technique: AA-BG Lamp Current (mA): 18
Signal Processing: PA Integration Time (sec): 5.0
Read Delay (sec): 0.0 Printer: DATA & SUPPL
Replicates: 2 Plot Full Scale: 1.00
BG Full Scale: 1.00

Calibration: AUTO Standard Units: µg/L Sample Units: µg/L

S1: 60. S2: 40. S3: 20.
S4:
S7: S8:
Reslope:

Figure 3. AutoSampler Page

Program Autosampler - Page 1

Element: Al

Date: 96/07/02

| Calib.: | AUTO Solutions | Location | Modifier 1 Loc.: 1 Modifier 2 Loc.: Vol.: Volume Blank Vol. | Vol.: 5 |
|---------|----------------------------------|----------|---|---------|
| | Std.Blank | 2 | 20 | |
| | Standard 1 | 3 | 20 | |
| | Standard 2 | 4 | 20 | |
| | Standard 3 | 5 | 20 | |
| | Standard 4 | | | |
| | Standard 5 | | | |
| | Standard 6 | | | |
| | Standard 7 | | | |
| | Standard 8 | | | |
| | Reslope | | | |
| | Sample Blank | | | |
| | Samples from 6 to 40 | | 20 | |
| | Restd/Reslop at Locations: 15 30 | | | |

Figure4. HGA Page

Program Element

HGA - Page

Date: 96/07/02

Element: Al

Wavelength: 309.3 nm

Slit: 0.7 L

Pre-Temp.: 1700

At-Temp.: 2500 M0: 10.0

Modif.: 0.05 mg Mg(NO3)2

| Step Number | Furnace Temperature | Ramp | Time Hold | Internal Gas Flow | Read On |
|-------------|---------------------|------|-----------|-------------------|---------|
| 1 | 150 | | 5 30 | 300 | |
| 2 | 1150 | | 5 60 | 300 | |
| 3 | 20 | | 1 15 | 300 | |
| 4 | 2500 | | 0 5 | 0 | -1.0 |
| 5 | 2650 | | 1 6 | 300 | |
| 6 | | | 1 1 | 300 | |
| 7 | | | 1 1 | 300 | |
| 8 | | | 1 1 | 300 | |
| 9 | | | 1 1 | 300 | |

Injection Temperature: 'C

Total Kjeldahl Nitrogen by Automated Wet Chemistry
using Perstorp by AlpKem

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

Cindi Brown

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Last TKN Analysis – Samples from March 2009

INDEX

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TABLES

1. Method Detection Limits.
2. Suggested Calibration Standards for Samples at Coweeta.
3. Single-Operator Precision and Bias for TKN determined from Quality Control Samples.

FIGURES

1. System diagram for Perstorp 3500.
2. Manifold setup for TKN.

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of TKN in Stream, Precipitation, Thrufall, and Lysimeter samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that are highly colored.

2. SUMMARY OF METHOD

2.1 This is a wet digestion method where the sample is heated to 380°C in the presence of sulfuric acid, potassium sulfate and mercury catalyst. These conditions break down the organic nitrogen bonds and convert them to ammonium sulfate. Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discrete. Ammonium reacts with salicylate and hypochlorite in an alkaline solution to form indophenol blue. Sodium Nitroprusside is added to intensify the blue-green color. The reaction is speeded up by running the solution through a heating bath coil at 37°C. After the reaction has developed color the solution goes to the detector measuring at 660nm. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance
a = absorptivity
b = length of light path
c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 620 - 660 nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.
- 4.2 Avoid using Ammonia based cleaning products in the lab.

5. SAFETY

- 5.1 Extra precaution should be used when handling the digested samples. Samples contain 4% Sulfuric Acid and a trace amount of Mercury. Wear lab coat, gloves, and eye protection when using Sulfuric Acid and Sodium Hydroxide. Always work under a hood when making up the reagent, vapors can also be harmful.
- 5.2 Wear protective clothing when using Sodium Hypochlorite.
- 5.3 Wear protective clothing when using Sodium Nitroferricyanide.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the procedure sheet for TKN digestion and the lab safety plan. Ask the lab safety officer if you have any questions.

6. APPARATUS AND EQUIPMENT

- 6.1 Perstorp Enviroflow 3500
 - 6.1.1 Autosampler:
Perstorp model 501 is a 300 position random access sampler. It can be operated in manual or remote mode.
 - 6.1.2 Dilutor:
Perstorp model 511 is an autodilutor interfaced to a computer. After a sample run is complete the computer recognizes peaks that have run above the highest calibration standard. The computer instructs the autosampler and the dilutor to rerun the off scale samples making a 10 X dilution.
 - 6.1.3 Peristaltic Pump:
Perstorp model 502 is a multispeed peristaltic pump with individual platens for 24 tubing channels. Pump speed and tubing inside diameters determine the flow rates for the sample and reagents.
 - 6.1.4 Mixing Manifolds:
Perstorp Supercartridge Jr. is a preconfigured mixing manifold for testing of Ammonium. The cartridge consist of polymeric tubing, fitting, reagent tees, coils and connectors.
 - 6.1.5 Cartridge Heater:
Perstorp model 503 cartridge heater is an electronic digitally operated temperature controller. It incorporates four heater cones to provide heat to Supercartridges when needed.
 - 6.1.6 Detector:
Perstorp model 510 is a variable wavelength, flow through absorbance detector. Wavelength is selected by a user adjustable concave holographic grating. The detector can operate from 360 nm to 800 nm with a tungsten halogen lamp. Output from the detector is sent to the computer and digitized.

- 6.1.7 Computer Interface:
Analog signals from the detectors are processed by a ER interface.
- 6.1.8 Computer Software:
Perstorp Winflow software package allows user control of the autosampler, diluter, data acquisition, calculation of results and analog signal display. Analysis parameters are entered via on screen tables and a series of prompting menus. Reports are generated to printer or disk.
- 6.1.9 Computer:
System requires at least a Pentium 90hz microprocessor, 16 meg of ram, floppy disk drive, cd rom drive, 1gig hard drive, color monitor, printer, mouse, serial port, and parallel port.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Ammonia free DI water. Filter the Working Buffer and Salicylate/Nitroferricyanide reagents prior to use.

- 7.1 Stock 10N Sodium Hydroxide (250mL)
Dissolve 100g NaOH in 200mL DI water and dilute to 250mL. Cool and store in a polyethylene container. Solution is stable for one month.
- 7.2 Stock Buffer, Sodium Phosphate Dibasic (500mL)
Dissolve 67g of Sodium Phosphate Dibasic Na_2HPO_4 in 400mL of DI water. Add 25mL of 10N Sodium Hydroxide and dilute to 500mL with DI water.
- 7.3 Working Buffer (500mL)
Mix 100mL of Stock Buffer and 100mL of DI water in 500mL flask. While stirring, add 125mL of DI water and 30mL 10N Sodium Hydroxide. Dilute to 500mL with DI water and mix well. Add .5mL Brij-35 and mix gently to prevent foaming. Prepare the working buffer daily.
- 7.4 Sodium Hypochlorite (200mL)
Dilute 12mL of NaOCl 5.25% (household bleach) to 100mL with DI water. Mix well and transfer to dark polyethylene bottle. Reagent is stable for one day.
- 7.5 Sodium Salicylate/Nitroferricyanide (250mL)
Dissolve 75g of Sodium Salicylate $\text{NaC}_7\text{H}_5\text{O}_3$ and .150g Sodium Nitroferricyanide $\text{Na}_2\text{Fe}(\text{CH})_5\text{NO} \cdot 2\text{H}_2\text{O}$ in 200mL DI water and dilute to 250mL. Store reagent in dark polyethylene bottle at room temperature. Make up reagent weekly.
- 7.6 DI water with Brij - 35, 30% w/v (250mL)
Add 3 drops of Brij - 35 to 250mL of DI water. This is used as the diluent and as the startup solution.
- 7.7 Sampler Wash, 3% - 4% H_2SO_4 (2L)
Prepare sampler wash solution with same acidity as digested samples. Coweeta TKN digest are usually between 3% to 4% H_2SO_4 . Add 70mL concentrated Sulfuric Acid to 1500mL of Ammonia free DI water. Mix well and dilute to 2L with DI water.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion calibrants, 1000mg/L NH₄, is purchased from Ricca yearly.
- 8.2 Working Standards:
Tare 500 mL flask on balance and make the following standards on w/w basis.
Ammonium: .10 mg/L, .20 mg/L, .50 mg/L, 1.00 mg/L

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 µmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 µmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO₄, Cl, NO₃, NH₄, PO₄, and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.
- 9.4 A standard curve is determined before every analysis with the Perstorp 3500. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. PROCEDURE AND CALCULATIONS

- 10.1 Startup:
1. Make up reagents and standards.
 2. Configure pump with proper tubes and connect to Ammonia cartridge.
 3. Turn power on and apply tension to pump platens.
 4. Connect all lines to H₂O and surfactant and observe stream.
 5. Connect reagent lines and observe stream.

- 10.2 Computer and Sampler startup:
1. Turn power on and run Winflow program.
 2. Select and click the Sample Table button. Edit an existing table or create a new one. Table should start with highest calibration standard (SYNC) sample followed by the lowest sample for carry over correction (CO) then a read baseline (RB). Calibration standards are next with duplicates from highest to lowest. Include QC standard after calibration standards and RB. Insert calibration verification (mid-range standard) after each 20 samples. For each sample cup indicate: location, sample ID, type, dilution, and weight. Rename, save and print the sample table.
 3. Click the Method Editor button and load an existing Methods file or create a new file. Verify all settings are correct. Refer to a previous method or the help files.
 4. Select the Data Collect button and verify or select Method and Sample Table in Run Setup. Start pump with reagents tubes in surfactant water.
 5. Enter an Operator ID and verify or enter a Filename for the run.
 6. Click on the Play button and monitor the baseline. When the baseline is stable, startup solution (water) then switch to reagents. Allow a few minutes for reagents to equilibrate and monitor for a steady baseline.
- 10.3 Sample run:
1. When a stable baseline has been achieved use the Sample Table print out to start loading the sample trays.
 2. Click the Fast Forward Start button to start data collection and sampler.
 3. Monitor the run until the SYNC peak has eluted and successfully marked the top of the peak at its steady state. Allow the run to continue. If run stops automatically then data file will be saved. If the run is stopped manually by clicking the Stop button then be sure to save the results once the completed run is displayed.
 4. Review the results. Calibration curve should yield a r^2 value greater than .98.
 5. Reports are produced by opening the File window and selecting the proper option. Reports may be edited, printed, and exported as needed.
- 10.4 Shutdown:
1. Connect all reagent lines to deionized water.
 2. Pump deionized water through the system for 15-20 minutes.
 3. Turn off the 509 power and unlatch all pump platens. Caution: Pump tubes may be seriously damaged if the platens remain latched with the pump power off.
 4. If the cartridge is to be stored away from the system or if it will not be used for a week or more, remove the reagent lines from the deionized water and pump the cartridge completely dry.
 5. Turn off the gas supply if not being used.
- 10.5 Troubleshooting:
1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow through system.
 2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operation parameters.
 3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

Chemistry

Reagent
Standards
pH
Temperature

Hydraulic

Pump tubing
Bubble size
Surfactant
Pump

Electrical/Mechanical

Circuit components
Optics/Lamps
Photometer/Detector
Cabling

4. Eliminate one variable at a time. Using good experimentation techniques, change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.
5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
6. Refer to the operation manual for further troubleshooting guide.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions. Table 3 summarizes the data.

12. REFERENCES

- 12.1 Perstorp Enviroflow 3500 Operation Manuals, Perstorp Analytical Corporation, 1992.
- 12.2 Methods for Chemical Analysis of Water and Waste, Method 351.2, EPA, 1983.
- 12.3 Standard Methods for the Examination of Water and Wastewater, Section 420, "Determination of Organic Nitrogen ", 16th edition, 1985.

Table 1. Method Detection Limits and Concentration Ranges for TKN

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|---------|-----------------------------|--------------------------|
| TKN | .086 | .1 - 1.0 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 9

Table 2. Suggested Calibration Standards for TKN Samples at Coweeta

| Analyte | Calibration Standards mg/L |
|---------|----------------------------|
| TKN | 0.10, 0.20, 0.50, 1.00 |

Table 3. Single Operator Precision and Bias for TKN determined from Quality Control Samples

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|---------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| TKN | .390 | 9 | .398 | .008 | .030 | 7.461 |

Relative Standard Deviation: 100 X (Sample Standard Deviation/Mean Value)

Figure 1.

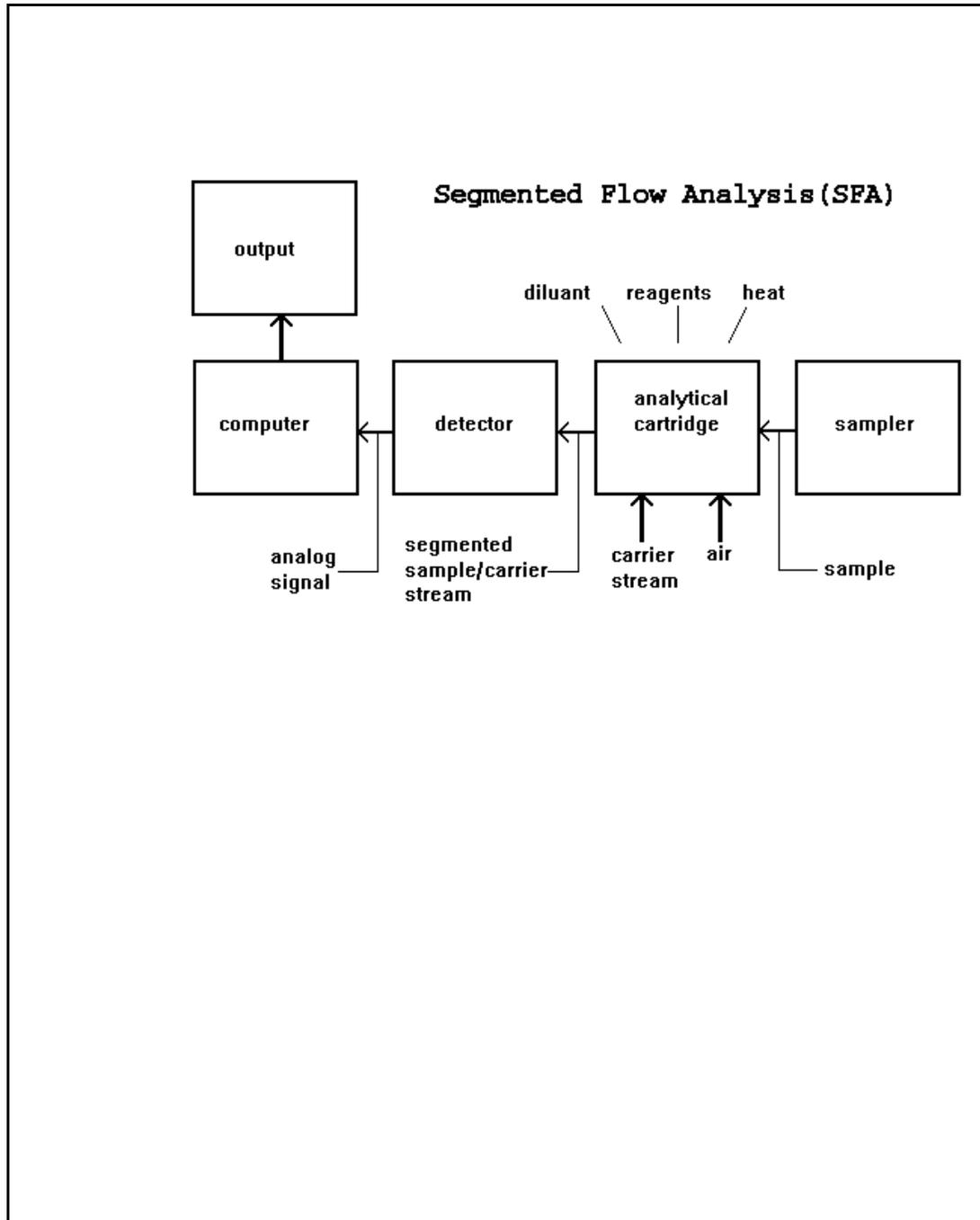
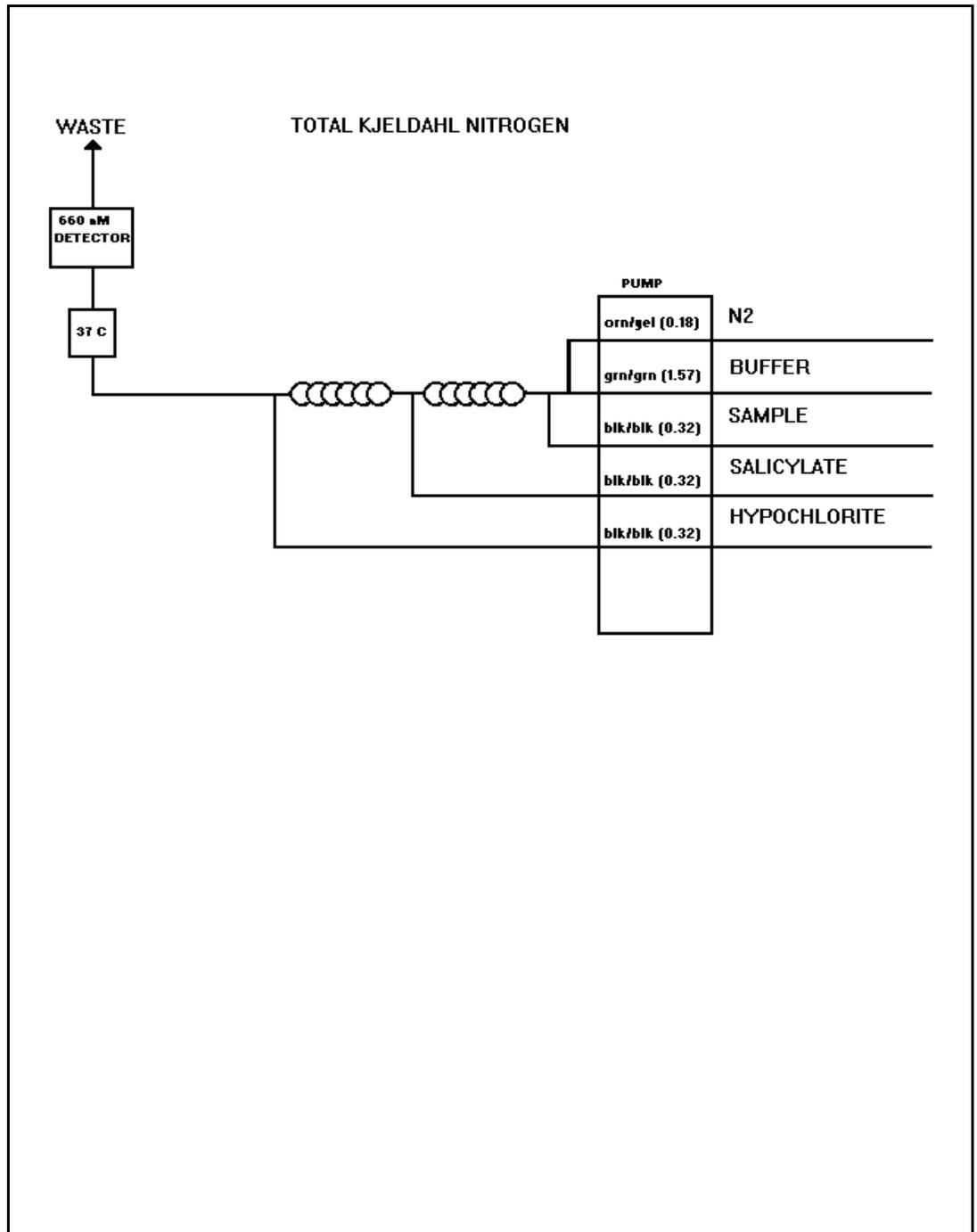


Figure 2.



Automated Wet Chemistry
for Ammonium
using Perstorp by AlpKem

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

Cindi Brown

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Instrument retired October 25, 2011

AlpKem

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TABLES

1. Method Detection Limits.
2. Suggested Calibration Standards for Samples at Coweeta.
3. Single-Operator Precision and Bias for Ammonium determined from Quality Control Samples.
4. Example of Sample ID Table.
5. Example of Sampler/Channel Setup Table.
6. Example of Standards Table

FIGURES

1. System diagram for AlpKem.
2. Manifold setup for Ammonium.
1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Ammonium in Stream, Precipitation, Thrufall, Lysimeter, and in extracted soil samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that are highly colored.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Ammonium reacts with alkaline phenol and hypochlorite to form indophenol blue. Sodium Nitroprusside is added to intensify the blue color. The reaction is speeded up by running the solution through a heating bath coil at 50⁰C. After the reaction has developed a color the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance
a = absorptivity

b = length of light path
c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentrations of the samples are calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 620 - 640 nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.
- 4.2 Avoid using Ammonia based cleaning products in the lab.

5. SAFETY

- 5.1 Extra precaution should be used when handling the liquid Phenol. Wear lab coat, gloves, and eye protection when using Phenol. Always work under a hood when making up the reagent, vapors can also be harmful.
- 5.2 Wear protective clothing when using Sodium Hypochlorite.
- 5.3 Wear protective clothing when using Sodium Nitroferricyanide.
- 5.4 Turn on the exhaust vent over the Autoanalyzer system. Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. APPARATUS AND EQUIPMENT

- 6.1 Perstorp Enviroflow 3500
- 6.1.1 Autosampler:
Perstorp model 501 is a 300 position random access sampler. It can be operated in manual or remote mode.
- 6.1.2 Dilutor:
Perstorp model 511 is a autodilutor interfaced to a computer. After a sample run is complete the computer recognizes peaks that have run above the highest calibration standard. The computer instructs the autosampler and the dilutor to rerun the off scale samples making a 10 X dilution.
- 6.1.3 Peristaltic Pump:
Perstorp model 502 is a multispeed peristaltic pump with individual platens for 24 tubing channels. Pump speed and tubing inside diameters determine the flow rates for the sample and reagents.
- 6.1.4 Mixing Manifolds:
Perstorp Supercartridge Jr. is a preconfigured mixing manifold for testing of Ammonium. The cartridge consists of polymeric tubing, fitting, reagent tees, coils and connectors.
- 6.1.5 Cartridge Heater:
Perstorp model 503 cartridge heater is an electronic digitally operated temperature controller. It incorporates four heater cones to provide heat to Supercartridges when needed.
- 6.1.6 Detector:
Perstorp model 510 is a variable wavelength, flow through absorbance detector. Wavelength is selected by a user adjustable concave holographic grating. The detector can operate from 360 nm to 800 nm with a tungsten halogen lamp. Output from the detector is sent to the computer interface and digitized.
- 6.1.7 Computer Interface:
Analog signals from the detectors are processed by an ER detector.
- 6.1.8 Computer Software:
Perstorp Winflow software package allows user control of the autosampler, diluter, data acquisition, calculation of results and analog signal display. Analysis parameters are entered via on screen tables and a series of prompting menus. Reports are generated to printer or disk.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Ammonia free DI water. Filter the Phenol and Nitroferricyanide reagents prior to use.

- 7.1 Stock 10N Sodium Hydroxide (250 mL)
Dissolve 100g NaOH in 200 mL DI water and dilute to 250 mL. Cool and store in a

polyethylene container. Solution is stable for one month.

- 7.2 Alkaline Phenol (250 mL)
Place stir bar in 250 mL flask with 200mL of DI water. While stirring, add 20 mL of 10N NaOH. Slowly add 23.5 mL of Liquid Phenol (88%) and dilute to 250 mL with DI water. Store reagent in dark polyethylene bottle at 4° C. Reagent should be a light straw color. Discard reagent if it turns brown or amber in color. Make up reagent weekly.
- 7.3 Sodium Hypochlorite (100 mL)
Dilute 50 mL of NaOCl 5.25% (household bleach) to 100 mL with DI water. Mix well and transfer to dark polyethylene bottle. Reagent is stable for one day.
- 7.4 Sodium Nitroferricyanide (250 mL)
Dissolve .125g $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$ in 200 mL DI water and dilute to 250 mL. Store reagent in dark polyethylene bottle at room temperature. Make up reagent weekly.
- 7.5 DI water with Brij - 35, 30% w/v (250 mL)
Add 3 drops of Brij - 35 to 250 mL of DI water. This is used as the diluent and as the startup solution. For soil resin sheets use .01N NaOH and Brij instead of DI water and Brij. For soil resin sheets make sampler wash .4N HCl acid.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion calibrants, 1000mg/L are purchased from Ricca yearly.
- 8.2 Working Standards:
Standards are made up in a volumetric flask. Ammonium: .010 mg/L, .050 mg/L, .100 mg/L, .200 mg/L, .600mg/L for Coweeta water samples. For N- Min samples make .050 mg/L, .100 mg/L, .50 mg/L, 1.00 mg/L in 2M KCl and use a volumetric flask. For soil resin sheets make standards in .5N HCl acid.

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989.
- 9.4 A standard curve is determined before every analysis with the Perstorp 3500. R squared must equal 0.98 or greater before samples are analyzed.

- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable range for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. PROCEDURE AND CALCULATIONS

10.1 Startup:

1. Make up reagents and standards.
2. Configure pump with proper tubes and connect to Ammonia cartridge.
- 3 Turn power on and apply tension to pump platens.
4. Connect all lines to H₂O and surfactant and observe stream.
5. Connect reagent lines and observe stream.

10.2 Computer and Sampler startup:

1. Turn power on and run Winflow program.
2. Select and click the Sample Table button. Edit an existing table or create a new one. Table should start with highest calibration standard (SYNC) sample followed by the lowest sample for carry over correction (CO) then a read baseline (RB). Calibration standards are next with duplicates from highest to lowest. Include QC standard after calibration standards and RB. Insert calibration verification (mid-range standard) after each 20 samples. For each sample cup indicate: location, sample ID, type, dilution, and weight. Rename, save and print the sample table.
3. Click the Method Editor button and load an existing Methods file or create a new file. Verify all setting are correct. Refer to a previous method or the help files.
4. Select the Data Collect button and verify or select Method and Sample Table in Run Setup. Start pump with reagents tubes in surfactant water.
5. Enter an Operator ID and verify or enter a Filename for the run.
6. Click on the Play button and monitor the baseline. When the baseline is stable, startup solution (water) then switch to reagents. Allow a few minutes for reagents to equilibrate and monitor for a steady baseline.

10.3 Sample run:

1. When a stable baseline has been achieved use the Sample Table print out to start loading the sample trays.
2. Click the Fast Forward Start button to start data collection and sampler.
3. Monitor the run until the SYNC peak has eluted and successfully marked the top of the peak at its steady state. Allow the run to continue. If run stops automatically then data file will be saved. If the run is stopped manually by clicking the Stop button then be sure to save the results once the completed run is displayed.
4. Review the results. Calibration curve should yield a r^2 value greater than .98.
5. Reports are produced by opening the File window and selecting the proper option. Reports may be edited, printed, and exported as needed.

10.4 Shutdown:

1. Connect all reagent lines to deionized water.
2. Pump deionized water through the system for 15-20 minutes.
3. Turn off the 509 power and unlatch all pump platens. Caution: Pump tubes may be seriously damaged if the platens remain latched with the pump power off.
4. If the cartridge is to be stored away from the system or if it will not be used for

a week or more, remove the reagent lines from the deionized water and pump the cartridge completely dry.

5. Turn off the gas supply if not being used.

10.5 Troubleshooting:

1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow through system.
2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operation parameters.
3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

Chemistry

Reagent
Standards
pH
Temperature

Hydraulic

Pump tubing
Bubble size
Surfactant
Pump

Electrical/Mechanical

Circuit components
Optics/Lamps
Photometer/Detector
Cabling

4. Eliminate one variable at a time. Using good experimentation techniques, change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.
5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
6. See manual for further trouble shooting solutions

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions. Table 3 summarizes the data.

12. REFERENCES

- 12.1 Perstorp Enviroflow 3500 Operation Manuals, Perstorp Analytical Corporation, 1992.
- 12.2 Methods for Chemical Analysis of Water and Waste, Method 350.1, EPA, 1983.
- 12.3 Standard Methods for the Examination of Water and Wastewater, Method 417 C, "Determination of Nitrogen as Ammonia", 16th edition, 1985.

Table 1. Method Detection Limits and Concentration Ranges for Ammonium

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|----------|-----------------------------|--------------------------|
| Ammonium | .001 | 0.01 - 1.00 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 9

Table 2. Suggested Calibration Standards for Ammonium Sample at Coweeta

| Analyte | Calibration Standards mg/L |
|----------|-------------------------------|
| Ammonium | 0.010, 0.05, 0.10, 0.50, 1.00 |

Table 3. Single Operator Precision and Bias for Ammonium determined from Quality Control Samples

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|----------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| Ammonium | .029 | 15 | .029 | .000 | .003 | 9.693 |

Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Table 4. Example of Sample ID table.

| TABLE NUMBER: 1 | | | | TABLE NAME: M-NH4-RL | | | |
|-----------------|------------------|------------|------------|----------------------|------------------|------------|------------|
| <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> | <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> |
| 1 | SYNC | 1 | 1 | 2 | W | 1 | 1 |
| 3 | S1 | 1 | 1 | 4 | S1 | 1 | 1 |
| 5 | S2 | 1 | 1 | 6 | S2 | 1 | 1 |
| 7 | S3 | 1 | 1 | 7 | S3 | 1 | 1 |
| 9 | S4 | 1 | 1 | 10 | S4 | 1 | 1 |
| 11 | S5 | 1 | 1 | 12 | S5 | 1 | 1 |
| 13 | CC | 1 | 1 | 14 | W | 1 | 1 |
| 15 | c1ad | 1 | 1 | 16 | c1as | 1 | 1 |
| 17 | c2ad | 1 | 1 | 18 | c2as | 1 | 1 |
| 19 | c3ad | 1 | 1 | 20 | c3as | 1 | 1 |
| 21 | c4ad | 1 | 1 | 22 | c4as | 1 | 1 |
| 23 | c5ad | 1 | 1 | 24 | c5as | 1 | 1 |
| 25 | c6ad | 1 | 1 | 26 | c6as | 1 | 1 |
| 27 | c7ad | 1 | 1 | 28 | c7as | 1 | 1 |

Table 5. Example of Sampler/Channel Setup Table

501 Sampler Setup

SAMPLE TIME= [30]
 WASH TIME= [35]
 SAMPLER DATA CHANNELS= [2]
 FIRST CHECK CALIBRANT POSITION= [13]
 NUMBER OF CHECK CALIBRANTS= [1]
 BASELINE CHECK INTERVAL= [20]
 BASELINE CHECK DURATION= [1]
 FIRST INSERTED BASELINE PRECEEDS CUP# [34]
 REPLICATE COUNT FOR ALL CALIBRANTS= [1]
 REPLICATE COUNT FOR ALL SAMPLES= [1]
 OPERATOR VERIFICATION OF CALIBRATION Y/N [Y]
 AUTO RERUN OF OFF-SCALE SAMPLES= [ON]
 # OF SAMPLES AFTER EACH OFF-SCALE TO RERUN= [1]
 FIRST DILUTION FOR OFF-SCALES= [10]

Channel Setup

CHANNEL #= [2]
CHANNEL NAME= NH4-N
START IGNORE TIME= [65]
INITIAL BASELINE LEAD TIME= [65]
CORRECTIONS CODE Y/N [Y]
CYCLE TIME= [65]
COLLECTION RATE= [2] POINTS / SEC.
CHANNEL OFF-SCALE WARNING= [ON]
OFF-SCALE WARNING LIMIT= [25]
CHANNEL ZERO SCALE WARNING= [OFF]
INVERT RAW DATA? Y/N [N]
NOMINAL VALUE OF CHECK CAL= [.05]
PERCENT DEVIATION FROM NOMINAL= [16]
OUT OF RANGE LIMIT. PERCENT= [10]
CHECK CALIBRANT ID= [CC]

Table 6. Example of Standards Table

Calibration Code: 1
Units: ppm
Calibration Mode: CF

Channel #: 2

| | | | |
|-----|-----|-----|---|
| S1 | 0 | S11 | 0 |
| S2 | .01 | S12 | 0 |
| S3 | .05 | S13 | 0 |
| S4 | .1 | S14 | 0 |
| S5 | .2 | S15 | 0 |
| S6 | 0 | S16 | 0 |
| S7 | 0 | S17 | 0 |
| S8 | 0 | S18 | 0 |
| S9 | 0 | S19 | 0 |
| S10 | 0 | S20 | 0 |

Figure 1.

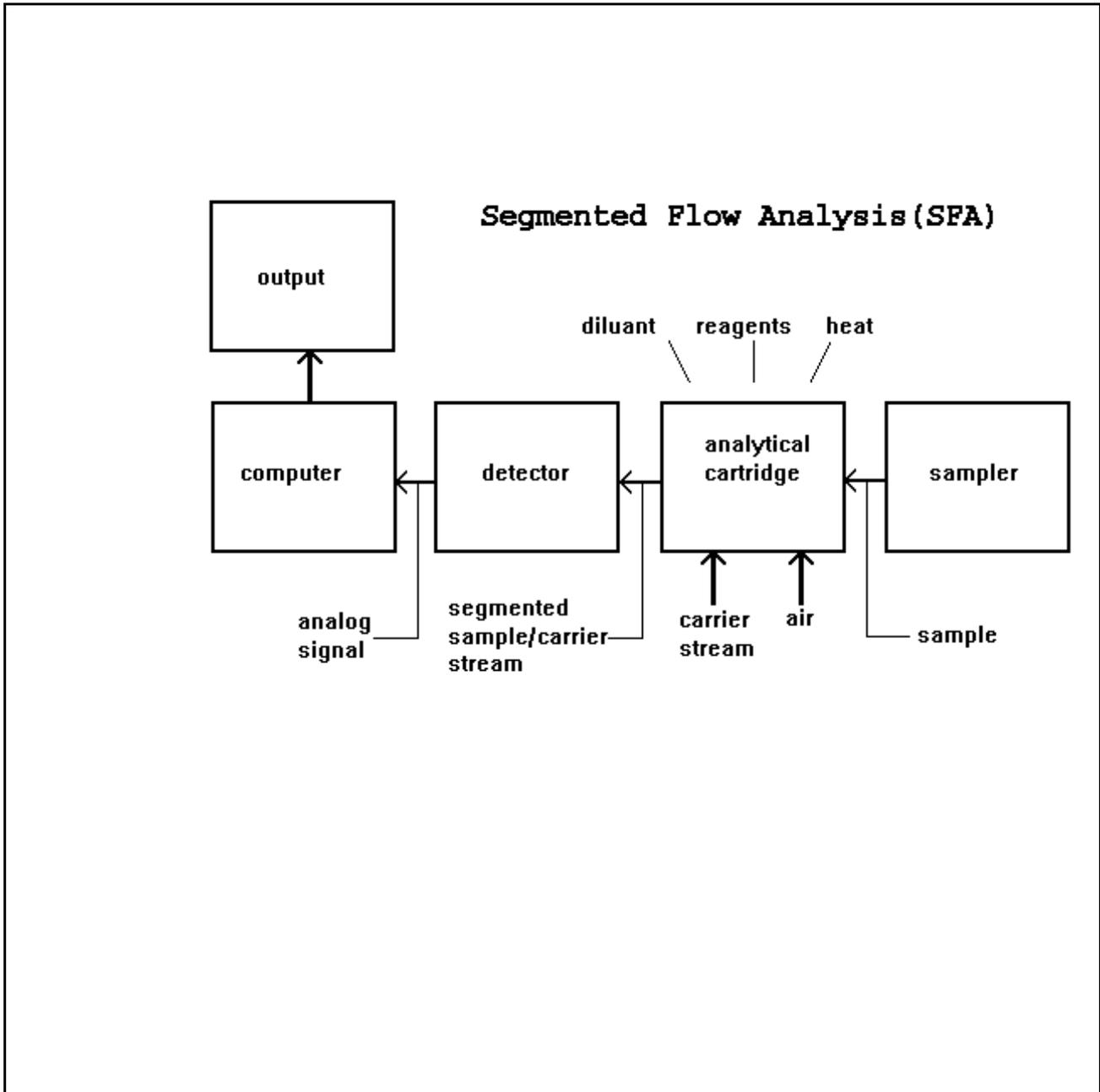
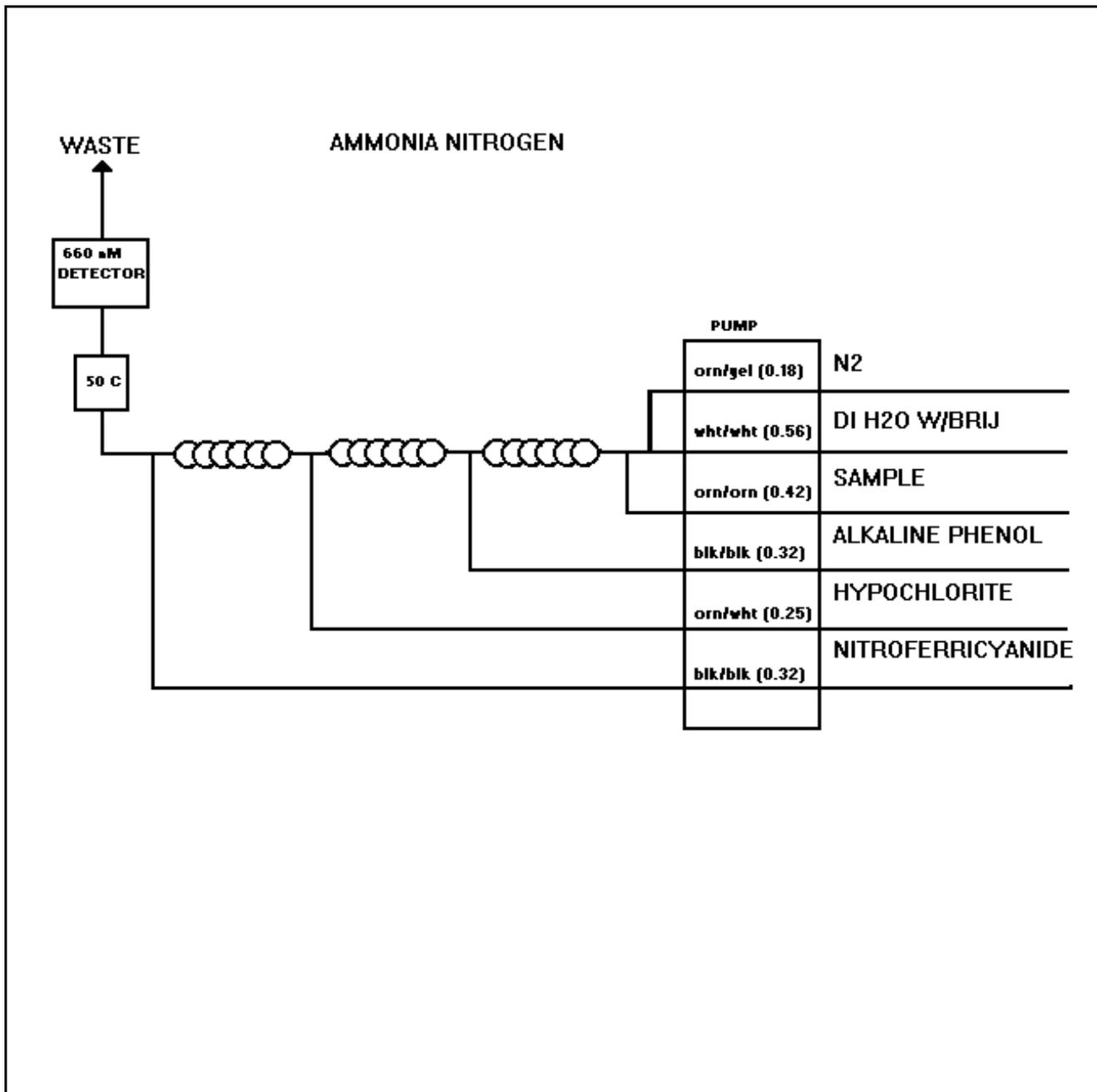


Figure 2.



Automated Wet Chemistry
Nitrate by Cadmium Reduction
using Perstorp by AlpKem

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

Cindi Brown

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Instrument retired October 25, 2011

INDEX

AlpKem

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| 05 | Safety |
| 06 | Apparatus and Equipment |
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| 08 | Calibration and Standardization |
| 09 | Quality Control |
| 10 | Procedure and Calculations |
| 11 | Precision and Bias |
| 12 | References |

TABLES

1. Method Detection Limits.
2. Suggested Calibration Standards for Samples at Coweeta.
3. Single-Operator Precision and Bias for Nitrate determined from Quality Control Samples.
4. Example of Sample ID Table.
5. Example of Sampler/Channel Setup Table.
6. Example of Standards Table

FIGURES

1. System diagram for Perstorp 3500.
2. Manifold setup for Nitrate.

AlpKem

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Nitrate in Stream, Precipitation, Thrufall, Lysimeter, and extracted soil samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that are highly colored.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Nitrate in the sample is reduced to Nitrite by passing through a cadmium coil reactor. The Nitrite then reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye. The reaction is measured at 520nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance

a = absorptivity

b = length of light path

c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.

3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

4.1 Highly colored samples that absorb in the wavelength range of 520 - 540 nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.

4.2 Samples high in turbidity must be filtered prior to analysis.

4.3 Samples high in iron or copper will need to be form a complex with EDTA.

4.4 Keep glassware that has been rinsed in HNO₃ separated for cations only.

5. SAFETY

5.1 Wear lab coat, gloves, and eye protection when using Hydrochloric Acid, Phosphoric Acid and Ammonium Hydroxide. Always work under a hood, vapors can be harmful.

5.2 Wear protective clothing when handling Cadmium coil.

5.3 When Cadmium coil has expired, store in tightly sealed container for later disposal at a hazardous waste treatment storage facility.

5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.

5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. APPARATUS AND EQUIPMENT

6.1 Perstorp Enviroflow 3500

6.1.1 Autosampler:

Perstorp model 501 is a 300 position random access sampler. It can be operated in manual or remote mode.

6.1.2 Dilutor:

Perstorp model 511 is an autodilutor interfaced to a computer. After a sample run is complete the computer recognizes peaks that have run above the highest calibration standard. The computer instructs the autosampler and the dilutor to rerun the off scale samples making a 10 X dilution.

6.1.3 Peristaltic Pump:

Perstorp model 502 is a multispeed peristaltic pump with individual platens for 24 tubing channels. Pump speed and tubing inside diameters determine the flow rates for the sample and reagents.

6.1.4 Mixing Manifolds:

Perstorp Supercartridge Jr. is a preconfigured mixing manifold for testing of Nitrate. The cartridge consist of polymeric tubing, fitting, reagent tees, coils and connectors.

- 6.1.5 Cartridge Heater:
Perstorp model 503 cartridge heater is a electronic digitally operated temperature controller. It incorporates four heater cones to provide heat to Supercartridges when needed.
- 6.1.6 Detector:
Perstorp model 510 is a variable wavelength, flow through absorbance detector. Wavelength is selected by a user adjustable concave holographic grating. The detector can operate from 360 nm to 800 nm with a tungsten halogen lamp. Output from the detector is sent to the computer and digitized.
- 6.1.7 Computer Interface:
Analog signals from the detectors are processed by a 12 channel A/D board.
- 6.1.8 Computer Software:
Perstorp Softpac Plus software package allows user control of the autosampler, diluter, data acquisition, calculation of results and analog signal display. Analysis parameters are entered via on screen tables and a series of prompting menus. Reports are generated to printer or disk.
- 6.1.9 Computer:
System requires at least a Pentium 90hz microprocessor, 16 meg of ram, floppy disk drive, cd rom drive, 1gig hard drive, color monitor, printer, mouse, serial port, and parallel port.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Nitrate/Nitrite free DI water. Filter the Sulfanilamide color and Buffer reagent prior to use.

- 7.1 Stock Imidizole Buffer, 0.1M)
Dissolve 6.81g NHCH:NCH:CH in 900 mL DI water in 1L flask. Adjust the pH to 7.5 with concentrated Hydrochloric Acid (2.5 – 2.75ml). Transfer to polyethylene container. Solution is stable for one month. For soil resin sheets(acidic) a .01N NaOH solution replaces water in the buffer solution. (imidizole buffer solution, Bran-Luebbe procedure)
- 7.2 Color Reagent (250 mL)
Place stir bar in 250 mL flask with 200 mL of DI water. While stirring, add 25 mL of concentrated Phosphoric Acid H_3PO_4 . Dissolve 10g of Sulfanilamide $C_6H_8N_2O_2S$ and .5g of N-1 Naphthylethyldiamine Dihydrochloride $C_{12}H_{14}N_2 \cdot 2HCl$ and dilute to 250 mL with DI water. Store reagent in dark polyethylene bottle at 4° C. Reagent is stable for two weeks.
- 7.3 .5N Hydrochloric Acid (250 mL)
Add 10.4 mL concentrated Hydrochloric Acid HCl to 200 mL DI water in volumetric flask. Mix well and dilute to final volume of 250 mL with DI water.
- 7.4 Copper Sulfate, 0.01M (500 mL)
Dissolve 1.25g of Copper Sulfate $CuSO_4 \cdot 5H_2O$ in 400 mL DI water and dilute to 500 mL.
- 7.5 DI water with Brij - 35, 30% w/v (250 mL)
Add 3 drops of Brij - 35 to 250 mL of DI water. This is used as the diluent and as the startup solution. Use a .4N HCl solution for the sampler wash when running soil resin sheet extractions.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion calibrants, 1000mg/L are purchased from Ricca yearly.
- 8.2 Working Standards:
Nitrate: .010 mg/L, .050 mg/L, .100 mg/L, .200 mg/L. For N-Min samples, make standards in 2M KCl and use volumetric flask for dilutions. For soil resin sheets, make standards in .4N HCl acid.

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO_4 , Cl, NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.
- 9.4 A standard curve is determined before every analysis with the Perstorp 3500. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the ERA concentrates. Acceptable range for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. PROCEDURE AND CALCULATIONS

- 10.1 Startup:
1. Make up reagents and standards.
 2. Configure pump with proper tubes and connect to Nitrate cartridge.
 3. Turn power on and apply tension to pump platens.
 4. Connect all lines to H_2O and surfactant and observe stream.
 5. Connect reagent lines and observe stream.
- 10.2 Computer and Sampler startup:
1. Turn power on and run Winflow program.
 2. Select and click the Sample Table button.
 3. Edit an existing table or create a new one. Table should start with highest calibration

standard (SYNC) sample followed by the lowest sample for carry over correction (CO) then a read baseline (RB).

4. Calibration standards are next with duplicates from highest to lowest. Include QC standard after calibration standards and RB. Insert calibration verification (mid-range standard) after each 20 samples. For each sample cup indicate: location, sample ID, type, dilution, and weight.
5. Rename, save and print the sample table.
6. Click the Method Editor button and load an existing Methods file or create a new file.
7. Verify all settings are correct. Refer to a previous method or the help files.
8. Select the Data Collect button and verify or select Method and Sample Table in Run Setup.
9. Enter an Operator ID and verify or enter a Filename for the run. Click on the Play button and monitor the baseline. Purge diluter if Method calls for it. When the baseline is stable on startup solution (water) then switch to reagents. Allow a few minutes for reagents to equilibrate and monitor for a steady baseline.

10.3 Calibration:

1. Load sampler in this order: SYNC (highest standard), water, standards (duplicates - lowest to highest), water, samples, water.
2. Computer will signal operator to review calibration curve after standards have run. Calibration curve should yield a r^2 value greater than .98. Edit calibration curve if needed and return to data collection screen.

10.4 Sample run:

1. When a stable baseline has been achieved use the Sample Table print out to start loading the sample trays.
2. Click the Fast Forward Start button to start data collection and sampler.
3. Monitor the run until the SYNC peak has eluted and successfully marked the top of the peak at its steady state. Allow the run to continue. If run stops automatically then data file will be saved. If the run is stopped manually by clicking the Stop button then be sure to save the results once the completed run is displayed.
4. Review the results. Calibration curve should yield an r^2 value greater than .98.
5. Reports are produced by opening the File window and selecting the proper option. Reports may be edited, printed, and exported as needed.

10.5 Shutdown:

1. Connect all reagent lines to deionized water.
2. Pump deionized water through the system for 15-20 minutes.
3. Turn off the 509 power and unlatch all pump platens. Caution: Pump tubes may be seriously damaged if the platens remain latched with the pump power off.
4. If the cartridge is to be stored away from the system or if it will not be used for a week or more, remove the reagent lines from the deionized water and pump the cartridge completely dry.
5. Turn off the gas supply if not being used.

10.6 Troubleshooting:

1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow through system.
2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test

- methodologies and flow diagrams to verify operation parameters.
3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

| | | |
|------------------|------------------|------------------------------|
| <u>Chemistry</u> | <u>Hydraulic</u> | <u>Electrical/Mechanical</u> |
| Reagent | Pump tubing | Circuit components |
| Standards | Bubble size | Optics/Lamps |
| pH | Surfactant | Photometer/Detector |
| Temperature | Pump | Cabling |
 4. Eliminate one variable at a time. Using good experimentation techniques change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.
 5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
 6. See operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions. Table 3 summarizes the data.

12. REFERENCES

- 12.1 Perstorp Enviroflow 3500 Operation Manuals, Perstorp Analytical Corporation, 1992.
- 12.2 Methods for Chemical Analysis of Water and Waste, Method 353.2, EPA, 1983.
- 12.3 Standard Methods for the Examination of Water and Wastewater, Method 418 F, "Determination of Nitrate/Nitrite by Automated Cadmium Reduction", 16th edition, 1985.

Table 1. Method Detection Limits and Concentration Ranges for Nitrate

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|---------|-----------------------------|--------------------------|
| Nitrate | .004 | 0.01 - 1.00 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 9

Table 2. Suggested Calibration Standards for Nitrate Sample at Coweeta

| Analyte | Calibration Standards mg/L |
|---------|-------------------------------|
| Nitrate | 0.010, 0.05, 0.10, 0.50, 1.00 |

Table 3. Single Operator Precision and Bias for Nitrate determined from Quality Control Samples

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|---------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| Nitrate | .050 | 10 | .049 | .001 | .001 | 7.753 |

Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Mean Bias: Sum of bias for each sample/number of replicates

Table 4. Example of Sample ID table.

| TABLE NUMBER: 1 | | | | TABLE NAME: M-NO3-RL | | | |
|-----------------|------------------|------------|------------|----------------------|------------------|------------|------------|
| <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> | <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> |
| 1 | SYNC | 1 | 1 | 2 | W | 1 | 1 |
| 3 | S1 | 1 | 1 | 4 | S1 | 1 | 1 |
| 5 | S2 | 1 | 1 | 6 | S2 | 1 | 1 |
| 7 | S3 | 1 | 1 | 7 | S3 | 1 | 1 |
| 9 | S4 | 1 | 1 | 10 | S4 | 1 | 1 |
| 11 | S5 | 1 | 1 | 12 | S5 | 1 | 1 |
| 13 | CC | 1 | 1 | 14 | W | 1 | 1 |
| 15 | c1ad | 1 | 1 | 16 | c1as | 1 | 1 |
| 17 | c2ad | 1 | 1 | 18 | c2as | 1 | 1 |
| 19 | c3ad | 1 | 1 | 20 | c3as | 1 | 1 |
| 21 | c4ad | 1 | 1 | 22 | c4as | 1 | 1 |
| 23 | c5ad | 1 | 1 | 24 | c5as | 1 | 1 |
| 25 | c6ad | 1 | 1 | 26 | c6as | 1 | 1 |
| 27 | c7ad | 1 | 1 | 28 | c7as | 1 | 1 |

Table 5. Example of Sampler/Channel Setup Table

501 Sampler Setup

SAMPLE TIME= [35]
 WASH TIME= [35]
 SAMPLER DATA CHANNELS= [1]
 FIRST CHECK CALIBRANT POSITION= [13]
 NUMBER OF CHECK CALIBRANTS= [1]
 BASELINE CHECK INTERVAL= [20]
 BASELINE CHECK DURATION= [1]
 FIRST INSERTED BASELINE PRECEEDS CUP# [34]
 REPLICATE COUNT FOR ALL CALIBRANTS= [1]
 REPLICATE COUNT FOR ALL SAMPLES= [1]
 OPERATOR VERIFICATION OF CALIBRATION Y/N [Y]
 AUTO RERUN OF OFF-SCALE SAMPLES= [ON]
 # OF SAMPLES AFTER EACH OFF-SCALE TO RERUN= [1]
 FIRST DILUTION FOR OFF-SCALES= [10]

Channel Setup

CHANNEL #= [1]
 CHANNEL NAME= NO3-N
 START IGNORE TIME= [70]
 INITIAL BASELINE LEAD TIME= [70]
 CORRECTIONS CODE Y/N [Y]
 CYCLE TIME= [70]
 COLLECTION RATE= [2] POINTS / SEC.
 CHANNEL OFF-SCALE WARNING= [ON]
 OFF-SCALE WARNING LIMIT= [100]

CHANNEL ZERO SCALE WARNING= [OFF]
INVERT RAW DATA? Y/N [N]
NOMINAL VALUE OF CHECK CAL= [.055]
PERCENT DEVIATION FROM NOMINAL= [11]
OUT OF RANGE LIMIT. PERCENT= [10]
CHECK CALIBRANT ID= [CC]

Table 6. Example of Standards Table

| | | | |
|-------------------|-----|-----|--------------|
| Calibration Code: | 1 | | |
| Units: | ppm | | Channel #: 1 |
| Calibration Mode: | CF | | |
| S1 | 0 | S11 | 0 |
| S2 | .01 | S12 | 0 |
| S3 | .05 | S13 | 0 |
| S4 | .1 | S14 | 0 |
| S5 | .2 | S15 | 0 |
| S6 | 0 | S16 | 0 |
| S7 | 0 | S17 | 0 |
| S8 | 0 | S18 | 0 |
| S9 | 0 | S19 | 0 |
| S10 | 0 | S20 | 0 |

Figure 1.

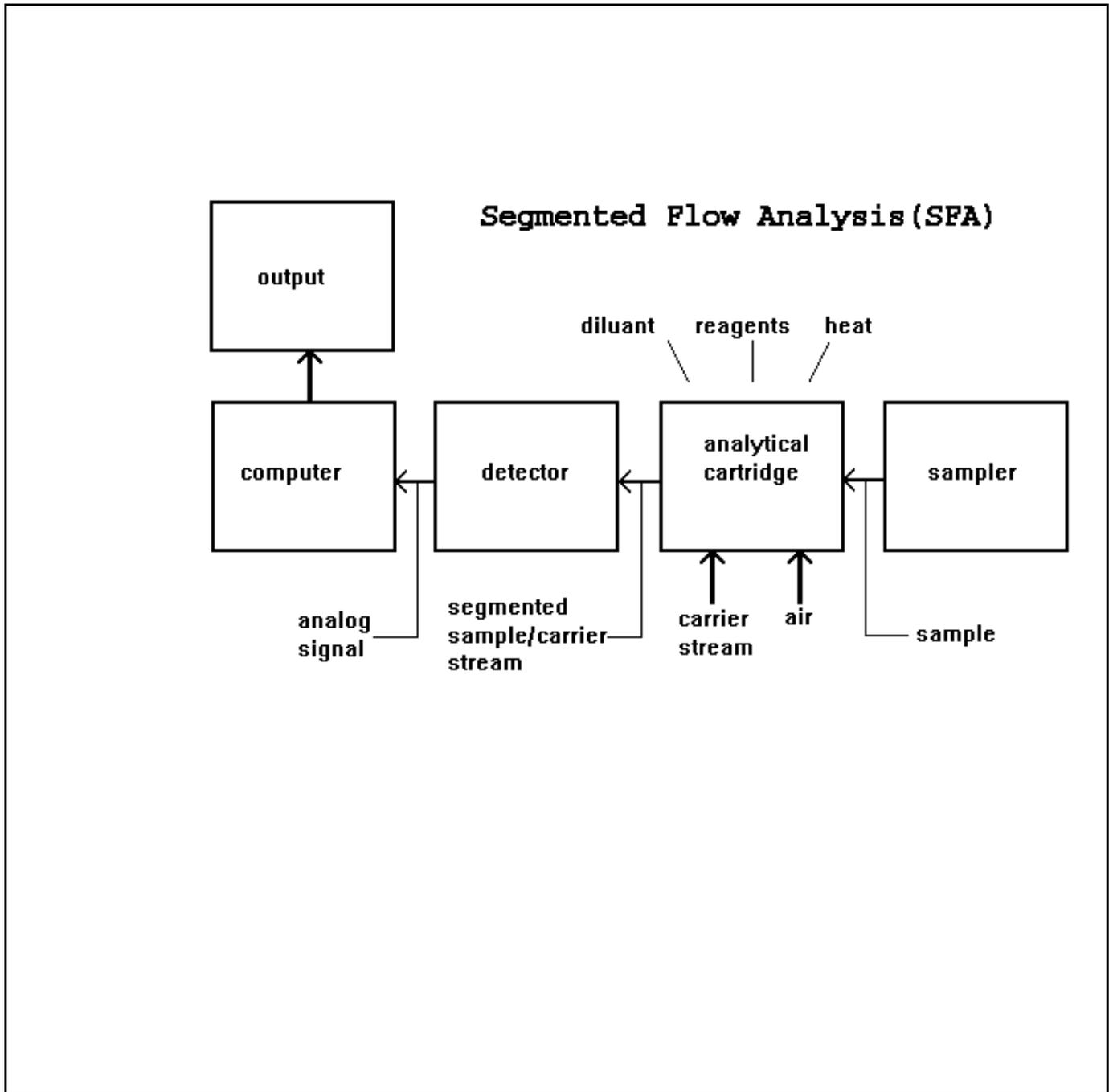
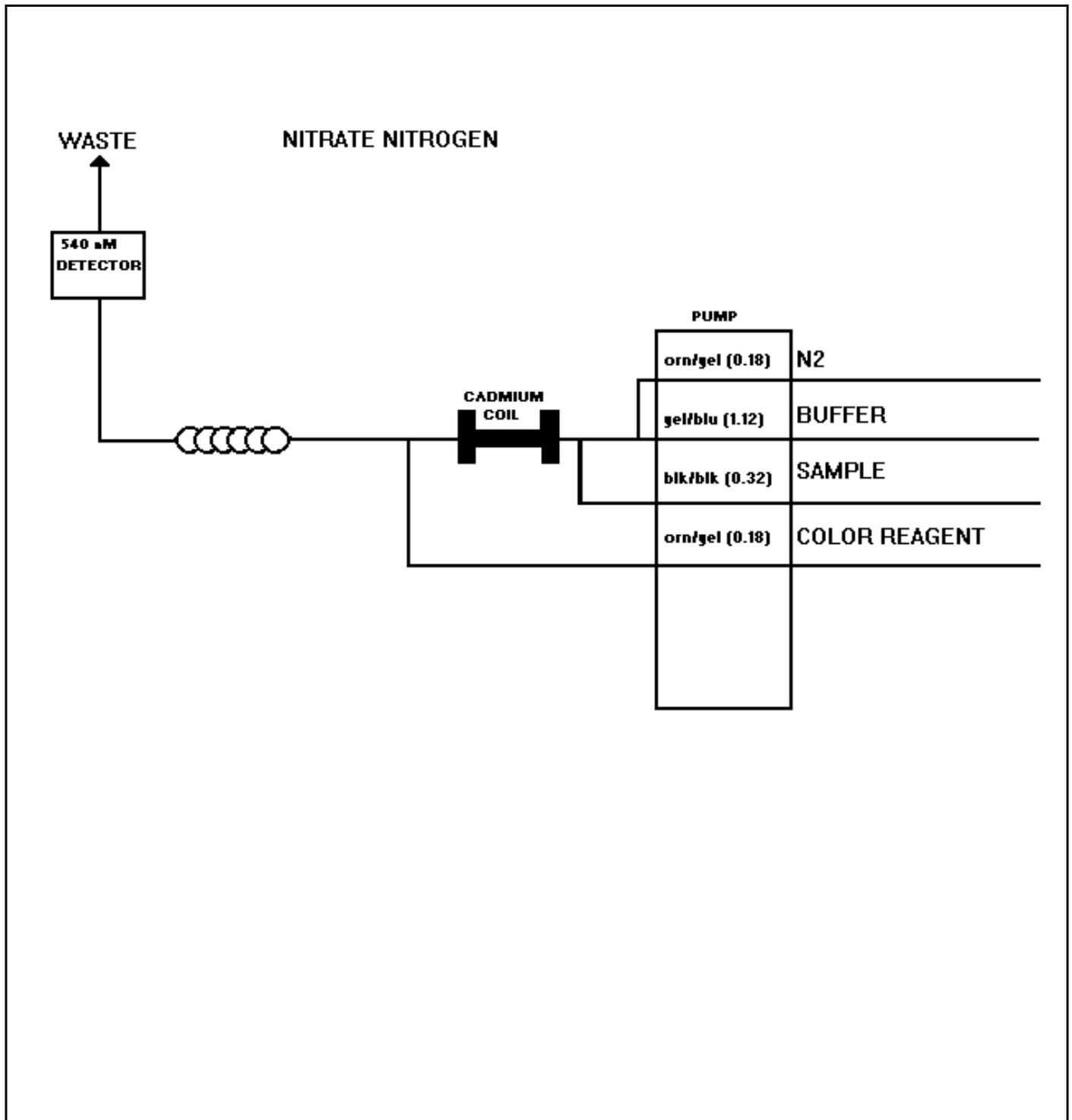


Figure 2.



Automated Wet Chemistry
for Orthophosphate
using Perstorp by AlpKem

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

Cindi Brown

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Instrument retired October 25, 2011

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I. AlpKem

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1. Method Detection Limits.
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1. System diagram for Perstorp 3500.
2. Manifold setup for Orthophosphate.

AlpKem

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Orthophosphate in Stream, Precipitation, Thrufall, Lysimeter, and extracted soil samples.
- 1.2 Orthophosphate measured by this method is Total Reactive Phosphorus. Refer to section 424 in Standard Methods for the Examination of Water and Wastewater for further information on fractions of Phosphorus.
- 1.3 Method detection limits are summarized in Table 1.

2. SUMMARY OF METHOD

- 2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. Orthophosphate in the sample reacts with Ammonium Molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ and Antimony Potassium Tartrate $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot \frac{1}{2}\text{H}_2\text{O}$ in acid media to form an Antimony-phosphomolybdate complex. The complex is reduced with Ascorbic Acid $\text{C}_6\text{H}_8\text{O}_6$ to form a blue color that is measured at 660nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance

a = absorptivity

b = length of light path

c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 640 - 660nm can cause false positive readings. Samples from coastal waters can present problems.
- 4.2 Samples high in turbidity must be filtered prior to analysis.
- 4.3 Silica concentrations greater than 10mg/L can cause positive interference. SiO₂ concentrations of 20mg/L would cause .005mg/L positive readings. Samples at Coweeta run below 20mg/L.

5. SAFETY

- 5.1 Wear lab coat, gloves, and eye protection when using Sulfuric Acid, Antimony Potassium Tartrate, and Ammonium Molybdate. Always work under a hood, vapors can be harmful.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. APPARATUS AND EQUIPMENT

- 6.1 Perstorp Enviroflow 3500
 - 6.1.1 Autosampler:
Perstorp model 501 is a 300 position random access sampler. It can be operated in manual or remote mode.
 - 6.1.2 Dilutor:
Perstorp model 511 is a autodilutor interfaced to a computer. After a sample run is complete the computer recognizes peaks that have run above the highest calibration standard. The computer instructs the autosampler and the dilutor to rerun the off scale samples making a 10 X dilution.
 - 6.1.3 Peristaltic Pump:
Perstorp model 502 is a multispeed peristaltic pump with individual platens for 24 tubing channels. Pump speed and tubing inside diameters determine the flow rates for the sample and reagents.
 - 6.1.4 Mixing Manifolds:
Perstorp Supercartridge Jr. is a preconfigured mixing manifold for testing of Nitrate. The cartridge consist of polymeric tubing, fitting, reagent tees, coils and connectors.
 - 6.1.5 Cartridge Heater:
Perstorp model 503 cartridge heater is a electronic digitally operated temperature controller. It incorporates four heater cones to provide heat to Supercartridges when needed.
 - 6.1.6 Detector:
Perstorp model 510 is a variable wavelength, flow through absorbance detector.

Wavelength is selected by a user adjustable concave holographic grating. The detector can operate from 360 nm to 800 nm with a tungsten halogen lamp. Output from the detector is sent to the computer and digitized.

- 6.1.7 Computer Interface:
Analog signals from the detectors are processed by a ER interface.
- 6.1.8 Computer Software:
Perstorp Winflow software package allows user control of the autosampler, diluter, data acquisition, calculation of results and analog signal display. Analysis parameters are entered via on screen tables and a series of prompting menus. Reports are generated to printer or disk.
- 6.1.9 Computer:
System requires at least a Pentium 90hz microprocessor, 16 meg of ram, floppy disk drive, cd rom drive, 1gig hard drive, color monitor, printer, mouse, serial port, and parallel port.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Phosphate free DI water. Filter the all reagents prior to use.

- 7.1 Sulfuric Acid, .5N (250 mL)
Add 35 mL of concentrated Sulfuric Acid H_2SO_4 to 200 mL of DI water. Mix well and dilute to final volume of 250 mL.
- 7.2 Stock Ammonium Molybdate Reagent (250 mL)
Dissolve 10g of Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 200 mL of DI water. Dilute to 250 mL with DI water. Store reagent in dark polyethylene bottle at 4° C. Reagent is not stable for more than two days. If reagent turns a faint blue, then remake.
- 7.3 Stock Antimony Potassium Tartrate (250mL)
Dissolve .75g of Antimony Potassium Tartrate $K(SbO)C_4H_4O_6 \cdot 1/2 2H_2O$ in 200 mL of DI water. Mix well and dilute to 250 mL with DI water. Store in a plastic bottle at 4°C for no more than one week.
- 7.4 Ascorbic Acid (250 mL)
Dissolve 4.4g of Ascorbic Acid $C_6H_8O_6$ in 200mL DI water with 12.5 mL of Acetone. Dilute to 250 mL with DI water. Store in a plastic bottle at 4°C for no more than one week.
- 7.5 Color Reagent (200 mL)
Stock Sulfuric acid, 5N-----100mL
Stock Antimony Potassium Tartrate Solution-----10mL
Stock Ammonium Molybdate Solution-----30mL
Stock Ascorbic Acid Solution-----60mL
Dowfax 2A1-----0.5mL

Add reagents in the order stated and mix after each addition. This will prevent the ascorbic acid from turning a darker color when the solution is first made. Prepare reagent daily.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion calibrants are 1000mg/L purchased from Ricca yearly.

- 8.2 Working Standards:
For Coweeta samples: .010 mg/L, .050 mg/L, .100 mg/L, .200 mg/L
For Double Acid Extraction samples: 5.0mg/L, 10.0mg/L, 20.0mg/L, 30.0mg/L

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO_4 , Cl, NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.
- 9.4 A standard curve is determined before every analysis with the Perstorp 3500. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. PROCEDURE AND CALCULATIONS

- 10.1 Startup:
1. Make up reagents and standards.
 2. Configure pump with proper tubes and connect to Silicate cartridge.
 3. Turn power on and apply tension to pump platens.
 4. Connect all lines to H_2O and surfactant and observe stream.
 5. Connect reagent lines and observe stream.
- 10.2 Computer and Sampler startup:
1. Turn power on and run Winflow program.
 2. Select and click the Sample Table button. Edit an existing table or create a new one. Table should start with highest calibration standard (SYNC) sample followed by the lowest sample for carry over correction (CO) then a read baseline (RB). Calibration standards are next with duplicates from highest to lowest. Include QC standard after calibration standards and RB. Insert calibration verification (mid-range standard) after each 20 samples. For each sample cup indicate: location, sample ID, type, dilution, and weight. Rename, save and print the sample table.

5. Click the Method Editor button and load an existing Methods file or create a new file. Verify all settings are correct. Refer to a previous method or the help files.
6. Select the Data Collect button and verify or select Method and Sample Table in Run Setup. Start pump with reagents tubes in surfactant water.
5. Enter an Operator ID and verify or enter a Filename for the run.
6. Click on the Play button and monitor the baseline. When the baseline is stable, put tubes in water then switch to reagents. Allow a few minutes for reagents to equilibrate and monitor for a steady baseline.

10.3 Sample run:

1. When a stable baseline has been achieved use the Sample Table print out to start loading the sample trays.
2. Click the Fast Forward Start button to start data collection and sampler.
3. Monitor the run until the SYNC peak has eluted and successfully marked the top of the peak at its steady state. Allow the run to continue. If run stops automatically then data file will be saved. If the run is stopped manually by clicking the Stop button then be sure to save the results once the completed run is displayed.
4. Review the results. Calibration curve should yield an r^2 value greater than .98.
5. Reports are produced by opening the File window and selecting the proper option. Reports may be edited, printed, and exported as needed.

10.4 Shutdown:

1. Connect all reagent lines to deionized water.
2. Pump deionized water through the system for 15-20 minutes.
3. Turn off the 509 power and unlatch all pump platens. Caution: Pump tubes may be seriously damaged if the platens remain latched with pump power off.
4. If the cartridge is to be stored away from the system or if it will not be used for a week or more, remove the reagent lines from the deionized water and pump the cartridge completely dry.
5. Turn off the gas supply if not being used.

10.5 Troubleshooting:

1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow through system.
2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operation parameters.
3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

| | | |
|------------------|------------------|------------------------------|
| <u>Chemistry</u> | <u>Hydraulic</u> | <u>Electrical/Mechanical</u> |
| Reagent | Pump tubing | Circuit components |
| Standards | Bubble size | Optics/Lamps |
| pH | Surfactant | Photometer/Detector |
| Temperature | Pump | Cabling |

4. Eliminate one variable at a time. Using good experimentation techniques, change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.

5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
6. See operation manual for further trouble shooting solutions.

11. PRECISION AND BIAS

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions. Tables 3 and 4 summarize the data.

12. REFERENCES

- 12.1 Perstorp Enviroflow 3500 Operation Manuals, Perstorp Analytical Corporation, 1992.
- 12.2 Standard Methods for the Examination of Water and Wastewater, Method 424 G, "Determination of Phosphorus by Automated Wet Chemistry", 16th edition, 1985.
- 12.3 Methods for Chemical Analysis of Water and Waste, Method 365.3, EPA, 1983.

Table 1. Method Detection Limits and Concentration Ranges for Orthophosphate

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|----------------|-----------------------------|--------------------------|
| Orthophosphate | .015 | 0.01 - 1.00 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 9

Table 2. Suggested Calibration Standards for Orthophosphate Sample at Coweeta

| Analyte | Calibration Standards mg/L |
|------------------------------|-------------------------------|
| Orthophosphate -- high range | 5.0, 10.0, 20.0, 30.0 |
| Orthophosphate -- low range | 0.010, 0.05, 0.10, 0.50, 1.00 |

Table 3. Single Operator Precision and Bias for Orthophosphate determined from Standards (high)

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|----------------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| Orthophosphate | 11.30 | 8 | 11.95 | .65 | .24 | 2.01 |

Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Mean Bias: Sum of bias for each sample/number of replicates

Table 4. Single Operator Precision and Bias for Orthophosphate determined from Standards (low)

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|----------------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| Orthophosphate | .113 | 9 | .119 | .006 | .005 | 3.85 |

Table 5. Example of Sample ID table.

| TABLE NUMBER: 1 | | | | TABLE NAME: M-O-PO4-RL | | | |
|-----------------|------------------|------------|------------|------------------------|------------------|------------|------------|
| <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> | <u>CUP#</u> | <u>SAMPLE ID</u> | <u>DIL</u> | <u>WGT</u> |
| 1 | SYNC | 1 | 1 | 2 | W | 1 | 1 |
| 3 | S1 | 1 | 1 | 4 | S1 | 1 | 1 |
| 5 | S2 | 1 | 1 | 6 | S2 | 1 | 1 |
| 7 | S3 | 1 | 1 | 7 | S3 | 1 | 1 |
| 9 | S4 | 1 | 1 | 10 | S4 | 1 | 1 |
| 11 | S5 | 1 | 1 | 12 | S5 | 1 | 1 |
| 13 | CC | 1 | 1 | 14 | W | 1 | 1 |
| 15 | c1ad | 1 | 1 | 16 | c1as | 1 | 1 |
| 17 | c2ad | 1 | 1 | 18 | c2as | 1 | 1 |
| 19 | c3ad | 1 | 1 | 20 | c3as | 1 | 1 |
| 21 | c4ad | 1 | 1 | 22 | c4as | 1 | 1 |
| 23 | c5ad | 1 | 1 | 24 | c5as | 1 | 1 |
| 25 | c6ad | 1 | 1 | 26 | c6as | 1 | 1 |
| 27 | c7ad | 1 | 1 | 28 | c7as | 1 | 1 |

Table 6. Example of Sampler/Channel Setup Table

501 Sampler Setup

SAMPLE TIME= [30]
 WASH TIME= [35]
 SAMPLER DATA CHANNELS= [1]
 FIRST CHECK CALIBRANT POSITION= [15]
 NUMBER OF CHECK CALIBRANTS= [1]
 BASELINE CHECK INTERVAL= [15]
 BASELINE CHECK DURATION= [1]
 FIRST INSERTED BASELINE PRECEEDS CUP# [29]
 REPLICATE COUNT FOR ALL CALIBRANTS= [1]
 REPLICATE COUNT FOR ALL SAMPLES= [1]
 OPERATOR VERIFICATION OF CALIBRATION Y/N [Y]
 AUTO RERUN OF OFF-SCALE SAMPLES= [ON]
 # OF SAMPLES AFTER EACH OFF-SCALE TO RERUN= [1]
 FIRST DILUTION FOR OFF-SCALES= [10]

Channel Setup

CHANNEL #= [1]
CHANNEL NAME= O-PO4
START IGNORE TIME= [65]
INITIAL BASELINE LEAD TIME= [65]
CORRECTIONS CODE Y/N [Y]
CYCLE TIME= [65]
COLLECTION RATE= [2] POINTS / SEC.
CHANNEL OFF-SCALE WARNING= [ON]
OFF-SCALE WARNING LIMIT= [100]
CHANNEL ZERO SCALE WARNING= [OFF]
INVERT RAW DATA? Y/N [N]
NOMINAL VALUE OF CHECK CAL= [.055]
PERCENT DEVIATION FROM NOMINAL= [11]
OUT OF RANGE LIMIT. PERCENT= [10]
CHECK CALIBRANT ID= [CC]

Table 7. Example of Standards Table

Calibration Code: 1
Units: ppm
Calibration Mode: CF

Channel #: 1

| | | | |
|-----|-----|-----|---|
| S1 | .05 | S11 | 0 |
| S2 | .2 | S12 | 0 |
| S3 | .5 | S13 | 0 |
| S4 | 1 | S14 | 0 |
| S5 | 3 | S15 | 0 |
| S6 | 0 | S16 | 0 |
| S7 | 0 | S17 | 0 |
| S8 | 0 | S18 | 0 |
| S9 | 0 | S19 | 0 |
| S10 | 0 | S20 | 0 |

Figure 1.

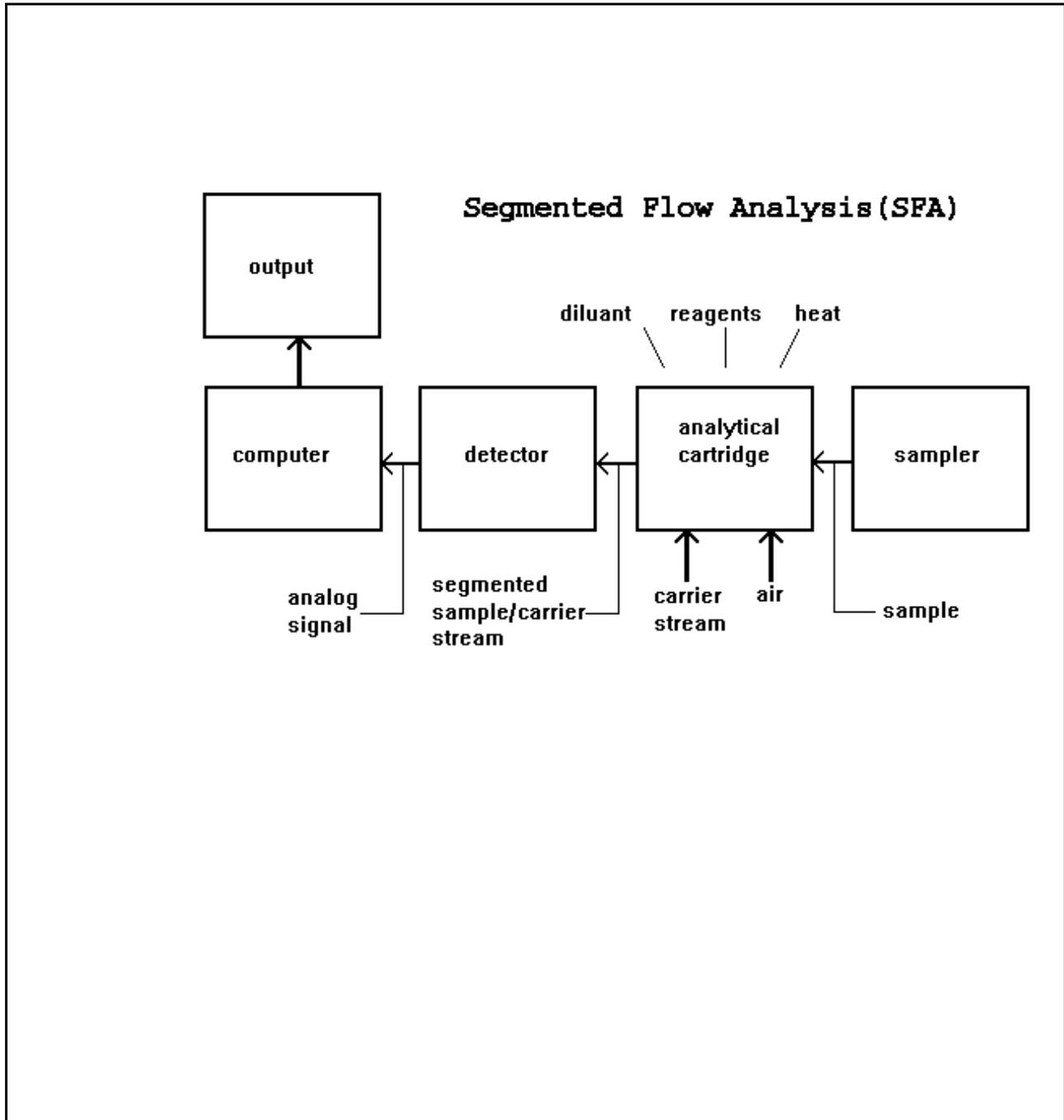
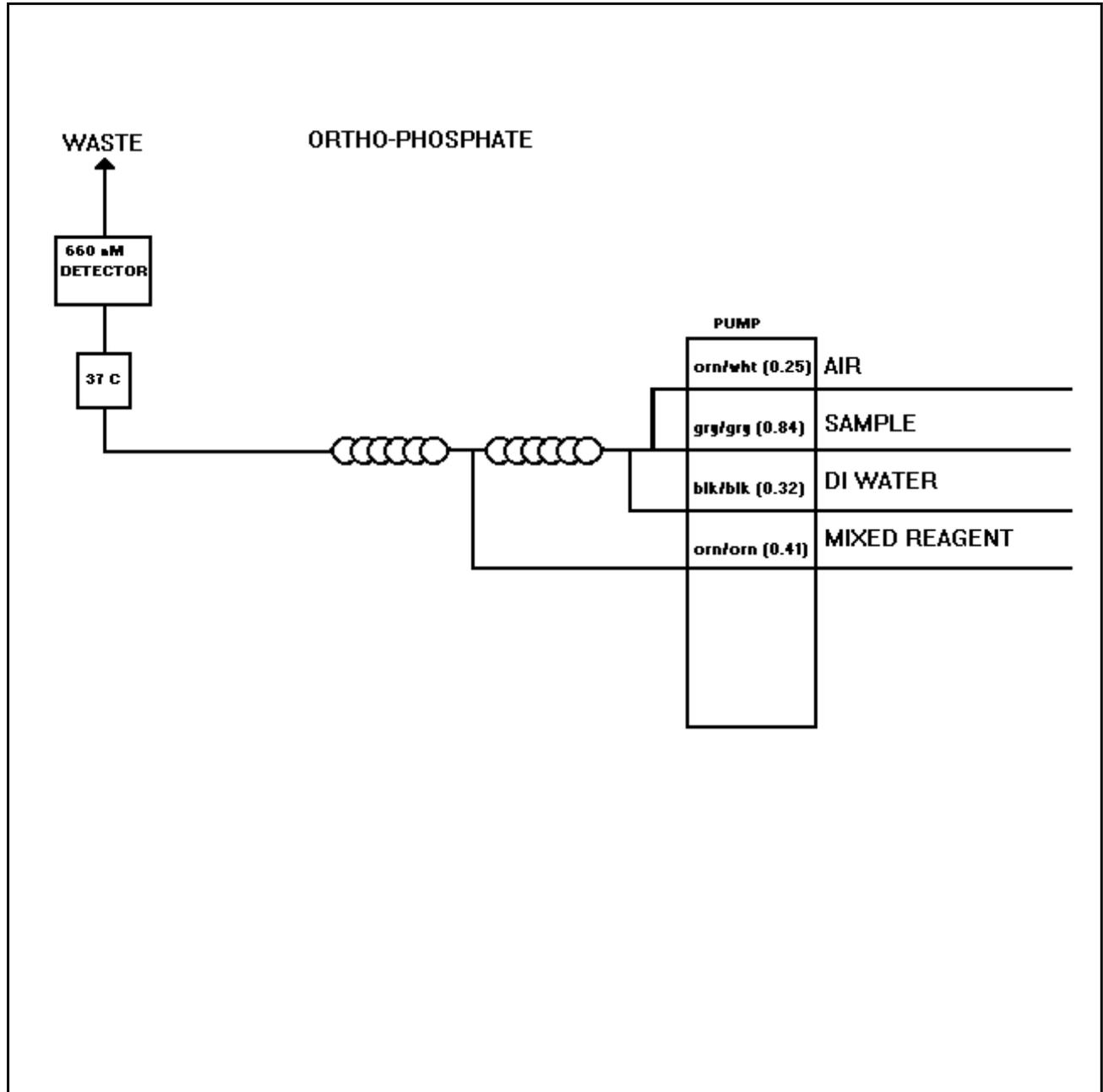


Figure 2.



Automated Wet Chemistry
for Silicate
using Perstorp by AlpKem

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
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James M. Deal

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Instrument retired October 25, 2011

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5. Example of Sample ID Table.
6. Example of Sampler/Channel Setup Table.
7. Example of Standards Table

FIGURES

1. System diagram for Perstorp 3500.
2. Manifold setup for Orthophosphate.

AlpKem

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Silica in Stream, Precipitation, Thrufall, and Lysimeter samples.
- 1.2 Method detection limits are summarized in Table 1.
- 1.3 This method is not recommended for samples that have been frozen.

2. SUMMARY OF METHOD

2.1 Samples are drawn from the automated sampler into a stream of reagents. Air bubbles are introduced to keep each sample discreet. The Silicate then reacts with Ammonium Molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ in acid media to form β -molybdosilicic acid. The complex is reduced by Ascorbic Acid $\text{C}_6\text{H}_8\text{O}_6$ to form Molybdenum Blue. The reaction is measured at 660nm when the solution goes to the detector. The detector is a spectrometer that utilizes a flow cell to measure changes in transmitted light intensity. Beer's law correlates transmitted light intensity to the concentration of ions in the sample.

$$\log (1/T) = abc$$

where: T = transmittance
a = absorptivity
b = length of light path
c = concentration of ion (mg/L)

Calibration curves are constructed from a series of known standard concentrations. The concentration of the samples are calculated based on the calibration curve.

3. DEFINITIONS

- 3.1 Absorbance -- The measurement of the absorption of a particular wavelength of light as it passes through a liquid.
- 3.2 Colorimetry -- the measurement of light transmitted by a colored complex as a function of concentration.
- 3.3 Monochromator -- a detector which measures absorbance of light at specific wavelengths using a diffraction grating to select a specific wavelength of light.
- 3.4 Peristaltic Pump -- a pumping system which moves liquids by compressing flexible tubing between a solid surface (platen) and a roller.
- 3.5 Segmented Flow Analysis (SFA) -- a technique where the samples are separated by bubbles of a gas. The bubbles also help mix the reagents as they move through the manifold coils.
- 3.6 Transmittance -- The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample

4. INTERFERENCES

- 4.1 Highly colored samples that absorb in the wavelength range of 640 - 660nm can cause false positive readings. Samples at Coweeta are usually clear so this is no problem.
- 4.2 Samples high in turbidity must be filtered prior to analysis.
- 4.3 Phosphates interfere but are suppressed by Oxalic Acid.
- 4.4 Do not freeze samples, silica will precipitate out of solution and cause false negative readings.

5. SAFETY

- 5.1 Wear lab coat, gloves, and eye protection when using Sulfuric Acid, Oxalic Acid Acetone, and Ammonium Molybdate. Always work under a hood, vapors can be harmful.
- 5.4 Wear protective clothing when working around the manifold, tubes could become clogged and pressure may increase enough to cause a tube to pop loose.
- 5.5 Read the lab safety plan and ask the lab safety officer if you have any questions.

6. APPARATUS AND EQUIPMENT

- 6.1 Perstorp Enviroflow 3500
 - 6.1.1 Autosampler:
Perstorp model 501 is a 300 position random access sampler. It can be operated in manual or remote mode.
 - 6.1.2 Dilutor:
Perstorp model 511 is an autodilutor interfaced to a computer. After a sample run is complete the computer recognizes peaks that have run above the highest calibration standard. The computer instructs the autosampler and the dilutor to rerun the off scale samples making a 10 X dilution.
 - 6.1.3 Peristaltic Pump:
Perstorp model 502 is a multispeed peristaltic pump with individual platens for 24 tubing channels. Pump speed and tubing inside diameters determine the flow rates for the sample and reagents.
 - 6.1.4 Mixing Manifolds:
Perstorp Supercartridge Jr. is a preconfigured mixing manifold for testing of Nitrate. The cartridge consist of polymeric tubing, fitting, reagent tees, coils and connectors.
 - 6.1.5 Cartridge Heater:
Perstorp model 503 cartridge heater is a electronic digitally operated temperature controller. It incorporates four heater cones to provide heat to Supercartridges when needed.

- 6.1.6 Detector:
Perstop model 510 is a variable wavelength, flow through absorbance detector. Wavelength is selected by a user adjustable concave holographic grating. The detector can operate from 360 nm to 800 nm with a tungsten halogen lamp. Output from the detector is sent to the computer and digitized.
- 6.1.7 Computer Interface:
Analog signals from the detectors are processed by an ER interface.
- 6.1.8 Computer Software:
Perstop Winflow software package allows user control of the autosampler, diluter, data acquisition, calculation of results and analog signal display. Analysis parameters are entered via on screen tables and a series of prompting menus. Reports are generated to printer or disk.
- 6.1.9 Computer:
System requires at least a Pentium 90hz microprocessor, 16 meg of ram, floppy disk drive, cdrom drive, 1gig hard drive, color monitor, printer, mouse, serial port, and parallel port.

7. REAGENTS AND CONSUMABLE MATERIALS

Prepare all reagents in Silicate free DI water. Filter the all reagents prior to use.

- 7.1 Sulfuric Acid, .05M (250mL)
Add .7mL of concentrated Sulfuric Acid H_2SO_4 to 200mL of DI water. Mix well and dilute to final volume of 250mL.
- 7.2 Ammonium Molybdate Reagent (250mL)
Place stir bar in 250mL flask with 200mL of .05M Sulfuric Acid. While stirring, add 2.5g of Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and dissolve. Dilute to 250mL with .05M Sulfuric Acid and add .25mL of Dowfax 2A1 wetting agent. Store reagent in dark polyethylene bottle at 4° C. Reagent is not stable for more than two days. If reagent turns a faint blue, then remake. Water may be contaminated with SiO_2 .
- 7.3 Oxalic Acid (1L)
Dissolve 50g of Oxalic Acid $C_6H_8O_6$ in 1L of DI water.
- 7.4 Ascorbic Acid (250mL)
Dissolve 4.4g of Ascorbic Acid $C_6H_8O_6$ in 200mL DI water with 12.5mL of Acetone. Dilute to 250mL with DI water. Store in plastic bottle at 4°C for no more than one week.

8. CALIBRATION AND STANDARDIZATION

- 8.1 Anion calibrants, 1000mg/L are purchased from Ricca yearly.
- 8.2 Working Standards:
For precipitation samples: .010 mg/L, .050 mg/L, .100 mg/L, .200 mg/L
For stream samples: 2.0 mg/L, 6.0 mg/L, 10.0 mg/L, 14.0 mg/L

9. QUALITY CONTROL

- 9.1 Deionized water blanks are poured up weekly with the DWS samples. These serve as a check on the DI water system, and the bottle washing. A random sample of washed bottles are checked with a conductivity meter and any reading above 1.0 μmho indicates that set of bottles needs to be rinsed again. DI water blanks are also poured up for the AI collection. Blanks are taken into the field and treated as samples. Another check of the DI system is weekly measurements of the DI conductivity, when the NADP sample is measured. Any reading above 1.0 μmho indicates a dirty cartridge and the cation exchange cartridges are changed.
- 9.2 Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
- 9.3 QC concentrates from NSI are sent to the lab quarterly. They are analyzed for SO_4 , Cl, NO_3 , NH_4 , PO_4 , and TKN. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989. Perkin-Elmer cation concentrate (Alternate Metals II) is used to check the Atomic Absorption Spectrophotometer. It is purchased yearly.
- 9.4 A standard curve is determined before every analysis with the Perstorp 3500. R squared must equal 0.98 or greater before samples are analyzed.
- 9.5 Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
- 9.6 Check limits of detection annually for all instruments.
- 9.7 Check calibration of balances twice a year.

10. PROCEDURE AND CALCULATIONS

- 10.1 Startup:
 1. Make up reagents and standards.
 2. Configure pump with proper tubes and connect to Silicate cartridge.
 3. Turn power on and apply tension to pump platens.
 4. Connect all lines to H_2O and surfactant and observe stream.
 5. Connect reagent lines and observe stream.
- 10.2 Computer and Sampler startup:
 1. Turn power on and run Winflow program.
 2. Select and click the Sample Table button. Edit an existing table or create a new one. Table should start with highest calibration standard (SYNC) sample followed by the lowest sample for carry over correction (CO) then a read baseline (RB). Calibration standards are next with duplicates from highest to lowest. Include QC standard after calibration standards and RB. Insert calibration verification (mid-range standard) after each 20 samples. For each sample cup indicate: location, sample ID, type, dilution, and weight. Rename, save and print the sample table.
 - II. Click the Method Editor button and load an existing Methods file or create a new file. Verify all settings are correct. Refer to a previous method or the help files.
 - III. Select the Data Collect button and verify or select Method and Sample Table in Run Setup. Start pump with reagents tubes in surfactant water.
 5. Enter an Operator ID and verify or enter a Filename for the run.
 6. Click on the Play button and monitor the baseline. When the baseline is stable on startup solution (water) then switch to reagents. Allow a few minutes for reagents to equilibrate and monitor for a steady baseline.

10.3 Sample run:

1. When a stable baseline has been achieved use the Sample Table print out to start loading the sample trays.
2. Click the Fast Forward Start button to start data collection and sampler.
3. Monitor the run until the SYNC peak has eluted and successfully marked the top of the peak at its steady state. Allow the run to continue. If run stops automatically then data file will be saved. If the run is stopped manually by clicking the Stop button then be sure to save the results once the completed run is displayed.
4. Review the results. Calibration curve should yield an r^2 value greater than .98.
5. Reports are produced by opening the File window and selecting the proper option. Reports may be edited, printed, and exported as needed.

10.4 Shutdown:

1. Connect all reagent lines to deionized water.
2. Pump deionized water through the system for 15-20 minutes.
3. Turn off the 509 power and unlatch all pump platens. Caution: Pump tubes may be seriously damaged if the platens remain latched with the pump power off.
4. If the cartridge is to be stored away from the system or if it will not be used for a week or more, remove the reagent lines from the deionized water and pump the cartridge completely dry.
5. Turn off the gas supply if not being used.

10.5 Troubleshooting:

1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check for correct connection of all control and signal lines. Check tubing connections and flow through system.
2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operation parameters.
3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

| <u>Chemistry</u> | <u>Hydraulic</u> | <u>Electrical/Mechanical</u> |
|------------------|------------------|------------------------------|
| Reagent | Pump tubing | Circuit components |
| Standards | Bubble size | Optics/Lamps |
| pH | Surfactant | Photometer/Detector |
| Temperature | Pump | Cabling |
4. Eliminate one variable at a time. Using good experimentation techniques change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.
5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
6. Refer to the operation manual for further troubleshooting guides.

11. PRECISION AND BIAS

11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions. Table 3 summarizes the data.

12. REFERENCES

- 12.1 Perstorp Enviroflow 3500 Operation Manuals, Perstorp Analytical Corporation, 1992.
12.2 Standard Methods for the Examination of Water and Wastewater, Method 425 E, "Determination of Silicate by Automated Wet Chemistry", 16th edition, 1985.

Table 1. Method Detection Limits and Concentration Ranges for Silica

| Analyte | Method Detection Limit mg/L | Concentration Range mg/L |
|----------|-----------------------------|--------------------------|
| Silicate | .010 | 0.01 - 14.00 |

Method Detection Limit: Sample Standard Deviation X Student's t value for one-tailed test at 99% confidence level with degrees of freedom = 9

Table 2. Suggested Calibration Standards for Silicate Sample at Coweeta

| Analyte | Calibration Standards mg/L |
|------------------------|-------------------------------|
| Silicate -- high range | 2.0, 6.0, 10.0, 14.0 |
| Silicate -- low range | 0.010, 0.05, 0.10, 0.50, 1.00 |

Table 3. Single Operator Precision and Bias for Silicate determined from Standards

| Analyte | True Value mg/L | Number of Samples | Mean Measured mg/L | Mean Bias, mg/L | Standard Deviation, mg/L | Relative Standard Deviation, % |
|----------|-----------------|-------------------|--------------------|-----------------|--------------------------|--------------------------------|
| Silicate | .100 | 15 | .103 | .003 | .003 | 2.609 |

Relative Standard Deviation: $100 \times (\text{Sample Standard Deviation} / \text{Mean Value})$

Mean Bias: Sum of bias for each sample/number of replicates

Figure 1

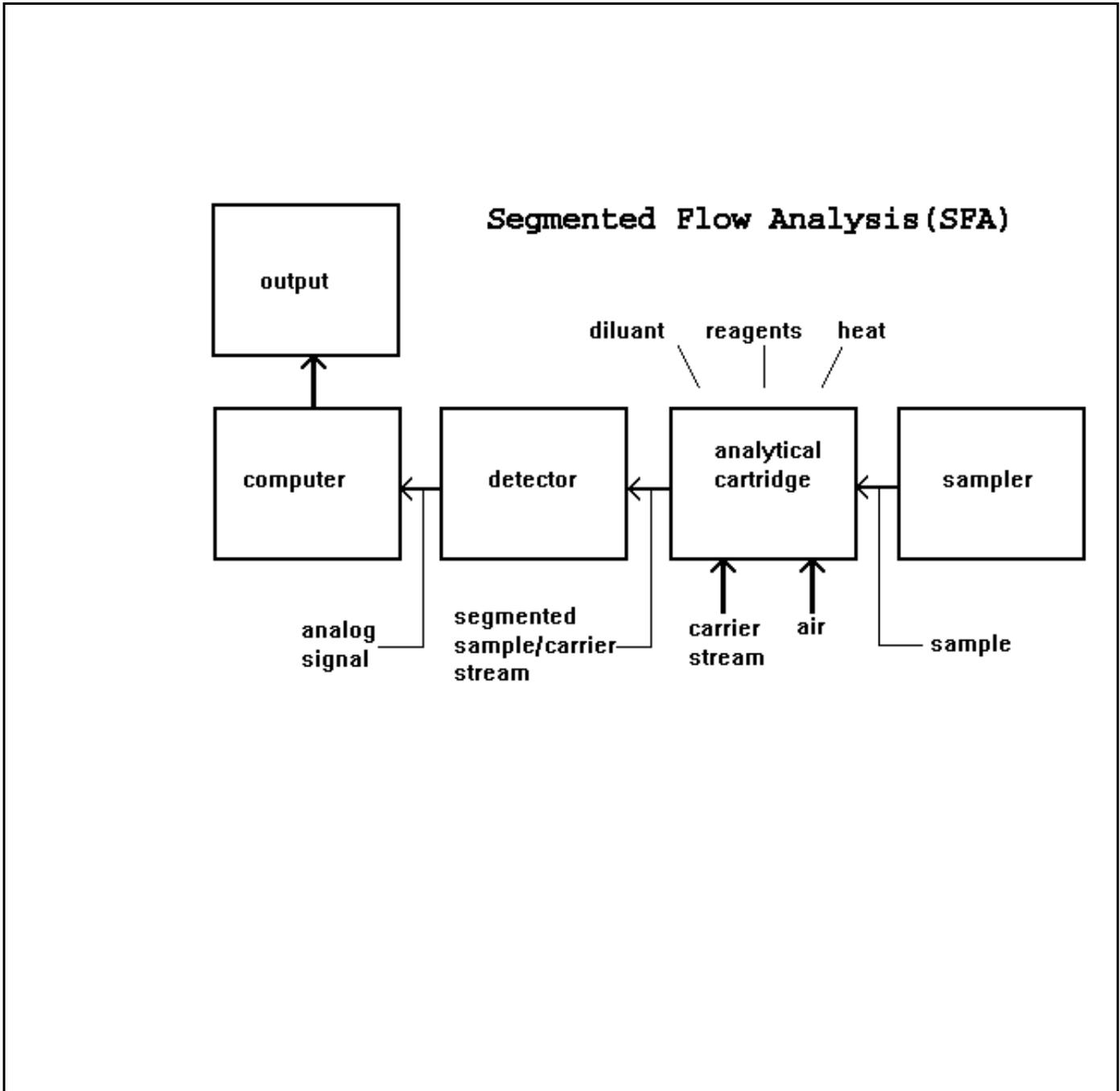
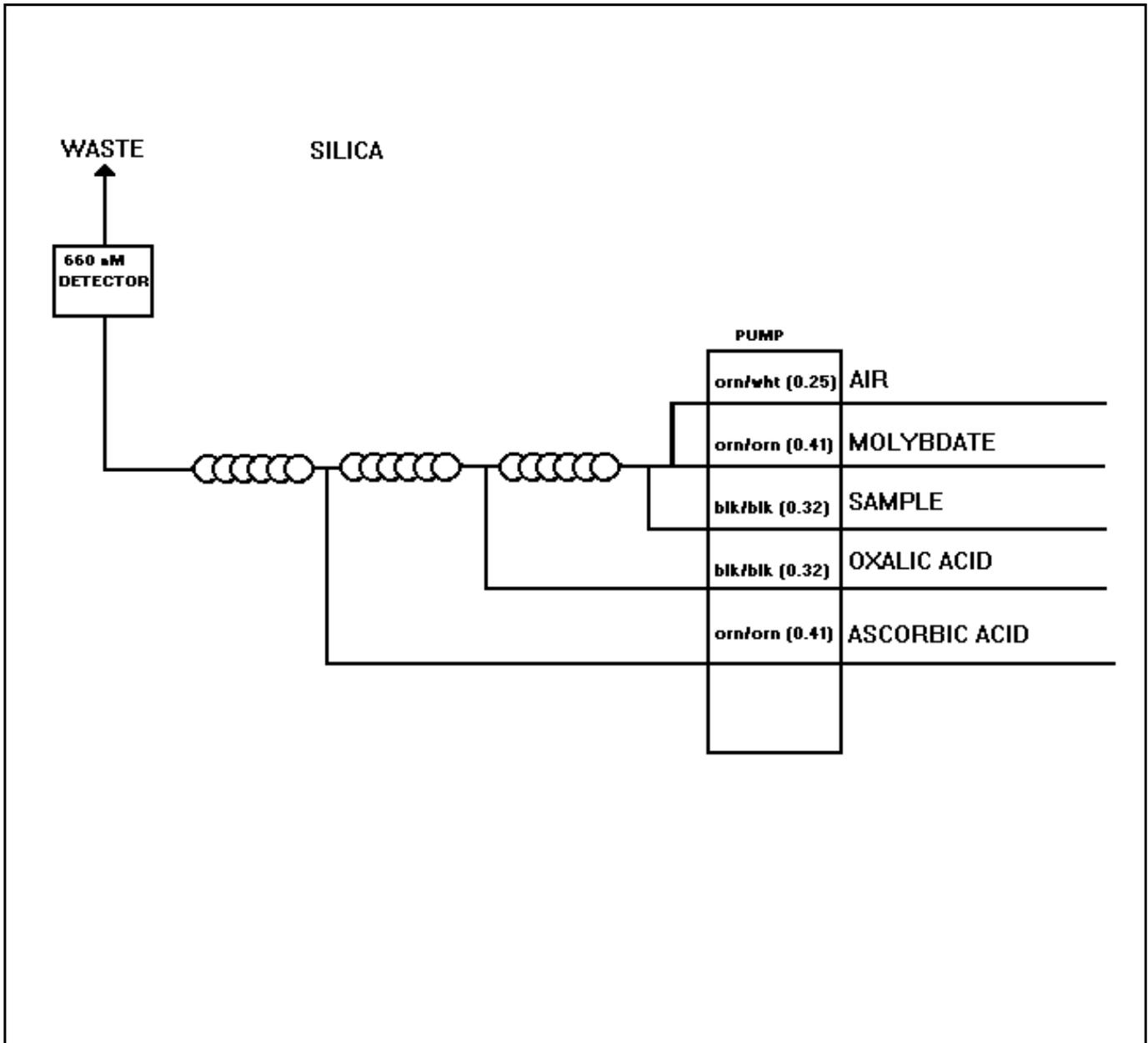


Figure 2.



Flow Injection Analysis
for Total Phosphorous
and ortho-Phosphate

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

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Chemist:

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Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

Instrument retired 2011

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TABLES

1. Method Detection Limits.

FIGURES

1. Manifold setup for Total Phosphorous.

1. SCOPE AND APPLICATION

- 1.1 This method is applicable to the determination of Total Phosphorous and Orthophosphate in Stream, Precipitation, Thrufall, Lysimeter, and extracted soil samples.
- 1.2 Method detection limits are summarized in Table 1.

2. SUMMARY OF METHOD

- 2.1 The digestion of phosphorous is converted to phosphate using persulfate with in line UV digestion. The ortho-phosphate reacts with ammonium molybdate and antimony potassium tartrate to convert to phosphomolybdate. Reduction occurs using ascorbic acid and a blue complex is formed which absorbs light at 880nm. The concentration of the analyte is proportional to the intensity of color produced. Ortho-Phosphate is quantified in the same manner skipping the digestion step. Calibration curves are constructed from a series of known standard concentrations. The concentration of the sample is calculated based on the calibration curve.

3. DEFINITIONS

- 1.1.1. Flow Injection Analysis – based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously changes due to the passage of the sample material through the flow cell
- 1.1.2. Colorimetry – a technique by which color is measured in a solution that contains an analyte to be determined. Typically, a calibration standard is combined with specific reagents that react with the analyte and form a chemical complex with a distinct color that can be measured. The intensity of the color is proportional to the concentration of the analyte in the calibration standard.
- 1.1.3. Flow Cell – light filter used to determine the intensity of the color created after the chemical mixes with the reagents
- 1.2. Diagram – graphic peaks generated during flow injection analysis

4. INTERFERENCES

- 1.2.1. Silicate is not a significant interference when using this method. 1000 mg/L SiO₂ gives a response of approximately 6 ug P/L.
- 1.2.2. Glassware contamination is a problem in low level phosphorous determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware
- 1.3. Noisy baselines can interfere with peak sensitivity
- 1.4. Reagents not being properly degassed leads to air spikes on diagram
- 1.5. Proper timing of peak start and peak width is necessary to properly measure peaks

5. SAFETY

- 1.1.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.
- 1.1.2 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS
 - Sulfuric Acid
 - Sodium Dodecyl Sulfate

6. APPARATUS AND EQUIPMENT

- 1.1 Random Access Sampler (RAS):
 1. Samples are loaded into 12ml reusable plastic vials
 2. Sampler operated in accordance with labels in software tray table
 3. Sampler time in sample and time in wash bath listed in method
 4. Sampler can hold 60 samples

Maintenance / Troubleshooting

- If it doesn't stop then check for felt on red sample cups
- If it's skipping cups either the bottom piece is dirty, it's not put in completely, the sensor near sample arm is dirty Ensure plate that guides trays isn't loose

- 1.2 Pump:
 1. Pumps left to right, there are arrows on plastic tube holders to guide placement Place the tensioner on the tube holder one click to the left to prevent tubes wearing out Cartridge holder can break
 2. Always unclamp cartridges when not running
 - i. Pump speed is always 35 To test pump speed it takes approximately 50 seconds for 10 pump revolutions

Maintenance / Troubleshooting

- Replace duraprene (pvc) pump tubes after three full days of running the instrument
- Santoprene tubes are replaced after running for a month the pump
- Always use diagram to ensure proper tubing size
- Waste line can clog with extended use

- 1.3 Heating Module:
 1. Consists of a temperature controller and a heating block
 2. The temperature controller allows the operator to manually adjust heat settings
 - i. The heating block consists of a heat rod, heat sensor, heat unit, and it is wrapped in tubing

Maintenance / Troubleshooting

- Front panel pops off for access
- Should only need to worry about the tubes, not the block
- Be careful when replacing, ensure the heat sticks are reinstalled
- Controllers can be removed and switched

1.4 Flow Cell:

1. The flow cell measures the intensity of the color created and determines the concentration of the analyte
2. The type of light filter is listed in each method

Maintenance / Troubleshooting

7-Make sure there's a flared seal and 3 O-rings

-When leaking, will come out through window

-Check once a month

-Leaks are almost always dealing with the seal

-Put in writing down

-Flow goes in bottom waste comes out top

-Filter narrows down light specific to chemistry, check methods for proper filter

-Filters will gradually degrade, clean with a Qtip and alcohol

-If not using filter for a long period put in desiccator

7. REAGENTS AND CONSUMABLES

1. Reagents

i. **Total Phosphorous method 10-115-01-30 E** (each volumetric has 500 mL DI to start)

1. *Stock Molybdate*: 40g ammonium molybdate tetrahydrate fill to 1L in volumetric using DI water, mix with magnetic stirrer
2. *Stock Antimony Potassium Tartrate*: 3.22g antimony potassium tartrate fill to 1L in volumetric using DI water, mix with stirrer
3. *Molybdate Color Reagent*: 25mL sulfuric acid, 213 mL ammonium molybdate solution, 72 mL antimony potassium tartrate solution fill to 1L in volumetric using DI water, mix with stirrer, degas
4. *Ascorbic Acid Reducing Solution*: 70g granular ascorbic acid, fill to 1L in volumetric using DI water, mix with stirrer, degas, then add 1.0g sodium dodecyl sulfate
5. *Sulfuric Acid Carrier Solution*: 30mL sulfuric acid, 9g potassium chloride fill to 1L in volumetric using DI water, mix with stirrer, degas
6. *Digestion Reagent 1*: 106.5 mL sulfuric acid, fill to 1L in volumetric using DI water, mix
7. *Digestion Reagent 2*: 26g potassium persulfate, fill to 1L in volumetric using DI water, mix with stirrer, degas

ii. **Orthophosphate Water method 10-115-01-1-A** (each volumetric has 500 mL DI to start)

1. Stock Molybdate and Antimony Potassium Tartrate same as total phosphorous
2. *Molybdate Color Reagent*: 35mL sulfuric acid, 213 mL ammonium molybdate solution, 72 mL antimony potassium tartrate solution fill to 1L in volumetric using DI water, mix with stirrer, degas
3. *Ascorbic Acid Reducing Solution*: 60g granular ascorbic acid, fill to 1L in volumetric using DI water, mix with stirrer, degas then add 1.0g sodium dodecyl sulfate
4. *Sulfuric Acid Carrier Solution*: 30mL sulfuric acid, 9g potassium chloride fill to 1L in volumetric using DI water, mix with stirrer, degas

5. *Sodium Hydroxide* – EDTA Rinse: 65g sodium hydroxide, 6g tetrasodium ethylenediamine tetraacetic acid, fill to 1L in volumetric using DI water, mix with stirrer
- iii. **Orthophosphate Soil Extraction method 12-115-01-1-N** (each volumetric has 500 mL DI to start)
 1. *Bray No. 1 Extraction Solution*: 12.7 mL of 2 M hydrochloric acid and 1.1 g ammonium fluoride, mix with magnetic stirrer
 2. *Mechlich No. 3 Extraction Solution*: to a 2L volumetric flask containing 800mL DI water, 40g ammonium nitrate, 22.98 mL acetic acid, 1.68 mL HNO₃, 1.12 ammonium fluoride, .58g EDTA
 3. Stock Molybdate and Antimony Potassium Tartrate same as total phosphorous
 4. *Molybdate Color Reagent*: 21mL sulfuric acid, 213 mL ammonium molybdate solution, 72 mL antimony potassium tartrate solution fill to 1L in volumetric using DI water, mix with stirrer, degas
 5. *Ascorbic Acid Reducing Solution*: 60g granular ascorbic acid, fill to 1L in volumetric using DI water, mix with stirrer, degas then add 2.0g sodium dodecyl sulfate
 6. *Sodium Hydroxide* – EDTA Rinse: 65g sodium hydroxide, 6g tetrasodium ethylenediamine tetraacetic acid, fill to 1L in volumetric using DI water, mix with stirrer
2. Sample Vials – 12ml sample vials: RAS sampler holds 60 samples and 16 standards

8. CALIBRATION AND STANDARDIZATION

1. Stock solutions are 1000ppm Ricca single analyte calibrants
2. Working standards in a 1000 mL volumetric
 - i. Total Phosphorous: .01, .1, .5
 - ii. Orthophosphate in water: .01, .8, 1.5
 - iii. Orthophosphate soil extractions: .4, 5, 12, 20

9. QUALITY CONTROL

1. Stock solutions are made up or purchased yearly. Two aliquots are set aside in order to check the stock concentration if necessary.
2. QC concentrates from NSI are sent to the lab quarterly. They are analyzed for TP and PO₄. A value within the 95% C.I. is considered acceptable. Samples are run in triplicate. X bar charts will be used for these results. See Standard Methods, 1989.
3. A standard curve is determined before every analysis with the Ion Chromatograph. R squared must equal 0.995 or greater before samples are analyzed.
4. Daily QC checks are done with solutions made from the NSI concentrates. Acceptable ranges for each ion are based on 95% confidence limits.
5. Check limits of detection annually for all instruments.
6. Check calibration of balances twice a year.

10. SOFTWARE

1. **Common files**

- i. Methods - save and reuse
- ii. Tray file – list of samples, can be made daily
- iii. Data file – all information for a give sample run
- iv. Runtime report – raw data
- v. DQM file: QC's, allows you to enter data parameters, can set up to stop and inform if QC's are out of range
- vi. When opening old data file make sure to open then load method and analyze

- vii. Review analyte calibration screen allows you to see what cal changes will do
 - viii. To actually change go to analyte screen placing cal stds in two levels allows you to delete one if it's bad
2. Method
- i. Graphical events – change peak base width / threshold to fix timing
 - ii. Description – overview of method (basic)
 - iii. Analyte Table – where you enter standards, cal handling, cal fit-type, force through zero, weighting, chemistry, inject to peak start, peak base width, width tolerance, threshold, method name
 - iv. Valve timing: turn on 1 or both valves, load time, period, inject period, method cycle period, sample reaches first valve time
 - v. Sampler timing – min. probe in wash, probe in sample period
 - vi. Pump timing – standby pump settings
 - vii. Display options – size of peak graph
 - viii. Review cal curves – see cal results, view possible changes without being able to apply
 - ix. Cal clear
 - x. Cal clear level
 - xi. Cal failure criteria – give warning or shut down sampling if cal is out of criteria
 - xii. Copy – copy method
 - xiii. Tray Level column tells what sample type (ex. 1-16 = cal std 0 = unknown)

11. PROCEDURE

1. Machine Startup

- i. Turn on instrument using power strip behind the battery backup
- ii. Make sure there is DI in brown bottle to fill wash bath
- iii. Check waste container and make sure it can hold the waste created during the sample run
- iv. Place reagent lines in DI water, clamp down lines onto pump
- v. Press manual run/stop button on pump to begin operation, speed should be 35
- vi. Let run for one minute then individually lift up each line out of the DI and check air bubble for consistent flow
- vii. For TP method set temperature of digestion block heater to 120 C there must always be liquid in pump tubes when the temperature is above 80 C, valve block heater is 50 (valve 1)
- viii. For o-PO4 method turn power off to digestion block, valve block heater is 37 (valve 2)
- ix. Check for leaks at all connection points on the machine
- x. When everything checks out put tubes into their proper reagent bottle

2. Software Startup

- i. Turn on computer
- ii. Click on start menu
- iii. Click ok
- iv. Login
- v. Click on lachat icon, listen for valves turning and ensure sample probe enters the wash bath
- vi. Click on file
- vii. Open method, open up the method you are using that day

3. Calibration and Sample Run

- i. Make reagents according to method, make calibrants and qc's using method and guidelines in wetlab - qc folder

- ii. In software pull up *analyte table* and verify settings are correct using method sheet, always put each of the three samples for each calibrant in its own column, by doing this you make it possible to delete one if necessary to correct the calibration curve
- iii. In software pull up *tray table* and enter samples, for calibration run make sure to run each qc 3 times, only enter data into columns you want sampled, any writing in a column will cause the machine to sample that column
- iv. Always run calibration separate from your samples, it is impossible to make changes to your calibration curve and apply it to samples if they are not separate
- v. Save files as the date and go alphabetically (a, b, c, etc.) to distinguish when you run more than one tray per day

4. **Shutdown**

- i. Remove lines from reagents and place in DI water
- ii. Turn In-Line sample prep module heater temperature to 70 or below
- iii. Run DI until the in-line sample prep module heater temperature is below 79
- iv. If running the next day you can leave DI in the lines, otherwise remove the lines from DI and let DI drain completely, make sure to take tube from wash bath bottle and turn sampler off and on to get the sampler out of the wash bath
- v. Stop pump and unclamp immediately, if sample lines are left clamped on the pump more than a few minutes without the pump running they will need to be replaced
- vi. If the waste tub is near full empty into hazardous waste container in the building behind the lab

12. **TROUBLESHOOTING**

1. Don't overlook the obvious. Look for the simplest and most obvious cause for a particular problem or solution. Check for correct power to all modules and the power switches are on. Check tubing connections and flow through system.
2. Rule out operator induced errors. Consult the system manual and manuals for each application for proper operating procedures and protocols. Review test methodologies and flow diagrams to verify operation parameters.
3. Isolate and define the problem. Categorize the problem or symptom as one of the following:

| <u>Chemistry</u> | <u>Hydraulic</u> | <u>Electrical/Mechanical</u> |
|------------------|------------------|------------------------------|
| Reagent | Pump tubing | Circuit components |
| Standards | Flow blockage | Optics/Lamps |
| pH | Leak | Flow Cell |
| Temperature | Pump | Cabling |

4. Eliminate one variable at a time. Using good experimentation techniques, change only one component or variable at a time and note the changes and results. In this manner, the problem may be cured or the solution more clearly defined.
5. Document the solution. Keep a log of your troubleshooting activity including problem, symptoms, causes, and solution. This information may be helpful for future troubleshooting sessions or for other users.
6. See manual for further trouble shooting solutions

13. **PRECISION AND BIAS**

- 11.1 Single operator precision and bias were obtained from the analysis of quality control check samples. QC samples were obtained from NSI Solutions and were diluted according to manufactures directions.

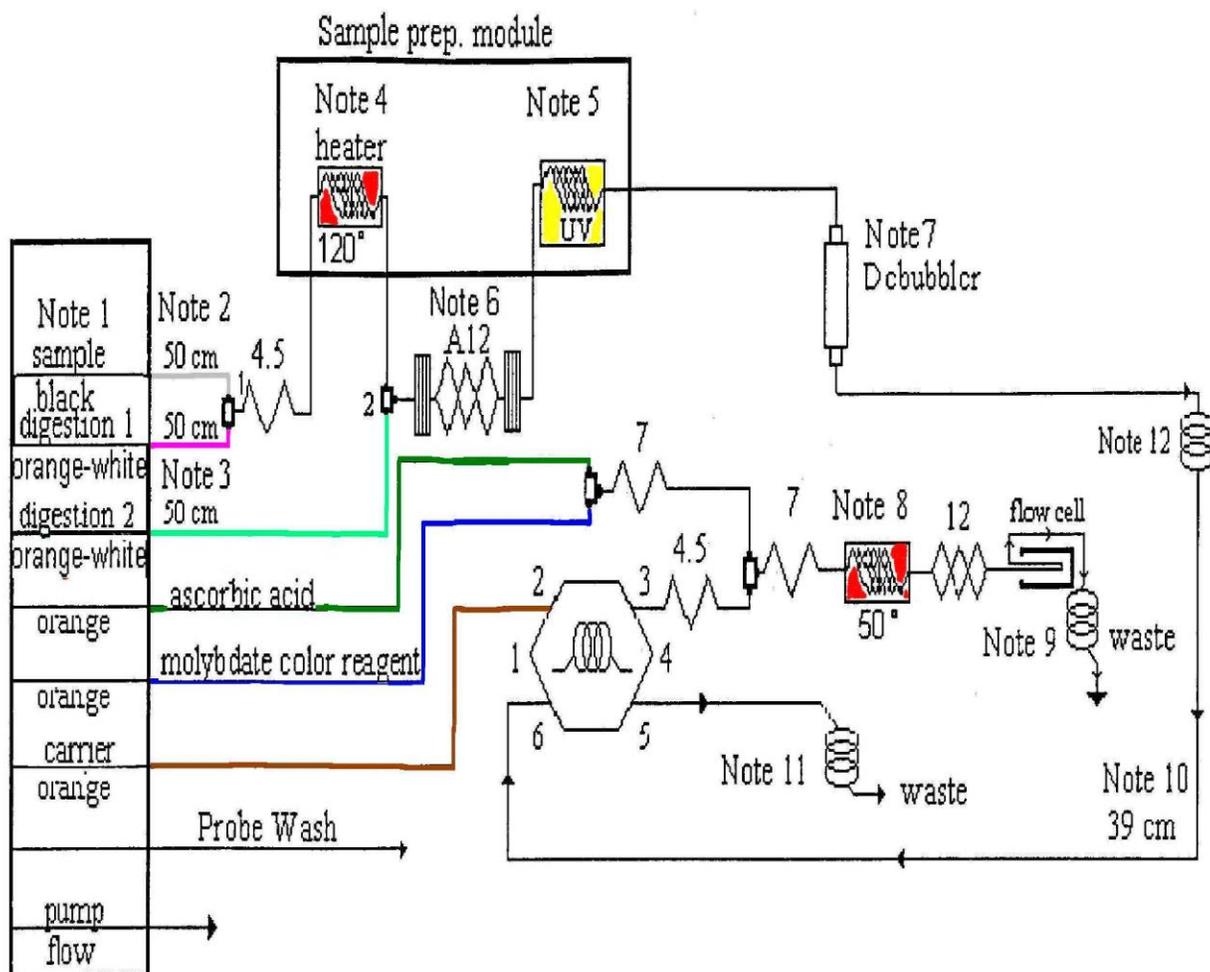
14. REFERENCES
User Manual QuikChem FIA+ 8000 Series, Lachat Instruments, 1999.
Methods for Chemical Analysis of Water and Waste, Method 350.1, EPA, 1983

Table 1 – Method Detection Limits

| .050ppm | Total P | O-PO4 |
|----------------|--------------------|--------------|
| QC | 0.050 | 0.044 |
| QC | 0.050 | 0.044 |
| QC | 0.050 | 0.045 |
| QC | 0.050 | 0.043 |
| QC | 0.050 | 0.044 |
| QC | 0.050 | 0.042 |
| QC | 0.050 | 0.047 |
| QC | 0.050 | 0.044 |
| QC | 0.050 | 0.048 |
| QC | 0.050 | 0.043 |
| avg | 0.050 | 0.044 |
| std | 0.000 | 0.002 |
| mdl | 0.001 | 0.005 |

Figure 1. Manifold Setup for Phosphorous

12.3 TOTAL PHOSPHORUS MANIFOLD DIAGRAM



Inductively Coupled Plasma Spectroscopy
Determination of
Potassium, Sodium, Calcium,
Magnesium, Aluminum, Sulfur and Phosphorous
Jobin Yvon Ultima II

October 2009

Coweeta Hydrologic Lab
3160 Coweeta Lab Road
Otto, N.C. 28763

Sponsoring Agency:

James M. Vose, Project Leader

U.S. Forest Service
University of Georgia

Laboratory Manager:

Cindi Brown

Chemist:

Cindi Brown

Laboratory Technician:

Carol Harper
Neal Muldoon
Sheila Gregory

The instrument was retired November 2012

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Inductively Coupled Plasma Spectroscopy

1. SCOPE AND APPLICATION

1.1 This method is applicable for the determination of K, Na, Mg, Ca, Al and P in sample matrices of water, 2% nitric acid, 0.5M HCl, and soil extract (See cation soil procedure).

2. SUMMARY OF METHOD

Inductively Coupled Plasma (ICP) Spectroscopy utilizes a high energy plasma not only to dissociate the sample but excite and ionize the atoms for atomic and ionic emission. The light emitted is focused onto a diffraction grating via a slit and a mirror. As the grating rotates a different wavelength is focused onto a second mirror and the exit slit. The light is then directed to a photomultiplier tube where the signal is amplified to a measurable amount. A calibration curve is developed for each element plotting intensity versus concentration.

3. INTERFERENCES

ICP was developed to eliminate interferences encountered using Atomic Absorption. However there are usually more than one wavelength associated with each element and some wavelengths from different elements can overlap and interfere. Therefore a profile must be developed for each line and background correction applied if necessary.

4. RANGE

K, Na, Ca, Mg, Al and P all have a working range up to 1000ppm.

5. APPARATUS AND EQUIPMENT

5.1 Jobin Yvon Ultima II Spectrophotometer (instrument was upgraded to an Ultima)

5.2 Jobin Yvon AS 421 autosampler

5.3 PolyScience water recirculator

5.4 LabCraft nitrogen generator

6. SAFETY

6.1 Never look directly at the plasma.

6.2 The exhaust must be on.

6.3 The quartz tubes, alumina injector, spray chamber and centering ring are soaked in aqua regia once a month. This must be done under the hood.

7. REQUIREMENTS

7.1 Gases
Liquid Argon
N₂, produced from N₂ generator using air from air compressor

7.2 Water recirculator

8. REAGENTS

8.1 All calibrants and QC's are made in the same matrix of the samples being analyzed. Trace metal acids are used when needed.

8.2 1000ppm Yttrium for setting the nebulizer pressure is purchased from Fisher Scientific.

8.3 ICP Instrument Check 1, cat# JYICP-QC1 purchased from Horiba, used to determine stability and LOD.

8.4 Aqua regia – Using trace metal acids make up a 3 to 1 HCl HNO₃ solution under the hood using caution.

9. STANDARDS AND CALIBRANTS

9.1 Calibrant – ICP custom mix #Q-5067 purchased from NSI Solutions Inc

9.2 QC calibration check -ICP custom mix #Q-5068 purchased from NSI Solutions Inc

9.3 Quarterly QC – Certified samples purchased from Environmental Resource Associates cat#ERA530

9.4 ICP Instrument Check 1, cat# JYICP-QC1 purchased from Horiba, used to determine stability and LOD

10. PROCEDURE

10.1 Turn on the instrument and N₂ generator and stabilize for 24 hours.

10.2 After instrument stabilizes, turn on water circulator and exhaust.

10.3 Spectrometer Start Up

- a. Turn on computer.
- b. Open up ICP 5.4.2 program.
- c. In the Automatism screen, start plasma.
- d. Go to rinse icon and set rinse.
- e. Go to instrument icon and then communication detail. Perform zero order and then reference check.
- f. Make up 1 to 100 dilution using ICP Instrument Check 1 and run stability check and LOD.
- g. Create a sequence by:
 1. Select sequence icon
 2. Use drop down menu to select method.
 3. Under location of samples tab, select tray.
 4. Under sequence tab, insert peak search, auto attenuate and calibration.

5. Insert samples and QCs.
6. Save giving file name date of analysis, ddmmyy.
- h. Run sequence by selecting arrow in upper left hand corner.

10.4 Shut down

- a. Once sequence is complete, go to automatism screen and stop plasma.
- b. Release tension on pump tubes.
- c. Close off argon.
- d. Turn off exhaust.
- e. Turn off water circulator.
- f. If instrument will not be used for a week or more:
 1. Turn off instrument.
 2. Turn off N2 generator by shutting down air compressor at the breaker.

10.5 Special Considerations

The temperature of the room should be kept at 70°F.

10.6 Maintenance

1. At least once per month soak the outer and inner quartz tubes, spray chamber, centering ring, and alumina injector tube in aqua regia overnight. After soaking, discard acid, flush with DI and soak for 1 hour in DI. Rinse again in DI and allow to dry before reassembling torch. The o-rings in the torch and spray chamber should be replaced at this time.

11. QUALITY CONTROL

- 11.1 A three point (or more) calibration curve is generated at the start of the run.
- 11.2 The calibration curve is checked using a certified standard. An accuracy of $\pm 10\%$ and a precision of 2% or less is maintained.
- 11.3 During the run the instrument recalibrates as dictated in the method.
- 11.4 Quarterly checks on the instrument are made using Environmental Resource Associates and NSI Solutions Inc certified QCs.

12. WASHING PROCEDURE FOR GLASSWARE AND CENTRIFUGE TUBES

- 12.1
 - a. Wash in Joy dishwashing liquid.
 - b. Rinse with tap water.
 - c. Soak overnight with 5% HNO₃.
 - d. Rinse five times in deionized water.

13. REFERENCES

- 13.1 How to Realize an Analysis in ICP with Version 5 Software, Jobin Yvon Horiba, Reference 31 088 494, February 2000.

Table 1 - PRECISION AND BIAS

| 2%HNO3 | K(mg/l) | Na(mg/l) | Ca(mg/l) | Mg(mg/l) | P(mg/l) |
|------------|--------------|--------------|--------------|--------------|--------------|
| 1 | 0.21 | 0.21 | 0.23 | 0.26 | 0.29 |
| 2 | 0.23 | 0.21 | 0.23 | 0.25 | 0.24 |
| 3 | 0.22 | 0.22 | 0.23 | 0.25 | 0.25 |
| 4 | 0.21 | 0.22 | 0.24 | 0.25 | 0.25 |
| 5 | 0.22 | 0.21 | 0.24 | 0.25 | 0.26 |
| 6 | 0.23 | 0.22 | 0.24 | 0.25 | 0.25 |
| 7 | 0.23 | 0.22 | 0.23 | 0.24 | 0.24 |
| 8 | 0.23 | 0.23 | 0.24 | 0.25 | 0.26 |
| 9 | 0.22 | 0.21 | 0.23 | 0.25 | 0.23 |
| 10 | 0.22 | 0.21 | 0.23 | 0.25 | 0.26 |
| avg | 0.222 | 0.216 | 0.234 | 0.250 | 0.253 |
| std | 0.008 | 0.007 | 0.005 | 0.005 | 0.016 |
| mdl | 0.022 | 0.020 | 0.015 | 0.013 | 0.046 |

| DI water | K(mg/l) | Na(mg/l) | Ca(mg/l) | Mg(mg/l) |
|------------|--------------|--------------|--------------|--------------|
| 1 | 0.256 | 0.253 | 0.264 | 0.245 |
| 2 | 0.240 | 0.253 | 0.267 | 0.245 |
| 3 | 0.235 | 0.249 | 0.296 | 0.245 |
| 4 | 0.243 | 0.247 | 0.262 | 0.245 |
| 5 | 0.247 | 0.250 | 0.266 | 0.243 |
| 6 | 0.236 | 0.246 | 0.301 | 0.245 |
| 7 | 0.241 | 0.247 | 0.269 | 0.243 |
| 8 | 0.253 | 0.245 | 0.258 | 0.244 |
| 9 | 0.242 | 0.246 | 0.270 | 0.244 |
| 10 | 0.241 | 0.250 | 0.261 | 0.246 |
| avg | 0.243 | 0.249 | 0.271 | 0.244 |
| std | 0.007 | 0.003 | 0.015 | 0.001 |
| mdl | 0.019 | 0.008 | 0.042 | 0.003 |

student t with nine degrees of freedom and a 99% confidence level = 2.821

Figure 1. – Sample ID Sheet

| Samples _____ | | Date _____ | | | | | |
|---|-------|------------|-------|------------|-------|------------|-------|
| 1-1 _____ | _____ | 1-12 _____ | _____ | 1-23 _____ | _____ | 1-34 _____ | _____ |
| 1-2 _____ | _____ | 1-13 _____ | _____ | 1-24 _____ | _____ | 1-35 _____ | _____ |
| 1-3 _____ | _____ | 1-14 _____ | _____ | 1-25 _____ | _____ | 1-36 _____ | _____ |
| 1-4 _____ | _____ | 1-15 _____ | _____ | 1-26 _____ | _____ | 1-37 _____ | _____ |
| 1-5 _____ | _____ | 1-16 _____ | _____ | 1-27 _____ | _____ | 1-38 _____ | _____ |
| 1-6 _____ | _____ | 1-17 _____ | _____ | 1-28 _____ | _____ | 1-39 _____ | _____ |
| 1-7 _____ | _____ | 1-18 _____ | _____ | 1-29 _____ | _____ | 1-40 _____ | _____ |
| 1-8 _____ | _____ | 1-19 _____ | _____ | 1-30 _____ | _____ | 1-41 _____ | _____ |
| 1-9 _____ | _____ | 1-20 _____ | _____ | 1-31 _____ | _____ | 1-42 _____ | _____ |
| 1-10 _____ | _____ | 1-21 _____ | _____ | 1-32 _____ | _____ | 1-43 _____ | _____ |
| 1-11 _____ | _____ | 1-22 _____ | _____ | 1-33 _____ | _____ | 1-44 _____ | _____ |
| | | | | | | | |
| 2-1 _____ | _____ | 2-12 _____ | _____ | 2-23 _____ | _____ | 2-34 _____ | _____ |
| 2-2 _____ | _____ | 2-13 _____ | _____ | 2-24 _____ | _____ | 2-35 _____ | _____ |
| 2-3 _____ | _____ | 2-14 _____ | _____ | 2-25 _____ | _____ | 2-36 _____ | _____ |
| 2-4 _____ | _____ | 2-15 _____ | _____ | 2-26 _____ | _____ | 2-37 _____ | _____ |
|  | | | | | | | |
| 2-10 _____ | _____ | 2-21 _____ | _____ | 2-32 _____ | _____ | 2-43 _____ | _____ |
| 2-11 _____ | _____ | 2-22 _____ | _____ | 2-33 _____ | _____ | 2-44 _____ | _____ |
| 3-1 _____ | _____ | 3-12 _____ | _____ | 3-23 _____ | _____ | 3-34 _____ | _____ |
| 3-2 _____ | _____ | 3-13 _____ | _____ | 3-24 _____ | _____ | 3-35 _____ | _____ |
|  | | | | | | | |
| 3-11 _____ | _____ | 3-22 _____ | _____ | 3-33 _____ | _____ | 3-44 _____ | _____ |

