Quality Assurance Manual

For the
Natural Resources Research Institute
Central Analytical Laboratory
University of Minnesota-Duluth
5013 Miller Trunk Highway
Duluth, MN 55811

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Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
9	Elaine M. Ruzycki	6/8/2015	Added non-routine methods to Appendix. Only updated QAM and SOPs to reflect additional Appendices.
8	Elaine M. Ruzycki	11/12/2014	Annual review; added SOPs 115,116, 117
7	Elaine M Ruzycki	3/25/2014	Annual review; added SOP 114 and appended non-routine methods
6	Elaine M Ruzycki	11/12/2012	Annual review and revised to address MDH non-compliance assessment - 387075
5	Elaine M Ruzycki	11/29/2011	Revised and reformatted to NELAC requirements
4	Elaine M Ruzycki	9/27/2011	Update personnel; update table T-3; add table of contents and equipment list
3	Elaine M Ruzycki	12/1/2009	Revised and reformatted per MDH request

Annual Review (A document review is required if no revisions have occurred over the past 12 months)

Signature	Title	Date
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Training Record and Signature Log

The following laboratory staff have read and agree to follow the current Quality Assurance Manual. In addition, these staff are responsible for signing and/or initialing laboratory records.

Signature	Name	Initials	Date
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	Turbidity, TSS, VSS, pH, ANC, specific conductivity, color, chloride, ortho-P, total P/total N, nitrite/nitrate-N, ammonium-N	
Appendix G	Physical (from NRRI/TR 2009-36)	
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1. Quality Policy

One of the fundamental responsibilities of water management is the establishment of continuing programs to insure the reliability and validity of data. Effective research in water pollution and management depends on a valid laboratory data base, which in turn may contribute to sound evaluations of both the progress of the research itself and the viability of available water pollution and management alternatives. Primary functions:

1. Continuous monitoring of the reliability (accuracy and precision) of the results reported (i.e., determination of quality).
2. Control of quality, meeting the requirements for reliability.

Quality assurance and quality control start with sample collection and are not complete until data is reviewed and accepted. Each method has a rigid, referenced protocol. Similarly, QA/QC associated with the test must include specific steps for monitoring the test and ensuring that its results are correct. All experimental variables that affect the final results should be considered, evaluated, and controlled.

Several types of samples are gathered and analyzed as part of continuing programs at the Natural Resources Research Institute (NRRI). These include water from precipitation, streams, wetlands, lakes, septic system effluent and sediments or other sources of particulate matter. Concentrations of many chemical constituents in water are in the low µg/l (ppb) range; therefore some of our methods are specifically adapted for very low nutrient and low ionic strength samples. Although some may not be certifiable by the Minnesota Department of Health, they are currently used extensively by limnological and oceanographic research centers.

2. Accredited Test Methods (Table 1).

Test	Method[a]	Category[b]
pH	SM 4500 H^+ B	CWP
Residue – non-filterable (TSS)	SM 2540-D	CWP
Residue – volatile suspended (VSS)	SM 2540-E	CWP
Turbidity	SM 2130 B	CWP
Color (true)	EPA 110.1	CWP
Chloride	SM 4500 Cl^- E	CWP
Specific conductivity (EC25)	SM 2510 B	CWP
Total Hardness*	SM 2340 C	CWP
Alkalinity (ANC)	SM 2320 B	CWP
[Nitrate + Nitrite]–N	SM 4500-$NO3^-$ F	CWP

Ortho- phosphorus as P (o-P)	SM 4500-P E 97	CWP
Total Phosphorus (TP)	SM 4500-P J/SM 4500-P E 97	CWP
Total *Kjeldahl* Nitrogen (TKN)	SM 4500 NO_3 F minus 4500-NO_2 B	CWP
Sulfate*	EPA 375.2	CWP

[a]Standard Methods
[b]Clean Water Program
*2015 certification - pending

The NRRI CAL performs a number of analyses (**Table 2**). For those analyses the laboratory is currently not accredited for the laboratory still follows all QA procedures. The CAL performs performance testing for these analyses if PT standards are available.

3. Job Descriptions

Laboratory Director/Program Manager - Administers the NRRI Central Analytical Laboratory (CAL). The Director provides supervision to all laboratory personnel to ensure adherence to lab documented procedures.

Quality Assurance Officer/Lab Manager - Responsible for the quality system and its implementation. The QA officer has direct access to the highest level of management at which decisions are made on lab policies and resources. This person also serves as lab manager and is responsible for hiring and training technicians, subcontracting sample analyses, communicating with clients, data management and managing and tracking lab income.

Senior Analysts – Responsible for all analyses performed in the lab, training technicians, sample collection, and data entry. Also responsible for reading and following SOPs, performing appropriate QC checks and informing the Lab Manager or Technical Director when problems occur.

Technical Director – Overall responsibility for the technical operation of the lab. The technical director is responsible for arranging and overseeing all support services including instrument service contracts and physical maintenance of the laboratory.

Deputy Technical Director – the Deputy Technical Director is responsible for the Technical Director's duties in his/her absence.

Laboratory Technicians – Technicians are responsible for reading and following SOPs, performing appropriate QC checks and informing the Lab Manager or Technical Director when problems occur.

4. Training and Education of Personnel

Training of CAL staff is done internally at NRRI-UMD by senior CAL staff. Specific training records are generally not kept; instead we track analyst performance using results from analyses performed. All analysts are required to read the QAM and the appropriate SOP(s) and must sign a document agreement form and these will be kept in the employee file. All analysts sign their work using three initials which are recorded in the QA logbook.

Note: The CAL is a very small laboratory consisting of Dr. Richard Axler (Sr Research Associate/Laboratory Director) Elaine Ruzycki (Research Fellow/QA Officer) and Jerry Henneck

(Scientist/Technical Director). Both Ruzycki and Henneck are responsible for the laboratory and field sampling activities of the CAL. As part of the UM system we also employ a number of undergraduate and graduate students to perform sample analyses and field sampling. Undergraduates are science or engineering majors or graduate students advised by Dr. Axler and other Aquatic Ecologists at the University of Minnesota-Duluth. Each is trained and supervised by both Ruzycki and Henneck with all of their work reviewed thoroughly before data is accepted. We track analyst performance using results from analyses performed and we maintain a file for each technician that includes a resume/CV, job application, courses taken as well as UM safety training documentation and emergency contact information. In addition all personnel receive additional laboratory training as part of standard minimum University of Minnesota Department of Environmental Health and Safety requirements.

All laboratory employees will be required to view a data integrity presentation on an annual basis. The NRRI CAL will use the presentation created by the Oregon Technical Advisory Committee entitled: Laboratory Ethics and Data Integrity (www.oelaonline.com*, 2008)*. Training records showing employee, date of training and their signature will be kept in the NRRI CAL QA files.

4. Internal Auditing Procedures

The NRRI CAL QAO will perform an annual review of the laboratory's quality system annually following the Managerial Review checklist (Appendix E). The results of the annual review will be approved by the Laboratory Director, QAO, and TD and archived in the NRRI CAL QA files. A record of the annual review will also be kept in the QAM.

The CAL QAO will periodically review current practices to ensure compliance with MN Rules for certification. Compliance with testing requirements for tribal, state and federal agencies and other clients will also be assessed periodically.

5. Sample Handling, Receipt, and Acceptance

Sample containers are provided by the CAL laboratory. Container cleanliness is verified by QA/QC procedures as specified in the laboratory's QAM and SOPs. Typical bottle preparation protocols are shown in **Table 3**.The laboratory verifies sample bottle cleanliness by running a specified number of bottle blanks on each shipment received and on each batch of sample bottles following laboratory cleaning and sterilization, if reused. A preservative is added to specific bottles, as required, or accompanies the bottles in a separate container. Preservatives used and their volumes and concentrations are specified in the laboratory QAM. In general, for nutrient samples, no preservatives are used other than chilling and then freezing at -20°C until analysis.

All samples are packed in an ice-filled cooler for transport to the laboratory. Samples are immediately brought to the Lab after sampling or in unusual circumstances stored in the NRRI walk-in cold room (4°C) overnight. Temperature blanks are included in the coolers provided by the laboratory to verify whether the appropriate sample temperature of ≤ 4°C has been maintained.

Upon arrival at the laboratory, we determine the condition of the samples. All samples received must either be recorded on NRRI CAL (**Appendix C**) or client provided COCs with date and time as well as condition upon arrival recorded. The samples are checked for leaks, appropriate preservation,

and checked to ensure that temperature has remained <4°C (typically just noting the presence of ice). The information is recorded on the sample identification sheet. The sample identification sheet information is then compared to the information on the sample bottles and any discrepancies are noted. The samples are then logged into the Analytical Lab ACCESS database and stored as noted above. The laboratory sample storage areas (freezers and walk-in cooler) are monitored daily using low/high temperature alarms. Temperatures are checked weekly using calibrated thermometers within each refrigerated unit.

Table 3. Typical bottle preparation protocols[1,2]		
pH-ANC-EC	250 ml	MQW soak 48 hours, rinse 3x MQW
Major Anions	125 ml	MQW soak 48 hours, rinse 3x MQW
DOC	125 ml	Rinse 1% HCL, 3x MQW , soak MQW 24 hours, rinse 2x MQW
Major cations and other metals†	60-125ml	Soak 15% HNO3 24 hours, rinse 4x MQW , soak MQW 24 hours, rinse 2x MQW
Nutrients (N and P-series)	125 ml	3x MQW rinse, soak MQW 24 hours, rinse 2x MQW
Cubitainers	1-4 liters	Rinse 3x MQW , soak MQW 48 hours, rinse 3x MQW
All samples	250 ml	Rinse 1% HCL, 3x MQW , soak MQW 24 hours, rinse 2x MQW
Other parameters	variable	See specific analyte

[1]If not specified, prep bottles according to Standard Methods 1995, 2005
[2]MQW = Millipore "Milli-Q" deionized water or equivalent (ASTM Type I, > 15 megohm/cm)
†Laboratory bottle blanks are analyzed for metals on each lot

The QA officer will archive all communications from and to CAL clients. We have developed a log of phone conversations; written communications and email communications are archived.

A summary of analyte specific handling and preservation procedures is in Table 2.

6. Sample Tracking

Each sample container has a label attached which is filled out in its entirety. The sample label includes the project ID, water body code or name, the site number, the date, and time of sample collection and sample processing information (raw, filtered or preservation information). Once sample identifications are verified and matched to the client's list they are logged into the CAL sample tracking system (a MS ACCESS database) and hard copies of COC (Appendix A) are filed. Files are saved on NRRI network backup system each week. Date of sample collection and in some cases, date of sample arrival at the laboratory, are used to keep track of specific analyte holding times.

Samples are logged into the CAL Laboratory Information Management System with the unique identifiers of project, site name, location, and sample date and time. We do not create separate entries for sample splits which routinely include either unfiltered (raw), filtered, or chlorophyll. We have instead instituted

the practice of including which sample container is used for an analysis. An entry is made into the notes section of each bench sheet indicating which bottle was used (e.g. the filtered sample was used for an ammonium analysis). This sample tracking method is more compatible with the research and water quality assessment activities of our laboratory.

Samples are preserved and refrigerated or frozen according to batch or date (depending on the project). A record is kept of the analyst and date for each parameter for each sample.

The majority of samples received or collected for analysis at NRRI do not require a formalized chain of custody. If the samples require a specific chain of custody, a procedure does exist for handling these samples. See Appendix C.

7. Sample Acceptance and Rejection

Occasionally, samples will be received that have not been properly preserved prior to receipt. In the majority of these cases the CAL will immediately notify the client of the problem and if necessary, discard the samples. In some cases, the analysis will continue with the client's permission, and the result flagged accordingly. This generally only occurs for research projects.

8. Equipment Maintenance and List Procedures

A preventive maintenance program is followed to ensure that instruments used for analysis are in optimum condition for operation. Personnel available within the lab are well trained in routine cleaning and maintenance of the instrumentation. This in-house maintenance is sometimes supported by service contracts with the instrument manufacturer for annual preventive maintenance or relatively quick response should a breakdown occur. Calibration and maintenance are archived in the QA/QC notebook and instrument logs. Equipment procedures list and calibration schedule are shown in **Table 4**.

A spreadsheet listing laboratory instrumentation that includes manufacturer, model, serial number, and date placed into service has been created and will be kept within the QA files.

Initial calibration for pH and turbidity meters will use a second source standard. We will use the instrument manufacturer-supplied second source sealed standard for turbidity and verified PT samples for pH. Standard lot numbers will be recorded with each analyte run.

Table 4. Equipment procedures list and calibration schedule.

Instrument	**Procedures**	**Calibration and Maintenance**
Sartorius Microbalance	SM 2540-D, SM 2540-E	Annual calibration by outside contractor and daily with calibrated weights
Latchat QuickCem FIA + 8000 Series	SM 4500-$NO3^-$ F , SM 4500 Cl^- E,	Daily per use, QCCS standards and blanks
Perkin Elmer Lambda 25 UV/VIS Spectrometer	EPA 110.1, EPA 365.3, SM 4500 P E 97,	Daily per use, QCCS standards and blanks
Corning 150 and 250 pH/ion meters	SM 4500 H+ B, SM 2320 B	Daily per use, commercially supplied pH 4, 7, 10 standards
HACH 2100P Turbidmeter	SM 2130 B	Daily per use, QCCS standards

		and blanks
Thermolyne Oven	SM 2540-D, SM 2540-E	Daily per use, annual with NIST thermometer
YSI 85/Hydrolab MS5 (sondes)	SM 2510 B	Daily per use

9. Internal Quality Control procedures

All raw data generated in the laboratory are recorded in bound notebooks, on project specific raw data sheets, and on analyte and project specific electronic spreadsheets. These data include sample identifiers, sample fraction (raw or filtered) calibration data, calculations, results, analyst notes, quality control data, date of analysis, and initials of the analyst. Completed notebooks are archived. Hard copies and electronic copies are archived by analyte and by project. All items are labeled, dated and signed by the analyst. When completed, the data are integrated into a final report per client requirements.

For out-of-control situations, a corrective action plan is in place. The initial action is to repeat the analyses of the samples bracketed by the unacceptable quality control sample. Replication of unacceptable results is investigated as a matrix effect by reviewing blank spikes or laboratory knowns. If the quality control samples are still unacceptable, the entire process is repeated. This includes sample preparation or extraction. If re-analysis is not possible due to the sample being past holding times or sample quantity is insufficient, documentation of the situation will be added to the raw data. In these cases, the report is flagged. If a corrective action requires additional documentation a corrective action form (**Appendix D**) may be completed and filed in the CAL QA file.

Quality assurance data sheet checks include scanning for apparent entry errors, measurement errors, omissions, and anomalies. Suspect data are flagged and/or excluded from use. Data may be presented in table, graph, and chart format. Unusual data are rechecked to verify their accuracy.

Recovery of Known Additions

"Recovery of Known Additions"or addition of an analyte spike to a sample is part of our regular methodology. Concentration of the known additions are 1 to 50 times the limit of detection (See Limit of Detection section) and fall within the linear range of the method. Concentrated solutions are used for additions so that volume change in the sample is negligible. Recovery is expressed as a percentage and is calculated as follows:

$$\% \text{ Recovery } = \left[\frac{\text{Spiked Sam ple - Sample}}{\text{Spike conc.}} \right] \times 100$$

Acceptable limits for sample spikes are ± 20 %.

Analysis of Reagent Blanks and Blank Spikes

Reagent blanks are run for each standard curve and may be inserted after samples with high concentrations to identify carryover to more dilute samples. At least one reagent blank per run is spiked with analyte at the same level as the samples and the percent recovery is calculated as per known additions to verify spiking level.

Analysis of Externally Supplied Standards

Quality control (QC) samples[1] with a certified known concentration are analyzed with each batch of samples. QC samples run at the beginning and end of each batch can show drift in the analytical run. These samples are prepared to fall within the linear range of the method. The assigned identification number or lot number of the external quality control standard are recorded with each data set to allow tracking in the event determined concentrations show drift beyond control limits.

Passing ranges for QC samples are posted in the quality assurance/quality control (QA/QC) data sheets and are updated for each new standard. A percent recovery is calculated by:

$$\%\ \text{Recovery} = \frac{\text{Determined concentration}}{\text{Certified concentration}} \times 100$$

Acceptable limits for these standards are 90-110% (± 10%). If ± 10% cannot be achieved, the instrument must be re-calibrated and the analysis repeated. An exception is made for Total Nitrogen (Method VI.1.01) where an acceptable QC recovery is ± 15%.

Calibration with Standards

Working standards are prepared by diluting a stock solution. For each run a minimum of three different concentrations spanning the expected range and a blank are prepared. Concentrations of individual samples are calculated from a linear regression of the measured parameter (e.g., spectral absorbance) against the standard concentration. If a sample concentration is greater than the highest standard of the standard curve the sample is diluted so its concentration falls within the mid range of the standard curve. Milli-Q water (MQW) is used for all blanks, standards, reagents, and final rinsing of glassware.

Mid-Point Check Standards

Mid-point check standards are read every 10 samples. Both the lowest and mid-point standard is inserted into each run.

Analysis of Duplicates

When samples have measurable levels of the constituent being determined, analysis of duplicate samples is used to estimate precision. In most cases, duplicates are run on 10% of the samples. See Table I-4 for frequency of duplicates. Acceptable limits are set at ± 15% unless the samples are at or near detection limits. If such a case occurs the QA officer can accept the results but must initial the results.

Standards and Reagents

When external QCCS from MDH approved vendors are received, CAL personnel will record manufacturer, certificate of analysis, lot #, date of receipt/preparation, preparer, method of preparation,

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[1] Purchased from MDH approved suppliers

storage conditions, and expiration date in the standards log book. In addition, new standards are cross checked against the previous batch to ensure the consistency of results. The same procedure is followed for the preparation of in-house standards.

Most reagents are discarded after daily use. If kept for > 24 hrs reagent the date of preparation and preparer initials are recorded on both the reagent label and in the standards log-book.

Maintenance of Control Charts and QA/QC Logs

Quality control charts are essential for quality control. There are several parameters that must be defined for use and maintenance of quality control charts.

Limit of Detection (LOD): This is the lowest concentration of an analyte that the analytical process can reliably detect. The LOD can further be defined as three times the variability (i.e., standard deviation) in the gross signal of the reagent blanks. The LOD is calculated every 25 runs or quarterly, whichever comes first. As the LOD is approached, the variance typically increases. This requires us to have what is termed the Limit of Quantification (LOQ) for a method.

Limit of Quantification: The LOQ is defined as the value for an analyte great enough to produce <15% RSD for its replication.

$$LOQ = 10(S.D.)$$

where 10(S.D.) is 10 times the standard deviation of the gross blank signal and the standard deviation is measured for a set of two replicates (in most cases).

Lower and Upper Control Limits (LCL and UCL):
To monitor drift in the calibration curves, warning limits are established and plotted. The Upper Control Limit (UCL) is the highest acceptable range of occurrence for a particular QA/QC parameter. The Lower Control Limit (LCL) is the lowest acceptable range of occurrence for a particular QA/QC parameter. UCL and LCL are calculated as:

$$UCL = \bar{X} + 3(S.D.)$$

$$LCL = \bar{X} - 3(S.D.)$$

where:

$\bar{X}$ = the mean value of the parameter
3(S.D.) = three times the standard deviation of the parameter

We apply control limits by plotting them as absolute boundaries for the acceptable range of occurrence.

Reporting limits (RL)
Reporting limits (RL) for the majority of analyses is 2 X the LOD.

The mean value ($\bar{X}$) becomes the central line of the control chart. Control charts are continuously updated and evaluated every 25 runs or quarterly, whichever comes first.

Percent Relative Standard Deviation of Replicates (% RSD): Ten percent of a run of samples are run in

duplicate and the % RSD, also called the coefficient of variation, is calculated by:

$$\%RSD = 100 \times \frac{(S.D)}{\overline{X}}$$

where:

S.D. = standard deviation of the replicates

$\overline{X}$ = mean of the replicates

Note that duplicate samples are split from a single sample. Higher % RSD (or RPD below) for true field replicates are not the responsibility of the CAL.

A % RSD below 15% for most replicates is considered acceptable. If the 15% RSD is exceeded, the samples will be reanalyzed. %RSD normally applies where the number of replicates is greater than 3.

Relative Percent Difference (RPD): While our standard laboratory convention is to analyze 10% of the samples in duplicate and use %RSD of the duplicates as a guide for accepting or rejecting the data, another measure of the variation of duplicates is RPD or:

$$RPD \frac{\left| x_1 - x_2 \right|}{\overline{X}} \times 100$$

Where:

X_1 = Value of duplicate 1, and
X_2 = Value of duplicate 2
X = mean of the duplicates

If the RPD exceeds 15%, the sample will be reanalyzed unless the samples are at or near detection limits. In those cases the QA officer has the option to accept the results with proper notations made.

Midpoint Check Standard: This refers to the placement of a mid range standard into the sample run. The value obtained should show less than 10% deviation from its true value.

"Out-of-control" Situations: In examining control charts and other QA/QC parameters, any of the following conditions would indicate an "out-of-control" situation.

1. Any point on a control chart beyond the control limits (i.e., LCL and UCL).
2. Seven successive points on the same side of the central line (mean) of the control chart.
3. A % RSD for duplicates greater than 15%, for a sample above the LOQ.
4. A QC check standard beyond its passing range.
5. Failure of a calibration curve where "failure" is typically defined as an $\mathbf{r}^2$ of 0.99 or less.
6. Midpoint checks standard failure.

The QA officer has the option to accept a run if fewer than 2 “out-of-control” situations occur but not if the QCCS standard is out of range. A failure of the QCCS standard automatically results in a rerun of the analyses. When an "out-of-control" situation occurs, the analysis must be stopped until the problem has been identified and resolved. A decision may be made to eliminate certain standards or spikes if the analyst can support this decision based on previous experience, knowledge of the aquatic system and an examination of other QA/QC information for that run.

Measurement and traceability

A database is in development that will allow NRRI CAL personnel to record and trace sources for standards and reagents for each procedure. Each source will be entered by manufacturer, chemical name, lot number and date first used. The sources will be tracked by date and each analyte run date will allow us to trace which source was used for each specific analysis. This database and all other QA electronic files are stored on the NRRI server that is backed-up weekly by IT staff and monthly by the QAO and TD.

Data Handling and Reporting

After completion of an analysis, the control charts are filled out. Electronic control sheets have been developed for each analyte. If a section is left blank, a comment section is filled out as to why. The comment section is also used to indicate any change in the analysis (i.e., path length, new standards and change in stock solutions). All control chart parameters are automatically updated and plotted using an EXCEL macro. Plots include LCL, UCL, LOQ, Special tests (spikes, method modifications, etc.) are included in the QC files, and reports are generated and reviewed quarterly (typically). All data is entered into electronic data sheets for QA tracking. Files are saved on NRRI network backup system each week.

Raw Data

Original raw data is stored, by analysis, under raw data files. A copy of this data is archived by project. A second copy of raw data is filed for laboratory reference and data entry. Field data is updated and readily available to lab managers and project heads. Project heads may request a copy of the raw data, but this data should not interfere with standard archiving procedures. Updates of spreadsheets are also filed by project. All lab books are centrally located when not in use. Notebooks do not leave the lab and data is entered ASAP. All raw data should have clear identification including sample ID, name of analyst, type of analysis, date of sample collection, QA/QC sample lot number and date of analysis. The QAO will periodically review each technician notebook for completeness.

Internal Audits

The data acquired from quality control (QC) procedures are used to estimate the quality of analytical data, to determine the need for corrective action, and to interpret results after corrective actions are implemented. Each method standard operating procedure (SOP) includes detailed QC procedures and QC limits. QC limits are generated where no method limits exist. QC limits for laboratory control samples (LCS) and matrix spikes (MS) are based on the historical mean recovery plus or minus three standard deviations units. Most analytical methods performed by NRRI CAL have on-going quality control charts that are updated following each

analyte run. Each analyst is responsible to report out of control situations to the QAO and in addition the QAO will review the control charts on a quarterly basis.

10. Verification Practices

Periodic (twice annually) audit and reference samples are received from a Minnesota Department of Health approved provider and analyzed following laboratory SOPs.

11. Data Validation

Within 48 hours of receipt of results of each chemical analysis, calculations and determinations of precision, completeness, and accuracy are made and corrective action implemented if needed. If data quality does not meet project specifications, the deficient data are flagged or discarded and the cause of failure evaluated (**Appendix D**). Any limitations on data use are detailed in the project reports and other documentation.

For the data to be considered valid, data collection procedures, handling of samples, and data analysis must be monitored for compliance with all requirements described in this QAM. Data are flagged and qualified if there is evidence of habitual violations of the procedures described in this QAM. Any limitations placed on the data are reported to the data end user in narrative form.

12. Corrective Actions

Corrective action is the process of identifying, investigating, approving, implementing and validating measures to counter unacceptable departures from policies and procedures or out of control QC performance which can affect data quality.

Deficiencies cited in the external assessment (such as an MDH inspection), internal quality audits, complaints, and managerial reviews are documented. Documentation is accomplished using the Corrective Action Form maintained by the laboratory, and signed by the QAO and TD. The Corrective Action form is included in **Appendix D**. Records shall be available to show that the root cause(s) of the deficiencies are investigated, including the results of the investigation. Records shall be available to document the intended corrective action. Records shall be available to show that the implemented corrective action is monitored for effectiveness. The QAO maintains these records. The TD will ensure that the corrective actions are discharged within the agreed upon time frame. When non-conformances and departures from SOPs cause doubt about the laboratory’s operations, the affected areas are promptly audited.

Method SOPs provide QC acceptance criteria and specific protocols for corrective actions. Any QC measure result that falls outside of acceptance limits requires corrective action. When testing discrepancies are detected such as out-of-control QC, the analyst will follow the specific protocol for corrective action as stated in the method SOP located in the Laboratory Manual. In the majority of cases where there is an out-of-control QC the analysis is re-run as soon as possible. In addition, any discrepancies are documented in the QA Log Book maintained by the QAO. The discrepancy will be identified, and the sample data associated with the discrepancy will be

flagged. The QAO will recommend corrective actions to be initiated by the analyst and ensure implementation and documentation of the corrective action. Each corrective action log entry is reviewed, signed, and dated by the QAO aor the TD. Corrective actions are performed prior to the reporting of the effected data.

In the event of the laboratory failing a performance test standard, the QA officer will review the procedures and results from the analysis and check for data entry errors. Identified errors will be corrected and recorded in the QA log book. The analysis will then be repeated with a new PT standard.

14. Subcontracting Testing

Certified laboratories may be subcontracted for testing not normally conducted by the CAL. Subcontractors for research projects will be selected based on specific project requirements.
There are instances where the NRRI CAL is unable to perform requested tests because the laboratory is uncertified, does not have the proper instrumentation or personnel, or because of instrument malfunction. Samples that need to be sent out to a contract laboratory will show transfer to the subcontracted lab in the CAL ACCESS Database and on the chain of custody form which will be archived in the client's file. A task reassignment form will be generated that includes the QAO signature authorizing the work, a list of sample ID's and requested analyses with turnaround time, the date/time the samples were sent out, cost information and the identity of the custodian responsible. Samples, along with copies of the field information and the task assignment form, and the COC are delivered to the contract lab. The delivery person and the recipient at the contract lab must sign the task assignment form indicating the transfer date and time. The task assignment form becomes part of the custody record.

15. Review of Requests, Tenders and Contracts

All new work is initiated by the Technical Director who delegates responsibilities for the new work according to available resources. Affected staff members meet prior to initiation of new work in order to determine if appropriate facilities and resources are available. The plan for any new testing shall be reviewed and approved by the technical director before commencing such work. If the review uncovers any potential conflicts, deficiencies, inappropriate accreditation status, and/or inability to perform the work, the laboratory shall notify the client. In cases where differences exist between the request/tender and contract they shall be resolved prior to starting work.

The review shall document that facilities and resources are organized to efficiently perform the work, including subcontracted work. The record of contract review includes pertinent discussions with the client regarding their requirements and results submitted during the contract period. For routine reviews of ongoing work a date and a signature of the laboratory official responsible for the contract is sufficient. For any new testing requirements, the designated official shall ensure that standard operating procedures and demonstration of capability to perform those tests prior to reporting results are available. The SOP(s) shall be under document control and a Demonstration of Capability statement(s) shall be on file. Copies are held in the

contract review file.

Clients are notified immediately in situations where the laboratory cannot conform to the contract and if there is a change in laboratory accreditation status.

16. Responding to Client Complaints

Responses to client queries and complaints will be dealt with as quickly as possible. A log of specific complaints and corrective action will be maintained in the laboratory QA/QC log book. E-mail communications with clients are archived.

17. Data Reporting to Clients

Data reports are generated as database exports into an Excel spreadsheet. These data reports are formatted and delivered per client request. Both an electronic version and hard copy are archived.

18. Procedures to protect confidential data

This has never been requested and would presumably be a very rare occurrence. If requested by a client, a data report will be stored as a file only accessible to NRRI CAL senior personnel and any generated reports will become the sole property of the client. Confidentiality agreements must follow the rules and regulations of the University of Minnesota and be approved by the appropriate University Office.

19. Records Retention and Document Control

All operating procedures, manuals including this quality manual, and documents are subject to document control. Controlled documents are indicated by the paper color indicated in the footer (i.e., blue). Uncontrolled copies are indicated by reproduction on any other color of paper. The quality assurance officer (QAO) controls the supply of paper used to produce controlled copies.

The purpose of the document control system is to ensure that only the most recent revisions are available to the appropriate personnel, revisions are timely, and receive the required approvals. All internal regulatory documentation, standard operating procedures are under document control. The QAO is responsible for the document control system and keeps a master list of the location of all documents and their current revision. The TD and the QAO approve all newly released documents and revised documents. Any employee can request a change to a document. Obsolete documents may be retained for legal reasons or for knowledge preservation. The QAO stores retained obsolete documents. All documents produced by the laboratory will contain the following information:

- effective date,
- revision number,
- document number,
- page numbers (including total number of pages), and
- document title.

Controlled documents will also include an approval signature page, a revision (change record) history page, and distribution list.

All SOPs and internal controlled documents are reviewed once per year. If a document is revised during the year the revision record in the document shall demonstrate review. If a document has not been revised during the year, the review record shall be the signature of the person responsible for the document and the date of the review.

Amendment of documents is allowed. The document control system allows for amendment of documents by hand pending re-issue. Such amendments are clearly marked, initialed and dated. A revised document is formally re-issued as soon as practically possible.

All data, including original observations, calculations and derived data, calibration records, QC records, and copies of the test reports, resulting from the analyses of samples are recorded and kept for five years (ten years for potable water samples) to allow historical reconstruction of the final result.

Electronic records are retained a minimum a 5 yrs but in most cases, indefinitely and backed up weekly on the NRRI server. All original raw data, bench sheets and laboratory notebooks are stored at the NRRI CAL indefinitely and never removed from the facility. Documents generated by the CAL are stored as both hard and electronic copies. The CAL ACCESS database is backed up weekly on the NRRI server as well as monthly on an external hard drive located at the laboratory.

20. Project Management Procedures

Overall project management at the NRRI CAL is performed by the Laboratory Director. The QA officer oversees day to day project management and reports to the Director.

21. Laboratory Safety

Workplace safety is important at NRRI. NRRI has an active safety committee that meets monthly to discuss ways of improving safety and problems such as accident reports that have occurred. Meetings are also held with project supervisors on a monthly basis to pass on information from the committee and provide them with information on topics from safe driving to ergonomics in the workplace. The committee is supported by the administration with a budget for materials and its own set of laboratory safety slide-tape presentations used for Employee Right to Know training (MERTKA).

The Central Analytical Laboratory (CAL) is an active participant in the programs of the committee and benefits from the safety awareness promoted there.

a. Safety Training and Documentation

Initial and annual training of NRRI laboratory personnel to meet the requirements of MERTKA and the OSHA Laboratory Standard is provided by their laboratory supervisor. All University of Minnesota staff, faculty, and students that work in laboratories or research areas are required, by law, to receive training about related safety issues and practices (mandated by both the federal Laboratory Safety Standard and the Minnesota Right to Know Act). New Employee Safety Training

(http://www.dehs.umn.edu/training_newlabsafety.htm) is the combination of three online tutorials designed to provide new employees, faculty and students their required safety training.

The Department of Environmental Health and Safety also provides an in-person version of this training, 5 times per year, called Laboratory Safety Training.

To fulfill the New Employee Research Safety Training requirement using the online version of the training, a researcher must complete:

1. Introduction to Research Safety
2. Chemical Safety
3. Chemical Waste Management

After completing these three tutorials, a researcher will know the general safety practices and protocols needed to be safe and compliant in the laboratory or research field.

Laboratory specific information such as the location of available safety equipment, location of material safety data sheets (MSDS), location of chemical storage and hazardous waste storage areas, etc. are also discussed with the employee. Supervisors are expected to train employees in the individual procedures they are performing and advise them of any hazards involved. Documentation of training is on a standard University form and records are kept in the employees file and by the UMD Health and Safety Officer.

Standard safety equipment is available to all laboratory employees. This includes chemical splash goggles, aprons, chemical protecting face shield, and rubber and autoclave gloves as personal protection gear. A hearing protection and respirator fit program are in place at the Institute if these are required for specific projects. Eye washes, chemical showers, fire blankets and fume hoods are available in laboratory areas.

Small lots of chemicals are stored in a room near the laboratories that is not actively used as a preparation or analytical area. Solvents, oxidizers and general chemicals are stored by group in separate cabinets. More solvent storage and corrosive chemical storage is in vented cabinets below the laboratory fume hoods. Working mixed reagents are usually stored near their point of use or in a compatible area if they are flammable (hood), produce fumes (hood) or are thermally labile (refrigerator). Case lots of acids or solvents are stored on the first floor of the building in rooms specifically designed for such storage.

Master collections of MSDS for the Institute are maintained in the office of the NRRI laboratory safety officer and the NRRI Library. MSDSs for specific procedures are also available in laboratory areas. MSDS's are available to all Institute employees.

The laboratory maintains a chemical list of all chemicals currently in the Institute laboratories. It lists the chemicals, quantities available, storage location and the National Fire Protection Association coding for hazard and flammability. It is provided to the Hermantown Volunteer Fire Department to aid them in knowing what chemicals are stored in the building as well as what the hazard may be. The list is updated annually.

Hazardous waste produced by laboratory procedures is bottled and held for short term storage in specific areas in the laboratory. It is then transferred to the Hazardous Waste Storage room on the first floor for packaging, manifesting and removal through the University of Minnesota Hazardous Waste Program. The Institute has an EPA Hazardous Waste Generator number. Assistance in recognizing and dealing with

hazards from stored chemicals as well as mixed wastes is always available from the University Environmental Health and Safety program if it is not available "in house."

The NRRI Analytical Chemistry Laboratory complies with the current OSHA laboratory standard. A copy of the modified University of Minnesota Chemical Hygiene Plan is on file in the office of the NRRI Laboratory Safety Officer, NRRI Center offices and the NRRI Library. The Chemical Hygiene Plan covers the general laboratory practices recommended by the University of Minnesota and more specific Institute policies and operating procedures for the laboratory.

b. Hazard Definitions

Chemical hazards - Any chemical, especially in concentrated form, that can have a detrimental effect on the health of an individual represents a chemical hazard. For purposes of this manual, chemical hazards have been divided into two categories:

1. Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

   Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

2. Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

3. Physical hazard **[*P]**: Hazards that can have detrimental health effects on an individual that are not related to the chemical properties but to ionizing radiation, heat, electrical and physical contact. Instruments with high temperature ovens have a thermal hazard and contact should be avoided. Radioactive materials require special handling and knowledge to avoid exposure to the ionizing radiation. Ultraviolet light from UV sources such as deuterium lamps may damage the retina of the eye if proper protective glasses are not worn. Electrical hazards are present any time an instrument is opened for trouble shooting or internal adjustments. When physical hazards are flagged, special knowledge and documented training are required.

c. Hazardous Waste

Hazardous wastes created by the procedures in this manual are disposed of through the University of

Minnesota Hazardous waste program. Analysts are responsible for properly identifying, labeling and storing hazardous waste. Supervisors are responsible for having laboratory personnel trained in proper handling of wastes.

1. Wastes created by any procedure should not be mixed with those from other procedures.
2. Waste containers should be compatible with the wastes they contain. In general, aqueous wastes should be stored in plastic containers and solvent wastes in metal or glass containers. Containers must have sealable caps and not capped with aluminum foil or other mismatched cover.
3. Aqueous wastes stored in laboratory satellite storage must be segregated by pH.
4. Solvent waste bottles must be capped. No solvents shall be evaporated in the fume hoods.
5. Waste containers must be labeled with hazardous waste labels before wastes are added. The labels must be fully filled out with the name of the generator, the generators telephone number, the starting date for the bottle and a complete list of components of the waste along with approximate concentrations. Chemical names must be complete. Abbreviations such as "MeOH" or "Mercury analysis waste" are unacceptable. Sample labels with the necessary information are included with many of the procedures in this manual.
6. Waste containers should only be filled to the shoulder of the bottle and not filled to the cap. A space must be left for expansion of the contents.
7. When containers are full, a manifest form should be filled out according to instructions in the **Hazardous Chemical Waste Management Manual**. The NRRI hazardous waste officer should be notified of the full container so that it can be checked for proper labeling and manifesting before being transported to hazardous waste storage for shipping. A copy of the manifest is attached to the waste container, a copy is given to the Hazardous Waste Officer and the remaining copies are forwarded to the UMD Hazardous Waste Officer.

d. **Waste Reduction**

This laboratory is concerned about the quantity of waste produced and the costs of disposal.

1. Analytical procedures that minimize the quantity of waste will be used as long as the limit of detection is adequate for the project.
2. Chemicals needed for procedures should be purchased on the basis of quantity needed rather than completely on price.
3. Excess chemicals should be made available to other projects.

22. References

Fishman, M.J. 1993. Methods of analysis by the U.S. Geological National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: USGS Open-File Report 93-125.

Janik, M., Earl Byron. Lake Tahoe Interagency Monitoring Program - Quality Assurance Manual. 1989. Division of Environmental Studies, University of California, Davis.

Reuter, J.E., C.R. Goldman. Water Quality of Selected Nevada Lakes with Special Emphasis on Investigating the Impacts of Nutrient Loading on Phytoplankton Growth in Pyramid Lake - Quality Assurance Plan. 1990. Limnological Research Laboratory, Div. of Environmental Studies - University of California, Davis.

Ruzycki, E., J. Ameel, J.R. Henneck. and R. Axler, 2009. *Analytical chemistry and quality assurance procedures for natural water, wastewater, and sediment samples.* Natural Resources Research Institute, Technical Report, **NRRI/TR-2009/36.**

Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1979. US EPA, Environmental Monitoring and Support Laboratory.

Standard Methods for the Examination of Water and Wastewater, 21st Edition. 2005.

Standard Methods for the Examination of Water and Wastewater, 18th Ed. 1992. American Public Health Association, Washington, D.C.

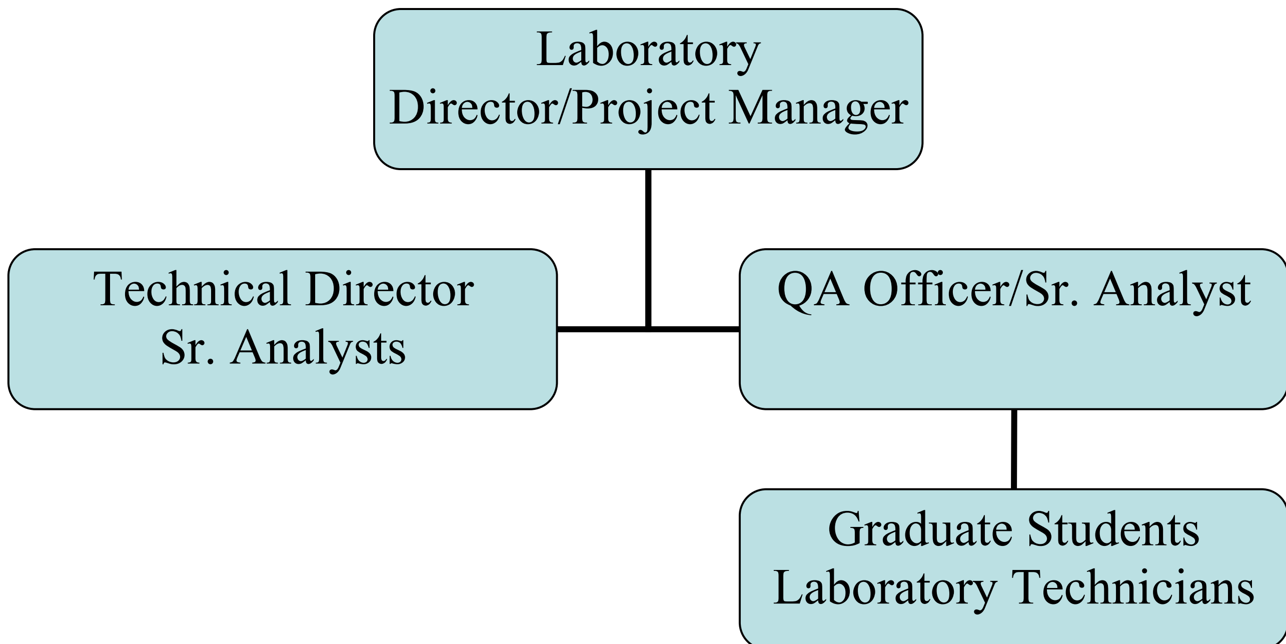
Hazardous Chemical Waste Management. 1996. 5th Edition, Department of Environmental Health and Safety, University of Minnesota.

Manual of Policies and Procedures for Radiation Protection. 1987 (rev 6/90). Department of Environmental Health and Safety, University of Minnesota.

Appendix A - NRRI CAL Laboratory Personnel and Organizational Chart

Name	**Title**	**Duties**
Richard Axler	Sr. Research Associate	Director/Project Manager
Elaine Ruzycki	Research Fellow	QA Officer/Sr. Analyst
Jerry Henneck	Scientist	Senior Analyst/Technical Director
Jeremy Erickson	Research Fellow	Senior Analyst

Note: NRRI as part of the University of Minnesota employs both undergraduate and graduate students.



Appendix B- Acronyms and Abbreviations

CAL: Central Analytical Laboratory
CSMP : Citizen Stream Monitoring Partnership
COC: chain of custody
DQO : Data Quality Objective
DI : Deionized
DIN : Dissolved inorganic nitrogen (= [nitrate+nitrite]-N + ammonium-N)
DO : Dissolved Oxygen
%DO : % DO saturation
EC25: specific electrical conductivity (EC normalized to 25^oC)
EDA : Environmental Data Access
EPA : Environmental Protection Agency
FD : Field Duplicate
H_2SO_4 : Sulfuric Acid
LIMS : Laboratory Information Management System
µ : Micron or Micro
µg/L : Microgram per liter (= 0.001 mg/L)
µS/cm : Microsiemens per centimeter (EC25 unit)
mg/L : Milligram per liter (=1000 µg/L)
MDH : Minnesota Department of Health
MPCA : Minnesota Pollution Control Agency
$Na_2S_2O_3$: Sodium Thiosulfate
NH_4-N : ammonium nitrogen
NIST : National Institute of Standards and Technology
NO_2-N : nitrite nitrogen
NO_3-N : nitrate nitrogen
NRRI-UMD: U. of Minnesota-Duluth Natural Resources Research Institute
NTU : Nephelometric Turbidity Units
PM : Project Manager
PO_4-P : phosphate phosphorus (aka: ortho-P [OP], soluble reactive-P [SRP], molybdenum reactive-P [MRP])
QA : Quality Assurance
QAC : Quality Assurance Coordinator
QAM : Quality Assurance Manual
QAPP : Quality Assurance Project Plan
QC : Quality Control
RPD : Relative Percent Difference
RSD : Relative Standard Deviation
SB : Sampler Blank
SLR : St. Louis River
SM : *Standard Methods* (*for the Examination of Water and Wastewater*)
SOP : Standard Operating Procedure
SWCD : Soil and Water Conservation District
STORET : STOrage and RETrieval
SU : Standard Unit (e.g. pH units)
TB : Trip Blank
TKN : Total Kjeldahl Nitrogen (total organic-N + total ammonium-N)
TN : Total Nitrogen
TP : Total Phosphorus
TSS : Total Suspended Solids
T-Tube : Transparency tube clarity in units of cms of visibility using a 100 or 120 cm tube.
WQ : Water Quality

Appendix C – Chain of custody form (attached)

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www.nrri.umn.edu/cwe/analyticallab.htm

Standard Operating Procedure

No. 101, Rev 3
Effective Date: 5/10/2012

TURBIDITY (Nephelometric) (SM 2130 B)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	5/10/2012	Annual review with revision of method validation procedure; updated to a controlled document

Reviewed by:

______________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
_______________________________________Date__________________
Richard Axler
Director Central Analytical Laboratory

1.0 Summary

This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Data are expressed in nephelometric turbidity units (NTU) but see Appendix A for instrument-specific turbidity codes (USGS, 2004).

All NRRI CAL turbidity measures are performed on surface waters where 40 NTUs is sometimes exceeded. We follow the instrument procedures using the manufacturer supplied sealed and second source standards that allow for direct, undiluted measurements of turbidities that exceed 40 NTUs. This protocol follows those developed by the USGS (2004) which states "**Note that dilution of environmental samples that contain particulate materials or exhibit other nonlinearity properties can introduce significant errors from subsampling; therefore, dilution is discouraged".**

2.0 Apparatus

1. Turbidimeter: HACH 2100P
2. Standard and Sample Cuvettes
3. Degassed Mili-Q water for zero standard and sealed standards set (5.46, 55.1, 551 NTU) if Formazin standards are not used.

3.0 Reagents

4,000 NTU Formazin Stock Suspension **[*T]** (HF Scientific (#70914), Hach (25M4A1001-119) or LabChem Inc. (Fischer LC26290-1)); RICCA 8830-32

4.0 Procedure

4.1 Standards Preparation (if instrument-supplied sealed standards are not used):

Measure Formazin Stock Suspension into the volumetric flask and bring up to volume with degassed Milli-Q water.

Standard Conc. (NTU)	Volume of Stock (mL)	Stock Conc (NTU)	Final volume (mL)
0.0	0.125	4000	1000
1.0	0.25	4000	1000
4.0	1.0	4000	1000
10	2.5	4000	1000

40	5.0	4000	500
80	10.0	4000	500
400	10.0	4000	100

Prepare at least 3 standards to cover the range anticipated.

Holding times: Hach recommends preparing dilutions <20 NTU daily while dilutions >20 can be held for 1 month. Standard Methods recommends preparing standards daily and EPA Regon 5 recommends preparing them weekly.

4.2 Calibration

When using sealed standards set:
Read a zero turbidity using degassed Milli-Q water followed by the sealed standard set (5.46, 55.1, 551 NTU)

Quality Control Standard: Initial calibration for turbidity meters will use a second source standard. We will use the instrument manufacturer-supplied second source sealed standard for turbidity. Standard lot numbers will be recorded with each analyte run.

4.3 Samples:

Rinse standard cuvette with an aliquot of 1 NTU standard. Pour approximately 15 mL of 1 NTU standard into standard cuvette. Wipe cuvette carefully with Kimwipes to remove fingerprints and smudges on the sides of the cuvette. Always handle the sample cell by the top.

Place cuvette into the turbidimeter and close cover on sample chamber.

Press: **I/O** to turn instrument on.

Select manual or automatic range by pressing the **RANGE** key. The display will show AUTO RNG when the instrument is in automatic range selection.

Press: **READ**
The display will show **-------NTU,** then the turbidity in NTU. Record the turbidity after the lamp symbol turns off.

If the reading of the sealed NTU standards are off by more than 5% the primary sealed standard must be used to recalibrate the instrument (see HACH 2100P manual for instructions).

Reading Samples

1. Allow samples to warm to room temperature to avoid fogging of curettes.
2. Shake raw sample well before pouring.
3. Read samples as per calibration standard described above.
4. Discard sample and rinse cuvette 3x with Milli-Q water. When reading next sample, be sure to place the cuvette in the same orientation in the turbidimeter.
5. Recheck standards every 5-10 samples.

See Table I-2 for hold times.

5.0 QA/QC

See QAM

6.0 Hazard

***T** Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

7.0 References

HACH Model 2100P Instrument and Procedure Manual (Cat. No. 46500-88)

Standard Methods for the Examination of Water and Wastewater, Method 2130A. 18th Ed. 1992. American Public Health Association, Washington, D.C.

U.S. Geological Survey (USGS). 2004. Turbidity (version 2): US Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6, section 6.7. (http://water.usgs.gov/owq/FieldManual/Chapter6/6.7_contents.html).

Appendix A. USGS TURBIDITY PARAMETER AND METHOD CODES (USGS 2004)	
NTU (Nephelometric Turbidity Units) Instruments--White or Broadband (400-680 nanometers) Light Source, 90-degree detection angle, one detector.	
NTRU (Nephelometric Turbidity Ratio Units) Instruments--White or Broadband (400-680 nanometers) Light Source, 90-degree detection angle, mutliple detectors with ratio compensation.	HACH, sensor model 2100 P
BU (Backscatter Units) Instruments--White or Broadband (400-680 nanometers) Light Source, 30- (plus or minus 15) degree detection angle (backscatter).	
AU (Attenuation Units) Instruments--White or Broadband (400-680 nanometers) Light Source, 180-degree detection angle (attenuation).	
NTMU (Nephelometric Turbidity Multibeam Units) Instruments--White or Broadband (400-680 nanometers) Light Source, Multiple light sources. Detectors at 90 degrees and possibly other angles to each beam.	
FNU (Formazin Nephelometric units) Instruments--Near Infra-Red (780-900 nanometers) or Monochrome light source. 90-degree detection angle, one detector.	Hydrolab, sensor model Datasonde 4 YSI Environmental, sensor model 6136
FNRU (Formazin Nephelometric Ratio Units) Instruments--Near Infra-Red (780-900 nanometers) or Monochrome light source. 90-degree detection angle, multiple detectors, ratio compensation.	
FBU (Formazin Backscatter Units) Instruments--Near Infra-Red (780-900 nanometers) or Monochrome light source. 30- (plus of minus 15) degree detection angle (backscatter).	
FAU (Formazin Attenuation Units) Instruments--Near Infra-Red or	

monochrome Light Source, 180-degree detection angle (attenuation).	
FNMU (Formazin Nephelometric Multibeam Units) Instruments (Including GLI Method 2)--Near Infra-Red (780-900 nanometers) or Monochrome light source, Multiple light sources. Detectors at 90 degrees and possibly other angles to each beam.	

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www.nrri.umn.edu/cwe/analyticallab.htm

Standard Operating Procedure

No. 102, Rev 3
Effective Date: 12/1/2012

Total Suspended Solids (TSS; Residue non-filterable) (SM 2540-D)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document

Reviewed by:

____ Date_________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
____ Date_________________
Richard Axler
Director Central Analytical Laboratory

1.0 Summary

A well mixed raw water sample is filtered through a weighed standard glass fiber filter and the residue retained on the filter is dried to a constant weight at 103-105EC. The increase in weight of the filter represents the total suspended solids (TSS).

Exclude large floating particles or submerged agglomerates of non-homogeneous materials from the sample if it is determined that this inclusion is not desired in the final result. Because excessive residue on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids, thoroughly wash the filter to ensure removal of the dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to excessive solids captured on the clogged filter. Samples must be filtered within 7 days and kept at 4EC. The limit of detection is based on the balance used in the analysis., Weights below 5 mg on a 4-place analytical balance should be considered unreliable. In such a case, more water should be filtered.

Note: Experience has shown that filter type is important. APHA Standard Methods (1992) specifically lists both Whatman 934-AH and Gelman A/E as acceptable glass fiber filters. It also states that Aother products that give demonstrably equivalent results@ may be used. As a result of inconsistencies of data from samples split with other laboratories, three filter types were compared in July, 1996, by filtering replicate portions of partially treated wastewater. These results gave a range of values apparently dependant on the filter type used.

Filter Type	Average TSS mg/L (n=3)	Relative standard deviation (%)
Whatman GF/C	56.1	8.5
Gelman A/E	44.4	3.3
Whatman 934-AH	34.1	11.6

This laboratory has traditionally used Whatman GF/C filters and has passed performance evaluation testing using those filters. However, from this data, it is important that filter type be recorded during any extended study and that the type not be changed during the study.

2.0 Apparatus

1. Filtration tower
2. Vacuum source
3. Aluminum weighing pans
4. 4.7 cm Whatman GF/C, Whatman GF/A, Whatman 9340AH or Gelman A/E filters

3.0 Reagents

None

4.0 Procedure

<u>Preparation of glass-fiber filter</u>*:* Insert filter with wrinkled side up on filtration apparatus. Apply vacuum and rinse filter with three successive 20-mL portions of deionized water (DIW). Continue suction to remove all traces of water, and discard rinse. Remove filter from filtration apparatus and transfer to a labeled aluminum weigh pan. Dry briefly at room temperature before drying the rinsed filters at 105EC for 2 hours. Lift the filter from the pan before placing it in the oven. This will prevent sticking.

2. If volatile solids are also to be measured, ignite filters at **550 ± 50EC [*P]** for 15 min in a muffle furnace. Cool to room temperature in a desiccator and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 0.5 mg between successive weighings. Store in desiccator until needed. Weigh immediately before use.
3. <u>Sample analysis</u>*:* Assemble filtering apparatus, seat filter and begin suction. Wet filter with a small volume of DIW to seat it. Filter a measured volume of well-mixed sample through the glass fiber filter. Wash with three successive 10-mL volumes of DIW, allowing complete drainage between washings and continue suction for about 3 min after filtration is complete. Carefully remove filter from filtration apparatus and transfer to an aluminum weigh pan as a support. Dry for at least 1 h at 103-105EC in an oven, cool in a desiccator to room temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less.

Calculation:

$$\text{Total suspended solids (TSS mg/L)} = \frac{(A-B) * 1000}{sample\ volume\ (mL)}$$

where:

A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

QA/QC

1. Record temperature of drying oven and/or muffle furnace.
2. Record balance used.
3. Record type of filter used and lot #.
4. Record weight of calibrated weight.
5. Include a blank in each run which involves filtering 1 L DIW onto a tared filter (i.e. same procedure as samples).

References

Standard Methods for the Examination of Water and Wastewater, Method 2541 D. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Hazards

***P** Physical hazard **[*P]**: Hazards that can have detrimental health effects on an individual that are not related to the chemical properties but to ionizing radiation, heat, electrical and physical contact. Instruments with high temperature ovens have a thermal hazard and contact should be avoided. Radioactive materials require special handling and knowledge to avoid exposure to the ionizing radiation. Ultraviolet light from UV sources such as deuterium lamps may damage the retina of the eye if proper protective glasses are not worn. Electrical hazards are present any time an instrument is opened for trouble shooting or internal adjustments. When physical hazards are flagged, special knowledge and documented training are required.

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Standard Operating Procedure

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Volatile Suspended Solids (VSS; Residue, volatile suspended) (SM 2540-E)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document

Reviewed by:

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Elaine M. Ruzycki
Laboratory Manager/QA Officer
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1.0 Summary

The residue from the total suspended solid method is ignited to constant weight at **550 ± 50°C [*P]**. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, sludges, industrial wastes, suspended periphyton, and bottom sediment. Negative errors in the volatile solids may be produced by loss of volatile matter during drying. Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error. In such cases, measure for suspect volatile components by another test, for example, total organic carbon. See Table I-2.

Note: Experience has shown that filter type is important. APHA Standard Methods (1992) specifically lists both Whatman 934-AH and Gelman A/E as acceptable glass fiber filters. It also states that Aother products that give demonstrably equivalent results@ may be used. As a result of inconsistencies of data from samples split with other laboratories, three filter types were compared in July, 1996, by filtering replicate portions of partially treated wastewater. These results gave a range of values apparently dependant on the filter type used.

Filter Type	Average TSS mg/L (n=3)	Relative standard deviation (%)
Whatman GF/C	56.1	8.5
Gelman A/E	44.4	3.3
Whatman 934-AH	34.1	11.6

This laboratory has traditionally used Whatman GF/C filters and has passed performance evaluation testing using those filters. However, from this data, it is important that filter type be recorded during any extended study and that the type not change during the study.

2.0 Apparatus

1. Filtration tower
2. Vacuum source
3. Aluminum weighing pans
4. 4.7 cm Whatman GF/C, Whatman GF/A, Whatman 9340AH or Gelman A/E filters
5. Muffle furnace capable of 550°C.

3.0 Reagents

None

4.0 Procedure

<u>Preparation of glass-fiber filter</u>: Insert filter with wrinkled side up on filtration apparatus. Apply vacuum and rinse filter with three successive 20-mL portions of deionized water (DIW). Continue suction to remove all traces of water, and discard rinse. Remove filter from filtration apparatus and transfer to a labeled aluminum weigh pan. Dry briefly at room temperature before drying the rinsed

filters at 105EC for 2 hours. Lift the filter from the pan before placing it in the oven. This will prevent sticking.

2. Ignite filters at **550 ± 50°C [*P]** for 15 min in a muffle furnace. Cool to room temperature in a desiccator and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight loss is less than 0.5 mg between successive weighings. Store in desiccator until needed. Weigh immediately before use.
3. *Sample analysis:* Assemble filtering apparatus, seat filter and begin suction. Wet filter with a small volume of DIW to seat it. Filter a measured volume of well-mixed sample through the glass fiber filter. Wash with three successive 10-mL volumes of DIW, allowing complete drainage between washings and continue suction for about 3 min after filtration is complete. Carefully remove filter from filtration apparatus and transfer to an aluminum weigh pan as a support. Dry for at least 1 h at 103-105°C in an oven, cool in a desiccator to room temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.5 mg, whichever is less.

Calculation:

$$\text{VSS mg/L} = \frac{(A-B) * 1000}{sample\ volume\ (mL)}$$

$$\text{Mg fixed solids} = \frac{(B-C) * 1000}{sample\ volume\ (mL)}$$

where:

A = weight of filter + dried residue, mg, and
B = weight of filter, mg.

QA/QC

1. Record temperature of drying oven and/or muffle furnace.
2. Record balance used.
3. Record type of filter used and lot #.
4. Record weight of calibrated weight within range of sample weights.
5. Include a blank in each run which involves filtering 1 L DIW onto a tared filter (i.e. same procedure as samples).

References

Standard Methods for the Examination of Water and Wastewater, Method 2541 D. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Hazards

***P** Physical hazard **[*P]**: Hazards that can have detrimental health effects on an individual that are not related to the chemical properties but to ionizing radiation, heat, electrical and physical contact. Instruments with high temperature ovens have a thermal hazard and contact should be avoided. Radioactive materials require special handling and knowledge to avoid exposure to the ionizing radiation. Ultraviolet light from UV sources such as deuterium lamps may damage the retina of the eye if proper protective glasses are not worn. Electrical hazards are present any time an instrument is opened for trouble shooting or internal adjustments. When physical hazards are flagged, special knowledge and documented training are required.

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Standard Operating Procedure

No. 104, Rev 3
Effective Date: 5/10/2012

pH in Dilute Natural Waters (SM 4500 H^+ B)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	5/10/2012	Inserted method validation procedure; updated to a controlled document

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Summary

The pH of a solution is a measure of its hydrogen ion activity and is the logarithm of the reciprocal of the hydrogen ion concentration. It is important to remember that a change of one pH unit represents a tenfold increase in $[H^+]$.

The pH of most natural waters falls in the range of 4.0 to 9.0 but most often in the range of 6.0 to 8.0. Most natural waters have a somewhat alkaline pH because of the presence of carbonate and bicarbonate. Changes in pH in surface waters may reflect biological activity, changes in natural chemistry as well as pollution.

Apparatus

1. pH meter (Corning 150 or 250, Beckman Φ11) equipped with temperature compensation and accurate to ±0.01 pH units.

2. Separate ceramic plug or fritted glass reference electrode and glass electrode (e.g. Orion Ross pH electrode (81-01-00 or 81-01BN) and Ross reference electrode (80-05-00)) for most critical pH measurement. The Ross combination electrode (81-02BN) can be used for less critical applications. (See also Precaution 9.)

3. Polyethylene containers for samples and buffers and other miscellaneous supplies.

4. Magnetic stirrer and stirring bars. A plywood block should be placed on the stirrer to prevent heat transfer to the stirred solutions.

Reagents

<u>Buffers, pH 4, 7, or 10</u>: Working buffers should be poured fresh before use. Do not use outdated buffers (check bottle for date).

<u>0.1 N H_2SO_4</u>: Dilute 2.8 mL concentrated H_2SO_4 to 1000 mL.

<u>Low ionic strength buffer (LISB)</u>: $10^{-3.8}$ sulfuric acid. Dilute 1.6 mL of 0.1N H_2SO_4 to 1000 mL. The pH will be approximately 3.82 for this solution. For the most critical analyses, the LISB solutions should be titrated regularly according to the methods of Galloway et al. 1978.

<u>Ross reference electrode filling solution</u>: (Orion 81-00-007)

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Procedure

1. Calibrate the pH meter according to the manufacturer's instructions using a two-point calibration with buffers that bracket the sample pH's (see Appendices 2 and 3). Record slope (see Precaution 10).
2. Use a magnetic stirrer and stir bar to stir the sample for 10 to 30 seconds before each reading. The stirring should be stopped and the pH stabilized for about 30 seconds before a reading is accepted.
3. Measure and record the pH of the LISB immediately after calibration. If the pH of the LISB exceeds 3.82 ± 0.05, the meter should be recalibrated and the LISB checked again. The LISB should be checked about every 10 samples.
4. Thoroughly rinse the pH electrodes with DIW between each sample or buffer. Rinse the electrodes with the sample or buffer before placing the electrode into the solution.
5. At the end of a sample set, read and record the pH of the LISB, pH 7 buffer and pH 4 buffer in that order.

Special precautions

1. Measurements should be made as soon as possible after samples are taken, preferably on samples less than 12 hours old. Samples should be stored at 4□C until analysis.
2. Store electrodes in pH 4 buffer.
3. Open the reference electrode filling hole to the atmosphere while measurements are being made.
4. Samples and buffers should be at the same temperature.
5. Electrodes must be rinsed repeatedly and thoroughly with distilled water between readings, especially after being immersed in buffers.
6. All pH measurements should be made on quiescent samples after reading has been stable for at least 30 seconds.
7. **Ross electrodes for analysis of low ionic strength samples should be used only for low ionic strength samples.**
8. Reference electrodes should be checked for solution level at least a day before they are to be used. The filling solution should be replaced about once a month and topped off to the filling hole level before use. Electrodes will stabilize more quickly if this is done the day before use and not the hour before.
9. The pH meter will show a gradual drift when reading low ionic strength waters. A slow drift is frustrating but normal. Try to take readings when the drift reaches the high end point. Erratic or rapid drift generally means a defective electrode. Replace the electrode, calibrate and try to measure the sample again. If the problem persists, report it to your supervisor.

10. The acceptable slope range is 54.4 mV/pH unit (92%) to 60.30 mV/pH unit (102%). Slopes below 92% indicate that the electrode may require cleaning or if cleaning does not help, the electrode should be replaced. Slopes above 102% indicate that the pH buffers are contaminated.

    Some pH meters display the slope as a percentage of the theoretical slop (59.12mV/pH unit). For example, a 98.5% slope is equivalent to a slope of 58.27 mV/pH unit.

Reference

Galloway, J.N., B.J. Cosby, and G.E. Likens. 1979. Acid precipitation: measurement of pH and acidity. Limnol. Oceanogr. 24:1161-1165.

McQuaker, N.R., P.D. Kluckner, and D.K. Sandberg. 1983. Chemical analysis of acid precipitation: pH and acidity determinations. Environ. Sci. Tech. 17:431-435.

QA/QC

Initial calibration for pH meters will use a second source standard. We use verified PT samples for pH. Standard lot numbers will be recorded with each analyte run.

See QA/QC program and Table I-4.

Appendix 1
Calibration of the Corning 150 pH meter

1. Plug in electrode(s) and ATC probe (if needed). Prepare the electrode by uncovering the filling hole. Top off with filling solution if necessary. Rinse the electrode with DIW and then pH 7 buffer. Place in beaker of buffer while setting up the meter.

2. Press **mode** to select **pH**. Press **cal** to start the calibration.

3. Select **2PT CAL**. The meter will prompt with **CAL 1 > 7.00**. Press the button by the flashing > if the meter will be calibrated with pH 4 and 7 buffers. Otherwise enter the pH of the high buffer using the keyboard. The meter will then prompt with **CAL 2 > 4.00**. Again accept the displayed value by pressing the button by the flashing > or enter the low buffer from the keyboard.

4. The meter will prompt with **MEASURE CAL 1**. Rinse and place the electrode(s) in the pH 7 (or high) buffer if it was not already done for step 1. Press = to start reading the buffer. The decimal point will blink while the meter is reading.

5. Stir the buffer for about 30 seconds. Stop the stirring and allow the pH reading to stabilize for 30 - 60 seconds. Once it is stable for 10 seconds, press =. The decimal will stop flashing and the meter will display a temperature corrected pH.

6. The meter will prompt with **MEASURE CAL 2**. Rinse the electrode(s) with DIW and then pH 4 (or low) buffer. Stir, stabilize and read the low buffer following step 5.

7. A brief delay will follow and the meter will display **SLOPE** = and the slope which should be **between 57.00 and 60.00**. Record the slope. If it is out of range, repeat the calibration steps 2 - 6. Note: The slope can be recalled by pressing **0**.

8. The meter will prompt with **MEASURE SAMPLE**.

9. Measure the pH of the **low ionic strength buffer (LISB)** by rinsing the electrode(s) with DIW followed by LISB. Stir, stop and stabilize the solution before reading the pH. LISB should read 3.82 ± 0.05. Record the pH. If it reads outside that range, re-calibrate the meter. (Low ionic strength buffer (LISB): ($10^{-3.8}$ N H_2SO_4 (approximate)) - dilute 1.6 mL of 0.1 N H_2SO_4 to 1000 mL)

10. Measure the pH of the samples. Rinse the electrode(s) between each with DIW and sample. Stir, stop and stabilize the readings as described above for each sample. Press = between each reading to stop the flashing decimal and put the meter in "standby" when the electrodes are removed from a solution.
11. When all of the samples have been measured, again measure and record the pH of the LISB, the high calibration buffer and the low calibration buffer in that order. Report any change greater than 0.05 pH units to your supervisor.
12. Rinse the electrode(s) in DIW, cover the filling hole in the reference electrode and store the electrode(s) in pH 4 buffer.
    Note: Do not leave electrode(s) exposed to air for an extended period of time. Store them in pH 4 buffer for long periods or DIW, sample or LISB for short periods.

Calibration of the Corning 250 pH meter

1. Plug in electrode(s) and ATC probe (if needed). Prepare the electrode by uncovering the filling hole. Top off with filling solution if necessary. Rinse the electrode with DIW and then pH 7 buffer. Place in beaker of buffer while setting up the meter.

2. Press **mode** to select **pH**. Press **cal** to start the calibration.

3. Select **2PT CAL**. The default calibration buffers are pH 7.00 and 4.00. If these do not bracket the expected range, see Note 1 below. The meter will prompt with **CAL 1 = 7.00** then **CAL 2 = 4.00** followed by **READ CAL 1**. Rinse the electrode(s) with DIW and pH 7.00 buffer. Press **READ**, stir the buffer for 30 seconds, stop the stirrer and allow the reading to stabilize for 30-60 seconds.
4. When the reading has stabilized near pH 7, press **READ** again to freeze (stop the flashing decimal) the display. After a short delay, a corrected pH close to 7.00 will be displayed and the meter will prompt **READ CAL 2**.

5. Rinse the electrode(s) in DIW and pH 4.00 buffer. Place them in the buffer and press **READ**. Stir for 30 seconds, stop the stirrer and allow the display to stabilize for 30-60 seconds. Press **READ** again to freeze the display. The meter will display **4.00 CAL 2** and **SLOPE** = after a short delay. Record the slope. If it is not in the range of 58.00 to 60.00, re-calibrate the meter. If a second calibration fails to bring the slope into this range, replace the electrode(s). After displaying **SLOPE** = the meter will prompt with **READ SAMPLE**.

   NOTE 1: To set the meter for buffers other than the default 7.00 and 4.00, enter **CAL** and request **2 PT CAL**. The display will show **CAL 1 = 7.00**, **CAL 2 = 4.00** and then **READ CAL 1**. Press **SET** until **DISP CAL 1 >** is displayed. Enter the pH of high buffer from the numeric keypad and press □. The display will request **DISP CAL 2 >**. Enter the pH of the low buffer and press □. The meter will then display **READ CAL 1**. Continue as with steps 4 and 5 above with the new buffers.

6. At **READ SAMPLE**, measure the pH of the low ionic strength buffer (LISB). Rinse the electrode(s) with DIW followed by LISB and place the electrode in the solution. Press **READ**. The decimal in the display should flash. Stir, stop and allow the solution to stabilize before reading the pH. LISB should read 3.82 ± 0.05. Record the pH. If it reads outside that range, re-calibrate the meter.

7. Measure the pH of the samples. Rinse the electrode(s) between each with DIW and sample. Stir, stop and stabilize the readings as described above for each sample. Press **READ** between each reading to stop the flashing decimal and put the meter in "standby" when the electrodes are removed from a solution.

8. When all of the samples have been measured, again measure and record the pH of the LISB, the high calibration buffer and the low calibration buffer in that order. Immediately report any change greater than 0.05 pH units to your supervisor.

9. Rinse the electrode(s) in DIW, cover the filling hole in the reference electrode and store the electrode(s) in pH 4 buffer.

   NOTE: Do not leave electrode(s) exposed to air for an extended period of time. Store them in pH 4 buffer for long periods or DIW, sample or LISB for short periods.

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Standard Operating Procedure

No. 105, Rev 3
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Alkalinity and Acid Neutralizing Capacity (ANC) (SM 2320 B)

Revision Record			
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2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	5/10/2012	Added note on method validation procedure

Reviewed by:

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1.0 Summary

Alkalinity of water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates, or other bases if these are present.

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Table IV-1.

For samples of low alkalinity (less than 20 mg $CaCO_3$/L [< 400 μ equiv/L]) use an extrapolation technique based on the near proportionality of the concentration of hydrogen ions to excess of titrant beyond the equivalence point (i.e., two end point or Gran Plot). The amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a simple extrapolation can be made to the equivalence point.

End points: When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The following pH values (Table IV-1) are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams $CaCO_3$ per liter. "Phenolphthalein alkalinity" is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the colored indicator, if any, used in the determination. The sharp end-point color changes produced by metacresol purple (pH 8.3) and bromcresol green (pH 4.5) make these indicators suitable for the alkalinity titration. See Appendix 1 for pH meter calibration.

Table IV.0.20-1.

	End Point pH for:	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity, mg $CaCO_3$/L:		
30	4.9	8.3
150	4.6	8.3
500	4.3	8.3
Silicates, phosphates known or suspected	4.5	8.3
Routine or automated analyses	4.5 - 4.2	8.3
2-end point	4.5 and 4.2	--
Industrial waste or complex system	4.5	8.3

Interferences

Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample. See Table I-2.

2.0 Apparatus

1. pH meter (Corning 150 & 250 meters) equipped with low ionic strength electrodes (Orion Ross pH and Reference Electrodes or combination electrode)
2. Microburet - 2 mL
3. Thermometer
4. Magnetic stirrer and stir bar
5. Top loading balance
6. Wood block to prevent stir plate from heating sample

3.0 Reagents

0.04N H_2SO_4 titrant: Dilute 2.27 mL **concentrated H_2SO_4 [*C]** to 2 liters with Milli-Q water. Standardize using NRRI method IV.0.25 against a 100 µeq /L THAM solution prepared by dissolving 1.2114g dried THAM in 100 m/L Milli-Q water in a 1000m/L volumetric flask. Dilute to the mark with MQW.

Low Ionic Strength Buffer (LISB): ($10^{-3.8}$ N H_2SO_4 (approximate)) - dilute 1.6 mL of 0.1 N H_2SO_4 to 1000 mL

4.0 Procedure

Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to Table IV-1. Titrate to the end-point pH without recording intermediate pH values and without undue delay using standardized titrant. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

Potentiometric titration of low alkalinity: For alkalinities less than 20 mg $CaCO_3$/L titrate 100 to 200 mL using a 10-mL microburet and 0.04 N standard acid solutions. Stop the titration at a pH in the range 4.2 to 4.5 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume. Stir while adding titrant. Do not stir when reading end point.

Calculations

1. Potentiometric titration to end-point pH:

$$Total\ alkalinity\ , mg\ CaCO3\ per\ L = \frac{[A *\ N * 50{,}000]}{mL\ sample}$$

where:

A = mL standard acid used and
N = normality of standard acid

Or

$$Total\ alkalinity, mg\ CaCO3\ per\ L = \frac{[A * t * 1000]}{mL\ sample}$$

where:

t = titer of standard acid, mg $CaCO_3$/mL.

Report pH of end point used as follows: "The alkalinity to pH ______ = ______ mg $CaCO_3$/L" and indicate clearly if this pH corresponds to an inflection point of the titration curve.

2. Potentiometric titration of low alkalinity (2-end point):

$$Total\ alkalinity\ , mg\ CaCO3\ per\ L = \frac{[(2B - C) *\ N * 50{,}000]}{mL\ sample}$$

or:

$$Total\ alkalinity\ , \mu eq\ per\ L = \frac{[(2B - C) *\ N * 999{,}100]}{mL\ sample}$$

where:

B = mL titrant to first recorded pH, (pH=4.5)
C = total mL titrant to reach pH 0.3 unit lower, and
N = normality of acid.

Alkalinity as mg $CaCO_3$/L can be converted to µeg/L by multiplying by 20.

QA/QC

A low-ionic-strength buffer (LISB) is read and recorded at the start and finish of a run. Record this value on QA/QC sheets. To make the LISB, see NRRI method IV.0.25.

Initial calibration for pH use a second source standard. We use verified PT samples for pH. Standard lot numbers will be recorded with each analyte run.

References

Standard Methods for the Examination of Water and Wastewater, Method 2541 D. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Hazards

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

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Standard Operating Procedure

No. 106, Rev 3
Effective Date: 12/1/2012

Specific Conductivity (EC@25°C) (SM 2510 B)

Revision Record			
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2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document; added QC language

Reviewed by:

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Richard Axler
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CONDUCTIVITY OR SPECIFIC CONDUCTANCE (EC25)

General

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valence, and relative concentrations, and on the temperature of measurement. Solutions of most inorganic acids, bases, and salts are relatively good conductors. Conversely, molecules of organic compounds that do not dissociate in aqueous solution conduct a current very poorly, if at all.

Specific conductance (EC25) is the conductivity at 25°C to compensate for temperature affects on ionic activity. Some instruments can be set to read EC25 directly (see YSI 85) while others require maintaining the sample at a constant temperature of 25°C while reading the conductivity. Formulas are available that can be used to adjust conductivity read at other temperatures to EC25 if sample temperature cannot be maintained at a constant temperature or a temperature compensated instrument is not available.

Apparatus

1. Sybron/Barnstead Conductivity Bridge, Model PM-70C-B
2. YSI 3400 Series Micro Conductivity Cell
3. Water Bath set at 25°C. Maintain temperature at 25°±0.2^{o}C.
4. Test tubes and rack.

or YSI 85 dissolved oxygen and conductivity instrument or equivalent

Note: The Sybron/Barnstead Conductivity Bridge is not a temperature compensated instrument. It is <u>very</u> important to adjust and maintain the temperature of the water bath as significant error will occur if the temperature is not maintained at 25°C. Water bath temperature should be monitored with both the water bath thermometer and a calibrated thermometer in a test tube of Milli-Q water in the rack with the sample tubes.

Reagents

<u>Stock Conductivity Solution, 0.0100N KCl</u>: Weigh 0.7455 g dried KCl and dilute to 1L in MQW = 1413 µmhos/cm (Note: 1 µmhos/cm = 1 µS/cm (S = siemans))
<u>Working Conductivity Solution, 14.94 µmhos/cm</u>: Dilute 1 ml of stock (0.01 N KCl) to 100 mL.

Standards and Calibration Checks

Four standards and calibration checks are used for each run:
1. Milli-Q water
2. Laboratory deionized water
3. Tap water
4. Conductivity standard
5. Certified quality control check standard (QCCS)

Procedure

Rinse each tube first with a small portion of standard or sample; pour two 15 to 20 ml replicates for each sample or standard. One will be used for a cell rinse and the other for measuring the conductance.

Place the rack of test tubes into the water bath at 25°C (cover w/foil to prevent evaporation). Let samples equilibrate to 25°C. Significant error will occur if the temperature is not maintained at 25°C.

Calibrate Conductivity Meter (Sybron/Barnstead Conductivity Bridge)

1. Disconnect probe; Turn on meter
2. Plug in shorting strap
   a. Set dial to 0.00
   b. Set multiplier to x1 kilohms
   c. Set sensitivity knob all the way to the right
3. Use screwdriver to adjust pointer to zero
   Remove shorting strap; Plug in Conductance and Resistance Calibrator (10.00 mho/cm)
4. Set dial to 1.00
5. Set multiplier to x10 micromhos/cm
6. Center sensitivity knob (1/2 way back to left)
7. Use screwdriver to adjust pointer to zero (exactly)

Reading samples/Standards

1. Connect probe wires, leave multiplier on x10 mmhos/cm
2. Use 1 tube to rinse probe then put probe in sample tube to read (make sure not to touch probe while taking reading)
3. Turn dial until the pointer is closest to zero. Be sure to find the closest point on either side of zero. Record that value and multiplication value.

QA/QC

1. Measure conductivity of Milli-Q water, DIW, tap water and QCCS.
2. Check conductance of a 0.001 N KCl solution.

See Table I-2 and I-4.

Method modification for use with the YSI 85.

The YSI 85 can be used for reading either conductivity (no temperature compensation) or EC25 (temperature compensated). It is a direct reading instrument that holds an internal calibration. Recalibration should only be attempted if a fresh QC standard varies more than ±10% from the certified value. Recalibrate using advanced procedures found in the YSI 85 manual.

1. Turn meter on. Allow to go through self-test.
2. Press MODE button until it reaches specific conductance. The large numbers on the display will be followed by either µS or mS (the instrument is autoranging) and the **small temperature display will flash on and off.** All readings will be temperature compensated. If the temperature display is not flashing, the mode button should be

pressed one more time to bring up specific conductance or temperature should be recorded for every reading so temperature compensation can be applied later.

3. Samples and standards should be transferred to beakers, 50mL centrifuge tubes or other containers large enough so that the entire probe can be submerged. The conductivity electrodes are in the small holes that are seen on the top of the probe. It is important that samples/standards flush this area completely and that the sample/standard level be maintained above these holes while readings are being taken.

4. Each samples/standard should be poured into two containers so the probe can be rinsed in the first and specific conductance read from the second.
5. Maintaining constant and accurate sample temperature for a batch of samples is not as important with this temperature compensated instrument as it is with the Sybron/Barnstead bridge but it doesn't hurt.
6. Read and record QA/QC samples as listed above.
7. Read and record EC25 and temperature for each sample. Be sure to record the range as the autorange will set the measurement to µS or mS.

Formula for temperature compensation.

If conductivity readings are taken that are not at 25°C and the temperature has been recorded, the following formula can be used to convert conductivity to specific conductance (EC25).

$$EC\ (\text{specific, i.e. at } 25°C) = EC\ (t) / [1 + 0.019*(t-25)]$$

Where: *EC*25=specific conductance (temperature compensated to 25°C)
EC=conductivity (not temperature compensated)
t=temperature of solution

Reference

Sybron/Barnstead Manual for Conductivity Bridge.

YSI Model 85 Operations Manual. YSI Incorporated, Yellow Springs, Ohio

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 120.1. EPA 600 4-79-020, Revised 1983.

Standard Methods for the Examination of Water and Wastewater, Method 2510B. 18th ed. American Public Health Association, Washington, D.C. 1992.

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Standard Operating Procedure

No. 107, Rev 3
Effective Date: 12/1/2012

Color, True (EPA 110.1)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document

Reviewed by:

_____________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
______________________________________Date_________________
Richard Axler
Director Central Analytical Laboratory

1.0 Summary

This method is a modified visual comparison method similar to method II.0.10 (Method 2120 B, Standard Methods, 1992) but it uses a standard curve based on the absorbance of the platinum cobalt standard read at 440 nm rather than visual comparison of standards using Nessler tubes. This procedure is not related to the more complex spectrophotometric color method, 2120C, in Standard Methods. Color determined on a filtered sample is referred to as true color. In some cases the contribution of the turbidity is of interest. Color measurement on unfiltered samples is referred to as apparent color.

2.0 Apparatus

1. Filtration tower
2. Vacuum source
3. Aluminum weighing pans
4. 4.7 cm Whatman GF/C, Whatman GF/A, Whatman 9340AH or Gelman A/E filters

Interferences

Since very slight amounts of turbidity interfere with the determination of color, samples should be filtered or centrifuged. This method is very pH dependent. Check and record pH of water to be analyzed and prepare the standard curve. Since biological activity may change the color characteristics of a sample, the determination of color should be made ASAP. Samples should be stored at 4EC. See Table I-2.

Color determined on a filtered sample is referred to as true color. In some cases the contribution of the turbidity is of interest. Color measurement on unfiltered samples is referred to as apparent color

3.0 Reagents

None

4.0 Procedure

For determination of true color, filter the sample through a membrane filter and place subsample of at least 15 mL in labeled container for analysis. Alternatively, samples may be centrifuged.

Samples should be analyzed within 48 hours of collection. If the samples are not analyzed immediately, store in refrigerator. Allow them to warm to room temperature before analysis. This prevents interference due to fogging of the cell.

1. Prepare standards by diluting platinum-cobalt standard stock solution of 500 Pt -Co units (Fisher SP120-500 or equivalent) with reagent grade water as follows:

Concentration Pt-Co Units	mL stock color standard/ 100 mL volumetric
5	1
10	2
25	5

50	10
100	20

Samples over 100 Pt-Co units should be diluted. Cover standards with parafilm to protect against reaction with airborne ammonia.

2. Turn on spectrophotometer lamp, set wavelength to 440 nm and let the instrument warm up for at least 20 minutes before zeroing.
3. Warm refrigerated samples to room temperature.

Calculations

1. Prepare a standard curve of absorbance against the platinum cobalt units (Pt-Co U) of the standards.
2. Report raw data as Aabsorbance at 440 nm and calculate sample color in Pt-Co units from the standard curve.
3. Report the data as either apparent or true color where apparent color is the color of an unfiltered sample or true color if the sample has been filtered or centrifuged.
4. According to Standard Method 2120-B, results giving 1-50 Pt-Co units should be reported to the nearest whole number. Results from 51-100 Pt-Co units should be rounded to the nearest 5 color units.

Calculation spreadsheet available

4. Use 4-cm cuvettes. Fill sample and reference cuvettes with distilled water, wipe cells with lab tissue, and place cells in respective compartments. Zero instrument.
5. Rinse sample cell with a few mL of sample before filling for reading.

QA/QC

1.

References

Standard Methods for the Examination of Water and Wastewater, Method 2541 D. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Hazards

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

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Standard Operating Procedure

No. 108, Rev 3
Effective Date: 12/01/2012

Chloride (SM 4500 Cl^- E)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/09/2012	Revised to a controlled document

Reviewed by:

____ Date_________________
Elaine M. Ruzycki
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____Date__________________
Richard Axler
Director Central Analytical Laboratory

1.0 Summary

Chloride ion reacts with mercuric thiocyanate to release thiocyanate ion, which combines with ferric ion to form a red complex that absorbs strongly at 480nm. The intensity of the color is proportional to the chloride concentration. See Table I-2.

Matrix: Waters, boiler water, wastewater with low solids content.
Range: Low level, 0.1 - 10 ppm
High level, 2.5 - 100 ppm
Rate: Low level, 55 samples/h
High level, 80 samples/h

Interferences Bromide, iodide, cyanide, thiosulfate, and nitrite. Color.

Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample. See Table I-2.

2.0 Apparatus

Lachat QuikChem 8000 Automatic Flow Injection Analyzer which include:

1. Random access sampler (RAS)
2. Proportioning Pump
3. Injection valve and sample loop
4. Colorimeter
   a. Flow cell, 10 mm
   b. Interference filter, 480 nm
5. Reaction Manifold: 10-117-07-1-B (high level), or 10-117-07-1-C (low level)
6. Omnion software system

3.0 Reagents

The combined reagent for chloride is available commercially from GFS Chemicals, Powell, OH, USA (Chloride Color Solution Mercuric Thiocyanate 0.06% solution, Item #1991, Catalog number 50041). The reagent can also be made in the laboratory but caution is advised as mercuric thiocyanate is highly toxic and ferric nitrate is an oxidizer and may cause burns. Consider the quantity of reagent needed. Proportion the amount of stock made to the amount or reagent required for the number of samples to be analyzed.

Mercuric thiocyanate stock **[*T]**: Dissolve 4.17 g mercuric thiocyanate ($Hg(SCN)_2$) in about 500mL of methanol in a 1L volumetric flask. Dilute to the mark with methanol and mix. (Note: Our mercuric thiocyanate is old and will not fully dissolve. This has no apparent affect on the analysis as long as the combined reagent is filtered as described below.)

Caution: Mercuric thiocyanate is toxic! Wear gloves!

Ferric Nitrate reagent stock , 0.5 M [*C]: Dissolve 202 g ferric nitrate ($Fe(NO_3)_3 \cong 9H_2O$) in about 800 mL of DI water in a 1L volumetric flask. Add 25 mL concentrated nitric acid (HNO_3) and dilute to the mark with DI water and mix.

Combined Color Reagent In a 500 mL volumetric flask, mix 75mL of mercuric thiocyanate stock with 75mL of ferric nitrate reagent and dilute to the mark with DI water. Vacuum filter the reagent through a 0.45 micron membrane filter (Millipore HAWP). Filtration of the color reagent has been found to decrease baseline noise.

> Note: Because the components of chloride analysis reagent are highly toxic and mercury contamination is likely, glassware and filtration apparatus should be marked, separated from other laboratory glassware and used only for chloride analysis procedures.

4.0 Procedure

Standards

Chloride stock, 1000 ppm: Dry several grams of NaCl at 105°C overnight. Dissolve 1.648 g of NaCl in approximately 800 mL of deionized water in a 1L volumetric flask and dilute to the mark with DI water.

Working standards.

Table IV.0.50-1. Working standards for Chloride analysis

Method file	CL_HIGH.MET	CL_LOW.MET	
QuickChem method	10-117-07-B	10-117-07-C	
Standard	mg/L	mg/L	
1	100	10.0	
2	50	8.0	
3	25	4.0	
4	10	1.0	
5	5.0	0.5	
6	2.5	0.25	
7	0.0	0.1	
8		0.0	

Table IV.0.50-2. Dilution scheme for working standards for high level chloride analysis (Method 10-117-07-1-B).

Standard	Standard Conc. mg/L	Volume Stock (mL)	Stock Conc. (mg/L)	Final Volume (mL)
1	100	10.0	1000	100
2	50	5.0	1000	100
3	25	2.5	1000	100
4	10	1.0	1000	100
5	5.0	0.5	1000	100
6	2.5	0.25	1000	100
7	0.0	B	B	B

Table IV.0.50-3. Dilution scheme for working standards for low level chloride analysis (Method 10-117-07-1-C).

Standard	Standard Conc. mg/L	Volume Stock (mL)	Stock Conc. (mg/L)	Final Volume (mL)
1	10.0	1.0	1000	100
2	8.0	0.8	1000	100
3	4.0	0.4	1000	100
4	1.0	0.1	1000	100
5	0.5	0.05	1000	100
6	0.25	0.025	1000	100
7	0.10	0.01	1000	100
8	0.00	B	B	B

Refer to LATCHAT Method 10-117-07-1-E

Carrier: degassed deionized water

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Standard Operating Procedure

No. 109, Rev 3
Effective Date: 12/01/2012

Ortho-Phosphate (o-P) Manual Ascorbic Acid method (SM 4500-P E 97)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document

Reviewed by:

_______________________________________ Date_________________
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Richard Axler
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Controlled document - yellow

1.0 Summary

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to intensely colored molybdenum blue by ascorbic acid.

Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. See Table IV-2.

Sample Collection

Deep water samples can be collected using a Van Dorn or Kemmerer sampler while stream and surface lake samples can be collected by dipping the sample bottle. Samples must be filtered through a 0.45 µm membrane filter ASAP. Hold time in hours, see Table I-2.

2.0 Apparatus

1. Spectrophotometer set at 880 nm.
2. Pair of 4 cm or 1 cm spectrophotometer cells
3. Test tubes and rack (16 x 125 mm tubes are cleaned and set aside for phosphorus analysis)
4. Vortex stirrer

3.0 Reagents

Acidic potassium antimonyl tartrate-ammonium molybdate combined reagent: Add 30.5 mL conc. H_2SO_4, 2.62 g **ammonium molybdate [*T]** and 0.075 g **potassium antimonyl tartrate [*T]** to 225 mL MQW.

Ascorbic acid 0.01 M: Dissolve 0.75 g ascorbic acid in 25 mL MQW. Prepare daily.

Stock Standards:

50 ppm phosphate-P stock: Dry primary standard grade KH_2PO_4 for 2 hours at 105EC. Dissolve 0.2197 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW.

5 ppm phosphate-P intermediate standard stock: Dilute 50 ppm phosphate stock 1 + 9 to make a 5 ppm

intermediate standard. or add 10 mL 50 ppm stock to a 50 mL volumetric flask and dilute to mark with MQW.

Working standards:

Prepare an individual calibration curve for each set of samples. The curve should cover the expected range of samples.

Standard Concentration	Volume of Stock	Concentration of Stock	Final Volume
10 ppb	0.2 mL	5 mg/L	100 mL
20 ppb	0.4 mL	5 mg/L	100 mL
50 ppb	0.2 mL	50 mg/L	200 mL
100 ppb	0.2 mL	50 mg/L	100 mL
200 ppb	0.4 mL	50 mg/L	100 mL
500 ppb	1.0 mL	50 mg/L	100 mL
1000 ppb	2.0 mL	50 mg/L	100 mL

For low levels, use 0, 10, 20, 50, 100 and 200 ppb standards and a 4 cm cell. For high levels, use 50, 200, 500 and 1000 ppb standards and a 1 cm cell.

General notes

New test tubes and glassware used in this procedure must first be washed with laboratory soap, thoroughly rinsed with deionized water (DIW) and followed with a rinse in 0.1 N HCl (8.3 mL concentrated HCl per liter DIW).

All test tubes and glassware should be filled with 0.1 N HCl for storage. Glassware and racks are set aside specifically for use for phosphorous analysis.

Prepare an individual standard curve for each set of samples.

The number of samples in a run should be 25 or less, not including QCCS and standards.

Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and subtract blank absorbance from absorbance of each sample.

4.0 Procedure

1. Pipet 10.0 mL standard, blank or sample into a clean, dry test tube.

2. Add 1.0 mL combined reagent and vortex.

3. Add 0.1 mL ascorbic acid and vortex.

4. After 20 minutes, vortex all samples then measure absorbance of each standard and sample at 880 nm using degassed MQW as the reference solution. Use the 4 cm cell unless the sample

concentrations are known to be high. Read all samples within 1 hour after the color develops.

5. Re-read standards and blank at the end of the run.

6. After run, rinse all glassware in DIW and fill with 0.1 N HCl for storage.

Calculation:

Enter initial and final standard absorbances into a regression using either a spreadsheet or calculator.

Calculate µg/L Ortho-P by entering sample absorbances into regression. Depending on light path, P range for this method is from < 5 ppb to 2000 ppb. If sample is below 5 ppb, report as below detection. If above 1200 ppb, dilute and re-run.

QA/QC

Use correct cell size depending on sample range.Verify wavelength setting before reading absorbances. See QA/QC program and Table I-4.

References

Standard Methods for the Examination of Water and Wastewater, Method 2541 D. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Hazards

***T**

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Standard Operating Procedure

No. 110, Rev 4
Effective Date: 4/15/2013

Total Phosphorus/Total Nitrogen Simultaneous Digest (SM 4500 NO_3^- F)

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Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document
4	Elaine M Ruzycki	4/15/2013	Updated safety information
5	Elaine M Ruzycki	6/5/2015	Added notes on nutrient nomenclature.

Reviewed by:

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_____________________________________Date_________________
Richard Axler
Director Central Analytical Laboratory

NUTRIENT ANALYSIS, NOMENCLATURE FOR FORMS OF NITROGEN AND PHOSPHORUS

Current literature in nutrient analysis contains an alphabet soup of acronyms for various forms of nitrogen and phosphorus depending on the author and the handling of the sample, e.g. raw water, filtered water, particulate, etc. Without getting into the actual reasons for the various types of partitioning or divisions, the following represents the standard nomenclature used in this laboratory.

Nitrogen

Nomenclature for nitrogen species is less variable than for phosphorus but confusion often still exits. We assume the following:

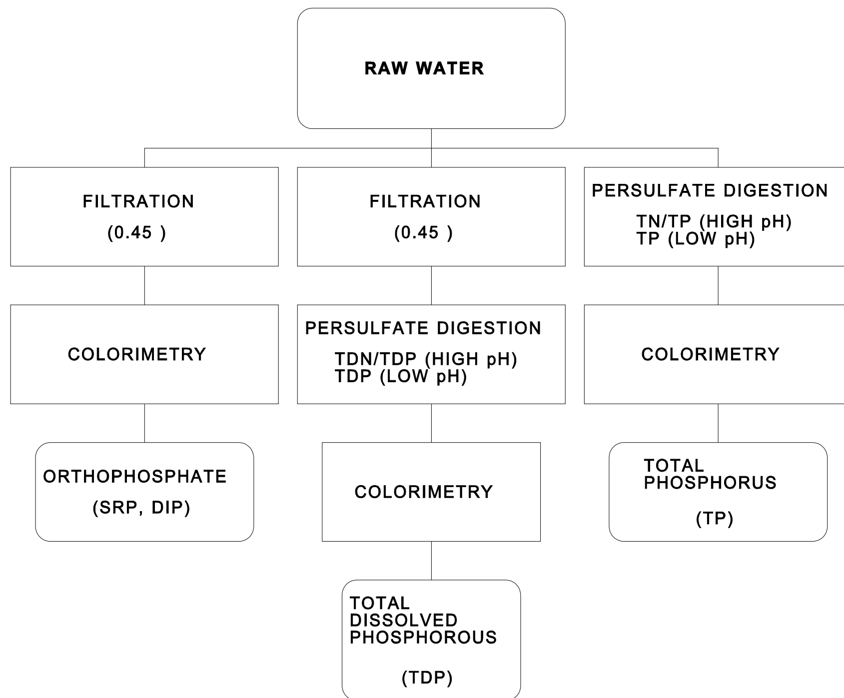
1. Dissolved = soluble
2. Total-N (TN) = dissolved-N (DN) + particulate-N (PN)
3. PN = particulate organic-N (PON), since particulate inorganic-N is virtually non-existent
4. DN = dissolved organic-N (DON) + dissolved inorganic-N (DIN)
5. DIN = nitrate-N + nitrite-N + ammonium-N (DIN = [NO_3^-+ NO_2^-]-N + [NH_4^+-N])
6. Often Anitrate-N@ refers to a combined analysis of [nitrate + nitrite] - N
7. We prefer to use the term ammonium-N (NH_4^+-N) because this is the predominant form within the range of pH values encountered in natural waters.
8. DON is determined by digesting and analyzing a filtrate as per TN and then subtracting DIN.
9. Total Kjeldahl Nitrogen (TKN) = PN + DON + NH_4^+-N and so we calculate TKN as TN - [NO_3^-+ NO_2^-]-N

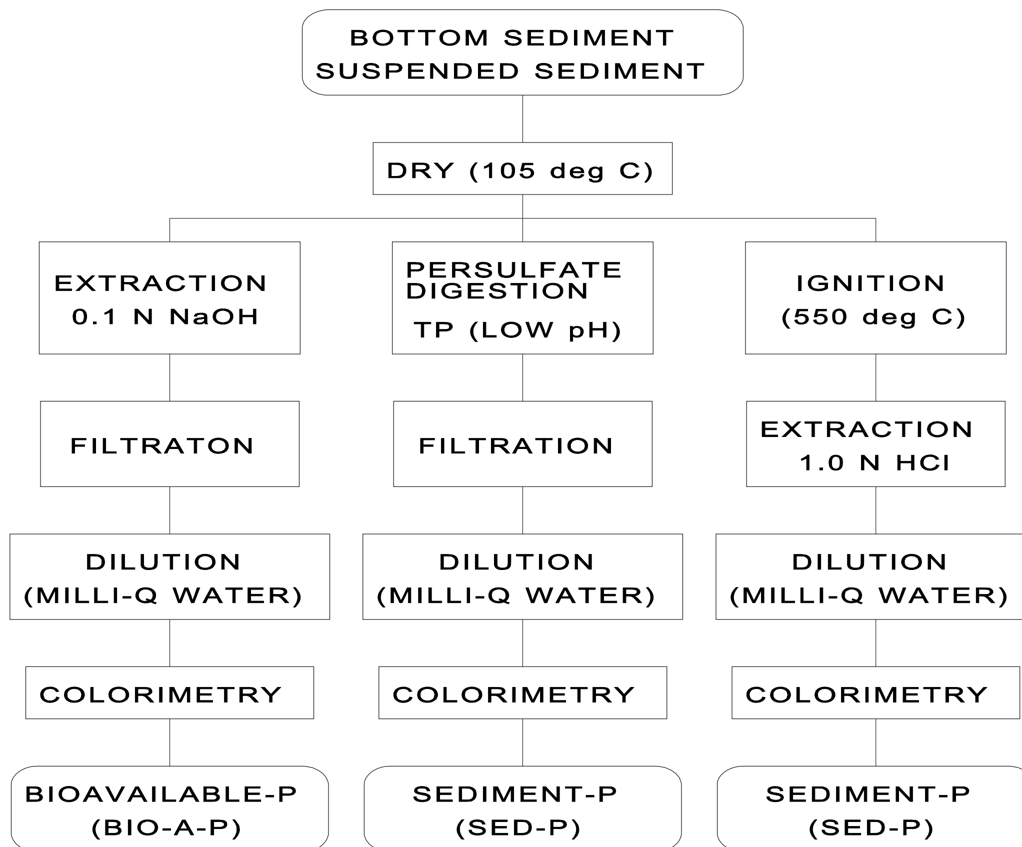
Phosphorus

The nomenclature for dissolved inorganic phosphorus is widely variable throughout the aquatic literature. We assume the following:

1. Dissolved = soluble
2. Dissolved inorganic-P (DIP) = soluble reactive-P (SRP)
   = dissolved reactive-P (DRP as in Standard Methods, 1995)
   = ortho-P (OP)
   = molybdenum reactive-P (MRP)
3. Total-P (TP) = dissolved-P (DP) + particulate-P (PP)
4. Dissolved-P = filtrate digested as per TP for analysis
5. Particulate-P can be analyzed directly from the residue on a glass fiber filter (usually Whatman GF/C or GF/F) or by calculation as TP - DP.

Fractionation schemes for phosphorus used in this laboratory for water samples and sediment or suspended materials are summarized in Figure IV.1.00-1 and IV.1.00-2


RAW WATER
FILTRATION
(0.45)
FILTRATION
(0.45)
PERSULFATE DIGESTION
TN/TP (HIGH pH)
TP (LOW pH)
COLORIMETRY
PERSULFATE DIGESTION
TDN/TDP (HIGH pH)
TDP (LOW pH)
COLORIMETRY
ORTHOPHOSPHATE
(SRP, DIP)
COLORIMETRY
TOTAL
PHOSPHORUS
(TP)
TOTAL
DISSOLVED
PHOSPHOROUS
(TDP)


BOTTOM SEDIMENT
SUSPENDED SEDIMENT
DRY (105 deg C)
EXTRACTION
0.1 N NaOH
PERSULFATE
DIGESTION
TP (LOW pH)
IGNITION
(550 deg C)
FILTRATON
FILTRATION
EXTRACTION
1.0 N HCl
DILUTION
(MILLI-Q WATER)
DILUTION
(MILLI-Q WATER)
DILUTION
(MILLI-Q WATER)
COLORIMETRY
COLORIMETRY
COLORIMETRY
BIOAVAILABLE-P
(BIO-A-P)
SEDIMENT-P
(SED-P)
SEDIMENT-P
(SED-P)

General

These notes apply to digestion of natural water samples for analysis of total nitrogen and total phosphorus. Organic nitrogen and ammonium are converted to nitrate and phosphorus compounds are liberated as ortho-phosphate.

Digestion is best performed within 12 hours although the holding time listed by the EPA for ortho-phosphate is 48 hours. If samples cannot be digested within the prescribed times, they can be preserved by freezing (Ameel, et al. 1993). This digestion prodedure has received a great deal of attention since its publication in the fall of 1993. This method is SM 4500-P J in SM 21st edition.

Apparatus

Digestion Tubes: 40 mL vials (Fisher 03-339-22N or equivalent) with autoclavable caps lined with Teflon7 liners (Scientific Specialties Service, Inc. Product number B68800-24 or equivalent). Soak vials and caps in10% HCl for 24 hours, then rinse 2X with Milli-Q water. Do not clean with soap.

Autoclave

Reagents

PPE: safety glasses, gloves and lab coat.

$K_2S_2O_8$ Reagent: Dissolve 20 grams **low nitrogen $K_2S_2O_8$ [*C]** in ~200 mL Milli-Q water (MQW) in 500 mL volumetric flask. Add MQW to the mark.

Persulfate recrystallization procedure: **Appendix A**

NaOH Reagent: **12 grams NaOH [*C]** (Mallinckrodt 7708 (pellets) or equivalent) in ~75 mL MQW in 100 mL volumetric flask. Add MQW to mark. (Note: Analyze any new batch of NaOH for N and P background. Some NaOH labeled Alow nitrogen@ has an unsuitably high background.)

Standards, Nitrogen stock

500 ppm nitrate-N stock: Dry reagent grade potassium nitrate (KNO_3)for 2 hours at 105°C. Dissolve 3.6090 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW.

50 ppm nitrate-N stock: Dry reagent grade potassium nitrate for 2 hours at 105°C. Dissolve 0.361 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW. lternative: Dilute 500 ppm N stock 1+9 with MQW.

500 ppm nitrite-N stock: Dry reagent grade sodium nitrite in a desiccator. Dissolve 0.616 g in about 50 mL MQW in a 250 mL volumetric flask. Dilute to the mark with MQW. Storage time is one month. This stock is diluted and digested for a cadmium column efficiency check.

Standards, Phosphorus stock

50 ppm phosphate-P stock: Dry primary standard grade KH_2PO_4 for 2 hours at 105°C. Dissolve 0.2195 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW.

5 ppm phosphate-P intermediate standard stock: Dilute 50 ppm phosphate stock 1 + 9 to make a 5 ppm intermediate standard.

Standards, spiking

200 ppm Urea N (low level TN): Dissolve 0.0429 g Urea in about 50 mL MQW in a 100 mL volumetric flask. Dilute to the mark with MQW. Add 0.015mL /15mL sample for a 200ppb spike.

600 ppm Urea N (high level TN): Dissolve 0.3218 g Urea in about 50 mL MQW in a 250 mL volumetric flask. Dilute to the mark with MQW. Add 0.2mL /15mL sample for an 8.0 ppm spike.

50 ppm P stock: The 50 ppm P stock prepared above can be used although an independently prepared stock of the same concentration is preferred.

Working standards, low level nitrogen:

Standard Conc. mg N/L	Vol. of Stock mL	Conc. of Stock mg/L	Final Volume mL
2.0	4.0	50	100
1.0*	4.0	50	200
0.5	1.0	50	100
0.1	0.2	50	100
0.05	0.1	50	100
0.02	0.04	50	100
0.01	0.02	50	100

* Concentration used for midpoint check standard

1.0 mg/L nitrite-N (column efficiency check standard). Add 0.200 mL of 500 ppm nitrite-N stock to a 100 mL volumetric flask. Add MQW to the mark.

Working standards, low level phosphorous:

Standard Conc. µg/L	Vol. of Stock mL	Conc. of Stock mg/L	Final Volume mL
500	1.000	50	100
200	0.400	50	100
100	0.200	50	100
50*	0.200	50	200
20	0.200 0.020	5 50	100

10	0.100 0.010	5 50	100

* Concentration used for midpoint check standard

Working standards, high level nitrogen:

Standard Conc. mg N/L	Vol. of Stock mL	Conc. of Stock mg/L	Final Volume mL
50.0	10.0	500	100
25.0*	5.0	500	100
10.0	2.0	500	100
2.5	0.5	500	100
1.0	0.4	500	200
0.5	0.1	500	100

* Concentration used for midpoint check standard

10.0 mg/L nitrite-N (column efficiency check standard). Add 2.00 mL of 500 ppm nitrite-N stock to a 100 mL volumetric flask. Add MQW to the mark.

Working standards, high level phosphorous:

Standard Conc. µg/L	Vol. of Stock mL	Conc. of Stock mg/L	Final Volume mL
500	1.000	50	100
200	0.400	50	100
100	0.200	50	100
50*	0.200	50	200
20	0.200 0.020	5 50	100
10	0.100 0.010	5 50	100

* Concentration used for midpoint check standard

These are the working standards most frequently used for the analysis of TN and TP. Alternate standards for other ranges may be found in the analytical procedure for that range. Standards other than those specified can be calculated but calculations should be checked by a supervisor. Expanded

ranges should also be checked for complete digestion using standards and spikes. TN has routinely been run up to 50mg/L and TP up to 25 mg/L for some projects.

Procedure

1. Pipet 15 mL of each standard or sample into digestion tube. Rinse the pipet tip once with 0.1N HCl followed by three rinses with MQW between sets of standards and between standards and samples.
2. Additional samples/standards to be included in any run are:
   a.. Check standards: at least one extra mid-range (1.0 mg/L N and 50 µg/L P) must be digested per tray of 24 samples.
   b. Column efficiency check: At least one 1.0 mg/L nitrite standard must be digested with each set of samples to determine the efficiency of the cadmium column for nitrate-N determination. Digesting 2 replicates is preferred.
   c. QCCS standards: At least one mid-range QCCS should be digested with the run. Digesting 2 or more replicates is preferred in case any digests must be re-run.
   d. Spikes: 10% of low level samples should be spiked by adding 15 µL of 200 ppm urea (+0.200 mg/L N) and 15 µL of 50 ppm P stock (+50 ppb P).
3. Add 5.0 mL $K_2S_2O_8$ to sample and standards followed directly by 250 µL NaOH.
4. Cover sample with foil and cap with Teflon7 lined cap.
5. Autoclave for 50 min. at 121^oC (250^oF) @ 17 psi.
6. If the digests are not going to be processed immediately, cool them to room temperature and then refrigerate (holding time after digestion is 28 days).
7. Just before analysis, warm the digests to room temperature. Just before analysis, add 250 µL of NaOH reagent and analyze by the methods listed below.
8. Analyze for PO_4-P per Methods IV.3.01 (high levels), IV.3.00 (low levels) or IV.3.10.
9. Analyze for (NO^-_3 + NO^-_2) -N as per IV.1.20.

QA/QC

See QA/QC program and Table I-4.

References

Ebina, J., T. Tsutsui and T. Shriai. 1983. Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. Water Research 17(12): 1721-1726.

Ameel, J. R. Axler, and C. Owen. 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. American Environmental Laboratory. Oct. 1993.

Standard Methods for the Examination of Water and Wastewater, Method 4500-N_{org} D. (proposed). 19th Ed. 1995. American Public Health Association, Washington, D.C.

Safety

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases,

phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

Waste Disposal

Waste cannot be sewered. All waste is to be poured into labeled hazardous waste. Waste is not to be stored for longer than 90 days

Appendix A: Persulfate Recrystallization

1. Dissolve 100 g of potassium persulfate in approximately 600 ml of Milli-Q previously heated to 60° C. Use a medium sized stir bar and a 1000 mL flask.

2. Filter the solution rapidly through a sintered glass funnel.

3. Rinse the 1000 mL flask.

4. Pour filtrate back into the flask used to heat the potassium persulfate solution.

5. Cool solution to about 4° C by placing the flask in ice water. Whirl the flask continuously to prevent the solution from freezing.

6. Filter the 4° C solution and wash with 1 or 2 squeezes of ice cold Milli-Q, save the white solid.

7. Discard the filtrate from the sidearm flask.

8. Rinse the flask used to cool the solution with Milli-Q

9. Fill the flask with 450ml of Milli-Q and heat to 60° C.

10. Add the crystals from step 5 and mix into solution.

11. Repeat steps 4 and 5. The white granules on top of the filter are crystals!

12. Dry crystals in vacuum over anhydrous calcium chloride. Rapid drying in a good vacuum and thus at a low temperature is essential as this will minimize the sulfuric acid formation on the crystals.

The yield is about 80%. The effect is illustrated by the blank obtained in the standard procedure: 0.178: mole for the original reagent, 0.020: mole after one recrystallization and 0.010: mole of N after two recrystallizations.

From:
Total phosphorous and total nitrogen, North Temperate Lakes Long Term Ecological Research. http://www.limnology.wisc.edu/totalp_totaln05.shtml

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EPA MN 00075
MDH 027-137-248

Standard Operating Procedure

No. 111, Rev 3
Effective Date: 12/1/2012

[Nitrate + Nitrite]–N by Autoanalyzer (SM 4500 NO_3^- F)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification
3	Elaine M Ruzycki	11/9/2012	Updated to a controlled document

Reviewed by:

______________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer

_______________________________________Date__________________
Richard Axler
Director Central Analytical Laboratory

General

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone also can be determined by removing the cadmium column.

Matrix: Surface water, wastewater
Range: Low level: 0.010 - 2.0 mg/L NO_3^- or NO_2^-
Mid level: 0.020 - 10.0 mg/L NO_3^- or NO_2^-
High level: 1.0 - 50.0 mg/L NO_3^- or NO_2^-
Throughput: Approximately 55 samples/h; 65 s/sample depending on range

Interferences

Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is soluble, the sample may be pre-filtered.

Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

Sample Handling and Preservation: Nitrite will be oxidized by air O_2 to nitrate in a few days. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4EC. When samples must be stored for more than 24 hours, they should be frozen or preserved with sulfuric acid (2 mL conc. H_2SO_4 per liter) and refrigerated. **CAUTION:** Samples must not be preserved with mercuric chloride or thiosulfate because this will degrade the cadmium column.

Because of the buffer used in this method, sulfuric acid - preserved samples do not have to be neutralized before determination. See Table I-2.

Apparatus

Lachat QuikChem 8000Automated Flow Injection Ion Analyzer which includes:

1. Random access sampler (RAS)
2. Proportioning Pump tubes will vary with range. See Table IV.1.20-1
3. Injection valve with loop. Size varies with range (see Table IV.1.20-1)
4. Colorimeter
   a. Interference Filter: 520 nm
   b. Flow Cell: 10 mm, 80 uL
5. Reaction Module 10-107-04-1-C with Cd column
6. Omnion Software System

Reagents

Reagent 1 Ammonium Chloride buffer, pH 8.5

While a liter of reagent can be made up with 85g of ACS grade ammonium chloride and 1.0 g disodium EDTA, lower backgrounds have been found using HCl and NH_4OH.

	per Liter
Water (MQW)	800 mL (800 g)
HCl, Concentrated	105 mL (126 g)
NH_4OH	95 mL (85 g)
Disodium ethylenediamine tetraacetic acid (EDTA)	1.0 g

In a hood, add components carefully to a beaker in the order listed. Caution! May produce strong vapors. Stir until dissolved. Check the pH with a pH meter and adjust the pH to 8.5 with 15 M sodium hydroxide or HCl if necessary.

> **15 M Sodium hydroxide** **[*C]**: Add 150 g NaOH slowly to 250 mL of water. CAUTION: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle. Used to adjust pH of buffer.

Reagent 2. Sulfanilamide color reagent.

	per Liter
Water (MQW)	876 mL (876 g)
Phosphoric Acid	100 mL (170 g)
Sulfanilamide	40 g
N-(1-naphthyl)ethylenediamine dihydrochloride	1.0 g

Add components in order to a dark 1 liter container. Shake until wetted and stir with stir bar for 20 min. This solution is stable for one month.

Cadmium-Copper Reduction Column: Columns are purchased from Latchat

Standard Stocks

50 ppm nitrate-N stock: Dry reagent grade potassium nitrate for 2 hours at 105°C. Dissolve 0.361 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW. Add 2 mL chloroform as a preservative. Store in refrigerator. This solution is stable for 6 months. New stock should be compared against the old stock before it is discarded.

500 ppm nitrate-N stock: Dry reagent grade potassium nitrate for 2 hours at 105°C. Dissolve 3.609 g in ~200 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW. Add 2 mL chloroform as a preservative. Store in refrigerator. This solution is stable for 6 months. New stock should be compared against the old stock before it is discarded.

50 ppm nitrite-N stock: Dry reagent grade sodium nitrite in a desiccator. Dissolve 0.2464 g in about 50 mL MQW in a 1000 mL volumetric flask. Dilute to the mark with MQW. Refrigerate. This stock is diluted for nitrite standards or for a cadmium column efficiency check. This solution is stable for about 1 month. New stock should be compared against the old stock before it is discarded.

500 ppm nitrite-N stock: Dry reagent grade sodium nitrite in a desiccator. Dissolve 0.616 g in about 50 mL MQW in a 250 mL volumetric flask. Dilute to the mark with MQW. Refrigerate. This stock is diluted for nitrite standards or for cadmium column efficiency checks. This solution is stable for about 1 month. New stock should be compared against the old stock before it is discarded.

Working standards

See range specific parameters below.

System Operation

1. Inspect system to ensure that all connections to the reaction module are correct. Check that the correct pump tubes and sample loop are installed for the range in use. Check that the correct interference filter (520 nm) is installed in the colorimeter head. Check that the column valve is turned so the column is off-line.
2. Turn on power to all modules.
3. Place reagent feedlines into proper containers. Raise tension levers on pump tube cassettes.
4. Pump system until all tubing is filled, then put column on line.
5. Place calibration standards and blank in sample tray in descending order of concentration followed by QCCS, unknowns and check standards. Be sure that samples are being placed in the locations designated in the tray table cup number. Cup numbers specified may not be sequential.
6. At end of run turn the column valve so it is off line, then place all feedlines in deionized water, flush system and pump dry.
7. Turn off pump, all modules, and release pump tube cassettes.
8. See Lachat manual for additional system operations.

Cadmium column efficiency check:

1. Before every run, visually check the cadmium column for gaps in the packing or air bubbles. If air bubbles are present, try to remove them by disconnecting the outlet end of the column and pump buffer through it at maximum pump speed while taping the column carefully but firmly with screwdriver handle. Work up the column until all of the bubbles have been flushed out. If this fails, the column must be repacked with copperized cadmium.

2. To check column efficiency, calibrate with nitrate standards. Then run a nitrate and a nitrite standard of the same concentration. Column efficiency is calibrated by:

$$E_{column} = \frac{C_{NO_3-N}}{C_{NO_2-N}} \times 100$$

Where: E_{column} = *% efficiency of the column*

C_{NO3-N} = *concentration of the NO_3-N standard*

C_{NO2-N} = *concentration of the NO_3-N standard*

If the efficiency is < 90%, the column should be repacked with fresh copperized cadmium.

QA/QC

For less than 10 samples run two duplicates, two spikes, two midpoint check standards and two QC samples. See QA/QC program and Table I-4.

Waste Disposal

Cadmium reduction produces three primary waste streams that should be collected and disposed of separately.

1. Cadmium metal: Copperized cadmium should be collected in a plastic container. Normally it will have buffer or copperizing solution with it. The solution should be decanted into the appropriate waste container.

2. Copperizing or cadmium column rinse solution: Collect solution from copperizing cadmium in a wide mouth plastic bottle. The label should read ApH # 2" and:

DDC #	CAS #	Cadmium Column Rinse Solution	
18PI	12125029A	Ammonium chloride	0.08 - 0.85%
02LI	7647010A	Hydrochloric Acid	1.00 - 4.00%
18PI	7758998A	Copper Sulfate	0.02 - 2.00%
08FB	67641A	Acetone	0.50 - 1.00%
05SO	60004B	Sodium EDTA	0.02 -0.10%
00	7732185A	Water	Balance

1. Mixed reagent waste from the Lachat and outdated reagents for the analysis can be combined in a 1-gal wide mouth plastic container. Often several gallons are accumulated

and transferred to a 5-gallon plastic pail for shipment. Containers should be labeled with ApH # 2" and:

DOC #	CAS #	NO_3-N Analysis Waste	Conc.
02 IA	76470	Hydrochloric Acid	2.70%
02 IA	76643	Phosphoric Acid	2.50%
18 BP	63741	Sulfanilamide	1.20%
02	13107	Sodium Hydroxide	0.24%
02 IA	12125	Ammonium Chloride	0.21%
10 BP	14652	N-1-	0.02%
18 BP	63819	EDTA	0.02%
00	77321	Water	Balanc

Working Standards.

Table IV.1.20-2. Working standards for specific Nitrate-Nitrite method ranges.

Method file	HIGHNO3.MET	NO3MIDJA.MET	NO3LOWJA.MET	
QuickChem method	10-107-04-1-F	10-107-04-1-O	10-107-04-1-C	
Standard	mg N/L	mg N/L	mg N/L	
1	50	10.0	2.00	
2	25	5.0	1.00	
3	10	2.0	0.50	
4	2.5	1.0	0.10	
5	1.0	0.5	0.05	
6	0	0.1	0.02	
7		0.05	0.01	
8		0.02	0.00	
9		0.00		

Table IV.1.20-3. Dilution scheme for Nitrate-nitrite and nitrite working standards.

Standard conc. mg N/L	Volume Stock (mL)	Stock Conc. (mg N/L)	Final Volume (mL)
50.0	10.00	500	100
25.0	5.00	500	100
10.0[1,2]	2.000	500	100
5.0	10.00 1.000	50 500	100
2.5	5.00 0.500	50 500	100
2.00	4.000	50	100
1.00[1,2]	4.000	50	200
0.50[1]	1.000	50	100
0.10	0.200	50	100
0.05	0.100	50	100
0.02	0.040	50	100
0.01	0.020	50	100
0.00	B	B	B

[1] Check standard - A standard placed every 10th position in the sampler to check for analytical drift.
[2] Nitrite standard concentration for column efficiency check

Working standards for total N will be supplied as digests.

If samples fall within a narrower range, more standards within this smaller range can be added and standards outside this range can be dropped.

References

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes. Method 353.2. EPA 600 4-79-020, Revised 1983.

Methods for Determination of Inorganic Substances in Water and Fluvial Sediments, Book 5, Chapter A1, U.S. Department of the Interior, U.S. Geological Survey

[c]LACHAT INSTRUMENTS - Method 10-107-04-01-B and C

Revised

C.J. Owen, 02/93; A. Bamford and J.J. Ameel, 07/98

Hazards

***C See Hazard section under Safety-QAM**
***T See Hazard section under Safety-QAM.**

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Standard Operating Procedure

No. 112, Rev 2
Effective Date: 9/1/2011

Low Level [NH_4]–N by FIA (Salicylate method, I-2522-90; USGS 1993)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
2	Elaine M Ruzycki	9/27/2011	Revised and reformatted for laboratory recertification

Reviewed by:

_________________________________ Date_______________
Elaine M. Ruzycki
Laboratory Manager/QA Officer

_________________________________ Date_______________
Richard Axler
Director Central Analytical Laboratory

General

When ammonium is heated with salicylate and hypochlorite in an alkaline phosphate buffer. An emerald green color is produced which is proportional to the ammonium concentration. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The color is intensified by adding sodium nitroprusside.

Matrix: Surface water, wastewater
Range: Low level: 5 to 300 µg N/L
Throughput: 60 samples/h; 60 s/sample

Sample Preservation

Samples should be run within 24 hours. Ammonia is volatile and will leave the sample slowly, even through polyethylene bottles. If samples cannot be run within 24 hours, they should be frozen. Alternatively, sample pH can be adjusted to < 2 with sulfuric acid and stored at 4°C. Filtered samples preserved in this manner can also be used for DOC. See Table I-2.

Apparatus

Lachat QuikChem 8000 Flow Injection analyzer including:

Chemistry Module 10-107-06-2-B
Sample loop: 150 cm x 0.8 mm i.d.
Flow cell: 10 mm
Interference filter, 660 nm
Heating coil: 650 cm x 0.8 mm i.d. at 60C
Omnion Software

Reagents

Important! Degassing with Helium: To prevent bubble formation in manifold tubing and flow cell, all reagents, carrier and rinse water should be degassed by bubbling vigorously with helium (20 lb/in2) through a fritted gas dispersion tube for one minute. Use dispersion tubes reserved for ammonia carrier and reagents. Degassing with general use tubes will result in unacceptable backgrounds.

Reagent 1. Buffer

	for 500 mL	for 1000 mL
1. Sodium hydroxide	15.0 g	30.0 g
2. Ethylenediaminetetraacetic acid, disodium salt dihydrate (EDTA)	12.5 g	25 g
3. Sodium phosphate, dibasic heptahydrate	33.5 g	67 g
4. Water (MQW)	483 mL	965 mL

Weigh the chemicals into a tared container, add water and mix until everything has dissolved. Use rate with a white/orange pump tube is 0.76 mL/min so 500 mL is generally enough buffer. Degas before use.

Reagent 2. Salicylate - Nitroprusside Color Reagent:

	for 500 mL	for 1000 mL
1. Sodium salicylate	72 g	144.0 g
2. Sodium nitroprusside (sodium nitroferricyanide dihydrate) [*T]	1.75 g	3.5 g
3. Water (MQW)	454 mL	908 mL

Weigh the chemicals into a tared, light-proof container, add water and mix until everything has dissolved. Alternatively, because sodium salicylate is fluffy and difficult to transfer to a narrow mouth container, prepare the reagent in a beaker and transfer it to the light-proof container once it has been mixed. Degas before use even if it has been stored for several days. Prepare fresh weekly. Use rate is 1.75 mL/min with a red pump tube when the pump is operated at 35.

Reagent 3. Hypochlorite Reagent:

	for 500 mL	for 1000 mL
1. Sodium hypochlorite, 5.25% (Clorox Bleach)	30 mL	60 mL
2. Water (MQW)	470 mL	940 mL

Mix regular Clorox Bleach (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and water, and degas with helium before use. Make up fresh reagent daily. This reagent can be discarded by sewering. Use rate is 1.28 mL/min with an orange pump tube.

Standards

Stock Standard, 25 mg/L NH4 +- N: In a 1 L volumetric flask dissolve 0.0955 g of NH4Cl that has been oven dried for 2 hours at 110°C. Dilute to mark with ammonia free MQW and invert to mix.

Spiking standard, 25 mg/L NH4 +- N: A separate spiking standard can be made up as above. For a 50ppb spike, add 0.01 mL of 25 mg/L standard to a 5 mL aliquot of sample.

Working standards: Range 0-300 µg N/L

Standard	Concentration	Vol. of Stock	Final Volume
1	300 µg N/L	3.00 mL	250 mL
2	200 µg N/L	2.00 mL	250 mL
3	100 µg N/L	1.00 mL	250 mL
4	50 µg N/L	0.50 mL	250 mL
5	20 µg N/L	0.200 mL	250 mL

6	10 µg N/L	0.100 mL	250 mL
7	5 µg N/L	0.050 mL	250 mL
8	0 µg N/L	0.000 mL	-----

Data system parameters for QuickChem 8000

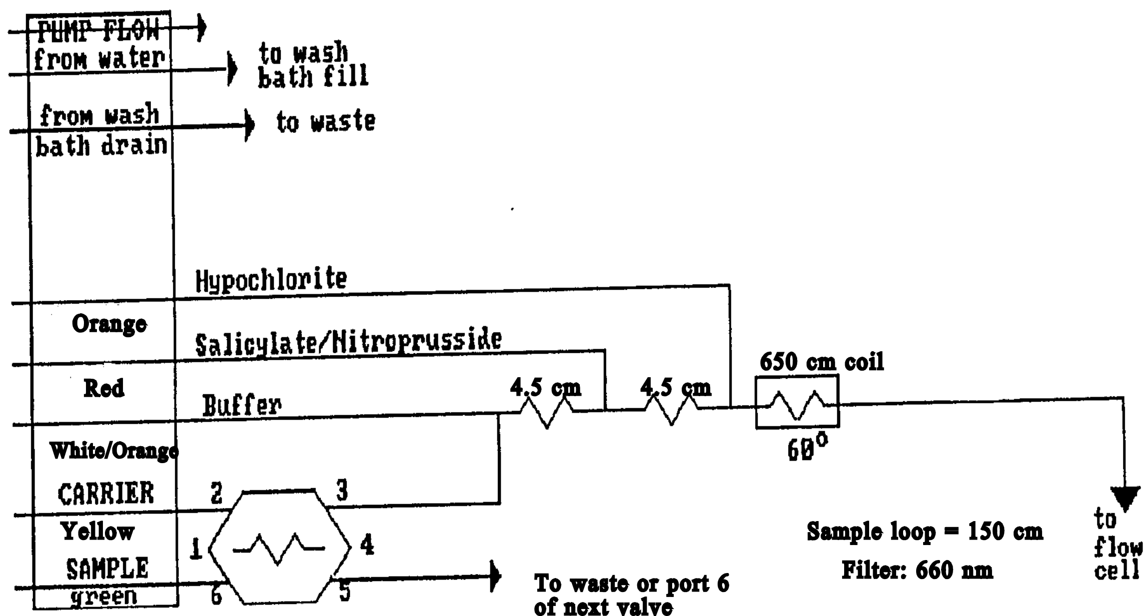
Timings are approximate and must be optimized.
Sample throughput: 60 samples/hour, 60 s/sample
Pump speed: 35
Cycle period: 60 s

Analyte data:
Concentration units: µg/L
Peak base width: 50 s
% width tolerance: 100
Threshold: 1000
Inject to peak start: 21 s
Chemistry: Direct

Calibration data:
Levels: See A working standards@ section
Calibration fit type: 1st order polynomial
Calibration rep. Handling: Average
Weighting method: None
Concentration scaling: None
Force through zero: No

Sampler timing:
Min. probe in wash period: 10 s
Probe in sample period: 24 s

Valve timing:
Load time: 0.0 s
Load period: 15 s
Inject period: 45 s



Manifold:Carrier is degassed MQW.
Manifold tubing is 0.8 mm i.d.
4.5 cm = 70 cm of 0.8 mm tubing on a 4.5 cm coil support.

Note: Use of a 100 cm x 0.5 mm ID back pressure loop after the flow cell is recommended.

System Operation

1. Inspect system to ensure that all connections to the reaction module are correct.
2. Turn on power to all modules. Set heater to 60C and allow it to warm up for at least 15 min.
3. Place reagent feedlines into proper containers. Raise tension levers on pump tube cassettes.
4. Pump system until a stable baseline is attained. Note: Never run the pump on Astand-by@ when running ammonia. Reagents may precipitate out at lower pump speeds and plug the heated coil.
5. Place calibration standards and blank in sample tray in descending order of concentration followed by QCCS, unknowns and check standards. Be sure that samples are being placed in the locations designated in the tray table cup number. Cup numbers specified may not be sequential.
6. At end of run, set heater control to 0°C, place all feedlines in deionized water, flush system and pump dry.
7. Turn off pump, all modules, and release pump tube cassettes.
8. See Lachat manual for system operations.

QA/QC

For less than 10 samples run two duplicates, two spikes, two midpoint check standards and two QC samples.

See QA/QC program and Table I-4.

Waste Disposal

Standard practice has been to collect several gallons of mixed salicylate waste in labeled plastic or glass 1-gallon containers and transfer them to a 5-gallon pail for transport and disposal through the University hazardous waste program. The container should be stored with other corrosive high pH wastes and labeled with A pH > 11 and:

DD C#	CAS #	NH4-N Analysis Waste, Salicylate method	Conc.
05	54217	Sodium Salicylate	3.7-
05	77828	Sodium Phosphate,	0.8-
05	63819	EDTA, di-sodium	0.3-
01	13107	Sodium Hydroxide	0.3-
01	76815	Sodium Hypochlorite	< 0.3%
18	14402	Sodium Nitroprusside	< 0.4%
Non	77321	Water	Balance

Never mix this waste with low pH wastes. Acidic conditions may release highly toxic hydrogen cyanide from sodium nitroprusside. Excess reagents 1 and 2 should be combined with the mixed

waste for disposal. Reagent 3 (diluted Clorox bleach) can be sewered.

Hazards

*C See Hazard section under Safety-QA/QC.
*T See Hazard section under Safety-QA/QC.

Reference

M.J. Fishman, 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory--Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 351.2. EPA-600/4-79-020, Revised March 1983.

[c]LACHAT INSTRUMENTS Method 10-107-06-2-A.

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EPA MN 00075
MDH 027-137-248

Standard Operating Procedure

No. 113, Rev 1
Effective Date: 4/15/2013

CHLOROPHYLL AND PHAEOPHYTIN DETERMINATION (SM 10200-H)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
	Elaine M Ruzycki	4/15/2013	Revised and reformatted for manual update; added safety information

Reviewed by:

_______________________________________ Date_________________
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Laboratory Manager/QA Officer
_______________________________________Date_________________
Richard Axler
Director Central Analytical Laboratory

General

Estimating the concentration of chlorophyll-*a* remains the most common method for assessing algal biomass. The concentration of chlorophyll-*a* has also been shown to relate to primary productivity (Wetzel 1983) and can be used to assess the physiological health of algae by examining its degradation product, phaeophytin. This degradation product has been shown to contribute 16-60% of

the chlorophyll-*a* content in seawater and freshwater. Despite numerous innovations such as HPLC, and dozens of conflicting reports regarding extraction and analytical protocols, most state resource and regulatory agencies still appear to rely on, and in fact require, methods listed in Standard Methods (1992) and in some cases EPA (1987). Therefore, spectrophotometry and fluorometry, utilizing 90% acetone extraction, remain the most commonly used methods. Spectrophotometry is most widely used but fluorometry is more sensitive and should be used when low levels of chlorophyll are anticipated.

Apparatus - Extraction

1. Prewashed 47 mm glass fiber fillers (GF/C or AE)
2. Gelman polycarbonate filtration tower
3. Vacuum pump
4. Centrifuge
5. DIW/acetone washed 15 mL Corex centrifuge tubes with caps

Apparatus - Spectrophotometry

1. UV/VIS spectrophotometer (1 nm spectral band width)
2. Optically matched 4 cm micro cuvettes

Reagents

1. **90% acetone [*T]:** (900 ml acetone + 100 mls MQW (MQW) + 0.1 ml conc. **NH_4OH [*C]**
2. Chlorophyll-*a* stock (10,000 ppb) Chlorophyll-*a* from *Anacystis nidulans* (Sigma chemical C6144) (see Working Standards)
3. 0.12 N HCl: (1.92 mL concentrated HCl + 200 mL MQW)
4. Saturated $MgCO_3$ solution: (1.0 g $MgCO_3$ + 100 mL MQW)

Prepare acetone reagent in a fume hood.
PPE: eye protection, gloves and lab coat.

Filtration Procedure

1. Concentrate chlorophyll by filtration as soon as possible after collection (< 24 hours). Filter an appropriate volume of water (depending on the specified analytical procedure and the estimated chlorophyll concentration) through a pre-washed 47mm glass fiber filter (Whatman GF/C or Gelman AE), using Gelman polycarbonate filtration towers, under low to moderate vacuum.
2. Add 0.15mL of a saturated $MgCO_3$ solution to the sample just prior to the end of filtration. The $MgCO_3$ acts to buffer the sample preventing premature phaeophytinization.
3. Fold the moist filter into quarters, wrap in aluminum foil and store frozen at -10EC.
4. Record volume of water filtered.

Extraction Procedure

Under subdued light.
1. Tear filters into quarters with forceps.

2. Place filter in a 15 ml centrifuge tube.
3. Add 10 mL 90% acetone.
4. Cap and extract overnight in the dark at 4° C.

Working Standards

*Make up stock and working standards under subdued light conditions. Keep stock solution in dark at all times.

Stock chlorophyll-*a* solution (10,000 ppb): Place 1 mg of purified chlorophyll-*a* in 100 mL of 90% acetone (make stock solution at least 24 hours prior to analysis).

400 ppb chlorophyll-*a* solution: Add 2.0 mL of stock solution to 50 mL of 90% acetone.

200 ppb chlorophyll-*a* solution: Add 1.0 mL of stock solution to 50 mL volumetric flask. Dilute to mark with 90% acetone.

Spectrophotometric Determination of Chlorophyll-*a* and Phaeophytin

Chlorophyll-*a* and phaeophytin concentrations in the extract are determined spectrophotometrically using the standard method for chlorophyll-*a* in the presence of phaeophytin (as detailed in Standard Methods 1992). A Perkin-Elmer Lambda 3B UV/VIS spectrophotometer with a 1 nm spectral band width and optically matched 4 cm micro-cuvettes are used.

1. Measure absorbance of a 90% acetone solution blank at 750 nm and at 664 nm to correct for primary pigment absorbance.
2. To measure phaeophytin, acidify the 4 mL of solution in the cuvette to a final molarity of 3 x 10^{-3} by adding 100 ul of 0.12N HCl to the 4 mL of extract in the cuvette. Allow acid to react for 60 seconds and record the absorbance at 665 nm and again at 750 nm. This is a correction made for the 665 nm wavelength (for phaeophytin).
3. Run working standard solutions of purified chlorophyll-*a* (Sigma Chemical Co. *Anacystis nidulans* by the procedure used for the blank).

Clarify sample filter extracts by centrifuging at 4,000 rpm for 20 minutes. Then read sample and standard
absorbances at
750 nm and 664
nm before
acidification (750_b and 664_b) and 750 nm and 665 nm after acidification (750_a and 665_a). Chlorophyll-*a* and phaeophytin concentrations are calculated by the following equations from Standard Methods (1992) :

Chlorophyll *a* (µg/L) =

$$\frac{26.7\ [E_{664b} - E_{665a}] * V_{ext}}{V_{sample} * L}$$

And

Phaeophytin (µg/L) =

$$\frac{26.7\,[1.7E_{665b} - E_{664b}] * V_{ext}}{V_{sample} * L}$$

where:

b = before acidification

a = after acidification

E_{664b} - [{$Abs_{664b(sample)}$-$Abs_{664b(blank)}$}-{$Abs_{750b(sample)}$-$Abs_{750b(blank)}$}]

E_{665a} - [{$A_{665a(sample)}$-$Abs_{665a(blank)}$}-{$Abs_{750a(sample)}$-$Abs_{750a(blank)}$}]

V_{ext} = Volume of 90% Acetone used in the extraction (mL)

V_{sample} = Volume of water filtered (L)

L = Cuvette path length (cm)

QA/QC

Verify that acid factors and correction factors are in range when running samples.

Safety

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

***T** Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute

or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

Waste Disposal

Waste cannot be sewered. All waste is to be poured into labeled hazardous waste bottle located in Room 413. Waste bottle must be capped at all times; solvent should not be allowed to evaporate up the fume hood. Waste is not to be stored for longer than 90 days

References

Standard methods for the examination of water and wastewater. 1992. 18th edition. American Public Health Association, Washington, D.C.

Axler, R. C. and C. J. Owen. 1994. Measuring chlorophyll and phaeophytin: whom should you believe? Lake and Reservoir Management 8:143-151.

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EPA. 1987. Handbook of methods for acid deposition studies: Laboratory analysis for surface water chemistry. EPA 600/4-87/026, September 1987, U.S. Environ. Protec. Agency, Washington, DC.

Wetzel, R.G. 1983. Limnology. 2nd Edition. Saunders College Publishing, Philadelphia, PA.

Revised

E. Ruzycki, 03/94; 07/98; 11/10, J.Ameel, 4/03

Hazards

***T See Hazard section under Safety-QA/QC.**

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www.nrri.umn.edu/cwe/analyticallab.htm

EPA MN 00075
MDH 027-137-248

Standard Operating Procedure

No. 114, Rev 1
Effective Date: 4/1/2014

Dissolved oxygen, specific conductivity, pH and temperature

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
1	Elaine M Ruzycki	4/1/2014	Developed for laboratory certification

Reviewed by:

______________________________________ Date_________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
_______________________________________Date__________________
Richard Axler
Director Central Analytical Laboratory

Summary

Water quality multiprobes (Hydrolab MiniSondes and YSI sondes) are used by the NRRI CAL to measure pH, dissolved oxygen (DO in mg/L), DO percent saturation, specific conductivity (EC @ 25°C uS/cm), temperature (°C), and depth (m) in lakes and streams. They can be used for short-term spot-sampling as the user moves from site to site throughout the course of a day, depth profiling, or long-term unattended monitoring at specified time intervals.

Methods

Dissolved oxygen	SM 4500 O-G 2001
Specific conductivity	SM 2510 B 1997
pH	SM 4500 H+ B 2000
temperature	USGS Water Temp 1975

Equipment, Reagents, and Supplies

1. pH standard (a.k.a. buffer) solutions: pH 4, 7, and 10
2. Conductivity standard solution (400 µS/cm standard: Dissolve 0.8284 g KCl in 4L MQW)
3. Tap and MQW water
4. MiniSonde® or YSI sonde
5. Surveyor (hand-held unit) and 5 meter cable or calibration cable/computer with Hydras 3LT or Hyperterminal/battery
6. Profiling communication cable
7. Sonde calibration cup, other communication cables, charger, probe/sensor protection cage
8. Hydrolab/YSI manuals

Calibration Procedure

(This is specifically for the Hydrolab sondes. YSI calibration uses the same standards but refer to manual for specific instructions).

1. Attach the power and data cable to the Sonde. Attach the 9-pin connector to a PC.

2. Start Hydras 3 LT. Wait for the software to scan for connected Sondes. Highlight the

multiprobe and press **OPERATE SONDE**. **Note:** If the Sonde appears to be connected and the software does not recognize the Sonde connection, remove and replace the connector cable and press RE-SCAN FOR SONDE. Repeat until Hydras 3 LT recognizes the Sonde.

3. Click on the Calibration Tab and click on the parameter to be calibrated.
4. Enter the calibration values and click **CALIBRATE**.

Calibration Preparation

The following is a general outline of the steps required to calibrate all the sensors.

- Select a calibration standard whose value is near the field samples.
- Clean and prepare the sensors.
- To ensure accuracy of calibration, discard used calibration standards appropriately.

1. Rinse sensors with tap water.
2. Pre-rinse sensors with the appropriate calibration standard
3. Attach the calibration cup.
4. Fill the cup with calibration standard
5. Shake the sonde to remove air bubbles

TEMPERATURE

The temperature sensor is factory-set and does not require recalibration. Temperature should be checked against a NIST thermometer annually.

SPECIFIC CONDUCTIVITY (EC @ 25 ^{o}C) Calibration

1. Pour the specific conductance standard (400 µS/cm) to within a centimeter of the top of the calibration cup.
2. Make sure there are no bubbles in the measurement cell of the specific conductance sensor.
3. Enter the SpCond standard for µS/cm using Hydras 3 LT software or a Surveyor.

pH

A two point calibration is most common. Cover all sensors with the pH standard. Always start with pH 7 standard, then 4 or 10; bracket expected field readings. If you're doing a three point calibration, start with pH 7 standard, then 10, then 4.

DISSOLVED OXYGEN (LDO)

The LDO sensor can be calibrated using water saturated air. Use this method when water saturated air is applied to the sensor.

1. Click the Calibration tab and select the LDO [SAT] tab.

2. Place the calibration cup with one end sealed so that the calibration cup opening is facing upwards. The sonde will be inserted down wards into this cup
3. Fill the calibration cup with approximately ½ inches of tap water (specific conductance less than 0.5 mS/cm) Water will not touch the top of the sensor cap.
4. Carefully remove any water droplets from the sensor cap and temperature probe with the corner of a tissue or clean cotton cloth. It is important that no evaporative cooling take place either on the sensor cap or the temperature probe during calibration.

Note:
It is important to maintain temperature stability during calibration. Care should be taken to keep the sonde out of direct sunlight or away from any other energy/heat source which will cause the temperature in the calibration cup to change during calibration. A reflective sun-shield is recommended if no natural shade is available. If the temperature in the calibration cup changes more than 0.5 °C during the calibration, it is recommended to recalibrate the sensor.

5. Gently set the sonde with sensors down into the calibration cup blocking any air exchange with the outside environment. Do not screw the calibration cup fully onto the sonde body as the compression of the o-ring will increase the pressure inside the calibration cup to above the barometric pressure and give a false 100% saturated reading. The goal is to block air exchange between the sealed calibration cup and the outside

6. Allow the dissolved oxygen and temperature readings to stabilize. As the temperature sensor has a smaller thermal mass than the luminescent dissolved oxygen sensor, it is best to allow the entire unit to stabilize for an additional 3–5 minutes after the temperature sensor stabilizes. At this point, the air inside the calibration cup should be fully saturated with water, hence the name "water saturated air."

7. Determine the barometric pressure for entry as the calibration standard.

8. Enter the barometric pressure in the field provided.

If using the local weather bureau BP, remember these numbers are corrected to sea level. To calculate the uncorrected atmospheric pressure BP', use on the following equations:

$BP' = BP - 2.5(Aft/100)$ or $BP' = BP - 2.5(Am/30.5)$
where:
BP' = Barometric Pressure at altitude
BP = Barometric Pressure at sea level
Aft = Altitude in feet
Am = Altitude in meters

Local barometric pressure in mbar (*BPmbar*) can be converted to local barometric pressure in mmHG (*BPmmHg*) using: $BPmmHG = 0.75 \times BPmbar$

DEPTH

Make sure nothing is blocking the depth port. Rinse out if necessary. Do not put anything down the port, the membrane damages easily. Put on the weighted sensor guard and turn the sonde upside down. Calibrate to zero. For more accurate results, calibrate in the field just prior to use.

SAFETY

- Conductivity and pH standards and D.O. and pH reference solutions are nontoxic, but can irritate eyes and other sensitive areas because of their high salt content.
- Wash hands thoroughly after calibration or after use in contaminated waters.
- When using a sonde in the field, be aware of your surroundings. Select an area in which you feel safe and secure from hazards.

QA/QC

- Record all calibration results on sonde-specific calibration sheets (appendix a).
- Sondes should be calibrated before each use and post-calibrated using standards afterward. A post-calibration is really a "post-check" since it is not necessary to recalibrate the sonde unless you're using it afterward.
- When traveling from site to site, make sure the sonde sensors are kept moist by replacing probe guard with calibration/storage cup filled with pH 4 buffer. If pH buffer is unavailable use tap or surface water.
- Although Hydrolab equipment is robust and made for heavy field use, it should be handled carefully at all times.
- Further quality control and quality assurance procedures will be addressed thoroughly on a project-by-project basis in the Quality Assurance Project Plan (QAPP) for each project.

Reference

Hydrolab DS5X, DS5, and MS5 water quality multiprobes User Manual. 2006. HACH Co, USA.

Appendix A. Sonde Calibration Record

Date: ____________
Meter Model and Serial #: ____________
Meter Purchased: ____________
Battery Voltage: ____________
Technician: ____________

Conductivity

Calibration Standard: ____________
Date purchased: ____________
Expiration date: ____________
Last calibration date:

Calibration results

Conductivity: ____________
Temperature: ____________
K:

Dissolved Oxygen

Last calibration date:

Calibration results

Percent saturation: ____________
DO: ____________
Temperature: ____________
Slope: ____________
Offset: ____________
Pressure (hPa)

Replacement Parts	**Date**
LDO sensor cap	____________
LDO iButton	____________

Notes:

pH

Calibration Standard (7):

Date purchased: ____________
Expiration date: ____________
Last calibration date: ____________
pH: ____________
Temperature: ____________
milliVolts (0 ±30 mV): ____________

Calibration Standard (4):

Date purchased: ____________
Expiration date: ____________
Last calibration date: ____________
pH: ____________
Temperature: ____________
milliVolts (180 ±30 mV): ____________
mV difference of pH 4-7 ____________
Span between should be about 165 -180 mV

Calibration Standard (10):

Date purchased: ____________
Expiration date: ____________
Last calibration date: ____________
pH: ____________
Temperature: ____________
milliVolts (-180 ±30 mV): ____________
mV difference of pH 7-10 ____________
Span between should be about 165 -180 mV

Slope mV/pH: (should be close to -58.0) ____________
Slope %: (about 98 to 103% at 25 °C) ____________
Offset mV: (should be close to 0.00) ____________
r^2: (should be close to 1.000) ____________

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Standard Operating Procedure

No. 115, Rev 1
Effective Date: 12/1/2014

Sulfate Methylthymol Blue Flow Injection Analysis (Low Method: 0-40ppm) (EPA Method 375.2)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
1	Elaine M Ruzycki	11/11/2014	Developed and formatted for laboratory certification

Reviewed by:

______________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
______________________________________Date_________________
Richard Axler
Director Central Analytical Laboratory

General
The Sulfate Methylthymol Blue (MTB) Flow Injection Analysis Method uses interactions between barium, sulfate, and MTB to estimate sulfate concentrations. At pH 13.0, barium complexes with MTB, creating a dark blue baseline. When the sample is injected into a known concentration of sulfate (the carrier) the sulfate displaces the MTB from the ethanolic barium-MTB. The displaced MTB has a grayish color. The NaOH reagent raises the pH and the intensity of the gray color (which is proportional to the sulfate concentration) is measured at 460 nm.
Interferences

1. Multivalent cations: Cations such as Ca2+ and Mg2+ are removed by the exchange column. A midrange standard with a known hardness (CaCO3) is used to check the column efficiency at removing the multivalent cations.
2. Strongly acidic samples: Highly acidic samples (pH<2) can displace multivalent cations from the column.
3. Turbidity: Turbid samples can be filtered or centrifuged.
4. Orthophosphate: At high pH barium forms a precipitate with orthophosphate. A recovery study should be done if samples are known to be high in orthophosphate.

Sample Collection

Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleansed and rinsed with reagent water (see QAM Table 3-major anions). Volume collected, approximately 125 mLs, should be sufficient to insure a representative sample, allow for replicate analysis (if required).

No chemical preservation required. Cool sample to 4°C.

Samples should be analyzed as soon as possible after collection. If storage is required, samples maintained at 4°C may be held for up to 28 days.

Apparatus
Lachat QuikChem Automated Flow Injection Ion Analyzer which includes:

1. Automatic Sampler
2. Proportioning Pump
3. Injection Module with a 100 cm X 0.8 mm i.d. sample loop
4. 200 cm restrictor coil
5. Colorimeter
   a. Interference Filter : 460 nm
   b. Flow Cell, 10 mm, 80 µl
6. Reaction Module 10-116-10-2-E and 10-116-10-2-E
7. QuickCalcII Software System

Reagents
(From QuikChem Method 10-116-10-2-E)
Carrier, 0.30 mg SO42-/L: Add 0.30 mL of 1000 mg/L stock sulfate solution (Standard 2) to a 1 L volumetric flask and dilute to the mark with water. Prior to the analysis degas with helium.
Barium Chloride Solution: Dissolve 1.526 g barium chloride dehydrate **[*T]** ($BaCl_2 \cdot 2H_2O$ in 995 ml water. Mix and Degas solution with helium prior to analysis.
Hydrochloric Acid: Mix 83 mL concentrated HCl **[*C]** to 917 mL water in a 1 L Pyrex container. Use the fume hood to avoid inhaling fumes. Degas with helium.
Barium- MTB Color Reagent: Measure 0.236 g methylthymol blue (3,3'-bis[N,N-di(carboxymethyl)aminomethyl]-thymol-sulfonephthalein,pentasodium salt, Lachet part number 50243) into a 500 mL volumetric flask. Add 50 mL of the Barium Chloride Solution reagent. Mix well before adding 4.0 mL of the Hydrochloric Acid reagent (will turn

the solution orange). Add 71 mL of water and mix well. Fill to the line with ethanol (specially denatured anhydrous alcohol, Aldrich 24,511-9). Make this solution at least one day before analysis and refrigerate. Prior to analysis warm solution to room temperature and degas with helium.
Sodium Hydroxide Solution: In a 1 L volumetric flask mix 7.2 g sodium hydroxide **[*C]** (NaOH) with 900 ml water and then dilute to the mark. The mixing will release heat. Store solution in a plastic bottle. Degas with helium prior to analysis.
Buffered EDTA: This solution is used for cleaning the manifold after sample analyses. In a 1 L volumetric flask add 6.75 g ammonium chloride (NH4Cl) to 500 mL water. Under a fume hood add 57 ml concentrated ammonium hydroxide (NH4OH), followed by 40 g tetrasodium EDTA dehydrate. Mix well and then dilute to the mark with water.

Standards

10000 ppm SO_4^{2-} stock: Dry anhydrous sodium sulfate (Na2SO4) at 105ºC for one hour. Add 1.479 g Na2SO4 to a 100 mL volumetric flask and dilute to the mark with water.
1000 ppm SO_4^{2-} stock: Perform a 1:10 dilution of the 10000 ppm SO_4^{2}stock solution by pipetting 10 mL of 10000 ppm SO_4^{2-} stock into a 1000 mL volumetric flask.
Working Standards

Standard concentration (ppm)	Volume of 10000 ppm stock (mL)	Volumetric flask size (mL)
0	-	-
2	0.1	500
5	0.1	200
10	0.1	100
20	0.5	250
40	1	250

Injection timing

Pump Speed: 35
Cycle Period: 55 s
Inject to start of peak period: 19 s
Min probe in wash period: 5 s
Probe in sample period: 30 s
Load Period: 25 s
Inject Period: 30 s
% Width Tolerance: 50
Peak Base: 53
Threshold: 10500
Chemistry: Direct/Bipolar
Calibration Rep Handling: Average
Calibration Fit Type: 3rd Order Polynomial
Sample Throughput: 65 samples/h, 55s/sample

Manifold

System Operation

1. Inspect the modules

Column Efficiency Check

Before engaging the column, pump the reagents through all of the manifold tubing. Once all of the air bubbles have

exited and a stable baseline is reached, place the column in line. Attach the column first to the valve and then to the manifold to avoid allowing bubbles to pass through the column.
To check the column efficiency, mix a 1:1 dilution of the 50 ppm standard and the calcium hardness solution (see reagents section). Column efficiency is calculated by

$$E_{column} = \frac{\text{True value}}{25 \text{ ppm}} \times 100$$

where: E_{column} = % efficiency of the column
True value = the concentration of the 1:1 dilution of 40 ppm standard and calcium hardness solution

QAQC
For less than 10 samples run two duplicates, two spikes, two midpoint check standards and two QC samples. See QA/QC program and Table I-5.
Spikes: 10 ppm (50 uL of 1000 ppm SO4 stock in 5 ml of sample)
Midpoint check: 20 ppm standard
QAQC check: 10 ppm

Column maintenance
Eventually the BioRex 70 ion exchange resin column will become depleted, allowing multivalent cations to complex with the free MTB. The column can either be repacked or the resin can be rejuvenated. The rejuvenated resin will approximately double in volume when it is converted back to the sodium form from the hydrogen form. Begin by preparing a 0.5 N NaOH solution (2.0 g of NaOH to 100 ml of DIW). Remove the column from the SO4 manifold. Run a black-black pumpline (small diameter pumpline) through the lachet and set the Lachat pump speed to 10. Turn the pump on and fill the pumpline with 0.5 N NaOH solution. Attach the black-black pump line to the column and then pump the 0.5 N NaOH solution through the column for 5 minutes. Following the 0.5 N NaOH, DIW should be run through column for 5 minutes followed by the SO4 method reagents.

Waste Disposal
Waste cannot be sewered. All waste is to be poured into labeled hazardous waste bucket located in Room 480. Waste is not to be stored for longer than 90 days. Mixed reagent waste from the Lachat and outdated reagents for the analysis can be combined in a 1-gal wide mouth plastic container or a 5-gal plastic pail for shipment. Containers should be labeled with "pH≤2" and:

References

LACHAT INSTRUMENTS- Method 10-116-10-2-E

O'Dell, James W (Ed). 1993. Method 375.2: Determination of Sulfate by Automated Colorimetry. Rev 2.0 USEPA, Cincinnati, OH

Safety

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing

proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

***T** Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

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Standard Operating Procedure

No. 116, Rev 1
Effective Date: 12/1/2014

Sulfate Methylthymol Blue Flow Injection Analysis (High Method: 50-300 ppm)

(EPA Method 375.2)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
1	Elaine M Ruzycki	11/11/2014	Developed and formatted for laboratory certification

Reviewed by:

_________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
_________________________________ Date________________
Richard Axler
Director Central Analytical Laboratory

General
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Interferences

1. Multivalent cations: Cations such as Ca2+ and Mg2+ are removed by the exchange column. A midrange standard with a known hardness (CaCO3) is used to check the column efficiency at removing the multivalent cations.
2. Strongly acidic samples: Highly acidic samples (pH<2) can displace multivalent cations from the column.
3. Turbidity: Turbid samples can be filtered or centrifuged.
4. Orthophosphate: At high pH barium forms a precipitate with orthophosphate. A recovery study should be done if samples are known to be high in orthophosphate.

Sample Collection

Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleansed and rinsed with reagent water (see QAM Table 3-major anions). Volume collected, approximately 125 mLs, should be sufficient to insure a representative sample, allow for replicate analysis (if required).

No chemical preservation required. Cool sample to 4°C.

Samples should be analyzed as soon as possible after collection. If storage is required, samples maintained at 4°C may be held for up to 28 days.

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3. Injection Module with a 100 cm X 0.8 mm i.d. sample loop
4. 100 cm restrictor coil
5. Colorimeter
   a. Interference Filter : 460 nm
   b. Flow Cell, 10 mm, 80 µl
6. Reaction Module 10-116-10-2-B
7. QuickCalcII Software System

Reagents
(From QuikChem Method 10-116-10-2-B)

Carrier, 0.30 mg SO42-/L: Add 0.30 mL of 1000 mg/L stock sulfate solution (Standard 2) to a 1 L volumetric flask and dilute to the mark with water. Prior to the analysis degas with helium.

Barium Chloride Solution: Dissolve 1.526 g barium chloride dehydrate **[*T]** ($BaCl_2 \cdot 2H_2O$ in 995 ml water. Mix and Degas solution with helium prior to analysis.

Hydrochloric Acid: Mix 83 mL concentrated HCl **[*C]** to 917 mL water in a 1 L Pyrex container. Use the fume hood to avoid inhaling fumes. Degas with helium.

Barium- MTB Color Reagent: Measure 0.236 g methylthymol blue (3,3'-bis[N,N-di(carboxymethyl)aminomethyl]-thymol-sulfonephthalein,pentasodium salt, Lachet part number 50243) into a 500 mL volumetric flask. Add 50 mL of the Barium Chloride Solution reagent. Mix well before adding 4.0 mL of the Hydrochloric Acid reagent (will turn the solution orange). Add 71 mL of water and mix well. Fill to the line with ethanol (specially denatured anhydrous alcohol, Aldrich 24,511-9). Make this solution at least one day before analysis and refrigerate. Prior to analysis warm solution to room temperature and degas with helium.

Sodium Hydroxide Solution: In a 1 L volumetric flask mix 7.2 g sodium hydroxide **[*C]** (NaOH) with 900 ml water and then dilute to the mark. The mixing will release heat. Store solution in a plastic bottle. Degas with helium prior to analysis.

Buffered EDTA: This solution is used for cleaning the manifold after sample analyses. In a 1 L volumetric flask add 6.75 g ammonium chloride (NH4Cl) to 500 mL water. Under a fume hood add 57 ml concentrated ammonium hydroxide (NH4OH), followed by 40 g tetrasodium EDTA dehydrate. Mix well and then dilute to the mark with water.

Standards

10000 ppm SO_4^{2-}stock: Dry anhydrous sodium sulfate (Na2SO4) at 105ºC for one hour. Add 1.479 g Na2SO4 to a 100 mL volumetric flask and dilute to the mark with water.

1000 ppm SO_4^{2-}stock: Perform a 1:10 dilution of the 10000 ppm SO_4^{2}stock solution by pipetting 10 mL of 10000 ppm SO_4^{2-} stock into a 1000 mL volumetric flask.

Working Standards

Standard concentration (ppm)	Volume of 10000 ppm stock (mL)	Volumetric flask size (mL)
0	-	-
50	0.1	500
100	0.1	200
150	0.1	100
250	0.5	250
300	1	250

Injection timing

Pump Speed: 35
Cycle Period: 55 s

Inject to start of peak period: 19 s
Min probe in wash period: 5 s
Probe in sample period: 30 s

Load Period: 25 s
Inject Period: 30 s
% Width Tolerance: 50
Peak Base: 53

Threshold: 10500
Chemistry: Direct/Bipolar
Calibration Rep Handling: Average
Calibration Fit Type: 3rd Order Polynomial
Sample Throughput: 65 samples/h, 55s/sample

Manifold

System Operation

1. Inspect the modules

Column Efficiency Check

Before engaging the column, pump the reagents through all of the manifold tubing. Once all of the air bubbles have exited and a stable baseline is reached, place the column in line. Attach the column first to the valve and then to the manifold to avoid allowing bubbles to pass through the column.
To check the column efficiency, mix a 1:1 dilution of the 50 ppm standard and the calcium hardness solution (see reagents section). Column efficiency is calculated by

$$E_{column} = \frac{\text{True value}}{25 \text{ ppm}} \times 100$$

where: E_{column} = % efficiency of the column
True value = the concentration of the 1:1 dilution of 40 ppm standard and calcium hardness solution

QAQC

For less than 10 samples run two duplicates, two spikes, two midpoint check standards and two QC samples. See QA/QC program and Table I-5.
Spikes: 10 ppm (50 uL of 1000 ppm SO4 stock in 5 ml of sample)
Midpoint check: 20 ppm standard
QAQC check: 10 ppm

Column maintenance

Eventually the BioRex 70 ion exchange resin column will become depleted, allowing multivalent cations to complex with the free MTB. The column can either be repacked or the resin can be rejuvenated. The rejuvenated resin will approximately double in volume when it is converted back to the sodium form from the hydrogen form. Begin by preparing a 0.5 N NaOH solution (2.0 g of NaOH to 100 ml of DIW). Remove the column from the SO4 manifold. Run a black-black pumpline (small diameter pumpline) through the lachet and set the Lachat pump speed to 10. Turn the pump on and fill the pumpline with 0.5 N NaOH solution. Attach the black-black pump line to the column and then pump the 0.5 N NaOH solution through the column for 5 minutes. Following the 0.5 N NaOH, DIW should be run through column for 5 minutes followed by the SO4 method reagents.

Waste Disposal

Waste cannot be sewered. All waste is to be poured into labeled hazardous waste bucket located in Room 480. Waste is not to be stored for longer than 90 days. Mixed reagent waste from the Lachat and outdated reagents for the analysis can be combined in a 1-gal wide mouth plastic container or a 5-gal plastic pail for shipment. Containers should be labeled with “pH≤2” and:

References

LACHAT INSTRUMENTS- Method 10-116-10-2-E

O'Dell, James W (Ed). 1993. Method 375.2: Determination of Sulfate by Automated Colorimetry. Rev 2.0 USEPA, Cincinnati, OH

Safety

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

***T** Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

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Standard Operating Procedure

No. 116, Rev 1
Effective Date: 12/1/2014

Hardness EDTA Titrimetric Method (SM 2340 C)

Revision Record			
Rev. #	**Author/Reviser/Reviewer**	**Review/Revision Date**	**Description of Review/Change**
1	Elaine M Ruzycki	11/11/2014	Developed and formatted for laboratory certification

Reviewed by:

______________________________________ Date________________
Elaine M. Ruzycki
Laboratory Manager/QA Officer
_______________________________________Date_________________
Richard Axler
Director Central Analytical Laboratory

1.0 Summary

This method is for determining total hardness as mg/L $CaCO_3$ in surface waters and suitable for concentration ranges of hardness greater than 5 mg/L: however, to avoid large titration volumes use a sample aliquot containing not more than 25 mg $CaCO_3$.

The EDTA titration method measures the calcium and magnesium ions and may be applied with appropriate modification to any kind of water. The procedure described affords a means of rapid analysis.

2.0 Sample Handling and Preservation

- Pour samples (RAW/unfiltered) samples into polyethylene bottles that have been pre-washed per QAM Table 3 (metals). To preserve the sample, add 150 µL of conc. nitric acid **[*C]** per 100 mLs. Mix.
- Store samples at 4°C. Preserved samples can be stored for at least six months.
- Before starting the test, warm the sample to room temperature and adjust the pH to approximately pH 7 with potassium hydroxide solution. Mix thoroughly.

3.0 Apparatus

- Analytical balance: capable of accurately weighing to nearest 0.0001g.
- Glassware: Class A volumetric flasks, beakers, and pipets
- 50 mL burette and stand
- Stir plate

4.0 Reagents

***Buffer*:** Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 mL conc. ammonium hydroxide **[*C]** (NH_4OH). Add .25 g magnesium salt of EDTA (available commercially) and dilute to 250 mL with distilled water.

Sodium hydroxide, NaOH, 0.1*N*.

***1+1 Hydrochloric acid* [*C]**: Slowly add 500 mL of con HCl to 500 mLs of MQW in a 1L volumetric flask.

***1+1 Nitric acid* [*C]:** Slowly add 500 mL of con HNO_3 to 500 mLs of MQW in a 1L volumetric flask.

***Standard EDTA titrant,* 0.01*M*:** Weigh 3.723 g analytical reagent-grade disodium ethylenediaminetetraacetate dihydrate, also called (ethylenedinitrilo)tetraacetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1000 mL.Standardize against standard calcium

solution as described in below. Because the titrant extracts hardness-producing cations from softglass containers, store in polyethylene bottles only.

Indicator: *Calmagite:* 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid. This is stable in aqueous solution and produces the same color change as Eriochrome Black T, with a sharper end point. Dissolve 0.10 g Calmagite in 100 mL distilled water. Use 1 mL per 50 mL solution to be titrated. Adjust volume if necessary.

***Standard calcium solution**:* Weigh 1.000 g anhydrous $CaCO_3$ powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL Erlenmeyer flask. Place a funnel in the flask neck and add, a little at a time, 1 **+** 1 HCl **[*C]** until all $CaCO_3$ has dissolved. Add 200 mL distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3*N* NH_4OH or 1 **+** 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water; 1 mL **+** 1.00 mg $CaCO_3$.

6.0 Procedure

Titration of sample: Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from time of buffer addition. Warm sample to room temperature. Dilute 25.0 mL sample to about 50 mL with distilled water in beaker. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1. The absence of a sharp end-point color change in the titration usually means that an inhibitor must be added at this point or that the indicator has deteriorated. Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears. Add the last few drops at 3- to 5-sintervals. At the end point the solution normally is blue. If sufficient sample is available and interference is absent, improve accuracy by increasing sample size.

Low-hardness sample: For natural waters of low hardness (less than 5 mg/L), take a larger sample, 100 to 1000 mL, for titration and add proportionately larger amounts of buffer, inhibitor, and indicator. Add standard EDTA titrant slowly from a microburet and run a blank, using redistilled, distilled, or deionized water of the same volume as the sample, to which identical amounts of buffer, inhibitor, and indicator have been added. Subtract volume of EDTA used for blank from volume of EDTA used for sample.

7.0 QA/QC

Analysis of the QCCS is recommended as a continuing calibration check. See NRRI QAM

8.0 DATA ANALYSIS AND CALCULATIONS

$$Total\ hardness\ , mg\ CaCO3\ per\ L = \frac{[A * \ B * 1000]}{mL\ sample}$$

Where:
A = mL of EDTA titrant used
B = mg CaCO3 equivalent to 1.00 mL EDTA titrant
Subtract vol of EDTA used for the blank from the vol of EDTA used for the sample.

References

Standard Methods for the Examination of Water and Wastewater, Method 2340 C. 21st Ed. 2005. American Public Health Association, Washington, D.C.

Safety

***C** Chemical hazard **[*C]**: The reactivity of the chemical or chemicals with each other or tissue involved through contact may create a reaction that is potentially injurious through the creation of heat, explosion, toxic gases and/or destruction of exposed tissue.

Examples of chemicals included would be corrosives (strong acids and bases, phenol), oxidizers (peroxides, and peroxide forming chemicals), water reactive (sodium), etc. Chemicals designated as having particularly hazardous properties should be handled only by experienced and trained individuals wearing proper protective clothing and equipment. Use of a fume hood for mixing reactive chemicals is advised. MSDS should be consulted for the hazard potential of unknown materials.

***T** Toxicity hazard **[*T]**: This is a type of chemical hazard but is flagged separately since the chemical may be extremely toxic to biological systems if ingested or inhaled as a gas or particulate. A toxicity flag may indicate the chemical itself is toxic or a reaction of chemicals may create a product that is toxic, e.g., adding nitroprusside waste to a bottle containing dilute or concentrated acid will produce cyanide gas. Special handling procedures are outlined in the method or a supervisor and the MSDS should be consulted for additional information if questions arise. Use a fume hood and proper protective equipment if chemicals are flagged.

Waste Disposal

Waste can be sewered with sufficient amount of tap water.

Physical Properties

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COLOR
(Visual Comparison Method)

General

The platinum-cobalt method is useful for measuring color of water derived from naturally occurring materials (i.e., vegetative residues such as leaves, barks, roots, humus and peat materials). Color is measured by visual comparison in Nessler tubes to the sample with platinum-cobalt standards. One unit of color is produced by 1 mg/l platinum in the form of the chloroplatinate ion.

Interferences

This method is straightforward but results may be skewed by the color vision of the analyst. Generally, the similar spectrophotometric method, NRRI method II.0.11, is used in this laboratory.

Since very slight amounts of turbidity interfere with the determination of color, samples should be filtered or centrifuged. This method is very pH dependent. Check and record pH of water to be analyzed and prepare the standard curve. Since biological activity may change the color characteristics of a sample, the determination of color should be made ASAP. Samples should be stored at 4EC. See Table I-2.

Color determined on a filtered sample is referred to as true color. In some cases the contribution of the turbidity is of interest. Color measurement on unfiltered samples is referred to as apparent color.

Apparatus

Matched set of 50 mL Nessler tubes and rack.

Reagents

Standard Chloroplatinate Solution: A 500 color unit platinum cobalt color stock standard is available from Fisher (SP-120-500), or prepare a stock by dissolving 1.246 g potassium chloroplatinate, K_2PtCl_2 (equivalent to 0.500 g metallic Pt), and 1 g crystalline cobaltous chloride, $CoCl_2$-H_2O, in 800 mL Milli-Q water containing 100 mL concentrated HCl. Dilute to 1000 mL (this equals 500 color units).

Standards
Prepare standards to cover the range of samples. The following series is suggested:

Color Units	mL color standard/50 mL	Color Units	mL color standard/50 mL
0	0.0	40	4.0
5	0.5	45	4.5
10	1.0	50	5.0
15	1.5	55	5.5
20	2.0	60	6.0
25	2.5	65	6.5
30	3.0	70	7.0
35	3.5	*	-

*Samples over 70 units should be diluted. Cover standards with parafilm to protect against reaction with airborne ammonia.

Procedure

Set up a series of Nessler tubes with standards in a rack with a white surface placed at such an angle that light is reflected upward through the columns. Fill a matched Nessler tube to the 50 mL mark with sample and compare the color to the standards by looking vertically downward through the tube toward the white or specular surface. Record the color of the best standard color match to the nearest 1 unit.

Calculations: Calculate color units by means of the following equation:

Where: A = estimated color of diluted sample;
V = mL sample taken for dilution

Report color results as follows:

Pt-Co color units	Record to nearest
1-50	1
51-100	5
101-250	10
251-500	20

Record sample pH.

QA/QC

See QA/QC program and Table I-4.

References

Standard Methods for the Examination of Water and Wastewater, Method 2120B. 18th Ed. 1992. American Public Health Association, Washington, D.C.

Revised

C.J. Owen, 02/93; J.J. Ameel, 01/01

COLOR, UV at 254 nm

General

This procedure has been used since July, 1996, at the request of Dr. Edward Swain of the Minnesota Pollution Control Agency Air Quality Division for acid deposition and mercury related studies.

Apparatus

UV/Vis Spectrometer capable of reading at 254 nm (Perkin Elmer Lambda 3B or equivalent)

Quartz cuvettes: Quartz must be used since it will pass UV wavelengths to 170 nm. (Note: Optical glass cuts off wavelengths below 330 nm and is not suitable.)

Procedure

Filter samples through [membrane filter] and place subsample of at least 15 mL in labeled container for analysis. Samples analyzed for true color could be used.

If the samples are not analyzed immediately, store in refrigerator. However, the samples should be allowed to warm to room temperature before measurement. Cold samples will cause the spectrophotometric cuvette to fog, leading to erroneous data.

Samples should be analyzed within 48 hours of collection.

1. No standards are required for this analysis.
2. Turn on spectrophotometer and UV lamp, set wavelength to 254 nm and let machine warm up for at least 20 minutes before zeroing.
3. Use 1-cm quartz cuvettes. Fill sample and reference cuvettes with distilled water, wipe cells with lab tissue, and place cells in respective compartments. Zero instrument. (Note: 4-cm quartz cuvettes can be used but absorbances must be divided by 4 to convert them to the equivalent 1-cm reading.)
4. Rinse sample cell with a few mL of sample before filling for reading.
5. Record absorbance. Rinse cuvette well between samples

Calculations

1. No calculations are necessary unless a 4-cm cuvette is used. Then divide all absorbances by 4 to convert data to the equivalent absorbance of a 1-cm cuvette
2. Report data as AAbsorbance at 254 nm@.

References

Pers Comm - Dr. Edward Swain, 1996. Minnesota Pollution Control Agency

Revised J.J. Ameel, 07/98

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CONDUCTIVITY OR SPECIFIC CONDUCTANCE (EC25)

General

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valence, and relative concentrations, and on the temperature of measurement. Solutions of most inorganic acids, bases, and salts are relatively good conductors. Conversely, molecules of organic compounds that do not dissociate in aqueous solution conduct a current very poorly, if at all.

Specific conductance (EC25) is the conductivity at 25EC to compensate for temperature affects on ionic activity. Some instruments can be set to read EC25 directly (see YSI 85) while others require maintaining the sample at a constant temperature of 25EC while reading the conductivity. Formulas are available that can be used to adjust conductivity read at other temperatures to EC25 if sample temperature cannot be maintained at a constant temperature or a temperature compensated instrument is not available.

Apparatus

1. Sybron/Barnstead Conductivity Bridge, Model PM-70C-B
2. YSI 3400 Series Micro Conductivity Cell
3. Water Bath set at 25EC. Maintain temperature at 25E∀0.2EC.
4. Test tubes and rack.

or YSI 85 dissolved oxygen and conductivity instrument

Note: The Sybron/Barnstead Conductivity Bridge is not a temperature compensated instrument. It is very important to adjust and maintain the temperature of the water bath as significant error will occur if the temperature is not maintained at 25EC. Water bath temperature should be monitored with both the water bath thermometer and a calibrated thermometer in a test tube of Milli-Q water in the rack with the sample tubes.

Reagents

Stock Conductivity Solution, 0.0100N KCl: Weigh 0.7455 g dried KCl and dilute to 1L in MQW = 1413 µmhos/cm (Note: 1 µmhos/cm = 1 µS/cm (S = siemans))
Working Conductivity Solution, 14.94 µmhos/cm: Dilute 1 ml of stock (0.01 N KCl) to 100 mL..

Standards and Calibration Checks

Four standards and calibration checks are used for each run:

1. Milli-Q water
2. Laboratory deionized water
3. Tap water
4. Conductivity standard
5. Certified quality control check standard (QCCS)

Procedure

Rinse each tube first with a small portion of standard or sample, pour two 15 to 20 ml replicates for each sample or standard. One will be used for a cell rinse and the other for measuring the conductance.

Place the rack of test tubes into the water bath at 25EC (cover w/foil to prevent evaporation). Let samples equilibrate to 25EC. Significant error will occur if the temperature is not maintained at 25EC.

Calibrate Conductivity Meter (Sybron/Barnstead Conductivity Bridge)

1. Disconnect probe; Turn on meter
2. Plug in shorting strap
   a. Set dial to 0.00
   b. Set multiplier to x1 kilohms
   c. Set sensitivity knob all the way to the right
3. Use screwdriver to adjust pointer to zero
   Remove shorting strap; Plug in Conductance and Resistance Calibrator (10.00 mho/cm)
4. Set dial to 1.00
5. Set multiplier to x10 micromhos/cm
6. Center sensitivity knob (1/2 way back to left)
7. Use screwdriver to adjust pointer to zero (exactly)

Reading samples/Standards

1. Connect probe wires, leave multiplier on x10 mmhos/cm
2. Use 1 tube to rinse probe then put probe in sample tube to read (make sure not to touch probe while taking reading)
3. Turn dial until the pointer is closest to zero. Be sure to find the closest point on either side of zero. Record that value and multiplication value.

QA/QC

1. Measure conductivity of Milli-Q water, DIW, tap water and QCCS.
2. Check conductance of a 0.001 N KCl solution.

See Table I-2 and I-4.

Method modification for use with the YSI 85.

The YSI 85 can be used for reading either conductivity (no temperature compensation) or EC25 (temperature compensated). It is a direct reading instrument that holds an internal calibration. Recalibration should only be attempted if a fresh QC standard varies more than ∀10% from the certified value. Recalibrate using advanced procedures found in the YSI 85 manual.

1. Turn meter on. Allow to go through self-test.
2. Press MODE button until it reaches specific conductance. The large numbers on the display will be followed by either ΦS or mS (the instrument is autoranging) and the **small temperature display will flash on and off.** All readings will be temperature compensated. If the temperature display is not flashing, the mode button should be pressed one more time to bring up specific conductance or temperature should be recorded for every reading so temperature compensation can be applied later.
3. Samples and standards should be transferred to beakers, 50mL centrifuge tubes or other containers large enough so that the entire probe can be submerged. The

conductivity electrodes are in the small holes that are seen on the top of the probe. It is important that samples/standards flush this area completely and that the sample/standard level be maintained above these holes while readings are being taken.

4. Each samples/standard should be poured into two containers so the probe can be rinsed in the first and specific conductance read from the second.
5. Maintaining constant and accurate sample temperature for a batch of samples is not as important with this temperature compensated instrument as it is with the Sybron/Barnstead bridge but it doesn=t hurt.
6. Read and record QA/QC samples as listed above.
7. Read and record EC25 and temperature for each sample. Be sure to record the range as the autorange will set the measurement to ΦS or mS.

Formula for temperature compensation.

If conductivity readings are taken that are not at 25EC and the temperature has been recorded, the following formula can be used to convert conductivity to specific conductance (EC25).

Where: *EC*25=specific conductance (temperature compensated to 25EC)
EC=conductivity (not temperature compensated)
T=temperature of solution

Reference

Sybron/Barnstead Manual for Conductivity Bridge.

YSI Model 85 Operations Manual. YSI Incorporated, Yellow Springs, Ohio

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 120.1. EPA 600 4-79-020, Revised 1983.

Standard Methods for the Examination of Water and Wastewater, Method 2510B. 18th ed. American Public Health Association, Washington, D.C. 1992.

Revised

C.J. Owen, 02/93; J.J. Ameel, 07/98, 4/04.

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RESIDUE, FILTERABLE (TDS)
(Gravimetric, Dried at 180EC)

General

This method is also known as total dissolved solids (TDS). Filterable residue is defined as those solids capable of passing through a glass fiber filter and dried to constant weight at 180EC. A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180EC. The method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of the determination is 10 to 20,000 mg/l. Non-Filterable Residue or Total Suspended Solids can be determined from the residue remaining on the filter (see Method II.0.40).

Sample Handling and Preservations

Preservation of the sample is not practical. Analysis should begin as soon as possible. Refrigeration or icing at 4EC to minimize microbiological decomposition of solids is recommended if samples cannot be analyzed immediately. See Table I-2.

Interferences

Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying, desiccation and rapid weighing. Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180EC to ensure that all the bicarbonate is converted to carbonate. Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.

Apparatus

1. Glass fiber filter discs, 4.7 cm, 2.4 cm, or 2.1 cm, without organic binder. (Whatman 934/AH, Gelman type A/E, Whatman GF/C, or equivalent)
2. Filter holder, membrane filter funnel or Gooch crucible adapter
3. Suction flask, 500 ml
4. Gooch crucibles, 25 ml (if 2.1 cm filter is used)
5. Evaporating dishes, porcelain, 100 ml volume (Vycor or platinum dishes may be substituted)
6. Drying oven, 180EC ∀ 2EC
7. Desiccator
8. Analytical balance capable of weighing to 0.1 mg

Procedure

1. Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. Apply vacuum and rinse the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard rinse.

2. Preparation of evaporating dishes: If Volatile Residue is also to be measured, heat the clean dish to 550 ∀ 50EC for one hour in a muffle furnace. If only Filterable Residue is to be measured, heat the clean dish to 180 ∀ 2EC for one hour. Cool in desiccator and store until needed. Weigh immediately before use.
3. Choose an aliquot of sample sufficient to contain a residue of at least 25 mg. To obtain a weighable residue, successive aliquots of sample may be added to the same dish.
4. Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 ml to the funnel by means of a 100 ml graduated cylinder. If total filterable residue is low, a larger volume may be filtered.
5. Filter the sample through the glass fiber filter, rinse with three 10 ml portions of distilled water and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.
6. Transfer 100 ml (or a larger volume) of the filtrate to the weighed evaporating dish and evaporate to dryness at temperatures below the boiling point.
7. Once the liquid has evaporated at lower temperature, dry the evaporated sample for at least one hour at 180 ∀ 2EC. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

Calculation: Calculate filterable residue as follows:

where:

A = weight of dried residue + dish in mg
B = weight of dish in mg
C = volume of sample used in mL

QA/QC

See QA/QC program and Table I-5.

Reference

Standard Methods for the Examination of Water and Wastewater, Method 2540 C. 18th Edition, American Public Health Association, Washington, D.C., 1992

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 160.1-2. EPA 600 4-79-020, Revised 1983.

Revised

C.J. Owen, 03/92; J.J. Ameel, 07/98

RESIDUE
TOTAL
(Gravimetric, Dried at 103-105EC)

General

This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of the determination is from 10 mg/l to 20,000 mg/l. A well mixed aliquot of the sample is quantitatively transferred to a pre-weighed evaporating dish and evaporated to dryness at 103-105EC. Total Residue is defined as the sum of the homogenous suspended and dissolved materials in a sample.

Sample Handling and Preservation

Preservation of the sample is not practical. Analysis should begin as soon as possible. Refrigeration or icing to 4EC to minimize microbiological decomposition of solids is recommended if samples cannot be analyzed immediately. See Table I-2.

Interferences

Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

Floating oil and grease, if present, should be included in the sample and dispersed by a blender device before aliquoting.

Apparatus

Evaporating dishes, porcelain, 90 mm, 100 mL capacity. (Vycor or platinum dishes may be substituted and smaller size dishes may be used if required.)

Procedure

1. Heat the clean evaporating dish to 103-105EC for one hour. If Volatile Residue is also to be measured, heat at **550 ∀ 50EC [*P]** for one hour in a muffle furnace. Cool in desiccator, weigh and store in desiccator until ready for use.
2. Transfer a measured aliquot of sample to the pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven.
3. Choose an aliquot of sample sufficient to contain a residue of at least 25 mg. To obtain a weighable residue, successive aliquots of sample may be added to the same dish.
4. If evaporation is performed in a drying oven, the temperature should be lowered to approximately 95EC to prevent boiling and splattering of the sample.
5. Dry the evaporated sample for at least 1 hour at 103-105EC. Cool in a desiccator and weigh. Repeat the cycle of drying at 103-105EC, cooling, desiccating and weighing until a constant weight is obtained or until loss of weight is less than 4% of the previous weight, or 0.5 mg, whichever is less.

Calculation: Calculate total residue as follows:

where:

A = weight of sample + dish in mg
B = weight of dish in mg
C = volume of sample in mL

QA/QC

See QA/QC program and Table I-4.

Reference

Standard Methods for the Examination of Water and Wastewater, Method 2590 B. 18th Edition, p. 93. 1992. American Public Health Association, Washington, D.C.

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 160.3-2. EPA 600 4-79-020, Revised 1983.

Revised

J.J. Ameel, 07/98

Hazards

***P See Hazard section under Safety-QA/QC.**

Biological and Organic Methods

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CHLOROPHYLL AND PHAEOPHYTIN DETERMINATION - Fluorometric

General

Estimating the concentration of chlorophyll-*a* remains the most common method for assessing algal biomass. The concentration of chlorophyll-*a* has also been shown to relate to primary productivity (Wetzel 1983) and can be used to assess the physiological health of algae by examining its degradation product, phaeophytin. This degradation product has been shown to contribute 16-60% of the chlorophyll-*a* content in seawater and freshwater. Despite numerous innovations such as HPLC, and dozens of conflicting reports regarding extraction and analytical protocols, most state resource and regulatory agencies still appear to rely on, and in fact require, methods listed in Standard Methods (1992) and in some cases EPA (1987). Therefore, spectrophotometry and fluorometry, utilizing 90% acetone extraction, remain the most commonly used methods. Spectrophotometry is most widely used but fluorometry is more sensitive and should be used when low levels of chlorophyll are anticipated.

Apparatus - Extraction

1. Prewashed 47 mm glass fiber fillers (GF/C or AE)
2. Gelman polycarbonate filtration tower
3. Vacuum pump
4. Centrifuge
5. DIW/acetone washed 15 mL Corex centrifuge tubes with caps

Apparatus -Fluorometry

1. Turner Designs TD-10 red sensitive fluorometer
2. 5-60 (10-050) excitation filter (deep blue)
3. 3-66 (10-052) reference filter (orange)
4. 2-64 (10-051) emission filter (deep red)
5. Borosilicate cuvette

Reagents

1. 90% acetone **[*T]**: (900 ml acetone + 100 mls MQW (MQW) + 0.1 ml conc. NH_4OH **[*C]**
2. Chlorophyll-*a* stock (10,000 ppb) Chlorophyll-*a* from *Anacystis nidulans* (Sigma chemical C6144) (see Working Standards)
3. 0.12 N HCl: (1.92 mL concentrated HCl + 200 mL MQW)
4. Saturated $MgCO_3$ solution: (1.0 g $MgCO_3$ + 100 mL MQW)

Filtration Procedure

1. Concentrate chlorophyll by filtration as soon as possible after collection (< 24 hours). Filter an appropriate volume of water (depending on the specified analytical procedure and the estimated chlorophyll concentration) through a pre-washed 47mm glass fiber filter (Whatman GF/C or Gelman AE), using Gelman polycarbonate filtration towers, under low to moderate vacuum.
2. Add 0.15mL of a saturated $MgCO_3$ solution to the sample just prior to the end of filtration. The $MgCO_3$ acts to buffer the sample preventing premature phaeophytinization.

3. Fold the moist filter into quarters, wrap in aluminum foil and store frozen at -10EC.
4. Record volume of water filtered.

Extraction Procedure

Under subdued light.

1. Tear filters into quarters with forceps.
2. Place filter in a 15 ml centrifuge tube.
3. Add 10 mL 90% acetone.
4. Cap and extract overnight in the dark at 4E C..

Working Standards

*Make up stock and working standards under subdued light conditions. Keep stock solution in dark at all times.

<u>Stock chlorophyll-*a* solution (10,000 ppb)</u>: Place 1 mg of purified chlorophyll-*a* in 100 mL of 90% acetone (make stock solution at least 24 hours prior to analysis).

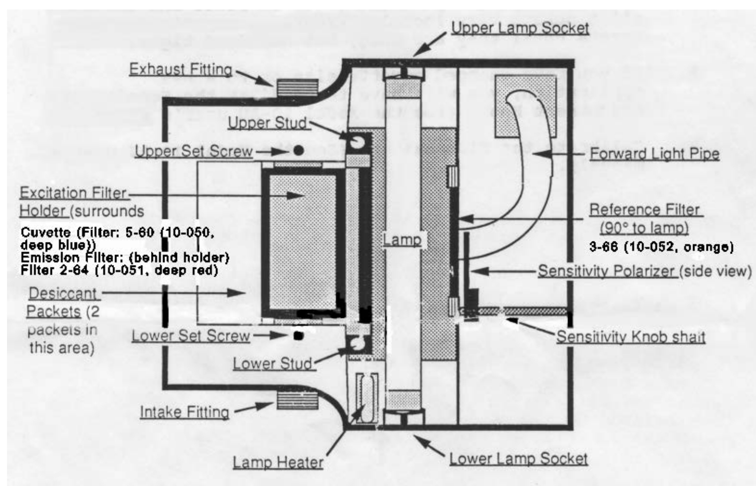
<u>500 ppb chlorophyll-*a* solution</u>: Add 2.5 mL of stock solution to 50 mL of 90% acetone.

<u>300 ppb chlorophyll-*a* solution</u>: Add 1.5 mL of stock solution to 50 mL volumetric flask. Dilute to mark with 90% acetone.

<u>150 ppb chlorophyll-*a* solution</u>: Add 0.75 mL of stock solution to 50 mL volumetric flask. Dilute to mark with 90% acetone.

Fluorometric Determination of Chlorophyll-*a* and Phaeophytin

Fluorometric assays with a Turner Designs TD-10 equipped for chlorophyll analysis requires a 5-60 (10-050) excitation filter, 3-66 (10-052) reference filter, 2-64 (10-051) emission filter and a red-sensitive photomultiplier tube.



The Turner Designs TD-10 is zeroed with 90% acetone at a gain setting of 10 and a multiplication factor of 100. The blank fluorescence is recorded at all other gains and multiplication factors. Responses are calibrated against pure chlorophyll-*a* before and after acidification. The acidification time is standardized at 60 seconds, as per the spectrophotometric method. Acid and fluorometer calibration factors are derived from these values prior to analyzing extracts:

$$r = \frac{Fb}{Fa}$$

$$C.F. = C_{a\,std} / Fb$$

where:

r = Acid factor

Fb = Fluorescence before acidification

Fa = Fluorescence after acidification

C.F. = Calibration factor

$C_{a\,std}$ = chlorophyll-*a* standard (ug/L)

Acid factors should fall in the range of 1.7 to 2.8 and correction factors should be stable from run to run.

Place a 4 mL aliquot of the sample extract into a borosilicate cuvette (note that fluorometric analysis does not require centrifugation) and place in the sample chamber. Take a reading after a 30 second stabilization, and then acidify with 100ul of a 0.12N HCl solution and record fluorescence after 60 seconds. Calculate chlorophyll-*a* and phaeophytin concentration using the following equations:

$$Total\ chlorophyll\ \left(\frac{ug}{L}\right) = C.F.* \left[\frac{Fb}{gain}\right] * \frac{\rho}{V_{\ sample}}$$

$$chlorophyll\ a\ \left(\frac{ug}{L}\right) = \frac{C.F.\left[\frac{r}{r} - 1\right] * (Fb - Fa) *\ \rho}{gain * V\ sample}$$

$$Phaeophytin\ \left(\frac{ug}{L}\right) = \frac{C.F.\left[\frac{r}{r} - 1\right] * [(r * Fa) - Fb) *\ \rho}{gain * V\ sample}$$

where:

C.F. = Calibration factor
r=Acid factor
Fb = Fluorescence before acidification

Fa = Fluorescence after acidification

Gain = Gain used to read sample fluorescence

V_{sample} = Volume of water filtered (L)

ρ = Standardized Gain x extract volume (mL)
Note: For Turner Designs T-10, ρ = 10 = Gain 1 H 10 mL extract volume

Fluorescence for the Turner Designs TD-10 is normalized to gain 1 by overmarking the front panel of the instrument (Turner Designs: Operating & Service Manual section 3.3.2) so that the panel reads as follows:

OLD	NEW
x31.6	MIN. CONC.
x10	x3.16
x3.16	x10
MIN. SENS.	x31.6

This allows the use of a single reading for the determination of fluorescence, by direct multiplication of the sensitivity and range settings by the meter value. This results in all "gain" values for the determination of total chlorophyll, chlorophyll-*a* and phaeophytin being normalized to "1."

QA/QC

Verify that acid factors and correction factors are in range when running samples.

References

Standard methods for the examination of water and wastewater. 1992. 18th edition. American Public Health Association, Washington, D.C.

Axler, R. C. and C. J. Owen. 1994. Measuring chlorophyll and phaeophytin: whom should you believe? Lake and Reservoir Management 8:143-151.

Axler, R., C. Rose, and C. Tikkanen. 1991. An assessment of phytoplankton nutrient deficiency in northern Minnesota acid sensitive lakes. NRRI Tech. Rep. NRRI/TR-91-18, Natural Resources Research Institute, University of Minnesota, Duluth, 55811. 114 pp.

Baker, K.S., R.C. Smith, and J.R. Nelson. 1983. Chlorophyll determinations with filter fluorometer: Lamp/filter combination can minimize error. Limnol. Oceanogr. 28:1037-1040.

Brown, J.M. and C.D. Goodyear. 1987. Acid Precipitation Mitigation Program: Research methods and protocols. U.S. Fish and Wildlife Service, Nat. Ecol. Res. Center, Leetown, WV. NERC 87/27.

EPA. 1987. Handbook of methods for acid deposition studies: Laboratory analysis for surface water chemistry. EPA 600/4-87/026, September 1987, U.S. Environ. Protec. Agency, Washington, DC.

Wetzel, R.G. 1983. Limnology. 2nd Edition. Saunders College Publishing, Philadelphia, PA.

Note: A Sequoia-Turner 450-003 "factory equipped" for high chlorophyll sensitivity with NB430 excitation filter, SC665 emission filter and a red-sensitive photomultiplier tube could also be used with the following modifications: The instrument is zeroed with 90% acetone on gain 5 and the fluorescence recorded for all other gains. The factor, $\rho = 50$ = gain 5 x 10 mL extract volume in the calculations.

Revised

E. Ruzycki, 03/94; 07/98, J.Ameel, 4/03

Hazards

***T See Hazard section under Safety-QA/QC.**

^{14}C PRIMARY PRODUCTIVITY METHOD (^{14}C-PPr)
Field Method (*In situ* incubation)

General

A solution of radioactive carbonate ($^{14}CO_3^{2-}$) is added to light and dark bottles that have been completely filled with sample as described for the dissolved oxygen method (NRRI Method IV.2.00). After *in situ* incubation, the plankton is collected on a membrane filter, treated with hydrochloric acid (HCl) to remove inorganic carbon 14, and the filter assayed for radioactivity. The quantity of carbon fixed is estimated from the fraction of radioactive carbon assimilated and the initial concentration of dissolved inorganic carbon.

Apparatus

1. 125 ml pyrex bottles w/ground glass stoppers
2. Aluminum foil
3. B-D Cornwall 1 cc syringe w/LuerLok 20 gauge needle
4. Electrical tape
5. Metered line with dog snaps and float
6. Millipore 0.45 µm HAWP filters
7. 45 mm filtering manifold
8. Scintillation vials w/caps
9. Liquid Scintillation Counter (Beckman LS 6000 SE or equivalent)
10. Black cloth

Reagents

$Na_2\,^{14}CO_3$ Solution
0.01 N HCl (0.83 ml conc. HCl/L)
ICN Cytoscint ES liquid scintillation cocktail or equivalent

General precautions

Always use green surgical gloves when handling radioactive materials. Double gloving is recommended.

All procedures using radioactive materials should be performed in a tray, cooler or dish pan lined with absorbent paper as secondary containment in the event of a spill.

Use equipment and glassware dedicated and labeled for handling radioactive materials.

Do not return dedicated glassware to general use unless it has been thoroughly checked for radioactive residue by the radiation safety officer.

Do not discard non-radioactive waste in containers marked for radioactive waste.

Follow Department of Transportation (DOT) procedures carefully when transporting radioactive materials to and from the field. See summary below.

Cleanup

Discard alkalized filtrate into a liquid rad waste jug and record the volume and approximate activity of the waste on a waste manifest. Place the solid waste (gloves, Kimwipes7, pipet tips, absorbent lab

paper, etc.) into the yellow solid waste trash can. Broken glass ampules go into the rad waste glass box and sharps (Pasteur pipettes) go in the rad-labeled sharps container. Manifold funnels receive final rinses with tap water and then DIW in the Rad Lab stainless steel sink (NRRI room 484). All work areas (lab benches, trays, sink, floor, door handles, and fume hood) and equipment (filter towers, manifolds, etc.) should be swipe tested with 42-47 mm diameter Whatman No. 1 filter paper and counted using Program 2: SWIPE TEST. Wipe tests should also include the packing materials used to transport the Isotopes to the field, the vehicle used to transport the materials and the use area in the field (boat or truck) where samples were inoculated with isotopes. Results should be reported to the radioactive lab safety officer (currently J. Ameel).

In the event of minor spills, carefully sop up the excess liquid with lab wipes (Kimwipes=) or paper towels, then wipe surfaces down with O.005N HCI and finish with tap water. Discard all potentially radioactive materials into the solid waste trash can. Finish by conducting swipe tests with 42-47 mm diameter Whatman No. 1 filter paper of all working areas including the sink, faucet knob and door handle. The swipes should be counted using Program 2: SWIPE TEST, and results should be reported to the radiation lab safety officer.

In the event of a major spill, contact the UMD Chemical Spill Response team by calling Mahjoub Labyad (726-7273) or Facilities Management, 726-8262 (days), 726-8147 (evenings) or 348-4748 (weekends & holidays).

Field Transport of ^{14}C or ^{3}H isotopes

Follow all applicable Federal and state Department of Transportation (DOT) regulations regarding transportation of small quantities of radioactive material. Ampulated isotope stock solution should be wrapped in paper towels inside small Rubbermaid7 containers which are then stored securely in the wooden field box along with incubation bottles and pipetts. The field box must be secured in the bed of a University truck. **No personal vehicles are to be used for transporting rad materials.** The box must contain a sheet documenting the type and quantity of radioisotope and it form (i.e. 4 ampules of ^{14}C-$NaCO_3$ each containing 6 mL x 10 ~uCi/mL= 60 uCi; Total isotope = 4 x 60 ~uCi = 240 ~uCi). The sheet must also contain the statement, **This package conforms to the conditions and limitations specified in 49CFR173.421for Radioactive Material, excepted package - limited quantity of material, UN2910**. The internal package must be labeled with a ARadioactive@ label but the field box does not have to be labeled on the outside.

Procedure

Fill two clear 125 mL bottles and one 125 mL bottle darkened with aluminum foil and painted white for each depth. Be careful not to expose water to direct or bright light. Hold samples in a light tight box while additional samples are collected. Use a black cloth to shield the bottles from the sun while working with the samples.

Working under the black cloth, inject each 125 ml bottle with approximately 5 μCi $Na_2{}^{14}CO_3$ solution. Use a B-D Cornwall 1 cc syringe w/LuerLok tip fitted with a small bore needle (20 gauge) or an adjustable pipette. Record the exact volume of $Na_2{}^{14}CO_3$ added to each bottle.

Tighten the ground glass stoppers and seal the dark bottles with aluminum foil and electrical tape to exclude light. Attach the bottles to the metered line by way of the loop of cooper wire around the neck of the bottle and the dog-snap on the metered line.

Lower the line and attached bottles into the water, and make secure to the float. Tether the float to the boat so as not to shade any of the bottles by the boat.

Incubate the bottles *in situ* for three to four hours, weather permitting. It is best to try and incubate the primary productivity bottles between 1000 and 1400 hours. Retrieve the bottles at the end of the incubation and place in order in the light-tight box. Filter the contents of the bottles as soon as possible.

Working in a mostly darkened lab, filter the PPr bottle contents through Millipore 0.45 µm HAWP filters. Use the six place filtering manifold with 47 mm magnetic filter bases. Rinse each bottle three times with about 5-10 ml deionized water from a wash bottle, pouring each rinse into the filter base. Waste must be contained in a dedicated trap.

When most of the liquid has passed through the filter, rinse the walls of the filter base with about 5-10 ml of 0.01-N HCl from a wash bottle. Follow the acid rinse with another small rinse of deionized water.

Continue to filter until excess water is removed, then with forceps transfer the filter into labeled scintillation vial.

Place the uncapped scintillation vial in the cardboard tray, with a properly labeled cap placed in an adjacent slot on the tray.

Let filters dry at room temperature overnight.

Add 15 ml CytoScint ES liquid scintillation cocktail to each vial and cap tightly.

Count the vials on the Liquid Scintillation Counter using Program 6: PPR METHOD.

Calculations

Primary productivity (P) is equal to algal productivity in the light (P_l) minus algal productivity in the dark (P_d). Calculations according to Lind, 1985, are:

where:

so:

This formula can be simplified for use in a spreadsheet as follows:

DIC is determined from alkalinity by NRRI Methods IV.0.20 and IV.0.30. Activity in µCi/mL is determined for each lot of ^{14}C-bicarbonate but is normally around 10 µCi/mL.

QA/QC

All personnel using radioactive material must successfully complete the University of Minnesota Radiation Protection Training Course and be trained by the NRRI Radiation Safety Officer (currently J. Ameel).

Reference

Vollenweider, R.A. (Ed.), 1977. Primary Production in Aquatic Environments. p. 48. IPB Handbook No. 12. Blackwell Scientific Publications.

Revised

C.P. Larsen, C.J. Owen, P. Aas, 03/92; J.J. Ameel, 05/01.

^{14}C PRIMARY PRODUCTIVITY METHOD (^{14}C-PPr)
Laboratory Method

General

This method is similar to the field method (see Section VII.0.20) only sample volumes are much smaller and incubation is carried out in an incubator under ambient light and temperature. Exposure to natural sunlight is also possible. The smaller sample volume allows for shorter incubation times and requires smaller amounts of isotope. In addition, the rate of photosynthesis (P) as a function of light intensity (I) (P vs I ratio) can be determined by setting up a light gradient by shading the vials with increasing number of screens.

Apparatus

1. glass scintillation (LSC) vials (both foil and polypropylene caps)
2. 45 mm filtering manifold
3. vacuum pump
4. Liquid Scintillation Counter (Beckman LS 6000 SE or equivalent)
5. light meter
6. fiberglass screening (black)

Reagents

$Na_2\,^{14}$-CO_3 solution
0.01 N HCl (0.83 mL conc. HCl/L)
ICN Cytoscint ES liquid scintillation cocktail or equivalent

General precautions

Always use green surgical gloves when handling radioactive materials. Double gloving is recommended.

All procedures using radioactive materials should be performed in a tray, cooler or dish pan lined with absorbent paper as secondary containment in the event of a spill.

Use equipment and glassware dedicated and labeled for handling radioactive materials.

Do not return dedicated glassware to general use unless it has been thoroughly checked for radioactive residue by the radiation safety officer.

Do not discard non-radioactive waste in containers marked for radioactive waste.

Follow Department of Transportation (DOT) procedures carefully when transporting radioactive materials to and from the field. See summary below.

Cleanup

Discard alkalized filtrate into a liquid rad waste jug and record the volume and approximate activity of the waste on a waste manifest. Place the solid waste (gloves, Kimwipes7, pipet tips, absorbent lab paper, etc.) into the yellow solid waste trash can. Broken glass ampules go into the rad waste glass box and sharps (Pasteur pipettes) go in the rad-labeled sharps container. Manifold funnels receive final

rinses with tap water and then DIW in the Rad Lab stainless steel sink (NRRI room 484). All work areas (lab benches, trays, sink, floor, door handles, and fume hood) and equipment (filter towers, manifolds, etc.) should be swipe tested with 42-47 mm diameter Whatman No. 1 filter paper and counted using Program 2: SWIPE TEST. Wipe tests should also include the packing materials used to transport the Isotopes to the field, the vehicle used to transport the materials and the use area in the field (boat or truck) where samples were inoculated with isotopes. Results should be reported to the radioactive lab safety officer (currently J. Ameel).

In the event of minor spills, carefully sop up the excess liquid with lab wipes (Kimwipes7) or paper towels, then wipe surfaces down with O.005N HCI and finish with tap water. Discard all potentially radioactive materials into the solid waste trash can. Finish by conducting swipe tests with 42-47 mm diameter Whatman No. 1 filter paper of all working areas including the sink, faucet knob and door handle. The swipes should be counted using Program 2: SWIPE TEST, and results should be reported to the radiation lab safety officer.

In the event of a major spill, contact Mahjoub Labyad in Environmental Health and Safety (726-7273, pager, 725-6751 or cell phone, 343-3130). **Do not leave the room until a radiation protection division representative has performed a survey for contaminated clothing and no radiation is detected and/or replacement clothing is provided. Failure to remain in the room may result in wide-spread radioisotopic contamination that will require an extensive and costly cleanup effort.**

Procedure

Measure 10-20 mL of sample into labeled LSC vials.
Innoculate all vials with ~ 0.1 mL $Na_2\,^{14}CO_3$ stock solution (10 µCi/mL). The amount of label can be varied depending on the amount of phytoplankton in the sample. Note time of inoculation.
Cap (with polypropylene lined caps), mix, and immediately place the vials into the appropriate screens or foil wrap (see Table 1).
Place vials in incubator and record start time (T_0).
Measure and record the light intensity at the same level the vials are placed. Rotate vials periodically to reduce the effect of uneven lighting.
After a 1-2 h incubation, cover all vials with foil to stop ^{14}C uptake.
Filter the samples on to a Millipore 0.45 um filter. Rinse the filter with 5 mL DIW, then 5 mL 0.01 N HCl, then 5 mL DIW again. Keep the room darkened during filtration.
Place the filter in a clean, labeled LSC vial and allow the filters to dry overnight (uncapped) in an undisturbed area.
Add 15 mL of scintillation cocktail and cap tightly(use foil lined caps).
Count the vials on the Liquid Scintillation Counter using Program 6: PPr method.

Calculations

To calculate PPr as µg Carbon/L/h, determine initial cell density of samples (chlorophyll-*a* determination, NRRI Method VII.0.10) and dissolved inorganic carbon (DIC) concentration (NRRI Methods IV.0.30 or IV.0.31.

$$PPr\ (ug/L/hr) = \frac{(DPM \times DIC \times 1000 \times 1.06)}{\text{Incubation time (hr)} \times \mu Ci/mL \times mL\ \text{inoculated} \times 2.22 \times 10^6}$$

QA/QC

All personnel using radioactive material must successfully complete the University of Minnesota Radiation Protection Training Course and be trained by the NRRI Radiation Safety Officer (currently J. Ameel).

Reference

Wetzel, R.G. and G.E. Likens. 1991. Limnological Analyses, 2nd edition. Springer-Verlag, New York, NY.

Revised
E.Ruzycki, 07/98; J.Ameel, 05/01

Table 1.

# screens	% I_0	# screens	% I_0
0	100	4	12
1	59	5	6.9
2	35	6	4.2
3	20	foil wrapped	0

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PHYTOPLANKTON BY RAPID ASSESSMENT METHOD

General

A general assessment of planktonic algal communities can provide insight into the biotic and abiotic factors influencing the structure of an aquatic system. Trends of taxonomic and morphological distributions have long been seen seasonally in lakes of various trophic status. Basic knowledge of the dominant algal species is essential in understanding an aquatic ecosystem. This rapid assessment method provides a simple, quick estimate of species distribution and contribution to phytoplankton biomass.

Special Considerations

A general knowledge of algal morphological characteristics is required with particular attention being paid to forms produced in response to various physiological and abiotic factors. Examples of these include heterocysts and akinetes in certain Cyanobacteria as well as the formation of resting cysts in many other species.

A knowledge of the sample source is also required. The volume of sample needed for algal community assessment must provide enough cells to get a good estimate of community composition and densities. In many oligotrophic lakes it may be necessary to concentrate a large volume of water by allowing the cells to settle (e.g. 1000 mL allowed to settle in a graduated cylinder, then siphoning off all but 100-200 mL which is saved. This 100 mL sample can be further subsampled then analyzed. Samples should be allowed to settle for a minimum of 24 hrs).

The recognition and recording of unidentifiable organisms is also important. If possible, morphological characteristics should be noted.

Sample

Sample date, location and depth should be noted. Raw water is preserved immediately with Lugol's Iodine (1 mL to 50 mL sample) or neutralized gluteraldehyde (0.5 to 1% final concentration). Samples should be stored under darkened conditions. Long-term storage will require gas impermeable containers.

Apparatus

- Utermohl settling chambers
- Graduate cylinders
- Petroleum jelly
- Inverted microscope capable of 100X to 400X magnification

Reagents

Lugol's iodine (dissolve 5 g iodine, 10 g KI and 10 mL glacial acetic acid in 100 mL distilled water). Add 1 mL to every 50 mL of raw water.

Procedure

Slide Prep

Determine the volume of sample needed to provide a good population of cells to analyze. Thirty-five milliliters is adequate for lakes of low productivity. If the chlorophyll-*a* concentration is 7-10 mg/L reduce volume to 25 mL, if 2-3 mg/L increase to approx. 50 mL. As noted above, it may sometimes be necessary to further concentrate sample by settling.

Mix sample well by inverting bottle vigorously for at least 1 minute to ensure an even distribution of cells.

Utermohl Method

Assemble the settling tower and counting chamber. Pour approximately 20 mL of water to check for leaks. Mix the sample well. Measure a representative volume and layer it over the water in the tower (layering helps to assure a random distribution of algal cells). Let the sample settle for at least 24 hours.

Algal Community Rapid Assessment

Scan the entire slide and identify and list genera and species (if possible) present in sample.

Estimate the percent biomass by volume for each taxon identified. Estimates should consider size, growth form (colonial or unicellular), and density. Record this estimate, then calculate the contribution of each taxon to the chlorophyll-*a* concentration (if available).

QA/QC

Replicate slides should be prepared and analyzed. If at all possible, replicates should periodically be counted by at least two different analysts.

References

Sandgren, C.D. and J.V. Robinson. 1984. A stratified sampling approach to compensating for non-random sedimentation of phytoplankton cells in inverted microscope settling chambers. Br. Phycol. J. 19:67-72.

Swain, E. 1992. (pers. comm.) Rapid Assessment Phytoplankton Protocol, Minnesota Pollution Control Agency.

Revised

E.M. Ruzycki, 06/15

PERMANENT PHYTOPLANKTON MOUNTS (HPMA)

General

This method follows the same sampling and filtering protocol as the Rapid Assessment Method but results in a permanent mount.

Special Considerations

See NRRI Method VII.0.30.

Sample

See NRRI Method VII.0.30

Apparatus

1. Bunsen burner
2. beaker tongs
3. ice bath
4. Pyrex beakers
5. 2 dropper bottles
6. fume hood
7. oven

Reagents

HPMA prepolymer resin (2-hydroxypropyl methacrylate): CAUTION-GLOVES AND FUME HOOD REQUIRED (see MSDS and manufacturer's information sheet).

1. Prepare an ice bath in plastic tub.
2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 125 mL beaker. **FUME HOOD AND GLOVES REQUIRED**.
3. Light Bunsen burner and set to high flame.
4. Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath then return to flame. **DO NOT LET MIXTURE BOIL**. Alternate heating and cooling until the mixture has the density of Karo syrup. Make sure the mixture is cool at this point or it will polymerize further. Transfer to a clean, glass jar for storage. Prepolymer will last up to a year if stored at room temperature and protected from UV light. It will last indefinitely if frozen.

CAUTION: THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST

EXPLOSIVELY IF ALLOWED TO GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PREPOLYMER.

Note: Beakers can be washed in ethanol by soaking for 24 to 48 hrs at least 2 times.

5. Fill two amber bottle with resin. Add crystalline iodine to one of the bottles (optional) until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. RESIN IS LIGHT SENSITIVE--COVER BOTTLES WITH FOIL.

Procedure

Prepare sample filter as for the Rapid Assessment Method.

Place wet filter face down on a 25 mm diameter cover glass and cover the back with a few drops of the prepolymer. Prepare several slides (you may want to use both the clear resin and the iodine resin). Place cover glass on tray or foil and heat for 12-24 hrs at 60EC until resin has infiltrated the cells and polymerized. **DO NOT APPLY MOUNTING MEDIA DIRECTLY TO THE FACE OF THE FILTER** (this is to avoid resuspension and non-random distribution of cells). Also do not slide filter around on the cover glass.

After polymerization the cover glass is secured, filter side down, on a slide with a drop of resin. Then place the slide in 60EC oven and polymerize for another 12-24 hrs.

References

Crumpton, W.G. 1987. A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. Limnol. Oceanogr. 32:1154-1159.

Revised

E.M. Ruzycki, C. Owen, 01/93.

DIRECT COUNTS OF AQUATIC BACTERIA BY DAPI STAINING

General

The most common method for determining the number of bacteria in aquatic samples is the *Direct Count method* using a fluorescent stain such as DAPI and membrane filtration. This stain attaches to bacterial DNA and fluoresces bright blue or yellow when viewed under fluorescent light (excitation = 365 nm, emission = 418 nm). This method can be used to count the total number of free living and attached bacterial cells in water samples, but cannot distinguish between active and dormant cells. This method is also good for determining the purity of water coming from the deionized water taps.

Apparatus

1. liquid scintillation vials (glass or plastic)
2. 0.2 µm Nuclepore or Poretics membrane filters (25 mm, prestained with Irgalan black)
3. Filter forceps
4. Eppendorf automatic pipets and pipet tips (10-100 µl, 100-1000 µl)
5. Petri dishes (for filter staining)
6. 25 mm Millipore filter towers
7. Side arm flasks and vacuum source
8. Microscope slides
9. 25 mm coverslips
10. Microscope with epifluorescence illumination and proper filters (excitation = 365 nm, emission = 418 nm)

Solutions

1. 37% formalin **(SENSITIZER AND SUSPECTED CARCINOGEN!) [*T]**
2. DAPI (4',6'-diamidino-2-phenylindole), M.W. 350.25, Sigma Chemical Co., see table 1 for concentrations. **(CAUTION: POSSIBLE MUTAGEN!) [*T]**
3. Irgalan black **[*T]** (20 g/L + 20 mL glacial acetic acid **[*C]:** filter through Whatman GF/C filter)
4. Low fluorescence immersion oil
5. 0.2 µm filtered water

Procedure

<u>Sample collection and preparation</u> Water samples (10 mL) are preserved in 37% formalin (0.5 mL) **[*T]** to achieve a 2% final concentration. Samples can be stored for up to two weeks at 4°C before counting. Water samples taken directly from the tap do not need to be preserved with formalin.

<u>Filter preparation</u> Counter stain the membrane filters with Irgalan black to reduce background fluorescence and improve visibility of the stained cells. Pour Irgalan black solution into a large petri dish and place filters in solution. Allow them to stain for several hours prior to use. When ready to use,

remove a filter from the irgalan black and soak in a petri dish containing D.I. water. The filters should now appear dark gray.

Slide preparation Make up a stock solution of DAPI **[*T]**. DAPI comes in a 1 mg vial. Do not remove any of the powdered DAPI from the vial, but add 260 µl of MQW to the vial and mix thoroughly to form the stock solution. Store this frozen and in the dark. **Caution. DAPI is a possible chromosomal mutagen. Take care not to contact the powder!**

When you are ready to do bacterial counts, make up a fresh working solution of DAPI daily. In order to conserve the stock solution, make only as much as you plan to use on any given day. You will need 200-500 µl of the working solution for every sample you plan to scan. To a foil wrapped or taped (i.e. light impermeable) scintillation vial, add 10 µl of the stock solution to 990 µl MQW. This is the working DAPI solution. Store this in the refrigerator until use.

Set up a 25 mm filtration tower. You will need to make an estimate of bacterial density before you begin staining and filtering the water sample. For example, I usually filter 2-5 mL of water when checking the D.I. system, which should be very dilute. For natural lake water samples, you should use 0.5 to 1 mL. For concentrated samples such as cultures or sewage treatment ponds, you will need much less (10-100 µl). Ideally, you should end up seeing around 20-30 bacteria per grid under the microscope. The volumes stated above should put you in that range.

Once you have decided on an appropriate volume for staining, place an Irgalan black stained filter on the filtration tower. Pipet the sample to be counted onto the filter. Let's assume you are doing a lake water sample and you want to filter 500 µl. After adding the sample to the tower, pipet 200 µl of the DAPI working solution into the filtration tower. Then add 1500 µl of MQW to the vial. Pipet this vigorously in order to mix the DAPI and the sample and to ensure uniform staining. You will need to use 100 µl of the working solution for each 1000 µl of sample. For example, if you were scanning sewage lagoon water, you may choose to add 50 µl of sample, 100 µl of DAPI working solution, and 950 µl of MQW. If you are checking the D.I. system, you obviously don't need to dilute with MQW , but you still need to add 100 µl of DAPI for each 1000 µl of sample.

Allow the sample to stain for 5 minutes in the filtration tower, and then filter the water under a gentle vacuum (<5 PSI).

Take a glass microscope slide, and place a drop of low fluorescence immersion oil near the middle of the slide. Use a cover slip or clean slide to spread the oil thinly. Tilt the slide to allow the oil to form a thin coat over most of the slide. With a forceps, remove the filter and gently drag it across the slide. The thin layer of oil on the slide will help to seat the filter evenly on the slide. It is very important to have the filter lie flat on the slide without creases or air bubbles. Now add one drop of immersion oil directly to the filter, taking care not to touch the filter. Place a cover glass on top of filter. Again, the oil on the filter will help seat the cover glass evenly on the slide. It is important to avoid air bubbles.

Counting bacteria Turn on the mercury lamp on the fluorescence scope. Allow it to warm up for several minutes prior to use. Add a drop of immersion oil to the top of the cover glass and place it on the microscope stage. Using the high power (1000x) objective, lower the objective to the slide. Once the slide hits the oil, you should see a "flash" of blue light. At this point it is often helpful to turn on the white substage light and slowly focus down until the surface of the filter is in focus. Turn off the white

light, and you should see your bacterial samples glowing bright blue (sometimes they are bright yellow) against a dark background.

Randomly count ten different grids on the filter. On occasion you may see larger organisms in the field of view. Since DAPI stains all DNA, these larger organisms will be very bright and may outshine any bacteria. If you happen upon such an organism, skip that field of view and randomly move to another. The stain will degrade rapidly while illuminated by the UV light, so if you see something of interest that you wish to show someone else, close the slider on the back part of the light tube. Similarly, if you plan to take a photograph of the field of view, the long exposure time can result in dimming of the image. You are better off pushing the slider halfway across to reduce the light intensity by 50%. This should give you a clearer picture.

Calculations The number of bacterial cells/mL can be calculated as follows:

cells/mL= [(ave. cell count)/(mL sample x 0.9524) x (conversion factor)]

conversion factor = [(area of funnel/magnified area of grid)]

***Note:** Multiplying by 0.9524 takes into account the diluting effect of the formalin. If water samples are not preserved in formalin, you may eliminate this term.

Table 1. Concentrations of DAPI solution.

Stock Concentration (store frozen)	1 mg/260 µl
Working Solution (store refrigerated)	10 µl Stock/1 mL
Stain solution	100 µl working/1 mL total sample (10 µM final concentration)

Table 2. Amounts of water to filter for different types of water. (Dilute all samples to 2 mL total volume).

Sample Type	Amount to filter
Bacterial culture	5-10 µl
Hypereutrophic water	50-100 µl
Productive Lakes	100-250 µl
Unproductive Lakes	500-2000 µl
D.I. water system	2-5 mL

References

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Revised

P. Aas 2/94; J.Ameel, E.Ruzycki, 4/95

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TOTAL ORGANIC CARBON IN SEDIMENT

General

The total carbon content of sediments can be divided into organic carbon and inorganic carbon. The latter is composed primarily of metal carbonates and bicarbonates that are easily converted to carbon dioxide with dilute acid. Acid treated sediment is dried and the acid resistant organic compounds are burned in a oxygen atmosphere to convert them to carbon dioxide as a measure of the total organic carbon present.

Equipment

1. Laboratory oven set at 70-80° C
2. Heating block to heat vials to 70-80° C
3. 2 dram glass shell vials, 19x51 mm. (Kimble 60965D-2 or equivalent)
4. Balance
5. Pasteur pipets
6. Leco CHN-800 carbon-hydrogen-nitrogen analyzer

Chemicals

1. 10% HCl Dilute ACS grade hydrochloric acid [C] 1+9 with MQW. Allow about 10 mL/sample.
2. EDTA (ethylenediaminetetraacetic acid) or other certified calibration standard

Procedure

The most cautious approach requires several weighing and drying steps if the samples have not been dried previously.

1. Record tare weight of the vial. Weigh about 2 gm wet weight of sediment into the vial. Record total weight.
2. Dry sample at 70°C for 24-48 hours. Cool in desiccator, weigh and record the total weight. Return the sample to the oven and repeat weighing until the weight difference is less than 5% for two consecutive weighing.
3. Use a spatula to grind the dried sediment in the vial. Re-weigh the vial to determine sample loss during the grinding.
4. Slowly add several drops of 10% HCl to the sediment with a Pasteur pipet. Bubbling or effervescence indicates the presence of inorganic carbonates. Wait until the effervescence stops and add several more drops of acid. Continue until acid addition causes no more effervescence. Do not add too much acid at once as the frothing may cause loss of sample.

5. Set up the block heater in a fume hood. Use a vial containing water to set the block heater so the sample temperature is 70-75°C. The block temperature may actually be 80-90°C. Place the vials in the block and heat until the sediment is dried and two consecutive weighings are within 5%. While this may require drying for 2-3 days, do not leave the vials heating and uncovered overnight. (Note: the laboratory oven is not used because of the corrosive nature of the HCl. Once the samples are nearly dry, they can again be heated in the oven.)
6. Use a spatula to grind the dried sediment in the vial and re-weigh the vial to determine sample loss due to the grinding.

7. Analyze the samples for percent carbon on the Leco CHN-800 following the CHN procedure IV.0.40 in this manual. Set the nitrogen flag to "OFF" using "SYSTEM UPDATE" since nitrogen concentrations are unnecessary. Each measurement requires 10-20 mg of dried sample. Analyze a QCCS sample such as Buffalo River sediment (SRM 2704) every 10-15 analyses and check instrument calibration every 20 samples with the standard (usually EDTA) used for calibration.

Calculations

The percent carbon in the dried sample is determined directly by the Leco CHN-800, NRRI Method IV.0.40.

Sample weights can be used to calculate percent solids or moisture in the sample or adjust the concentration if a wet basis TOC is required.

References

United States Environmental Protection Agency. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. Technical Report EPA /CE-81-1. May 1981.

Revised

J. Ameel, 4/95;07/98.

DRY WEIGHT AND ASH FREE DRY WEIGHT

General

This gravimetric approach involves collecting particulate matter on a pre-rinsed and ashed filter, then drying it to a constant weight. For ash free dry weight (AFDW), the dried material is oxidized (ashed) in a muffle furnace and re-weighed. The loss upon oxidation is referred to as AFDW. Note that we consider AFDW to be equivalent to ash free dry mass (AFDM).

Note: The AFDW determination does not distinguish algal material from other organic material (e.g., bacteria, fungi, etc) in the sample, nor does it account for the physiological state of the organic material (i.e. detritus, and/or other senescent material). Therefore if one is interested in algal biomass alone this method may prove unsatisfactory, especially if there is a large fraction of non-algal material in the sample.

Apparatus

1. filtration tower
2. aspiration pump or other vacuum source
3. aluminum weigh pans
4. 4.7 cm Whatman GF/C or Gelman A/E filters
5. drying oven, 103-105EC
6. muffle furnace, 550∀50EC
7. analytical balance

Procedure

Filter preparation: Insert filter with wrinkled side up on filtration apparatus. Apply vacuum and wash with three successive 20 mL portions of MQW. Continue suction to remove all traces of water, discard the washings. Remove filter and transfer to a labeled aluminum weigh pan. Let dry to a constant weight (103-105EC). If AFDW is to be determined ignite filters at 550∀ 50EC for 15 min in a muffle furnace. Cool in a desiccator to room temperature and weigh. Store in desiccator until needed. Weigh immediately before use.

Sample analysis: Assemble filtering apparatus, seat filter and begin suction. Wet filter with a small volume of MQW to seat it. Filter a measured volume of a well-mixed sample through the filter. Wash with three successive 10 mL volumes of MQW. Carefully remove filter from filtration apparatus and transfer to an aluminum weigh pan. Dry at least 1 h at 103-105EC in an oven, cool in desiccator to room temperature, and weigh. Repeat until a constant weight is obtained or until the weight loss is less than 4% of the previous weight or 0.54 mg whichever is less. For AFDW; after a dry weight is obtained ignite the filter and sample at 550∀ 50EC for 1 h in a muffle furnace. Cool and weigh until a constant weight is achieved.

Calculation

where:

A = weight of filter + dried residue, mg
B = weight of filter, mg

where:

C = weight of filter + residue before ignition, mg
D = weight of filter + residue after ignition, mg

QA/QC

1. Record temperatures of drying oven and muffle furnace
2. Record balance used
3. See QA/QC program and Table I-5.

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Standard Methods for the Examination of Water and Wastewater, Method 10300 C,D. 19th Ed. 1995. American Public Health Association, Washington, D.C.

Steinman, A.D. and G. A. Lamberti. 1996. Biomass and pigments of benthic algae. p297. In AMethods in Stream Ecology@. Hauer, F.R. and G.A. Lamberti (eds). Academic Press , San Diego, CA.

Revision

E. Ruzycki, 07/98

BOD

5-DAY TEST

General

Principle: Dissolved oxygen (DO) is measured initially and after a 5-day incubation at 20EC, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined immediately after the dilution is made, all oxygen uptake, including that occurring during the first 15 min, is included in the BOD measurement.

Grab samples: If analysis is begun within 2 h of collection, cold storage is unnecessary. When this is not possible because the sampling site is distant from the laboratory, store at or below 4EC and report length and temperature of storage with the results. In no case start analysis more than 24 h after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 h of collection. Warm chilled samples to 20EC before analysis.

Apparatus

Incubation bottles: 250- to 300-mL capacity. Clean bottles and stoppers with a detergent, 0.1 N HCl acid soak, rinse thoroughly, and drain before use. Use a waterseal as a precaution against drawing air into the dilution bottle during incubation. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during incubation.

Air incubator or water bath, thermostatically controlled at 20 ∀ 1EC. Exclude all light to prevent possibility of photosynthetic production of DO.

D.O. meter and probe (YSI model 50 or equivalent). The azide method for DO measurement (NRRI Method IV.2.00) can be used if a DO meter is not available.

Reagents

Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4X7H_2O$, and 1.7 g NH_4Cl in about 500 mL Milli-Q water (MQW) and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.

Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4X7H_2O$ in MQW and dilute to 1 L.

Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in MQW and dilute to 1 L.

Ferric chloride solution: Dissolve 0.25 g $FeCl_3X6H_2O$ in MQW and dilute to 1 L.

Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL MQW, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL MQW. This solution is not stable; prepare daily.

Nitrification inhibitor: 2-chloro-6-(trichloro methyl) pyridine ("N-serve").

Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103EC for 1 h. Add 150 mg glucose and 150 mg glutamic acid to MQW and dilute to 1 L. Prepare fresh immediately before use.

Seed: Polyseed□or conditioned bacterial source (see below).

Procedure

Preparation of dilution water: Place desired volume of MQW water in a suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, $FeCl_3$, and NH_4Cl solutions/L of water.

Before use, bring dilution water temperature to 20EC. Saturate with DO by shaking in a partially filled bottle or by aerating with organic-free filtered air. Alternatively, store in cotton-plugged bottles long enough for water to become saturated with DO. Protect water quality by using clean glassware, tubing, and bottles.

Storage is not recommended because nitrifying organisms may develop. Prepare dilution water fresh daily.

Add sufficient seeding material to produce a DO uptake of 0.05 to 0.1 mg/L in 5 days (d) at 20EC. Incubate a BOD bottle full of dilution water for 5 d at 20EC. Determine initial and final DO as in Determination of initial DO section. The DO uptake in 5 d at 20EC should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

Glucose-glutamic acid check: Because the BOD test is a bioassay its results can be influenced greatly by the presence of toxicants or by use of a poor seeding material. Periodically check dilution water quality, seed effectiveness, and analytical technique by making BOD measurements on pure organic compounds and samples with known additions. Use a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a "standard" check solution.

Determine the 5-d 20EC BOD of a 2% dilution of the glucose-glutamic acid standard check solution using the techniques outlined in Seeding through Determination of final DO section. Evaluate data as described in Precision and Bias.

Seeding

Seed source: It is necessary to have present a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise-undisinfected effluents from biological waste treatment plants, and surface waters receiving wastewater discharges contain satisfactory microbial populations. Some samples do not contain a sufficient microbial population (for example, oligotrophic lakewater samples or wastes with extreme pH values). For such samples seed the dilution water by adding a population of microorganisms. The preferred seed is effluent from a biological treatment system processing the waste. Optionally, use a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD values that increase with time of adaptation to a steady high value indicate successful seed adaptation.

Seed control: Determine BOD of the seeding material as for any other sample. This is the *seed control*. From the value of the seed control and a knowledge of the seeding material dilution (in the dilution water) determine seed DO uptake. Ideally, make dilutions of seed such that the largest quantity results in

at least 50% DO depletion. A plot of DO depletion, in milligrams per liter, versus milliters seed should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.1 mg/L. To determine a sample DO uptake subtract seed DO uptake from total DO uptake. The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L. Techniques for adding seeding material to dilution water are described below.

Sample pretreatment: Samples containing caustic alkalinity or acidity, residual chlorine compounds or other toxic substances require special treatment. Refer to Standard Methods when working with such samples. Samples containing more than 9 mg DO/L at 20EC may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturation at 20EC by bringing sample to about 20EC in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

Sample temperature adjustment: Bring samples to 20 ∀ 1EC before making dilutions.

Nitrification inhibition: If nitrification inhibition is desired add 3 mg 2-chloro-6-(trichloro methyl) pyridine (TCMP) to each 300-mL bottle before capping or add sufficient amounts to the dilution water to make a final concentration of 10 mg/L. (NOTE: Pure TCMP may dissolve slowly and can float on top of the sample. Some commercial formulations dissolve more readily but are not 100% TCMP; adjust dosage accordingly.) Samples that may require nitrification inhibition include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen inhibition in reporting results.

Dilution technique: Dilutions that result in a residual DO of at least 1 mg/L and a DO uptake of at least 2 mg/L after 5 d incubation produce the most reliable results. Make several dilutions of prepared samples to obtain DO uptake in this range. Experience with a particular sample will permit use of a smaller number of dilutions. A more rapid analysis, such as COD, may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following dilutions: 0.0 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters.

Prepare dilutions directly in BOD bottles. Prepare replicate bottles for dilution water blanks, seed controls and glucose standards, and include at least one set of replicate samples in each analysis.

Using a graduated cylinder, add the desired sample volume to individual BOD bottles of known capacity. Prepare commercial seed inoculum as per manufacturer's suggested procedure. Add appropriate amounts of seed material to the individual BOD bottles. Fill bottles with enough dilution water so that insertion of stopper will displace all air, leaving no bubbles. For dilutions greater than 1:100 make a primary dilution in a graduated cylinder before making final dilution in the bottle. If the membrane electrode method is used for DO measurement, prepare one BOD bottle for each dilution. Determine initial DO on this bottle and replace any displaced contents with dilution water to fill the bottle. Stopper tightly, water-seal, and incubate for 5 d at 20EC. Rinse DO electrode between determinations to prevent cross-contamination of samples. Read blanks first and glucose standards last.

Determination of initial DO: If the sample contains materials that react rapidly with DO, determine initial DO immediately after filling BOD bottle with diluted sample. If rapid initial DO uptake is insignificant, the time period between preparing dilution and measuring initial DO is not critical.

Use the azide modification of the iodometric method (Standard Methods 4500-O.C, 18th Ed.) or the membrane electrode method (Standard Methods 4500-O.G, 18th Ed.) to determine initial DO on all sample dilutions, dilution water blanks, and, where appropriate, seed controls.

Dilution water blank: Use a dilution water blank as a rough check on quality of unseeded dilution water and cleanliness of incubation bottles. Together with each batch of samples incubate duplicate bottles of unseeded dilution water. Determine initial and final DO. The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L.

Incubation: Incubate at 20EC ∀ 1EC BOD bottles containing desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks. Water-seal bottles as described in Dilution technique section. Place sample in the dark.

Determination of final DO: After 5 day s incubation, determine DO in sample dilutions, blanks, and checks.

Calculation

When dilution water is not seeded:

When dilution water is seeded:

where:

D_1	=	DO of diluted sample immediately after preparation, mg/L,
D_2	=	DO of diluted sample after 5 d incubation at 20EC, mg/L,
P	=	decimal volumetric fraction of sample used,
B_1	=	DO of seed control before incubation, mg/L,
B_2	=	DO of seed control after incubation, mg/L, and
f	=	ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control).

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification is inhibited.

If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated above. If the dilution water does not meet these criteria, proper corrections are difficult and results become questionable.

Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months: 14
Number of triplicates: 421
Average monthly recovery: 204 mg/L
Average monthly standard deviation: 10.4 mg/L

In a series of interlaboratory studies, each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, $\bar{X}$, and standard deviation, S, from these studies were:

$$\bar{X} = 0.658 \text{ (added level, mg/L)} + 0.280 \text{ mg/L}$$
$$S = 0.100 \text{ (added level, mg/L)} + 0.547 \text{ mg/L}$$

For the 300-mg/L mixed primary standard, the average 5-day BOD would be 198 mg/L with a standard deviation of 30.5 mg/L.

Control limits: Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, for each laboratory, establish its control limits by performing a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ∀ 3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If control limits are outside the range of 198 ∀ 30.5, re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water.

Working range and detection limit: The working range is equal to the difference between the maximum initial DO (7 to 9 mg L) and minimum DO residual of 1 mg/L multiplied by the dilution factor. A lower detection limit of 2 mg/L is established by the requirement for a minimum DO depletion of 2 mg/L.

QA/QC

Reference solution of glucose/glutanic acid run with each batch.
Seeded and unseeded dilution water blanks.
Read and record temperature of incubator.
See QA/QC program and Table I-5.

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Revised

C.A. Tikkanen, C.J. Owen, 03/92; J. Henneck, 07/98

SEDIMENT OXYGEN DEMAND

General

The sediment oxygen demand procedure measures the oxygen consumption rate of sediment. Sediments are generally in a reduced chemical state and have a potential to remove oxygen from overlying water. This results from the migration of dissolved oxygen to the sediment water interface followed by subsequent chemical reaction and/or the migration of reduced chemical species (ferrous iron, manganous manganese, sulfide) from the sediments to the overlying water followed by subsequent oxidation. In addition to chemical processes, biological organisms in the sediment consume oxygen which has diffused into the sediments from overlying water.

Apparatus

1. dissolved oxygen meter
2. BOD probe with stir attachment
3. AC adjustable voltage regulator
4. petri dishes
5. weighted petri dish cover with attached string
6. wide-mouth cylindrical jars with screw caps and seals (one quart mason jars work well)

Procedure

1. Weigh a subsample of moist, blended sediment and place in a petri dish. Alternatively, place a known volume of sediment in the petri dish.
2. Place the petri dish in the bottom of the jar and cover with the weighted, removable cover.
3. Fill the jar with the desired incubation water of known dissolved oxygen (DO) mg/L (generally saturation at room temp.).
4. Carefully remove the weighted cover so as not to disturb the sediment.
5. Top the jar off with incubation water, and seal.
6. With each batch of samples also include an incubation water control, which consists of a jar filled with incubation water only.
7. Incubate the jars in the dark at a constant temperature.
8. Record dissolved oxygen readings at various time intervals. The number and frequency of readings will depend on the oxygen uptake rate of specific samples. To measure dissolved oxygen, remove the sealed cap and carefully replace the weighted petri dish cover over the sediment sample. Insert the BOD probe to a depth of approximately 2-3 cm. Turn on the stirring attachment but adjust the voltage supply to achieve a desirable reduction in stirring velocity (36% of normal speed seems to work well). Reduced stir speed helps to avoid sediment resuspension. Record time, temperature, and dissolved oxygen for each sample and control.
9. After measurement, carefully remove petri dish cover, top jar off with incubation water, and re-seal.

Calculations

Calculate the initial amount of oxygen in the jar based on its volume and on the initial oxygen concentration as follows:

$$A_o = (V_o)(C_o)$$

where:

A_o = amount of oxygen, mg
V_o = volume of water used, L
C_o = oxygen concentration at time zero, mg/L

The rate of oxygen uptake can then be calculated based on the change in the amount of oxygen in the chamber:

where:

M = rate of oxygen uptake, mg/m^2/day
A_1 = calculated amount of oxygen in the chamber at time 1, mg
A_2 = calculated amount of oxygen in the chamber at time 2, mg
t_1 = elapsed time from the start of the test to time 2, d
t_2 = elapsed time from the start of the test to time 1, d
s = sediment area, m^2

QA\QC

See QA/QC program and Table I-5.

Reference

E.P.A. and USACE, Procedures for Handling and Chemical Analysis of Sediment and Water Samples. 3-399.

Revised

C.A. Tikkanen, R. Axler, 03/92; E. Ruzycki, 08/98

Inorganics – Non-Metallic

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ACIDITY (TITRIMETRIC)

General

This method is applicable to rain, surface and other waters of pH less than 8.3. The method measures the concentration of strong and weak acids that react with hydroxyl ions. This includes the dissolved gases that are present. Samples are titrated with 0.02 N carbonate free NaOH solution. The end point is determined with a pH meter. Results are reported as microequivalents (μeq) per liter.

The range of this method depends on the volume of sample titrated and upon the precision that the increments of titrant can be measured. If only 10 mL of sample is available for analysis, it is necessary to use a 50 μl syringe for dispensing the titrant in order to achieve a precision of less than 10 μeq/L. Samples with an initial pH between 4.3 and 8.3 are subject to error due to the loss or gain of dissolved gases during sampling, storage and analyses. See Table I-2 for sample preservation and holding times and Appendix A for calibration of pH meters.

Note: for samples analyzed for the EPA Clean Water Program, EPA Method 305.1 is required.

Apparatus

1. pH meter and electrode(s).
2. Micro buret or microsyringes.
3. Teflon or glass stir bars and magnetic stirrer.
4. Beakers or flasks.

Reagents

Standard sodium hydroxide solution, 1 N: Dissolve **40g NaOH [*C]** in 250 mL distilled water. Cool and dilute to 1 liter with CO_2 free distilled water. Store in a polyethylene bottle fitted with a soda lime tube or tight cap to protect from atmospheric CO_2.

Standard sodium hydroxide titrant, 0.02 N: Dilute 20.0 mL of **1 N NaOH [*C]** with CO_2-free distilled water to 1 liter. Store in rubber stoppered bottle. Protect from atmospheric CO_2 by using a soda lime tube. Standardize against a 0.02 N potassium hydrogen phthalate solution (KHP) prepared by dissolving 4.085 g of anhydrous $KHC_8H_4O_4$ in CO_2-free distilled water. Dilute KHP to 1000 mL with CO_2-free distilled water.

> Note: Never store hydroxide solutions in a glass stopped bottle or volumetric flask as the hydroxides will etch the joint and cause it to bind irreversibly. Always transfer sodium hydroxide solutions to plastic bottles with plastic or rubber caps.

CO_2-free water: Analytical grade deionized water from the Millipore Milli-Q (or equivalent) system has been found to be CO_2 free. A commercially available system of mixed bed anion-cation resins (e.g. Culligan system with ~1 megohm-cm resistivity) has produced acceptable blanks. Alternatively, water may be boiled for ~5 min. under a N_2 atmosphere.

Procedure

1. Pipet an appropriate aliquot of sample into beaker or flask containing a small teflon or glass stirring bar. Use extreme care to minimize the sample surface disturbance.
2. Immerse pH electrode(s) into sample and stir at a rate that does not cause sample surface disturbance.

3. Titrate with 0.02 N NaOH to pH 8.3. Titration should be made as quickly as possible to prevent absorption of atmospheric CO_2. Record volume of titrant.

Calculation

$$\text{Acidity}, \frac{\text{ueq}}{\text{L}} = \left[\frac{\text{mL}_B}{\text{mL}_S}\right] * \text{N}_B * 10^6$$

Where:

$\mu eq/L$ = microequivalents per liter
mL_B = mL of NaOH titrant
mL_S = mL of sample
N_B = normality of titrant

QA/QC

See QA/QC program and Table I-4.

References

APHA method. Standard Methods for the Examination of Water and Wastewater, 2310 A. 18th Ed. 1992. American Public Health Association, Washington DC.

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 305.2. 1983. EPA 600 4-79-020.

Revised

C.J. Owen, 03/92: J.J. Ameel, 07/97

Hazard

***C See Hazard section under Safety-QA/QC.**

ALKALINITY AND ACID NEUTRALIZING CAPACITY (ANC) (TITRIMETRIC)

General

Alkalinity of a water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates, or other bases if these are present.

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points, see Table IV-1.

For samples of low alkalinity (less than 20 mg $CaCO_3$/L [< 400 µ equiv/L]) use an extrapolation technique based on the near proportionality of the concentration of hydrogen ions to excess of titrant beyond the equivalence point (i.e., two end point or Gran Plot). The amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a simple extrapolation can be made to the equivalence point.

End points: When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. The following pH values (Table IV-1) are suggested as the equivalence points for the corresponding alkalinity concentrations as milligrams $CaCO_3$ per liter. "Phenolphthalein alkalinity" is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the colored indicator, if any, used in the determination. The sharp end-point color changes produced by metacresol purple (pH 8.3) and bromcresol green (pH 4.5) make these indicators suitable for the alkalinity titration. See Appendix 1 for pH meter calibration.

Table IV.0.20-1.

	End Point pH for:	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity, mg $CaCO_3$/L:		
30	4.9	8.3
150	4.6	8.3
500	4.3	8.3
Silicates, phosphates known or suspected	4.5	8.3
Routine or automated analyses	4.5 - 4.2	8.3
2-end point	4.5 and 4.2	--
Industrial waste or complex system	4.5	8.3

Interferences

Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample. See Table I-2.

Apparatus

1. pH meter (Corning 150 & 250 meters) equipped with low ionic strength electrodes (Orion Ross pH and Reference Electrodes or combination electrode)
2. Microburet - 2 mL
3. Thermometer
4. Magnetic stirrer and stir bar
5. Top loading balance
6. Wood block to prevent stir plate from heating sample

Reagents

0.04N H_2SO_4 titrant: Dilute 2.27 mL **concentrated H_2SO_4 [*C]** to 2 liters with Milli-Q water. Standardize using NRRI method IV.0.25 against a 100 µeq /L THAM solution prepared by dissolving 1.2114g dried THAM in 100 m/L Milli-Q water in a 1000m/L volumetric flask. Dilute to the mark with MQW.

Procedure

Potentiometric titration to preselected pH: Determine the appropriate end-point pH according to Table IV-1. Titrate to the end-point pH without recording intermediate pH values and without undue delay using standardized titrant. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant.

Potentiometric titration of low alkalinity: For alkalinities less than 20 mg $CaCO_3$/L titrate 100 to 200 mL using a 10-mL microburet and 0.04 N standard acid solution. Stop the titration at a pH in the range 4.2 to 4.5 and record volume and exact pH. Carefully add additional titrant to reduce the pH exactly 0.30 pH unit and again record volume. Stir while adding titrant. Do not stir when reading end point.

Calculations

a. Potentiometric titration to end-point pH:

where:

A = mL standard acid used and

N = normality of standard acid

or

where:

t = titer of standard acid, mg $CaCO_3$/mL.

Report pH of end point used as follows: "The alkalinity to pH ______ = ______ mg $CaCO_3$/L" and indicate clearly if this pH corresponds to an inflection point of the titration curve.

b. Potentiometric titration of low alkalinity (2-end point):

or:

where:

B = mL titrant to first recorded pH, (pH=4.5)
C = total mL titrant to reach pH 0.3 unit lower, and
N = normality of acid.

Alkalinity as mg $CaCO_3$/L can be converted to µeg/L by multiplying by 20.

QA/QC

See QA/QC program and Table I-4.

A low-ionic-strength buffer (LISB) is read and recorded at the start and finish of a run. Record this value on QA/QC sheets. To make the LISB, see NRRI method IV.0.25.

Reference

Standard Methods for the Examination of Water and Wastewater, Method 2320B. 18th Ed., 1992. American Public Health Association, Washington, D.C. (Acceptable for both the EPA Clean Water Program and Safe Drinking Water Program)

U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, Method 310.1, 1983 EPA/600/4-79-OZO-Revised March 1983.

Revised

C.J. Owen, 03/92; J.J. Ameel, 01/01

Hazard

***C See Hazard section under Safety-QA/QC.**

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ALKALINITY - Gran plot method

General

Alkalinity is due mainly to bicarbonate (HCO_3^-), carbonate ($CO_3^=$), organic anions, and hydroxide (OH^-). It is determined by titration with a strong mineral acid (H_2SO_4). Ten measurements of pH and volume of titrant are obtained beyond the equivalence point. These data points are then extrapolated back to the equivalence point using a Gran plot. This is the most precise analytical method for potentiometric acid-base titration (Rossotti and Rossotti, 1965 and Galloway et.al., 1979).

Projects involving acid precipitation and low ionic strength surface waters require precision of measurement beyond that required for day-to-day water chemistry of surface waters. This method contains information for Afine tuning@ alkalinity by compensating for small errors from CO_2 by measuring partial pressures (pCO_2). Minor errors that may result from drift in pH readings are checked by standardizing a dilute H_2SO_4 solution giving a low pH that is considered relatively stable (low ionic strength buffer (LISB)). These procedures are listed in the method as Aoptional@ for reference purposes. The LISB is used as part of the normal laboratory procedure as a check on pH calibration and instrument drift but it is not calibrated as rigorously as required for acid precipitation studies.

Reagents

1. 0.04 N H_2SO_4 titrant (approximate) - dilute 2.27 mL conc H_2SO_4 to 2000 mL using deionized Milli-Q water (MQW)
2. 0.1 N H_2SO_4 (approximate) - dilute 2.8 mL concentrated H_2SO_4 to 1000 mL with MQW
3. Low ionic strength buffer (LISB): ($10^{-3.8}$ N H_2SO_4 (approximate)) - dilute 1.6 mL of 0.1 N H_2SO_4 to 1000 mL
4. 0.1N NaOH (approximate) - dissolve and dilute 4 g NaOH to 1000 mL

Apparatus

1. pH meter equipped with low ionic strength electrodes (Orion Ross pH and Reference Electrodes)
2. Calibrated microburet - 2 mL
3. Buret - 50 mL (for standardization)
4. Magnetic stirrer and stir bar
5. Top loading balance
6. Thermometer (optional)
7. Bubbling system (pump and fritted gas dispersion cylinder) (optional)

Standardization of Reagents

0.040 N H_2SO_4 titrant: Dry tris(hydroxymethyl)aminomethane (THAM) for two hours at 100° -120°C, then cool in desiccator. Dissolve and dilute 1.2114 g of dried THAM in 100 mL for a 100 meq/L THAM solution. Dilute 1 mL of 100 meq/L THAM to 1000 mL to obtain the 100 μeq/L THAM used for standardization. Weigh out 100 g aliquots of the 100 μeq/L THAM on the top loading balance. With the acid in the 2 mL microburet, titrate the base, keeping the tip of microburet below the surface of the solution. Take 10 readings of pH and volume beyond the equivalence point between pH 4.5 and 3.6. Do four determinations and use the Gran plot to determine the equivalence point volume. A Quattro

spreadsheet, AGRANplot template2.wb3", is available at \\TYR\projects\an_lab to calculate the normality of the acid titrant or the alkalinity of a sample. The normality of the titrant is found by:

Optional standardization:

$10^{-3.8}$N H_2SO_4 (optional): To standardize LISB, dry tris-(hydroxymethyl) aminomethane (THAM) for two hours at 100° -120°C, then cool in desiccator. After cooling, accurately weigh 19.20 mg THAM on the Cahn electrobalance. Dissolve and dilute to 1000 mL for a $10^{-3.8}$ N THAM solution. Weigh 25 mL aliquots of the THAM solution (1 g = 1 mL) using the top loading balance. With the acid in the 50 mL buret, titrate the base. Take 10 readings of both pH and volume of acid used beyond the equivalence point (pH 4.5 to 3.6). (pK of THAM = 5.90) Construct a plot of the Gran function ($F=(V_o+V)10^{-pH}$, where V_o = initial volume of base, V = volume of titrant) vs. mL of acid used. Extrapolate to F = 0 to obtain the equivalence point volume. The normality of the acid can then be determined by:

0.1N NaOH (optional): To standardize NaOH for pCO_2 determination, dry potassium acid phthalate (KHP) for two hours at 110° -120°C, then cool in desiccator. After cooling, accurately weigh 20.4230 g KHP. Dissolve and dilute to 1000 mL for a 0.100 N KHP solution. Take approximately 50 mL aliquots of the KHP solution, and add 3 drops of phenolphthalein indicator. Use a 50 mL buret filled with the 0.1 N NaOH to titrate the acid to the first permanent pink end point. The normality of the base is obtained by:

Standard procedure

1. After the pH meter has been standardized with pH 7 and pH 4 buffers, always check the pH of the low ionic strength buffer ($10^{-3.8}$ N H_2SO_4). (Note: Always record the slope of the meter, the normality of the titrant and the buret number at the beginning of any run.)
2. Warm samples to room temperature
3. Weigh 35 - 50 g of sample on the top loading balance. Place stir bar in solution, stir for 30 seconds, stop and record initial pH when drift ceases.
4. Titrate to pH 4.4 with standardized 0.040 N H_2SO_4. Add titrant while stirring. Stop and allow pH to stabilize before recording pH and titrant volume. Note: If a 2-endpoint pH is to be determined as well as a Gran plot, the first reading should be at pH 4.5. One of the remaining Gran plot readings should be 0.3 pH units below pH 4.5.
5. Titrate nine additional points between pH 4.4 and 3.6 recording pH and titrant volume for each. Be careful in reading the buret.
6. Calculate alkalinity using the formulas below or the Quattro spread sheet, AGRANplot template2.WB3".

7. At the end of a run, check and record the pH of LISB, pH 7 buffer and pH 4 buffers.

Optional procedures

After the pH meter has been standardized with pH 7 and pH 4 buffers, always check the pH of the low ionic strength buffer ($10^{-3.8}$ N H_2SO_4). If the pH is not equal to pConc., then the difference must be subtracted from or added to each of the pH measurements.

pCO_2 Determination: Take approximately 100 mL, as determined on the top loading balance, of freshly distilled deionized water and determine its pH. Add 1.0 mL of 0.1N NaOH to the water and again determine its pH. Take the temperature of the solution. With the electrodes still immersed in the solution, bubble with the bubbling apparatus and stir the solution for about 45-50 minutes or until the pH of the solution has stabilized. The pH should stabilize around 8.3. Record the pH value. After the pH has stabilized, the solution can be titrated with the 0.04 N H_2SO_4 titrant in the 2 mL microburet. Take 10 readings within the pH range of 4.4 to 3.6. After each acid addition, the solution should be bubbled and stirred for approximately 15 seconds. Again, use the Gran plot to determine the equivalence point volume. The alkalinity of the solution (which should be the same as the Na^+ conc. in the solution) is found by:

and the pCO_2 is:

where:

A = Alkalinity of standard bicarbonate (equiv/L)

$[H^+] = 10^{-pH}$ of bicarbonate solution at air equilibrium

$K_1 = 10^{(6.57-0.00926 \times T)}$

$K_2 = 10^{-(10.614-0.0117 \times T)}$

$K_H = 10^{-(1.13+0.0131 \times T)}$

$K_w = 10^{(-4470.99/T+273.15) + 6.0875 - 0.01706(T+273.15)}$

T = °C

Sample procedure (optional)

If the sample has been cooled, allow it to come to room temperature. Weigh out approximately 100 mL of sample on the top loading balance and record the weight to the nearest 0.1 g. Take an initial pH and temperature reading of the sample. Bubble and stir the sample for approximately 15 minutes or until the pH of the sample has stabilized. After bubbling, and before acid addition, the pH and the temperature of the sample is again taken. The average of the two temperature readings is used to determine the concentrations of HCO_3^-, $CO_3^=$, and H_2CO_3. Titrate the sample with the 0.02N H_2SO_4 titrant and take 10 readings within the pH range of 4.4 and 3.6, bubbling and stirring the sample for approximately 15 seconds after each acid addition. Use the Gran plot to determine the equivalence point volume and the

alkalinity. The pCO_2 (as determined from above), the average temperature reading, and the initial pH after bubbling can be used to determine the concentration of the species HCO_3^-, $CO_3^=$, and H_2CO_3.

$$H_2CO_3 = K_H \cdot pCO_2$$

$$HCO_3^- = K_1 K_H \cdot pCO_2/H^+$$

$$CO_3^= = K_1 K_2 K_H \cdot pCO_2/(H^+)^2$$

$$OH^- = K_w/H^+$$

Total dissolved inorganic carbon (DIC in umoles/L) is calculated from:

$$DIC = HCO_3- + CO_3^= + H_2CO_3$$

and the carbonate alkalinity (Alk_{carb}) from:

$$Alk_{carb} = HCO_3^- + 2CO_3^= + OH^- - H^+$$

The noncarbonate Alkalinity ($Alk_{noncarb}$) is calculated from:

$$Alk_{noncarb} = Alk_{total} - Alk_{carb}$$

A computer program may be used to compute each of the terms above.

References

Galloway, J.N., Cosby, B.J., Jr., and Likens, G.E. 1979. Acid Precipitation: Measurement of pH and acidity. Limnol. Oceanogr. 24, 1161-1165.

Rossotti, F.J.C., and Rossotti, H. 1965. Potentiometric titrations using Gran plots. J. Chem. Ed. 42, 375-378.

Revised

R.B. Cook, J.J. Ameel, C Kelly, 08/84; J.J. Ameel, 07/98

DISSOLVED INORGANIC CARBON
GAS STRIPPING METHOD

General

A sample containing CO_2, HCO_3- and CO_3= is acidified to convert carbonate and bicarbonate to H_2CO_3. Agitation of the sample with helium partitions gaseous CO_2 out of solution into the gas phase. A loop controlled volume of the He_2-CO_2 mixture at atmospheric pressure is then swept through a water vapor trap into an infrared detector.

Nitrogen can be used as the partitioning gas with decreased precision.

Samples for dissolved inorganic carbon (DIC) are collected and sealed in 50 mL polypropylene syringes. They should be stored immediately at 4EC and run within 24 hours of collection. Contact with air should be minimized since mixing of the sample with air will change the equilibrium of the sample due to the CO_2 in the air. The samples should not be acidified, filtered or frozen. The operating range for this procedure is ~10 - 2000 umoles/L (0.12 - 24.0 mg/L) total inorganic C. See Table I-2.

Apparatus

1. Infrared detector (Horiba PIC-2000 or equivalent).
2. Nitrogen or compressed helium (partitioning gas) are to operate sample valve
3. Gas sampling loop of 0.5 - 2.00 mL volume.
4. Strip Chart Recorder, Fisher Recordall Series 5000 or equivalent.
5. 50 mL polypropylene syringes - for sample collection.
6. Syringe for dispensing 1 mL volume of acid.
7. Plastic 3-way Luer-lok stopcock (Baxter/SP S8965-1).

Reagents

CO_2-free water: Analytical grade deionized water from the Millipore Milli-Q (or equivalent) system has been found to be CO_2 free. A commercially available system of mixed bed anion-cation resins (e.g. Culligan system with ~1 megohm-cm resistivity) has produced acceptable blanks. Alternatively, water may be boiled for ~5 min. under a N_2 atmosphere.

1 N Sulfuric acid: Dilute 28 mL of **concentrated H_2SO_4 [*C]** to 1 liter with distilled water.

DIC stock standard: 100 mg C/L stock. Dissolve 0.700 g of dry $NaHCO_3$ in CO_2-free water. Take to 1000 mL with minimum aeration. (1.0 mL = 0.100 mg C). Prepare daily.

Instrument Operation

1. Turn on nitrogen carrier to the Horiba IR detector. Set flow to 150 mL/min.
2. Turn on power to the IR detector and allow it to warm up for at least 1 hour. NOTE: If sample sets are expected over a period of weeks, do not turn the detector off. Maintain a low nitrogen flow of 20 mL/min while it is on.
3. Replace the Drierite in the drying tube in the sample line to the valve. This scrubs acidic water vapor from the sample to prevent damage to the IR cell.

4. Check the Zero on the IR detector with the **Range** switch in **Check** (first switch position with no number). Adjust the **Zero** control to the meter "0". The meter should read "0" when the

control is set at 7.2 ∀ 0.3. If the control reading exceeds this range, the detector should be set to zero by:

a. Set the **Range** switch to **Check**. Be sure that nitrogen is flowing through the detector at 150 mL/min.
b. Turn **Zero** and **Span** fully clockwise to 10.
c. Adjust the optical trimmer to the null point (lowest meter reading). This is the small black lever at the back of the IR cell.
d. Adjust the chopper motor thumb screws to low null. The chopper motor thumb screws have knurled heads and are located on the stainless steel block just ahead of the optical trimmer lever. These must be turned together but in opposite directions. They normally will not have to be adjusted.
e. Move the optical trimmer to the right until the meter reads 50% full scale.
f. Zero the meter with the **Zero** control.
NOTE: One check for a dirty IR flow cell is to turn the **Zero** to 10 and check for a 50% meter reading. If the needle does not return to that setting, the cell may require cleaning.

5. Turn on the strip chart recorder. Set the range to 1 volt and chart speed to 0.5 cm/min (5 cm/min) 10). Turn the range switch to **Check** and use the **Zero** control to set the recorder zero to "0" or "5" depending on your preference. A setting of "5" will allow for negative drift from the detector. Reset the **Range** to 1 volt.
6. Select the detector range and set the span by injecting high standard. Adjust the detector span to maximize the peak on the recorder to 80-90% full scale. The display meter operates only in the **Check** position. The **Range** switch positions 1, 2 and 3 are roughly equivalent to 20, 50 and 200 ppm carbon.
7. Instrument shut down: Turn off the nitrogen to the sample valve. Reduce the carrier flow to 20-50 mL/min if the instrument will be left on or turn the carrier flow off if the instrument will be turned off. If additional sample sets will be run, leave power to the instrument. Otherwise, turn it off.

Procedure

1. Prepare a series of standards to cover the range of samples to be analyzed using CO_2-free water to dilute the stock CO_2 standard. For standards in the 0-14 mg C/L we use:

Standard Concentration (mg C/L)	mL 100 mg/L stock per 250 mL
0	0
2	5
4	10
6	15
8	20
10	25
12	30
14	35

2. Rinse 50 mL syringe with blank, standard or sample. Draw up about 25 mL of solution. Allow all samples and standards to equilibrate at room temperature for ~1 hour. The partitioning of CO_2 between liquid and gas phases is temperature dependent, so it is essential that samples and standards be analyzed at the same temperature.
3. Place 3-way stopcock on end of standard or sample syringe. Open valve and discard sample in excess of 20 mL.
4. Inject 1 mL of 1 N H_2SO_4 through the side connection on the 3-way valve using the acid dispensing syringe.
5. Close off the acid using the 3-way valve. Wipe the end of the valve with a Kim-wipe to remove any acid residue and inject helium at a slow rate (<100 mL/min) into the syringe until the plunger is at the 50 mL mark. Remove from the helium source, allow the pressure to reach ambient and close the 3-way valve.
   NOTE: It is important to maintain identical sample and gas volumes in each syringe.
6. Agitate the sample vigorously. Shake at least 40 times and be consistent from sample to sample.
7. Hold the syringe vertically and open the 3-way valve. Expel a small amount of the gas mixture to remove any liquid trapped in the valve. Wipe it off with a Kim-wipe and connect the syringe to the sample loop.
8. Flush the sampling system and loop with about 15 mL of the gas mixture from the high standard syringe. **Be careful not to inject liquid into the loop.**
9. Inject the contents of the loop into the carrier flow of the IR detector. Typically, up to three aliquot of gas can be injected from a syringe (~ 5 mL each).
10. Repeat steps 3-9 with each standard and sample.
11. Data reduction:
    a. Measure peak heights. Average multiple peak heights taken on the same sample or standard.
    b. Calculate the regression line for concentration versus peak height using the regression function on the HP11c calculator. Record the slope, intercept and correlation coefficient for the curve.
    c. Calculate sample concentrations based on the curve.

QA/QC

1. Inject a minimum of two aliquots of gas from the syringe of each standard and sample.
2. Run a mid-range standard after every 5 samples. They should vary less than 10%.
3. Run 10% of the samples in duplicate or as required by the project.
4. See QA/QC program and Table I-4.

Reference

Stainton, M.P., Capel, M.J. and Armstrong, F.A.J. 1977. The Chemical Analysis of Fresh Water, 2nd Ed. Fish. Mar. Serv. Misc. Spec. Publ. 25:166.

Revised

J.J. Ameel and R.P. Axler, 01/93; J.J. Ameel, 07/98

Hazard

***C See Hazard section under Safety-QA/QC.**

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DISSOLVED INORGANIC CARBON
CALCULATION METHOD

General

Dissolved inorganic carbon (DIC) of lake water is the sum of all carbon present as CO_2, HCO_3^-, and CO_3^{-2}. DIC is of interest in evaluating the inorganic carbon available for photosynthesis and in evaluating properties of fresh water for municipal and industrial uses.

The total inorganic carbon of fresh water can be estimated by calculation from measurements of total alkalinity, pH and temperature and factors from Table 1. Increased accuracy, especially for soft or low ionic strength water, can be obtained by directly measuring DIC by acidification of the sample, gas stripping and subsequent analysis by either IR absorption (Method IV.0.30) or GC. The calculation method also does not apply to highly saline water.

Apparatus: None

Procedure

Measure pH and temperature of the water. Determine alkalinity by an appropriate method (single end point, 2-endpoint or Gran plot).

Calculation

Use pH and temperature to determine pH factor from Table 1 on the next page. Multiply the pH factor times total alkalinity as mg $CaCO_3$/L.

> $C_{available}$, mg C/L = (total alkalinity, mg $CaCO_3$/L) X (pH factor from Table 1)
>
> Note: When calculating water column DICs in a lake, the temperature at the sampling depth must be used.

References

Saunders, G.W., F.B. Trama and R.W. Bachmann. 1962. Evaluation of a modified C-14 technique for shipboard estimation of photosynthesis in large lakes. Great Lakes Res. Div. Publ. No. 8. University of Michigan, Ann Arbor.

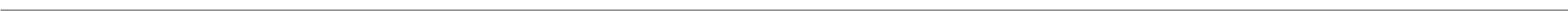
Wetzel, R.G. and G.E. Likens. 1995. Limnological Analyses, 2nd ed. Springer-Verlag, New York., N.Y. 391 p.

Revised

J.J. Ameel, E. . Ruzycki, 07/98

TABLE 1 pH factors for the conversion of total alkalinity to milligrams of carbon per liter (from Saunders, Trama, and Bachmann, 1962)

	Temperature (°C)					
pH	*0*	*5*	*10*	*15*	*20*	*25*
5.0	9.36	8.19	7.16	6.55	6.00	5.61
5.1	7.49	6.55	5.74	5.25	4.81	4.51
5.2	6.00	5.25	4.61	4.22	3.87	3.63
5.3	4.78	4.22	3.71	3.40	3.12	2.93
5.4	3.87	3.40	3.00	2.75	2.53	2.38
5.5	3.12	2.75	2.43	2.24	2.06	1.94
5.6	2.53	2.24	1.98	1.83	1.69	1.59
5.7	2.06	1.83	1.62	1.50	1.39	1.31
5.8	1.69	1.50	1.34	1.24	1.15	1.09
5.9	1.39	1.24	1.11	1.03	0.96	0.92
6.0	1.15	1.03	0.93	0.87	0.82	0.78
6.1	0.96	0.87	0.77	0.73	0.70	0.67
6.2	0.82	0.74	0.68	0.64	0.60	0.58
6.3	0.69	0.64	0.59	0.56	0.53	0.51
6.4	0.60	0.56	0.52	0.49	0.47	0.45
6.5	0.53	0.49	0.46	0.44	0.42	0.41
6.6	0.47	0.44	0.41	0.40	0.38	0.37
6.7	0.42	0.40	0.38	0.37	0.35	0.35
6.8	0.38	0.37	0.35	0.34	0.33	0.32
6.9	0.35	0.34	0.33	0.32	0.31	0.31
7.0	0.33	0.32	0.31	0.30	0.30	0.29
7.1	0.31	0.30	0.29	0.29	0.29	0.28
7.2	0.30	0.29	0.28	0.28	0.28	0.27
7.3	0.29	0.28	0.27	0.27	0.27	0.27
7.4	0.28	0.27	0.27	0.26	0.26	0.26
7.5	0.27	0.26	0.26	0.26	0.26	0.26
7.6	0.27	0.26	0.26	0.25	0.25	0.25
7.7	0.26	0.26	0.25	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.25	0.25	0.24	0.24
8.1	0.25	0.25	0.24	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24	0.24	0.24
8.5	0.24	0.24	0.24	0.24	0.24	0.24
8.6	0.24	0.24	0.24	0.24	0.24	0.24
8.7	0.24	0.24	0.24	0.24	0.24	0.24
8.8	0.24	0.24	0.24	0.24	0.23	0.23
8.9	0.24	0.24	0.23	0.23	0.23	0.23
9.0	0.24	0.23	0.23	0.23	0.23	0.23
9.1	0.23	0.23	0.23	0.23	0.23	0.23
9.2	0.23	0.23	0.23	0.23	0.23	0.23
9.3	0.23	0.23	0.23	0.22	0.22	0.22
9.4	0.23	0.23	0.22	0.22	0.22	0.22



CHLORIDE BY ARGENTOMETRIC METHOD
(Titrimetric)

General

In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed. See Table I-2.

Range: 0.15 - 10 mg Cl^- in the portion titrated. Maximum sample portion required is 100 mL. Analysis is on 100 mL or a portion of sample diluted to 100 mL.

Interferences

Bromide, iodide and cyanide register as equivalent chloride concentrations. Sulfide, thiosulfate and sulfite ions interfere byt can be removed by treatment with hydrogen peroxide. Orthophosphate in excess of 25 mg/L interferes by precipitatin as silver phosphate. Iron in excess of 10 mg/L interferes by masking the end point.

Apparatus

1. 250 mL Erlenmeyer flask
2. 50 mL buret

Reagents

Potassium chromate indicator solution: Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

Standard silver nitrate titrant, 0.0141 M (0.0141 N): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL. Standardize against NaCl (1.00 mL = 500 µg Cl^-). Store in a brown bottle.

Standard sodium chloride, 0.0141 M (0.0141 N): Dissolve 824.0 mg NaCl (dried at 140EC) in distilled water and dilute to 1000 mL; 1.00 mL = 500 µg Cl.

Special reagents for removal of interference

Aluminum hydroxide suspension: (Note: make up only if needed for removal of color from samples.) Dissolve 125 g aluminum potassium sulfate $AlK(SO_4)_2X12H_2O$ or aluminum ammonium sulfate $AlNH_4(SO_4)_2X12H_2O$ in 1 L distilled water. Warm to 60EC and add 55 mL conc ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

Phenolphthalein indicator solution

Sodium hydroxide, NaOH, 1N

Sulfuric acid, H_2SO_4, 1N

Hydrogen peroxide, H_2O_2, 30% **CAUTION: H_2O_2 IS A STRONG OXIDIZER. WEAR SAFETY GOGGLES AND GLOVES WHEN HANDLING.**

Procedure

Sample preparation: Use a 100 mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL $Al(OH)_3$ suspension, mix, let settle, and filter. If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.
Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition. Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

Calculation

where:

A = mL titration for sample,
B = mL titration for blank, and,
N = normality of $AgNO_3$.

QA/QC

See QA/QC program and Table I-4.

References

Standard Methods for the Examination of Water and Wastewater, Method 4500-Cl^- B. 18th Ed. 1992. American Public Health Association, Washington, D.C. (Approved for EPA Clean Water Program)

Revised

C.J. Owen, 03/92; J.J. Ameel, 01/01

FLUORIDE BY ION SPECIFIC ELECTRODE
(Potentiometric)

General

Fluoride is of interest in dilute surface waters because it complexes with Al. This method will measure flouride from about 0.8 to 500 ppm. The water sample is combined with a "total ionic strength adjuster" (TISAB) to provide a constant ionic strength for a background, decomplex fluoride and adjust the solution pH. Measurement requires a pH meter with an mV scale, a fluoride electrode and a reference electrode. Measurements are plotted using 2- or 4-cycle semilogarithmic paper or a logarithmic curve matching program on a calculator or computer.

Apparatus

1. pH meter with mV scale. Corning 250 or equivalent.
2. Fluoride ion specific electrode. Orion 94-09-00 or equivalent.
3. Reference electrode. Orion 90-01-00 filled with Orion filling solution 90-00-01.
4. Magnetic stirrer. A piece of wood or other insulator should be used to thermally isolate the sample from the stirrer to prevent the motor from heating the sample.
5. Plastic labware. Fluoride reacts with silica in glass, so all operations should take place in plastic. If plastic labware is unavailable, contact time with glass should be minimized.

Reagents

100 ppm Sodium fluoride stock: 221.0 mg anhydrous sodium fluoride dissolved in distilled water and diluted to 1000 mL. Store the stock standard in a plastic bottle (1 mL = 100 µg F).

Total ionic strength adjuster (TISAB): Place about 500 mL distilled water in a 1-liter beaker. Add 57 mL glacial acetic acid, 58 g NaCl and 4 g CDTA (cyclohexylene dinitrilotetraacetic acid or 1,2-diaminocyclohexane N, N, N, N - tetraacetic acid). Stir to dissolve. Adjust to pH 5.0 to 5.5 with 5 M NaOH. About 150 mL of NaOH is required. Cool to room temperature. Pour into a 1000 mL volumetric flask and dilute to the mark. Store in 1 liter plastic bottle.

5 M NaOH solution: Place 200 g NaOH pellets [*C] in about 500 mL of distilled water and stir to dissolve. A magnetic stir bar and plate may be necessary to completely dissolve.

CAUTION: Beaker will become very hot! Place in water bath to cool to room temperature. Pour into 1000 mL volumetric and fill to mark. Store in 1 liter plastic bottle. **Do not store in glass or a volumetric flask.**

Procedure

Electrode preparation: Fill the Orion 90-01-00 reference electrode with Orion 90-00-1 filling solution. Connect both electrodes to the pH meter. Set the meter for reading millivolts.

Checking electrode slope (Should be done daily): Place 25 mL of distilled water and 25 mL of TISAB in a plastic beaker on the magnetic stirrer. Add a clean magnetic stirring bar to the beaker. Place the electrode in the solution at a depth of 1 inch.

Pipet 0.5 mL of 100 ppm stock into the solution and stir thoroughly. Let it stabilize until reading remains the same for 15 seconds. Press the stand-by switch (Aread@ or A=@ button) and record the electrode potential in mV. Return the instrument to stand-by between readings.

Add 5 mL of the 100 ppm stock to the solution, stir and again read the potential in mV. The electrode is operating correctly if the difference between the first potential and the second is between 54-60 mV when the solution is between 20 and 25E C. If the potential difference is 54.0 or less, see the troubleshooting check list in the electrode instruction manual.

Working standards

Prepare daily five standards by dilution of the 100 ppm stock. For lake water the following dilutions should be adequate to cover the expected concentration range. Transfer to plastic beakers as soon as possible.

Standard Concentration mg F/L	mL 100 mg F/L Stock	Final Volume
0.01	0.01	100 mL
0.05	0.05	100 mL
0.1	0.1	100 mL
0.2	0.2	100 mL
0.5	0.5	100 mL

Preparation of samples: All standards and samples should be at the same temperature. A 1EC difference in temperature will result in a 2% measurement error. Keep them in a constant temperature water bath between 20 and 25EC.

Samples must be above pH 5 to avoid complexing by hydrogen ions and below pH 7 to avoid hydroxide interference. TISAB is used to maintain these pH conditions.

The TISAB will also break up fluoride complexes with iron and aluminum. If the concentration of either iron or aluminum is greater than 3 to 5 ppm then a more concentrated TISAB IV must be used. Consult the Orion Electrode manual for the preparation of TISAB IV. See Table I-2 for further information on hold times and preservation.

Preparation of a standard curve: Place 25 mL of each standard in plastic beakers. Mix 25 mL of each standard with 25 mL of TISAB.

Before each reading, the electrode should be dipped in a small aliquot of the sample or standard.

Place a magnetic stir bar and the electrodes in the lowest standard and allow to stabilize. Set the meter function switch to mV. Stir and allow to stabilize. Read while stirring. Read each of the remaining standards and record in mV.

Prepare a standard curve by plotting the millivolt readings (linear axis) against concentration (log axis) on standard semilogarithmic paper, or by regression of mV on log concentration using a calculator.

Reading samples: Add 25 mL TISAB to 25 mL of each of the samples. Read each sample in the same way as the standards. If the millivolts read greater than the standards, dilute the sample and read again.

Recalibrate the standard curve every 4 hours of use. If the ambient temperature has not changed, simply place the electrodes in the standard closest to sample readings. Millivolt readings for that standard must be ∀ 10% to continue current standard curve. If more than 10%, recalibrate.

For the standard set of 10-14 samples, recalibrate after every sample set. Check standard curve after every 5 samples. Do a spike every sample set (0.05 ppm Spike = 25 µl of 100 ppm F).

QA/QC

See QA/QC program and Table I-4.

References

APHA method. Standard Methods for the Examination of Water and Wastewater, 4500-F-C. 18th Ed. 1992. American Public Health Association, Washington DC. (Approved for EPA Clean Water Program and Safe Drinking Water Program)

Kissa, Erik. 1983. Determination of fluoride at low concentrations with the ion-selective electrode. Anal. Chem. 55, 1445-1448.

Orion Instruction Manual for the fluoride electrode.

Hewlett-Packard 11C Owner's handbook, pg. 63.

Revised

C.J. Owen, S.H. Randall, J.J. Ameel, 03/92; J.J. Ameel, 07/98

Hazards

***C See Hazard section under Safety-QA/QC.**

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pH OF CLOSED SYSTEM

(Closed head space pH)

Because pH can be affected to some degree by CO_2 in the air, closed headspace pH measurement will minimize air contact. Samples should be collected in 60cc syringe, sealed in the field, and stored on ice for transport to the laboratory. Measurement of pH should be as soon as possible after collection, preferably within 12 hours.

Apparatus

pH meter: Corning 150 or 250
pH electrode: Orion Ross combination electrode
Closed head space chamber (See Fig. IV-3)
Micro stir bar
Magnetic stirrer with wooden block

Procedure

NOTE: Samples and buffers should be at room temperature since temperature compensation probes cannot be used.

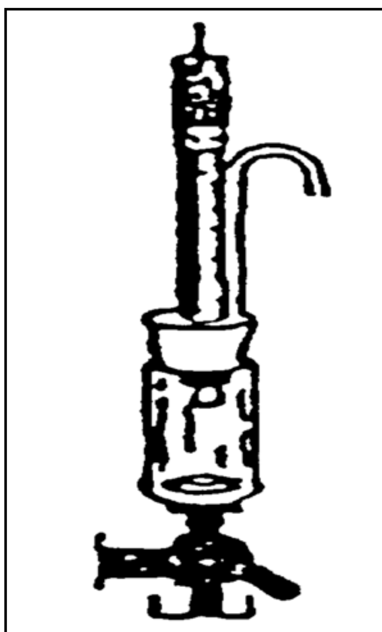

Figure IV-3.

1. Calibrate meter with pH 7 and 4 buffers. Record slope. Check calibration with low ionic strength buffer (LISB). Record pH of the LISB. See Appendix 1 or 2 for calibration of the meter.

2. Place rubber ring with waste tubing over the end of the electrode.

3. Attach sample chamber to the rubber ring so the electrode is in the sample chamber thus creating a closed system (See Figure IV-3). Stand the sample chamber on the stir plate, turn the stir plate on, making sure the stir bar will turn inside the sample chamber.

1. With the stop-cock open, inject about 15 mL of sample into the sample chamber, forcing excess sample already in the sample chamber to a waste container. Be sure to force all air out of the chamber with the solution.

2. Stir the sample for about 30 sec. Turn the stir plate off, let stabilize for about 30 sec to 1 min. (If the pH does not vary by more than 0.02 pH units in one direction throughout a 1 min. interval, the reading is considered stable.) Record this value.

3. Inject about 5 mL of sample into the chamber, repeat step 5.

4. Repeat these two steps (5 and 6) until there are 3 readings in a row within 0.02 pH units (or until the sample runs out)..

5. When measurement of a sample is completed, draw the sample back into the syringe, remove the sample syringe from the stop-cock, cool and discard the sample. Rinse the electrode and sample chamber with DIW and continue with the next sample starting at step 4.
6. When all samples have been measured, remove the sample chamber and rubber ring from the electrode, measure and record the pH of the LISB, pH 7 buffer and pH 5 buffer in that order.

QA/QC

See QA/QC program and Table I-4.

Revised

C.M. Host, C.J. Owen, 03/92; J.J. Ameel, 07/98