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U.S. Army
Environmental
Center

TEST PLAN FOR THE PHYTOREMEDIATION STUDIES OF LEAD-CONTAMINATED SOIL FROM THE SUNFLOWER ARMY AMMUNITION PLANT

Desoto, Kansas

Volume II of II

Prepared for
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401

Prepared by
Tennessee Valley Authority
Environmental Research Center
Muscle Shoals, Alabama 35662-1010

October 1996

TVA Contract No. RG-99712V
Report No. SFIM-AEC-ET-CR-96198

Test Plan
For the Phytoremediation Studies of Lead-Contaminated Soils from the Sunflower Army Ammunition Plant, Desoto, Kansas

Volume II of II

Prepared for
U.S. Army Environmental Center
Environmental Technology Division
Aberdeen Proving Ground, MD 21010-5401
POC: Ms. Darlene Bader

Prepared by
Tennessee Valley Authority
Tennessee Valley Authority Environmental
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Muscle Shoals, Alabama 35662-1010

October 1996

NOTICE

This "Test Plan for the Phytoremediation Studies of Lead-Contaminated Soil from the Sunflower Army Ammunition Plant, Desoto, Kansas" was prepared by employees of the Tennessee Valley Authority (TVA) loaned to the U.S. Army Environmental Center (USAEC) at Aberdeen Proving Grounds, Maryland, 21010-5401, pursuant to the provisions of TVA Contract RG-99712V and Military Interdepartmental Purchase Order Request (MIPR) MIPR9526 dated August 8, 1996.

Under that agreement and MIPR, TVA provided the services mutually agreed upon as loaned employees. In regard to the services provided by the TVA employees, sections d and e of the contract and MIPR state as follows:

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>
> e. It is expressly understood that for all purposes under this MIPR the TVA employees will be acting as loaned employees and will be under the complete supervision and control of the Army at all times and that TVA shall not and cannot supervise or control such employees during the time that they are providing services to the Army. It is further understood and agreed that neither TVA nor any of the loaned employees warrant or guarantee the advice under this agreement and that the Army is solely responsible for determining the suitability and acceptability of such advice and consultations for any purpose. Neither TVA, its agents and employees, nor the loaned employees assume any liability, or responsibility to the Army, its agents, employees, or contractors, or any third party for any costs, charges, damages, (either direct or consequential), demands, claims, or causes of action for any personal injuries (including death) or damage to property, real or personal, or delays arising out of or resulting from any such action or failures to act on the part of such loaned employees whose services are provided under this MIPR.

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TABLE OF CONTENTS

ABBREVIATIONS

AA	-Atomic Absorption
AAP	-Army Ammunition Plant
Cd	-Cadmium
Cr	-Chromium
Cu	-Copper
CDTA	-Cyclohexane - 1,2 - Diaminetetraacetic Acid
CERCLA	-Comprehensive Environmental Response, Compensation, and Liability Act
DANT	-Diaminonitrotoluene
DNT	-Dinitrotoluene
DoD	-Department of Defense
DTPA	-Dieethylenetrinitrilopentaacetic acid
EAAL	-Environmental Applications Analytical Laboratory
EDTA	-Ethylenedinitrilotetraacetic acid
EDX	-Energy Dispensive X-Ray
EGTA	-Ethylenebis(oxyethylenetrinitrlo)tetraacetic acid
ERC	-Environmental Research Center
FIA	-Flow Injection Analyzer
Hg	-Mercury
HPLC	-High Performance Liquid Chromatography
IC	-Ion Chromatography
ICP	-Inductively Coupled Plasma
MDL	-Method Detection Limit
NC	-Nitrocellulose
NG	-Nitroglycerin
NH_4-N	-Ammonia Nitrogen
Ni	-Nickel
NO_3-N	-Nitrate Nitrogen
NQ	-Nitroguanidine
PEL	-Permissible Exposure Limit
Pb	-Lead
P	-Phosphorus
PO_4	-Orthophosphate
PO_4-P	-Orthophosphate - Phosphorus
QA	-Quality Assurance
QC	-Quality Control
Se	-Selenium
SFAAP	-Sunflower Army Ammunition Plant
TKN	-Total Kjeldahl Nitrogen
TOC	-Total Organic Carbon
TVA	-Tennessee Valley Authority
TVAE	-Tennessee Valley Authority Environmental
U.S.	-United States
USACE	-United States Army Corp. of Engineers
USAEC	-United States Army Environmental Center
USEPA	-United States Environmental Protection Agency
USIOC	-United States Army Industrial Operations Command
Zn	-Zinc

APPENDIX B

METHODS AND PROCEDURES

Appendix B-1 – Lab Procedures for Chain of Custody

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : SP-0001

Title: Sample Chain of Custody

Signature	Title	Date
Prepared by: William J. Rogers	QA Officer	11/26/96
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	11/26/96
Concurred:		
Concurred:		
Approved: Joseph J. Hoagland	Manager	11/27/96

Revision	R0	R1	R2			
Control Date	29-Sep-89	10-Jan-96	29-Nov-96			

Copy No: ___ has been issued to holder on ________

"Sample Chain of Custody"

1.0 PURPOSE

This procedure provides instructions for sample custody from collection to final disposition.

2.0 SCOPE

This procedure applies to all samples collected under a sampling plan which requires documentation of sample custody.

3.0 SUMMARY

Requirements for documentation of sample collection and sample custody are specified.

4.0 REFERENCES

4.1 U. S. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," SW-846, 3rd Edition, Most Recent Update (September 1994)

4.2 "Preparation Aids for the Development of Category II Quality Assurance Project Plans," EPA/600/8-91/004, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Develoent, U.S. Environmental Protection Agency, Cincinnati, OH 45268

4.3 "Preparation Aids for the Development of Category III Quality Assurance Project Plans," EPA/600/8-91/005, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Enviromental Protection Agency, Cincinnati, OH 45268

4.4 "Sample Receipt, Log-in, and Data Handling", GLP-0016, Tennessee Valley Authority, Analytical Laboratory of Environmental Applications, Muscle Shoals, AL.

5.0 RESPONSIBILITIES

5.1 The laboratory team leader shall ensure that this procedure is followed.

5.2 The sampler shall follow this procedure to ensure sample integrity in the field.

5.3 The person transporting the samples shall follow the procedure to ensure sample integrity in transit.

5.4 The person receiving the samples shall follow this procedure to ensure sample integrity upon receipt and immediately following.

5.5 Laboratory analysts shall follow this procedure during sample analysis.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Sample containers shall be cleaned to specifications of the sampling plan, or in their absence, to good commercial practice.

6.1.2 Sample containers shall have preservative added before sampling as required by the sampling plan.

6.2 Limitations and Actions

6.2.1 If the sampling organization has its own sampling procedure, sample custody procedure, labels, or custody forms, they may be substituted for the contents of this procedure as permitted by the sampling plan.

6.2.2 The number of persons handling samples from the time of sampling to receipt by the laboratory should be held to a minimum.

6.2.3 Sample containers shall be labeled by attaching tie-on tags, adhesive labels, or by writing on sample containers with indelible markers. Sample containers shall be labeled with sufficient information that they may be traced to sample collection logs, field sheets, or custody records. Choice of adhesive labels or indelible ink should take into consideration that samples may come into contact with melted ice or condensed moisture during shipment or storage.

6.2.4 Individual samples shall be sealed or sample shipping containers shall be sealed with a tamper-proof seal when they will be relinquished by TVA to a common carrier or if the sampling plan requires it. If the samples will remain in the custody of TVA employees from the time of sampling through transport to the laboratory or under lock and key (as in a locked vehicle or storage container) during this time, use of seals is not required. However, even if seals are not required, their use is strongly urged on shipping containers if the sample is to change hands several times in transport.

6.3 Requirements

6.3.1 Apparatus/Equipment

This procedure specifies no additional apparatus or equipment in addition to any sampling plan.

6.3.2 Materials

6.3.2.1 Sample containers specified in the sampling plan shall be utilized.

6.3.2.2 Labels - Samples labels shall have an adhesive which does not readily release when containers become damp.

6.3.2.3 Custody Forms - Sample chain of custody forms shall be used to record custody of samples after sampling from relinquishment by the sampling organization through transport to receipt by the laboratory. The following information shall be supplied on the custody form:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Requested analysis
g. Sampling location
h. Comments
i. Signature of sample collector.

In addition the form shall contain an area so that each relinquishment and receipt of samples may be documented.

Example custody forms are attached as appendices 10.1 and 10.2. Other forms specific to a given project may be developed as long as they contain the minimum information specified above.

> Note: If sample collection time and location are already recorded on a field sheet or sampling log, that information need not be repeated on this form provided a copy of the sampling information is transmitted to the laboratory with the custody sheet.

6.3.2.4 Tamper-evident seals - These seals shall be individually numbered or otherwise marked so that they could not be removed and replaced without it being detected. Two styles have been useful for samples or sample containers.

6.3.2.4.1 Adhesive seals advertised as meeting forensic science requirements, such as Kapak brand seals.

6.3.2.4.2 Padlock-style plastic seals for hasps.

6.3.2.5 Field Logbooks or Field Sheets - Sampling activities may be documented in field logbooks or field sheets designed for that purpose. When these are used, they shall contain:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Reference to the sampling procedure
g. Sampling location
h. Comments
i. Signature of sample collector.

7.0 PROCEDURE

7.1 Field Operations

7.1.1 Prior to sampling, label sample containers with an adhesive label or with indelible marker. (Note: If the sampling conditions require it, labels may be affixed after sampling and cleaning the outside of the container.)

"Sample Chain of Custody"

7.1.2 Document sample information in a field log, field sheet, or the custody sheet if the first two are not provided.

7.1.3 Seal the sample container with an adhesive seal if the sampling plan requires it.

7.1.4 Complete a "Sample Chain of Custody" form.

7.1.4.1 If field logs or field sheets contain collection time and location, these items may be omitted from the form. In that case, draw a diagonal line in that column and attach a copy of the field logs or sheet so that the laboratory may have pertinent sampling information.

7.1.4.2 If a numbered seal is to be used on the shipping container, note that number in the comments section of the custody form.

7.1.4.3 If the shipping container is to be sealed, sign and date the "relinquished" area of the form.

7.1.5 Place the original copy of the paperwork in a plastic bag inside the shipping container. Retain one copy for field files. Transmit a third copy by separate courier, mail or fax to the laboratory.

7.1.6 Place the samples in a shipping container. As required by the sampling plan, place ice (or commercial substitute) and a temperature test bottle in the container as well. Seal the shipping container if the sampling plan requires it. See also 6.2.4.

7.1.7 Deliver the container to be transported to the laboratory.

7.2 Laboratory Receipt (Reference also GLP-0016)

7.2.1 Inspect the seals. Open the shipping container. Inspect the sample custody form to ensure that it is correctly completed. Sign as receiver. Compare the shipping container contents to the information on the form.

7.2.2 If the "relinquished" blank is not completed and the person delivering the samples is present, have that person sign the "relinquished by." Otherwise write "Not completed", date and initial. If a person signs "relinquished by," provide that person a copy of the paperwork.

"Sample Chain of Custody"

7.2.2 As required by the sampling plan, measure the temperature of any samples or temperature blanks and record that information on the custody sheet.

7.2.3 Communicate any errors, broken seals, missing seals, broken samples, differing identification numbers, extra samples, missing samples or misidentification to field personnel. Document all discussions by memorandum or database sample comment file. Document all problems and their resolution by memorandum or database sample comment file. If seals show signs of tampering, bring this to the attention of the group leader or team leader.

7.2.4 Refer to GLP-0016 for further sample receipt and log-in instructions.

7.2.6 Following logging, store the samples in a locked, refrigerated storage area as required by the sampling plan or project plan.

7.3 Laboratory Custody

7.3.1 Samples in locked storage areas, being prepared, being processed, or in autosampler trays are considered to be in the custody of the laboratory. When sampling plans require it, laboratory work areas shall be locked when unattended.

7.4 Sample Disposal

7.4.1 When customers request it, samples shall be returned to them following analysis.

7.4.2 Otherwise, dispose of samples after the time period specified in the sampling plan or project plan. If these do not specify a date, samples should be kept no longer than three months after all analyses are complete.

7.4.3 If the sampling plan requires it, document sample disposal in the workorder file, or custody records.

8.0 SAFETY

8.1 Wear rubber gloves and protective eyewear when handling samples unless it is known that the samples are innocuous.

8.2 Avoid contact with samples. Be aware of broken containers, corrosives, irritants, biohazards, flammability, pyrophoricity, reactivity, radioactivity and toxicity. Inspect labels and shipping information for warnings. When hazards are known, label samples with hazard information if that is not already provided by the customer.

8.3 In case of skin contact, wash thoroughly with soap and water.

8.4 In case of eye contact, hold the eyes open and wash for at least 15 minutes in an eyewash. Call for help.

8.5 Flammable liquids must be refrigerated only in explosion-proof refrigerators to avoid the risk of explosion caused by sparks in the electrical contacts of the compressor.

8.6 In handling samples, be aware of spills on outside of containers. Clean the exterior of containers as needed.

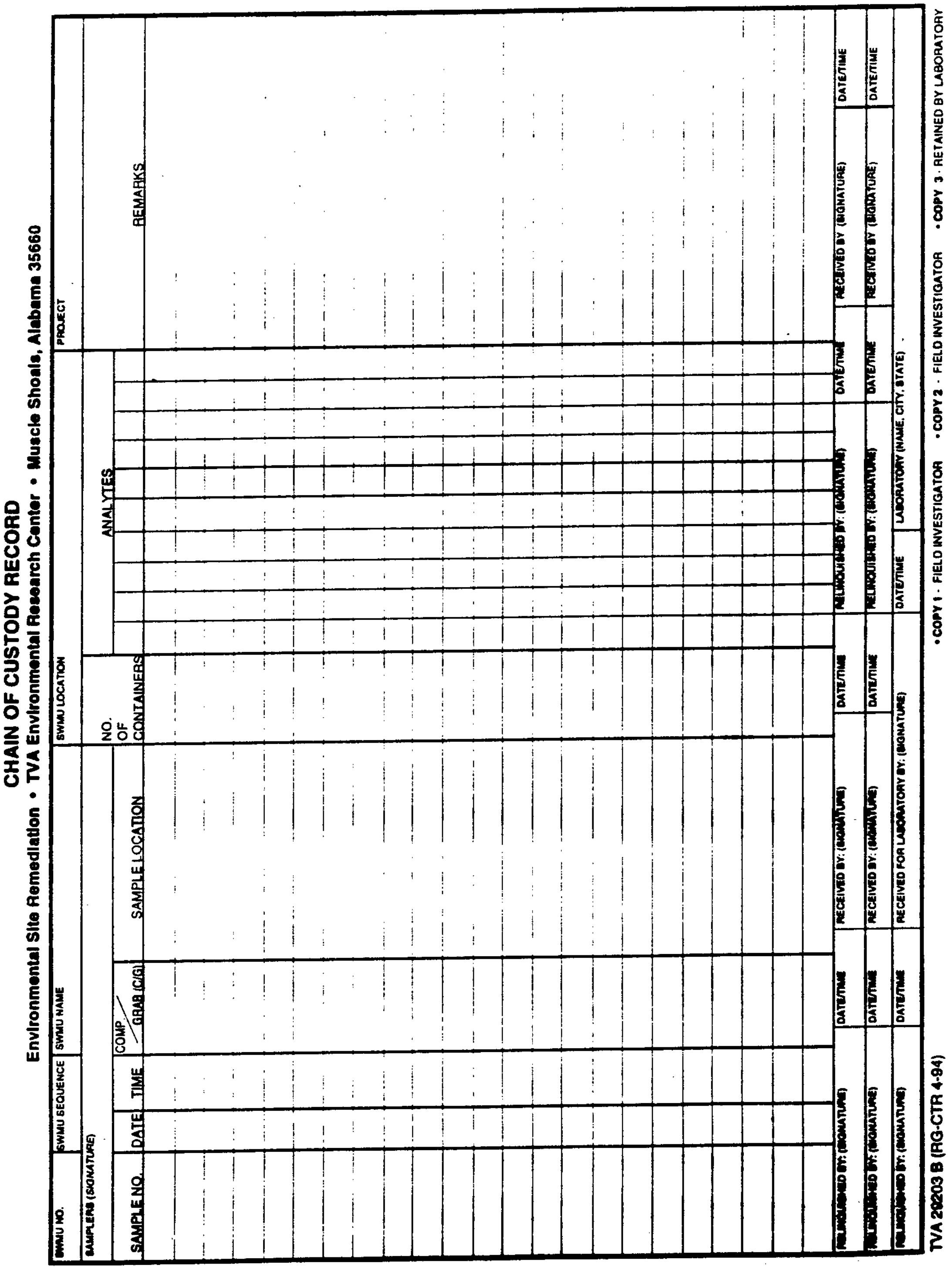
9.0 NOTES

None

"Sample Chain of Custody"

10.0 ATTACHMENTS AND APPENDICES

10.1 Chain of Custody Record - TVA 29203 B (RC-CTR 4-94)



CHAIN OF CUSTODY RECORD

Environmental Site Remediation • TVA Environmental Research Center • Muscle Shoals, Alabama 35660

SWMU NO.
SWMU SEQUENCE
SWMU NAME
SWMU LOCATION
PROJECT
SAMPLERS (SIGNATURE)

SAMPLE NO.	DATE	TIME	COMP/GRAB (C/G)	SAMPLE LOCATION	NO. OF CONTAINERS	ANALYTES	REMARKS

RELINQUISHED BY: (SIGNATURE) DATE/TIME RECEIVED BY: (SIGNATURE) DATE/TIME RELINQUISHED BY: (SIGNATURE) DATE/TIME RECEIVED BY (SIGNATURE) DATE/TIME
RELINQUISHED BY: (SIGNATURE) DATE/TIME RECEIVED BY: (SIGNATURE) DATE/TIME RELINQUISHED BY: (SIGNATURE) DATE/TIME RECEIVED BY (SIGNATURE) DATE/TIME
RELINQUISHED BY: (SIGNATURE) DATE/TIME RECEIVED FOR LABORATORY BY: (SIGNATURE) DATE/TIME LABORATORY (NAME, CITY, STATE)

TVA 29203 B (RG-CTR 4-94) • COPY 1 - FIELD INVESTIGATOR • COPY 2 - FIELD INVESTIGATOR • COPY 3 - RETAINED BY LABORATORY

10.2 Sample custody form - General

Sample Chain of Custody
Tennessee Valley Authority
Environmental Applications CTR-1K Muscle Shoals, AL

Project ______________________________ Date of Collection ______________________________

	Sample ID	Collection Time*	Number of Containers	Analyses Requested	Location*	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

	Signatures	Date and Time
Collector		
Relinquishing		
Receiving		

* These columns need not be completed if field sampling sheets containing the same information are attached

END OF PROCEDURE

Appendix B-2 – Lab Procedures for Soil pH: Method ASA 12-2.6

Soil pH
ASA 12-2.6

Procedure:

1. Calibrate the pH meter according to manufacturer's instructions using two buffers to bracket the expected range of measurements. Buffers should be approximately three pH units apart.

2. Where available, check the calibration with a third buffer.

3. Prepare a slurry of soil and water in the ratio of 10.0 g to 10.0 ml.

4. Stir the slurry vigorously with a glass rod and place the electrode into the slurry. Allow the electrode to come to equilibrium and measure the pH.

5. Record information about the calibration buffers (manufacturer, expiration date, known value), the check buffer and its measurement, and sample measurements.

References:

"pH, Method 150.1 (Electrometric)," *Methods for Chemical Analysis of Water and Wastes* - Revised March 1983, U. S. Environmental Protection Agency, Cincinnati, OH, PB84-128677.

"Glass Electrode - Calomel Electrode pH Meter Method," Section 12-2.6 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-3 – Lab Procedures for Total Organic Carbon (TOC): Method 415 Series

Total Organic Carbon - Method 415.1 with Dohrmann DC-190

1.0 Procedure

Perform Total Organic Carbon analysis in accordance with "Organic Carbon, Total", Method 415.1 (Combustion or Oxidation) and in accordance with chapters 6 and 10 of the operating manual for the Dohrmann DC-190 high temperature organic carbon analyzer as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

ORGANIC CARBON, TOTAL

Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680
Dissolved 00681

1. Scope and Application
   1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.
   1.2 The method is most applicable to measurement of organic carbon above 1 mg/1.
2. Summary of Method
   2.1 Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion or wet chemical oxidation. The CO_2 formed can be measured directly by an infrared detector or converted to methane (CH_4) and measured by a flame ionization detector. The amount of CO_2 or CH_4 is directly proportional to the concentration of carbonaceous material in the sample.
3. Definitions
   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:
      A) soluble, nonvolatile organic carbon; for instance, natural sugars.
      B) soluble, volatile organic carbon; for instance, mercaptans.
      C) insoluble, partially volatile carbon; for instance, oils.
      D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.
   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

Approved for NPDES
Issued 1971
Editorial revision 1974

4. Sample Handling and Preservation
   4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples.
   NOTE 1: A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.
   4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.
   4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified ($pH \leq 2$) with HCl or H_2SO_4.
5. Interferences
   5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.
   5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.
6. Apparatus
   6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.
   6.2 Apparatus for total and dissolved organic carbon:
      6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.
      6.2.2 No specific analyzer is recommended as superior.
7. Reagents
   7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.
   7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.
   NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.
   7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.
   7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as	
		Bias, %	Bias, mg/liter
4.9	3.93	+15.27	+0.75
107	8.32	+ 1.01	+1.08

(FWPCA Method Study 3, Demand Analyses)

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water", Standard D 2574–79, p 469 (1976).
2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 532, Method 505, (1975).

SECTION 6
OPERATION

INTRODUCTION

This section contains instructions for routine operation along with detailed descriptions on how to operate and calibrate the different modes.

6.1 ROUTINE OPERATION

SUMMARY

- Daily Start-Up
- Daily Operation
- Daily and Long-term Shutdown

DAILY START-UP

Check utility supply.

Enough carrier gas for a day's operation.

Acid reservoir at least 1/3 full.

Replenish IC chamber.

Confirm the IC chamber is half full (gas off).

Fill the IC chamber by using the "Acid to IC chamber" function (press **MAIN 2 5**). Each use of this function will result in 20 pulses and is equivalent to 2 ml of acid.

Turn on gas.

Press **CARRIER** .

For Boat Users :

Connect the 1/8 inch PTFE line from the boat module furnace to the DC-190 dehumidifier (see Figure 4.8).

Check system status.

(Press **MAIN 1** to view the status menu.)

Flow rate = 180 - 220 cc/min.

Dryer temperature = 0 - 10 $^{\circ}$C

Furnace Temperature = Furnace set point (Furnace light is green.) For most applications, the temperature should be 680°C.

Confirm or change set-up number on display (see Section 6.8).

Check set-up.

(See Section 6.2 for help in choosing set-up.)

Modes last used are lit up. Make any changes for the day and print the set-up parameters. System is ready for analysis.

DAILY OPERATION

Press START when ready.	It is good practice to run a check standard at the beginning of the day before analyzing unknowns, especially if any conditions have been changed. Update calibration if needed. See Section 6.3 for notes on operating and calibrating.

DAILY SHUTDOWN

Check the RUN status.	The unit should not be in a RUN mode.
For Boat Users :	Disconnect the 1/8 inch PTFE line which runs from the boat furnace to the dehumidifier.
Shut off the gas.	Press CARRIER .

NOTE: The furnace and the NDIR should be left on unless the unit is going to be relocated or will not be used for a long time. Frequently turning the furnace on/off reduces the life of the heater element. The NDIR requires at least 2 hours for stabilization after power up.

6.2 SELECTING THE ANALYSIS PARAMETERS

Most analysis have three parameters:

1) Analysis mode.
2) Inlet mode.
3) Volume.

NOTE: The ASM and RSM operating modes have other parameters which must be selected. See Sections 6.4 and 6.5 for guidelines in selecting these parameters.

SELECT THE ANALYSIS MODE

Use Table 6.1 to match your application to an analysis mode. The default mode is **NPOC**. To set another mode, press the corresponding button.

Table 6.1
ANALYSIS MODE SELECTION

ANALYSIS MODE	APPLICATION	METHOD
NPOC	Any water sample.	IC purged from sample at sparging station. Inject into TC port. $\text{NPOC} \xrightarrow[\text{Furnace}]{\text{TC}} \text{CO}_2$
TOC	Any water sample. Method of choice when sample has no volatiles.	TOC = TC - IC Two (2) injections per analysis. DC-190 calculates the difference. See See TC and IC descriptions.
IC	Any sample where dissolved CO_2 or carbonate concentration is of interest.	Sample injected into IC port. $\text{IC} \xrightarrow[\text{Chamber}]{\text{IC}} \text{CO}_2$
TC	Any water sample.	Sample injected into TC port. $\text{TC} \xrightarrow[\text{Furnace}]{\text{TC}} \text{CO}_2$
POC	Water sampler where volatile organics or other purgeables are of interest.	Sample is sparged at POC sparge station. LiOH scrubber removes IC from sparged gas. POC Gas --> IC Scrubber --> $\text{POC} \xrightarrow[\text{Furnace}]{\text{TC}} \text{CO}_2$
Boat Option, TC	Solids, sludges, slurries and waters with particulates greater than 0.5 mm.	Sample introduced onto platinum boat. Boat pushed into 183 furnace. $\text{Sample} \xrightarrow{800^{\circ}\text{C}} \text{CO}_2$

SELECT THE INLET MODE

The default inlet mode is **SYRINGE** . To select a different mode, refer to the following Table, then press the button corresponding to the new inlet mode.

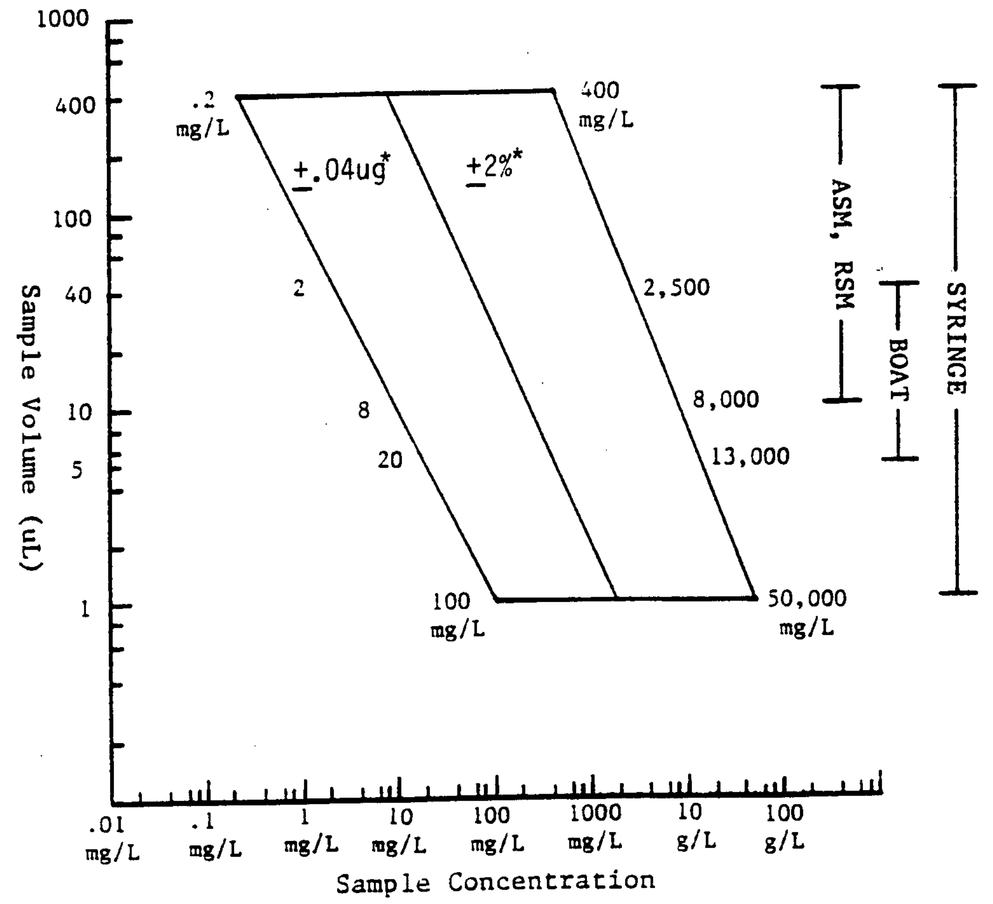
Table 6.2
INLET MODE SELECTION

ANALYSIS MODE	INLET MODE	DEFAULT		POSSIBLE VOLUME (ul)
		Volume (ul)	Range (mgC/L)	
NPOC TOC IC TC	Syringe	50	1 - 2000	1 - 400 20 - 200 *
	ASM	50	1 - 2000	10 - 400
TOC IC TC	RSM	50	1 - 2000	10 - 400
TC NPOC	Boat	40	2 - 4000	5 - 40
POC	N/A	10 mL	.01 - 20	2 - 10 mL

* This is the range for the manual micropipettor which is used with the **SYRINGE** mode.

SELECT VOLUME

The default volume and corresponding concentration range for each inlet mode are shown in the previous table. If the default concentration range is unsuitable, a better sample volume may be selected using Figure 6.1. Enter the new sample volume on the inlet mode menu.



* Expected precision. See Section 1.4.

FIGURE 6.1 Concentration Range vs. Sample Volume

EXAMPLE:	Expected sample concentration range = 5 to 5,000 ppm. From Figure 6.1, 20 ul gives 4 to 6,000 ppm. (Note the logarithmic scales.) 20 ul is compatible with all inlet modes, except POC.

6.3 MANUAL OPERATION

Use these instructions for syringe or micropipettor operation in NPOC, TOC, IC, TC or POC modes. The following table shows the general operation sequence for syringe injections. Specific notes for each analysis mode follow the table.

GENERAL OPERATION SEQUENCE - ALL MODES

- If it is desired to save the current operating parameters before making any changes, select a new set-up number (see Section 6.7).
- Choose set-up.
- Have the syringe filled and ready. (Have the septum installed as shown in Figure 6.2.)
- Press **START** .
- Inject the sample. (Review the injection technique for the mode selected.)
- At the conclusion of the analysis, the screen will display the final ppm value along with:

  Continue Y/N ?

  (This question must be answered before the system will perform any other action.)
- Press **YES** to make more injections.
- Press **NO** to end the run.
- Press **STOP** to end the run after the current analysis. To terminate the run, immediately press **STOP** five times.

ABOUT SYRINGES

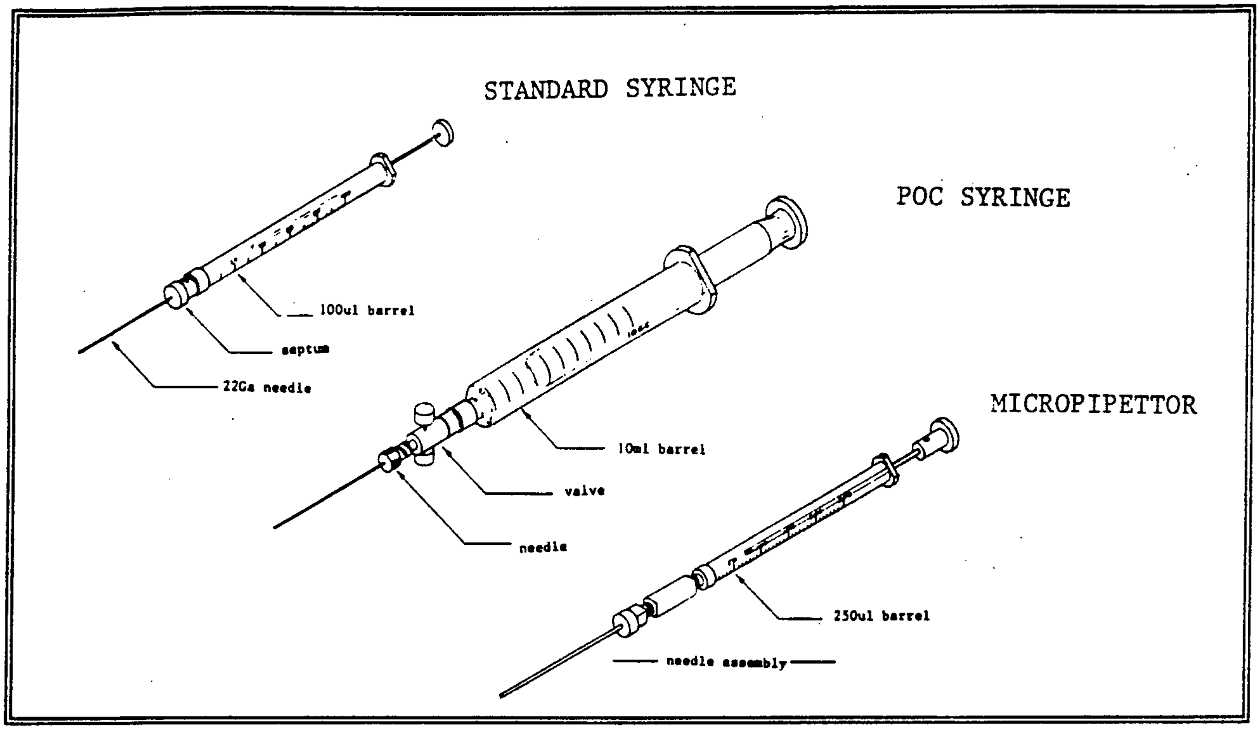


FIGURE 6.2. SYRINGE ASSEMBLIES

Assemble the syringes and micropipettor as shown in Figure 6.2. Always have a grey septum attached to the syringe or pipettor.

It is important for reliable sample introduction to use blunt-point needles such as those supplied with the DC-190. Side-port needles should not be used except on the POC syringe.

The 100 uL syringe (P/N 060-871) provided with the DC-190 has a 22S gauge (0.006 inch I.D.) needle. The 22 gauge (0.016 inch I.D.) replacement needles (P/N 060-872) are provided in the DC-190 operating kit for sample types requiring a larger I.D. needle.

Also available are a micropipettor barrel (250 uL syringe barrel, P/N 060-875) and a micropipettor needle (P/N 888-297). The micropipettor is used for samples containing particulates up to 0.5 mm diameter or samples which are incompatible with (react with or corrode) a stainless steel needle. The micropipettor probe should be used with a 250 uL syringe barrel only.

TC or IC

Injection Technique

As soon as the INJECT light comes on, press **OPEN/CLOSE**

Immediately insert the syringe into the injection port that has the illuminated LED.

Make seal during injection by pressing the grey septum against the port.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.

Inject at 50 ul/sec rate.

Withdraw the syringe and immediately press **OPEN/CLOSE** to close the port.

For 1 - 10 ul volumes, wait 5 seconds in between injecting and withdrawing syringe.

Micropipettor Users:

When using a micropipettor, wipe off the outside of the probe after drawing up the sample.

For volumes below 50 uL, the injection rate is crucial to obtaining reproducible results. Make the injection rapidly without jarring the syringe. (**HINT:** After withdrawing the syringe, look at the tip. If it is wet on the outside, inject faster; if it is partially empty, inject slower.)

Wait 10 seconds after injecting before withdrawing the pipettor <u>for all volumes</u>.

Sample Pretreatment

None, unless the samples are inhomogeneous or contain large particulates (> 0.5 mm diameter).

TOC

(This is a combination of the TC and IC modes.)

Injection Technique

Use the same technique as for the TC and IC modes.

Make two injections per analysis.

The first injection goes in the TC port.

Have the syringe filled and ready for the second injection which is made to the IC port. Look for the prompt from the display.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.

NOTE: When high pH samples are expected, treat combustion tube with 2 injections of 100 ul of pH1 HCl or HNO_3 solution.

NPOC

(This is the default analysis mode.)

Injection Technique

Use the same technique as for the TC and IC modes.

Inject into the TC port only.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.

Sample Pretreatment

The sample must be sparged prior to injection to remove the IC.

To sparge the sample:

- Pour about 10 mL of sample into a 20 mL vial (P/N 889-726).
- Screw the vial into Sparger A or Sparger B.
- Press A or B, and then 1 to start sparging.
- The sample will be automatically acidified. Each unit of "Add acid" is equivalent to 100 ul.
- Sparging will stop automatically at the end of sparge time.
- Remove the vial and cap it until the analysis is run.

Two samples can be sparged simultaneously.

Samples containing large particulates (> 0.5 mm) must be pretreated as directed in Section 10.2.

POC

⚠WARNING

Acid hazard. Check installation before sparging or priming. See manual sect. 5.

Priming and sparging steps involve acid pumping into the appropriate vessels. Make sure the plumbing is properly connected to avoid acid injury to persons or property.

Injection Technique

As soon as the INJECT light comes on, inject the sample into the POC sparger through the injection port.

When the analysis is over, withdraw the remaining sample from the sparger with the syringe.

Sample Pretreatment

None.

How to Fill the Syringe

Remove the plunger from the syringe and close the syringe valve and needle. Open the sample or standard container, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 10 ml.

This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample container, the analyst should fill a second syringe at this time in case the first analysis is unsuccessful.

6.4 AUTOSAMPLER OPERATION

INTRODUCTION

The DC-190 Autosampler (ASM) option is designed for unattended operation for many hours. The sample tray holds 32 8 mL vials. Automatic acid addition and sparging are provided by the sparge tower to remove inorganic carbon for NPOC analysis. The sample probe may be rinsed with either water and/or sample between analyses. The ASM can handle samples with particulates up to 0.5 mm and the sample may be stirred with gas before the sample is drawn to insure uniform sampling. Cross-contamination is minimized by the use of non-wetting materials for all sample contacting parts. Sample vials may be marked as blanks or standards for automatic calibration of the system during the ASM run.

The ASM offers an autoranging capability which will adjust the sample volume to maintain the peak integral within the range of the detector. Since the dynamic range of the DC-190 system is very wide (10,000 to 1), activation of the autoranging will normally be a very rare event. When this feature is active, the DC-190 will check the first replicate of a vial in the ASM mode to verify that the peak integral is within range. If the peak integral is below range, the result will be printed, but ignored in future statistical calculations. The injection will then be repeated, but with a volume 5 times larger than the original injection. If the peak integral is over range, a similar procedure is followed with a volume one fifth the original volume. The volume adjustment will be repeated until the peak integral is within range. If an adjustment would result in a volume outside the 10 to 400 uL range, the volume will be set to either 10 or 400 uL as appropriate and no further adjustment will be made. The original injection volume will be restored at the beginning of the next sample vial. The accuracy of the autoranged data may suffer somewhat because the ASM was not calibrated with the new volume. The inaccuracy without autoranging is potentially much worse, however, than with autoranging. If desired, the results of autoranged data may be rechecked later.

Below is a table of expected and observed volumes for the ASM. These values are approximate and will vary from instrument to instrument. This volume variation only affects autoranged data. This will not apply to normal calibrated ASM data because the same volume is used for analysis.

VOLUME (uL)	
Expected	Observed
10	10.3
20	19.5
40	35.4
80	70
100	92
200	194
400	400

OPERATION

- Refer to DAILY START-UP in Section 6.1 to prepare the analyzer for operation.

- If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

- Refer to Section 6.2 and select the analysis mode and volume desired. See Table 6.3 for guidelines to set the other operating parameters.

- Place the vials in the sample tray beginning with tray position 1. Refer to Table 6.4 and mark the vials as blanks, standards, or samples as appropriate. Mark the first empty tray position after the samples as indicated in Table 6.4 to terminate the run.

- Clean and fill the rinse bottle with DI water if water rinses were called for on the Rinse\stir menu.

- Check that the acid bottle is at least 1/3 full of acid solution if set up for NPOC analysis.

- Check that the printer is ready and has sufficient paper.

- Press **START** .

- There are two ways to end the run before completion. Press **STOP** to end the run after the current analysis. To terminate the run immediately, press **STOP** five times. After an immediate bail out, the ASM may have to be returned to its resting position. The sparge arm may be raised by selecting "Raise sparge arm" (**1**) on the "Sparge arm menu" (**MAIN 2 5 3 3**). The sample arm may be returned to the rinse bottle position by selecting "Move arm to rinse" (**4**) on the "Sample arm" menu (**MAIN 2 5 3 2**). Always check the "Furnace/IC ports" menu (**MAIN 2 5 5**) to be sure the inlet ports are shut (even if the indicator lights next to the ports are not lit).

TABLE 6.3

ASM OPERATION PARAMETER GUIDELINES

# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats, with 3 being the default.
Sparge time (min)	The default time (3 minutes) should be satisfactory for almost all samples as long as the pH is in the proper range (see "Acid volume" below). This option is applicable to the NPOC mode only.
Acid volume	The pH must be adjusted to a value less than 4. It may be necessary to check a few samples after acid addition and make adjustments by trial and error until the acid addition matches the particular samples being analyzed. The default is 1 (each unit of acid volume is equivalent to 100 ul). This option is applicable to the NPOC mode only.
<u>The following selections are on the "Rinse and /or stir" menu:</u>	
# of rinses w/water	This option specifies the number of times the ASM sample probe and loop will be rinsed with water between each vial.
# of rinses w/sample	Similar to the above option except that the ASM will rinse with sample before the first injection from each vial.
Sample stir time (sec)	Specifies the time that the sample will be stirred before the sample is drawn into the sample loop. The allowed range is 0 - 30 seconds (default = 0). In most applications, 15 seconds will provide effective stirring. Stirring is accomplished by bubbling gas out of the sample probe to suspend particulates and obtain a more uniform sample.
Auto-range	When set to "Yes", the DC-190 will automatically adjust the injection volume. "No" is the default setting. See the INTRODUCTION to this section for details on this feature.
CG off after	The default "No" means the carrier gas (CG) will not be turned off at the end of an ASM run. A "Yes" will cause the carrier gas to be turned off 10 minutes after the end of an ASM run. During this period, the red light in the **START/STOP** button will blink as if the run is still in progress.

TABLE 6.4
ASM VIAL MARKERS

VIAL	PEG POSITION		INDICATION
	INNER*	OUTER**	
No	No	No	Skip Position
Yes	No	No	Sample
Yes	Yes	No	Blank
Yes	No	Yes	Standard
Yes	Yes	Yes	Rinse Sample***
No	No	Yes	Terminate Run

* Peg hole closer to center of sample tray.

** Peg hole closer to sample vial.

*** Sample is used for rinse only (no analysis).

NOTE: If the printer runs out of paper or jams during a run "Print last run" (**MAIN 2 3**) will reprint the run data from a buffer. This allows data otherwise lost to be retreived. The buffer which retains the data is not large enough, however, to hold a complete run of data in all cases. This buffer has sufficient capacity to hold data from approximately 32 vials with 3 replicates per vial in modes where each replicate requires one line to print (TC, IC, or NPOC). In the TOC mode, each replicate requires three lines to print. In this mode, the buffer will only hold approximately 10 vials with 3 replicates per vial. The buffer is filled on a first in first out basis so that the data remaining at the end of the run will be the last data point back until the buffer is full.

6.5 OPERATION OF THE RSM OPTION

The RSM option allows the continuous sampling of a sample stream which is tapped to flow through the RSM sample cell. The ASM will perform the designated number of replicates on the sample stream and then wait for a designated time period. The sampling cycle is then repeated. The TC, IC, and TOC analysis modes may be performed using the RSM option. However, if the sample stream IC and TC levels are not constant, the accuracy of the TOC analysis may suffer due to the time lag between the IC and TC portions of the analysis.

* Adjust the sample flow rate to the sample cell by slowly opening the needle valve (counter clockwise) until the water level stabilizes slightly above the drain port of the sample cell.

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Select **TC**, **IC**, or **TOC** (see Section 6.2 for selection guidelines) and then **RSM** to set the analysis mode. Verify that the operating parameters are set to the desired values. Use the guidelines in Table 6.5.

* Calibrate the DC-190 according to the RSM calibration procedure in Section 6.8.

* Press **START** to begin the analysis. The RSM will continue until manually stopped.

* To stop the analysis, press **STOP** (same button as **START**). This will stop the DC-190 at the end of an analysis in progress or immediately during the time between runs. To stop the run immediately during an analysis, press the **STOP** button 5 times.

TABLE 6.5

RSM OPERATING PARAMETER GUIDELINES

Sample volume	See Figure 6.1
# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats with 3 being the default.
Time between runs	This is the time from the conclusion of the last replicate of a group to the beginning of the first replicate of the next group. The allowable range is 0 to 54 minutes with a default of 0 minutes.

6.6 OPERATION OF THE BOAT OPTION

Use the boat sampler for slurries, sludges, solids, and suspensions. Operate in either the **TC** or **NPOC** mode. Refer to "Installation and Operation of the 183 Boat Sampling Module" (P/N 915-240) for sample introduction instructions (Section V, Parts 5A and 5B). The DC-190 calculates ppmC from liquids or solids.

SAMPLE TYPE	SAMPLE INTRODUCTION	CONCENTRATION UNITS
Liquids, light slurries, suspensions	See 183 Instructions for **Liquids**	mg/L
Solids, heavy slurries	See 183 Instructions for **Solids**	ug/g

- If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).
- Press **BOAT TC** or **NPOC** .
- Press **1** until the appropriate units are displayed.
- Introduce the sample into the boat - see "Installation and Operation of the 183 Boat Sampling Module".
- Press **START** and follow the 183 instructions.
- If ug/g units are selected, enter the sample weight when asked - "Sample weight (mg)?".
- **SOLIDS ONLY:** Enter the sample weight when asked - "Sample weight (mg)?".

6.7 CALIBRATION

The DC-190 offers a choice of either one point or two point calibration. Two point calibration is equivalent to subtracting the blank value automatically. The DC-190 system always calculates a two point linear calibration. If only a single point calibration is desired, the System Blank may be set to 0 before updating the Calibration Factor. In this case the System Blank will remain 0 after updating the Calibration Factor resulting in a single point calibration. Since the system blank for IC is normally insignificant, its value is set to zero and IC analysis always has one point calibration. When two-point calibration is used, both calibration factor and system blank are recalculated each time either the calibration factor or system blank is updated. In TOC mode, the system uses TC value for calibration and blank update.

The DC-190 system provides a common calibration set (calibration factor and system blank) for SYRINGE, ASM, and RSM modes. POC and BOAT modes have their own calibration sets. When changing inlet mode from SYRINGE to ASM or RSM, calibration stays the same. When changing inlet mode from SYRINGE, ASM, or RSM to POC or Boat, calibration changes accordingly. The multiple set-up function (see Section 6.8) provides capability to store and retrieve up to 5 calibration sets.

Since SYRINGE and ASM/RSM calibrations are not necessarily the same, calibration for these modes should be done separately. Use the multiple set-up function to store the different calibration sets.

SUMMARY

1. System Blank
2. Calibrating Syringe, POC, or Boat Modes
3. Calibrating The ASM Mode
4. Calibrating The RSM Option
5. Omitting Outlier Data
6. Calibration Equations

SYSTEM BLANK

System blank is defined as the response contributed by the analyzer when carbon-free water sample is injected and analyzed. In reality, it is very difficult to produce and preserve the carbon-free water. Thus the true system blank and the carbon content of the water sample cannot be accurately distinguished. However, the carbon content of high purity water can be below the defection limit (.2ppmC) and the response with such water may be assumed as the system blank. When it exists, the blank value is subtracted from every analysis except in IC mode where blank is always assumed to be zero.

The system blank becomes increasingly important for analyses below 10 mgC/L as shown :

MODE	VOLUME	TYPICAL BLANK (mgC/L)
TC NPOC	400 ul	.10 - .40
IC	400ul	0*
POC	10ml	0 - .03
BOAT	40ul	2.0 - 4.0

Factors affecting the blank :

- Cleanliness of syringes, spargers and IC chamber.
- Sample handling.
- Age and sample history of TC and boat combustion tubes.
- Dehumidifier temperature.

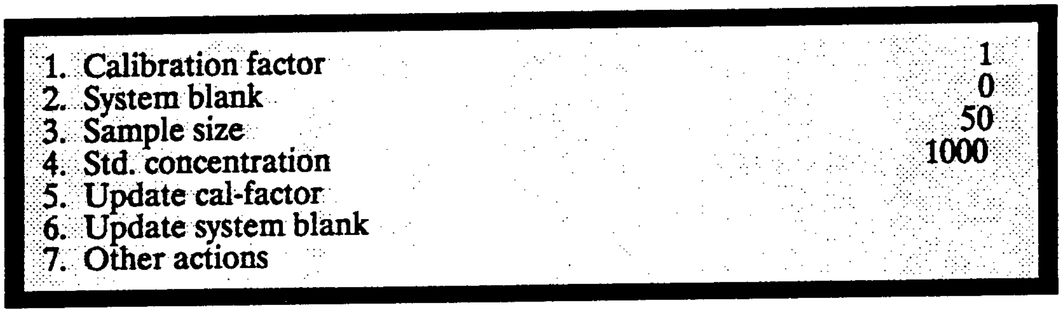
CALIBRATING THE SYRINGE, POC, or BOAT INLET MODES

See "SYSTEM BLANK" earlier in this section for guidelines to determine whether a two point calibration is needed for the samples to be analyzed.

- Analyze a standard in the analysis mode to be used. An average of at least two determinations is recommended. Respond **NO** to the prompt "Continue yes/no?" when satisfied with the results.

- Outlier data can be omitted at this point if desired. See the section "OMITTING OUTLIER DATA" at the end of this section for details on how to do this.

- Press **CALIBRATE** to review the calibration menu :



```
1. Calibration factor                 1
2. System blank                       0
3. Sample size                       50
4. Std. concentration              1000
5. Update cal-factor
6. Update system blank
7. Other actions
```

- Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.

- Press **5** to update the Calibration Factor. The new calibration factor will be calculated and displayed on the menu.

- To complete a two point calibration, if desired, repeat the above procedure with a blank sample. Use the cleanest reagent water available (less than 0.150 mgC/L). Press **6** to update the System Blank.

- The DC-190 is now calibrated for the selected analysis mode.

Analyze a check standard with each sample set. If the reported value deviates from the expected value by more than 2%, re-calibrate the system.

Note To Boat Users:	It is easy to use a liquid standard to calibrate the DC-190 even when using "ug/g" units to analyze solid samples. For example, to obtain 10 mg of sample, simply inject 10 ul of standard. This relationship holds as long as the density of the standard is 1 g/mL, which will be true for most water-based standards.

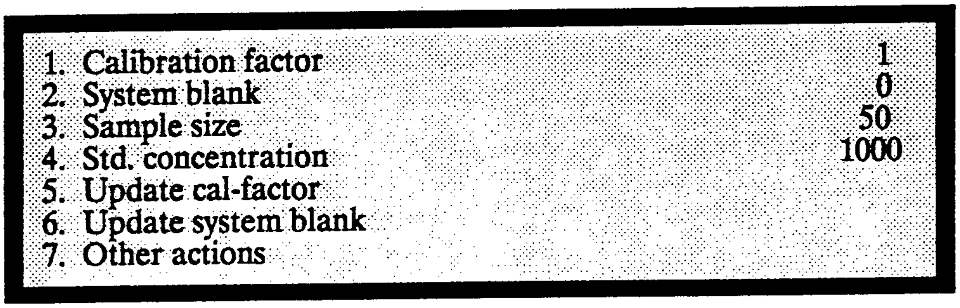
CALIBRATING THE ASM INLET MODE

- Select the ASM operating paramaters as described in Section 6.4 and press **START** to begin analyzing the standard.

- Place the vials of standard in the first tray positions. It is recommended that two vials of standard be placed next to each other at the beginning of the ASM sample tray. Place a peg in the outer hole next to the second vial to mark it as a standard for calibration (see Table 6.4).

- If blanks are to be determined, place two or three vials of blank immediately following the vials of standard. In most circumstances, two vials are sufficient. For best accuracy at low levels, three vials are recommended. Place a peg in the inner hole next to the last of the two or three blank vials to instruct the DC-190 to determine a new blank value (see Table 6.4).

- Press **CALIBRATE** to review the calibration menu:



```
1. Calibration factor                          1
2. System blank                                0
3. Sample size                                50
4. Std. concentration                       1000
5. Update cal-factor
6. Update system blank
7. Other actions
```

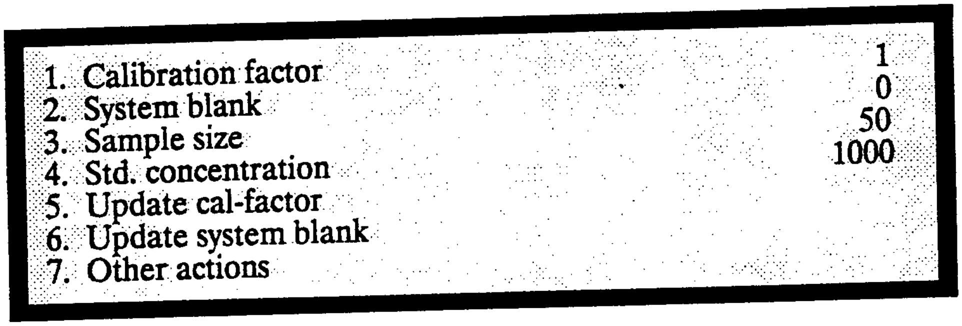
- Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.

- Place the sample vials in the sample tray following the standard and blank vials, and run the analysis according to the operation instructions in Section 6.4. The DC-190 will automatically calculate and use the calibration factor and blank value.

CALIBRATING THE RSM OPTION

The RSM mode is easiest to calibrate using a vial of the desired standard rather than by pumping the standard through the RSM sample cell. This method is described in the following steps:

- Lift the sample cell from its holder and secure it in the clip located to the left of the black cell holder.
- Place an ASM vial (P/N 080-140) containing the standard solution into the black cell holder.
- Select the RSM operating paramaters as described in Section 6.5 and press **START** to begin analyzing the standard.
- Since the RSM does not stop automatically, it is necessary to manually stop it by pressing **STOP** (the same button as **START**) during the last desired replicate of the standard. The DC-190 will then stop at the end of the current analysis.
- Outlier data can be omitted at this point if desired. See the Section "OMITTING OUTLIER DATA" at the end of this Section for details on how to do this.
- Press **CALIBRATE** to review the calibration menu:



```
1. Calibration factor                  1
2. System blank                        0
3. Sample size                        50
4. Std. concentration               1000
5. Update cal-factor
6. Update system blank
7. Other actions
```

- Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If subtraction of the blank is not desired, make sure the System Blank is set to 0. Make any necessary changes.
- Press **5** "Update cal factor" to calculate and store a new calibration factor.
- Repeat the above procedure with a blank sample and press **6** "Update system blank" on the "Calibration" menu if an update of the system blank is desired.

OMITTING OUTLIER DATA

The DC-190 provides the ability to reject outlier data when operated in the manual modes (Syringe, Boat, and POC) and the RSM mode (no provision for outlier rejection is made in the ASM mode). A new average and standard deviation are calculated after the data is rejected. This feature saves having to re-run a data set due to a bad data point when updating the Calibration Factor or System Blank. The DC-190 will not allow the number of replicates to be reduced to less than 2 as a result of data rejection. Data rejection is accomplished by the following steps:

* Complete the run by responding **NO** in one of the manual modes or **STOP** in the RSM mode (see the calibration instructions for the mode in use) to the prompt at the end of the analysis. Three or more replicates must have been generated.

* Select the "Auxiliary functions" menu (**MAIN 2**) and press **1** "Omit an outlier".

* At the prompt, enter the number of replicates to reject. Each replicate to be rejected will be prompted for separately. Enter a replicate number after each prompt.

* New statistics will be displayed on the screen and printer. An update of the Calibration Factor or System Blank will now be based on the new average value.

* If the "Omit an outlier" menu item is selected again after the current data set has been edited, the DC-190 will start the data rejection over and ignore the previous data editing.

CALIBRATION EQUATIONS

The following equations are used in the DC-190 system.

The equation for determining a calibrated result is:

$$y = (Fx - b) / V$$

where:
y = Concentration (calibrated) of sample.
x = NDIR peak with background subtracted. Normally invisible to the user. The displayed value, y, may be made to equal x by setting F, b, and V to the appropriate values (1, 0, and 1, respectively)
F = "Calibration Factor". This is the slope of the linear fit line.
b = Intercept. This is an internal parameter which is invisible to the user.
SB = "System Blank" = b/V.
V = Sample volume (or mass).

The quantities F and SB are the ones displayed on the calibration menu and are the ones which can be edited directly.

The Calibration Factor and Blank are calculated by:

$$F_n = F_o (C_s / y_s)$$

$$b_n = b_o (F_n / F_o)$$

where:
C_s = Concentration of the standard.
o = Old value.
n = New value.
s = Value for Standard.

These are the equations used internally by the DC-190 system. Both F_n and b_n are recalculated each time either the Calibration Factor or the System Blank is updated. It should be noted that if the old value b_o is already 0, the new value b_n and therefore SB will also be 0. This provides a means to have the system effectively do a one point calibration update when it calculates a new Calibration Factor. These equations may also be used to manually calculate the values and enter them on the "Calibration" menu directly.

6.8 USING THE MULTIPLE PARAMETER SETS

The DC-190 provides the capability to store 5 complete sets of operating parameters. This capability allows the user to return to a previously defined set of operating parameters without having to re-enter the parameters. The parameter set includes the inlet mode, the analysis mode, the parameters appropriate to the analysis/inlet mode as well as the Calibration Factor and System Blank..

One of the parameter sets is always the "working" set-up. This is the parameter set associated with the current set-up number. Any run started will now use the parameter values currently contained in the working parameter set. As changes are made to the operating parameters, these changes are made to the working set-up.

When a new set-up number is selected, the parameter values in the previous set-up are saved as they were at the time of the new selection. The working parameter set now takes the values associated with the new set-up number. Any run started will use the new parameter values and any parameter changes are now made to the new parameter set.

Returning to the previous set-up number will restore the operating parameters to the state they were in when the set-up number was last used.

If it is desired to save the current set of parameter values for future re-use, a new set-up number should be selected before starting to define a new parameter set.

To determine the set-up number:	Display the "System status" menu (**MAIN 1**). Line 5 "Analysis set-up" indicates the current Set-up number.
To change to another set-up number:	Select the "System status" menu (**MAIN 1**) and then "Analysis set-up" (**5**) and enter the new Set-up number. This saves the current parameter set.

To print the current parameter set:	Press the analysis mode button with the lit LED and then select the "Print set-up" option on the displayed operating parameter menu.
To print all the parameter sets:	Display the "System status" menu (**MAIN** **1**). Press **6** "Print set-up selections".

USING THE CLIPBOARD

A clipboard is provided in the DC-190 system which allows the Calibration Factor and System Blank to be copied from one parameter set to another. This feature can save time and effort when changing from parameter set to another after the system has been calibrated. Use the following steps:

- Select the "Other actions" section of the "Calibration" menu (**CALIBRATE 7**).

- Verify that the "Analysis set-up" shown on line 4 is the one from which to copy the calibration factors. If not, select **4** "Analysis set-up" and enter the desired set-up number.

- Select **2** to save the calibration factors.

- Enter the number of the new parameter set on line 4 and select **3** to copy the calibration factors.

The new parameter set now contains the same Calibration Factor and System Blank as the one copied.

DC-190 Operation Guide

DAILY START-UP

1. Gas @ 30 Psig.

2. Check that the acid bottle is 1/3 full.

3. Confirm that the IC chamber is 1/2 full **(gas off)**

4. Fill IC chamber by using the prime acid function.

5. Press $\boxed{\text{CARRIER}}$ Check that gas is flowing in IC chamber.

6. Ensure there is water in the dehumidifier.

7. Observe green lights on carrier & furnace.

8. Check for: flow rate 180-220cc/min, dehumidifier temp. 0-10°C, and furnace temp. 680°C. (Most applications)

9. Confirm or change Set-up number on display. **(Section 6.8)**

10. Check analysis and inlet mode.

11. Print Set-up.

12. If using the Boat, connect Teflon tubing to inlet part of dehumidifier. **(Fig. 4.15)**

13. If using ASM, clean the rinse bottle and fill it with acidified DI water. **(Few Drops of H_3PO_4)**

14. Observe for stable baseline **(Peak to Peak <.2mV)** before starting analysis.

DAILY SHUT-DOWN

1. Check that system is <u>not</u> in the **RUN** mode.

2. Push $\boxed{\text{CARRIER}}$ to turn off gas.

3. Leave furnace at operating temperature. **(Normally 680°C)**

4. Disconnect the Teflon tubing from the dehumidifier to boat at the boat inlet.

5. For total shut down turn **OFF** main power switch in the rear.

OPER. & CAL

1. Select analysis mode **(Table 6.1)**

2. Select inlet mode **(Table 6.2)**

3. Confirm or change volume. **(Fig. 6.1)**

4. For **CALIBRATION**, press $\boxed{\text{CAL}}$ to confirm or change concentration. **(Section 6.7)**

5. For manual injection, see **Section 6.3** for injection technique.

6. For ASM, confirm or change other parameters. **(Table 6.3)**

7. Refer to **Table 6.3** for ASM vial markers.

8. For RSM, see **Section 6.5** .

9. To complete **CALIBRATION**, see **Section 6.7.**

MAINTENANCE

Daily checks:
1. Printer paper
2. Gas supplies
3. IC chamber 1/2 full & acidified
4. Water in dehumidifier tube
5. Acid bottle 1/3 full
6. Gas flow 180-220cc/min
7. Temp. at set point
8. Dehumidifier temp 0-10°C

Weekly checks:
1. Daily checks plus
2. Replace septum in POC sparger every 40 injections.
3. Inspect TC inlet valve
4. Inspect combustion tube. Wipe inside area near top with wet Q-tips if necessary.
5. Inspect IC inlet valve
6. Clean IC reactor
7. Drain dehumidifier water & replace with acidified water. Flush several times if necessary.

Monthly checks:
1. Daily & weekly and/or:
2. Inspect & replace LiOH if necessary.
3. After ~160 hrs of operation, rinse catalyst, and combustion tube, replace silver wool **(Section 7.1)**. Condition catalyst at 900°C for 1/2 hr with DI injections.
4. Inspect O-rings in TC inlet and bottom connector. Replace if necessary.

DO'S & DONT'S

1. **DO** Check the bottom connector when checking the combustion tube.

2. **DO** Use a Soap Film Bubble meter to check output gas flow rates.

3. **DO** leave furnace at 680°C except for long term shut down.

4. **DO** Condition new catalyst. 100ul of water every 5 min. for 2 hours at 900°C.

5. **DON'T** use Pyrex wool in the combustion tube.

6. **DO** clean combustion tube weekly if used heavily. DI injections @ 900°C for 1/2 hrs. Use good water–should stabilize at 1 to 3ppm or better.

7. **DO** check valve seal & O-rings monthly when inspecting TC & IC ports.

8. **DO** re-align TC & IC ports with ASM probe after inspections.

9. **DO** study flow diagram Figs 8-1 & 8.2.

10. **DO** acidify ASM rinse bottle

11. **DON'T** use ASM stirring time > 30 sec.

12. **DO** inject acidified water daily into TC port if non-acidified samples are analyzed. (3, 100ul inj. of pH1, HCl or HNO_3)

13. **DO** rinse (section 7.1) and condition catalyst (section 5.3) when catalyst is contaminated.

14. **DON'T** raise drain line higher than 1 1/2" above lab bench.

SECTION 10

STANDARDS PREPARATION AND SAMPLE HANDLING

10.1 STANDARDS PREPARATION

REAGENT WATER

Use:
Standards preparation, system blanks, sample dilution, cleaning, etc.

Requirements:
Deionized or distilled.
ASTM Type II reagent water or equivalent.

TOC level: Less than 0.2 mgC/L.

ACID SOLUTION

Use:
Automatic acid feed for IC chamber, sparge stations, autosampler.

Requirements:
Reagent water.
Phosphoric (H_3PO_4), sulfuric (H_2SO_4), or nitric (HNO_3) acid, concentrated, reagent grade.

Do not use hydrochloric acid (HCl).

Preparation:
Final volume: 100 ml.

20% Phosphoric Acid Solution:
Add 20 ml acid to 80 ml reagent water. Transfer to the acid bottle (4 oz borosilicate with open top screw cap).

If phosphoric acid is not available, 10% sulfuric acid or 5% nitric acid can be substituted.

Replace monthly.

TC and IC STOCK SOLUTIONS

Use:

Dilute to appropriate concentration for calibration or system check-out.

Requirements:

Reagent water.

Reagent-grade concentrated acid (H_3PO_4 or H_2SO_4) for TC stock only.

Standard compounds are reagent-grade, and must be dried to a constant weight. (See the table in the next page.)

Preparation:

Final volume: 100 mL.

Standard compound choice:
For system performance check and troubleshooting purposes, use a compound listed below. For routine analyses, use one of these, or any compound which might be more appropriate for your application.

Weigh the specified amount of the compound into a 100 ml volumetric flask. Add about 75 ml reagent water to dissolve the compound. Add about 0.1 ml acid to TC solutions to adjust pH below 3. Then fill to the mark.

Store stock solutions in amber borosilicate bottles with Teflon-lined closures at 4°C.

Replace monthly.

TC STOCK SOLUTIONS **(Choose one):**

Compound	Weight (g/100mL)	Concentration	Add Acid?
Potassium Biphthalate KHP ($C_8H_5KO_4$)	2.126	10,000 mgC/L	Yes
Sucrose ($C_{12}H_{22}O_{11}$)	2.375	10,000 mgC/L	Yes

IC STOCK SOLUTIONS **(Choose one):**

Compound	Weight (g/100mL)	Concentration	Add Acid?
Na_2CO_3 (Anhydrous)	0.883	1,000 mgC/L	No
$NaHCO_3$	0.699	1,000 mgC/L	No

Use this formula to determine the weight required to make 100 ml stock solutions using other compounds:

$$\mathbf{g\ Compound} = \frac{\mathbf{mw \times \%C}}{\mathbf{N \times 12.01}}$$

where:

mw = molecular weight of compound
%C = concentration of standard in % carbon
N = number of carbon atoms per molecule
12.01 = atomic weight of carbon

For example

For a 1% (10,000 mgC/L) solution of sucrose (mw = 342.29):

$$\frac{342.29 \times 1\%}{12 \times 12.01} = 2.375 \text{ g.}$$

TC and IC WORKING STANDARDS

Use:

Calibration or system check-out.
Choose the standard concentration to match the working range of your samples.

Requirements:

Reagent water.
Clean volumetric flasks and volumetric pipets.

Preparation:

Final volume: Depends on concentrations.

Use larger volumes as concentration decreases. Make 1 liter volume at 10 mgC/L. Do not make final volume smaller than 100 ml.

TC solutions only: Maintain at pH 3 or lower.

Store standard solutions in amber borosilicate bottles with Teflon-lined closures at 4°C. **Minimize exposure to atmosphere.**

Bottle volume: Between 100 - 200 mL, depending upon the concentration.

Replace weekly.

System Performance Check: (Initial Start-Up)

Make 100 ml of 1000 mgC/L TC standard and 100 ml of 100 mgC/L IC standard.

POC STANDARD

Use:

Calibrate POC sparger.

Requirements:

Very clean 1 liter volumetric flask.

Reagent water.

Stir plate and Teflon coated stirbar.

Reagent grade compound.

Preparation:

Final volume: 1000 ml.

Compound Choice:

Benzene or chloroform is strongly recommended. Other compounds can be used if reliable results can be demonstrated. Use only benzene or chloroform for system performance check and troubleshooting.

WARNING!

BENZENE	DANGER! Extremely flammable. Suspected human carcinogen. Harmful if swallowed, inhaled or absorbed through the skin. May affect the blood system.
CHLOROFORM	Warning! Suspected human carcinogen. Harmful if inhaled or swallowed. Skin and eye irritant and may produce toxic vapors if burned.

Please consult material safety data sheets for more precautions regarding these compounds.

Fill the 1 liter flask to the mark with reagent water. Add the stir bar and gently agitate water on stirplate for 1 - 2 minutes to degas. Inject a microliter quantity of the compound. Use the table or formula in the following page to determine the proper quantity to inject. The syringe needle should be well immersed in the water. Cap the flask and gently agitate the solution until it comes to equilibrium (approximately 5 minutes).

COMPOUND	VOLUME TO INJECT	CONCENTRATION
Benzene (C_6H_6)	12 ul	9.92 mgC/L
Chloroforom ($CHCl_3$)	67 ul	9.72 mgC/L

To make other concentrations or standards, use this formula:

$$\textbf{Concentration of POC Standard } C = \frac{V \times D \times F}{L}$$

where:

C = Concentration of standard (mgC/L)
V = Microliters of POC solvent injected
D = Density of POC solvent (mg/ul)
F = Fraction of carbon per molecule by weight
L = Volume in liters of water

10.2 SAMPLE HANDLING

Good laboratory practice is important in obtaining reliable analysis for carbon content of samples. Since carbon is everywhere in nature, it is very easy to contaminate a sample. Follow these guidelines for sample handling during collection, pretreatment, and analysis.

Syringe Handling: Dedicate a syringe to a particular carbon range. When the syringe gets contaminated (indicated by sample or standard not completely wetting the inner barrel), draw chromic acid into the syringe a few times, then rinse well with reagent water.

Sample Bottles: It is preferable to store and collect samples in glass containers. Plastic bottles should only be used if it is established that the specific type of container to be used does not contribute contaminating organics.

The sample collection bottles should be cleaned well before collecting the sample. The amount of cleaning necessary is dependent on the expected concentration of carbon in the sample. As a rule of thumb, the following levels are suggested:

*** <u>Greater than 100 mgC/L</u>**

- Wash bottle in hot, soapy water.
- Rinse with clean water.
- Plastic cap may be used, but try to use Teflon-lined cap.
- Analyze samples within 2 weeks.
- Treat standard bottles and sparge vials the same way.

*** Less than 100 mgC/L**

- Use amber bottle.
- Wash in hot, soapy water.
- Rinse with clean water.
- Swirl with chromic/sulfuric acid cleaning solution.
- Rinse with reagent water.
- Use Teflon-lined cap.
- Store sample at 4°C.
- Analyze within two weeks.
- Treat standard bottles and sparge vials the same way.

Sample Pretreatment:

If a sample contains particulates larger than 0.5 mm or insoluble matter, homogenize with a blender or tissuemizer until the average particle size is less than 0.5 mm. Analyze these samples with the micropipettor or autosampler.

If the average particle size cannot be reduced to below 0.5 mm by homogenizing, dilute the sample with reagent water and blend again, or analyze the sample using the boat sampler.

*** Below 100 mgC/L:**

Minimize the sample handling and the blend time in order to minimize contamination and loss of volatiles. Analyze a blank with the same pretreatment as a sample.

Appendix B-4 – Lab Procedures for Total Kjeldahl Nitrogen (TKN): Method 351 Series

Nitrogen, Total Kjehldahl - Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

1.0 Procedure

Perform analysis for Total Kjehldahl Nitrogen (Method 351.2) in accordance with procedures for the Technicon II AutoAnalyzer, or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

NITROGEN, KJELDAHL, TOTAL

Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

STORET NO. 00625

1. Scope and Application
    1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogeneous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/1 TKN. The range may be extended with sample dilution.
2. Summary of Method
    2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.
3. Definitions
    3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.
    3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
    4.1 Samples may be preserved by addition of 2 ml of conc H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.
5. Apparatus
    5.1 Block Digestor–40
    5.2 Technicon Manifold for Ammonia (Figure 1)
    5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)
6. Reagents
    6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10 ml conc H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.
    6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled water and 200 ml of conc H_2SO_4. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

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6.3 Sulfuric Acid Solution (4%): Add 40 ml of conc. sulfuric acid to 800 ml of ammonia free distilled water, cool and dilute to 1 liter.

6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 ml of ammonia-free distilled water and dilute to 1 liter.

6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.

6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) in about 800 ml of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.

6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (6.5) to 200 ml of stock buffer solution (6.6) and mix. Add xx ml sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.

6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.

6.9 Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled water.

6.10 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml = 1.0 mg NH_3-N.

7. Procedure

Digestion

7.1 To 20 or 25 ml of sample, add 5 ml of digestion solution (6.2) and mix (use a vortex mixer).

7.2 Add (4–8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.

7.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

7.4 Cool sample and dilute to 25 ml with ammonia free water.

Colorimetric Analysis

7.5 Check the level of all reagent containers to ensure an adequate supply.

7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

7.7 Flush the Sampler IV wash receptacle with about 25 ml of 4.0% sulfuric acid (6.3).

7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

TABLE 1

CONCENTRATION RANGES
(NITROGEN)

	Dilution loops						
	Initial sample		Resample		Approx. std. cal. setting	Range PPM N (±10%)	ml stock NaOH per liter working buffer solution
No.	Sample line	Diluent line	Resample line	Diluent line			
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0–2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0–10	80

7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

7.10 After a stable baseline has been obtained start the Sampler.

8. Calculations

8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/1, the precision was ±0.07, ±0.03 and ±0.15, respectively.

9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/1, the recoveries were 99 and 99%, respectively.

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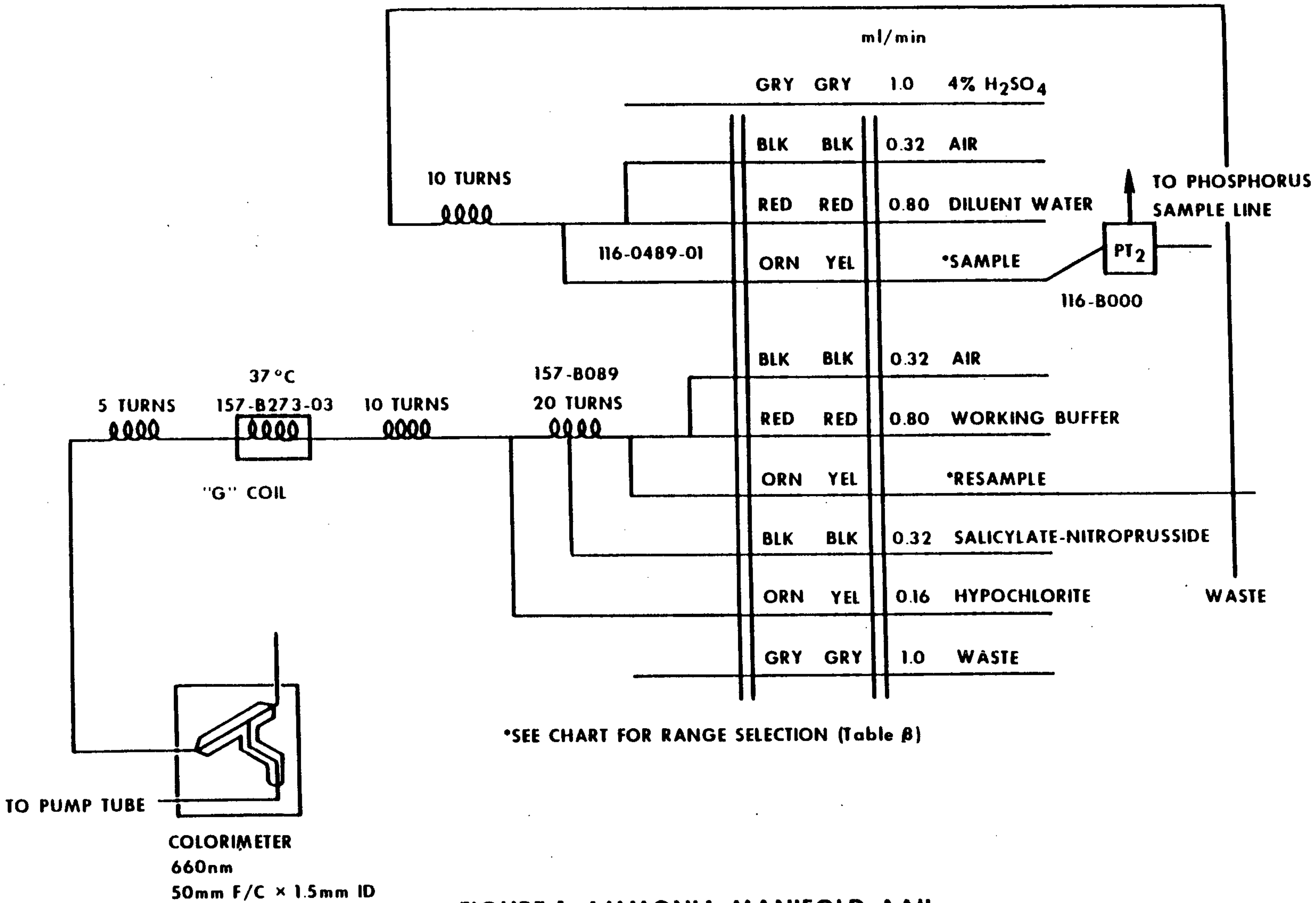


*SEE CHART FOR RANGE SELECTION (Table β)

FIGURE 1. AMMONIA MANIFOLD AAII

QuikChem METHOD 10-107-06-2-D

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS

6645 WEST MILL ROAD

MILWAUKEE, WI 53218, USA

LACHAT
INSTRUMENTS

QuikChem Method 10-107-06-2-D

Total Kjeldahl Nitrogen in Waters

0.2 to 20 .0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.
2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers

Revised by Karin Wendt 18 October 1994 written by D. Diamond and copyrighted on 24 June 92 by**Lachat Instruments**, **6645 West Mill Road, Milwaukee, WI 53218.** USA. Phone: (414) 358-4200 FAX: 1-414-358-4206.

Contents

QUIKCHEM METHOD 10-107-06-2-D

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.

1.3. The applicable range is 0.2 to 20 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. The sample is heated in the presence of sulfuric acid, H_2SO_4, for two and one half hours. The residue is cooled, diluted with water an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.

2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

  6.3.1. Sampler

  6.3.2. Multichannel proportioning pump

  6.3.3. Reaction unit or manifold

  6.3.4. Colorimetric detector

  6.3.5. Data system

6.4. Special apparatus

  6.4.1. Heating Unit

  6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

  6.4.3. 5 mL and 20 mL repipet dispensers

  6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation, the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degasssed.

Reagent 1. Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 40.0 mL water and **10 mL** concentrated **sulfuric acid** (H_2SO_4). Then add **8.0 g red mercuric oxide** (HgO). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

Reagent 2. **Digestion Solution**

In a **1** L volumetric flask, add **133.0 g potassium sulfate** (K_2SO_4) and **200 mL concentrated sulfuric acid** (H_2SO_4) to approximately **700 mL water**. Add **25.0 mL Reagent 1**. Dilute to the mark with water and invert to mix. Prepare fresh monthly.

Reagent 3. **Buffer**

By Volume: In a **1** L volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. **Salicylate Nitroprusside**

By Volume: In a **1 L** volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about **800 mL water**. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

By Weight: To a tared **1 L** dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] and **908 g water**. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.

Reagent 5. **Hypochlorite Solution**

By Volume: In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

By Weight: To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. **Diluent 5.0% (V/V) Sulfuric Acid**

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 1000 mg N/L

In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500.

Standard 2. Working Stock Standard 20.0 mg N/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily) Concentration mg N/L	A 20.00	B 10.00	C 5.00	D 2.00	E 1.00	F 0.50	G 0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	125	62.5	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (20.0 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (1000 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20 g** of Standard 1 (1000 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard.

$$\textbf{Labeled non-digested standard concentration} = \frac{\textbf{final digestate volume}}{\textbf{initial sample volume}} \times \textbf{standard concentration}$$

Working Standards Prepare Daily) Concentration mg N/L	A 20.00	B 10.00	C 5.00	D 2.00	E 1.00	F 0.500	G 0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with Reagent 6	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with Reagent 6.	250.0	125	62	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to $\mathrm{pH} < 2$ and cooled to $4^{O}C$ at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at $4^{O}C$ and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being > 10X MDL. If any determined concentration exceeds the known values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \quad X100$$

Where,

R = percent recovery

C_s = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid. standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

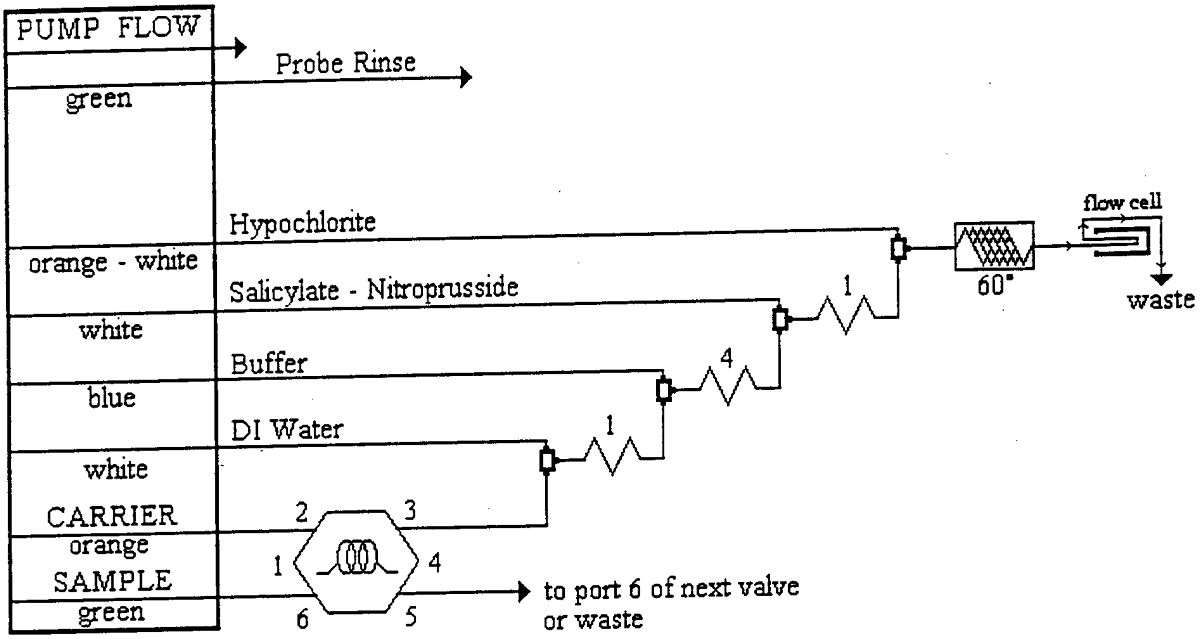
15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



QC8000 Sample Loop = 13.5 cm
AE Sample Loop = 8 cm

Interference Filter = 660 nm

CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve, flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d**. This is **5.2 uL/cm.**

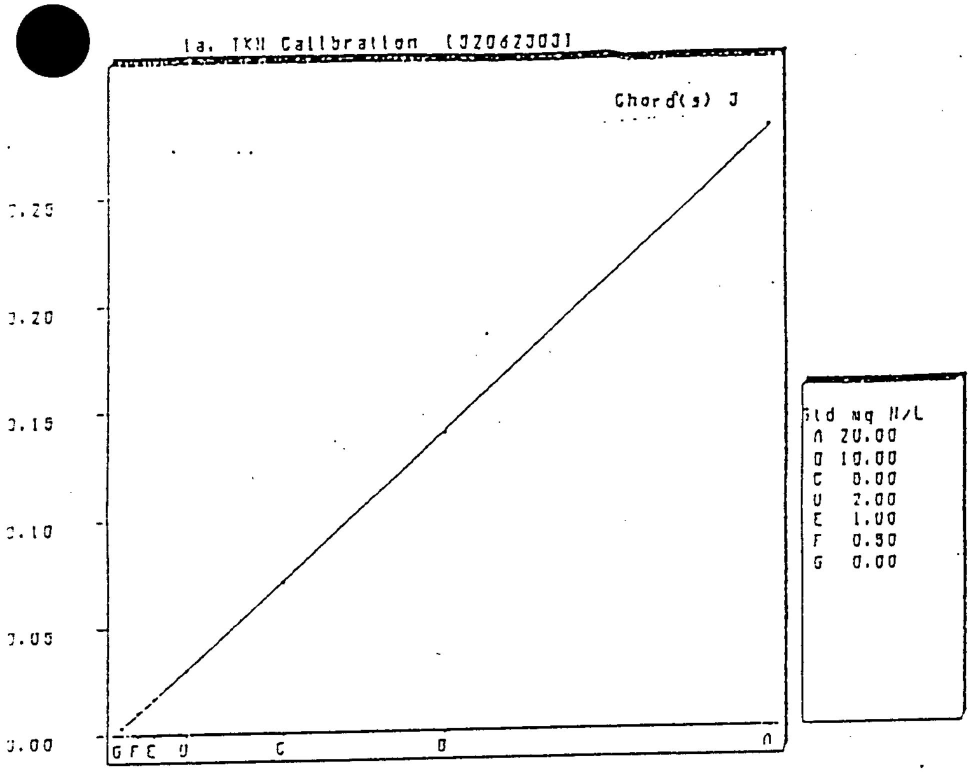
MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2 DATA SYSTEM PARAMETERS FOR THE QUIKCHEM AE.

Sample throughput:		90 samples/hour; 60 s/sample
Pump speed:		35
Cycle Period:		45 s
Inject to start of peak period:		38 s
Presentation, Data Window		
Top Scale Response:		0.32 abs
Bottom Scale Response:		0.00 abs
Segment/Boundaries:	A:	20.00 mg N/L
	E:	1 mg N/L
	F:	0.00 mg N/L

Series 4000/System IV Settings: Gain = 420 x 1

17.3 QUIKCHEM AE SUPPORT DATA



```
                    Calibration Statistics Report
                                                  06/24/92 03:48 pm
Cal Ref: 92062303
 Method: TKN-F

                         Channel: TKN

                     Correlation Coefficients

Seg Stds    Full    Chord 1   Chord 2   Chord 3  Chord 4   Chord 5
 1  A-E    0.9999   0.9992    1.0000    1.0000   0.9999   0.4219 ?
 2  E-G    0.9979   0.9902 ?  1.0000    1.0000   0.9904   0.6500 ?

                 Percent Standard Deviation in Slope

 1  A-E     0.7      1.5       0.2       0.1      0.6      67.
 2  E-G     1.6     51.5 ?     0.0       0.0      4.8      72.
```

```
*******************************************************************************
       QuikChem AE Calibration Report for Calibration 92062303
                          Method: TKN
            This calibration was done on 06/23/92 at  07:51 pm
            This report prepared on 06/24/92 at  03:44 pm
*******************************************************************************

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard A, TKN               mg N/L    20.000    20.042      -0.21           0.2795

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard B, TKN               mg N/L    10.000     9.913       0.87           0.1334

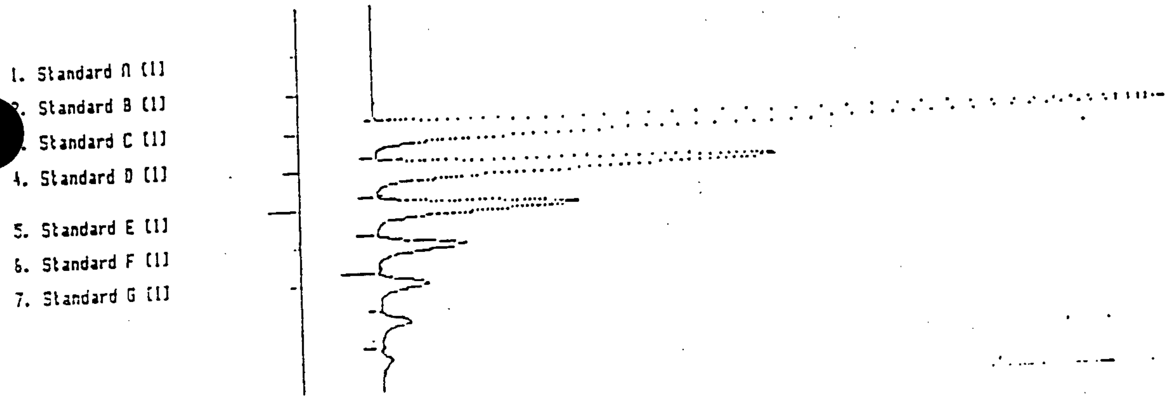
                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard C, TKN               mg N/L     5.000     4.994       0.12           0.0714

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard D, TKN               mg N/L     2.000     2.007      -0.34           0.0301

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard E, TKN               mg N/L     1.000     1.000       0.00           0.0168

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard F, TKN               mg N/L     0.500     0.500      -0.01           0.0091

                                         --- Average Concentrations ---  Baseline Corrected
                  Analyte      Units     Known   Determined  % Residual  Average Absorbance
Standard G, TKN               mg N/L     0.000    -0.000      ***.**          0.0033
```



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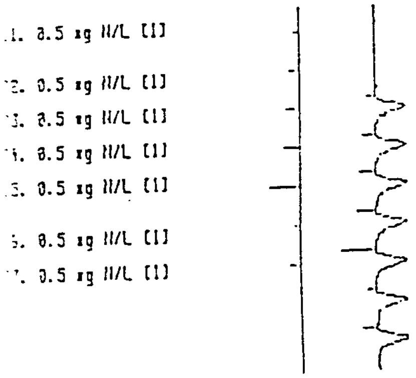
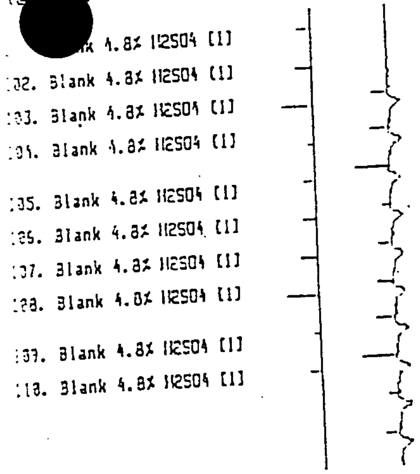
ack 1 (Ref: 92062300)  06/23/1992, 08:08 pm
```



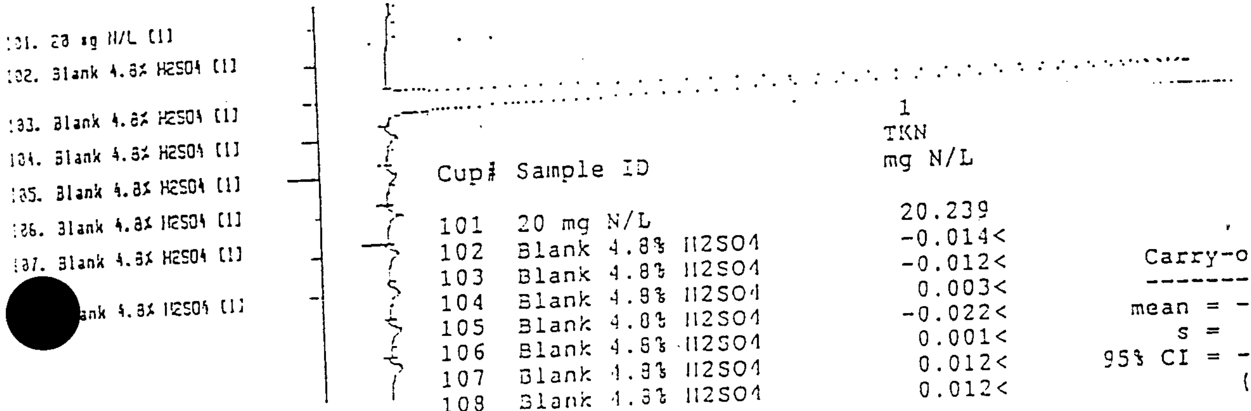
```
                              1
                              TKN
                              mg N/L
Cup# Sample ID
                                                 MDL
101  0.5 mg N/L               0.492<          ----------
102  0.5 mg N/L               0.496<        mean = 0.494
103  0.5 mg N/L               0.506            s = 0.0092
104  0.5 mg N/L               0.492<    (3.14) s = 0.029 mg N/L
105  0.5 mg N/L               0.479<
106  0.5 mg N/L               0.487<
107  0.5 mg N/L               0.503
```

```
Ra[illegible] 1 (Ref: 92062306)  06/23/1992, 07:51 pm
```



```
                                  1
                                  TKN
                                  mg N/L
Cup# Sample ID
                                                 EMDL DATA
101  Blank 4.8% H2SO4             0.010<         ---------
102  Blank 4.8% H2SO4            -0.004<        Mean = 0.0059
103  Blank 4.8% H2SO4             0.007<           s = 0.0091
104  Blank 4.8% H2SO4             0.012<    (4.65) s = 0.04 mg N/L
105  Blank 4.8% H2SO4            -0.003<
106  Blank 4.8% H2SO4            -0.000<
107  Blank 4.8% H2SO4             0.021<
108  Blank 4.8% H2SO4             0.008<
109  Blank 4.8% H2SO4             0.015<
110  Blank 4.8% H2SO4            -0.007<
```

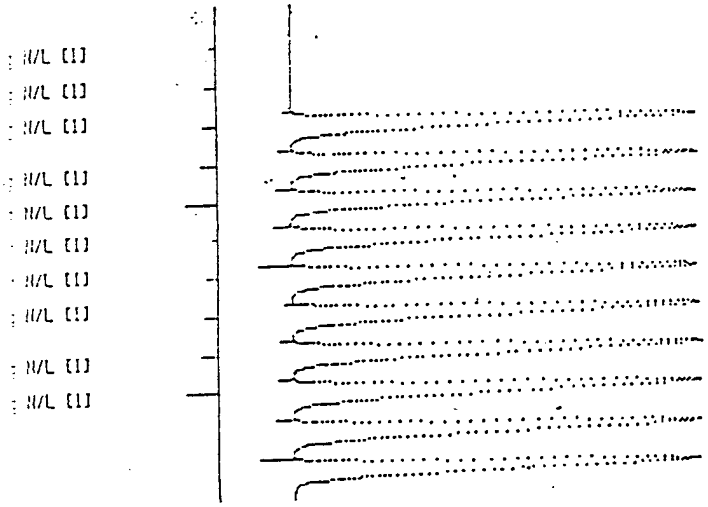
```
Rack 1 (Ref: 92062310)  06/23/1992, 08:29 pm
```



```
                                  1
                                  TKN
                                  mg N/L
Cup# Sample ID

101  20 mg N/L                   20.239
102  Blank 4.8% H2SO4            -0.014<           Carry-over
103  Blank 4.8% H2SO4            -0.012<           ----------
104  Blank 4.8% H2SO4             0.003<        mean = -0.0029
105  Blank 4.8% H2SO4            -0.022<           s =  0.0133
106  Blank 4.8% H2SO4             0.001<     95% CI = -0.015 to 0.009
107  Blank 4.8% H2SO4             0.012<              (passes)
108  Blank 4.8% H2SO4             0.012<
```

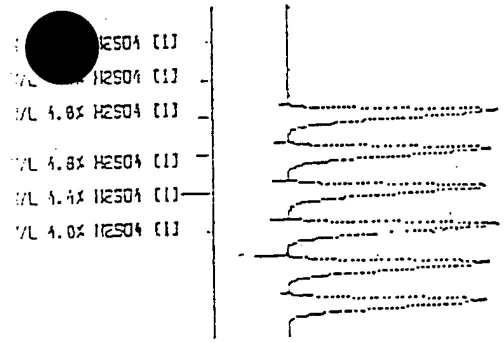
(Ref: 92062309)   06/23/1992, 08:15 pm



```
                                  1
                                 TKN
Cup# Sample ID                  mg N/L

101  10.0 mg N/L                10.017
102  10.0 mg N/L                10.022
103  10.0 mg N/L                 9.971          Precision
104  10.0 mg N/L                 9.989          ---------
105  10.0 mg N/L                10.001       mean = 10.06
106  10.0 mg N/L                10.096          s = 0.065
107  10.0 mg N/L                10.126       %RSD = 0.64
108  10.0 mg N/L                10.102
109  10.0 mg N/L                10.068
110  10.0 mg N/L                10.065
```

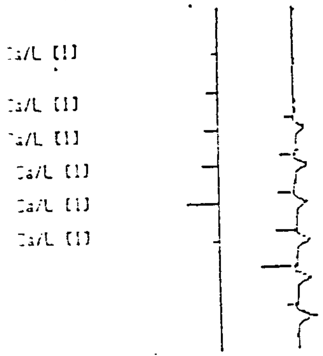
(Ref: 92062307)   06/23/1992, 08:00 pm



```
                                        1
                                       TKN
Cup# Sample ID                        mg N/L

101  5.0 mg N/L 4.8% H2SO4            5.039           Acid Effect
102  5.0 mg N/L 4.4% H2SO4            4.893           -----------
103  5.0 mg N/L 4.0% H2SO4            4.753     mean (4.8%) = 5.025 mg N/L
104  5.0 mg N/L 4.8% H2SO4            5.011     mean (4.4%) = 4.859 mg N/L
105  5.0 mg N/L 4.4% H2SO4            4.825     mean (4.0%) = 4.775 mg N/L
106  5.0 mg N/L 4.0% H2SO4            4.757
```

(Ref: 92062311)   06/23/1992, 08:38 pm



```
                           1
                          TKN
Cup# Sample ID           mg N/L

101  20 mg Ca/L         -0.002<        Calcium Interference
102  20 mg Ca/L          0.001<      --------------------
103  20 mg Ca/L          0.021<    <0.10 mg N/L at 100 mg Ca/L
104  100 mg Ca/L         0.044<
105  100 mg Ca/L         0.051<
106  100 mg Ca/L         0.080<
```

17.4 DATA PARAMETERS FOR THE QUIK CHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width:	39 s
% Width Tolerance:	100
Threshold:	11537
Inject to Peak Start:	42 s
Chemistry:	Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None

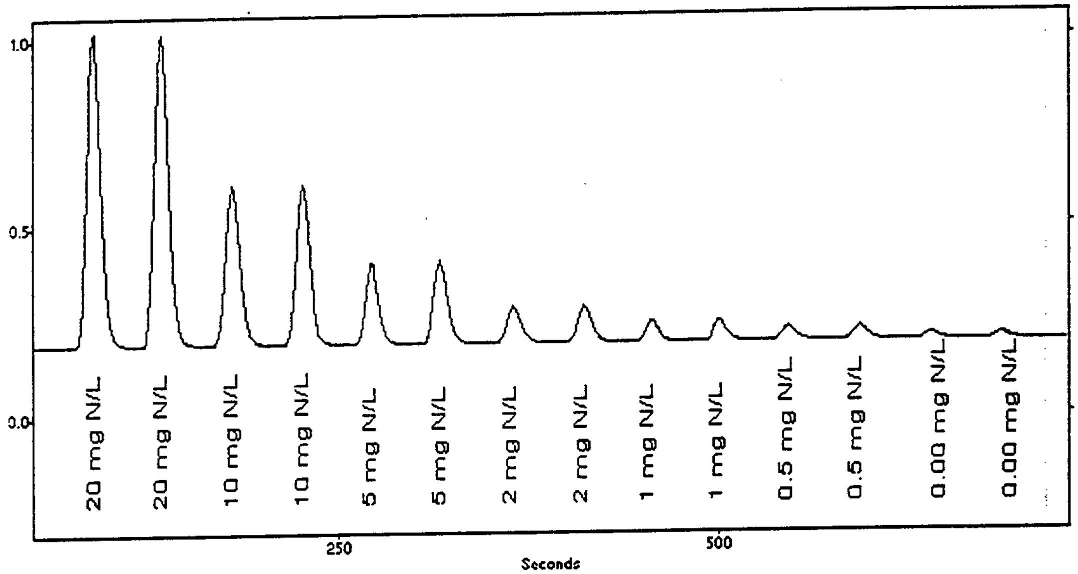
Sampler Timing:

Min. Probe in Wash Period:	14 s
Probe in Sample Period:	20 s

Valve Timing:

Load Period:	20 s
Inject Period:	25 s
Load Time:	0.0 s

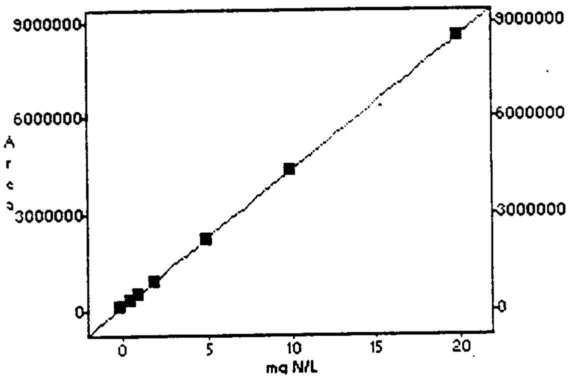
17.5 QUIKCHEM 8000 SUPPORT DATA



ACQ. TIME: Aug 22, 1994 13:25:57
DATA FILENAME: C:\OMNION\DATA\1010762E\082294C2.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

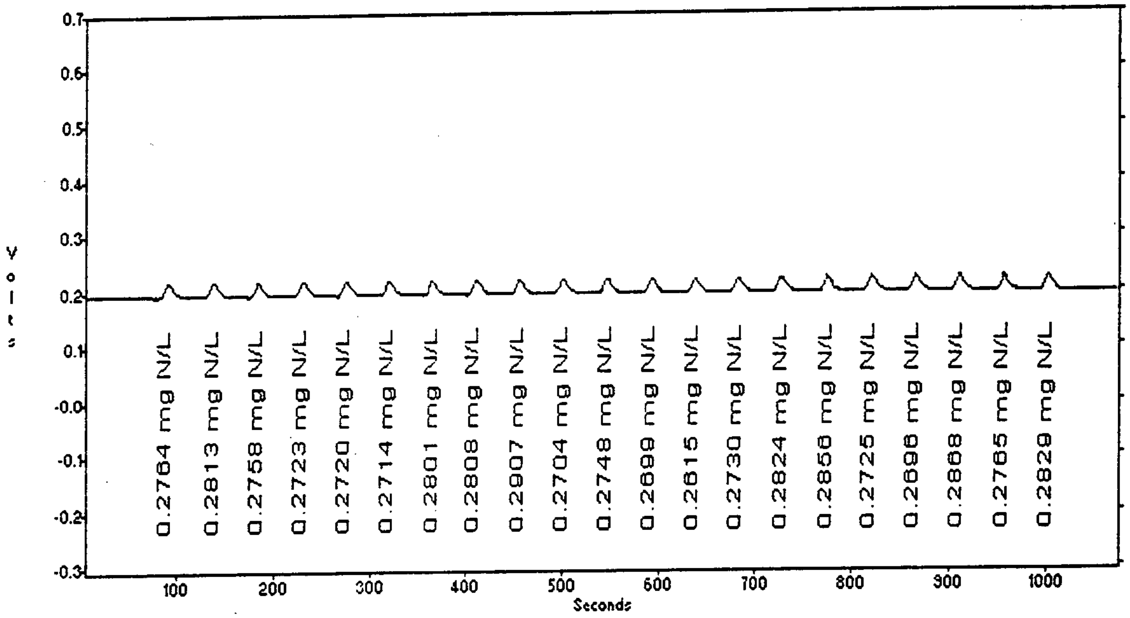
Calibration Graph and Statistics

Level	Area	mg CN⁻/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8596849	20	20.000	8596849	8634613	26703.2	0.3	-0.0
2	4383597	10	10.020	438597	4373046	7460.7	0.2	-0.2
3	2248447	5	4.960	2248447	2246723	1218.7	0.1	0.8
4	990856	2	1.991	990856	978804	8522.1	0.9	0.9
5	574638	1	0.997	574638	566821	5527.9	1.0	0.3
6	366814	0.5	0.504	366814	364718	1481.9	0.4	-0.9
7	167977	0	0	167977	165993	1403.2	0.8	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 2.369e-006Area-3.646e-001
$R^2 = 1.000$

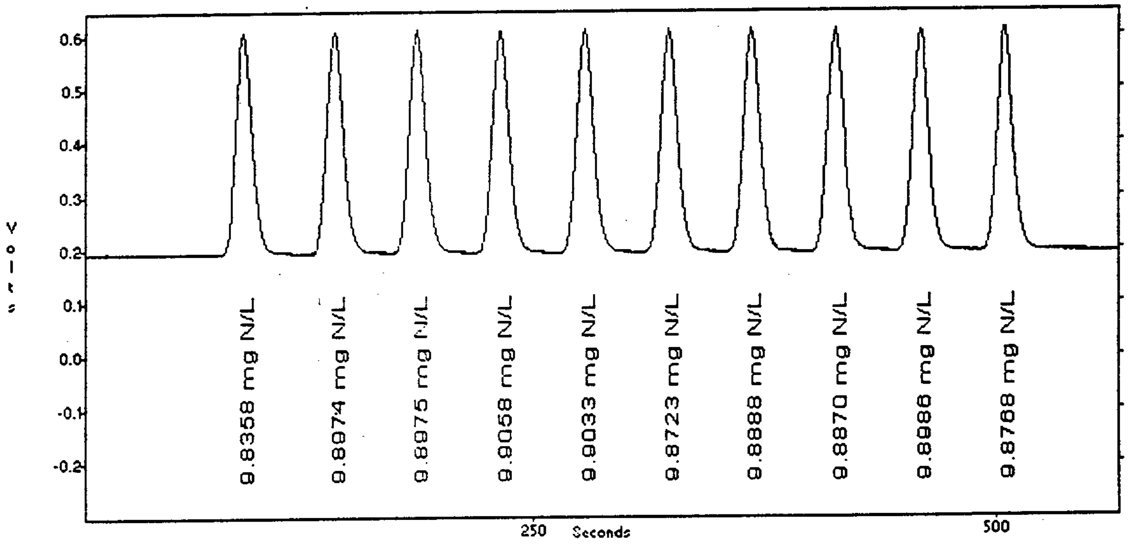
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME: Aug 22, 1994 13:54:02
DATA FILENAME: C:\OMNION\DATA\1010762E\082294M1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

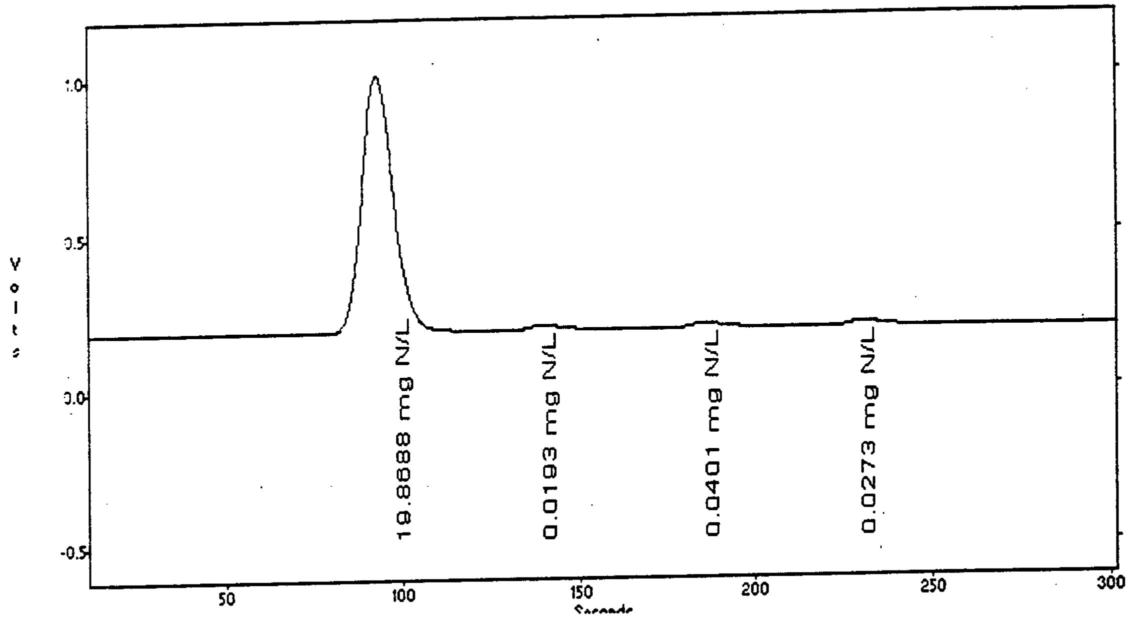
Figure 3. Precision



Precision = 0.211 % RSD

ACQ. TIME: Aug 22, 1994 14:21:28
DATA FILENAME: C:\OMNION\DATA\1010762E\082294P1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

Figure 4. Carryover



ACQ. TIME: Aug 22, 1994 14:32:42
DATA FILENAME: C:\OMNION\DATA\1010762E\082294R1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

QuikChem METHOD 10-107-06-2-E

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS
6645 WEST MILL ROAD
MILWAUKEE, WI 53218, USA

LACHAT

INSTRUMENTS

QuikChem Method 10-107-06-2-E

Total Kjeldahl Nitrogen in Waters

0.1 to 5.0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.
2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)
3. 5 mL and 20 mL Repipet Dispensers

Revised by Karin Wendt 18 October 1994 written by D. Diamond and copyrighted on 24 June 92 by**Lachat Instruments. 6645 West Mill Road. Milwaukee, WI 53218,** USA. Phone: (414) 358-4200 FAX: 1-414-358-4206.

Contents

QUIKCHEM METHOD 10-107-06-2-E

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.

1.3. The applicable range is 0.1 to 5 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. The sample is heated in the presence of sulfuric acid, H_2SO_4, for two and one half hours. The residue is cooled, diluted with water an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known. basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia. and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.

2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation. the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degasssed.

Reagent 1. **Mercuric Sulfate Solution**

To a 100 mL volumetric flask add approximately 40.0 mL water and **10 mL** concentrated **sulfuric acid** (H_2SO_4). Then add **8.0 g red mercuric oxide** (HgO). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

Reagent 2. **Digestion Solution**

In a **1 L** volumetric flask, add **133.0 g potassium sulfate** (K_2SO_4) and **200 mL concentrated sulfuric acid** (H_2SO_4) to approximately **700 mL water**. Add **25.0 mL Reagent 1.** Dilute to the mark with water and invert to mix. Prepare fresh monthly.

Reagent 3. **Buffer**

By Volume: In a **1 L** volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. **Salicylate Nitroprusside**

By Volume: In a **1 L** volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about **800 mL water**. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

By Weight: To a tared **1 L** dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] and **908 g water**. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.

Reagent 5. Hypochlorite Solution

By Volume: In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

By Weight: To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. Diluent 5.0% (V/V) Sulfuric Acid

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 250 mg N/L

In a **1 L** volumetric flask dissolve **0.9540 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500 (use 1.18g).

Standard 2. Working Stock Standard 5.0 mg N/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~ 250 g) divide by **factor** below with DI water.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (5.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (250 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20 g** of Standard 1 (250 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg N/L for a 5.00 mg N/L non-digested standard. If non-digested standards are used to calibrate, the "labeled" concentrations should be entered in the data system.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \text{ X standard concentration}$$

Preparation of Non-digested Working Standards

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 4** diluted to 100 mL with **Standard 3**	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 4** diluted to final weight (~250 g) divide by **factor** below with **Standard 3**.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to pH < 2 and cooled to $4^{O}C$ at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at $4^{O}C$ and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being > 10X MDL. If any determined concentration exceeds the known values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \text{ X100}$$

Where,

R = percent recovery

C_s = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system. such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the

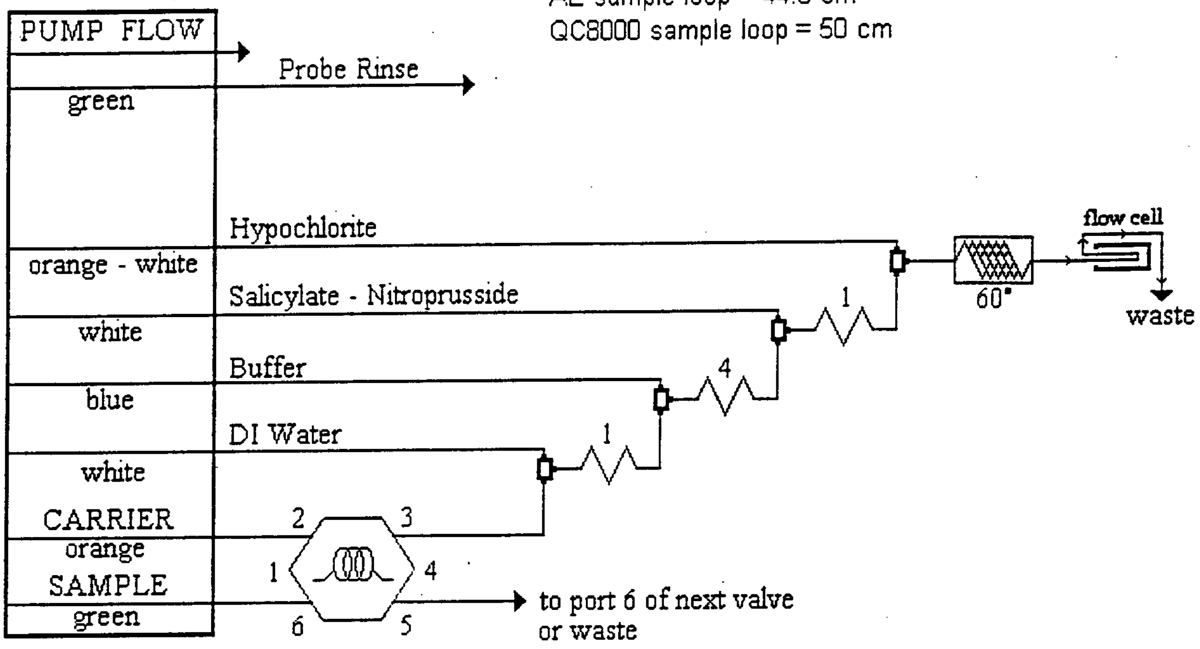
"Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2
2. ASTM, Water(I), Volume 11.01, Method D3590-89, Test Methods for Kjeldahl Nitrogen in Water, p. 447
3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1
4. Code of Federal Regulations 40, Chapter 1, Part 136, Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



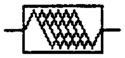
AE sample loop = 44.5 cm
QC8000 sample loop = 50 cm

Interference Filter = 660 nm

CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve, flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d.** This is **5.2 uL/cm.**

MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2. DATA SYSTEM PARAMETERS FOR QUIKCHEM AE

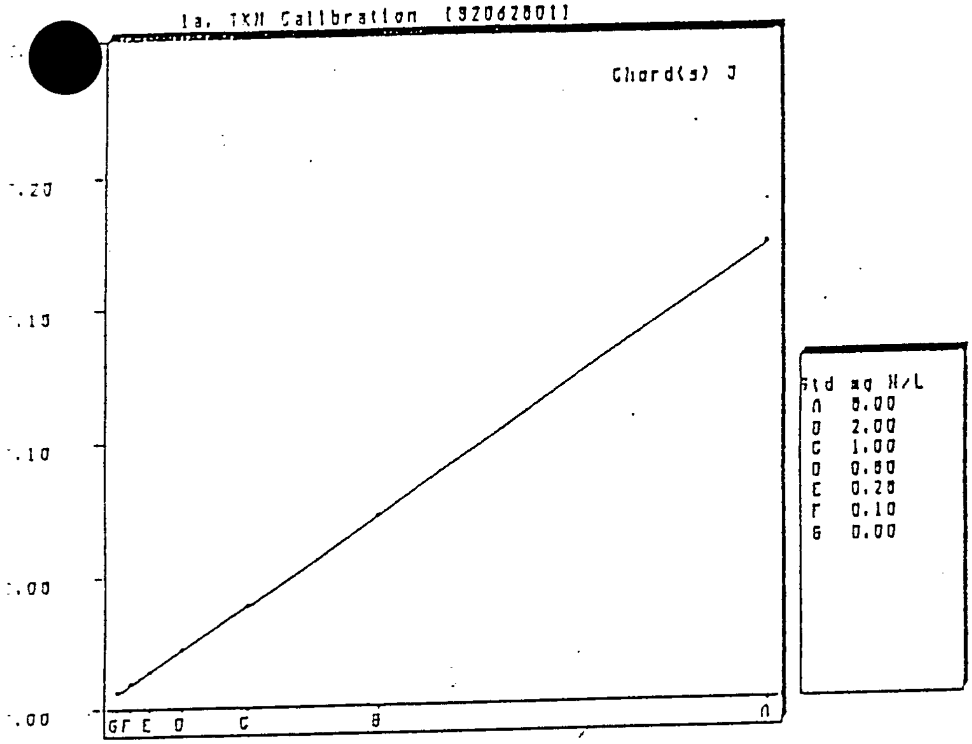
Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s
Inject to start of peak period:	38 s

Presentation, Data Window

Top Scale Response:		0.25 abs
Bottom Scale Response:		0.00 abs
Segment/Boundaries:	A:	5.00 mg N/L
	C:	1 mg N/L
	G:	0.00 mg N/L

Series 4000/System IV Settings: Gain = 570 x 1

17.3. QUIKCHEM AE SUPPORT DATA



```
                         CalibrationStatistics Report
Cal Ref: 92052501                                   06/23/92 01:43 pm
 Method: TKN-F

                              Channel: TKN

                         Correlation Coefficients

Seq Stds    Full     Chord 1    Chord 2    Chord 3   Chord 4    Chord 5
 1  A-C    1.0000    0.9992     0.9997     1.0000    0.9999     1.0000
*2  C-G    0.9906    0.8384 ?   0.9980     0.9998    0.9959     0.4112 ?

                     Percent Standard Deviation in Slope

 1  A-C     0.3       2.0        1.0        0.5       0.0        0.4
 2  C-G     2.2      25.3        2.6        0.0       3.7       59.1 ?
```

```
--------------------------------------------------------------------------
      QuikChem AE Calibration Report for Calibration 92062501
                          Method: TKN
            This calibration was done on 06/23/92 at  12:25 pm
            This report prepared on 06/23/92 at  01:31 pm
--------------------------------------------------------------------------

                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard A, TKN                  mg N/L  5.000     5.003       -0.05          0.1715

                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard B, TKN                  mg N/L  2.000     1.989        0.54          0.0711

                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard C, TKN                  mg N/L  1.000     1.004       -0.36          0.0345

                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard D, TKN                  mg N/L  0.500     0.496        0.78          0.0229

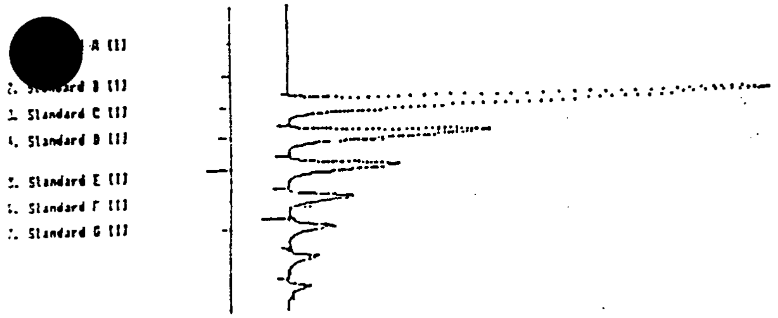
                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard E, TKN                  mg N/L  0.250     0.242        3.27          0.0137

                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard F, TKN                  mg N/L  0.100     0.102       -1.77          0.0072

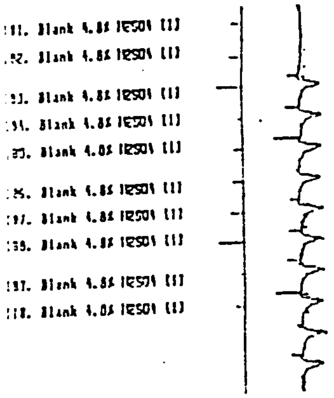
                                         —— Average Concentrations ——   Baseline Corrected
                Analyte          Units   Known   Determined  % Residual  Average Absorbance
Standard G, TKN                  mg N/L  0.000     0.006       ***.**         0.0061

————————— End of Calibration Report For Calibration 92062501 —————————
```

```
Calibration 06/25/1992, 12:17 pm
```



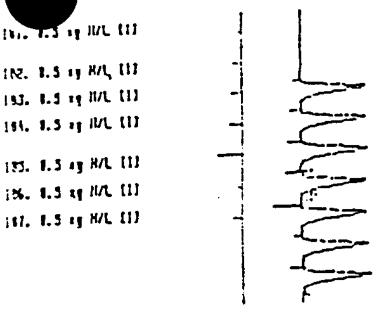
```
Rack 1 (Ref: 92062502)  06/25/1992, 12:25 pm
```



```
                                      1
                                     TKN
                                   mg N/L
Cup  Sample ID

101  Blank 4.0% H2SO4              0.013<
102  Blank 4.0% H2SO4              0.012<        EMDL Determination
103  Blank 4.0% H2SO4              0.009<        ------------------
104  Blank 4.0% H2SO4              0.003<          mean = 0.009
105  Blank 4.0% H2SO4              0.049<             s = 0.016
106  Blank 4.0% H2SO4             -0.007<     4.65 (s) = 0.076 mg N/L
107  Blank 4.0% H2SO4              0.010<
108  Blank 4.0% H2SO4              0.009<
109  Blank 4.0% H2SO4              0.009<
110  Blank 4.0% H2SO4             -0.013<
     --------- End of Report for Tray 92062502.RS -----------
```

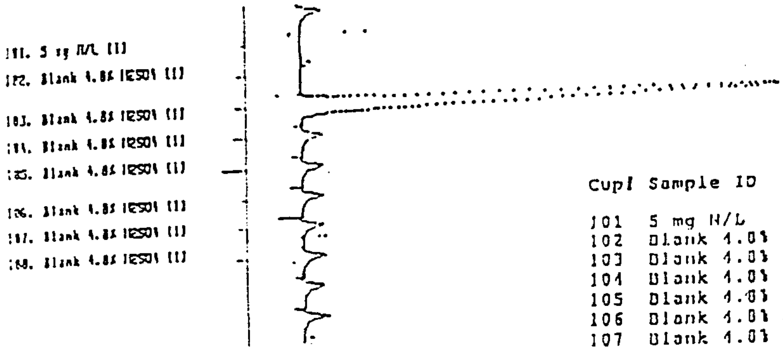
```
 (Ref: 92062504)  06/25/1992, 12:41 pm
```



```
                                      1
                                     TKN
Cup  Sample ID                     mg N/L

101  0.5 mg N/L                     0.489          MDL Study
102  0.5 mg N/L                     0.486         -----------
103  0.5 mg N/L                     0.488       mean = 0.487
104  0.5 mg N/L                     0.491          s = 0.008
105  0.5 mg N/L                     0.470   (3.14) s = 0.024
106  0.5 mg N/L                     0.492
107  0.5 mg N/L                     0.490
     --------- End of Report for Tray 92062504.RS -----------
```

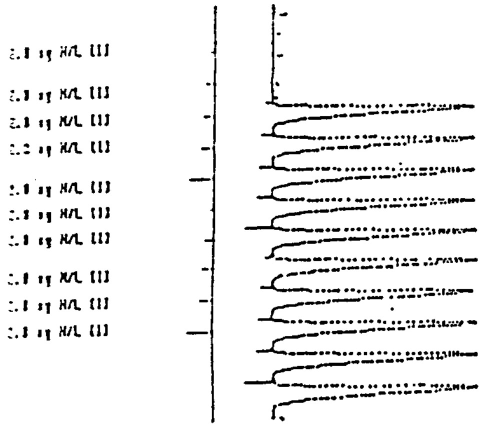
```
Rack 1 (Ref: 92062506)  06/25/1992, 12:54 pm
```



```
                                      1
                                     TKN
Cup  Sample ID                     mg N/L

101  5 mg N/L                       4.052
102  Blank 4.0% H2SO4               0.003<      Carry-over Study
103  Blank 4.0% H2SO4               0.002<      ----------------
104  Blank 4.0% H2SO4               0.001<      mean = 0.001
105  Blank 4.0% H2SO4              -0.001<         s = 0.005
106  Blank 4.0% H2SO4               0.009<   95% CI ( -0.004 to 0.006)
107  Blank 4.0% H2SO4               0.004<          passes
108  Blank 4.0% H2SO4              -0.009<
     --------- End of Report for Tray 92062506.RS -----------
```

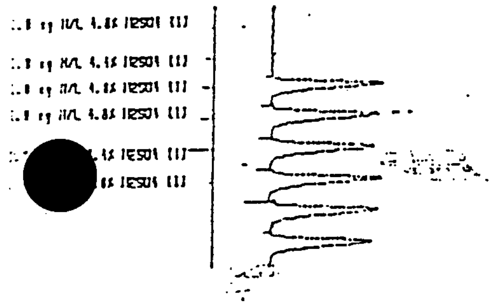
Ch 1 (Ref: 92062507) 06/25/1992, 01:04 pm



```
                                   1
                                   TKN
Cup# Sample ID                     mg N/L

101  2.0 mg N/L                      1.923        Precision at Midscale
102  2.0 mg N/L                      1.926        ---------------------
103  2.0 mg N/L                      1.925          mean = 1.929
104  2.0 mg N/L                      1.926             s = 0.023
105  2.0 mg N/L                      1.947          %RSD = 1.18
106  2.0 mg N/L                      1.912
107  2.0 mg N/L                      1.883
108  2.0 mg N/L                      1.933
109  2.0 mg N/L                      1.955
110  2.0 mg N/L                      1.963
    --------- End of Report for Tray 92062507.RS -----------
```

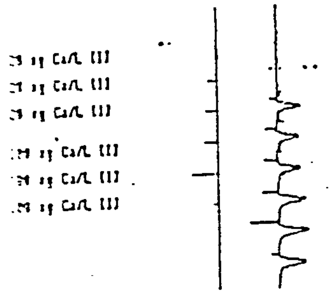
Ch 1 (Ref: 92062503) 06/25/1992, 12:35 pm



```
                                  1
                                  TKN
Cup# Sample ID                    mg N/L

101  1.0 mg N/L 4.0% H2SO4        0.966         Acid Effect
102  1.0 mg N/L 4.4% H2SO4        0.960         -----------
103  1.0 mg N/L 4.8% H2SO4        0.894      mean (4.0%) = 0.971
104  1.0 mg N/L 4.0% H2SO4        0.975      mean (4.4%) = 0.943
105  1.0 mg N/L 4.4% H2SO4        0.926      mean (4.8%) = 0.891
106  1.0 mg N/L 4.8% H2SO4        0.888
    --------- End of Report for Tray 92062503.RS -----------
```

Ch 1 (Ref: 92062508) 06/25/1992, 01:14 pm



```
                                  1
                                  TKN
Cup# Sample ID                    mg N/L

101  20 mg Ca/L                  -0.013<
102  20 mg Ca/L                   0.005<     Calcium Interference Study
103  20 mg Ca/L                   0.020<     --------------------------
104  100 mg Ca/L                  0.052<       mean (20 mg/L) = 0.004
105  100 mg Ca/L                  0.058<     mean (100 mg/L) = 0.059
106  100 mg Ca/L                  0.068<         <MDL at 100 mg/L
    --------- End of Report for Tray 92062508.RS -----------
```

17.4. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width:	31 s
% Width Tolerance:	100
Threshold:	25000
Inject to Peak Start:	42 s
Chemistry:	Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None

Sampler Timing:

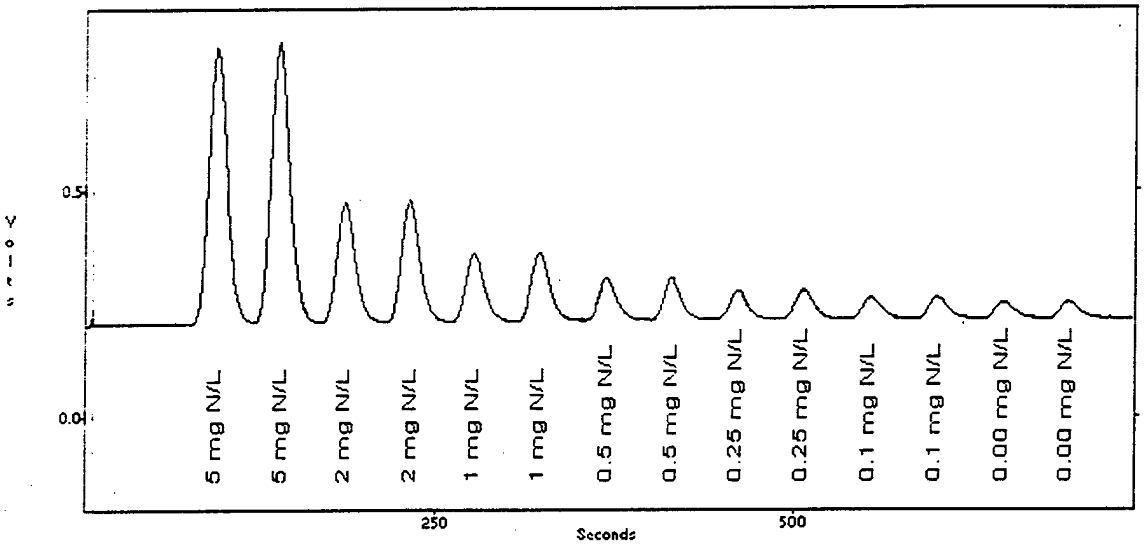
Min. Probe in Wash Period:	14 s
Probe in Sample Period:	20 s

Valve Timing:

Load Period:	20 s
Inject Period:	25 s
Load Time:	0.0 s

17.5 QUIKCHEM 8000 SUPPORT DATA

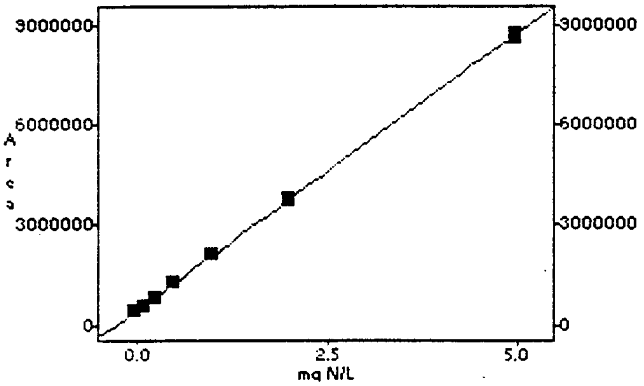
Figure 1. Calibration Graph and Statistics for Total Kjeldahl Nitrogen



ACQ. TIME: Aug 15, 1994 15:00:17
DATA FILENAME: C:\OMNION\DATA\1010762E\081594C1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

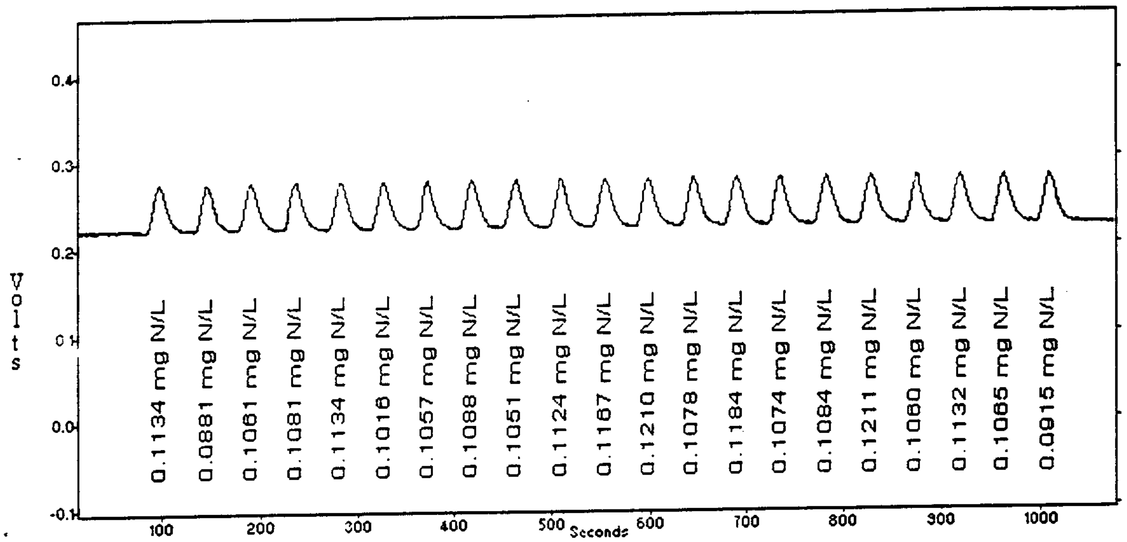
Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8778128	5	5.000	8851078	8705178	1003166.9	1.2	0.0
2	3809886	2	2.002	3832259	3787513	31640.2	0.8	-0.1
3	2159521	1	1.006	2157189	2161852	3296.9	0.2	-0.6
4	1326319	0.5	0.5035	1335357	1317280	12782.8	1.0	-0.7
5	896683	0.25	0.2445	890758	902609	8380.0	0.9	2.2
6	643806	0.1	0.108	648879	638732	7174.7	1.1	8.0
7	498016	0	0	5071570	488876	12926.6	2.6	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 6.032e-007Area-2.964e-001
$R^2 = 1.000$

Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME: Aug 18, 1994 8:52:31
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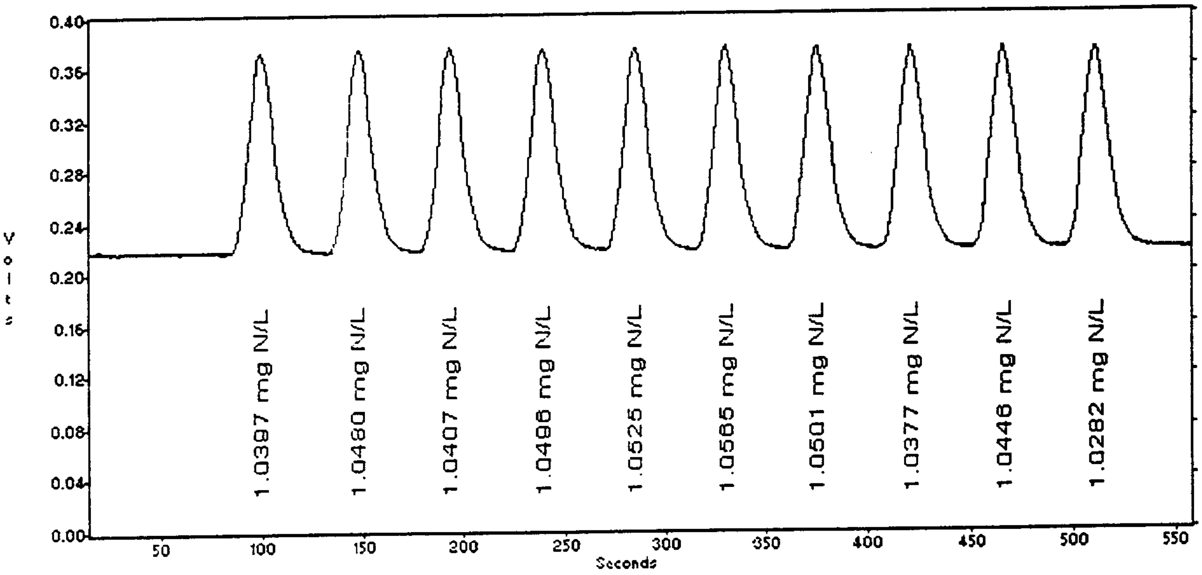
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

Figure 3. Precision



Precision = 0.796 % RSD

ACQ. TIME: Aug 15, 1994 15:50:24
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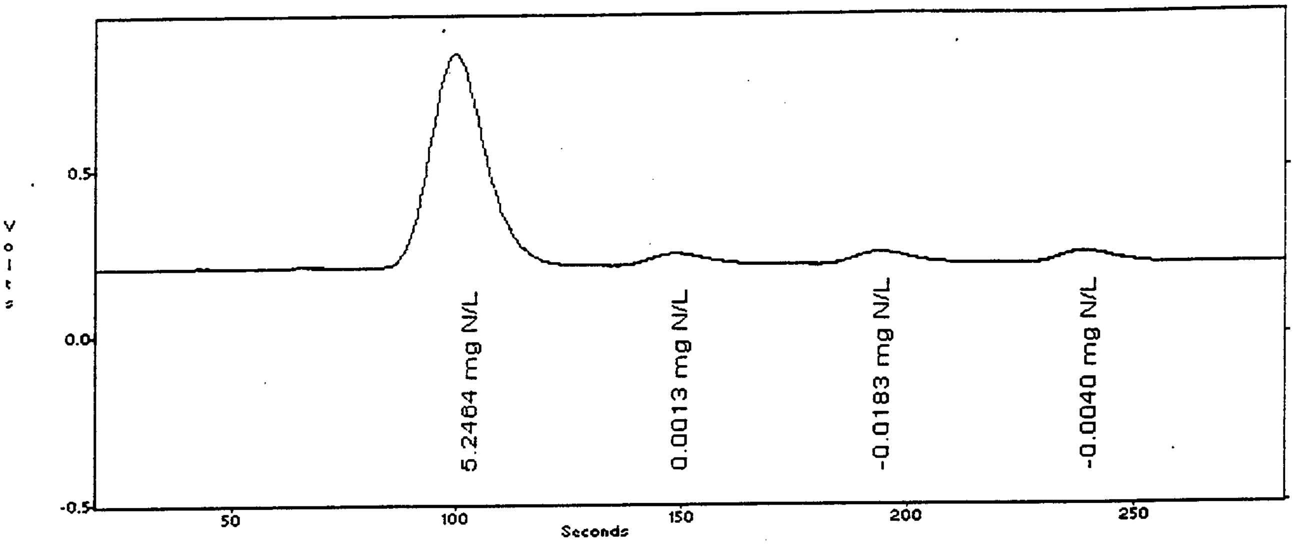
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

Figure 4. Carryover



Carryover passed

ACQ. TIME:	Aug 15, 1994 16:01:01
DATA FILENAME:	C:\OMNION\DATA\1010762E\081594R1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762E\1010762E.met

Technicon™ AutoAnalyzer™ II

INDUSTRIAL METHOD No. 334-74W/B$^+$ **RELEASED: JANUARY, 1976/REVISED: MARCH 1977**

INDIVIDUAL/SIMULTANEOUS* DETERMINATION OF NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

RANGE: Nitrogen 1-50 mg/l; 20-1000 mg/l
Phosphorus 1-50 mg/l; 20-1000 mg/l
BD-20/BD-40 (DIALYZER)

GENERAL DESCRIPTION

NITROGEN

The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at a pH of 12.8-13.0. The ammonia-salicylate complex is read at 660 nm.

PHOSPHORUS

The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm.

The acid digest samples are prepared by digestion with the Technicon™ BD-40 or BD-20 Block Digestor. Refer to Manual No. TA4-0323-11 for sample preparation.

PERFORMANCE AT 40 SAMPLES PER HOUR

MANUALLY PREPARED STANDARDS

NITROGEN	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 1.00 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.6%	at 500 mg/l ±0.4%
Detection Limit	1.0 mg/l	20 mg/l

PHOSPHORUS	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 0.60 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.5%	at 500 mg/l ±0.6%
Detection Limit	1.0 mg/l	20 mg/l

*See Operating Note 7.

TECHNICON INDUSTRIAL SYSTEMS / TARRYTOWN, N.Y. 10591
A DIVISION OF TECHNICON INSTRUMENTS CORPORATION

PRINTING CODE:
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4732-1-6/R3-7-1.5

REAGENTS

Unless otherwise specified, all reagents should be of ACS quality or equivalent.

GENERAL REAGENTS

TRITON X-100 SOLUTION (50% in Methanol)

Triton X-100** (Technicon No. T21-0188)	50 ml
Methanol (CH_3OH)	50 ml

Preparation:

Add 50 ml of Triton X-100 to 50 ml of methanol and mix thoroughly.

SYSTEM WASH WATER SOLUTION
(For System Shut-Down and Start-Up Only)

Triton X-100 Solution	1.0 ml
Distilled Water	1000 ml

Preparation:

Add 1.0 ml of Triton X-100 solution to one liter of distilled water and mix.

SAMPLER IV WASH RECEPTACLE SOLUTION

Distilled Water

Note: This reagent contains *no* wetting agent.

NITROGEN REAGENTS

STOCK SODIUM HYDROXIDE SOLUTION, 20%

Sodium Hydroxide Solution, 50% w/w	400 g
Distilled Water, q.s.	1000 ml

Preparation:

To 600 ml of distilled water, add 400 g of sodium hydroxide solution, 50% w/w. Cool to room temperature and dilute to one liter with distilled water.

STOCK SODIUM POTASSIUM TARTRATE SOLUTION, 20%

Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)	200 g
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 200 g of sodium potassium tartrate in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly.

STOCK BUFFER SOLUTION 0.5*M*

Sodium Phosphate, Dibasic, crystal ($Na_2HPO_4 \cdot 7H_2O$	134 g
[Sodium Phosphate, Dibasic, anhydrous (Na_2HPO_4)]	[71 g]
Sodium Hydroxide Solution, 50% w/w	40 g
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 134 g of sodium phosphate, dibasic, crystal (or 71 g of sodium phosphate, dibasic, anhydrous) in about 800 ml of distilled water. Add 40 g of sodium hydroxide solution, 50% w/w, dilute to one liter with distilled water and mix thoroughly.

WORKING BUFFER SOLUTION

Stock Buffer Solution, 0.5*M*	200 ml
Stock Sodium Potassium Tartrate Solution, 20%	250 ml
Stock Sodium Hydroxide Solution, 20%	250 ml
Distilled Water, q.s.	1000 ml
Brij-35,*** 30% Solution (Technicon No. T21-0110)	1.0 ml

Preparation:

Combine the reagents in the stated order: add 250 ml of stock sodium potassium tartrate solution, 20%, to 200 ml of stock buffer solution, 0.5*M*, with swirling. Slowly, with swirling, add 250 ml of sodium hydroxide solution, 20%. Dilute to one liter with distilled water, add 1.0 ml of Brij-35, 30% solution, (20-25 drops) and mix thoroughly.

SULFURIC ACID/SODIUM CHLORIDE SOLUTION

Sulfuric Acid, 95-98% (H_2SO_4)	7.5 ml
Sodium Chloride (NaCl)	100 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% Solution	1.0 ml

Preparation:

Dissolve 100 g of sodium chloride in about 600 ml of distilled water. Add 7.5 ml of sulfuric acid and dilute to one liter with distilled water. Add 1.0 ml of Brij-35 (about 20 drops) and mix thoroughly.

SODIUM SALICYLATE/SODIUM NITROPRUSSIDE SOLUTION

Sodium Salicylate ($NaC_7H_5O_3$)	150 g
Sodium Nitroprusside [$Na_2Fe(CN)_5NO \cdot 2H_2O$]	0.30 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% solution	1.0 ml

**Trademark of Rohm and Haas Company.

***Trademark of Atlas Chemical Industries, Inc.

Preparation:

Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 ml of distilled water. Filter through fast filter paper into a one liter volumetric flask and dilute to volume with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly. Store in a light-resistant container.

SODIUM HYPOCHLORITE SOLUTION, 0.315%

Sodium Hypochlorite Solution, 5.25%	6.0 ml
Distilled Water, q.s.	100 ml
Brij-35, 30% Solution	0.1 ml

Preparation:

Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with distilled water. Add 0.1 ml (2 drops) of Brij-35 and mix thoroughly. Prepare fresh daily. [Any commercial bleach solution (e.g. Clorox) containing 5.25% available chlorine is satisfactory.]

PHOSPHORUS REAGENTS

SULFURIC ACID SOLUTION, 4.0*N*

Sulfuric Acid, 95-98% (H_2SO_4)	111 ml
Distilled Water, q.s.	1000 ml
Triton X-100 Solution	1.0 ml

Preparation:

While swirling, cautiously add 111 ml of sulfuric acid to about 600 ml of distilled water. Cool to room temperature and dilute to one liter with distilled water. Add 1.0 ml of Triton X-100 solution and mix thoroughly.

SODIUM CHLORIDE SOLUTION, 0.25%

Sodium Chloride (NaCl)	2.5 g
Distilled Water, q.s.	1000 ml
Aerosol-22****	5.0

Preparation:

Dissolve 2.5 g of sodium chloride in about 600 ml of distilled water. Dilute to one liter with distilled water. Add 5.0 ml of Aerosol-22 and mix thoroughly.

MOLYBDATE/ANTIMONY SOLUTION

Ammonium Molybdate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$	10.0 g
Antimony Potassium Tartrate $[K(SbO)C_4H_4O_6 \cdot 1/2H_2O]$	0.15 g
Sulfuric Acid, 95-98% (H_2SO_4)	60 ml
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 10.0 g of ammonium molybdate and 0.15 g of antimony potassium tartrate in about 800 ml of distilled water. While swirling, cautiously add 60 ml of sulfuric acid. Cool to room temperature, dilute to one liter with distilled water and mix thoroughly. Transfer to a light-resistant container. This solution is stable for about one month.

ASCORBIC ACID SOLUTION, 1.0%

Ascorbic Acid ($C_6H_8O_6$) – OR – Araboascorbic Acid ($C_6H_8O_6$)	2.0 g
Distilled Water, q.s.	200 ml

Preparation:

Dissolve 2.0 g of ascorbic acid or araboascorbic acid in about 150 ml of distilled water. Dilute to 200 ml with distilled water and mix thoroughly. Transfer to a light-resistant container. If kept refrigerated and tightly stoppered when not in use, this solution is stable for at least two days.

OPERATING NOTES

1. **Start-Up**
   a. Check the level of all reagents to ensure an adequate supply.
   b. Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers.
   c. When reagents have been pumping for at least five minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate for 10 minutes.

   NOTE: If a precipitate appears after the addition of salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid is caused by a low pH. Before restarting the system, check the concentration of the sulfuric acid solution and/or the working buffer solution.

   d. To prevent precipitation of salicylic acid in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen colorimeter TO WASTE tube separate from all other lines or keep tap water flowing in the waste tray.

2. **Shut-Down**
   a. Remove the salicylate and molybdate/antimony lines from their containers and allow them to pump air. When the air bubbles enter the analytical system, place all reagent lines (excluding the Sampler IV Wash Receptacle Solution line) in the System Wash Water Solution.
   b. After 15 minutes, stop the proportioning pump and remove the platen.

****Trademark of American Cyanamid Company.

3. **System Operation**
   a. Be sure the plastic cover of the analytical cartridge is in place when operating the system.

   b. At STD CAL settings of 6.00 or more, the system may be operated in the DAMP 1 position, if necessary.

4. **Manifold Connections**

   To avoid the possibility of airborne contamination, the air lines of the nitrogen channel should be attached to an air scrubber containing dilute sulfuric acid (10% v/v).

5. **Reagent Background Color**
   a. Place all lines in the system wash water container and start the proportioning pump. After making the necessary adjustments on the colorimeters set the STD CAL control of the nitrogen colorimeter to 1.00 and the STD CAL control of the phosphorus colorimeter to 2.90. Adjust the water baseline on both colorimeters to zero with the BLANK control.

   b. Following the start-up procedure, place all reagent lines in the proper order in their respective containers and allow the system to equilibrate.

   c. The reading of the reagents compared to distilled water should not be more than 14 units (0.140 absorbance) for the nitrogen channel and not more than 5 units (0.25 absorbance) for the phosphorus channel. If the absorbance of either channel is much higher than the above values, one or more of the reagents or the water used to make up the reagents is probably contaminated.

6. **Concentration Ranges**
   a. All concentration ranges refer to the concentration of components in the digestion tube after diluting to volume with distilled water.

   b. **Nitrogen Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the flowcell and the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 1 and flow diagram).
      2. For any one manifold configuration, an approximate five-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 1.00 or higher.

   c. **Phosphorus Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure and flow diagram).
      2. For any one manifold configuration, an approximate three-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 2.00 or higher.

7. **Manifold Configurations**
   a. **Individual Determination of N or P**

   When N or P is being determined individually, the PT fitting is omitted and the sample line is attached directly to the sample probe of the Sampler IV.

   b. **Simultaneous Determination of N and P**

   When N and P are being determined simultaneously, both initial sample lines are connected to a PT stream-splitter fitting which is in turn connected to the sample probe on the Sampler IV.

8. **Sample Probe and PT Stream-Splitter**

   Because stainless steel is susceptible to attack by sulfuric acid solutions, this method utilizes special Kel-F sample probe (Technicon No. 17-0745) and a special PT stream-splitter with platinum nipples (Technicon No. 116-B331).

9. **Phosphorus Channel (only)**
   a. **Cleansing Procedure**

   Before initially operating the system, the following procedure should be performed to cleanse the system. Once a week thereafter, this procedure should be repeated during system start-up.

   With the exception of the ascorbic acid and molybdate/antimony lines, place all phosphorus reagent lines into their respective containers. Start the proportioning pump and allow five minute pumping time. Place both the ascorbic acid and molybdate/antimony lines in sodium hydroxide solution, 20% for five minutes, then into hydrogen peroxide, 50% for five minutes, then into distilled water. After five minutes follow the start-up procedure (Operating Note 1) and allow the system to equilibrate.

b. **Conditioning Procedure**

After the initial cleansing of the system is performed, condition the phosphorus channel as described below. Once this channel has been conditioned, there is no need to repeat the procedure; only the cleansing procedure need be performed once each week during start-up.

Following the Start-Up procedure (Operating Note #1), place all reagent lines for phosphorus in their respective containers and allow the system to equilibrate. Place three sample cups containing midscale standard solution on the Sampler IV tray (with a stop-pin at the third cup) and start the sampler. Aspirate the set of standards three times, allowing five minutes of wash between each set. After the Recorder traces the last standard peak, wait ten minutes and adjust the baseline tracing to zero using the BASELINE control.

10. **Crude Protein Determination – AOAC**

When this methodology is utilized to assay acid digestates for the determination of Crude Protein in Feeds by the official AOAC procedure, the following hardware changes **must** be incorporated into the system:

a. **Sampler IV** – Sampler IV cam must be 40/hour with a sample-to-wash ratio of 2:1 (cam is included in the accessories and spares kit).

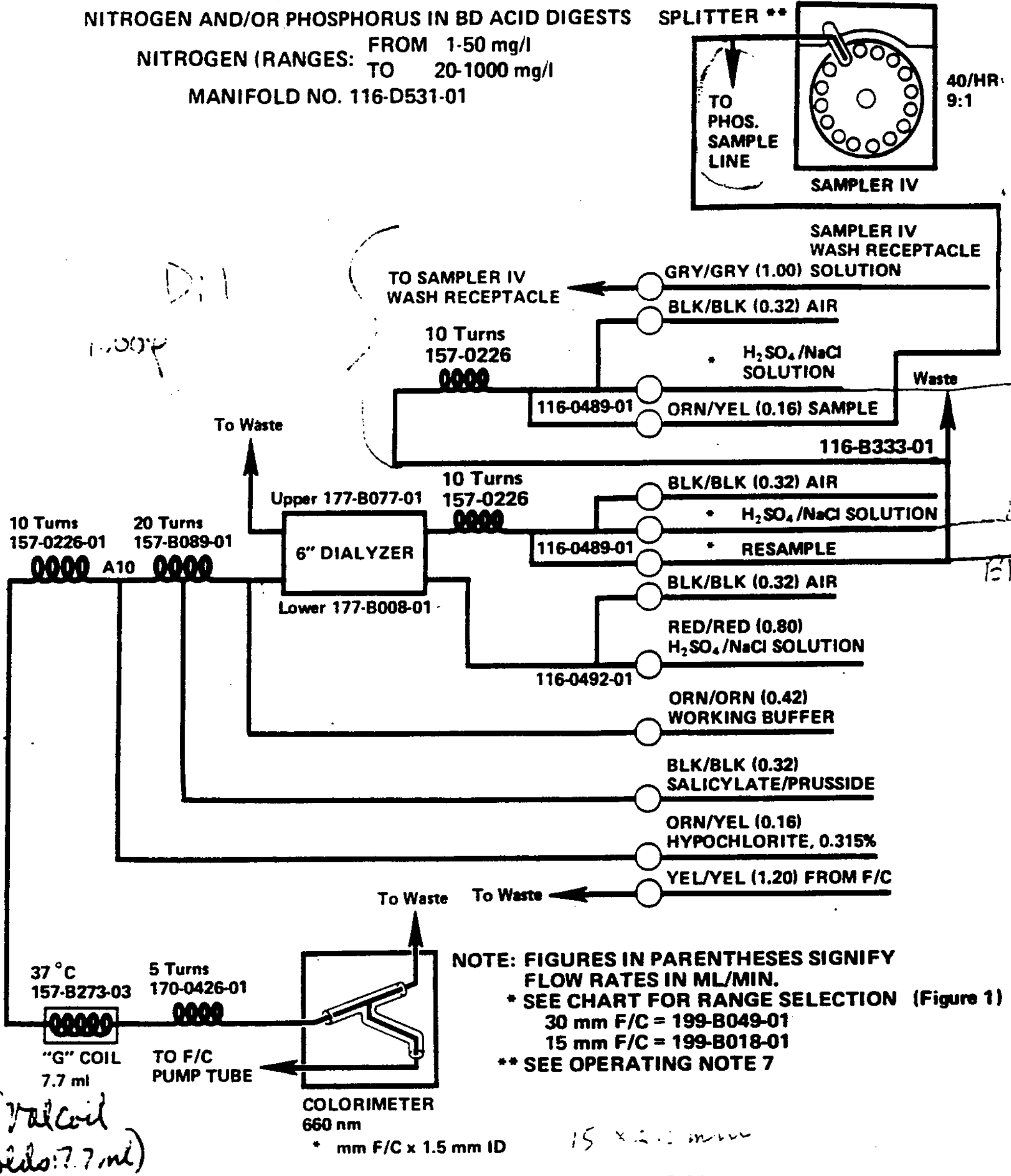
). **Analytical Cartridge** – dilution loop pump tubes **must** be of the following size:

INITIAL SAMPLE DILUTION	
Sample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	1.20 ml/min (Yel/Yel)

RESAMPLE DILUTION	
Resample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	0.80 ml/min (Red/Red)

c. **Colorimeter** – must be equipped with 15 mm pathlength flowcell (1.5 or 2.0 mm ID).

INDIVIDUAL/SIMULTANEOUS
NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS
NITROGEN (RANGES: FROM 1-50 mg/l
TO 20-1000 mg/l
MANIFOLD NO. 116-D531-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.
* SEE CHART FOR RANGE SELECTION (Figure 1)
30 mm F/C = 199-B049-01
15 mm F/C = 199-B018-01
** SEE OPERATING NOTE 7

(ValCoil holds: 7.7 ml)

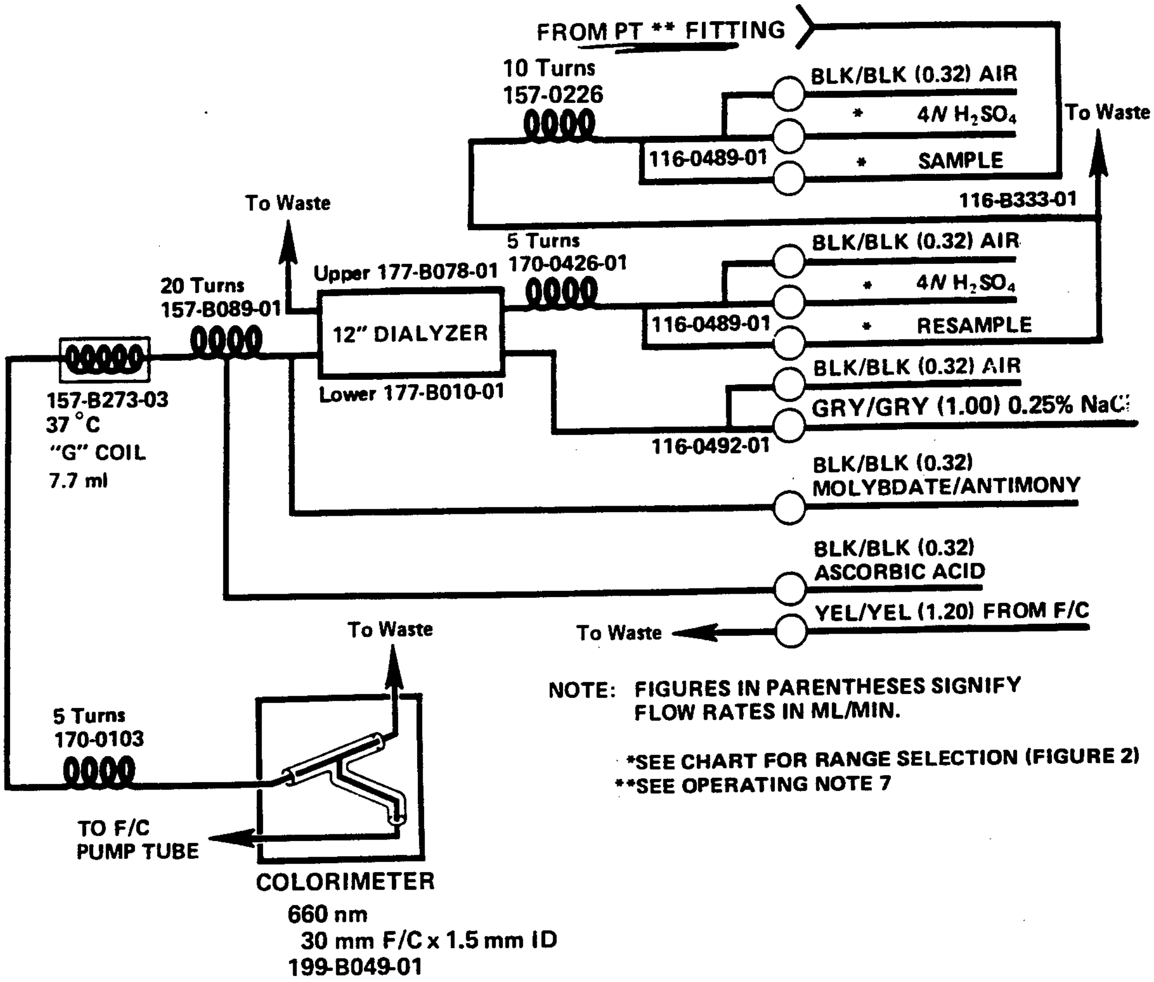
15 × 2.0 mm

Figure 1. CONCENTRATION RANGES (NITROGEN)

DILUTION LOOPS				FLOWCELL PATH LENGTH (mm)	APPROX. STD CAL SETTING	RANGE PPM N (±10%)
INITIAL SAMPLE		RESAMPLE				
SAMPLE LINE	H_2SO_4/NaCl LINE	RESAMPLE LINE	H_2SO_4/NaCl LINE			
.16 (Orn/Yel)	1.20 Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	30	7.30 1.00	.7-35 1.4-170
.16 (Orn/Yel)	1.20 Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30 1.00	1.2-60 -300
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30 1.00	2-100 10-500
.16 (Orn/Yel)	1.20 (Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	15	7.30 1.00	1.2-60 6-300
.16 (Orn/Yel)	1.20 (Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30 1.00	2.4-120 12-600
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30 1.00	4-200 20-1000

INDIVIDUAL/SIMULTANEOUS NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

PHOSPHORUS RANGES: FROM 1-50 mg/l
TO 20-1000 mg/l
MANIFOLD NO. 116-D541-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

*SEE CHART FOR RANGE SELECTION (FIGURE 2)
**SEE OPERATING NOTE 7

Figure 2. CONCENTRATION RANGES
(PHOSPHORUS)

DILUTION LOOPS				APPROX. STD CAL SETTING	RANGE mg/l P (±10%)
INITIAL SAMPLE		RESAMPLE			
SAMPLE LINE	4N H_2SO_4 LINE	RESAMPLE LINE	4N H_2SO_4 LINE		
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.32 (Blk/Blk)	0.80 (Red/Red)	7.30 2.20	1-50 3-150
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	2-100 6-300
0.16 (Orn/Yel)	1.60 (Blu/Blu)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	6-300 20-1000

AutoAnalyzer Application

Industrial Method - #376-75W/B

DIGESTION AND SAMPLE PREPARATION FOR THE ANALYSIS OF TOTAL KJELDAHL NITROGEN AND/OR TOTAL PHOSPHORUS IN WATER SAMPLES USING THE BRAN + LUEBBE BD-40 BLOCK DIGESTOR

The following procedure is recommended for the analysis of nitrogen and/or phosphorus in water samples. Samples are digested using a Bran + Luebbe BD-40 Block Digestor and assayed using a Bran + Luebbe AutoAnalyzer II Continuous Flow Analytical System.

This procedure should be utilized in conjunction with the Operation Manual for the Block Digestor BD-20/40 (Bran + Luebbe Publication No. TA4-0323-11) and the methodology for Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests (Industrial Method No. 329-74W - Revised 11/78).

Introduction

When analyzing water samples with the BD-40, the water present in the digestion tubes must be evaporated before heating the tubes at a temperature that is high enough to affect digestion. When water is added to sulfuric acid, the boiling point of the resultant mixture is considerably lower than that of sulfuric acid alone. If this mixture is placed in the block at a temperature significantly higher than its boiling point, the tube contents will bump out resulting in loss of sample, contamination of adjacent tubes, and most importantly, possible bodily injury.

The automatic (temperature-programmed) mode of the BD-40 is utilized to first evaporate the water and then to raise and maintain the block temperature high enough to affect digestion. At the end of the programmed cycle, the unit automatically shuts down.

Since the concentration levels of N and P in the samples encountered are frequently very low, accuracy and precision can be insured only if good analytical technique is employed during all steps of the procedure -- from sample preparation to assay of the digested samples. Every precaution should be observed to avoid contamination of sample tubes, pipets, reagents, spatulas, etc. The use of de-ionized, distilled water or its equivalent is required throughout the procedure. Contaminated water is the most frequently encountered source of difficulty in running this procedure.

Concentration Ranges and Manifold Configuration

Samples

The choice of manifold configuration depends on the concentration of N and/or P in the sample.

Depending on the manifold configuration and STD CAL setting, the BD-40 related analytical cartridges can accommodate N and/or P in the ranges from 0.024 - 1.2 mg/l to 1.50 - 75 mg/l in the undigested sample.

The methodology (No. 329-74W) gives three configurations for the nitrogen and phosphorus cartridges and the concentration ranges for each of the configurations. Each configuration can accommodate approximately a five-fold change in concentration by varying the STD CAL control from 100 to 700. The range represents the detection limit (2% of full scale) and the full scale concentration for a particular STD CAL setting.

Samples containing higher levels of nitrogen or phosphorus should be diluted with distilled de-ionized water prior to digestion.

Referring back to Figures 1 and 2 of Method 329-74W: Because of the possibility of contamination, it is recommended that configuration #1 be used only for samples containing 15 mg N or P/1 or less; all samples greater than 15 mg/l should be assayed on configuration #2 or #3. The choice of cartridge configuration is best illustrated by example.

Consider a group of samples containing 1 - 5 mg N/l and 5 - 50 mg P/1. The configuration of choice for nitrogen would be #2 adjusted with the STD CAL control to 5 mg/l full scale deflection. This would yield peaks ranging from 20% to full scale. The choice for phosphorus would be configuration #3 adjusted for a full scale deflection of 50 mg/l. This would yield peaks ranging from 10% to full scale.

Occasionally, the range of N or P in the samples will not be as narrow as stated above; i.e., a few samples may be too low or too high for the range that was chosen for the majority of the samples. If so, the sample volume per tube may be adjusted up or down or the full scale deflection may be adjusted by means of the STD CAL.

Whenever possible, a cartridge configuration should be chosen such that adjustment of the STD CAL control will accommodate all the values encountered. Note that the STD CAL setting should not be changed while samples are being assayed; i.e., sample peaks can be compared to standard peaks only when both are run at the same STD CAL setting. If a STD CAL adjustment is anticipated, be sure to have on hand standards which will fall into the anticipated range.

Standards

The recommended standards for use with the BD-40 are aqueous solutions of ammonium sulfate [$(NH_4)_2SO_4$] for nitrogen and potassium dihydrogen phosphate (KH_2PO_4) for phosphorus. The volumes of standard solution to be used depend on the concentration range of the sample.

It is recommended that two standards for each parameter to be run: one at 30 - 40% of full scale and one at 70 - 80% of full scale.

Standards should be handled in exactly the same manner as samples; i.e., they should be pipetted into the BD tubes and carried through the entire digestion procedure.

A series of working standard solutions which can accommodate all the ranges of the method can be prepared utilizing the following stock solutions:

Stock Solution A (2.0 mg N/ml)

Ammonium Sulfate $(NH_4)_2SO_4$	0.9434 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.9434 g of ammonium sulfate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

Stock Solution B (2.0 mg P/ml)

Potassium Dihydrogen Phosphate (KH_2PO_4)	0.8788 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.8788 g of potassium dihydrogen phosphate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

The preparation of standards can be performed most readily if pipets ranging from 1 to 10 ml are available.

In Table #1, the extreme left column indicates the milliliters of stock solution to be diluted to one liter to obtain working standard solutions which will yield the concentration values in the second column. The same volumes diluted to 100 ml, or 10x those volumes diluted to one liter, will yield concentrations 10x these concentration values.

The preparation of standard solutions is best illustrated by an example. Using the example cited previously in the section on Samples, the N range was 1 - 5 mg/l and the P range was 5 - 50 mg/l.

For the N channel, an 80% of full scale standard would be 4.0 mg/l. From Table #1 4.0 mg/l can be obtained by using 8 ml per tube of a working standard solution containing 10 mg/l (Row 5, Column 8). Using 3 ml per tube of the same working solution will give 1.5 mg/l (Row 5, Column 3) or 30% of full scale.

Since 8 ml and 3 ml per tube were chosen for the nitrogen standards, the appropriate amount of P must also be present in those aliquots to accommodate the phosphorus channel. An 80% deflection for phosphorus corresponds to 40 mg P/l and a 30% deflection corresponds to 15 mg/l. Checking Column 8, 40 mg/l (10x chart value) can be obtained by using 8 ml stock solution B. The 30% value will automatically fall in range using the 3 ml aliquot. Hence, using 3 ml and 8 ml of a working standard solution prepared by diluting 5 ml of Stock Solution A plus 50 ml of Stock Solution B to one liter will yield the required N and P values.

The following general procedure may be used for preparation of standard solutions. Once the manifold configuration and concentration range have been chosen, choose a value (or 10x a chart value) from Table 1 which corresponds to an 80% deflection and which requires 5 ml per tube or more working standard solution. Using 5 ml or more for the 80% deflection insures that a smaller volume can be found on the chart which approximates the 30% deflection.

On a simultaneous system, either parameter may be determined first. Once the chart value has been chosen for one parameter, choose the value (or 10x a chart value) from the same column that most closely approximates an 80% deflection for the other parameter. Since the values are proportional to volume, the 30% values will automatically fall into range with each other.

Blanks

A duplicate blank determination (all reagents less sample) should be performed with each rack of samples by carrying the blank tubes through the entire digestion procedure.

Operating Procedure

Samples, Standards and Blanks

Samples and standards are pipetted directly into the digestion tubes. Samples should be pipetted in 20 ml aliquots. The amount of standard is determined by the level of the component(s) of interest.

Refer to Section II for guidelines on standard volumes and manifold configuration. Samples may be assayed singly or in duplicate, depending on workload. It is recommended that standards and blanks be assayed in duplicate.

While samples and standards are being prepared, pre-heat the block to 200°C by setting the HIGH TEMP dial to 200°C and depressing the MANUAL button.

Boiling Aids

Plain (not selenized) Hengar chips are utilized to promote smooth boiling during digestion. The addition of 2 - 3 chips per tube is recommended. The use of glass beads or perforated glass beads is not satisfactory to obtain smooth boiling.

Hengar chips are available from Arthur H. Thomas Company, Vine & Third Streets, Philadelphia, PA. 19105. As an alternative, some users report a preference for acid-washed Chemware TFE (teflon) boiling stones. TFE boiling stones are available from Markson Science, Inc., Box 767, Delmar, California 92014.

Catalyst

Red mercuric oxide is recommended as a catalyst for the digestion of water samples. Because mercury can interfere in both chemistries, the amount added per tube is limited to an amount determined by the manifold configuration being utilized; i.e., the more the sample is diluted, the greater the amount of mercury that can be utilized. The amount of mercury recommended is 10 mg/tube. The mercury is most conveniently utilized as a solution of HgO in 10% sulfuric acid.

Preparation

Into a 100 ml volumetric flask, weigh 8.0 of red mercuric oxide. Add about 75 ml of 10% sulfuric acid and stir until dissolved. Dilute to 100 ml with 10% sulfuric acid and mix thoroughly.

Digestion (Salt/Acid/Catalyst) Mixture

To insure uniform blank values from tube to tube, it is recommended that potassium sulfate, sulfuric acid and catalyst be added to each tube as a single mixture rather than as separate components. The procedure below may be used to prepare the digestion mixture.

Prepare and store the mixture in a stoppered container to minimize the possibility of airborne contamination. The mixture may be prepared in as large a quantity as is practical to handle and store.

Preparation

Carefully add 200 ml of concentrated sulfuric acid to 700 ml of de-ionized distilled water. Dissolve 133 g of potassium sulfate into this mixture, add 25 ml of mercuric sulfate catalyst solution and dilute to one liter with de-ionized distilled water.

For most applications, 5 ml of digestion mix per tube is satisfactory.

The utilization of a plunger-type repetitive dispensing device offers a rapid, convenient method of adding the digestion mixture to the tubes. When a plunger-type dispenser is utilized, the dispenser must be broken down, cleaned with water and air dried every three days. Failure to clean the plunger every three days can cause the plunger to freeze in the barrel of the dispenser due to crystallization of potassium sulfate.

Digestion

After samples, standards and reagents have been added to the digestion tubes, the water must be evaporated before high temperature digestion can be performed.

Place the loaded rack into the pre-heated block (200°C) and attach the end plates to the rack. The plates should remain in place until the rack is removed from the block. End plates promote water evaporation during low temperature operation and insure proper refluxing of the acid during high temperature digestion.

When the loaded rack is placed in the block, set the programmer as follows and then depress the AUTO button:

Total Cycle Time:	2 1/2 hours
High Temp °C:	380 °C
Low Temp Time:	1 hour
Low Temp °C:	200 °C

Under these conditions, the until will operate for a total cycle of 2 1/2 hours: 1 hour at 200 °C, about 1 hour to heat up to 380 °C and about 1/2 hour at 380 °C. At the end of 2 1/2 hours, the until will automatically shut down.

Cooling and Dilution

At the end of the program cycle (2 1/2 hours), remove the rack from the block, place it on an asbestos pad or in the cooling rack and remove the metal end plates. Allow the tubes to cool for about 5 minutes before diluting with 20 ml of de-ionized distilled water. Tubes are cool enough to dilute when the white acid fumes have dissipated and the upper half of the tube is cool enough to handle comfortably. The tubes should not be allowed to cool to the point of K_2SO_4 precipitation.

With the aid of a vortex type tube mixer, add to each tube, while swirling, 20 ml of deionized distilled water using a repetitive pipetter. Add the water in one continuous portion at a moderate rate and angle the tube away from the face. Allow the tube contents to mix thoroughly.

The tube contents should be at room temperature before analyzing. The tubes may be cooled rapidly by placing the entire rack into a sink partially filled with cold water.

Analysis

After cooling to room temperature, the digests may be analyzed using Bran + Luebbe Methodology No. 329-74W -- Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests, Revised 11/78.

Transfer to glass sample cups, which have been previously acid washed and dried.

Before analyzing the entire set of samples, standards and blanks, run a few standard cups through the system and with the STD CAL control, adjust the standard peaks to the proper chart reading.

TABLE 1: CONCENTRATION OF STANDARD IN DIGESTS

*Ml Stock Solution A or B	Working Standard Conc. Mg N or P/L	Ml Working Standard Solution Per Tube and Resulting Digest Concentration in Mg N or P/L									
		1	2	3	4	5	6	7	8	9	10
1	2	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
2	4	.2	.4	.6	.8	1.	1.2	1.4	1.6	1.8	2
3	6	.3	.6	.9	1.2	1.5	1.8	2.1	2.4	2.7	3
4	8	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4
5	10	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5
6	12	.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6
7	14	.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7
8	16	.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8
9	18	.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9
10	20	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10

*Dilute to 1000 ml to get chart values

Dilute to 100 ml to get 10X chart values

Stock Solution A = Nitrogen

Stock Solution B = Phosphorus

Appendix B-5 – Lab Procedures for Total Metals: Method 6010A

Method 6010A -Inductively Coupled Plasma-Atomic Emission Spectroscopy

1.0 Procedure

Perform analysis for metals and certain other elements amenable to ICP analysis in accordance with Method 6010A from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method:

method blank
reagent blank
calibration check sample.

For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine.

Where possible, for each batch analyze one matrix spike sample.

For each batch analyze a matrix spike duplicate or sample duplicate.

METHOD 6010A

INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROSCOPY

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-atomic emission spectroscopy (ICP) determines trace elements, including metals, in solution. The method is applicable to all of the elements listed in Table 1. All matrices, including ground water, aqueous samples, TCLP and EP extracts, industrial and organic wastes, soils, sludges, sediments, and other solid wastes, require digestion prior to analysis.

1.2 Elements for which Method 6010 is applicable are listed in Table 1. Detection limits, sensitivity, and optimum ranges of the metals will vary with the matrices and model of spectrometer. The data shown in Table 1 provide estimated detection limits for clean aqueous samples using pneumatic nebulization. Use of this method is restricted to spectroscopists who are knowledgeable in the correction of spectral, chemical, and physical interferences.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples must be solubilized or digested using appropriate Sample Preparation Methods (e.g. Methods 3005-3050). When analyzing for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis.

2.2 Method 6010 describes the simultaneous, or sequential, multielemental determination of elements by ICP. The method measures element-emitted light by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the lines are monitored by photomultiplier tubes. Background correction is required for trace element determination. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction, ~~is~~ not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in Section 3.0 should also be recognized and appropriate corrections made; tests for their presence are described in Step 8.5.

TABLE 1.
RECOMMENDED WAVELENGTHS AND ESTIMATED INSTRUMENTAL DETECTION LIMITS

Detection Element	Wavelength[a] (nm)	Estimated Limit[b] (ug/L)
Aluminum	308.215	45
Antimony	206.833	32
Arsenic	193.696	53
Barium	455.403	2
Beryllium	313.042	0.3
Cadmium	226.502	4
Calcium	317.933	10
Chromium	267.716	7
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Lead	220.353	42
Lithium	670.784	5
Magnesium	279.079	30
Manganese	257.610	2
Molybdenum	202.030	8
Nickel	231.604	15
Phosphorus	213.618	51
Potassium	766.491	See note c
Selenium	196.026	75
Silver	328.068	7
Sodium	588.995	29
Strontium	407.771	0.3
Thallium	190.864	40
Vanadium	292.402	8
Zinc	213.856	2

[a] The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see Step 3.1). In time, other elements may be added as more information becomes available and as required.

[b] The estimated instrumental detection limits shown are taken from Reference 1 in Section 10.0 below. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

[c] Highly dependent on operating conditions and plasma position.

3.0 INTERFERENCES

3.1 Spectral interferences are caused by: (1) overlap of a spectral line from another element at the analytical or background measurement wavelengths; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuum or recombination phenomena; and (4) stray light from the line emission of high-concentration elements. Spectral overlap can be compensated for by computer-correcting the raw data after monitoring and measuring the interfering element. Unresolved overlap requires selection of an alternate wavelength. Background contribution and stray light can usually be compensated for by a background correction adjacent to the analyte line.

Users of all ICP instruments must verify the absence of spectral interference from an element in a sample for which there is no instrument detection channel. Recommended wavelengths are listed in Table 1 and potential spectral interferences for the recommended wavelengths are given in Table 2. The data in Table 2 are intended as rudimentary guides for indicating potential interferences; for this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed.

3.1.1 Element-specific interference is expressed as analyte concentration equivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interference element. For example, assume that As is to be determined (at 193.696 nm) in a sample containing approximately 10 mg/L of Al. According to Table 2, 100 mg/L of Al would yield a false signal for As equivalent to approximately 1.3 mg/L. Therefore, the presence of 10 mg/L of Al would result in a false signal for As equivalent to approximately 0.13 mg/L. The user is cautioned that other instruments may exhibit somewhat different levels of interference than those shown in Table 2. The interference effects must be evaluated for each individual instrument since the intensities will vary with operating conditions, power, viewing height, argon flow rate, etc. The user should be aware of the possibility of interferences other than those specified in Table 2 and that analysts should be aware of these interferences when conducting analyses.

3.1.2 The dashes in Table 2 indicate that no measurable interferences were observed even at higher interferent concentrations. Generally, interferences were discernible if they produced peaks, or background shifts, corresponding to 2 to 5% of the peaks generated by the analyte concentrations.

3.1.3 At present, information on the listed silver and potassium wavelengths is not available, but it has been reported that second-order energy from the magnesium 383.231-nm wavelength interferes with the listed potassium line at 766.491 nm.

TABLE 2.

ANALYTE CONCENTRATION EQUIVALENTS ARISING FROM INTERFERENCE AT THE 100-mg/L LEVEL

		Interferent[a,b]									
Analyte	Wavelength (nm)	Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Tl	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

[a] Dashes indicate that no interference was observed even when interferents were introduced at the following levels:

Al - 1000 mg/L	Mg - 1000 mg/L
Ca - 1000 mg/L	Mn - 200 mg/L
Cr - 200 mg/L	Tl - 200 mg/L
Cu - 200 mg/L	V - 200 mg/L
Fe - 1000 mg/L	

[b] The figures recorded as analyte concentrations are not the actual observed concentrations; to obtain those figures, add the listed concentration to the interferent figure.

3.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or high acid concentrations. Differences in solution volatility can also cause inaccuracies when organic solvents are involved. If physical interferences are present, they must be reduced by diluting the sample or by using a peristaltic pump. Another problem that can occur with high dissolved solids is salt buildup at the tip of the nebulizer, which affects aerosol flow rate and causes instrumental drift. The problem can be controlled by wetting the argon prior to nebulization, using a tip washer, or diluting the sample. Changing the nebulizer and removing salt buildup at the tip of the torch sample injector can be used as an additional measure to control salt buildup. Also, it has been reported that better control of the argon flow rate improves instrument performance; this is accomplished with the use of mass flow controllers.

3.3 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. If observed, they can be minimized by careful selection of operating conditions (incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. Chemical interferences are highly dependent on matrix type and the specific analyte element.

4.0 APPARATUS AND MATERIALS

4.1 Inductively coupled argon plasma emission spectrometer:

4.1.1 Computer-controlled emission spectrometer with background correction.

4.1.2 Radio frequency generator compliant with FCC regulations.

4.1.3 Argon gas supply - Welding grade or better.

4.2 Operating conditions - The analyst should follow the instructions provided by the instrument manufacturer. For operation with organic solvents, use of the auxiliary argon inlet is recommended, as are solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased RF power to obtain stable operation and precise measurements. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be established for each individual analyte line on that particular instrument. All measurements must be within the instrument linear range where spectral interference correction factors are valid. The analyst must (1) verify that the instrument configuration and operating conditions satisfy the analytical requirements and (2) maintain quality control data confirming instrument performance and analytical results.

4.3 Class A volumetric flasks

4.4 Class A volumetric pipets

4.5 Analytical balance - capable of accurate measurement to 4 significant figures.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is in question analyze for contamination. If the concentration is less than the MDL then the reagent is acceptable.

5.1.1 Hydrochloric acid (conc), HCl.

5.1.2 Hydrochloric acid (1:1), HCl. Add 500 mL concentrated HCl to 400 mL water and dilute to 1 liter in an appropriate beaker.

5.1.3 Nitric acid (conc), HNO_3.

5.1.4 Nitric acid (1:1), HNO_3. Add 500 mL concentrated HNO_3 to 400 mL water and dilute to 1 liter in an appropriate beaker.

5.2 Reagent Water. All references to water in the method refer to reagent water unless otherwise specified. Reagent water will be interference free. Refer to Chapter One for a definition of reagent water.

5.3 Standard stock solutions may be purchased or prepared from ultra-high purity grade chemicals or metals (99.99 to 99.999% pure). All salts must be dried for 1 hour at 105°C, unless otherwise specified.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

Typical stock solution preparation procedures follow. Concentrations are calculated based upon the weight of pure metal added, or with the use of the mole fraction and the weight of the metal salt added.

Metal

$$\text{Concentration (ppm)} = \frac{\text{weight (mg)}}{\text{volume (L)}}$$

Metal salts

$$\text{Concentration (ppm)} = \frac{\text{weight (mg)} \times \text{mole fraction}}{\text{volume (L)}}$$

5.3.1 Aluminum solution, stock, 1 mL = 1000 ug Al: Dissolve 1.0 g of aluminum metal, weighed accurately to at least four significant figures, in an acid mixture of 4 mL of (1:1) HCl and 1 mL of concentrated HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional

10 mL of (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.2 Antimony solution, stock, 1 mL = 1000 ug Sb: Dissolve 2.70 g $K(SbO)C_4H_4O_6$ (mole fraction Sb = 0.3749), weighed accurately to at least four significant figures, in water, add 10 mL (1:1) HCl, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.3 Arsenic solution, stock, 1 mL = 1000 ug As: Dissolve 1.30 g of As_2O_3 (mole fraction As = 0.7574), weighed accurately to at least four significant figures, in 100 mL of water containing 0.4 g NaOH. Acidify the solution with 2 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water

5.3.4 Barium solution, stock, 1 mL = 1000 ug Ba: Dissolve 1.50 g $BaCl_2$ (mole fraction Ba = 0.6595), dried at 250°C for 2 hours, weighed accurately to at least four significant figures, in 10 mL water with 1 mL (1:1) HCl. Add 10.0 mL (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.5 Beryllium solution, stock, 1 mL = 1000 ug Be: Do not dry. Dissolve 19.7 g $BeSO_4 \cdot 4H_2O$ (mole fraction Be = 0.0509), weighed accurately to at least four significant figures, in water, add 10.0 mL concentrated HNO_3, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.6 Cadmium solution, stock, 1 mL = 1000 ug Cd: Dissolve 1.10 g CdO (mole fraction Cd = 0.8754), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.7 Calcium solution, stock, 1 mL = 1000 ug Ca: Suspend 2.50 g $CaCO_3$ (mole Ca fraction = 0.4005), dried at 180°C for 1 hour before weighing, weighed accurately to at least four significant figures, in water and dissolve cautiously with a minimum amount of (1:1) HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.8 Chromium solution, stock, 1 mL = 1000 ug Cr: Dissolve 1.90 g CrO_3 (mole fraction Cr = 0.5200), weighed accurately to at least four significant figures, in water. When solution is complete, acidify with 10 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.9 Cobalt solution, stock, 1 mL = 1000 ug Co: Dissolve 1.00 g of cobalt metal, weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.10 Copper solution, stock, 1 mL = 1000 ug Cu: Dissolve 1.30 g CuO (mole fraction Cu = 0.7989), weighed accurately to at least four significant figures), in a minimum amount of (1:1) HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.11 Iron solution, stock, 1 mL = 1000 ug Fe: Dissolve 1.40 g Fe_2O_3 (mole fraction Fe = 0.6994), weighed accurately to at least four significant figures, in a warm mixture of 20 mL (1:1) HCl and 2 mL of concentrated HNO_3. Cool, add an additional 5.0 mL of concentrated HNO_3, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.12 Lead solution, stock, 1 mL = 1000 ug Pb: Dissolve 1.60 g $Pb(NO_3)_2$ (mole fraction Pb = 0.6256), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10 mL (1:1) HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.13 Lithium solution, stock, 1 mL = 1000 ug Li: Dissolve 5.324 g lithium carbonate (mole fraction Li = 0.1878), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HCl and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.14 Magnesium solution, stock, 1 mL = 1000 ug Mg: Dissolve 1.70 g MgO (mole fraction Mg = 0.6030), weighed accurately to at least four significant figures, in a minimum amount of (1:1) HNO_3. Add 10.0 mL (1:1) concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.15 Manganese solution, stock, 1 mL = 1000 ug Mn: Dissolve 1.00 g of manganese metal, weighed accurately to at least four significant figures, in acid mixture (10 mL concentrated HCl and 1 mL concentrated HNO_3) and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.16 Molybdenum solution, stock, 1 mL = 1000 ug Mo: Dissolve 2.00 g $(NH_4)_6Mo_7O_{24}.4H_2O$ (mole fraction Mo = 0.5772), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.17 Nickel solution, stock, 1 mL = 1000 ug Ni: Dissolve 1.00 g of nickel metal, weighed accurately to at least four significant figures, in 10.0 mL hot concentrated HNO_3, cool, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.18 Phosphate solution, stock, 1 mL = 1000 ug P: Dissolve 4.393 g anhydrous KH_2PO_4 (mole fraction P = 0.2276), weighed accurately to at least four significant figures, in water. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.19 Potassium solution, stock, 1 mL = 1000 ug K: Dissolve 1.90 g KCl (mole fraction K = 0.5244) dried at 110°C, weighed accurately to at least four significant figures, in water, and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.20 Selenium solution, stock, 1 mL = 1000 ug Se: Do not dry. Dissolve 1.70 g H_2SeO_3 (mole fraction Se = 0.6123), weighed accurately to at least four significant figures, in water and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.21 Silver solution, stock, 1 mL = 1000 ug Ag: Dissolve 1.60 g $AgNO_3$ (mole fraction Ag = 0.6350), weighed accurately to at least four significant figures, in water and 10 mL concentrated HNO_3. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.22 Sodium solution, stock, 1 mL = 1000 ug Na: Dissolve 2.50 g NaCl (mole fraction Na = 0.3934), weighed accurately to at least four significant figures, in water. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.23 Strontium solution, stock, 1 mL = 1000 ug Sr: Dissolve 2.415 g of strontium nitrate ($Sr(NO_3)_2$) (mole fraction 0.4140), weighed accurately to at least four significant figures, in a 1-liter flask containing 10 mL of concentrated HCl and 700 mL of water. Dilute to volume in a 1,000 mL volumetric flask with water.

5.3.24 Thallium solution, stock, 1 mL = 1000 ug Tl: Dissolve 1.30 g $TlNO_3$ (mole fraction Tl = 0.7672), weighed accurately to at least four significant figures, in water. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.25 Vanadium solution, stock, 1 mL = 1000 ug V: Dissolve 2.30 g NH_4O_3 (mole fraction V = 0.4356), weighed accurately to at least four significant figures, in a minimum amount of concentrated HNO_3. Heat to increase rate of dissolution. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.3.26 Zinc solution, stock, 1 mL = 1000 ug Zn: Dissolve 1.20 g ZnO (mole fraction Zn = 0.8034), weighed accurately to at least four significant figures, in a minimum amount of dilute HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1,000 mL volumetric flask with water.

5.4 Mixed calibration standard solutions - Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks (see Table 3). Matrix match with the appropriate acids and dilute to 100 mL with water. Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards to ensure that the elements are compatible and stable together. Transfer the mixed standard solutions to FEP fluorocarbon or previously unused polyethylene or polypropylene bottles for storage. Fresh mixed standards should be prepared, as needed, with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample (see Step 5.8) and monitored weekly for stability. Some typical calibration standard combinations are listed in Table 3. All mixtures should then be scanned using a sequential spectrometer to verify the absence of interelement spectral interference in the recommended mixed standard solutions.

<u>NOTE</u>: If the addition of silver to the recommended acid combination results in an initial precipitation, add 15 mL of water and warm the flask until the solution clears. Cool and dilute to 100 mL with water. For this acid combination, the silver concentration should be limited to 2 mg/L. Silver under these conditions is stable in a tap-water matrix for 30 days. Higher concentrations of silver require additional HCl.

TABLE 3.
MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As, Mo
IV	Al, Ca, Cr, K, Na, Ni,Li,& Sr
V	Ag (see Note to Step 5.4), Mg, Sb, and Tl
VI	P

5.5 Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, and the reagent blank is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

5.5.1 The calibration blank is prepared by acidifying reagent water to the same concentrations of the acids found in the standards and samples. Prepare a sufficient quantity to flush the system between standards and samples.

5.5.2 The method blank must contain all the reagents and in the same volumes as used in the processing of the samples. The method blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

5.6 The instrument check standard is prepared by the analyst by combining compatible elements at concentrations equivalent to the midpoint of their respective calibration curves (see Step 8.6.1.1 for use). The instrument check standard should be prepared from a source independent from that used in the calibration standards.

5.7 The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate

5.7 The interference check solution is prepared to contain known concentrations of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at approximate concentrations of 10 times the instrumental detection limits. In the absence of measurable analyte, overcorrection could go undetected because a negative value could be reported as zero. If the particular instrument will display overcorrection as a negative number, this spiking procedure will not be necessary.

5.8 The quality control sample should be prepared in the same acid matrix as the calibration standards at 10 times the instrumental detection limits and in accordance with the instructions provided by the supplier.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the material in Chapter Three, Metallic Analytes, Steps 3.1 through 3.3.

7.0 PROCEDURE

7.1 Preliminary treatment of most matrices is necessary because of the complexity and variability of sample matrices. Water samples which have been prefiltered and acidified will not need acid digestion as long as the samples and standards are matrix matched. Solubilization and digestion procedures are presented in Sample Preparation Methods (Methods 3005A-3050A).

7.2 Set up the instrument with proper operating parameters established in Step 4.2. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration).

7.3 Profile and calibrate the instrument according to the instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in Step 5.4. Flush the system with the calibration blank (Step 5.5.1) between each standard or as the manufacturer recommends. (Use the average intensity of multiple exposures for both standardization and sample analysis to reduce random error.) The calibration curve should consist of a blank and three standards.

7.4 Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than 5% (or the established control limits, whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this condition.

7.5 Flush the system with the calibration blank solution for at least 1 minute (Step 5.5.1) before the analysis of each sample (see Note to Step 7.3). Analyze the instrument check standard (Step 5.6) and the calibration blank (Step 5.5.1) after each 10 samples.

8.0 QUALITY CONTROL

8.1 All quality control data should be maintained and available for easy reference or inspection. Refer to Chapter One for additional quality control procedures.

8.2 Dilute and reanalyze samples that are more concentrated than the linear calibration limit or use an alternate, less sensitive line for which quality control data is already established.

8.3 Employ a minimum of one method blank per sample batch to determine if contamination or any memory effects are occurring. A method blank is a volume of reagent water acidified with the same amounts of acids as were the standards and samples.

8.4 Analyze one replicate sample for every twenty samples or per analytical batch, whichever is more frequent. A replicate sample is a sample brought through the whole sample preparation and analytical process in duplicate. Refer to Chapter One for a more detailed description of an analytical batch.

8.5 It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests, as outlined in Steps 8.5.1 and 8.5.2, will ensure the analyst that neither positive nor negative interferences are operating on any of the analyte elements to distort the accuracy of the reported values.

8.5.1 Serial dilution: If the analyte concentration is sufficiently high (minimally, a factor of 10 above the instrumental detection limit after dilution), an analysis of a 1:4 dilution should agree within ± 10% of the original determination. If not, a chemical or physical interference effect should be suspected.

8.5.2 Post digestion spike addition: An analyte spike added to a portion of a prepared sample, or its dilution, should be recovered to within 75% to 125% of the known value. The spike addition should produce a minimum level of 10 times and a maximum of 100 times the instrumental detection limit. If the spike is not recovered within the specified limits, a matrix effect should be suspected.

CAUTION: If spectral overlap is suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

8.6 Check the instrument standardization by analyzing appropriate check standards as follows.

8.6.1 Verify calibration every 10 samples and at the end of the analytical run, using a calibration blank (Step 5.5.1) and a check standard (Step 5.6).

8.6.1.1 The results of the check standard are to agree within 10% of the expected value; if not, terminate the analysis, correct the problem, and reanalyze the previous ten samples.

8.6.1.2 The results of the calibration blank are to agree within three standard deviations of the mean blank value. If not, repeat the analysis two more times and average the results. If the average is not within three standard deviations of the background mean, terminate the analysis, correct the problem, recalibrate, and reanalyze the previous 10 samples.

8.6.2 Verify the interelement and background correction factors at the beginning and end of an analytical run or twice during every 8-hour work shift, whichever is more frequent. Do this by analyzing the interference check solution (Step 5.7). Results should be within ± 20% of the true value obtained in Step 8.6.1.1.

8.6.3 Spiked replicate samples are to be analyzed at a frequency of 5% or per analytical batch, whichever is more frequent.

8.6.3.1 The relative percent difference between replicate determinations is to be calculated as follows:

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where:

RPD = relative percent difference.
D_1 = first sample value.
D_2 = second sample value (replicate).

(A control limit of ± 20% RPD shall be used for sample values greater than ten times the instrument detection limit.)

8.6.3.2 The spiked replicate sample recovery is to be within ± 20% of the actual value.

9.0 METHOD PERFORMANCE

9.1 In an EPA round-robin Phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been spiked with various metal concentrates. Table 4 lists the true values, the mean reported values, and the mean percent relative standard deviations.

9.2 In a single laboratory evaluation, seven wastes were analyzed for 22 elements by this method. The mean percent relative standard deviation from triplicate analyses for all elements and wastes was 9 ± 2%. The mean percent recovery of spiked elements for all wastes was 93 ± 6%. Spike levels ranged from 100 ug/L to 100 mg/L. The wastes included sludges and industrial wastewaters.

10.0 REFERENCES

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2. Test Methods: Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater; U.S. Environmental Protection agency. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information: Cincinnati, OH, 1982; EPA-600/4-82-057.

3. Patel, B.K.; Raab, G.A.; et al. Report on a Single Laboratory Evaluation of Inductively Coupled Optical Emission Method 6010; EPA Contract No. 68-03-3050, December 1984.

4. Sampling and Analysis Methods for Hazardous Waste Combustion; U.S. Environmental Protection Agency; Air and Energy Engineering Research Laboratory, Office of Research and Development: Research Triangle Park, NC, 1986; Prepared by Arthur D. Little, Inc.

5. Bowmand, P.W.J.M. Line Coincidence Tables for Inductively Coupled Plasma Atomic Emission Spectrometry, 2nd ed.; Pergamon: 1984.

6. Rohrbough, W.G.; et al. Reagent Chemicals, American Chemical Society Specifications, 7th ed.; American Chemical Society: Washington, DC, 1986.

7. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

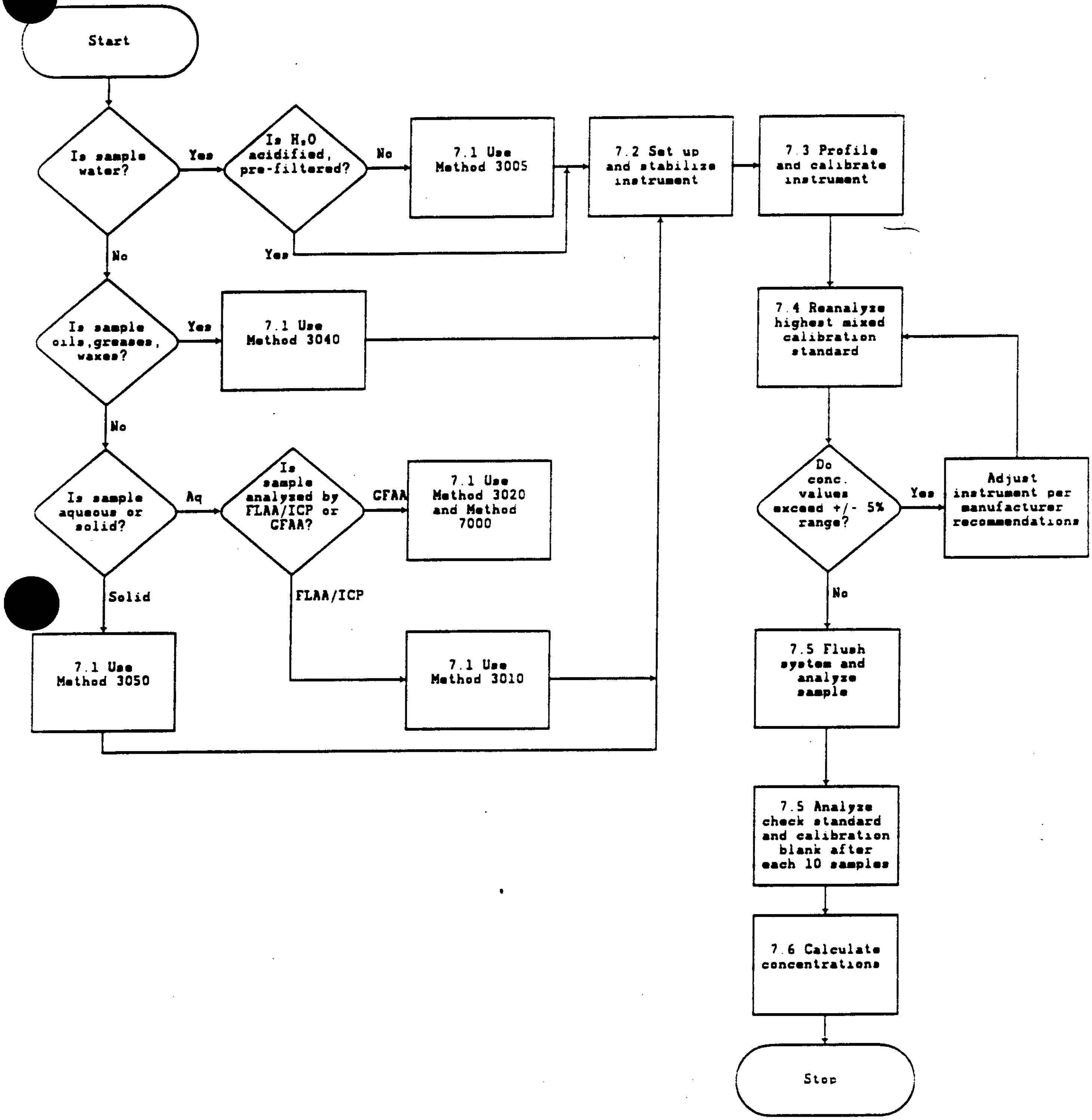
TABLE 4.
ICP PRECISION AND ACCURACY DATA[a]

	Sample No. 1			Sample No. 2			Sample No. 3		
Element	True Value (ug/L)	Mean Reported Value (ug/L)	Mean SD[b] (%)	True Value (ug/L)	Mean Reported Value (ug/L)	Mean SD[b] (%)	True Value (ug/L)	Mean Reported Value (ug/L)	Mean SD[b] (%)
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
V	750	749	1.8	70	69	2.9	170	169	1.1
As	200	208	7.5	22	19	23	60	63	17
Cr	150	149	3.8	10	10	18	50	50	3.3
Cu	250	235	5.1	11	11	40	70	67	7.9
Fe	600	594	3.0	20	19	15	180	178	6.0
Al	700	696	5.6	60	62	33	160	161	13
Cd	50	48	12	2.5	2.9	16	14	13	16
Co	700	512	10	20	20	4.1	120	108	21
Ni	250	245	5.8	30	28	11	60	55	14
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se[c]	40	32	21.9	6	8.5	42	10	8.5	8.3

[a] Not all elements were analyzed by all laboratories.

[b] SD = standard deviation.

[c] Results for Se are from two laboratories.


Start
Is sample water?
Yes
Is H_2O acidified, pre-filtered?
No
7.1 Use Method 3005
Yes
7.2 Set up and stabilize instrument
7.3 Profile and calibrate instrument
No
Is sample oils, greases, waxes?
Yes
7.1 Use Method 3040
No
Is sample aqueous or solid?
Aq
Is sample analyzed by FLAA/ICP or GFAA?
GFAA
7.1 Use Method 3020 and Method 7000
FLAA/ICP
7.1 Use Method 3010
Solid
7.1 Use Method 3050
7.4 Reanalyze highest mixed calibration standard
Do conc. values exceed +/- 5% range?
Yes
Adjust instrument per manufacturer recommendations
No
7.5 Flush system and analyze sample
7.5 Analyze check standard and calibration blank after each 10 samples
7.6 Calculate concentrations
Stop

Appendix B-6 – Lab Procedures for Exchangeable P: Method ASA 24-5.2

Phosphorus Soluble in Dilute Hydrochloric Acid and Sulfuric Acid
or
Mehlich I (North Carolina Double Acid) P Determination in Soil

ASA 24-5.2

Reagents:

1. Extraction Solution: Add 12 ml of concentrated H_2SO_4 and 73 ml of concentrated HCl to approximately 15 liters of deionized water. Make to 18 liters. This solution is approximately 0.05 N HCl and 0.025 N H_2SO_4. Smaller quantities may be made in the same ratio.

Procedure:

1. Weigh 12.5 g of soil to a 125-ml Erlenmeyer flask.

2. Add 50.0 ml of extracting solution.

3. Shake on oscillating shaker at 180 oscillations per minute for exactly 5 minutes.

4. Filter through Whatman 42 filter paper into a 50-ml Erlenmeyer flask.

5. Submit the filtrates for analysis by inductively coupled plasma (ICP), atomic absorption, or spectrometric methods.

References:

"Phosphorus Soluble in Dilute Hydrochloric Acid and Sulfuric Acid," Section 24-5.2 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-7 – Lab Procedures for Exchangeable K, Ca, and Mg: Method ASA 9-3.1

Determination of Exchangeable Cations in Soils Without Determining Total CEC

Ammonium Acetate Extraction

ASA 9-3.1

Reagent:

1. 1N Ammonium Acetate - Dissolve 231.34 g of reagent grade ammonium acetate in 2 liters of deionized water. Make to a 3 liter volume. Place beaker on a stirrer, insert electrodes in the solution and adjust pH to 7.0 with concentrated ammonium hydroxide or glacial acetic acid. For an 18 liter volume dissolve 1388.04 g of ammonium acetate. (Other volumes may be made in the same ratio.)

Procedure:

1. Weigh 5 g of soil (-2 mm, which is -9 mesh) into 125 ml Erlenmeyer flask.

2. Add 50 ml of 1N ammonium acetate, shake for 30 minutes on oscillating shaker on low setting (180/min).

3. Let stand at least 6 hours, preferably overnight, occasionally swirling the flasks.

4. Filter through Whatman 40 filter paper into 50 ml Erlenmeyer flask.

5. Submit the filtrates for analysis by inductively coupled plasma (ICP) or atomic absorption.

6. Convert soil ppm to centimols (cmol) per kg (report to a hundredth of a cmol).

Examples:

Cation	Divide soil ppm by
Ca	400
Mg	242
K	391
Mn	549

References:

"Replacement of Exchangeable Cations, Ammonium Acetate Method" Section 9-3.1 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-8 – Lab Procedures for Exchangeable Al: Method ASA 9-4.2

Exchangeable Aluminum by One Normal Potassium Chloride Extraction

ASA 9-4.2

Reagents: 1N KCl - Dissolve 74.0 grams potassium chloride in about 800 ml of deionized water. Dilute to 1 liter.

Procedure:

1. Weigh 5 grams soil into a 250 ml centrifuge tube.
2. Add 50 ml 1N KCl to each sample.
3. Shake for 30 minutes at 180/min setting.
4. Centrifuge for 5 minutes at 1500 rpm.
5. Filter through Whatman 42 filter paper into a 50ml Erlenmeyer flask.
6. Submit the sample for aluminum analysis by ICP.

References:

Can. J. Soil Sci. 70:263-275

"Exchangeable Acidity, Potassium Chloride Method," Section 9-4.2 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-9 – Lab Procedures for DTPA - Extractable Fe and Mn: Method ASA 17-4.3

DTPA Extraction of Soils

ASA 17-4.3

Reagent:

DTPA Extraction Solution (0.005M DTPA, 0.01M Calcium Chloride, 0.1M TEA)

1. Add 600 ml deionized water to a 1 liter volumetric flask.
2. Add 14.9 g TEA (Triethanolamine) and dissolve (add 16.5 ml if liquid form used).
3. Add 1.970 g of diethylene triamine pentaacetic acid and dissolve.
4. Add 1.470 g of calcium chloride and dissolve.
5. Bring volume to about 970 ml with deionized water.
6. Transfer to a beaker and adjust to pH of 7.3 with 6N HCl (about 13 ml required).
7. Return to volumetric flask and bring to volume.

Procedure:

1. Place 10 g dry soil in 125 ml Erlenmeyer flask.
2. Add 20 ml of DTPA extracting solution.
3. Shake for 2 hours on an oscillating shaker on low setting (180/min).
4. Filter extract through previously folded Whatman 42 filter paper into a 50 ml Erlenmeyer flask.
5. Submit the filtrates for analysis by inductively coupled plasma (ICP), atomic absorption, or spectrometric methods.

References:

"Availability Indices," Section 17-4.3 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Appendix B-10 – Lab Procedures for Total Metals: Method 3050A

Method 3050A -Acid Digestion of Sediments, Sludges, and Soils

1.0 Procedure

Prepare solid samples for further analysis by AA or ICP in accordance with Method 3050A from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method:

- method blank
- reagent blank
- calibration check sample.

For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine.

Where possible, for each batch analyze one matrix spike sample.

For each batch analyze a matrix spike duplicate or sample duplicate.

METHOD 3050A

ACID DIGESTION OF SEDIMENTS, SLUDGES, AND SOILS

1.0 SCOPE AND APPLICATION

1.1 This method is an acid digestion procedure used to prepare sediments, sludges, and soil samples for analysis by flame or furnace atomic absorption spectroscopy (FLAA and GFAA, respectively) or by inductively coupled argon plasma spectroscopy (ICP). Samples prepared by this method may be analyzed by ICP for all the listed metals, or by FLAA or GFAA as indicated below (see also Step 2.1):

FLAA

Aluminum
Barium
Beryllium
Cadmium
Calcium
Chromium
Cobalt
Copper
Iron
Lead
Magnesium
Manganese
Molybdenum
Nickel
Osmium
Potassium
Silver
Sodium
Thallium
Vanadium
Zinc

GFAA

Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Iron
Lead
Molybdenum
Selenium
Thallium
Vanadium

NOTE: See Method 7760 for FLAA preparation for Silver.

2.0 SUMMARY OF METHOD

2.1 A representative 1- to 2-g (wet weight) sample is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed with either nitric acid or hydrochloric acid. Hydrochloric acid is used for flame AA and ICP analyses and nitric acid is used for furnace AA work. Dilute hydrochloric acid is used as the final reflux acid for (1) the ICP analysis of As and Se, and (2) the flame AA or ICP analysis of Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Os, Pb, Tl, V, and Zn. Dilute nitric acid is employed as the final dilution acid for the furnace AA analysis of As, Be, Cd, Cr, Co, Fe, Pb, Mo, Se, Tl, and V. The diluted samples have an approximate acid concentration of 5.0% (v/v). A separate sample shall be dried for a total % solids determination.

3.0 INTERFERENCES

3.1 Sludge samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed to aid in determining whether Method 3050 is applicable to a given waste.

4.0 APPARATUS AND MATERIALS

4.1 Conical Phillips beakers - 250-mL, or equivalent.

4.2 Watch glasses ribbed or equivalent.

4.3 Drying ovens - That can be maintained at 30° C.

4.4 Thermometer - That covers range of 0-200°C.

4.5 Filter paper - Whatman No. 41 or equivalent.

4.6 Centrifuge and centrifuge tubes.

4.7 Analytical Balance - Capable of accurately weighing to the nearest 0.01 g.

4.8 Electric Hot Plate or equivalent - Adjustable and capable of maintaining a temperature of 90-95°C.

4.9 Glass Funnel or equivalent.

4.10 Graduated cylinder or equivalent.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

5.2 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.3 Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.4 Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.4 Hydrogen peroxide (30%), H_2O_2. Oxidant should be analyzed to determine level of impurities.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and water. Plastic and glass containers are both suitable. See Chapter Three, Step 3.1.3, for further information.

6.3 Nonaqueous samples shall be refrigerated upon receipt and analyzed as soon as possible.

7.0 PROCEDURE

7.1 Mix the sample thoroughly to achieve homogeneity. For each digestion procedure, weigh to the nearest 0.01 g and transfer to a conical beaker 1.00-2.00 g of sample. For samples with low percent solids a larger sample size may be used as long as digestion is completed.

7.2 Add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass. Heat the sample to 95°C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the watch glass, and reflux for 30 minutes. Repeat this last step to ensure complete oxidation. Using a ribbed watch glass, allow the solution to evaporate to 5 mL without boiling, while maintaining a covering of solution over the bottom of the beaker.

7.3 After Step 7.2 has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the beaker.

7.4 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.

NOTE: Do not add more than a total of 10 mL 30% H_2O_2.

7.5 If the sample is being prepared for (a) the ICP analysis of As and Se, or (b) the flame AA or ICP analysis of Ag, Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Os, Pb, Tl, V, and Zn, then add 5 mL of concentrated HCl and 10 mL of water, return the covered beaker to the hot plate, and reflux for an additional 15 minutes without boiling. After cooling, dilute to a 100 mL volume with water. Particulates in the digestate that may clog the nebulizer should be removed by filtration, by centrifugation, or by allowing the sample to settle.

7.5.1 Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

7.5.2 Centrifugation - Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

7.5.3 The diluted sample has an approximate acid concentration of 5.0% (v/v) HCl and 5.0% (v/v) HNO_3. The sample is now ready for analysis.

7.6 If the sample is being prepared for the furnace analysis of As, Be, Cd, Co, Cr, Fe, Mo, Pb, Se, Tl, and V, cover the sample with a ribbed watch glass and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL. After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle.

7.6.1 Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

7.6.2 Centrifugation - Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

7.6.3 The diluted digestate solution contains approximately 5% (v/v) HNO_3. For analysis, withdraw aliquots of appropriate volume and add any required reagent or matrix modifier. The sample is now ready for analysis.

7.7 Calculations

7.7.1 The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

7.7.2 If percent solids is desired, a separate determination of percent solids must be performed on a homogeneous aliquot of the sample.

8.0 QUALITY CONTROL

8.1 All quality control measures described in Chapter One should be followed.

8.2 For each batch of samples processed, preparation blanks should be carried throughout the entire sample preparation and analytical process. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

8.3 Replicate samples should be processed on a routine basis. Replicate samples will be used to determine precision. The sample load will dictate frequency, but 5% is recommended. Refer to Chapter One for the proper protocol when analyzing replicates.

8.4 Spiked samples or standard reference materials must be employed to determine accuracy. A spiked sample should be included with each batch of samples processed and whenever a new sample matrix is being analyzed. Refer to Chapter One for the proper protocol when analyzing spikes.

8.5 The concentration of all calibration standards should be verified against a quality control check sample obtained from an outside source.

9.0 METHOD PERFORMANCE

9.1 No data provided.

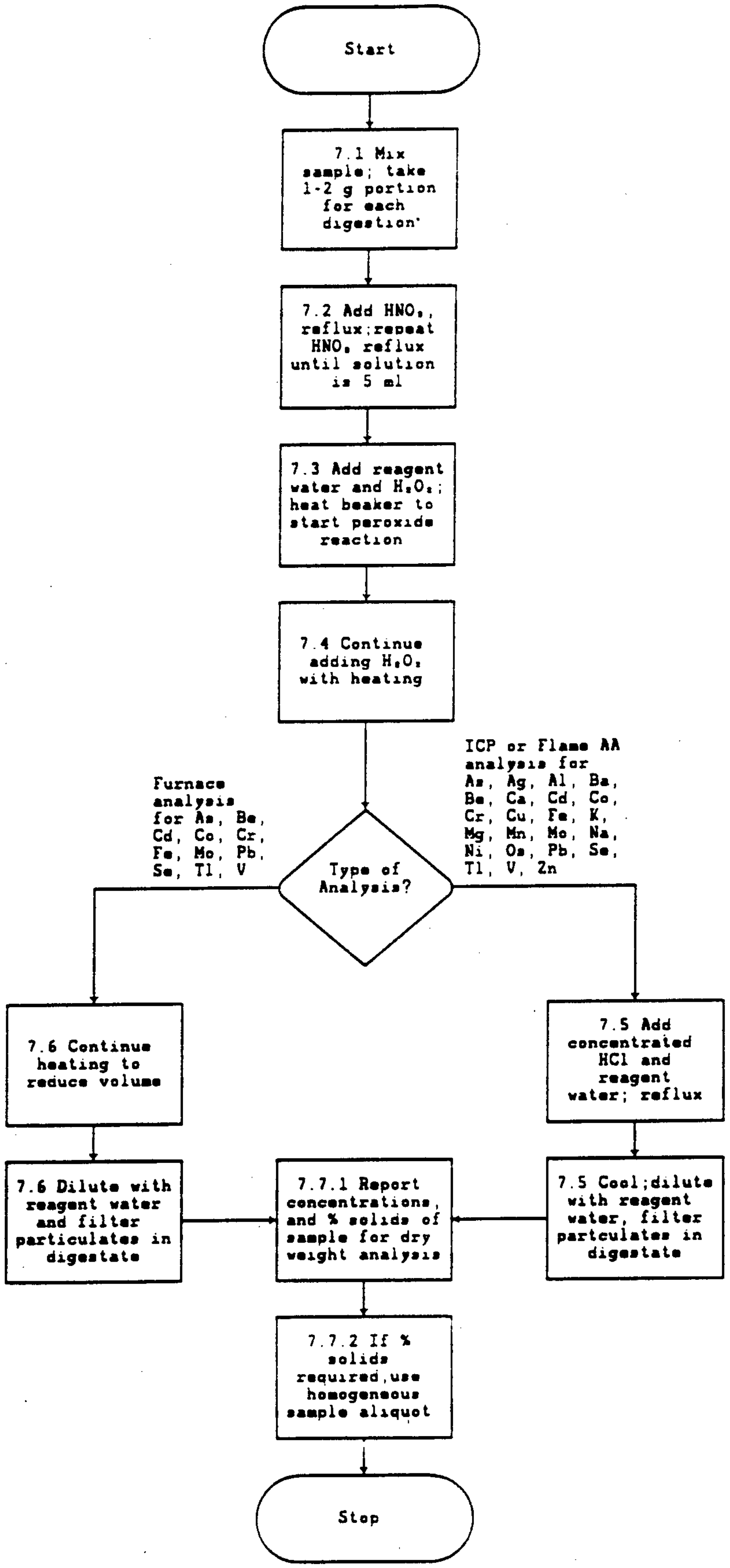
10.0 REFERENCES

1. Rohrbough, W.G.; et al. Reagent Chemicals, American Chemical Society Specifications, 7th ed.; American Chemical Society: Washington, DC, 1986.

2. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

3. Edgell, K.; USEPA Method Study 37 - SW-846 Method 3050 Acid Digestion of Sediments, Sludges, and Soils. EPA Contract No. 68-03-3254, November 1988.

METHOD 3050A
ACID DIGESTION OF SEDIMENTS, SLUDGES, AND SOILS



Appendix B-11 – Lab Procedures for Total Metals: Method 3005A

Method 3005A -Acid Digestion of Waters for Total Recoverable or Dissolved Metals for Analysis by FLAA or ICP Spectroscopy

1.0 Procedure

Prepare liquid samples for further analysis by AA or ICP in accordance with Method 3005A from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method:

- method blank
- reagent blank
- calibration check sample.

For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine.

Where possible, for each batch analyze one matrix spike sample.

For each batch analyze a matrix spike duplicate or sample duplicate.

METHOD 3005A

ACID DIGESTION OF WATERS FOR TOTAL RECOVERABLE OR DISSOLVED METALS FOR ANALYSIS BY FLAA OR ICP SPECTROSCOPY

1.0 SCOPE AND APPLICATION

1.1 Method 3005 is an acid digestion procedure used to prepare surface and ground water samples for analysis by flame atomic absorption spectroscopy (FLAA) or by inductively coupled argon plasma spectroscopy (ICP). Samples prepared by Method 3005 may be analyzed by AAS or ICP for the following metals:

- Aluminum
- Antimony**
- Arsenic*
- Barium
- Beryllium
- Cadmium
- Calcium
- Chromium
- Cobalt
- Copper
- Iron
- Lead
- Magnesium
- Manganese
- Molybdenum
- Nickel
- Potassium
- Selenium*
- Silver
- Sodium
- Thallium
- Vanadium
- Zinc

* ICP only
**May be analyzed by ICP, FLAA, or GFAA

1.2 When analyzing for total dissolved metals filter the sample, at the time of collection, prior to acidification with nitric acid.

2.0 SUMMARY OF METHOD

2.1 Total recoverable metals - The entire sample is acidified at the time of collection with nitric acid. At the time of analysis the sample is heated with acid and substantially reduced in volume. The digestate is filtered and diluted to volume, and is then ready for analysis.

2.2 Dissolved metals - The sample is filtered through a 0.45-µm filter at the time of collection and the liquid phase is then acidified at the time of collection with nitric acid. Samples for dissolved metals do not need to be digested as long as the acid concentrations have been adjusted to the same concentration as in the standards.

3.0 INTERFERENCES

3.1 The analyst should be cautioned that this digestion procedure may not be sufficiently vigorous to destroy some metal complexes.

Precipitation will cause a lowering of the silver concentration and therefore an inaccurate analysis.

4.0 APPARATUS AND MATERIALS

4.1 Griffin beakers of assorted sizes or equivalent.

4.2 Watch glasses or equivalent.

4.3 Qualitative filter paper and filter funnels.

4.4 Graduated cylinder or equivalent.

4.5 Electric hot plate or equivalent - adjustable and capable of maintaining a temperature of 90-95°C.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Reagent Water. Reagent water shall be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.3 Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine level of impurities. If method blank is < MDL, then acid can be used.

5.4 Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, then acid can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and water. Both plastic and glass containers are suitable.

6.3 Sampling

6.3.1 Total recoverable metals - All samples must be acidified at the time of collection with HNO_3 (5 mL/L).

6.3.2 Dissolved metals - All samples must be filtered through a 0.45-µm filter and then acidified at the time of collection with HNO_3 (5 mL/L).

7.0 PROCEDURE

7.1 Transfer a 100-mL aliquot of well-mixed sample to a beaker.

7.2 For metals that are to be analyzed, add 2 mL of concentrated HNO_3 and 5 mL of concentrated HCl. The sample is covered with a ribbed watch glass or other suitable covers and heated on a steam bath, hot plate or other heating source at 90 to 95°C until the volume has been reduced to 15-20 mL.

CAUTION: Do not boil. Antimony is easily lost by volatilization from hydrochloric acid media.

7.3 Remove the beaker and allow to cool. Wash down the beaker walls and watch glass with water and, when necessary, filter or centrifuge the sample to remove silicates and other insoluble material that could clog the nebulizer. Filtration should be done only if there is concern that insoluble materials may clog the nebulizer; this additional step is liable to cause sample contamination unless the filter and filtering apparatus are thoroughly cleaned and prerinsed with dilute HNO_3.

7.4 Adjust the final volume to 100 mL with reagent water.

8.0 QUALITY CONTROL

8.1 All quality control measures described in Chapter One should be followed.

8.2 For each analytical batch of samples processed, blanks should be carried throughout the entire sample preparation and analytical process. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

8.3 Replicate samples should be processed on a routine basis. A replicate sample is a sample brought through the whole sample preparation and analytical process. Replicate samples will be used to determine precision. The sample load will dictate the frequency, but 5% is recommended. Refer to Chapter One for the proper protocol when analyzing replicates.

8.4 Spiked samples or standard reference materials should be employed to determine accuracy. A spiked sample should be included with each batch. Refer to Chapter One for the proper protocol when analyzing spikes.

9.0 METHOD PERFORMANCE

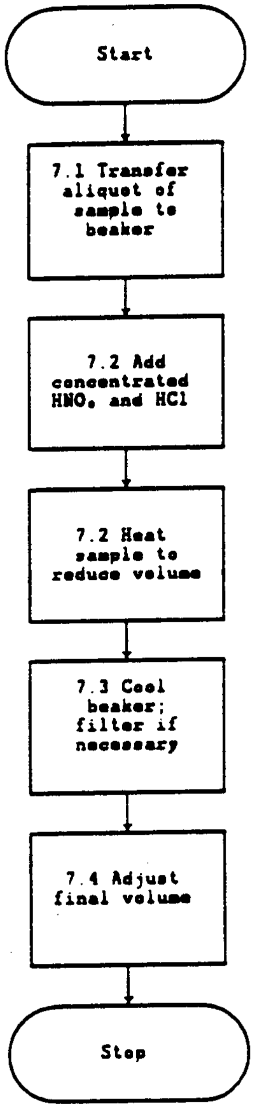
9.1 No data provided.

10.0 REFERENCES

1. Rohrbough, W.G.; et al. Reagent Chemicals, American Chemical Society Specifications, 7th ed.; American Chemical Society: Washington, DC, 1986.

2. 1985 Annual Book of ASTM Standards, Vol. 11.01; "Standard Specification for Reagent Water"; ASTM: Philadelphia, PA, 1985; D1193-77.

METHOD 3005A
ACID DIGESTION OF WATERS FOR TOTAL RECOVERABLE OR DISSOLVED METALS FOR ANALYSIS BY FLAA OR ICP SPECTROSCOPY



Appendix B-12 – Lab Procedures for Total Metals (Hg): Method 7470 and 7471A

Method 7470A -Mercury in Liquid Waste (Manual Cold-Vapor Technique)
Method 7471A - Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)

1.0 Procedure

Perform analysis of liquid samples for mercury in accordance with Method 7470A from SW-846 as attached. Perform analysis of solid or semisolid samples for mercury in accordance with Method 7471A from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method:

method blank
reagent blank
calibration check sample.

For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine.

Where possible, for each batch analyze one matrix spike sample.

For each batch analyze a matrix spike duplicate or sample duplicate.

METHOD 7470A

MERCURY IN LIQUID WASTE (MANUAL COLD-VAPOR TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7470 is a cold-vapor atomic absorption procedure approved for determining the concentration of mercury in mobility-procedure extracts, aqueous wastes, and ground waters. (Method 7470 can also be used for analyzing certain solid and sludge-type wastes; however, Method 7471 is usually the method of choice for these waste types.) All samples must be subjected to an appropriate dissolution step prior to analysis.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, the liquid samples must be prepared according to the procedure discussed in this method.

2.2 Method 7470, a cold-vapor atomic absorption technique, is based on the absorption of radiation at 253.7-nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration.

2.3 The typical detection limit for this method is 0.0002 mg/L.

3.0 INTERFERENCES

3.1 Potassium permanganate is added to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as sodium sulfide do not interfere with the recovery of added inorganic mercury from reagent water.

3.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/L had no effect on recovery of mercury from spiked samples.

3.3 Seawaters, brines, and industrial effluents high in chlorides require additional permanganate (as much as 25 mL) because, during the oxidation step, chlorides are converted to free chlorine, which also absorbs radiation of 253.7 nm. Care must therefore be taken to ensure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine sulfate reagent (25 mL). In addition, the dead air space in the BOD bottle must be purged before adding stannous sulfate. Both inorganic and organic mercury spikes have been quantitatively recovered from seawater by using this technique.

3.4 Certain volatile organic materials that absorb at this wavelength may also cause interference. A preliminary run without reagents should determine if this type of interference is present.

4.0 APPARATUS AND MATERIALS

4.1 Atomic absorption spectrophotometer or equivalent: Any atomic absorption unit with an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Instruments designed specifically for the measurement of mercury using the cold-vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

4.2 Mercury hollow cathode lamp or electrodeless discharge lamp.

4.3 Recorder: Any multirange variable-speed recorder that is compatible with the UV detection system is suitable.

4.4 Absorption cell: Standard spectrophotometer cells 10 cm long with quartz end windows may be used. Suitable cells may be constructed from Plexiglas tubing, 1 in. O.D. x 4.5 in. The ends are ground perpendicular to the longitudinal axis, and quartz windows (1 in. diameter x 1/16 in. thickness) are cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 2-in. x 2-in. cards. One-in.-diameter holes are cut in the middle of each card. The cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.

4.5 Air pump: Any peristaltic pump capable of delivering 1 liter air/min may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

4.6 Flowmeter: Capable of measuring an air flow of 1 liter/min.

4.7 Aeration tubing: A straight glass frit with a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

4.8 Drying tube: 6-in. x 3/4-in.-diameter tube containing 20 g of magnesium perchlorate or a small reading lamp with 60-W bulb which may be used to prevent condensation of moisture inside the cell. The lamp should be positioned to shine on the absorption cell so that the air temperature in the cell is about 10°C above ambient.

4.9 The cold-vapor generator is assembled as shown in Figure 1 of reference 1 or according to the instrument manufacturers instructions. The apparatus shown in Figure 1 is a closed system. An open system, where the mercury vapor is passed through the absorption cell only once, may be used instead of the closed system. Because mercury vapor is toxic, precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system either to vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing medium, such as:

1. Equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4; or

2. 0.25% Iodine in a 3% KI solution.

A specially treated charcoal that will adsorb mercury vapor is also available from Barnebey and Cheney, East 8th Avenue and North Cassidy Street, Columbus, Ohio 43219, Cat. #580-13 or #580-22.

4.10 Hot plate or equivalent - Adjustable and capable of maintaining a temperature of 90-95°C.

4.11 Graduated cylinder or equivalent.

5.0 REAGENTS

5.1 Reagent Water: Reagent water will be interference free. All references to water in this method will refer to reagent water unless otherwise specified.

5.2 Sulfuric acid (H_2SO_4), concentrated: Reagent grade.

5.3 Sulfuric acid, 0.5 N: Dilute 14.0 mL of concentrated sulfuric acid to 1.0 liter.

5.4 Nitric acid (HNO_3), concentrated: Reagent grade of low mercury content. If a high reagent blank is obtained, it may be necessary to distill the nitric acid.

5.5 Stannous sulfate: Add 25 g stannous sulfate to 250 mL of 0.5 N H_2SO_4. This mixture is a suspension and should be stirred continuously during use. (Stannous chloride may be used in place of stannous sulfate.)

5.6 Sodium chloride-hydroxylamine sulfate solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in reagent water and dilute to 100 mL. (Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.)

5.7 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g of potassium permanganate in 100 mL of reagent water.

5.8 Potassium persulfate, 5% solution (w/v): Dissolve 5 g of potassium persulfate in 100 mL of reagent water.

5.9 Stock mercury solution: Dissolve 0.1354 g of mercuric chloride in 75 mL of reagent water. Add 10 mL of concentrated HNO_3 and adjust the volume to 100.0 mL (1 mL = 1 mg Hg). Stock solutions may also be purchased.

5.10 Mercury working standard: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug per mL. This working standard and the dilutions of the stock mercury solution should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask, as needed, before addition of the aliquot.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

6.3 Aqueous samples must be acidified to a pH <2 with HNO_3. The suggested maximum holding times for mercury is 28 days.

6.4 Nonaqueous samples shall be refrigerated, when possible, and analyzed as soon as possible.

7.0 PROCEDURE

7.1 Sample preparation: Transfer 100 mL, or an aliquot diluted to 100 mL, containing <1.0 g of mercury, to a 300-mL BOD bottle or equivalent. Add 5 mL of H_2SO_4 and 2.5 mL of concentrated HNO_3, mixing after each addition. Add 15 mL of potassium permanganate solution to each sample bottle. Sewage samples may require additional permanganate. Ensure that equal amounts of permanganate are added to standards and blanks. Shake and add additional portions of potassium permanganate solution, if necessary, until the purple color persists for at least 15 min. Add 8 mL of potassium persulfate to each bottle and heat for 2 hr in a water bath maintained at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate. After a delay of at least 30 sec, add 5 mL of stannous sulfate, immediately attach the bottle to the aeration apparatus, and continue as described in Paragraph 7.3.

7.2 Standard preparation: Transfer 0-, 0.5-, 1.0-, 2.0-, 5.0-, and 10.0-mL aliquots of the mercury working standard, containing 0-1.0 ug of mercury, to a series of 300-mL BOD bottles. Add enough reagent water to each bottle to make a total volume of 100 mL. Mix thoroughly and add 5 mL of concentrated H_2SO_4 and 2.5 mL of concentrated HNO_3 to each bottle. Add 15 mL of $KMnO_4$ solution to each bottle and allow to stand at least 15 min. Add 8 mL of potassium persulfate to each bottle and heat for 2 hr in a water bath maintained at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. When the solution has been decolorized, wait 30 sec, add 5 mL of the stannous sulfate solution, immediately attach the bottle to the aeration apparatus, and continue as described in Paragraph 7.3.

7.3 Analysis: At this point the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 liter/min, is allowed to run continuously. The absorbance will increase and reach a maximum within 30 sec. As soon as the recorder pen levels off (approximately 1 min), open the bypass valve and continue the aeration until the absorbance returns to its minimum value. Close the bypass valve, remove the stopper and frit from the BOD bottle, and continue the aeration. Because of instrument variation refer to the manufacturers recommended operating conditions when using this method.

7.4 Construct a calibration curve by plotting the absorbances of standards versus micrograms of mercury. Determine the peak height of the unknown from the chart and read the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed.

7.5 Calculate metal concentrations (1) by the method of standard additions, or (2) from a calibration curve. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 ug/g dry weight).

8.0 QUALITY CONTROL

8.1 Refer to section 8.0 of Method 7000.

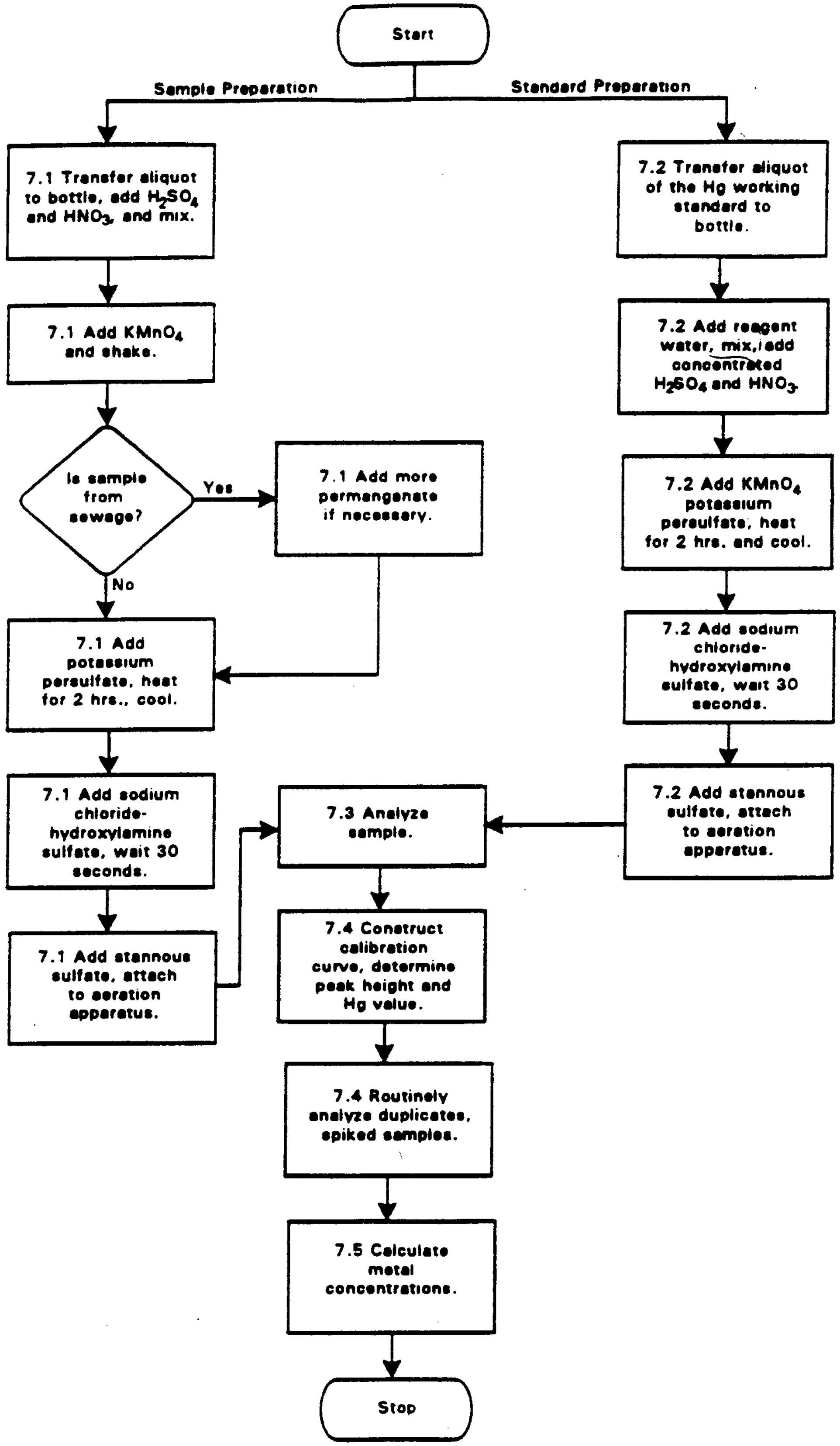
9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 245.1 of Methods for Chemical Analysis of Water and Wastes.

10.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 245.1.

METHOD 7470A
MERCURY IN LIQUID WASTE (MANUAL COLD-VAPOR TECHNIQUE)



METHOD 7471A

MERCURY IN SOLID OR SEMISOLID WASTE (MANUAL COLD-VAPOR TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7471 is approved for measuring total mercury (organic and inorganic) in soils, sediments, bottom deposits, and sludge-type materials. All samples must be subjected to an appropriate dissolution step prior to analysis. If this dissolution procedure is not sufficient to dissolve a specific matrix type or sample, then this method is not applicable for that matrix.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, the solid or semi-solid samples must be prepared according to the procedures discussed in this method.

2.2 Method 7471, a cold-vapor atomic absorption method, is based on the absorption of radiation at the 253.7-nm wavelength by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured as a function of mercury concentration.

2.3 The typical instrument detection limit (IDL) for this method is 0.0002 mg/L.

3.0 INTERFERENCES

3.1 Potassium permanganate is added to eliminate possible interference from sulfide. Concentrations as high as 20 mg/Kg of sulfide, as sodium sulfide, do not interfere with the recovery of added inorganic mercury in reagent water.

3.2 Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/Kg had no effect on recovery of mercury from spiked samples.

3.3 Samples high in chlorides require additional permanganate (as much as 25 mL) because, during the oxidation step, chlorides are converted to free chlorine, which also absorbs radiation of 253 nm. Care must therefore be taken to ensure that free chlorine is absent before the mercury is reduced and swept into the cell. This may be accomplished by using an excess of hydroxylamine sulfate reagent (25 mL). In addition, the dead air space in the BOD bottle must be purged before adding stannous sulfate.

3.4 Certain volatile organic materials that absorb at this wavelength may also cause interference. A preliminary run without reagents should determine if this type of interference is present.

4.0 APPARATUS AND MATERIALS

4.1 Atomic absorption spectrophotometer or equivalent: Any atomic absorption unit with an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed. Instruments designed specifically for the measurement of mercury using the cold-vapor technique are commercially available and may be substituted for the atomic absorption spectrophotometer.

4.2 Mercury hollow cathode lamp or electrodeless discharge lamp.

4.3 Recorder: Any multirange variable-speed recorder that is compatible with the UV detection system is suitable.

4.4 Absorption cell: Standard spectrophotometer cells 10 cm long with quartz end windows may be used. Suitable cells may be constructed from Plexiglas tubing, 1 in. O.D. x 4.5 in. The ends are ground perpendicular to the longitudinal axis, and quartz windows (1 in. diameter x 1/16 in. thickness) are cemented in place. The cell is strapped to a burner for support and aligned in the light beam by use of two 2-in. x 2-in. cards. One-in.-diameter holes are cut in the middle of each card. The cards are then placed over each end of the cell. The cell is then positioned and adjusted vertically and horizontally to give the maximum transmittance.

4.5 Air pump: Any peristaltic pump capable of delivering 1 L/min air may be used. A Masterflex pump with electronic speed control has been found to be satisfactory.

4.6 Flowmeter: Capable of measuring an air flow of 1 L/min.

4.7 Aeration tubing: A straight glass frit with a coarse porosity. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return.

4.8 Drying tube: 6-in. x 3/4-in.-diameter tube containing 20 g of magnesium perchlorate or a small reading lamp with 60-W bulb which may be used to prevent condensation of moisture inside the cell. The lamp should be positioned to shine on the absorption cell so that the air temperature in the cell is about 10°C above ambient.

4.9 The cold-vapor generator is assembled as shown in Figure 1 of reference 1 or according to the instrument manufacturers instructions. The apparatus shown in Figure 1 is a closed system. An open system, where the mercury vapor is passed through the absorption cell only once, may be used instead of the closed system. Because mercury vapor is toxic, precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system either to vent the mercury vapor into an exhaust hood or to pass the vapor through some absorbing medium, such as:

1. equal volumes of 0.1 M $KMnO_4$ and 10% H_2SO_4, or
2. 0.25% iodine in a 3% KI solution.

A specially treated charcoal that will adsorb mercury vapor is also available from Barneby and Cheney, East 8th Avenue and North Cassidy Street, Columbus, Ohio 43219, Cat. #580-13 or #580-22.

4.10 Hot plate or equivalent - Adjustable and capable of maintaining a temperature of 90-95°C.

4.11 Graduated cylinder or equivalent.

5.0 REAGENTS

5.1 Reagent Water: Reagent water will be interference free. All references to water in this method refer to reagent water unless otherwise specified.

5.2 Aqua regia: Prepare immediately before use by carefully adding three volumes of concentrated HCl to one volume of concentrated HNO_3.

5.3 Sulfuric acid, 0.5 N: Dilute 14.0 mL of concentrated sulfuric acid to 1 liter.

5.4 Stannous sulfate: Add 25 g stannous sulfate to 250 mL of 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use. A 10% solution of stannous chloride can be substituted for stannous sulfate.

5.5 Sodium chloride-hydroxylamine sulfate solution: Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in reagent water and dilute to 100 mL. Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

5.6 Potassium permanganate, mercury-free, 5% solution (w/v): Dissolve 5 g of potassium permanganate in 100 mL of reagent water.

5.7 Mercury stock solution: Dissolve 0.1354 g of mercuric chloride in 75 mL of reagent water. Add 10 mL of concentrated nitric acid and adjust the volume to 100.0 mL (1.0 mL = 1.0 mg Hg).

5.8 Mercury working standard: Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 ug/mL. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask, as needed, before adding the aliquot.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and reagent water. Plastic and glass containers are both suitable.

6.3 Non-aqueous samples shall be refrigerated, when possible, and analyzed as soon as possible."

7.0 PROCEDURE

7.1 Sample preparation: Weigh triplicate 0.2-g portions of untreated sample and place in the bottom of a BOD bottle. Add 5 mL of reagent water and 5 mL of aqua regia. Heat 2 min in a water bath at 95°C. Cool; then add 50 mL reagent water and 15 mL potassium permanganate solution to each sample bottle. Mix thoroughly and place in the water bath for 30 min at 95°C. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate.

> CAUTION: Do this addition under a hood, as Cl_2 could be evolved. Add 55 mL of reagent water. Treating each bottle individually, add 5 mL of stannous sulfate and immediately attach the bottle to the aeration apparatus. Continue as described under step 7.4.

7.2 An alternate digestion procedure employing an autoclave may also be used. In this method, 5 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3 are added to the 0.2 g of sample. Add 5 mL of saturated $KMnO_4$ solution and cover the bottle with a piece of aluminum foil. The samples are autoclaved at 121°C and 15 lb for 15 min. Cool, dilute to a volume of 100 mL with reagent water, and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Purge the dead air space and continue as described under step 7.4. Refer to the caution statement in section 7.1 for the proper protocol in reducing the excess permanganate solution and adding stannous sulfate.

7.3 Standard preparation: Transfer 0.0-, 0.5-, 1.0-, 2.0-, 5.0-, and 10-mL aliquots of the mercury working standard, containing 0-1.0 ug of mercury, to a series of 300-mL BOD bottles or equivalent. Add enough reagent water to each bottle to make a total volume of 10 mL. Add 5 mL of aqua regia and heat 2 min in a water bath at 95°C. Allow the sample to cool; add 50 mL reagent water and 15 mL of $KMnO_4$ solution to each bottle and return to the water bath for 30 min. Cool and add 6 mL of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Add 50 mL of reagent water. Treating each bottle individually, add 5 mL of stannous sulfate solution, immediately attach the bottle to the aeration apparatus, and continue as described in Step 7.4.

7.4 Analysis: At this point, the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to a rate of 1 L/min, is allowed to run continuously. The absorbance, as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 sec. As soon as the recorder pen levels off (approximately 1 min), open the bypass valve and continue the aeration until the absorbance returns to its minimum value. Close the bypass valve, remove the fritted tubing from the BOD bottle, and continue the aeration.

7.5 Construct a calibration curve by plotting the absorbances of standards versus micrograms of mercury. Determine the peak height of the unknown from the chart and read the mercury value from the standard curve. Duplicates, spiked samples, and check standards should be routinely analyzed.

7.6 Calculate metal concentrations: (1) by the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration read-out. All dilution or concentration factors must be taken into account. Concentrations reported for multiphased or wet samples must be appropriately qualified (e.g., 5 ug/g dry weight).

8.0 QUALITY CONTROL

8.1 Refer to section 8.0 of Method 7000.

9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 245.5 of Methods for Chemical Analysis of Water and Wastes.

9.2 The data shown in Table 1 were obtained from records of state and contractor laboratories. The data are intended to show the precision of the combined sample preparation and analysis method.

10.0 REFERENCES

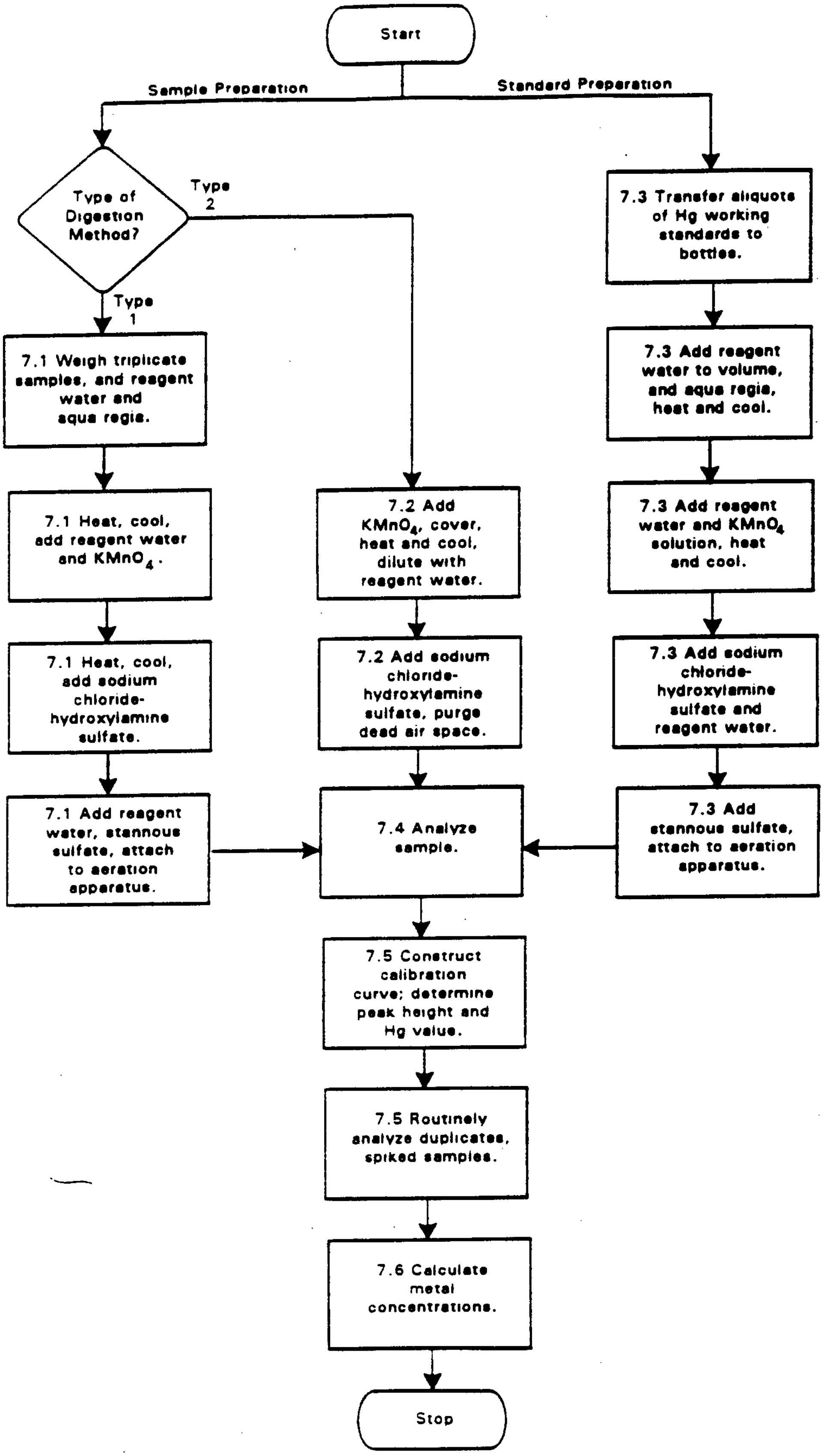
1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 245.5.

2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

TABLE 1. METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	Not known	12, 12 ug/g
Wastewater treatment sludge	Not known	0.4, 0.28 ug/g

METHOD 7471A
MERCURY IN SOLID OR SEMISOLID WASTE (MANUAL COLD-VAPOR TECHNIQUE)



Appendix B-13 – Lab Procedures for Total Metals (Se): Method 7740

Method 7740 -Selenium (Atomic Absorption, Furnace Technique)

1.0 Procedure

Perform analysis for selenium in accordance with Method 7740 from SW-846 as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform the quality control analyses specified in the method:

- method blank
- reagent blank
- calibration check sample.

For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine.

Where possible, for each batch analyze one matrix spike sample.

For each batch analyze a matrix spike duplicate or sample duplicate.

METHOD 7740

SELENIUM (ATOMIC ABSORPTION, FURNACE TECHNIQUE)

1.0 SCOPE AND APPLICATION

1.1 Method 7740 is an atomic absorption procedure approved for determining the concentration of selenium in wastes, mobility-procedure extracts, soils, and ground water. All samples must be subjected to an appropriate dissolution step prior to analysis.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis by Method 7740, samples must be prepared in order to convert organic forms of selenium to inorganic forms, to minimize organic interferences, and to convert samples to suitable solutions for analysis. The sample-preparation procedure varies, depending on the sample matrix. Aqueous samples are subjected to the acid-digestion procedure described in this method. Sludge samples are prepared using the procedure described in Method 3050.

2.2 Following the appropriate dissolution of the sample, a representative aliquot is placed manually or by means of an automatic sampler into a graphite tube furnace. The sample aliquot is then slowly evaporated to dryness, charred (ashed), and atomized. The absorption of lamp radiation during atomization will be proportional to the selenium concentration.

2.3 The typical detection limit for this method is 2 ug/L.

3.0 INTERFERENCES

3.1 Elemental selenium and many of its compounds are volatile; therefore, samples may be subject to losses of selenium during sample preparation. Spike samples and relevant standard reference materials should be processed to determine if the chosen dissolution method is appropriate.

3.2 Likewise, caution must be employed during the selection of temperature and times for the dry and char (ash) cycles. A nickel nitrate solution must be added to all digestates prior to analysis to minimize volatilization losses during drying and ashing.

3.3 In addition to the normal interferences experienced during graphite furnace analysis, selenium analysis can suffer from severe nonspecific absorption and light scattering caused by matrix components during atomization. Selenium analysis is particularly susceptible to these problems because of its low analytical wavelength (196.0 nm). Simultaneous background correction is required to avoid erroneously high results. High iron levels can give overcorrection with deuterium background. Zeeman background correction can be useful in this situation.

3.4 If the analyte is not completely volatilized and removed from the furnace during atomization, memory effects will occur. If this situation is detected, the tube should be cleaned by operating the furnace at full power at regular intervals in the analytical scheme.

3.5 Selenium analysis suffers interference from chlorides (>800 mg/L) and sulfate (>200 mg/L). The addition of nickel nitrate such that the final concentration is 1% nickel will lessen this interference.

4.0 APPARATUS AND MATERIALS

4.1 250-mL Griffin beaker.

4.2 10-mL volumetric flasks.

4.3 Atomic absorption spectrophotometer: Single- or dual-channel, single- or double-beam instrument with a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190-800 nm, and provisions for simultaneous background correction and interfacing with a strip-chart recorder.

4.4 Selenium hollow cathode lamp, or electrodeless discharge lamp (EDL): EDLs provide better sensitivity for the analysis of Se.

4.5 Graphite furnace: Any graphite furnace device with the appropriate temperature and timing controls.

4.6 Strip-chart recorder: A recorder is strongly recommended for furnace work so that there will be a permanent record and so that any problems with the analysis, such as drift, incomplete atomization, losses during charring, changes in sensitivity, etc., can easily be recognized.

4.7 Pipets: Microliter with disposable tips. Sizes can range from 5 to 1,000 uL, as required.

5.0 REAGENTS

5.1 ASTM Type II water (ASTM D1193): Water should be monitored for impurities.

5.2 Concentrated nitric acid (HNO_3): Acid should be analyzed to determine levels of impurities. If a method blank made with the acid is <MDL, the acid can be used.

5.3. Hydrogen peroxide (30%): Oxidant should be analyzed to determine levels of impurities. If a method blank made with the oxidant is <MDL, the oxidant can be used.

5.4 Selenium standard stock solution (1,000 mg/L): Either procure a certified aqueous standard from a supplier and verify by comparison with a second standard, or dissolve 0.3453 g of selenious acid (actual assay 94.6% H_2SeO_3, analytical reagent grade) or equivalent in Type II water and dilute to 200 mL.

5.5 Nickel nitrate solution (5%): Dissolve 24.780 g of ACS reagent grade $Ni(NO_3)_2 \cdot 6H_2O$ or equivalent in Type II water and dilute to 100 mL.

5.6 Nickel nitrate solution (1%): Dilute 20 mL of the 5% nickel nitrate to 100 mL with Type II water.

5.7 Selenium working standards: Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Withdraw appropriate aliquots of the stock solution, add 1 mL of concentrated HNO_3, 2 mL of 30% H_2O_2, and 2 mL of the 5% nickel nitrate solution. Dilute to 100 mL with Type II water.

5.8 Air: Cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The source may be a compressor or a cylinder of industrial-grade compressed air.

5.9 Hydrogen: Suitable for instrumental analysis.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and Type II water. Plastic and glass containers are both suitable.

6.3 Special containers (e.g., containers used for volatile organic analysis) may have to be used if very volatile selenium compounds are to be analyzed.

6.4 Aqueous samples must be acidified to a pH of <2 with nitric acid.

6.5 Nonaqueous samples shall be refrigerated, when possible, and analyzed as soon as possible.

7.0 PROCEDURE

7.1 Sample preparation: Aqueous samples should be prepared in the manner described in Steps 7.1.1 to 7.1.3. Sludge-type samples should be prepared according to Method 3050. The applicability of a sample-preparation technique to a new matrix type must be demonstrated by analyzing spiked samples and/or relevant standard reference materials.

7.1.1 Transfer 100 mL of well-mixed sample to a 250-mL Griffin beaker; add 2 mL of 30% H_2O_2 and sufficient concentrated HNO_3 to result in an acid concentration of 1% (v/v). Heat for 1 hr at 95°C or until the volume is slightly less than 50 mL.

7.1.2 Cool and bring back to 50 mL with Type II water.

7.1.3 Pipet 5 mL of this digested solution into a 10-mL volumetric flask, add 1 mL of the 1% nickel nitrate solution, and dilute to 10 mL with Type II water. The sample is now ready for injection into the furnace.

7.2 The 196.0-nm wavelength line and a background correction system must be employed. Follow the manufacturer's suggestions for all other spectrophotometer parameters.

7.3 Furnace parameters suggested by the manufacturer should be employed as guidelines. Because temperature-sensing mechanisms and temperature controllers can vary between instruments or with time, the validity of the furnace parameters must be periodically confirmed by systematically altering the furnace parameters while analyzing a standard. In this manner, losses of analyte due to overly high temperature settings or losses in sensitivity due to less than optimum settings can be minimized. Similar verification of furnace parameters may be required for complex sample matrices.

7.4 Inject a measured uL-aliquot of sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace pipetting errors.

7.5 Analyze all EP extracts, all samples analyzed as part of a delisting petition, and all samples that suffer from matrix interferences by the method of standard additions.

7.6 Run a check standard after approximately every 10 sample injections. Standards are run in part to monitor the life and performance of the graphite tube. Lack of reproducibility or significant change in the signal for the standard indicates that the tube should be replaced.

7.7 Duplicates, spiked samples, and check standards should be analyzed every 20 samples.

7.8 Calculate metal concentrations: (1) by the method of standard additions, (2) from a calibration curve, or (3) directly from the instrument's concentration read-out. All dilution or concentration factors must be taken into account.

8.0 QUALITY CONTROL

8.1 All quality control data should be maintained and available for easy reference or inspection.

8.2 Calibration curves must be composed of a minimum of a blank and three standards. A calibration curve should be made for every hour of continuous sample analysis.

8.3 Dilute samples if they are more concentrated than the highest standard or if they fall on the plateau of a calibration curve.

8.4 Employ a minimum of one blank per sample batch to determine if contamination or any memory effects are occurring.

8.5 Verify calibration with an independently prepared check standard every 15 samples.

8.6 Run one spike duplicate sample for every 10 samples. A duplicate sample is a sample brought through the entire sample preparation and analytical process.

8.7 The method of standard additions (see Method 7000, Section 8.7) shall be used for the analysis of all EP extracts, on all analyses submitted as part of a delisting petition, and whenever a new sample matrix is being analyzed.

9.0 METHOD PERFORMANCE

9.1 Precision and accuracy data are available in Method 270.2 of Methods for Chemical Analysis of Water and Wastes.

9.2 The data shown in Table 1 were obtained from records of state and contractor laboratories. The data are intended to show the precision of the combined sample preparation and analysis method.

10.0 REFERENCES

1. Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055, December 1982, Method 270.2.

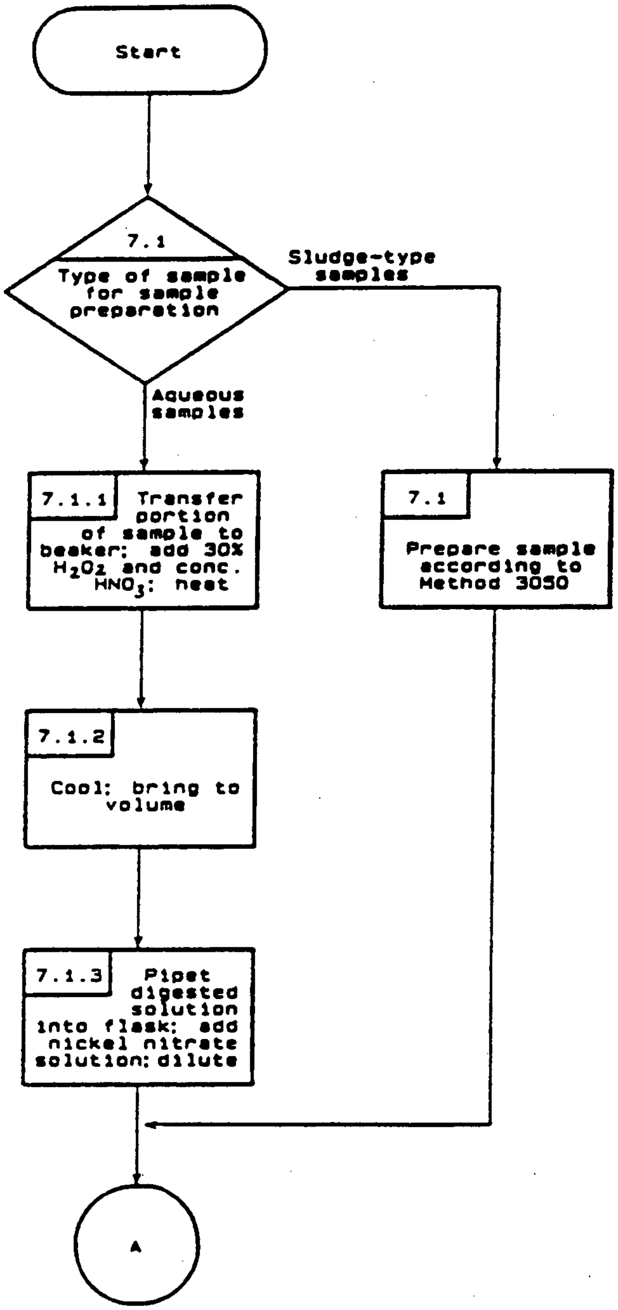
2. Gaskill, A., Compilation and Evaluation of RCRA Method Performance Data, Work Assignment No. 2, EPA Contract No. 68-01-7075, September 1986.

TABLE 1. METHOD PERFORMANCE DATA

Sample Matrix	Preparation Method	Laboratory Replicates
Emission control dust	3050	14, 11 ug/g

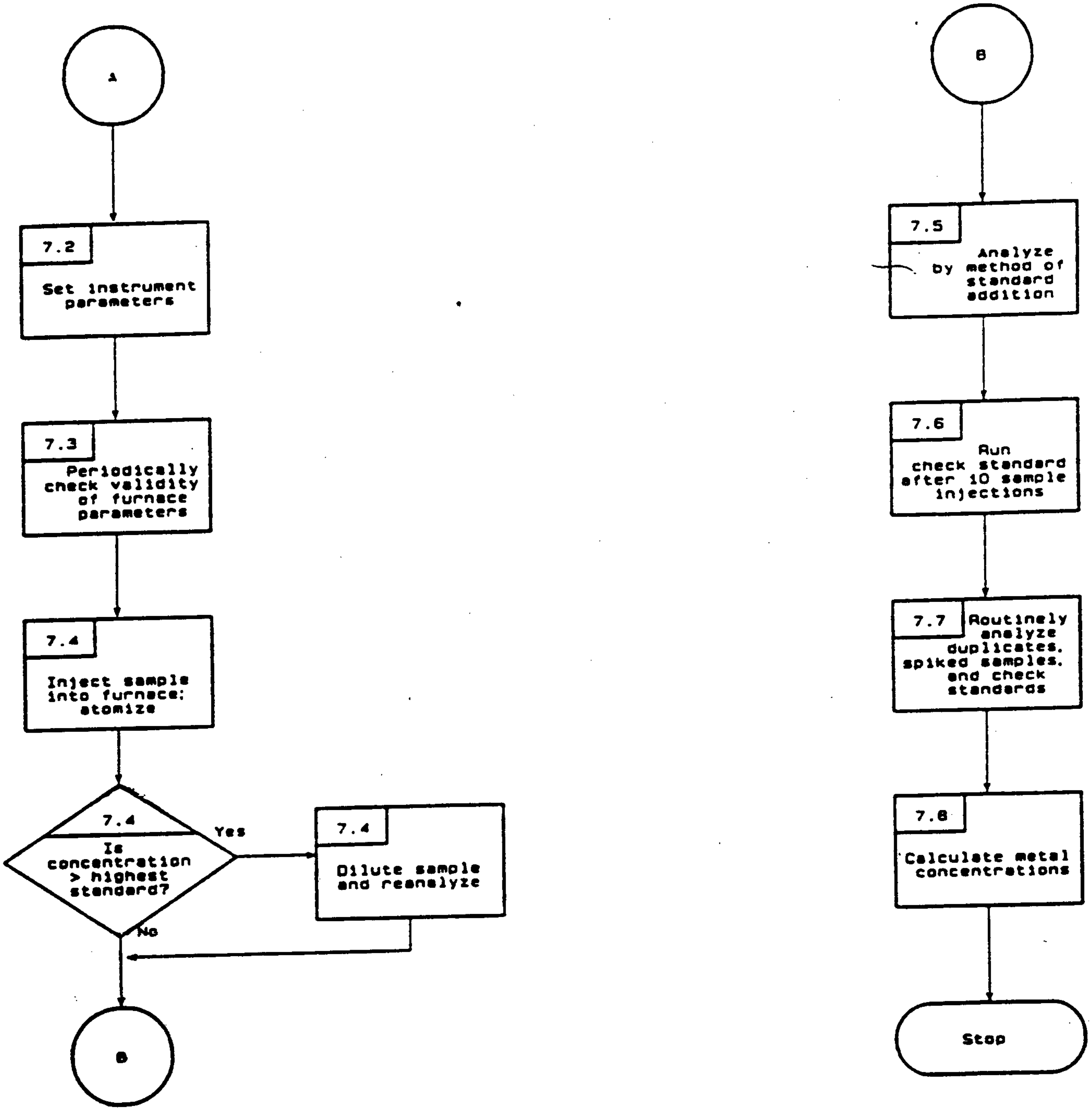
METHOD 7740

SELENIUM (ATOMIC ABSORPTION, FURNACE METHOD)



METHOD 7740

SELENIUM (ATOMIC ABSORPTION, FURNACE METHOD)
(Continued)



Appendix B-14 – Lab Procedures for Plant-available Pb: Method ASA 21-5

EDTA Extraction of Lead from Soils
ASA 21-5

Reagent:

0.1 M EDTA Solution

Dissolve 37.2 g EDTA (Ethylenedinitrilo Tetraacetic Acid Disodium Salt, Dihydrate) in approximately 800 ml deionized water in a volumetric flask. Bring the volume to one liter with deionized water.

Procedure:

1. Place 10 g dry soil in 125 ml Erlenmeyer flask.

2. Add 20 ml of EDTA extracting solution.

3. Shake for 2 hours on an oscillating shaker on low setting (180/min).

4. Filter extract through previously folded Whatman 42 filter paper into a 50 ml Erlenmeyer beaker.

5. Submit the filtrates for analysis by inductively coupled plasma (ICP) or atomic absorption.

References:

"Selective Extraction," Section 21-5 in *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Second Edition, A. L. Page Editor, American Society of Agronomy, Inc. 1982

Miller, J. E., J. J. Hassett, and D. E. Koeppe, 1975, Commun. Soil Sci. Plant Analy. 6:339-347

Appendix B-15 – Lab Procedures for Total Metals: Sequential Extraction for Soil

1.0 PURPOSE

This procedure describes an analytical process for partitioning of soil bound particulate trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn) into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and residual.

2.0 SCOPE

This procedure applies to soil samples from studies of phytoremediation of lead contaminated soils.

3.0 SUMMARY

A two gram sample of soil or sediment is subjected to extraction by five different chemical reagents each progressively more reactive to the sample (magnesium chloride, then sodium acetate, then hydroxylamine hydrochloride in acetic acid, then nitric acid and hydrogen peroxide and finally hydrofluoric and perchloric acids). Complementary measurements are then performed on the individual leachates and on the residual solids following each extraction to evaluate the selectivity of the various metals (Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn) toward specific geochemical phases.

4.0 REFERENCES

4.1 "Sequential Extraction for the Speciation of Particulate Trace Metals", Tessier, A., P.G.C. Campbell and M. Bisson. 1979. Anal. Chem. 51:844-850.

4.2 Method 6010A, "Inductively Coupled Plasma - Atomic Emission Spectroscopy", Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods, SW-846, 3rd Edition, Most Recent Update (July 1992 with proposed methods dated November 1992)

4.3 "Standard Specification for Reagent Water", ASTM D1193-91, 1996 Annual Book of ASTM Standards, Volume 11.01, Water and Environmental Technology, p.116-118

5.0 RESPONSIBILITIES

5.1 The laboratory supervisor, or his designee, shall ensure that this procedure is followed during the sequential extraction for the speciation of particulate trace metals.

5.2 The laboratory group leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure and is responsible for the training of new personnel on this procedure. Data shall be reviewed by the laboratory group leader or his designee.

5.3 The analyst shall follow this procedure and report any abnormal results or nonconformance to the laboratory group leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 All sample containers must be prewashed with detergents, acids and ASTM Type II water. Plastic and glass containers are both suitable.

6.1.2 Samples shall be refrigerated upon receipt and analyzed as soon as possible.

6.1.3 All samples shall be air dried at room temperature to a constant weight and ground to pass through a #10 sieve.

6.2 Limitations and Actions

For this procedure, a batch is defined as a group of no more than 20 samples extracted at the same time with the same set of reagents.

6.3 Requirements

.6.3.1 Apparatus/Equipment

6.3.1.1 Analytical balance: capable of weighing to 0.1 mg

6.3.1.2 Centrifuge: capable of centrifuging at 10,000 rpm

6.3.1.3 Centrifuge tubes: polypropylene, 50 ml

6.3.1.4 pH meter with appropriate electrode

6.3.1.5 Platinum crucibles

6.3.1.6 Magnetic stirrer and stirring bars

6.3.1.7 Laboratory oven

6.3.1.8 Normal laboratory glassware

6.3.2 Reagents and Standards

6.3.2.1 ASTM Type II water (ASTM D1193): Water shall be monitored for impurities by conductivity (conductivity of less than 1.0 µmho/cm at 25°C).

6.3.2.2 Magnesium chloride: reagent grade

6.3.2.3 Magnesium chloride, 1M: weigh 95.23 g of reagent grade magnesium chloride into a 1 liter volumetric flask and dilute to volume with ASTM Type II water

6.3.2.4 Glacial acetic acid: reagent grade

6.3.2.5 Sodium acetate: reagent grade

6.3.2.6 Sodium acetate, 1M: weigh 82.04 g of reagent grade sodium acetate into a 1 liter volumetric flask and dilute to volume with ASTM Type II water

6.3.2.7 Carbonate extracting solution: 1 M sodium acetate adjusted to pH 5.0 with glacial acetic acid

6.3.2.8 Hydroxylamine hydrochloride: reagent grade

6.3.2.9 Hydroxylamine hydrochloride, 0.04 M in 24% acetic acid: Weigh 2.780 g of hydroxylamine hydrochloride into a 1 liter flask and dissolve in 500 ml ASTM Type II water. Add 250 ml glacial acetic acid and make to volume with ASTM Type II water.

6.3.2.10 Nitric acid: concentrated, reagent grade

6.3.2.11 Nitric acid, 0.02 M: add 1.27 ml of concentrated, reagent grade nitric acid to 500 ml of ASTM Type II water in a 1 liter flask, swirl to mix and make to volume with ASTM Type II water

6.3.2.12 Hydrogen peroxide, 30%: reagent grade

6.3.2.13 Hydrogen peroxide, 30% adjusted to pH 2: Add concentrated reagent grade nitric acid to 30% reagent grade hydrogen peroxide until the pH drops to 2.0

6.3.2.14 Ammonium acetate: reagent grade

6.3.2.15 Ammonium acetate, 3.2 M in 20% nitric acid: Add 246.66 g of reagent grade ammonium acetate to 500 ml ASTM Type II water in a 1 liter volumetric flask and swirl to dissolve. Add 200 ml concentrated reagent grade nitric acid, swirl and make to volume with ASTM Type II water.

6.3.2.15 Hydrofluoric acid: reagent grade

6.3.2.16 Perchloric acid: concentrated, reagent grade

6.3.2.17 Hydrochloric acid: concentrated, reagent grade

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 Weigh a 2 gram sample of dried (room temperature) soil or sediment into a 50 ml polypropylene centrifuge tube.

7.1.2 Add 16 ml of magnesium chloride solution and stir on a magnetic stirrer for 1 hour.

7.1.3 Centrifuge at 10,000 rpm for 30 minutes.

7.1.4 Remove supernatant with a pipette and submit this solution for analysis of trace metals by ICP. This is the exchangeable fraction.

7.1.5 Add 16 ml of ASTM Type II water to the centrifuge tube, suspend the solids by stirring and centrifuge at 10,000 rpm for 30 minutes.

7.1.6 Remove this wash solution with a pipette and discard it.

7.1.7 Add 16 ml of 1 M sodium acetate adjusted to pH 5.0 with acetic acid.

7.1.8 Stir continuously for 5 hours.

7.1.9 Centrifuge at 10,000 rpm for 30 minutes.

7.1.10 Remove the supernatant with a pipette and submit this solution for analysis of trace metals by ICP . This is the fraction bound to carbonates.

7.1.11 Add 16 ml of ASTM Type II water and suspend the solids by stirring.

7.1.12 Centrifuge at 10,000 rpm for 30 minutes.

7.1.13 Remove this wash solution with a pipette and discard it.

7.1.14 Add 40 ml of 0.04 M hydroxylamine hydrochloride in 25% acetic acid and stir to suspend solids.

7.1.15 Place in a laboratory oven set at 96°C and heat with occasional agitation for 6 hours.

7.1.16 Cool and centrifuge at 10,000 rpm for 30 minutes.

7.1.17 Remove the supernatant with a pipette and submit this sample for analysis of trace metals by ICP. This fraction is defined as the fraction bound to Fe-Mn oxides.

7.1.18 Add 16 ml of ASTM Type II water and stir to suspend solids.

7.1.19 Centrifuge at 10,000 rpm for 30 minutes.

7.1.20 Remove the wash solution with a pipette and discard it.

7.1.21 Add 6 ml of 0.02 M HNO_3 and 10 ml of H_2O_2 adjusted to pH 2 with HNO_3 and heat in a laboratory oven at 85^0C for 2 hours with occasional agitation.

7.1.22 Add a second aliquot of 10 ml of 30% H_2O_2 (pH 2 with HNO_3) and heat an additional 3 hours in a laboratory oven at 85^0C with intermittent agitation.

7.1.23 Cool and add 10 ml of 3.2 M ammonium acetate in 20% HNO_3 and dilute to 40 ml.

7.1.24 Stir continuously for 30 minutes.

7.1.25 Centrifuge at 10.000 rpm for 30 minutes.

7.1.26 Remove the supernatant with a pipette and submit for analysis of trace metals by ICP. This is the fraction bound to organic matter.

7.1.27 Add 16 ml of ASTM Type II water and stir to suspend solids.

7.1.28 Remove wash solution with a pipette and discard it.

7.1.29 Transfer the residue to a platinum crucible.

NOTE: The steps 7.1.30, through 7.1.33 must be performed in a perchloric acid hood.

7.1.30 Add 1 ml $HClO_4$ and 15 ml HF and evaporate to near dryness without boiling.

7.1.31 Add a second aliquot of 1 ml $HClO_4$ and 15 ml HF and again evaporate to near dryness without boiling.

7.1.32 Add 1 ml $HClO_4$ and heat until the appearance of white fumes.

7.1.33 Cool and add 7 ml ASTM Type II water and 4 ml concentrated reagent grade HCl.

7.1.34 Warm to dissolve solids, transfer to a 50 ml volumetric flask and make to volume with ASTM Type II water.

7.1.35 Submit this solution for analysis of trace metals by ICP. This is the residual fraction.

7.2 Quality Control Sample Requirements

7.2.1 One duplicate sample will be analyzed for every batch.

7.2.2 One method blank will be analyzed for every batch.

7.2.3 A matrix spike will be analyzed for each batch for each of the five sequential extractions. To 10 ml of each extract solution, 1 ml of a 100 mg/L standard will be added. (The spike concentration will then be 9.09 mg/L.)

Note: Smaller quantities may be used in the same ratio if sample size does not permit using 10 ml.

8.0 SAFETY

8.1 Concentrated perchloric acid can react explosively with organic material such as paper or plant tissue. Caution is advised. Work with perchloric acid in a perchloric acid hood which has been specifically designed for operations with that chemical.

8.1 General laboratory safety rules shall be observed.

9.0 NOTES

None

10.0 ATTACHMENTS AND APPENDICES

None

END OF PROCEDURE

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Sequential Extraction Procedure for the Speciation of Particulate Trace Metals

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An analytical procedure involving sequential chemical extractions has been developed for the partitioning of particulate trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe, and Mn) into five fractions: exchangeable, bound to carbonates, bound to Fe–Mn oxides, bound to organic matter, and residual. Experimental results obtained on replicate samples of fluvial bottom sediments demonstrate that the relative standard deviation of the sequential extraction procedure is generally better than ±10%. The accuracy, evaluated by comparing total trace metal concentrations with the sum of the five individual fractions, proved to be satisfactory. Complementary measurements were performed on the individual leachates, and on the residual sediments following each extraction, to evaluate the selectivity of the various reagents toward specific geochemical phases. An application of the proposed method to river sediments is described, and the resulting trace metal speciation is discussed.

Most studies dealing with particulate metals in natural water systems (i.e., metals associated with suspended matter or bottom sediments) concern total metal concentration. Relatively few attempts have been made to evaluate the speciation of particulate metals, i.e., the partitioning among the various forms in which they might exist. Use of total concentration as a criterion to assess the potential effects of sediment contamination implies that all forms of a given metal have an equal impact on the environment; such an assumption is clearly untenable.

Conceptually, the solid material can be partitioned into specific fractions which can be extracted selectively by using appropriate reagents; considering the similarities between sediments and soils, extraction procedures can be borrowed or adapted from the methods of soil chemical analysis (*1*). Several experimental procedures, varying in manipulative complexity, have recently been proposed for determining the speciation of particulate trace metals. These procedures can be grouped into (i) methods designed to effect the separation between residual and nonresidual metals only (*2–5*), and (ii) more elaborate methods making use of sequential extractions (*6–14*). The former methods normally involve a single extraction and offer a better contrast between anomalous and background samples than does the determination of the total metal concentration. Despite their rapidity and relative simplicity, these techniques suffer from the difficulty of finding a single reagent effective in dissolving quantitatively the nonresidual forms of metal without attacking the detrital forms. The use of sequential extractions, although more time consuming, furnishes detailed information about the origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport of trace metals.

The purpose of this study was to develop and examine the merits of a method of sequential "selective" extractions for partitioning particulate trace metals into chemical forms likely to be released in solution under various environmental conditions. The trace metals Cd, Co, Cu, Ni, Pb, Zn, Fe, and Mn were studied. For convenience, the experiments were performed with bottom sediments, which are more easily obtained in workable quantities than is suspended matter. Although iron and manganese are not generally referred to as trace metals in the literature, they have been so designated in the following sections in order to simplify the presentation of the results.

SELECTION OF FRACTIONS

In defining the desired partitioning of trace metals, care was taken to choose fractions likely to be affected by various environmental conditions; the following five fractions were selected.

Fraction 1. Exchangeable. Numerous studies (*15–*[illegible] performed on sediments or on their major constituents (clays, hydrated oxides of iron and manganese, humic acids) have demonstrated the adsorption of trace metals; changes in water ionic composition (e.g., in estuarine waters) are likely to affect sorption–desorption processes.

Fraction 2. Bound to Carbonates. Several workers [illegible] *11*, *24*, *25*) have shown that significant trace metal concentrations can be associated with sediment carbonates; this fraction would be susceptible to changes of pH.

Fraction 3. Bound to Iron and Manganese Oxides. It is well established (*26*) that iron and manganese oxides exist as nodules, concretions, cement between particles, or simply as a coating on particles; these oxides are excellent scavengers for trace metals and are thermodynamically unstable under anoxic conditions (i.e., low Eh).

Fraction 4. Bound to Organic Matter. Trace metals may be bound to various forms of organic matter: living organisms, detritus, coatings on mineral particles, etc. The complexation and peptization properties of natural organic matter (notably humic and fulvic acids) are well recognized, as is the phenomenon of bioaccumulation in certain living organisms. Under oxidizing conditions in natural waters, organic matter can be degraded, leading to a release of soluble trace metals.

Fraction 5. Residual. Once the first four fractions have been removed, the residual solid should contain mainly primary and secondary minerals, which may hold trace metals within their crystal structure. These metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature.

EXPERIMENTAL

Sampling. Sediment samples were collected at two sampling stations, Saint-Marcel and Pierreville, located in the lower reaches of the Yamaska and Saint-Francois rivers (Québec, Canada), respectively. An Ekman dredge was used to collect a large amount of sediment; without emptying the dredge, a sample was taken from the center with a polyethylene spoon to avoid contamination by the metallic parts of the dredge. The samples were stored at 4 °C for ≤3 days, then dried at 105 °C in a forced air oven. They were subsequently ground in an agate mortar, homogenized and stored at 4 °C until needed.

Leaching Procedures and Reagents. After a [illegible] evaluation of the available literature, the following chemical extraction methods were retained for further study; the quantities indicated below refer to 1-g sediment samples (dry weight of original sample used for the initial extraction).

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(i) *Exchangeable*. The sediment was extracted at room temperature for 1 h with 8 mL of either magnesium chloride solution (1 M $MgCl_2$, pH 7.0) or sodium acetate solution (1 M NaOAc, pH 8.2) with continuous agitation.

(ii) *Bound to Carbonates*. The residue from (i) was leached at room temperature with 8 mL of 1 M NaOAc adjusted to pH 5.0 with acetic acid (HOAc). Continuous agitation was maintained and the time necessary for complete extraction was evaluated.

(iii) *Bound to Fe-Mn Oxides*. The residue from (ii) was extracted with 20 mL of either 0.3 M $Na_2S_2O_4$ + 0.175 M Na-citrate + 0.025 M H-citrate, as prescribed by Anderson and Jenne (27), or 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc. The latter experiments were performed at 96 ± 3 °C with occasional agitation and the time needed for complete dissolution of the free iron oxides was evaluated.

(iv) *Bound to Organic Matter*. A method described by Gupta and Chen (9) was adopted. To the residue from (iii) were added 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3, and the mixture was heated to 85 ± 2 °C for 2 h with occasional agitation. A second 3-mL aliquot of 30% H_2O_2 (pH 2 with HNO_3) was then added and the sample was heated again to 85 ± 2 °C for 3 h with intermittent agitation. After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added and the sample was diluted to 20 mL and agitated continuously for 30 min. The addition of NH_4OAc is designed (9) to prevent adsorption of extracted metals onto the oxidized sediment.

(v) *Residual*. The residue from (iv) was digested with a $HF-HClO_4$ mixture according to the procedure described below for total metal analysis.

The "selective" extractions were conducted in centrifuge tubes (polypropylene, 50 mL) to minimize losses of solid material. Between each successive extraction, separation was effected by centrifuging (Sorvall, Model RC2-B) at 10000 rpm (12000 *g*) for 30 min. The supernatant was removed with a pipet and analyzed for trace metals (see below), whereas the residue was washed with 8 mL of deionized water; after centrifugation for 30 min, this second supernatant was discarded. The volume of rinse water used was kept to a minimum to avoid excessive solubilization of solid material, particularly organic matter.

Deionized water used in preparing stock solutions and in each step of the leaching procedure was obtained from a Millipore Milli-Q3RO/Milli-Q2 system. All glassware used for the experiments was previously soaked in 14% HNO_3 (v/v) and rinsed with deionized water. All reagents used in this study were of analytical grade or better and were checked for possible trace metal contamination; of all the reagents used, only sodium dithionite showed serious contamination (Zn).

Trace Metal Analysis. Trace metal concentrations were determined by atomic absorption spectrophotometry (Varian Techtron Model AA-5) involving direct aspiration of the aqueous solution into an air-acetylene flame. The following techniques were used for the first four fractions. For the trace metals Cd, Co, Cu, Ni, Pb, and Zn, a standard addition technique was employed because matrix effects, presumably due to material leached from the sediments, were observed; these effects would have contributed in many cases to an error of as much as 15%. For the metals present in high concentrations (Fe and Mn) the supernatant solution was diluted (20 to 50 ×) with deionized water and the concentrations were obtained directly from appropriate calibration curves prepared with the components of the extraction solution diluted by the same factor.

For total or residual trace metal analysis, the solid was digested with a 5:1 mixture of hydrofluoric and perchloric acids. For a 1-g (dry weight) sample, the sediment was first digested in a platinum crucible with a solution of concentrated $HClO_4$ (2 mL) and HF (10 mL) to near dryness; subsequently a second addition of $HClO_4$ (1 mL) and HF (10 mL) was made and again the mixture was evaporated to near dryness. Finally, $HClO_4$ (1 mL) alone was added and the sample was evaporated until the appearance of white fumes. The residue was dissolved in 12 N HCl and diluted to 25 mL. The resulting solution was then analyzed by flame atomic absorption spectrophotometry for trace metals using the standard addition technique.

Complementary Measurements. To assess the selectivity of each of the extraction steps, complementary measurements were performed on both the leachates (concentrations of Si, Al, Ca, S, inorganic and organic carbon) and the sediments before and after the various chemical treatments (X-ray diffraction; concentrations of S, inorganic and organic carbon).

Silica, aluminum, and calcium concentrations in the leachates were determined by atomic absorption spectrophotometry using a nitrous oxide-acetylene flame. For Al and Si, the leachate 5 was obtained by fusion with lithium metaborate followed by dissolution of the fused material in 1.2 N HCl. Sulfide concentrations were measured with a selective ion electrode (Orion Model 96-16A) using the sulfide antioxidant buffer solution (SAOB), as prescribed by the manufacturer. The SAOB solution was added to the supernatant immediately after centrifugation and separation from the sediments. Dissolved inorganic and organic carbon concentrations were determined with a Beckman Total Organic Carbon Analyzer (Model 915A).

Particulate sulfur and carbon were determined with a Leco Total Sulfur Analyzer (Model 1R-32) and a Leco Total Carbon Analyzer (Model 1R-12), respectively. Inorganic carbon was assumed to be the difference between total and organic carbon concentrations, the latter being measured after acid treatment (HCl) of the sediment to dissolve the carbonate fraction. X-ray diffraction analyses were performed on the whole sediment as well as on the 2-16 μm and <2-μm fractions; separation of the fractions was effected by differential centrifugation, according to Stoke's law.

RESULTS AND DISCUSSION

Choice of Reagents and Leaching Conditions. In the choice of reagents for the sequential extractions, particular emphasis was placed on the potential selectivity of each leaching solution. Procedures currently used in soil chemical analysis were considered, together with those developed in recent studies of trace metal partitioning in sediments and suspensions.

Many reagents, including ammonium acetate (*1*, *9*, *25*, *28*-*30*), sodium acetate (*28*), and magnesium chloride (*8*, *13*), have been employed to liberate *exchangeable* metals. Of these, NH_4OAc (pH 7.0) has been the most extensively used in soil and sediment analysis. However, according to many workers (*1*, *28*, *30*), it may also attack carbonates. According to Chapman (*28*), the solubility of $CaCO_3$ is much lower in 1 M sodium acetate at pH 8.2, a reagent currently used in measuring the cation-exchange capacity of soils, than in the neutral ammonium acetate. For this reason, NH_4OAc was not considered in the present study; the merits of 1 M NaOAc (pH 8.2) and $MgCl_2$ (pH 7.0), as suggested by Gibbs (*8*, *13*), were examined and are discussed below.

Reagents recommended for dissolving selectively the *carbonate* fraction in sediments generally make use of sodium acetate and/or acetic acid at acidic pH values (*6*, *9*, *24*, *31*, *32*). The procedure selected involves 1 M NaOAc and adjustment of the pH to 5.0 with HOAc. Grossman and Millet (*33*) reported that organic carbon and free iron concentrations in noncalcareous soil samples were unchanged after contact with this buffer for nine weeks; other workers (*6*, *9*, *32*) have demonstrated that lower pH values lead to a partial attack of Fe and Mn oxides. The time required for carbonate dissolution will depend upon such factors as particle size, percentage and type of carbonate present, and sample size (*31*). To evaluate the optimum time for leaching the carbonate fraction, sediment samples were first extracted during 1 h for exchangeable metals with either 1 M NaOAc (pH 8.2) or 1 M $MgCl_2$ (pH 7.0), and then further leached with 1 M NaOAc (pH 5.0, adjusted with HOAc); the calcium concentration was measured in centrifuged supernatants after different time intervals. Figure 1 indicates that for both sediments dissolution of calcium was complete within 5 h. This extraction time is likely to be sufficient for most samples of finely divided suspended solids. However, for coarse bottom sediments with high carbonate content, longer leaching times and frequent pH adjustment might be necessary.

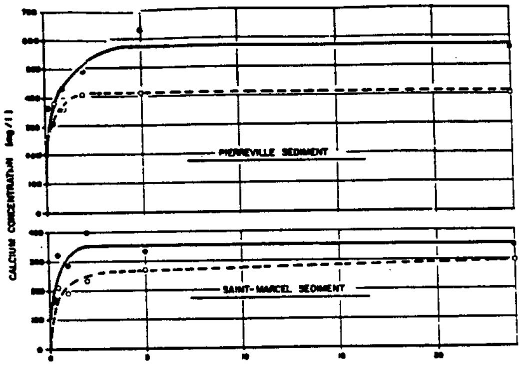

Figure 1. Effect of leaching time on calcium concentrations in the NaOAc–HOAc (pH 5.0) extracting solution for sediments previously leached for exchangeable metals with 1 M $MgCl_2$ at pH 7.0 (●—●) or with 1 M NaOAc at pH 8.2 (O···O)

Figure 1 shows that for both sediments a lesser amount of calcium is found in fraction 2 when NaOAc rather than $MgCl_2$ is used in the preliminary extraction to leach exchangeable metals. This effect can probably be ascribed to the complexing ability of acetate, i.e., $Ca^{2+} + OAc^- = CaOAc^+$; log $K = 1.24$ (*34*); thermodynamic calculations, taking into account the acetate complex formation, predict even more pronounced differences than those observed in Figure 1. For this reason, and also because it has recently been found (*35*) to be an effective reagent for desorbing specifically adsorbed trace metals, $MgCl_2$ was finally selected as the initial reagent in the sequential extraction procedure.

The most successful methods for leaching *iron and manganese oxides* involve the combined action of reagents reducing these metals to their ferrous and manganous forms, respectively, and of agents capable of keeping in solution the relatively large amounts of metals liberated; the two couples most often used are hydroxylamine hydrochloride–acetic acid (*2, 4, 7, 9, 24, 36*) and sodium dithionite–citrate (*8, 9, 13, 31, 37, 38*). With the latter combination, using concentrations prescribed by Anderson and Jenne (*27*), we observed substantial precipitation of trace metals, presumably due to the formation of sulfide as result of the disproportionation of dithionite. Examination of the results of Gupta and Chen (*9*), who employed both combinations in parallel experiments, reveals that trace metal concentrations were consistently lower in the leachates obtained with dithionite–citrate than in those obtained with hydroxylamine hydrochloride–acetic acid; the differences were greatest for those trace metals which form the most insoluble sulfide salts. Furthermore, several additional problems are raised by the utilization of the dithionite–citrate couple: (i) dithionite is highly contaminated with zinc and its purification by a chelation-extraction procedure proves difficult (*12*); (ii) frequent clogging of the burner is observed upon analysis of the aqueous extracts by flame atomic absorption spectrophotometry, due to the high salt content of the extraction solution; to minimize this problem, a chelation–extraction step is required before analysis (*38*). Consideration of these problems led us to eliminate the dithionite–citrate couple from further study.

To evaluate the optimum time for reducing and dissolving the Fe and Mn oxides, sediment samples, previously extracted for exchangeable metals ($MgCl_2$) and carbonates (NaOAc + HOAc, pH 5.0), were leached with the $NH_2OH{\cdot}HCl$ + HOAc solution, and the iron concentration was measured in the centrifuged supernatants after different time intervals. The results (Figure 2) indicate that for both sediments extraction of reducible iron was essentially complete after 6 h. In subsequent experiments, a leaching time of 6 h was adopted, as longer times would have increased the possibility of attacking fractions 4 and 5.

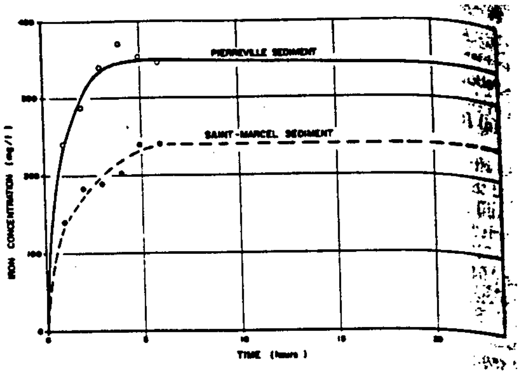

Figure 2. Effect of leaching time on iron concentrations in the 0.04 M $NH_2OH{\cdot}HCl$–25% (v/v) HOAc extracting solution for sediments previously leached for exchangeable metals with 1 M $MgCl_2$ and for the carbonate fraction with NaOAc–HOAc (pH 5.0)

Hydrogen peroxide in an acidic medium is generally used to oxidize *organic matter* in soil (*1, 31*) and sediment (*6, 7, 9, 12, 14*) analysis, even though oxidation of all forms of organic matter may not be complete (*1*). More efficient methods for destroying organic matter do exist (e.g., concentrated nitric acid used separately or in combination with hydrochloric or perchloric acids), but they usually suffer from a lack of specificity in the sense that they may also effect a partial attack of silicate lattices. In the present study, a method involving treatment with hot hydrogen peroxide in a nitric acid medium was adopted (*9*).

Procedures for dissolving primary and secondary minerals (*residual fraction*) usually involve either alkaline fusion (*1, 6, 8, 13, 39*) or dissolution with mixtures of hydrofluoric acid (*1, 7, 9, 11, 12, 25, 32*) and some other strong acid (e.g., nitric or perchloric acid). To ensure complete decomposition of the silicates, a large excess of fusing salt must be used, leading to high salt concentrations in the solution to be analyzed for trace metals; this can cause instability and high background readings in atomic absorption spectrophotometry. For these reasons, we selected the HF–$HClO_4$ digestion procedure described previously.

The overall procedure finally adopted thus involves five successive extractions with: (i) $MgCl_2$ (pH 7.0); (ii) NaOAc/HOAc (pH 5.0); (iii) $NH_2OH{\cdot}HCl$ in 25% HOAc (pH ~2); (iv) H_2O_2/HNO_3 (pH ~2) and subsequently NH_4OAc; (v) HF and $HClO_4$.

Precision and Accuracy. The analytical precision for each of the extraction steps, and for the overall procedure, was tested by subjecting six sub-samples of each of the Saint-Marcel and Pierreville sediments to the sequential procedure described above; the results obtained, together with the detection limit (expressed in micrograms metal per gram of dry sediment), are shown in Table I. Note that the detection limit, defined as twice the base-line noise, could be lowered by using higher sediment/solution ratios. As anticipated, the precision is generally low when the concentration approaches the detection limit but improves for higher concentrations; coefficients of variation of 10% or lower are typically observed for metal concentrations amounting to five times or more the detection limit. Precision, while acceptable in most cases for the whole sediment, is expected to improve when the sequential procedure is applied to suspensions or to the fine fraction of bottom sediments (e.g., <20 μm).

Low reproducibility of the results is observed for lead in the Pierreville sediment. Leaching and analytical procedures are probably less responsible for this result than is

Table I. Detection Limit, Precision, and Accuracy of the Sequential Extraction Procedure as Determined on Two Sediment Samples[a]

	fraction[c]	detection limit	mean and standard deviation[b] sediment No. 1 (Saint-Marcel)	 sediment No. 2 (Pierreville)
Cd	1	0.1	<0.1	<0.1
	2	0.3	<0.3	<0.3
	3	0.1	<0.1	0.15 ± 0.05
	4	0.2	<0.2	<0.2
	5	0.1	<0.1	<0.1
	Σ			
	M_T	0.1	<0.1	<0.1
Co	1	0.5	<0.5	<0.5
	2	2.0	<2.0	<2.0
	3	0.5	3.6 ± 0.5	6.4 ± 0.7
	4	1.4	<1.4	<1.4
	5	1	5.4 ± 0.4	9.2 ± 0.8
	Σ		12.9 > $\overline{X}$ > 9.4	19.6 > $\overline{X}$ > 16.3
	M_T	1	13.2 ± 1.9	19.5 ± 1.8
Cu	1	0.1	0.15 ± 0.05	0.2 ± 0
	2	0.5	3.2 ± 0.3	8.2 ± 0.5
	3	0.4	4.0 ± 0.3	9.1 ± 0.8
	4	0.5	5.0 ± 0.7	11.3 ± 1.5
	5	1	7.9 ± 0.5	16.4 ± 1.0
	Σ		20.2 ± 1.0	45.2 ± 1.1
	M_T	1	25.0 ± 4.9	48.6 ± 4.0[d]
Ni	1	0.2	<0.2	<0.2
	2	1.1	1.1 ± 0.3	1.9 ± 0.5
	3	0.5	6.9 ± 0.8	13.2 ± 0.7
	4	0.6	1.4 ± 0.5	2.7 ± 0.3
	5	1	17.3 ± 2.6	38.4 ± 3.7
	Σ		26.5 ± 2.4	56.2 ± 4.0
	M_T	1	28.8 ± 1.5	60.2 ± 1.5
Pb	1	0.6	<0.6	<0.6
	2	0.5	2.6 ± 0.4	9.4 ± 5.5[d]
	3	1.0	3.8 ± 0.6	10.3 ± 2.9[d]
	4	1.2	2.7 ± 0.6	7.8 ± 1.7[d]
	5	1.4	7.8 ± 1.2	10.1 ± 2.0[d]
	Σ		16.9 ± 1.4	37.7 ± 11.1[d]
	M_T	1.4	18.2 ± 2.9	42.5 ± 6.6[d]
Zn	1	0.1	<0.1	<0.1
	2	0.5	11.2 ± 0.6	7.9 ± 2.0
	3	0.1	22.2 ± 0.5	33.5 ± 0.6
	4	0.1	3.9 ± 0.2	9.0 ± 1.3
	5	1	49.9 ± 7.8	71.7 ± 4.8
	Σ		87.2 ± 7.2	122.1 ± 5.4
	M_T	1	88 ± 2	127 ± 5
Fe	1	0.4	0.4 ± 0.1	0.4 ± 0.1
	2	0.4	732 ± 33	476 ± 21
	3	50	4630 ± 100	6910 ± 120
	4	80	620 ± 60	1150 ± 30
	5	10	30300 ± 1000	39500 ± 2400
	Σ		36300 ± 1100	48000 ± 2500
	M_T	10	36100 ± 260	50400 ± 360
Mn	1	2.5	99 ± 2	38 ± 0
	2	9	90 ± 21	314 ± 20
	3	10	106 ± 5	265 ± 10
	4	1.2	10.9 ± 0.8	30 ± 1
	5	2	413 ± 21	456 ± 29
	Σ		720 ± 30	1103 ± 37
	M_T[e]	2	768 ± 17	1090 ± 30

[a] Detection limit, mean value, and standard deviation are all expressed in μg/g of sediment, dry weight. [b] Unless otherwise indicated, results for six replicate determinations. [c] Following the sequence described in the text: 1, exchangeable, 2, carbonate . . . , Σ represents the sum of the five fractions and M_T represents the total metal concentration. [d] A value differing from the mean by more than three times the standard deviation was excluded. [e] Results for three replicate determinations.

Successive Leachates

parameter	fraction	leached from sediment, % sediment No. 1 (Saint-Marcel)	leached from sediment, % sediment No. 2 (Pierreville)
Si	exchangeable	0.02	0.01
	bound to carbonates	0.03	0.06
	bound to Fe–Mn oxides	0.45	0.73
	bound to organic matter	0.31	0.47
	residual	99.2	98.7
Al	exchangeable	0.03	0.03
	bound to carbonates	0.06	0.14
	bound to Fe–Mn oxides	1.6	2.2
	bound to organic matter	1.5	2.9
	residual	96.8	94.7
Ca	exchangeable	2.4	4.9
	bound to carbonates	17.9	26.3
	bound to Fe–Mn oxides	3.4	5.4
	bound to organic matter	3.6	4.6
	residual	72.7	58.8
S	exchangeable	<2	<2
	bound to carbonates	<2	<2
inorganic carbon	exchangeable	2.4	2.8
organic carbon	exchangeable	1.3	1.7

heterogeneity of this sediment with respect to Pb, as only one of the two sediments shows a high variability. The high variability reported here for Pb in the Pierreville sediment was also observed during preliminary experiments, despite repeated grinding of the sediment in an effort to improve its homogeneity. Similar problems of low reproducibility for "acid leachable" (pH 1.5) lead in suspended sediments have been reported (*40*) and were attributed to the presence of relatively large (>8 μm) lead-rich particles originating from aerosols produced by the combustion of leaded gasolines; such particles are observed close to highways (*41*). Wagemann et al. (*30*) also reported scattered lead results following leaching of sediments and suspensions with saturated EDTA or 0.1 M HCl solutions.

Comparison of the sum of the metal concentrations in the individual fractions with the total metal concentration $[M]_T$ (Table I) shows good agreement for all trace metals. Note that the variability discussed previously for specific fractions of Pb in the Pierreville sediment is also found in the $[Pb]_T$ determinations for this sediment.

Selectivity. Selectivity of the extraction reagents toward specific geochemical phases is an important performance criterion for a sequential extraction procedure designed to determine the speciation of trace metals. In the following discussion we shall attempt to evaluate the degree to which the desired sediment phases are solubilized, and also the degree to which the remaining phases resist attack. Two types of evidence are considered: (i) the sediment components that are solubilized during each successive extraction and thus are found in the various leachates along with the trace metals, and (ii) the changes in overall sediment composition after treatment with each successive reagent. A knowledge of the principal characteristics of the two sediment samples is a prerequisite to this type of approach. The results of X-ray diffraction on the whole sediments indicate that quartz, plagioclase, and K-feldspar are the major phases whereas chlorite, amphibole, and mica are minor constituents; two other minor phases were identified in the 2–16 μm fraction (dolomite) and in the <2-μm fraction (smectites). Inorganic carbon (0.2–0.4% C), organic carbon (0.5–0.7% C), and sulfide (0.04–0.05% S) all represent small percentages of the total sediment composition.

Fraction 1. Exchangeable Metals. The low levels of Al, S, and organic carbon found in leachate 1 (Table II) im that $MgCl_2$ treatment does not affect silicates, sulfides, organic matter. Thermodynamic calculations indicate Fe and Mn oxides should not be significantly solubilized pH 7.0, and concentrations of Fe in leachate 1 are indeed low. The slight dissolution of carbonates (2–3%) cou reduced by shortening the leaching time.

Fraction 2. Metals Bound to Carbonates. Dissolution the sediment carbonate fraction is essentially complete evidenced by the disappearance of the dolomite X-ray di fraction peak following treatment with the acetate buffer (p 5.0). Further evidence is provided by the kinetics of leaching step (Figure 1); increasing the leaching time from to 24 h does not result in higher calcium concentrations the leachate.

The low levels of Si, Al, and S found in leachate 2 (Table II) indicate that attack of silicate and sulfide minerals by the NaOAc–HOAc reagent is minimal. Furthermore, the organic carbon content of the sediments is undiminished after treatment with the acetate buffer (Table III), suggesting th the organic fraction is also unaffected (cf. *31*). The appreciable iron and manganese concentrations found in leachate probably result from the dissolution of divalent salts. Ferr and manganous carbonate are the logical candidates (42, given the pH values and inorganic carbon concentratio prevalent in natural environments.

Assuming that the trace metals found in fraction 2 origin solely from dissolution of dolomite, the only carbonate ph identified, one can calculate the following concentrations these metals in $CaMg(CO_3)_2$: 190 and 300 ppm Cu, 70 ppm Ni, 150 and 350 ppm Pb, and 660 and 290 ppm where the two values refer to Saint-Marcel and Pierrev sediments respectively. These concentrations are far in ex of those reported for carbonate sedimentary rock (*44*), for sea sediments (*44*), or for calcium carbonate of biogenic or (*45*). This raises the possibility that in the preceding traction the neutral 1 M $MgCl_2$ did not completely desorb specifically adsorbed trace metals, the adsorption–desorp processes being strongly pH dependent; lowering the pH 5.0 in the subsequent extraction would then release th maining specifically adsorbed trace metals as well

ions Found in the
ɔm sediment, %
sediment No. 2
ierreville)
0.01
0.06
0.73
0.47
98.7
0.03
0.14
2.2
2.9
94.7
4.9
26.3
5.4
4.6
58.8
<2
<2
2.8
1.7

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pm Pb, and 660 and 290 ppm Zn,
r to Saint-Marcel and Pierreville
ese concentrations are far in excess
iate sedimentary rock (*44*), for deep
alcium carbonate of biogenic origin
sibility that in the preceding ex-
igCl$_2$ did not completely desorb the
metals, the adsorption–desorption
ɔH dependent; lowering the pH to
raction would then release the re-
orbed trace metals as well as the

Table III. Sulfide and Organic Carbon Concentrations Remaining in Sediments after Successive Leachings

treatment	[sulfide] % S sediment No. 1 (Saint-Marcel)	[sulfide] % S sediment No. 2 (Pierreville)	[organic carbon] % C sediment No. 1 (Saint-Marcel)	[organic carbon] % C sediment No. 2 (Pierreville)
none	0.043	0.040	0.5	0.6
NaOAc–HOAc (pH = 5.0)	*a*	*a*	0.5	0.7
$NH_2OH \cdot HCl$–HOAc (pH ≃ 2)	0.047	0.045	0.5	0.7
H_2O_2–HNO_3 (pH ≃ 2)	0.018	0.017	0.1	0.1

[a] Not measured.

carbonate-bound fraction. Examination of curves of trace metal adsorption as a function of pH (*22*, *35*) suggests that lowering the pH from 7.0 to 5.0 might release in solution a large fraction of specifically adsorbed trace metals from various solid substrates.

Fraction 3. Metals Bound to Fe–Mn Oxides. Increasing the leaching time from 6 to 24 h did not result in higher iron concentrations in leachate 3 (Figure 2), indicating that the extraction of reducible iron oxide phases is complete; as manganese oxides are known to be more easily leached than iron oxides (*46*), it is probable that their extraction is also complete. The low levels of Fe and Mn found in the subsequent leachate (fraction 4, Table I) are an additional indication of the completeness of the extraction.

Thermodynamic calculations suggest that metal sulfides should be solubilized to a large extent at the pH of the extracting solution; however, as shown in Table III, sulfur levels are unchanged in the sediments after extraction with $NH_2OH \cdot HCl$–HOAc. These results imply that the reduced sulfur present in the sediments studied does not exist as amorphous metal sulfides, but rather as well-crystallized phases (e.g., pyrite) or as organosulfur compounds. The organic carbon content of the sediments is also undiminished after treatment with the hydroxylamine hydrochloride–acetic acid reagent, indicating that organic matter is not significantly leached. The possible liberation of some metals from labile organic complexes cannot however be excluded.

The low levels of Si and Al found in leachate 3 indicate that only slight attack of the major silicate components occurs during treatment with $NH_2OH \cdot HCl$–HOAc. A relative decrease of the smectites X-ray diffraction peak is noted, suggesting partial attack of these minerals. Contrary to the results of Chester and Hughes (*24*), however, no evidence of chlorite transformation can be observed from X-ray diffraction analysis.

Fraction 4. Metals Bound to Organic Matter. Oxidation of organic matter by acidified hydrogen peroxide, although extensive, is incomplete; sulfide minerals are also extracted to a large extent, as shown in Table III. According to Jackson (*1*), the remaining organic matter should consist of paraffin-like material and resistant structural (nonhumified) organic matter residues. Stronger oxidizing solutions usually rely upon the use of strong acids, which may seriously attack silicate material. Thus the choice of H_2O_2 represents a compromise between complete oxidation and alteration of silicate material.

The low levels of Si and Al found in leachate 4 suggest that attack of the major silicate phases is indeed minimal. However, a relative decrease in the smectites and chlorite X-ray diffraction peaks is observed, suggesting a partial alteration of these minerals (Figure 3); mica is also subject to partial transformation, as evidenced by the appearance of a small shoulder on the X-ray diffraction peak.

Fraction 5. Residual Metals. The residue remaining after the four preceding extractions consists essentially of detrital silicate minerals, resistant sulfides, and a small quantity of refractory organic material. Treatment with the HF–$HClO_4$ reagent results in complete dissolution.

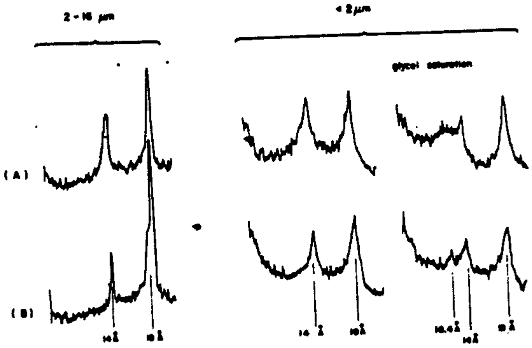

Figure 3. X-ray diffraction spectra for the Saint-Marcel sediment: (A) without any treatment; (B) after the first four extractions. Peaks represent mica (10 Å) chlorite (14 Å) and smectites (16.4 Å)

Speciation. Having considered in some detail the analytical performance of the proposed sequential extraction procedure, we shall now briefly comment on the trace metal partitioning observed in the sediment samples studied (Table I). Each successive fraction is discussed below: where two concentration values are given, the first refers to the Saint-Marcel sediment and the second to the Pierreville sample.

Fraction 1. Exchangeable Metals. Concentrations found for exchangeable cadmium, cobalt, nickel, lead, and zinc are lower than their respective detection limits; low concentrations of copper (0.15 and 0.2 ppm) and iron (0.4 ppm) are observed, as are relatively high concentrations of manganese (99 and 38 ppm). For the latter element, fraction 1 accounts for 14–3% of the total metal concentration. From available evidence on the extraction of exchangeable metals from bottom sediments or suspended solids (*8*, *9*, *12*, *13*, *25*, *30*), these results are to be expected; exchangeable Cd, Co, Cu, Ni, Pb, Zn, and Fe are generally found to represent a minor fraction of the total metal concentration of the solid. The relatively high concentrations of exchangeable manganese suggest that the metal exists in a reduced form; it is recognized (*42*) that in most natural waters Mn(II) oxidation is a much slower process than Fe(II) oxidation. Our results for exchangeable Mn differ significantly from the findings of Gupta and Chen (*9*) who reported undetectable concentrations on coastal marine sediments. Possible explanations for this difference include the high ionic strength of seawater, which should favor desorption of Mn, and the higher pH, which should increase the rate of Mn(II) oxidation (*42*).

Fraction 2. Metals Bound to Carbonates. Concentrations found for cadmium and cobalt in fraction 2 are lower than their respective detection limits; the levels of nickel and iron, though detectable, remain low (<4%) compared to the total metal concentrations. For copper, zinc, and manganese, however, the observed concentrations represent an appreciable proportion of the total metal: 16–18% Cu; 13–16% Zn;

Expressed as Percent of Fe-Mn Oxides[a]

sediment	Cd	Co	Cu	Ni	Pb	Zn
Saint-Marcel	<0.0022	0.040	0.044	0.073	0.042	0.247
Pierreville	0.0011	0.048	0.068	0.098	0.094	0.249

[a] For the calculation, $Fe(OH)_3$ and MnO_2 were taken as representative empirical formulas for the Fe and Mn oxides.

13–28% Mn. These results are essentially similar to those reported by Gupta and Chen (*9*) for marine sediments using a 1 M HOAc extraction.

Fraction 3. Metals Bound to Fe–Mn Oxides. With the exception of cadmium, the trace metal levels in fraction 3 are relatively high and represent a large fraction of the total metal concentrations: ~40% Co; >20% for Cu, Ni, and Pb; 13–14% Fe; 15–24% Mn. Assuming that $Fe(OH)_3$ and MnO_2 are representative empirical formulas for the Fe and Mn oxides, respectively, and that the observed trace metals originate only from Fe–Mn oxides, one can calculate the trace metal concentrations in the Fe–Mn oxide phases (Table IV); the results illustrate the strong scavenging efficiency of Fe–Mn oxides for trace metals (*26*).

Fraction 4. Metals Bound to Organic Matter. Levels of cadmium and cobalt found in fraction 4 are once again lower than their respective detection limits. The remaining metals are present in detectable quantities, but only in the case of copper and lead does fraction 4 represent a significant proportion of the total metal concentration: ~25% Cu; 16–21% Pb. The absolute concentrations follow the order Fe > Mn > Cu > Zn > Pb > Ni for each sediment (Table I).

Fraction 5. Residual Metals. Without exception, trace metal concentrations found in fraction 5 are higher than those observed in any of the preceding extractions. In the case of cobalt, nickel, zinc, iron, and manganese, the residual fraction accounts for more than 50% of the total metal concentration. This overwhelming importance of fraction 5, amply borne out by the results of others (*7–9*, *12*, *13*, *32*), illustrates clearly the difficulty of distinguishing between background and anomalous levels of trace metal contamination when only total metal analyses are performed.

CONCLUSIONS

The precision and accuracy of the sequential extraction procedure are generally good, the limiting factor being the inherent heterogeneity of the sediment; analytical performance should thus improve when the procedure is applied to suspensions or to sized fractions of bottom sediment.

The results obtained for fractions 3 and 4 (Fe–Mn oxides and organic matter) indicate that these phases have a scavenging action for trace metals that is far out of proportion to their own concentration. As these fractions constitute important sources of potentially available trace metals (*47*), they should be considered explicitly when estimating the bio-availability of a particular metal.

For the moment, it is obvious that the distribution of a given metal between various fractions does not necessarily reflect the relative scavenging action of discrete sediment phases, but rather should be considered as operationally defined by the method of extraction. Before metal concentrations measured in a particular fraction can be ascribed with reasonable certainty to well defined geochemical forms or phases, it will be necessary to develop more selective extraction reagents, notably for sulfide bound trace metals. Spiking experiments, involving the addition of known geochemical phases to sediment matrices, would provide valuable information concerning the selectivity of the proposed sequential extraction procedure. Application of the procedure to a wide range of

Sequential extraction procedures do, however, off major advantage that they simulate to a certain extent environmental conditions to which the sediment subjected; deductions can then be made about the trac levels likely to be observed under these conditions environment. Possible applications include the evaluat the effects of dredging operations and the prediction of metal behavior in estuarine waters or in anoxic lacu systems.

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Correlation of Electron Capture Response Enhancements Caused by Oxygen with Chemical Structure for Chlorinated Hydrocarbons

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The enhancement of response to 32 simple chlorinated molecules of a constant-current electron capture detector (ECD) caused by the addition of oxygen to its carrier gas has been determined. The effects of size and isomeric differences for alkyl, vinyl, allyl, and phenyl chlorides are examined. Also, the oxygen-induced enhancements of several chlorofluoromethanes are reported. From these data, general trends of structural differences emerge which facilitate the prediction of where oxygen-doping in ECD analyses might be gainfully applied. These data are discussed in terms of a mechanism which was previously proposed and is further developed here into a detailed model of the instrument's response. Response enhancements are attributed to assistance provided by O_2^- in the overall electron capture process. It appears that relative rates of the reaction of O_2^- with halocarbons can be derived from ECD data obtained in the manner described here.

The electron capture detector (ECD) in its usual configuration responds exceedingly sensitively to polychlorinated molecules, but relatively poorly to hydrocarbons having only one or a few chlorine atoms (*1*). We have recently shown (*2*, *3*), however, that the intentional addition of oxygen to the carrier gas of a gas chromatograph with electron capture detection (GC-ECD) can cause large increases in its response especially to monochlorinated hydrocarbons, thereby increasing the utility of the ECD to this class of compounds. We have shown, for example, that the addition of oxygen to the carrier gas of a constant-current, pulsed ECD leads directly to an improved analysis of methyl chloride in ambient air (*3*). Another potentially valuable use of oxygen doping may be as an aid in the qualitative identification of all ECD-active compounds, since the magnitude of an oxygen-induced response enhancement has appeared to be quite reproducible for a given compound and varies greatly from one compound type to another. A recent report (*4*) of an improved analysis of CO_2 in the atmosphere using an ECD and intentionally O_2-doped carrier gas suggests the applicability of this method to measurement problems beyond those of the halocarbons.

In this article, we report additional measurements of oxygen-induced response enhancements where a systematic variation of the chlorinated substrate molecules from C_1 to C_6 chlorocarbons has been made. The effect of the position of a chlorine atom or atoms on saturated, olefinic, and phenyl hydrocarbon skeletons is examined. The mechanism previously proposed (*2*) to account for response enhancements will be further developed here to provide a quantitative description of the response of a constant-current, oxygen-doped ECD to sample molecules. It will also be suggested that the gas-phase, relative rates of the reactions of O_2^- with ECD-active compounds can be determined from the measurement of oxygen response enhancements with an electron capture detector.

EXPERIMENTAL

Standards. All halocarbons studied were reagent grade obtained from commercial suppliers. Further purification was unnecessary because the method of introduction to the ECD is gas chromatography which provides the necessary separation from impurities. Standards were prepared by the successive dilution of the compounds of interest into either nitrogen gas or into hexane or benzene. Standards of compounds having boiling points less than 100 °C were prepared as gases and those above 100 °C, as liquids. The concentrations of standards were adjusted to produce small, but easily quantified peaks in their analysis by GC-ECD. The procedure for gaseous standard preparation has been described in detail previously (*3*).

Instruments. The gas chromatograph is a Varian 3700 Aerograph with constant-current, pulse-modulated operation of a ^{63}Ni detector. The detector activity is 7.5 mCi and its volume is 0.3 mL. A 10-ft by $^1/_8$-in. stainless steel column packed with 10% SF-96 on chromosorb W was generally used at oven temperatures of from 30 to 60 °C depending on the compound studied. For a few of the least volatile compounds examined, a 1.5-ft by $^1/_8$-in. column packed with 3% OV-17 was used at temperatures up to 110 °C. Where peak areas were desired for the determination of relative molar ECD responses, an Autolab minigrator was used. All chromatograms were recorded on a strip chart recorder.

The normal carrier gas flow was modified as previously described (*3*) to allow the controlled addition of oxygen to the carrier gas. This was accomplished by incorporating a 5-L stainless exponential dilution sphere in the carrier gas stream into which aliquots of purified air were added. The carrier gas was ultra high purity nitrogen (Matheson) specified to be greater than 99.999% pure and the air was breathing quality (Chemetron), both of which were passed through traps containing activated charcoal and 13X

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Appendix B-16 – Lab Procedures for Total Metals: Sequential Extraction for Plants

1.0 PURPOSE

The purpose of this procedure is to sequentially extract plant tissue in order to identify the tissue fraction containing lead and other heavy metals

2.0 SCOPE

This procedure applies to vegetation including leafy matter, stems, and roots. The extracts and solids resulting from this procedure are then scheduled for analysis of metals and chelate compounds.

3.0 SUMMARY

Fresh plant material is extracted with water, hot ethanol, and 0.2 M HCl. The 0.2 M HCl extract is mixed with acetone and a solid phase is separated. Extracts, precipitates, and the plant tissue residue from the process are scheduled for analysis of metals and chelate compounds.

4.0 REFERENCES

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5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the laboratory group leader to review the results of the procedure.

5.3 It is the responsibility of the laboratory analyst to follow this procedure and to report any abnormal results or unusual occurrences to the laboratory group leader.

6.0 REQUIREMENTS

6.1 Prerequisites

None

6.2 Limitations and Actions

None

6.3 Requirements

6.3.1 Apparatus/Equipment

6.3.1.1 Centrifuge capable of 1000 rpm.

6.3.1.2 Homogenizer - a laboratory grade tissue homogenizer.

6.3.2 Reagents and Standards

6.3.2.1 95% Ethanol - Reagent Grade

6.3.2.2 0.2 M HCl - Mix 16.5 ml reagent grade hydrochloric acid with 800 ml deionized water. Dilute to one liter. (Larger or smaller quantities may be mixed in the same proportion.)

6.3.2.3 Acetone - Reagent Grade

6.3.2.4 50% Acetone - a mixture of equal volumes of reagent grade acetone and deionized water.

7.0 PROCEDURE

7.1 Water Extraction

7.1.1 Macerate 2 to 3 g of fresh plant tissue with 10-15 ml deionized water in a homogenizer for 5 min

7.1.2 Centrifuge resulting slurry

7.1.3 Wash residue twice with 10-15 ml DEIONIZED WATER, centrifuging after each wash

7.1.4 Analyze supernatant for total metals (Pb, Cd, Cu, Ni, Zn, Cr, Se, Hg) and chelate; remaining plant residue will be used in step 2

7.2 Ethanol Extraction

7.2.1 Immerse plant residue from step 1 in 15 ml boiling 95% Ethanol for several seconds

7.2.2 Centrifuge and decant the Ethanol

7.2.3 Wash residue twice with 10-15 ml Ethanol, with each wash followed by centrifugation

7.2.4 Combine all Ethanol supernatants

7.2.5 Analyze supernatant for total metals (Pb, Cd, Cu, Ni, Zn, Cr, Se, Hg) and chelate; remaining plant residue will be used in step 3.

7.3 Extraction with 0.2 M HCl and Precipitation With Acetone

7.3.1 Extract residue from step 2 three times with 5-7 ml of 0.2 M HCl

7.3.2 Centrifuge and decant the liquid

7.3.3 Combine the supernatants from the HCl extractions. Set the plant residue aside.

7.2.4 Add an equal volume of acetone to the HCl extraction supernatant; a precipitate will form

7.2.5 Centrifuge to spin down the precipitate and decant the liquid

7.2.6 Wash the precipitate twice with 50% acetone; add the supernatant from these washes back to the HCl extraction supernatant

7.2.7 Schedule the HCl extraction supernatant, the precipitate, and the remaining plant residue for total metals (Pb, Cd, Cu, Ni, Zn, Cr, Se, Hg)

8.0 SAFETY

8.1 Wear gloves, safety glasses, and a lab coat when mixing solutions and performing this procedure.

8.2 Wear goggles when diluting concentrated acid in water.

9.0 NOTES

None

10.0 ATTACHMENTS AND APPENDICES

10.1 "Chemicals Aspects of Metal Hyperaccumulation" - Two pages

10.2 "The Distribution of Some Inorganic Elements in Plant Tissue Extracts." - Six pages

End of Procedure

Chemical Aspects of Metal Hyperaccumulation

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Over the last 20 years extensive work has documented the occurrence of hyperaccumulators of several metals (particularly Ni), and has shown how they are distributed, both geographically and throughout the plant kingdom. Some effort has also been devoted to answering questions about the way in which the metals are sequestered in accumulator plants.

Approaches have mainly relied on the extraction and isolation of metal-rich fractions from fresh or freeze-dried leaf material, although some work has also been done with metal-rich exudates such as those from the laticifers of accumulators from families such as the Sapotaceae and Euphorbiaceae. In the case of Ni accumulators, the metal-rich fractions have been studied by ion exchange and electrophoresis, and in several instances the organic ligands associated with the metal have been identified by GC-MS or HPLC. Several noteworthy points have emerged from this work.

1. Although 1000 µg/g has been taken as the threshold in defining Ni hyperaccumulation (see Reeves (1992) for the most recent published list of species), there are now more than 100 species in which Ni concentrations of over 10,000 µg/g have been recorded, and values of 30-40,000 µg/g (3-4% of the dry mass) are sometimes found. (See Table 1 for examples.) Such levels could not be attained if the major part of the Ni were present in a molecule of high molar mass.

Table 1. Extreme examples of nickel hyperaccumulation (leaf tissue, dry matter basis).

Family	Species	Origin	Ni conc. (%)
Brassicaceae	*Alyssum argenteum*	Italy	2.94%
Brassicaceae	*Thlaspi alpinum* ssp. *sylvium*	C. Europe	3.10%
Cunoniaceae	*Geissois pruinosa*	New Caledonia	3.40%
Euphorbiaceae	*Phyllanthus serpentinus*	New Caledonia	3.81%
Stackhousiaceae	*Stackhousia tryonii*	Australia	4.13%

2. The above conclusion is borne out by MW studies using gel filtration, and by sequential extraction experiments along the lines suggested by Bowen et al. (1962). Extraction of leaf material with 95% ethanol removes only a small proportion of the Ni, denying the presence of small to medium-sized neutral molecules such as Ni analogs of chlorophyll. The major part of the Ni is water-soluble (Table 2) and most of the remainder can be brought into solution with dilute HCl. Water-soluble Ni may increase to 80-92% if fresh, rather than freeze-dried, material is used.

3. Purification of the major Ni-containing fractions, e.g. by gel filtration, has led to solutions in which there is negligible N content (ruling out significant complexing with aminoacids or phytochelatins), but containing large amounts of citrate and/or malate, and smaller amounts of other organic acids (Pelosi et al. 1976; Lee et al. 1977, 1978; Vergnano Gambi & Gabbrielli 1987; Homer et al. 1991). Traces of other cations (Ca^{2+}, Mg^{2+}, K^{+}) are generally still present in these fractions.

4. Ion exchange and electrophoresis have shown the presence of Ni in anionic, neutral and cationic forms in varying proportions, depending on the plant and on the conditions used.

5. The stability constants for binding of Ni^{2+} to citrate and malate are not particularly large. Thus, both in the course of plant metabolism, and during extraction and isolation, it is possible that the Ni complexes will undergo partial decomposition and re-formation. This implies a danger of artifactual observations resulting from separation processes (including experiments as in (4) above).

Table 2. Sequential extraction of nickel from leaves of hyperaccumulators

	% of total Ni in various fractions							
Species	a	b	c	d	e	f	g	h
Psychotria douarrei	0.4	65.0	27.6	0.4	4.2	<0.1	1.8	0.6
Walsura monophylla	1.6	67.8	12.8	3.4	6.2	0.3	6.9	1.0
Dichapetalum gelonioides ssp. *tuberculatum*	1.1	77.0	14.6	1.6	4.0	<0.1	1.5	0.2

a 95% ethanol: lipids, pigments, small neutral molecules
b H_2O: ionic and polar low M.W. compounds
c 0.2 M HCl/acetone: supernatant: acid-soluble polar compounds
d precipitate: proteins, pectates
e residue from c with 0.5 M $HClO_4$/acetone : supernatant: degraded cellulose, lignin, remaining polar compounds
f precipitate: nucleic acids
g residue from e with 2M NaOH: degraded polysaccharides
h final residue: cellulose, lignin, insol. cell wall materials

6. Arguments have been presented (Still & Williams 1980) for the involvement of N-containing ligands in the processes responsible for the selectivity for Ni over other elements such as Fe, Co, Mn, Mg. The observations of (3) above do not mean that N ligands are unimportant: such ligands may play a role in assisting Ni transport across root membranes, for example, even though at any instant a negligible proportion of the total plant Ni may be bound in this way. The focus on the study of leaf material may also have diverted attention away from regions where N-ligands have a role. It is difficult to see how the ubiquitous hydroxycarboxylic acids alone can account for the metal-specificity and species-specificity of Ni hyperaccumulation.

7. The significance of amino acids and their Ni complexes in Ni hyperaccumulation is still unclear -- several studies carried out to date give conflicting conclusions about the relevance of the amino acid profile to Ni uptake.

8. The importance of Ni hyperaccumulators for bioremediation remains to be determined. Such plants should certainly allow the removal of excess Ni from contaminated soils, and multielement accumulators such as certain *Thlaspi* species may allow multielement removal. However, a greater understanding of the basis of the selectivity may also lead to development of synthetic materials capable of reproducing the selective extraction behavior shown by these plants.

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The Distribution of some Inorganic Elements in Plant Tissue Extracts

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WITH TWO FIGURES IN THE TEXT

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SUMMARY

Tomato plants were fed with calcium 45, cobalt 60, copper 64, iron 59, gnesium 28, manganese 54, molybdenum 99, potassium 42, sodium 24, tung- 187, and zinc 65 in sand or water culture. Fresh tissues were then extracted h a series of reagents, and the percentage radioactivity determined in each action. Apart from calcium and iron, a measurable proportion of the assimilated ctivities were found in a form soluble in ethanol: the nature of the soluble cobalt and zinc complexes was investigated. Almost all the elements were removed by treatment with dilute hydrochloric and perchloric acids, but significant amounts of iron and cobalt were left in the residue.

INTRODUCTION

Although the mineral metabolism of plants has been widely studied, few orkers have investigated the chemical states of inorganic elements in plant sues. It is generally assumed that the bulk of such elements is present as which can diffuse more or less freely into plant cells. On the other hand, eral investigators (e.g. Epstein, 1956; Fried and Noggle, 1958), have found cessary to postulate the existence of uncharged complexes to account for ctive transport of ions across cell membranes. In addition, there is little that several of the essential trace elements, notably iron, copper, bdenum, and zinc, as well as magnesium, are complexed in specific me systems where they perform a vital role (Nason, 1958). Magnesium, example, is an essential constituent of chlorophyll.

he investigations described below were intended to improve our knowledge of the chemical states of certain elements inside plants. They can only regarded as preliminary observations, and, as frequently happens in such s, they raise more problems than they solve.

EXPERIMENTAL

Tomato plants (*Lycopersicon esculentum*, var. 'Moneymaker') were grown in ter or sand culture. A radioactive isotope was then added to the culture lution and the plant was allowed to take it up for a suitable period. Fresh f tissue was then removed, and treated successively with a series of solvents h which it was hoped to extract distinct chemical fractions. All fractions

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w[illegible]en counted with the same geometry, and in this way the distr o[illegible] element throughout the fractions could be established. For treatm with isotopes of Mn, Mg, K, Na, and Zn, the plants were grown in 150 m black polythene bottles containing the culture solution recommended b Hewitt (1952). At the time of administration the plants were just coming in flower and had a fresh weight of approximately 10–20 g. For Ca, Co, Cu, F Mo, and W, and for Na and K in separate experiments, the plants were grow in 10-inch pots containing approximately 10 kg. sand, using the same cultur solution. At the time of administration of the radioisotope the plants were fruit and had a fresh weight of approximately 700 g.

Most of the radioactive elements were made by neutron activation in th Harwell reactor BEPO in a flux of 10^{12} neutrons/cm.²/sec. Details of th preparation and purification are given below.

Calcium 45 (t½ 153 days). 2·375 g. spec. pure calcium carbonate was seal in silica and activated for 45 days. It was then dissolved in 80 per cent. nitr acid and 10 mg. each of strontium and barium carriers were added. Th precipitate was spun down and rejected, and the supernatant liquid w neutralized with 18 N. ammonia. This was scavenged with ferric hydroxid after adding a few drops of cobalt carrier, and the gelatinous precipitate w filtered. The supernatant was treated with excess 10 per cent. sodium carbonate to precipitate calcium carbonate. This precipitate was well washed with water, and then dissolved in 2 N. hydrochloric acid and finally brough to pH 7 with ammonia. The beta energy was determined by absorber measurements as a check on radiochemical purity.

Cobalt 60 (t½ 5·2 years). 0·0078 g. spec. pure tricobaltic tetroxide wa sealed in silica and activated for 45 days. It was then dissolved in hot aqua regia, diluted, neutralized, and tested for impurities with a gamma spectrometer after 14 days' decay.

Copper 64 (t½ 12 hours). 0·012 g. spec. pure cupric oxide was sealed in polythene and activated for 12 hours. It was then dissolved in hot 16 N. nitric acid, diluted to 2 N., and copper precipitated by adding sodium sulphite and potassium thiocyanate. The precipitate was washed and dissolved in 16 N nitric acid. This was made alkaline with ammonia after adding a few mg. iron carrier, and centrifuged. The supernatant liquid was then neutralized with acetic acid and fed to the plant 2·5 hours after removal from the reactor.

Iron 59 (t½ 45 days). 2·3 g. spec. pure triferric tetroxide was activated fo 45 days. It was then dissolved in 12 N. hydrochloric acid, diluted to 6 N. and extracted repeatedly with diethyl ether until the aqueous phase was colourless. The ether was evaporated off and the residue treated with 2 N. ammonia. After centrifugation, the precipitate was dissolved in the minimum of nitric acid and brought into neutral solution by adding ammonia and ammonium citrate. The radiochemical purity was tested with a gamma spectrometer.

Magnesium 28 (t½ 21 hours). 16 microcuries of this isotope were obtained commercially.

Manganese 54 (t½ 300 days). 50 microcuries of this isotope were obtained

commercially and shown to be radiochemically pure with a g[illegible] spectrometer.

Molybdenum 99 (t½ 2·7 days). 0·2 g. spec. pure ammonium molybdate was activated for 2·7 days. It was dissolved in water and its radiochemical purity tested with a gamma spectrometer after decaying for one-half life to free it from other radioisotopes of molybdenum.

Potassium 42 (t½ 12 hours). 0·11 g. spec. pure potassium carbonate was activated for 12 hours. It was dissolved in Hewitt's culture solution at pH 7 and fed to the plant 2 hours after removal from the reactor. Its radiochemical purity was tested with a gamma spectrometer.

Sodium 24 (t½ 15 hours). 0·019 g. spec. pure sodium bicarbonate was activated for 15 hours. It was dissolved in Hewitt's culture solution at pH 7 and fed to the plant 2 hours after removal from the reactor. Its radiochemical purity was tested with a gamma spectrometer.

Tungsten 187 (t½ 24 hours). 0·003 g. spec. pure tungstic oxide was activated for 24 hours. It was dissolved in hot 16 N. ammonia, scavenged with ferric hydroxide, neutralized with tartaric acid, and diluted with Hewitt's culture solution. It was fed to the plant 5 hours after removal from the reactor, and its radiochemical purity was tested with a gamma spectrometer.

Zinc 65 (t½ 245 days). 500 microcuries of this isotope were obtained commercially. Its radiochemical purity was tested with a gamma spectrometer.

TABLE I

Uptake times and counting techniques for radioisotopes

Isotope	Uptake time	Counting technique	*Max β energy* (MeV)	*γ energy* (MeV)
^{45}Ca	5 hours	Geiger	0·25	—
^{60}Co	2 days	Scintillation	0·31	1·2, 1·3
^{64}Cu	3 hours	Scintillation	0·66	1·34
^{59}Fe	2 days	Geiger and Scintillation	0·46	1·1, 1·3
^{28}Mg	24 hours	Scintillation	0·45	1·35
^{54}Mn	4 days	Scintillation	—	0·84
^{99}Mo	2 days	Geiger	1·23	0·74
^{42}K	4 hours	Geiger and Scintillation	3·5	1·50
^{24}Na	3 hours	Geiger	1·4	2·75
^{187}W	4 hours	Scintillation	1·3	0·69
^{65}Zn	7 days	Scintillation	0·32	1·11

The uptake times were limited largely by the half-lives of the isotopes used; they are shown in Table I.

The extraction technique used was based on work by Hewitt and Notton (1960). Mature leaves were harvested by cutting them off with scissors, and a small aliquot removed for counting. About 2 g. of leaf lamina was pared off, immersed in boiling 95 per cent. ethanol for a few seconds, and then macerated with 10 ml. 95 per cent. ethanol in a top-drive homogenizer for 5 minutes. The resulting slurry was centrifuged, and the residue washed twice with ethan[illegible]e filtered alcoholic extracts, containing most of the lipids and o small molecules, were combined and designated as the *ethanol fraction*.

The remaining powder was extracted three times with 5 ml. portions of 0·2 N. hydrochloric acid. The acid fractions were combined, and an equal volume of acetone was added to precipitate any extracted proteins and pectates. This was spun down and washed twice with 50 per cent. acetone. The supernatant liquid and gelatinous precipitate will be referred to as the *hydrochloric acid fraction* and *acetone insoluble fraction* respectively. The precipitate was not dialysed. The remaining tissue was extracted with 0·5 N. perchloric acid at 80° C. The acid extracts were again combined and treated with an equal volume of acetone to precipitate nucleic acid. This was spun down and washed twice with 50 per cent. acetone. The supernatant liquid and gelatinous precipitate will be referred to as the *perchloric acid fraction* and *nucleic acid fraction* respectively.

The remainder was boiled with 2 N. sodium hydroxide for about 10 minutes, which dissolved or degraded most of the remaining proteins and polysaccharides in it. On centrifugation two final fractions were obtained, which will be referred to as the *soda fraction* and the *residue* respectively. The latter consisted mostly of cellulose and lignin: for liquid gamma counting it was dissolved in fuming nitric acid.

In some cases an aliquot of active tissue was extracted three times with diethyl ether. Whenever the ethereal extract gave an adequate counting rate, it was further fractionated by the procedure of Bowen and Thick (1960). This fractionation yielded four portions containing organic acids, phenols, bases, and neutral materials, which were counted separately.

The counting techniques adopted are shown in Table I, but all the samples apart from those containing ^{45}Ca could have been counted with either a scintillation or Geiger counter. The scintillation counter incorporated a single-channel analyser which could be set to count over the best energy range for each isotope. In practice, scintillation counting was easier because the liquid fractions could be made up to 25 ml. in polythene bottles and stood directly on the sodium iodide crystal. Liquid Geiger counting was not employed, as counter contamination can prove troublesome when counting a large number of samples of very different activities. For beta counting, liquid samples were neutralized and evaporated and when the volume was reduced to 1 ml. they were transferred to aluminium counting trays for drying. The Geiger counter had the advantage of a lower background than the scintillation counter, and it frequently had a higher geometrical efficiency.

RESULTS

Preliminary experiments with inactive tomato tissue showed how much of the dry weight was removed in each successive extraction step. The data are summarized in Table II.

The results of nine separate experiments carried out with the elements listed above are shown in Table III. Count rates have been corrected for lost counts, radioactive decay, and (in the case of ^{45}Ca) for self-[illegible]ption. All data are the means of two replicates which generally agree within ± 20 per cent. The accuracy claimed for the figures in Table III is certainly no better than this.

TABLE II

Percentage dry weight of tomato leaf tissue extracted in different fractions

Fraction	Percentage dry weight extracted
Ethanol . . .	26·5
Hydrochloric acid .	15·5
Acetone insoluble .	2·2
Perchloric acid . .	14·4
Nucleic acid . .	18·7
Soda . . .	10·2
Residue . . .	12·4

TABLE III

Percentage distribution of elements in tomato leaf extracts

	Fraction						
Element	Ethanol	Hydrochloric acid	Acetone insoluble	Perchloric acid	Nucleic acid	Soda	Residue
Calcium . .	1·2	36·8	39·7	20·4	0·4	0·6	0·8
Cobalt . .	37·0	44·8	2·0	9·1	1·8	4·5	0·9•
Cobalt (seed-tissue) . .	31·8	12·7	12·7	16·7	14·3	10·9	1·0•
Copper . .	9·1	72·3	1·1•	13·7	1·2•	1·5•	1·2•
Iron . . .	0·5•	27·3	5·2	48·9	8·7	8·6	0·8
Magnesium .	11·6	75·5	0·2•	9·9	0·3•	1·1•	1·4
Manganese . .	2·3	94·2	0·02•	3·4	0·005•	0·09	0·04•
Molybdenum .	13·6	30·7	2·1	48·2	1·5	2·3	1·6
Potassium . .	17·0	71·3	3·0	5·3	0·2•	3·2	0•
Sodium . .	27·2	68·1	2·7	1·9	0•	0•	0·1•
Tungsten . .	9·1•	61·1	5·5•	18·8	2·3•	0•	3·2•
Zinc . . .	5·8	81·6	2·8•	4·9•	0·5•	3·5•	0·9•

• Counts not significantly above background.

Leaf tissue was used in all cases, but in the case of cobalt, 5 g. of seed tissue was fractionated in parallel.

There was virtually no difference between the figures for sodium and potassium distribution in plants grown in water culture and those grown in and culture. This was assumed to be true for the remaining elements.

The results of fractionating ethereal extracts of active tissue are given in Table IV. These extracts were made for only five elements: calcium, copper, ron, manganese and molybdenum. The data have been expressed as percentage total count, so that they are directly comparable with those of Table III.

TABLE IV

Distribution of elements in fractions of ether extracts from tomato leaves, expressed as percentage total count

Element	Fraction			
	Acids	Phenols	Bases	Neutral material
Calcium . . .	0·2*	0·13*	0·17*	N.D.
Copper . . .	6·2	1·5*	1·0*	1·5*
Iron . . .	0·05*	0·04*	0·6	N.D.
Manganese . .	0·54	0·15	0·14	0·16
Molybdenum . .	0·005*	0·005*	0·02	0·0006*

* Counts not significantly above background.

In cases where the count rate was not significantly different from the b ground, the results are marked with an asterisk.

Further work on cobalt. In the case of cobalt, 37 per cent. of the acti from leaf tissue and 32 per cent. of that from seed tissue was found in ethanol fraction. At first it was suspected that this cobalt was present vitamin B_{12}, which is extremely soluble in ethanol. It was decided to inve gate this possibility using paper chromatography (Scott and Ericson, 195 Fresh leaf extract was spotted on to a 27-cm. strip of Whatman No. 1 pap in parallel with cobalt sulphate spiked with ^{60}Co, and inactive vitamin B as cyanocobalamin. It was then eluted for 17 hours, using as solvent butanol saturated with water, containing 3 per cent. acetic acid and 2·5 p cent. sodium cyanide. As shown in Fig. 1, the extract activity did not el with the same characteristics as either the free cobalt ion or vitamin B_{12}.

Further work on zinc. An aliquot of the ethanol extract of the plant co taining ^{65}Zn was spotted on to a 27-cm. strip of Whatman No. 1 paper parallel with an aliquot of zinc chloride spiked with ^{65}Zn, and an aliquot the ethanol extract of an inactive plant, also spiked with ^{65}Zn. It was elu for 5·5 hours using 95 per cent. ethanol as a solvent. As shown in Fig. 2, t active extract contained zinc in two chemical states, one of which eluted w an Rf of about 0·39 and the other which remained at the origin and w probably the solvated zinc ion. The ethanol extract of an inactive plant d not possess the power of complexing zinc into a form which moved on t chromatogram. The zinc complex was not coloured and did not move w the pigments, which had a mean Rf of 0·82.

DISCUSSION

Calcium. This element is believed to exist in plant tissues largely as fr cations, or as calcium pectate in cell walls: calcium oxalate crystals are fou in a few plants. Only a very small percentage was extracted by ethanol a by ether. The bulk of the element was removed by the acid extractions, a a remarkably high percentage was precipitated with the acetone insolub fraction. This almost certainly represents calcium pectate, which is know

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be thrown out of solution by such treatment. However, Long and Levitt (1952) have reported significant uptake of ^{45}Ca by mitochondria, microsomes d free protein in potato tuber tissues. In agreement with the findings of

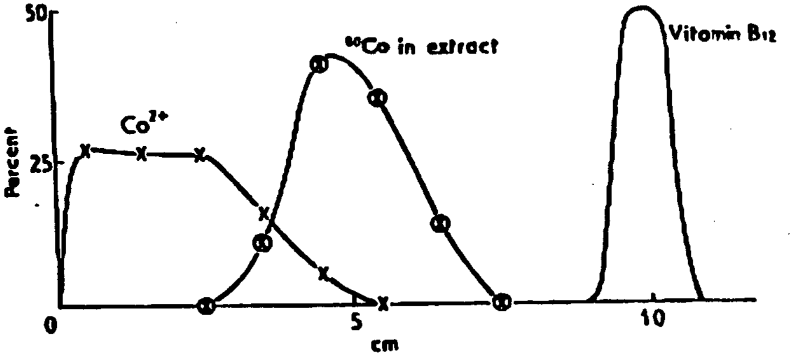
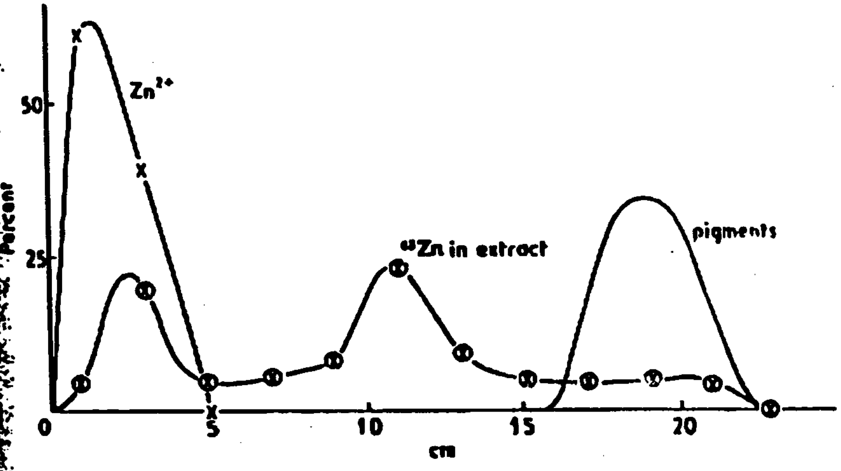


FIG. 1. Chromatographic behaviour of Co^{2+}, ethanol extract of tomato leaf containing ^{60}Co, and vitamin B_{12}.



. 2. Chromatographic behaviour of Zn^{2+} (with or without ethanol extract of tissue), ethanol extract of tomato leaf containing ^{65}Zn, and ethanol-soluble pigments.

ulson and Bowen (1952) and Loring and Waritz (1957) little calcium was nd in the nucleic acid fraction. Steffensen and LaChance (1960) claim t the element is a natural constituent of nuclei and chromosomes, but it y well be bound to histone rather than to DNA. In the tissue studied we imate 40 per cent. of the calcium was present as pectate and the remainder gely as free Ca^{2+} ions.

Sodium and potassium. These elements almost certainly exist as free cations cytoplasm, vacuoles and intercellular spaces. It is therefore not surprising t less than 10 per cent. remained unextracted after the hydrochloric acid ment. The most remarkable feature of the figures for these t in Table III are the high percentages soluble in ethanol. Since the 10 ml. 95 per cent. ethanol used was diluted by the water present in the 2 g. of ti the concentration of the final extract was approximately 75 per cent. etha This solvent dissolves appreciable amounts of sodium and potassium s (Seidell, 1940), and this dilution effect could account for the results. O would not expect sodium or potassium salts to dissolve in absolute ethanol in ether, but the complex of potassium with salicylaldehyde is soluble covalent solvents (Brewer and Sidgwick, 1925; Brewer, 1931). Epstein (19 and Fried and Noggle (1958) quote convincing circumstantial evidence t these elements are transported across cell walls as covalent complexes. T bacterium *Escherichia coli* strongly binds potassium (Cowie *et al.*, 1949), b the complexes are extremely labile as they are broken down by treatm with 50 per cent. ethanol: these complexes may be salts of hexose phosph (Roberts *et al.*, 1949). Potassium is strongly bound to mitochondria (Stanb and Mudge, 1953; Gamble, 1957; Thiers and Vallee, 1957) and is neces for certain enzymes (Miller and Evans, 1957; McCollum, Hageman a Tyner, 1958).

Magnesium. The relatively high percentage of magnesium extracted b ethanol may be largely present combined in chlorophyll. The remainder w soluble in acid solvents and there was no obvious concentration in eith protein or nucleoprotein fractions, though magnesium is required for a nu ber of enzymes (Nason, 1958) and is an important constituent of chromoso (Steffensen and LaChance, 1960) and RNA (Loring and Waritz, 1957).

Manganese. Almost all the manganese fed to plants was found in the aci soluble fractions. Negligible amounts were found in the residue, aceto insoluble, and nucleic acid fractions, but small quantities were soluble ethanol and in ether. Most of the ether-soluble fraction appeared to cont manganese complexed to an organic acid, but there are probably seve complexes present (Cotzias, 1958). Our results contrast with the mangane distribution found in animal tissue, where a substantial fraction of t manganese was bound to protein in mitochondria, and a smaller fraction w found in nuclei (Maynard and Cotzias, 1955; Thiers and Vallee, 1957). T former authors reported that radioactive manganese was easily removed fr mitochondria by washing.

Iron. This element was scarcely extracted from leaf tissue by ethanol ether, though the small amount soluble in ether appeared to be coupled to organic base, possibly a haem. 90 per cent. of the element was extracted the two acid treatments, and an appreciable amount appeared in the so acetone insoluble, and nucleic acid fractions. The former may repres protein-bound iron. Levitt and Todd (1952) found as much as 27 per c of the iron in potato tuber tissue was associated with crude protein. Iron i essential constituent of the proteins ferritin and cytochrome-C (Beinert Maier-Leibnitz, 1948), and autoradiographic studies have shown that i incorporated in cell nuclei (Poulson and Bowen, 1952). Here it is p

present as cytochrome-C (Dounce, 1952), and most of it is iently labile to be washed out by normal hydrochloric acid (Possingham and Brown, 1958). In our experiments the iron was evidently not all washed out by the acid treatments: the insoluble fraction may be present as a complex with RNA, as it appears to be in tobacco mosaic virus (Loring and Waritz, 1957) and yeast (Tu, 1961). Schmid and Gerloff (1961) have shown that iron in tobacco stem exudate is exclusively present as a complex anion which contains very little nitrogen or phosphorus: much of the iron in our hydrochloric acid fraction may have been in this form.

Cobalt. The unexpectedly high solubility of leaf and seed tissue cobalt in ethanol has already been commented on. This cobalt was present as neither free cobalt ion nor vitamin B_{12}, though the latter is an important product of biosynthesis by some micro-organisms (Chaiet, Rosenblum, and Woodbury, 1950). Similar cobalt complexes, which are supposed to be formed from the free ion and proteins or peptides, have been reported by Rosenfeld and Tobias (1950) in mouse liver cells, and by Ballantine and Stevens (1951) in sodium chloride extracts of the mould *Neurospora crassa*, the green alga *Chlorella vulgaris*, and the higher plants barley, oat, musk-melon, and tomato: here 10–40 per cent. of the cobalt taken up appeared as non-dialysable protein, and in the case of *Neurospora* nearly half of this was attached to microsomal particles. In addition a cobalt complex, insoluble in ethanol but soluble in dilute acids, has been isolated from the red alga *Rhodymenia palmata* (Scott and Ericson, 1955). The investigation of these complexes is a matter for future research, especially important in view of the essentiality of the element for some, if not all, groups of plants (Holm-Hansen *et al.*, 1954: Ahmed and Evans, 1960). Cobalt is an activator for a number of cytoplasmic and nuclear enzymes (Nason, 1958). More cobalt appeared to be associated with seed proteins than with leaf proteins in this work.

Copper. Copper is an activator of several essential enzymes (Nason, 1958), and autoradiographic studies of insect tissue have shown that it concentrates in cytoplasmic granules and at the surface of the nuclear membrane (Poulson and Bowen, 1952). Copper is 300 times as abundant in cytoplasm as in nuclei according to Rosenfeld and Tobias (1950). Levitt and Todd (1952) found 3 per cent. of the copper in potato-tuber tissue to be associated with crude protein, using colorimetric analysis. In the present work we found a negligible amount in the fractions supposed to contain protein and the element was almost entirely extracted by the acid treatments. However, significant percentages were soluble in ethanol and ether, and the bulk of the activity in the latter case was associated with an acidic substance. This finding should be further investigated.

Zinc. This is another essential co-enzyme (Nason, 1958), which has been shown to associate with crude protein by Levitt and Todd (1952). Rosenfeld and Tobias (1950) investigated the distribution of this element in mouse liver tissue, and found the relative percentage uptakes in cytoplasm, cytoplasmic protein, and nuclei to be 22·4, 0·32, and 0·006 respectively. Our results also

sho[illegible] significant concentration of zinc in the two fractions containin
protein. The nature of the zinc complex which is soluble in ethanol and wh
moves on a chromatogram is being further investigated.

Molybdenum and tungsten. These elements differ from those descri
above in that they were supplied to the plants, and presumably taken up
anions (molybdates and tungstates). The tungsten uptake was poor in
experiments, although tomato plants can take up large amounts of this e
ment without physiological damage (Bowen, unpublished). However, t
general pattern of their distribution among the fractions is similar. T
elements are extracted to a limited extent by ethanol, and the remainder
almost completely removed by treatment with acids. Small amounts occ
in the protein and nucleoprotein fractions, though molybdenum is an activato
for at least three essential enzymes (Nason, 1958). The very small amount
molybdenum extracted by ether appears to be associated with organic ba
(Table IV). Autoradiography has shown that molybdenum is concentra
in the interveinal areas of leaves (Stout and Meagher, 1948) but little is know
of its chemical state there.

CONCLUSIONS

The results of this preliminary study of the distribution of radio-eleme
in tissue fractions have raised a number of interesting questions. In particul
the ethanol-soluble complexes of cobalt, zinc, and several other elemen
merit further study, as does the nature of the association of cobalt and ir
with proteins and iron with nucleoprotein. The fractionation procedur
could be considerably modified and extended, particularly if a high-speed
centrifuge were used to separate intracellular particles of different sizes
notably mitochondria and microsomes, from debris and soluble material
this has been attempted only for inactive tissues (Thiers and Vallee, 1957).

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Appendix B-17 – Lab Procedures for Soil Moisture Analysis: Method ASA 21.2.2.2

Soil Moisture, Oven Drying Method

ASA Physical Method 21-2.2.2

1.0 Purpose

To determine the moisture loss of a soil sample by oven drying overnight at 105 °C.

2.0 Scope

This procedure applies to soil, sand, silt, rock, and soil organic matter.

3.0 Summary

A sample is dried overnight at 105 °C. Moisture content is determined by weight loss.

4.0 References

Chapter 21-2.2 "Gravimetry With Oven Drying." *Methods of Soil Analysis, Part I, Physical and Mineralogical Methods*, Second Edition, 1986. Arnold Klute, Editor. American Society of Agronomy, Inc. Soil Science Society of America Inc. Publisher, Madison, Wisconsin, USA.

ASTM D 2216-92, "Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil and Rock"

ASTM D 2974-87 (Reapproved 1995) "Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils"

5.0 Responsibilities

5.1 The Laboratory Manager shall ensure that this procedure is followed during the analysis of samples.

5.2 The Laboratory Group Leader shall review and approve data produced under this procedure.

5.3 The laboratory analyst shall follow this procedure and laboratory safety guidelines. The analyst shall record all data, calculate results, and sign a written report of the analysis.

6.0 Requirements

6.1 Prerequisites

None

6.2 Limitations and Actions

For extremely dry soils, the quantity weighed should be increased in step 7.1.3 to 50g.

6.3 Requirements

6.3.1 Apparatus/Equipment

6.3.1.1 Laboratory oven with forced air, thermostatted to control temperature to plus or minus 5 °C.

6.3.1.2 Desiccator with active dessicant (Drierite, or Anhydrone)

6.3.1.3 Tongs or insulated gloves

6.3.1.4 Analytical Balance - capable of weighing to 0.0001 g.

6.3.2 Reagents and Standards

None

6.4 Quality Control Sample Requirements

Run a duplicate sample and method blank for every batch of 20 samples or subset thereof.

7.0 Procedure

7.1 Procedure Instructions

7.1.1 Thoroughly mix a portion of soil. Remove stones larger than 1 cm diameter. Remove roots and leaves. Break up any lumps or adhesions.

7.1.2 Dry a beaker or weighing dish for 30 minutes at 105 °C. Allow to cool in a desiccator with active dessicant.

7.1.3 Obtain the tare weight of the container then the weight plus 10 to 20g soil (record weight to 0.0001g).

7.1.4 Place the moist sample and container in the drying oven overnight (approximately 16 hours) at 105 °C uncovered.

7.1.5 Remove the container from the oven and place it in a desiccator with active dessicant to cool.

7.1.6 Weigh the dried sample and container.

7.2 Calculations and Recording Data

7.2.1 Calculate the water content of the material to the nearest 0.1% as follows:

$$w = [(M_{cws} - M_{cs})/(M_{cs} - M_c)] * 100$$

where

w = water content, %
M_{cws} = mass of container and wet specimen in grams
M_{cs} = mass of container and dry specimen in grams
M_c = mass of container

7.2.2 Calculate the percent solids to the nearest 0.1% as follows:

$$\text{Percent solids} = 100 - w$$

7.2.3 Record data on the form provided in 10.1.

Note: A spreadsheet may be used to calculate the data.

8.0 Safety

8.1 Follow general laboratory safety rules. Exercise care in removing hot items from the oven. Use tongs or insulated gloves.

8.2 Excercise caution to not spill hot soil containing organic matter into Anhydrone (magnesium perchlorate) which is a strong oxidizing agent.

9.0 Notes

None

10.0 Attachments and Appendices

10.1 Soil Percent Moisture Worksheet

Percent Moisture
Oven Drying Water Worksheet

Initial Date/Time ____________ Initial Oven Temp ________

Final Date/Time ____________ Final Oven Temp ________

Workorder		=========	=========	=========	=========	=========
Fraction #						
Gross wt (GW)						
Tare wt (TW)						
Dried wt (DW)						

Sample
Moisture Loss

%moisture
%solid

Entered by ____________ Date ________

Reviewed by ____________ Date ________

Sample Description

END OF PROCEDURE

Appendix B-18 – Lab Procedures for Soil Moisture Retention/Release Curves: Method ASA 8-2.3

Water Retentivity
(Moisture Release Curves)
ASA Method 8-2.3

1.0 Procedure

Perform analysis for water retentivity (Moisture Release Curves) in accordance with ASA Method 8-2.3 as attached and in accordance with manufacturer's instructions on the Soilmoisture Model 1500 "15 Bar Ceramic Plate Extractor" as attached.

2.0 Recordkeeping

Retain all worksheets, calculations, graphs, and notes.

3.0 Quality Control Samples

The only quality control sample possible with this physical characterization method is to run a duplicate sample.

4.0 References

Chapter 8-2 "Water Retentivity." *Methods of Soil Analysis, Part I, Physical and Mineralogical Methods*, Second Edition, 1986. Arnold Klute, Editor. American Society of Agronomy, Inc. Soil Science Society of America Inc. Publisher, Madison, Wisconsin, USA.

8–2.3 Method [2]

8–2.3.1 SPECIAL APPARATUS

1. Membrane apparatus: Pressure-plate and pressure-membrane apparatus like those shown in Fig. 8–3 are commercially available and are usually about 28 cm. in diameter. Soil on the membrane is contained in rings of approximately 1-cm. height and 6-cm. diameter that hold about 25 g. of sample. Rubber rings must be used on acetate membranes.
2. Source of regulated air pressure: A source of compressed air at adjustable regulated pressure is required, such as that supplied by Soilmoisture Equipment Co., Santa Barbara, Calif.

8–2.3.2 PROCEDURE FOR TESTING APPARATUS

To check ceramic pressure-plate apparatus for defects, install the plates in the chamber, cover the plates with water, close the chamber, and apply the maximum appropriate air pressure. Measure the outflow rate as soon as the outflow becomes relatively free of air bubbles. Since this is a qualitative test, do not wait for a steady outflow rate. Commercial plates of approximately 28-cm. diameter, with 1- and 2-bar bubbling pressure, have a conductance of about 15 cc. min.^{-1} bar^{-1}, while plates with 15-bar bubbling pressure have a conductance in the range of 0.5 to 2 cc. min.^{-1} bar^{-1}. Plate conductance is not critical except for retentivity measurements at low suction values. In this case, higher conductance gives appreciably faster results.

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[2] U. S. Salinity Laboratory Staff (1954).

Next check the plates for bubbling pressure. This is the pressure difference that will cause streaming of air through a wet plate. Release the air pressure, empty excess water from the chamber, and apply the maximum air pressure to be used in the retentivity measurements. After a few minutes, the outflow of water will cease, and there should ideally be no bubbling of air. Actually a bubbling rate as high as 2 or 3 cc. of air per minute can be tolerated. Air bubbling at the outflow tube can come through the plate, but it can also come from leaks in the mounting or from joints in the outflow tube that are inside the chamber.

After observing air bubbling at the outflow tube, submerge the chamber in water, or observe air pressure change in the chamber with the supply source shut off, to make sure the chamber is air-tight. Air leaks from the chamber may produce evaporative losses that will dry the samples below the equilibrium value that would otherwise have been attained by membrane suction.

8–2.3.3 PROCEDURE FOR RETENTIVITY MEASUREMENTS

It is convenient to have 75 to 100 g. of air-dried soil. Reduce all aggregates to <2-mm. diameter by rubbing the soil through a 2-mm. round-hole sieve with a rubber stopper. Place the sieved fraction on a mixing cloth, and pull the cloth in such a way as to produce mixing. (Some pulling operations will produce segregation instead of mixing, and special care must be exercised to obtain a homogeneous sample.) Flatten the sample until the pile is 2 to 4 cm. deep.

For water retentivity, two or three representative subsamples having a fairly definite volume are required. Use a separate paper cup for each subsample. Mark with a pencil line around the inside of the cup the height of soil needed to give the desired volume of subsample. This volume should be somewhat less than the volume of soil required to make the soil-retainer ring on the membrane level full. Use a thin teaspoon or scoop (not a knife or spatula), and lift small amounts of soil from the pile. Place successive spoonfuls in successive cups, and progress around the pile until the cups are filled to the desired level. Transfer a small enough quantity of soil in each operation to keep the larger particles from rolling off the spoon or scoop. Roll-off should be avoided because it makes the extracted subsample nonrepresentative. Place the sample-retainer rings on the porous plate. To avoid particle-size segregation, dump *all* the subsample from each container into a ring, and level the soil without spilling any outside the ring. A wide-mouth powder funnel, used as a tremie, is convenient for this sample transfer operation. Allow the samples to stand at least 16 hours with an excess of water on the membrane. Close the pressure chamber, and apply pressure. Connect the outflow tube from the pressure chamber to the bottom of a 25- or 50-ml. buret.

For a pressure chamber with an acetate membrane and a rubber diaphragm for holding the samples against the membrane, proceed as follows: Apply the air pressure first to the soil chamber. After a short time, usually 1 or 2 hours, the water outflow rate falls off markedly. By this time, the wet samples acquire some bearing strength. Then apply an excess of pressure of about 1/4 bar to the diaphragm chamber in accordance with the manufacturer's instructions.

Samples 1-cm. high can be removed any time after 48 hours from initiating the extraction or when readings on the outflow buret indicate that liquid water outflow has ceased from all samples on each membrane. Some soils will approach equilibrium in 18 to 20 hours. Before releasing the air pressure in the chamber, put a pinch clamp on the outflow tube. This reduces backflow of water to the samples after the pressure is released. To avoid changes in the water content of the samples after opening the chamber, transfer the samples quickly to metal boxes for drying. Determine the water content by drying the samples to constant weight at 105°C. Express the water content in terms of percentage on a dry-weight basis. Retentivity data should be accompanied by information on the temperature and ambient air pressure of the soil while on the membrane. Information on the structure and history of the sample should also be given.

8-2.3.4 COMMENTS

For some purposes, the ratio R of the weight of the coarse separate (>2 mm.) to the weight of the fine separate (≤ 2 mm.) should be recorded. Mineral soil material >2-mm. diameter complicates retentivity measurements and retains a negligible amount of water. When desired, the retentivity of the whole soil P_W can be calculated from the retentivity of the fine separate P_F by the equation, $P_W = P_F[1/(1 + R)]$.

Some air transfer always occurs through wet pressure membranes. According to Henry's law, the solubility of air in water is proportional to the pressure. Consequently, the concentration of dissolved air in the membrane water on the soil side is always higher than on the outflow side. This air moves through the membrane during liquid outflow and appears as bubbles in the outflow buret. When liquid outflow ceases, dissolved air moves through the membrane by molecular diffusion, and air bubbles will continue to appear in the outflow system, but at a reduced rate. This may amount to several cubic centimeters per minute for Visking cellulose membranes, 28 cm. in diameter, at a pressure of 15 bars. The maximum possible error from this air transfer can be calculated by assuming that all the water required to humidify these air bubbles comes from the soil samples.

The time required for soil samples to attain hydraulic equilibrium with a membrane increases approximately with the square of the height of the sample. This should be taken into consideration if it is planned to put core samples on a membrane.

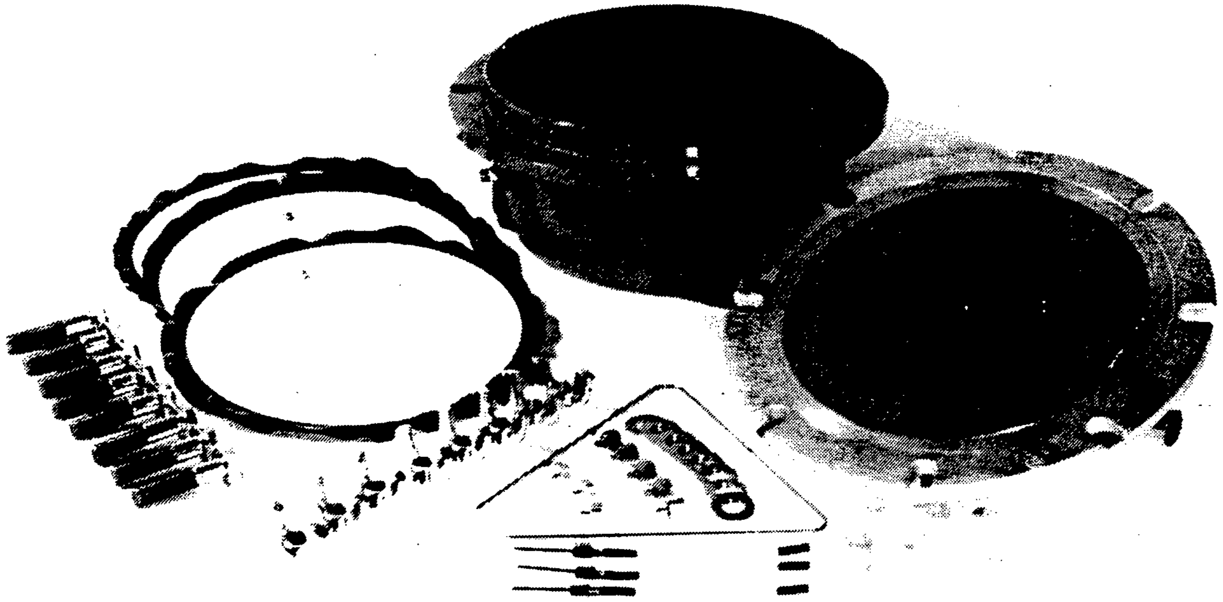
Ceramic plates with rubber backing for use in pressure chambers up to

15 bars are less troublesome to use than cellulose membranes. Microbial action in some soils, iron rust from the chamber, sand grains near the gasket seal, and other things can cause disabling leaks in cellulose membranes. Pressure chambers for acetate membranes, however, do have the diaphragm for pressing the sample against the membrane to prevent loss of contact that might be caused by shrinkage of fine-textured samples.

Principal errors in retentivity measurements come from nonrepresentative subsamples; evaporative loss from samples during approach to equilibrium, as occurs on tension tables, or as caused by air leaks from pressure chambers; pressure or temperature fluctuations causing hysteresis effects; failure to attain outflow equilibrium; inadequate prewetting of samples; wetting of samples from backflow; or drying by evaporation during removal of the samples from the membrane. With skill, a coefficient of variation of 1 or 2% is attainable, and the measured value is independent of the type of apparatus or membrane.

1500 OPERATING INSTRUCTIONS

15 BAR CERAMIC PLATE EXTRACTOR



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SOILMOISTURE EQUIPMENT CORP.
P.O. Box 30025
Santa Barbara, CA 93105 U.S.A.

SOILMOISTURE

UNPACKING AND ASSEMBLY OF THE EXTRACTOR

REMOVE FROM BOX

THE 15 BAR CERAMIC PLATE EXTRACTOR IS SHIPPED WITH LID ASSEMBLED TO THE PRESSURE VESSEL. THE 15 BAR CERAMIC PLATE CELLS AND TRIANGULAR SUPPORT FOR BOTTOM PRESSURE PLATE CELL ARE PACKED INSIDE THE EXTRACTOR. OUTFLOW TUBE ASSEMBLIES, PLUG BOLTS AND PLASTIC SPACERS ARE PACKED SEPARATELY OUTSIDE OF THE EXTRACTOR. AFTER LIFTING UNIT FROM PACKING CRATE, SET THE EXTRACTOR DIRECTLY ON ITS FEET.

REMOVE ALL PACKING MATERIAL AND TAPE FROM AROUND CLAMPING BOLTS AND OVER OUTLET PORTS IN THE SIDE OF THE EXTRACTOR. THE PRESSURE INLET FITTING TO THE EXTRACTOR IS CAPPED WITH A THREAD PROTECTOR WHICH MUST BE REMOVED BEFORE CONNECTING HOSE IS ATTACHED.

REMOVE LID

THE EXTRACTOR LID IS READILY REMOVED BY UNDOING THE EIGHT CLAMPING BOLTS AROUND THE PERIPHERY OF THE UNIT. THE WING NUTS ON THE CLAMPING BOLTS SHOULD NOT BE COMPLETELY REMOVED. IT IS NECESSARY ONLY TO UNDO THE WING NUTS SEVERAL TURNS. THE BOLTS CAN THEN BE SLIPPED OUT OF THE SLOTS. THE BOLTS HAVE SPECIAL RECTANGULAR HEADS WHICH FIT INTO A CONSTRAINING GROOVE IN THE BOTTOM OF THE LOWER CLAMPING RING. IN REPLACING THE CLAMPING BOLTS, ALWAYS BE SURE THAT THEIR HEADS ARE PROPERLY FITTED INTO THE CONSTRAINING GROOVE.

AFTER THE CLAMPING BOLTS ARE REMOVED, THE LID CAN BE LIFTED OFF. IF THE LID APPEARS TO "STICK", LIFT FORCIBLY AT ONE EDGE TO BREAK CONTACT BETWEEN SEALING "O" RINGS AND LID. IT IS IMPORTANT TO HANDLE THE LID CAREFULLY SO THAT THE SEALING AREA ON THE UNDERNEATH SIDE IS NEVER SCRATCHED OR OTHERWISE DAMAGED SINCE SUCH DAMAGE WOULD PREVENT THE UNIT FROM SEALING PROPERLY. THE "O" RING ITSELF FITS INTO A GROOVE AT THE TOP EDGE OF THE PRESSURE VESSEL WALL AND IS EASILY REMOVED AND/OR REPLACED.

REMOVE PRESSURE PLATE CELLS

THE 15 BAR CERAMIC PLATE CELLS MAY NOW BE REMOVED ALONG WITH THE TRIANGULAR SUPPORT AND ALL PACKING MATERIAL. HANDLE THE CERAMIC PLATE CELLS WITH CARE TO AVOID SHARP BLOWS WHICH MAY CAUSE CRACKING OR BREAKING.

MOUNT OUTFLOW TUBE AND PLUG BOLTS

THE METAL OUTFLOW TUBE FITTING IS SHIPPED ASSEMBLED TO THE OTHER INTERNAL CONNECTING TUBES. REMOVE THE RUBBER SLEEVE FROM THE OUTFLOW TUBE FITTING BEFORE SCREWING FITTING INTO OUTLET PORT IN VESSEL WALL. SIX OUTLET PORTS ARE PROVIDED IN THE WALL OF THE VESSEL. FOUR ARE AROUND THE TOP EDGE AND TWO OTHERS ARE SPACED DOWN THE WALL OF THE VESSEL FOR USE WHEN THE THREE PRESSURE CELLS ARE BEING RUN AT THE SAME TIME. FIVE PLUG BOLTS ARE PROVIDED FOR SEALING THE UNUSED OUTLET PORTS.

THE PRESSURE SEAL AT THE OUTLET PORT IS MADE BY A SMALL NEOPRENE RUBBER "O" RING RECESSED INTO THE HEAD OF THE OUTFLOW TUBE ASSEMBLY AND PLUG BOLT. BEFORE INITIALLY INSERTING THE OUTFLOW TUBE ASSEMBLY OR PLUG BOLT, APPLY A SMALL AMOUNT OF STOPCOCK GREASE OR VASELINE ON THE EXPOSED PORTION OF THE "O" RING TO LUBRICATE IT AS IT SLIDES AGAINST THE WALL OF THE VESSEL WHEN SCREWED INTO PLACE.

ONLY A SMALL AMOUNT OF TORQUE IS REQUIRED TO MAKE THE OUTLET PORT SEAL. A STANDARD 1/2" SIZE WRENCH WILL FIT THE OUTLET FITTINGS AND PLUG BOLTS. IN TIGHTENING

SE FITTINGS IT IS ONLY NECESSARY TO BRING THE OUTER EDGE OF THE FITTING INTO CONTACT WITH THE FLAT "SPOT FACED" SURFACE ON THE PRESSURE VESSEL WALL. THIS PROVIDES THE PROPER COMPRESSION ON THE "O" RING TO MAKE THE SEAL. FURTHER TIGHTENING WILL ONLY SERVE TO DAMAGE THE FITTING AND SHORTEN THE LIFE OF THE "O" RING SEAL.

MOUNT PM HINGE

IF A PM HINGE, CAT. NO. 1080, IS TO BE USED IN CONJUNCTION WITH THE EXTRACTOR IT WILL BE NECESSARY TO HAVE A CAT. NO. 1081 ADAPTER PLATE. THIS PLATE FITS ON TOP OF THE EXTRACTOR LID UNDERNEATH THE TOP CLAMP OF THE PM HINGE AND PROVIDES THE PROPER SPACING TO MATCH THE CLAMP HEIGHT. INSTRUCTIONS FOR INSTALLATION OF THE PM HINGE ARE BASICALLY THE SAME AS FOR THE PRESSURE MEMBRANE EXTRACTOR WHICH ARE INCLUDED WITH THE HINGE. IT IS USUALLY DESIRABLE TO MOUNT THE PM HINGE AT THE BACK OF THE 15 BAR CERAMIC PLATE EXTRACTOR WITH PRESSURE INLET FITTING SPACED 45° TO THE RIGHT.

CLOSING AND OPENING LID WITH PM HINGE

WHEN THE PM HINGE IS USED IT IS NECESSARY TO APPLY ADDITIONAL TORQUE ON THE TWO WING NUTS ON EITHER SIDE OF THE HINGE IN ORDER TO COMPRESS THE COUNTERBALANCING SPRING IN THE HINGE WHEN THE LID IS CLOSED. THE FOLLOWING PROCEDURE SHOULD BE ADHERED TO FOR MAXIMUM EASE AND EFFICIENCY OF OPERATION. FIRST APPLY A THIN COAT OF HEAVY GREASE (SUCH AS WHEEL BEARING GREASE--OBTAINABLE AT ANY GASOLINE SERVICE STATION) ON THE UNDERSIDE OF EACH WING NUT AND TOP OF EACH WASHER. WHEN THE LID IS CLOSED INSERT FIRST TWO CLAMPING BOLT ASSEMBLIES, ONE ON EITHER SIDE OF THE HINGE AND IMMEDIATELY ACENT TO IT. TIGHTEN FIRST ONE WING NUT UNTIL IT IS SNUG AND THEN TIGHTEN THE R ONE UNTIL SNUG. WORK BACK AND FORTH TIGHTENING FIRST ONE AND THEN THE OTHER UNTIL THE LID IS DOWN AGAINST THE TOP OF THE EXTRACTOR VESSEL. NOW INSERT THE SIX REMAINING CLAMPING BOLT ASSEMBLIES AND TIGHTEN ALL WING NUTS UNTIL THEY ARE FIRM.

WHEN THE EXTRACTOR IS BEING OPENED AFTER A RUN THE PROCESS IS JUST REVERSED. FIRST, LOOSEN AND REMOVE ALL CLAMPING BOLT ASSEMBLIES EXCEPT THE TWO ON EITHER SIDE OF THE HINGE. THEN LOOSEN ONE OF THESE REMAINING BOLTS ABOUT 1/8 TURN INITIALLY, AND THEN LOOSEN THE OTHER ABOUT 1/8 TURN. WORK BACK AND FORTH SLIGHTLY LOOSENING FIRST ONE AND THEN THE OTHER UNTIL THEY TURN EASILY (AFTER ABOUT 2-3 FULL TURNS), AND CAN BE REMOVED.

PLACEMENT OF TRIANGULAR SUPPORT

THE TRIANGULAR SUPPORT MUST BE PLACED IN THE EXTRACTOR VESSEL ON THE BOTTOM BEFORE ANY PRESSURE PLATE CELLS ARE INSTALLED. THE PURPOSE OF THE TRIANGULAR SUPPORT IS TO KEEP THE LOWER PRESSURE PLATE CELL OFF OF THE BOTTOM OF THE EXTRACTOR. THIS IS NECESSARY BECAUSE UNDER CERTAIN CIRCUMSTANCES IF IT IS NOT USED A SEAL CAN BE MADE BETWEEN THE OUTER EDGE OF THE RUBBER BACKING ON THE PRESSURE PLATE CELL AND THE FLAT BOTTOM OF THE EXTRACTOR. UNDER THESE CIRCUMSTANCES WHEN THE AIR PRESSURE IS APPLIED A LARGE PRESSURE DIFFERENTIAL WILL DEVELOP BETWEEN THE TOP AND BOTTOM OF THIS CERAMIC PLATE AND BREAK IT. BE SURE THE TRIANGULAR SUPPORT IS ALWAYS IN THE BOTTOM OF THE EXTRACTOR BEFORE THE PRESSURE PLATE CELLS ARE INSTALLED.

INSTALLING THE PRESSURE PLATE CELLS

THE FIRST PRESSURE PLATE CELL IS PLACED DIRECTLY ON THE TRIANGULAR SUPPORT AT THE OM OF THE EXTRACTOR AND CONNECTION IS MADE THROUGH THE LOWEST OUTLET PORT. THE SECOND PRESSURE PLATE CELL IS SET ON THREE PLASTIC SPACERS WHICH ARE PLACED ON THE

IRST CERAMIC PLATE CELL NEAR THE OUTER EDGE AND LOCATED ABOUT 120° FROM EACH OTHER. CONNECTION TO THIS CELL IS MADE THROUGH THE MIDDLE OUTLET PORT. THE THIRD CELL IS MOUNTED SIMILARLY TO THE SECOND AND CONNECTION IS MADE THROUGH ONE OF THE OUTLET PORTS AT THE UPPER EDGE OF THE VESSEL WALL.

THE FLEXIBLE OUTER EDGE OF THE RUBBER DIAPHRAGM MAY BE USED TO LIFT THE PRESSURE PLATE CELLS IN AND OUT OF THE EXTRACTOR.

TUBE CONNECTIONS TO CERAMIC PLATE CELLS

THE CERAMIC PLATES IN GENERAL ARE NOT FLAT, AND HAVE A TENDENCY TO BE CONVEX. FOR THIS REASON IT IS NECESSARY TO SUPPORT THE PLATE WITH THE FINGERS DIRECTLY BEHIND THE OUTLET STEM WHEN THE RUBBER SLEEVE CONNECTION IS MADE. IN MAKING THE INTERNAL TUBE CONNECTIONS AT THE CELL AND AT THE OUTLET PORT, BE SURE THAT THE HARD NYLON TUBING RUNS THROUGH THE SLEEVE AND UP TO OR INTO THE METAL CONNECTING PARTS. THE NYLON TUBING WILL WITHSTAND THE HIGH EXTRACTION PRESSURES WITHOUT COLLAPSING WHEREAS THE RUBBER SLEEVES MAY NOT. IF THESE CONNECTIONS ARE NOT MADE IN SUCH A WAY THAT THE TUBING CANNOT COLLAPSE, AN ERRONEOUS EQUILIBRIUM READING WILL RESULT. THE ONLY OUTWARD EVIDENCE OF THIS MALFUNCTION WILL BE A RUSH OF AIR FROM THE OUTFLOW TUBE WHEN THE PRESSURE IN THE CHAMBER IS RELEASED, AS IS DONE AT THE END OF A RUN.

PRESSURE CONNECTION

THE SINGLE PRESSURE INLET TO THE EXTRACTOR IS A STANDARD 50/20 ADAPTER LOCATED PART WAY UP THE WALL OF THE VESSEL AND OPPOSITE TO THE OUTLET PORTS. A STANDARD T. NO. 1091 CONNECTING HOSE WILL COUPLE DIRECTLY TO THIS FITTING. THREAD SIZE OF THE ESSURE FITTING IS 9/16-18. THE PRESSURE SEAL AT THE HOSE CONNECTION IS MADE WHEN THE ROUND "NOSE" OF THE BRASS STEM INSIDE THE HOSE NUT IS PRESSED AGAINST THE RECESSED CONICAL SURFACE OF THE 50/20 ADAPTER. THIS IS A METAL TO METAL SEAL AND IS VERY EFFECTIVE. THE SCREW THREADS ON THE FITTING AND NUT ONLY SERVE AS A MEANS OF HOLDING THE PARTS IN CONTACT. THE THREADS THEMSELVES DO NOT MAKE A SEAL. ONLY A SMALL AMOUNT OF TORQUE IS REQUIRED AND SHOULD BE USED IN CONNECTING THE HOSE.

HANDLING AND CARE OF THE 15 BAR CERAMIC PLATE CELLS AND EXTRACTOR VESSEL

CONSTRUCTION OF PRESSURE PLATE CELL

EACH PRESSURE PLATE CELL CONSISTS OF A 15 BAR CERAMIC PLATE APPROXIMATELY 10-1/4" IN DIAMETER WHICH IS SEALED ON ONE SIDE BY A THIN NEOPRENE DIAPHRAGM. AN INTERNAL SCREEN KEEPS THE DIAPHRAGM FROM CLOSE CONTACT WITH THE PLATE AND PROVIDES A PASSAGE FOR FLOW OF WATER. AN OUTLET STEM RUNNING THROUGH THE CERAMIC PLATE CONNECTS THIS PASSAGE TO THE OUTFLOW TUBE ASSEMBLY.

THE 15 BAR CERAMIC IS QUITE STRONG, HOWEVER, CARE SHOULD BE TAKEN TO AVOID SHARP BLOWS. IT IS ALSO IMPORTANT TO AVOID LARGE MECHANICAL LOADING.

CHECK OUT OF PRESSURE CELLS

BEFORE MAKING A RUN IT IS DESIRABLE TO CHECK OUT THE PRESSURE PLATE CELLS TO ACQUAINT THE OPERATOR WITH THEIR CHARACTERISTICS AND TO DETERMINE THAT THEY HAVE NOT BEEN DAMAGED IN SHIPMENT. PRIOR TO SHIPMENT EACH CELL IS TESTED FOR WATER OUTFLOW RATE AND AIR DIFFUSION RATE AND A PERMANENT RECORD IS MADE FOR EACH CELL.

IN MAKING THIS TEST THE GENERAL PROCEDURES AS GIVEN IN "MAKING A RUN FOR MOISTURE RETENTION STUDIES" (SEE PAGE 6) SHOULD BE FOLLOWED WITH THE EXCEPTION THAT ONLY WATER

[illegible] BE USED. LET AN EXCESS OF WATER STAND ON THE SURFACE OF THE CERAMIC PLATE CELLS FOR SEVERAL HOURS TO THOROUGHLY WET THE PLATE. APPROXIMATELY 150 ML OF WATER WILL BE REQUIRED TO FILL THE PORES OF EACH PLATE. NEXT MOUNT ONE OR MORE OF THE WETTED PLATES IN THE EXTRACTOR AND MAKE THE OUTFLOW CONNECTIONS. CAREFULLY ADD WATER ON TO THE SURFACE OF EACH CELL SO THAT THE SURFACE IS COMPLETELY COVERED TO THE MAXIMUM DEPTH PERMITTED BY THE OUTER EDGE OF THE NEOPRENE DIAPHRAM. CLOSE THE EXTRACTOR AND BUILD UP THE PRESSURE TO 15 BARS (220 PSI). AS THE PRESSURE BUILDS UP INSIDE THE EXTRACTOR THERE WILL BE A RUSH OF AIR FROM THE OUTFLOW TUBES. THIS IS CAUSED BY THE REDUCTION OF THE INTERNAL VOLUME OF THE CELL AS THE DIAPHRAGM AND SCREEN COLLAPSE UNDER THE PRESSURE IN THE EXTRACTOR. IF THE INTERNAL OUTFLOW TUBING CONNECTIONS ARE "TIGHT" AND THE CELL HAS NOT BEEN CRACKED OR DAMAGED, THIS AIR FLOW WILL STOP AFTER SEVERAL MINUTES AND THERE WILL BE A STEADY FLOW OF WATER. SMALL BUBBLES OF AIR WILL COME OUT IN THE FLOW OF WATER AT REASONABLY REGULAR INTERVALS. THIS IS AIR WHICH IS SLOWLY DIFFUSING THROUGH THE CERAMIC PLATE, AND IS TO BE EXPECTED. THE OUTFLOW RATE IN ML/MIN SHOULD BE MEASURED SOON AFTER FLOW STARTS, WHILE THE ENTIRE SURFACE OF THE CERAMIC IS COVERED WITH WATER.

AFTER A PERIOD OF TIME ALL OF THE WATER ON THE CERAMIC PLATE WILL HAVE BEEN CONDUCTED THROUGH AND FLOW OF WATER WILL STOP. THE SLOWLY DIFFUSING AIR WILL GRADUALLY CONDUCT SMALL AMOUNTS OF WATER SURROUNDING THE INTERNAL SCREEN TO THE OUTSIDE.

TO MEASURE THE RATE OF DIFFUSION A SHORT LENGTH OF RUBBER TUBING CAN BE CONNECTED TO THE OUTFLOW TUBE AND THE END INSERTED UNDER AN INVERTED GRADUATE WHICH HAS BEEN PREVIOUSLY FILLED WITH WATER. THE FLOW RATE OF THE AIR SHOULD BE LESS THAN 1/10 ML OF AIR AT ATMOSPHEREIC PRESSURE PER MIN WITH THE EXTRACTOR PRESSURE AT 220 PSI. IF THE [illegible] RATE OF AIR IS APPRECIABLY HIGHER THAN THIS, IT INDICATES THAT THERE IS A LEAK IN [illegible] TUBING CONNECTION OR THAT THE CELL IS CRACKED OR NOT SEALED PROPERLY.

DRYING CELL AFTER RUN

WHEN A PRESSURE CELL IS TO BE DRIED FOR STORAGE AFTER A RUN, IT IS IMPORTANT TO KEEP EVAPORATION DEPOSITS ON THE SURFACE TO A MINIMUM. THIS IS EASILY ACCOMPLISHED BY COVERING THE SURFACE OF THE CERAMIC PLATE WITH A THIN LAYER OF FINE DRY SOIL AND ALLOWING IT TO SET FOR SEVERAL DAYS UNTIL DRY. THE SOIL IS THEN REMOVED AND THE CELL IS STORED. BY THIS MEANS EVAPORATION DEPOSITS ARE FORMED ON THE SOIL PARTICLES RATHER THAN THE SURFACE OF THE CERAMIC PLATE.

REMOVAL OF EVAPORATION DEPOSITS FROM PRESSURE PLATE CELL

IF AFTER A PERIOD OF TIME THE FLOW RATE OF THE CELL DROPS DUE TO DEPOSITS, THESE CAN BE REMOVED. CALCIUM CARBONATE DEPOSITS ON THE SURFACE OF THE CERAMIC CAN BE REMOVED BY CAREFULLY SANDING THE SURFACE WITH A FINE OR MEDIUM GRADE OF GARNET OR SANDPAPER.

DEPOSITS IN THE PORES OF THE CERAMIC CAN BE REMOVED BY FLUSHING THROUGH THE PRESSURE PLATE CELL UNDER PRESSURE IN THE EXTRACTOR A 10% SOLUTION OF HYDROCHLORIC OR OTHER INORGANIC ACIDS. THIS SHOULD BE FOLLOWED BY SIMILAR FLUSH OF CLEAR WATER.

BACTERIAL ACTION ON PRESSURE PLATE CELLS

FOR MOST SOILS AND WORK BACTERIAL ACTION IN THE PRESSURE PLATE CELL DOES NOT PRESENT A PROBLEM. HOWEVER, TO MINIMIZE THIS CONDITION, THE INTERNAL SCREEN IS OF [illegible]R IN ORDER TO RETARD BACTERIAL ACTION.

IN THOSE CASES WHERE BACTERIAL ACTION IS OF IMPORTANCE THE PRESSURE PLATE CELLS CAN BE FLUSHED UNDER PRESSURE PERIODICALLY WITH A SOLUTION OF COPPER SULFATE OR

RCURIC CHLORIDE IN THE MANNER DESCRIBED FOR REMOVAL OF EVAPORATION DEPOSITS.

CARE OF EXTRACTOR VESSEL

THE EXTRACTOR VESSEL AND TOP ARE RUGGEDLY CONSTRUCTED, WELL PLATED FOR PROTECTION AND SHOULD REQUIRE LITTLE ATTENTION.

IN HANDLING THE EXTRACTOR BE SURE TO PROTECT FROM DAMAGE THOSE AREAS WHERE THE "O" RING SEAL IS MADE. KEEP SOIL PARTICLES CLEAR OF THE "O" RING AND THE SEAT IN THE WALL OF THE PRESSURE VESSEL.

THE VESSEL IS ADDITIONALLY COATED ON THE INSIDE WITH AN ASPHALT BASE PAINT, "GILA COAT", MANUFACTURED BY THE W. P. FULLER PAINT CO. IN THE EVENT RUSTING DEVELOPS IN THE VESSEL IT CAN BE RECOATED AS NECESSARY WITH THIS OR A COMPARABLE MATERIAL.

GAS PRESSURE SOURCE, PRESSURE REGULATION, LABORATORY SETUP

PRESSURE REQUIREMENTS

THE 15 BAR CERAMIC PLATE EXTRACTOR REQUIRES A SOURCE OF REGULATED GAS PRESSURE OF 220 PSI OR MORE IN ORDER TO MAKE MOISTURE EXTRACTIONS FROM SOIL SAMPLES THROUGH THE WILTING POINT (15 BARS).

EXISTING PRESSURE SUPPLY

IF THE LABORATORY ALREADY HAS A REGULATED PRESSURE SOURCE FOR PRESSURE MEMBRANE IPMENT, THEN THIS SAME SUPPLY CAN BE USED FOR THE 15 BAR CERAMIC PLATE EXTRACTOR. SSURE CONNECTION FOR THE 15 BAR CERAMIC PLATE EXTRACTOR IS MADE TO THE SAME LINE THAT SUPPLIES AIR TO THE "EXTRACTION CHAMBER" ON THE PRESSURE MEMBRANE EXTRACTOR. APPROPRIATE SHUT OFF AND VENT VALVES SHOULD BE PROVIDED FOR THE NEW EXTRACTOR.

INITIAL SETUP

IF AN INITIAL SETUP IS BEING MADE FOR THE 15 BAR CERAMIC PLATE EXTRACTOR, THE PRESSURE SOURCE CAN BE EITHER A COMPRESSOR OR COMPRESSED GAS IN TANKS.

THE PM COMPRESSOR, CAT. NO. 500, PROVIDES A CONVENIENT, LOW COST PRESSURE SOURCE FOR ALL OF THE GAS PRESSURE EXTRACTORS AND CAN BE USED AS A PRESSURE SOURCE FOR THIS EXTRACTOR.

COMPRESSED NITROGEN OR AIR (2000 PSI) IN TANKS CAN BE USED, PARTICULARLY WHERE THE EXTRACTOR IS OPERATED ON A LIMITED BASIS. WHERE TANK GAS IS USED IT IS VERY IMPORTANT TO MAKE SURE THAT ALL PIPING IS LEAK FREE, SINCE A SMALL LEAK CAN WASTE A LARGE VOLUME OF GAS OVER A PERIOD OF A RUN.

THE INTERNAL VOLUME OF THE 15 BAR CERAMIC PLATE EXTRACTOR IS APPROXIMATELY 1/4 CU. FT. IF CONTINUOUS EXTRACTIONS ARE BEING MADE AT THE 15 BAR LEVEL AN AIR COMPRESSOR WILL BE DESIRABLE AS THE PRESSURE SOURCE.

PRESSURE REGULATION

THE TYPE OF PRESSURE REGULATOR REQUIRED WILL DEPEND ON THE TYPE OF PRESSURE SOURCE AND ON THE ACCURACY REQUIRED FOR THE STUDIES BEING CONDUCTED.

ACCURACY OF REGULATION IS DEPENDENT ON THE CONSTRUCTION OF THE REGULATOR AND ALSO VARIATIONS IN THE PRESSURE FROM THE SOURCE OF SUPPLY. WITH REGULATORS SUITABLE FOR USE WITH THIS EQUIPMENT ONE CAN EXPECT VARIATIONS IN THE SOURCE PRESSURE TO BE REFLECTED IN THE REGULATED PRESSURE IN THE RATIO OF ABOUT 1/12 to 1/25. IN OTHER WORDS, A

GE IN THE PRESSURE FROM THE COMPRESSED AIR SOURCE OF 25 PSI WILL CHANGE THE REGULATED PRESSURE BY 1 TO 2 PSI, DEPENDING ON THE MAKE OF THE REGULATOR. IN CASES WHERE EXTREME ACCURACY IS DESIRED THIS VARIATION CAN BE ELIMINATED BY THE PROCESS OF "DOUBLE REGULATION". THIS IS DONE SIMPLY BY PUTTING TWO REGULATORS IN SERIES. THE FIRST REGULATOR IS SET AT A SOMEWHAT HIGHER PRESSURE THAN THE SECOND IN ORDER TO SUPPLY REASONABLY CONSTANT PRESSURE TO THE SECOND REGULATOR. PRESSURE FROM THE SECOND REGULATOR IN TURN WILL BE VERY CONSTANT WITH SOURCE PRESSURE VARIATIONS REDUCED IN THE RATIO OF AT LEAST 1/100.

FOR ROUTINE DETERMINATIONS OF THE 15 BAR PERCENTAGE A SETUP USING A SINGLE HIGH PRESSURE REGULATOR IS ADEQUATE. AT LOWER PRESSURES IT WILL BE DESIRABLE TO MAKE USE OF A MORE SENSITIVE REGULATOR.

TO PROVIDE GOOD REGULATION THROUGHOUT THE WHOLE RANGE FROM 0 THROUGH 15 BARS TWO REGULATORS SHOULD BE USED. THE HIGH PRESSURE REGULATOR FOR THE HIGH RANGE WITH SIMPLE VALVING SO THAT THE PRESSURE FROM THE HIGH PRESSURE REGULATOR CAN BE DIVERTED TO THE LOW PRESSURE REGULATOR FOR WORK IN THE LOW RANGE, THUS MAKING USE OF THE PRINCIPLE OF "DOUBLE REGULATION" IN THE LOW PRESSURE RANGE.

WHERE COMPRESSED GAS IN TANKS IS USED AS A PRESSURE SOURCE, THE HOKE REGULATOR CAT. NO. 510B15 SERVES WELL AS THE HIGH PRESSURE REGULATOR. THIS CAN BE COUPLED WITH THE NORGREN REGULATOR CAT. NO. 20AG-X2G WITH 0-125 LB. COMPOUND SPRING FOR USE IN THE LOW PRESSURE RANGE.

WHERE A COMPRESSOR IS USED AS THE PRESSURE SOURCE, THE NORGREN REGULATOR CAT. NO. 20AG-X2G WITH 0-250 LB. SPRING CAN BE USED FOR THE HIGH PRESSURE WORK. THIS URN CAN BE COUPLED WITH THE NORGREN REGULATOR CAT. NO. 20AG-X2G WITH 0-125 LB. OUND SPRING FOR THE LOW PRESSURE WORK.

AIR FILTER USE WITH COMPRESSOR

IT IS DESIRABLE TO INSTALL AN AIR FILTER, SUCH AS NORGREN CAT. NO. 30AF-N2 AHEAD OF THE REGULATORS WHERE A COMPRESSOR IS USED AS THE PRESSURE SOURCE. THE FILTER HELPS TO KEEP SMALL DIRT PARTICLES OUT OF THE REGULATORS. WHEN THE REGULATED PRESSURE TENDS TO DRIFT APPRECIABLY FROM ITS SET VALUE IT IS USUALLY DUE TO AN IMPROPERLY SEATED VALVE IN THE REGULATOR; AND THIS IS FREQUENTLY DUE TO ACCUMULATION OF DIRT ON THE VALVE SEAT. INSTRUCTIONS FOR PROPER CARE AND MAINTENANCE OF REGULATORS ARE PROVIDED WITH THE REGULATORS.

PRESSURE GAUGE

FOR ACCURATE READOUT OF THE REGULATED PRESSURE A PRECISION PRESSURE GAUGE IS REQUIRED. THE ASHCROFT LABORATORY TEST GAUGE CAT. NO. 1082A WITH 0-300 PSI RANGE IS SUITABLE FOR USE WITH THE 15 BAR CERAMIC PLATE EXTRACTOR.

SOURCE OF PRESSURE REGULATING EQUIPMENT

PRESSURE CONTROL EQUIPMENT AND MANIFOLD FITTINGS CALLED OUT CAN BE OBTAINED THROUGH LOCAL DEALERS OR IF MORE CONVENIENT THROUGH SOILMOISTURE EQUIPMENT CO.

COMPLETE MANIFOLDS ASSEMBLED AND TESTED SUITABLE FOR USE WITH THIS EQUIPMENT CAN BE OBTAINED FROM SOILMOISTURE EQUIPMENT CO. WRITE FOR FURTHER DETAILS.

G A RUN FOR MOISTURE-RETENTION STUDIES

WORKING WITH THE 15 BAR CERAMIC PLATE EXTRACTOR, IS BASICALLY THE SAME AS WORKING

THE PRESSURE PLATE EXTRACTOR, CAT. NO. 1200.

HANDLING OF SOIL SAMPLES

PROPER HANDLING OF THE SOIL SAMPLES IS NECESSARY FOR ACCURATE, CONSISTENT RESULTS. FOR THIS, WE RECOMMEND THAT THE USER REFER TO THE PROCEDURES AS CALLED OUT IN THE AGRICULTURE HANDBOOK NO. 60 OF THE U.S. DEPARTMENT OF AGRICULTURE, DIAGNOSIS AND IMPROVEMENT OF SALINE AND ALKALI SOILS. THIS HANDBOOK COVERS DETAILED PROCEDURES FOR THE 1/10, 1/3, AND 15 BAR PERCENTAGE DETERMINATIONS, AND ON THE DEVELOPMENT OF MOISTURE-RENTENTION CURVES.

WHERE MOISTURE EQUILIBRIUM STUDIES ARE BEING RUN IT IS DESIRABLE TO KEEP SAMPLE HEIGHTS SMALL IN ORDER TO KEEP THE TIME TO REACH EQUILIBRIUM REASONABLE. THE TIME REQUIRED TO REACH EQUILIBRIUM VARIES AS THE SQUARE OF THE SAMPLE HEIGHT.

PREPARE DUPLICATE 25 GM. SAMPLES THAT HAVE BEEN PASSED THROUGH A 2 MM ROUND-HOLE SIEVE, FOR EACH SOIL TYPE TO BE RUN. PLACE SOIL SAMPLE RETAINING RINGS, CAT. NO. 1093, ON THE CERAMIC PLATE TO RECEIVE THE GROUP OF SAMPLES. EACH CERAMIC PLATE CELL WILL ACCOMODATE 12 SAMPLES WHEN RETAINED IN THESE RINGS. IN ORDER TO AVOID PARTICLE-SIZE SEGREGATION, DUMP ALL OF THE SOIL SAMPLE FROM EACH CONTAINER INTO ONE RING. POURING OUT PART OF THE SAMPLE AND LEAVING PART IN THE CONTAINER WILL GIVE A NONREPRESENTATIVE SAMPLE. LEVEL THE SAMPLES IN THE RING, COVER WITH SQUARES OF WAXED PAPER, AND ALLOW THE SAMPLES TO STAND AT LEAST 16 HOURS WITH AN EXCESS OF WATER ON THE PLATE.

IT IS DESIRABLE TO CONNECT THE NYLON TUBE AND RUBBER SLEEVE TO THE OUTLET STEM ON THE PRESSURE PLATE CELL PRIOR TO PLACING OF THE SAMPLES.

LOADING THE EXTRACTOR

WHEN THE SAMPLES ARE READY FOR THE EXTRACTOR REMOVE THE EXCESS WATER FROM THE CERAMIC PLATES WITH A PIPETTE OR SYRINGE, MOUNT THE CELLS IN THE EXTRACTOR AND CONNECT UP THE OUTFLOW TUBES. BE SURE THE TRIANGULAR SUPPORT IS IN THE BOTTOM OF THE VESSEL.

USE THE PLASTIC SPACERS TO SEPARATE THE PRESSURE PLATE CELLS. CLOSE ALL UNUSED OUTLET PORTS WITH THE PLUG BOLTS THAT ARE PROVIDED. BE SURE "O" RING IS IN PLACE, MOUNT LID, AND SCREW DOWN CLAMPING BOLTS.

CONNECTION TO A BURETTE

IT IS DESIRABLE TO PROVIDE A MEANS FOR DETERMINING WHEN EQUILIBRIUM HAS BEEN REACHED. THIS CAN BE EASILY DONE BY CONNECTING EACH OUTFLOW TUBE TO THE TIP OF A BURETTE WITH A PIECE OF SMALL DIAMETER TUBING. GAS DIFFUSING THROUGH THE CERAMIC PLATE PASSES CONTINUOUSLY IN SMALL BUBBLES THROUGH THIS SMALL OUTFLOW TUBE, AND KEEPS THE EXTRACTED LIQUID TRANSPORTED TO THE BURETTE. THE BURETTE CAN BE READ PERIODICALLY AND THE APPROACH TO EQUILIBRIUM CAN THUS BE FOLLOWED. IF THE PRESSURE IN THE EXTRACTOR IS MAINTAINED CONSTANT, NO MEASURABLE AMOUNT OF CHANGE IN THE BURETTE READING WILL BE OBSERVED OVER A PERIOD OF MANY HOURS OR DAYS AFTER EQUILIBRIUM IS ONCE ATTAINED.

TURNING ON THE PRESSURE

BUILD UP THE PRESSURE IN THE EXTRACTOR TO THE EQUILIBRIUM VALUE SOMEWHAT SLOWLY. THIS PROCEDURE WILL PERMIT YOU TO MAKE THE MOST ACCURATE SETTING ON THE EQUILIBRIUM V[illegible].

AS THE PRESSURE BUILD UP INSIDE THE EXTRACTOR THERE WILL BE A RUSH OF AIR FROM THE OUTFLOW TUBES. THIS IS CAUSED BY THE REDUCTION OF THE INTERNAL VOLUME OF THE

[illegible]SURE PLATE CELL AS THE DIAPHRAGM AND SCREEN COLLAPSE UNDER THE PRESSURE IN THE EXTRACTOR.

IF THE RUN IS FOR DETERMINATIONS OF THE 15 BAR PERCENTAGE, THE PRESSURE IN THE EXTRACTOR IS SET AT 15 BARS OR 220 PSI.

WHERE THE PM COMPRESSOR IS USED AS AN AIR SOURCE, IT IS POSSIBLE THAT THE COMPRESSOR TANK PRESSURE WILL BE REDUCED BELOW THE REQUIRED LEVEL IF THE EXTRACTOR PRESSURE IS SET IMMEDIATELY AT 220 PSI SINCE THE VOLUME OF THE EXTRACTOR IS LARGE COMPARED WITH THE AIR STORAGE TANK. WHEN THIS OCCURS, SIMPLY TURN THE TIMER DIAL ON THE COMPRESSOR, MANUALLY, IN THE CLOCKWISE DIRECTION UNTIL THE COMPRESSOR STARTS. A SINGLE RUN CYCLE ON THE COMPRESSOR WILL BUILD THE PRESSURE UP ABOVE THE PRESSURE VALUE REQUIRED.

REMOVAL OF SAMPLES

SAMPLES MAY BE REMOVED WHEN READINGS ON THE OUTFLOW BURETTES INDICATE FLOW HAS STOPPED AND EQUILIBRIUM ATTAINED. MOST SOILS WILL APPROACH HYDRAULIC EQUILIBRIUM WITHIN 18 TO 20 HOURS.

AT THE CLOSE OF A RUN THE EXTERNAL TUBES RUNNING FROM THE OUTFLOW TUBE ASSEMBLIES SHOULD BE REMOVED OR PINCHED OFF TO PREVENT POSSIBLE BACK FLOW OF WATER WHEN THE PRESSURE IN THE EXTRACTOR IS RELEASED.

IMMEDIATELY AFTER THE PRESSURE REGULATOR IS SHUT OFF AND THE PRESSURE EXHAUSTED FROM THE EXTRACTOR, THE CLAMPING BOLTS AND LID ARE REMOVED. SAMPLES ARE TRANSFERRED TO MOISTURE BOXES AS SOON AS POSSIBLE AFTER RELEASE OF PRESSURE IN ORDER TO AVOID CHANGES IN THE MOISTURE CONTENT.

ACTION OF GAS PRESSURE ON SOIL SAMPLES AND USES OF EXTRACTOR

AS SOON AS AIR PRESSURE INSIDE THE VESSEL IS RAISED ABOVE ATMOSPHERIC PRESSURE, THE HIGHER PRESSURE INSIDE THE VESSEL FORCES EXCESS WATER THROUGH THE MICROSCOPIC PORES IN THE 15 BAR CERAMIC PLATES. THE HIGH PRESSURE AIR, HOWEVER, WILL NOT FLOW THROUGH THE PORES SINCE THEY ARE FILLED WITH WATER AND THE SURFACE TENSION OF THE WATER AT THE GAS-LIQUID INTERFACE AT EACH OF THE PORES SUPPORTS THE PRESSURE MUCH THE SAME AS A FLEXIBLE RUBBER DIAPHRAGM. WHEN THE AIR PRESSURE IS INCREASED INSIDE THE EXTRACTOR THE RADIUS OF CURVATURE OF THIS INTERFACE DECREASES. HOWEVER, THE WATER FILMS WILL NOT BREAK AND LET AIR PASS THROUGHOUT THE WHOLE PRESSURE RANGE OF THE EXTRACTOR, FROM 0 TO 15 BARS.

AT ANY GIVEN AIR PRESSURE IN THE CHAMBER, SOIL MOISTURE WILL FLOW FROM AROUND EACH OF THE SOIL PARTICLES AND OUT THROUGH THE CERAMIC PLATE UNTIL SUCH TIME AS THE EFFECTIVE CURVATURE OF THE WATER FILMS THROUGHOUT THE SOIL ARE THE SAME AS AT THE PORES IN THE CERAMIC PLATE. WHEN THIS OCCURS, AN EQUILIBRIUM IS REACHED AND THE FLOW OF MOISTURE CEASES. WHEN THE AIR PRESSURE IN THE EXTRACTOR IS INCREASED, FLOW OF SOIL MOISTURE FROM THE SAMPLES STARTS AGAIN AND CONTINUES UNTIL A NEW EQUILIBRIUM IS REACHED. AT EQUILIBRIUM, THERE IS AN EXACT RELATIONSHIP BETWEEN THE AIR PRESSURE IN THE EXTRACTOR AND THE SOIL SUCTION (AND HENCE THE MOISTURE CONTENT) IN THE SAMPLES. FOR EXAMPLE, IF THE AIR PRESSURE IN THE EXTRACTOR IS MAINTAINED A 1 BAR OR ATMOSPHERE (15 PSI), THE SOIL SUCTION IN THE SAMPLES AT EQUILIBRIUM WILL BE AT 1 BAR. IF THE AIR PRESSURE IS MAINTAINED AT 15 BARS OR ATMOSPHERES (220 PSI) THE SOIL SUCTION AT EQUILIBRIUM WILL BE AT 15 BARS, WHICH IS THE APPROXIMATE WILTING POINT FOR ALL SOILS.

THE 15 BAR CERAMIC PLATE EXTRACTOR CAN BE USED FOR ALL TYPES OF STUDIES INVOLVING

HE MOISTURE RELATIONSHIPS IN SOILS. ALL TYPES OF SOIL SAMPLES MAY BE USED WITH THE XCEPTION OF FINE CLAY SOILS THAT EXPERIENCE CONSIDERABLE SHRINKAGE AS MOISTURE IS REMOVED. THIS TYPE OF SOIL WILL SHRINK AWAY FROM THE CERAMIC PLATE IN 15 BAR EXTRACTIONS AND THE REDUCED FLOW AREA WILL NOT PERMIT THE SAMPLE TO REACH EQUILIBRIUM.

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FOR MOISTURE EQUILIBRIUM STUDIES THROUGHOUT THE WHOLE PLANT GROWTH RANGE FROM 0 TO 15 BARS, THE 15 BAR CERAMIC PLATE EXTRACTOR PROVIDES A NEW DIMENSION IN EASE OF HANDLING AND EFFICIENCY OF OPERATION.

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A COMPLETE STOCK OF ACCESSORIES AND REPLACEMENT PARTS FOR THE 15 BAR CERAMIC PLATE EXTRACTOR IS MAINTAINED FOR PROMPT DELIVERY. DETAILS AND PRICES ARE EITHER CARRIED IN THE CURRENT CATALOG OR MAY BE OBTAINED BY WRITING TO:

NOTICE

IT IS ADVISABLE TO ALWAYS USE THE CAT. NO. 1595, RIGHT ANGLE OUTFLOW TUBE ADAPTER, WHEN STACKING TWO OR MORE PRESSURE PLATE CELLS IN THE EXTRACTOR.

HE STEM ON THE ADAPTER IS PUSHED INTO THE HOLE IN THE RUBBER CONNECTING SLEEVE, BE RE NYLON CONNECTING TUBE IS BUTTED UP NEXT TO THE STEM. THE RUBBER SLEEVE IS USED ONLY TO MAKE A SEAL AND CANNOT BE RELIED UPON TO SUPPORT THE HIGH EXTRACTION PRESSURES.

THE HOLE IN THE RIGHT ANGLE ADAPTER HAS AN INTERNAL "O" RING WHICH MAKES A PRESSURE SEAL WHEN IT IS SLIPPED OVER THE OUTLET STEM FROM THE CERAMIC PLATE CELL. THESE ADAPTERS ARE EXTREMELY EASY TO CONNECT AND DISCONNECT FROM THE PRESSURE PLATE CELLS, WHEN THE CELLS ARE LOADED AND UNLOADED FROM THE EXTRACTOR. THE ADAPTERS ELIMINATE ANY POSSIBLE KINKING OF THE OUTFLOW TUBE ASSEMBLY AND HENCE PREVENT ANY PINCHING OFF OF THE OUTFLOW TUBE THAT CAN RESULT IN ERRONEOUS EQUILIBRIUM VALUES AND POSSIBLE DAMAGE OR BREAKAGE, UNDER CERTAIN CONDITIONS, TO THE PRESSURE PLATE CELLS.

NOTICE

IT IS NOT ADVISABLE TO USE THE CAT. NO. 1590, 15 BAR CERAMIC PLATE CELLS, SUPPLIED WITH THIS UNIT FOR DETERMINATION OF THE 1/10 BAR AND 1/3 BAR MOISTURE PERCENTAGES OF SOILS. DUE TO THE VERY SMALL PORE SIZE OF THE 15 BAR CERAMIC THE FLOW RATE THROUGH THE CERAMIC PLATE IS VERY LOW AT PRESSURE DIFFERENTIALS ACROSS THE PLATE OF 1/10 BAR (1.5 PSI) AND 1/3 BAR (5 PSI). THIS RESULTS IN EXTREMELY LONG EQUILIBRIUM TIMES AND EQUILIBRIUM MOISTURE CONTENT VALUES WILL TEND TO BE HIGHER THAN ACTUAL. FOR THE MEASUREMENT OF THE 1/10 BAR AND 1/3 BAR MOISTURE PERCENTAGE AS WELL AS ALL OTHER WORK IN THE 0 TO 1 BAR RANGE IT IS ADVISABLE TO USE THE CAT. NO. 1290 PRESSURE PLATE CELLS. THESE PRESSURE PLATE CELLS HAVE MUCH LARGER PORE SIZE THAN THE 15 BAR CERAMIC PLATE CELLS, AND IN THE 0 TO 1 BAR RANGE EQUILIBRIUM VALUES WILL BE REACHED MUCH FASTER. HE CAT. NO. 1290 PRESSURE PLATE CELL WILL FIT INTO THE CAT. NO. 1500, 15 BAR CERAMIC LATE EXTRACTOR, AND THE SAME OUTFLOW TUBE CONNECTORS CAN ALSO BE USED WITH THESE CELLS.

INSTRUCTIONS FOR SETUP OF THE CAT. NO. 700-23 MANIFOLD

THE CAT. NO. 700-23 MANIFOLD SUPPLIED IS COMPLETELY ASSEMBLED AND TESTED AND READY FOR MOUNTING ON THE LABORATORY WALL. THE 3/4" THICK PLYWOOD BASE WHICH SUPPORTS THE VARIOUS COMPONENTS CAN BE DRILLED AT ANY CONVENIENT LOCATION FOR MOUNTING WITH WOOD SCREWS OR BOLTS IN THE LABORATORY WALL. NORMALLY, THE CAT. NO. 500-A PM COMPRESSOR IS SET ADJACENT TO THE LABORATORY BENCH AND THE PRESSURE CONTROL MANIFOLD. THE ATTACHED ENGINEERING DRAWING SHOWS A TYPICAL LABORATORY SETUP FOR THIS MANIFOLD. AS INDICATED ON THE ENGINEERING DRAWING, A CAT. NO. 710 CONNECTING HOSE COMBINATION IS USED FOR PRESSURE CONNECTION BETWEEN THE COMPRESSOR AND MANIFOLD. THIS CONNECTING HOSE COMBINATION HAS THE ELBOW FITTING WHICH SCREWS INTO THE BACK PRESSURE OUTLET ON THE COMPRESSOR TANK. THESE ARE 1/4" NPT PIPE THREADS AND A SUITABLE PIPE DOPE OR TAPE SHOULD BE USED TO MAKE A PRESSURE SEAL. A SHUT-OFF VALVE IS CONNECTED TO THIS ELBOW FITTING AND CAN BE USED FOR SHUTTING OFF THE AIR SUPPLY AT THE TANK. THE FLEXIBLE NEOPRENE HOSE THEN CONNECTS THIS SHUT-OFF VALVE WITH THE INLET FITTING ON THE MANIFOLD. THE THREAD SIZE OF THIS CONNECTING HOSE AS WELL AS THE CONNECTING HOSE USED TO CONNECT THE VARIOUS EXTRACTORS TO THE MANIFOLD IS 9/16-18. PRESSURE SEAL AT THE HOSE CONNECTION IS MADE WHEN THE ROUND "NOSE" OF THE BRASS STEM INSIDE THE HOSE NUT IS PRESSED AGAINST THE RECESSED CONICAL SURFACE OF THE PRESSURE FITTINGS. THIS IS A METAL-TO-METAL SEAL AND IS VERY EFFECTIVE. THE SCREW THREADS ON THE FITTING AND HOSE NUT ONLY SERVE AS A MEANS OF HOLDING THE PARTS IN CONTACT. THE THREADS THEMSELVES DO NOT MAKE A SEAL. ONLY A SMALL AMOUNT OF TORQUE IS REQUIRED AND SHOULD BE USED IN CONNECTING THE HOSES.

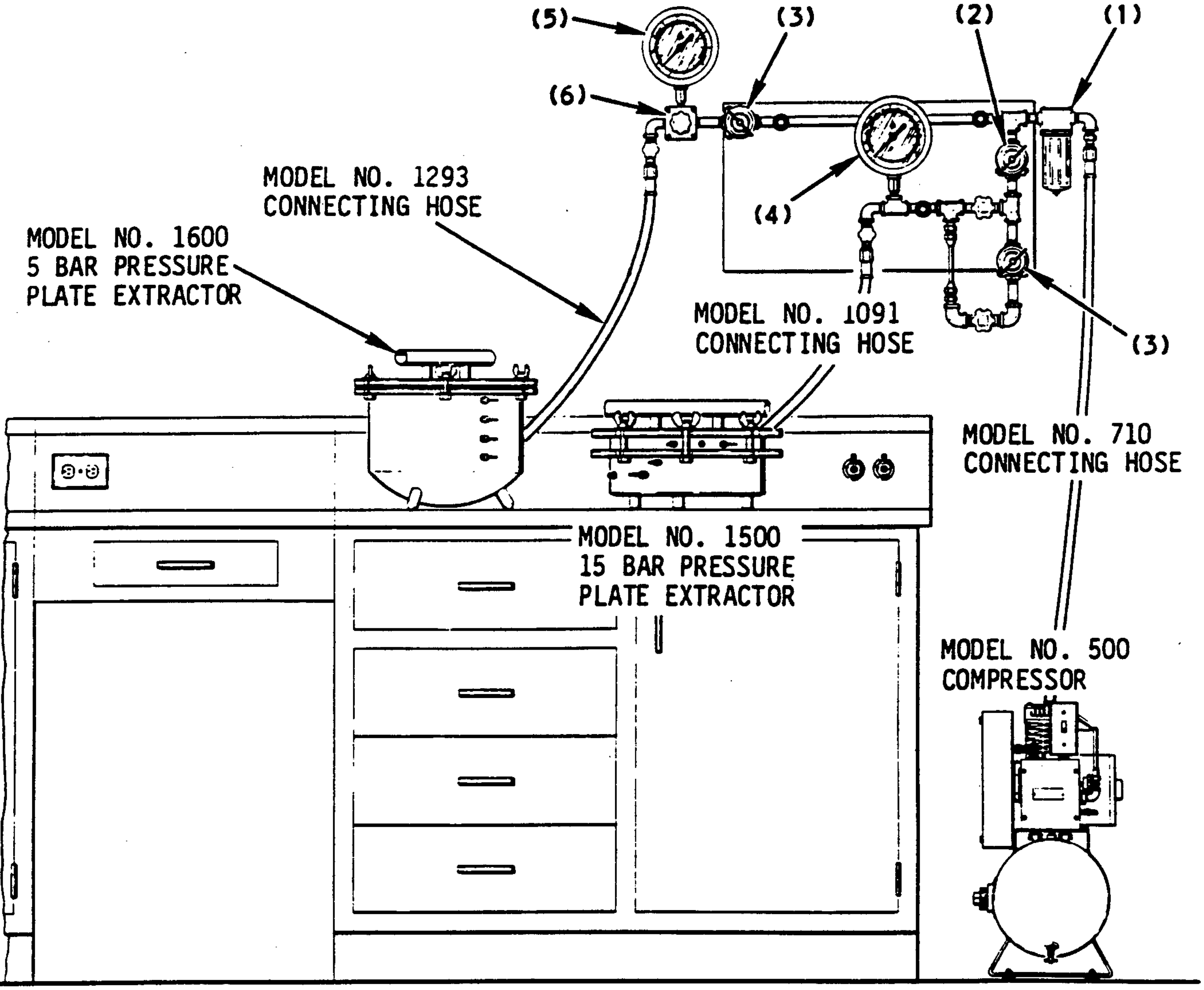
THE CAT. NO. 700-23 MANIFOLD IS A COMBINATION MANIFOLD COMBINING THE 700-2 STATION AND THE 700-3 STATION. THE 700-2 STATION IS FOR OPERATION OF THE CAT. NO. 1500 15 BAR CERAMIC PLATE EXTRACTOR. THE CAT. NO. 700-3 STATION PROVIDES VERY PRECISE LOW PRESSURE REGULATION IN THE PRESSURE RANGE FROM 0 TO 50 PSI AND IS USED FOR OPERATION OF THE CAT. NO. 1200 PRESSURE PLATE EXTRACTOR, CAT. NO. 1250 VOLUMETRIC PRESSURE PLATE EXTRACTOR, CAT. NO. 1400 AND 1450 TEMPE PRESSURE CELLS, AND THE CAT. NO. 1600 5 BAR EXTRACTOR.

THE CAT. NO. 700-2 STATION CONSISTS OF A CAT. NO. 11-002-017, 0 TO 250 PSI, PRESSURE REGULATOR; A CAT. NO. 11-002-013, 0 TO 125 PSI, PRESSURE REGULATOR; A CAT. NO. 1082-A, 0 TO 300 PSI, TEST GAUGE; AND ALL NECESSARY VALVES AND FITTINGS. IN SETTING EXTRACTION PRESSURES IN THE RANGE FROM 125 PSI TO 225 PSI, VALVE A, REFERENCE THE ATTACHED ENGINEERING DRAWING, IS OPENED, AND VALVE B IS CLOSED. ALL PRESSURE REGULATION IS THEN DONE WITH THE ONE CAT. NO. 11-002-017 REGULATOR. THE REGULATOR IS TURNED CLOCKWISE FOR HIGHER PRESSURE VALUES AND THE PRESSURE IS READ DIRECTLY ON THE TEST GAUGE. FOR LOW EXTRACTION PRESSURES IN THE RANGE FROM 0 TO 125 PSI, VALVE A IS CLOSED AND VALVE B IS OPENED. THE HIGH PRESSURE REGULATOR, CAT. NO. 11-002-017, IS SET FOR A PRESSURE VALUE IN EXCESS OF 125 PSI, AND USUALLY IN THE RANGE OF 125 TO 150 PSI. THIS HIGH PRESSURE REGULATOR THEN SUPPLIES PRESSURE TO THE 11-002-013 LOW PRESSURE REGULATOR. THIS LOW PRESSURE REGULATOR IS THEN SET FOR THE EXTRACTION PRESSURE DESIRED AND THE PRESSURE IS READ OUT ON THE TEST GAUGE.

THIS SYSTEM FOR LOW PRESSURE REGULATION IS KNOWN AS "DOUBLE REGULATION" AND IS FREQUENTLY USED TO PROVIDE VERY ACCURATE CONTROL OF PRESSURE. ALL REGULATORS REFLECT IN THEIR OUTPUT PRESSURE VARIATIONS PRESENT IN THE PRESSURE FROM THE SOURCES OF SUPPLY. BY PLACING TWO REGULATORS IN SERIES, SUCH AS MENTIONED ABOVE, VARIATIONS IN THE OUTPUT PRESSURE FROM THE FIRST REGULATOR ARE CONSIDERABLE REDUCED BY THE SECOND REGULATOR SO THAT THE OUTPUT PRESSURE FROM THE SECOND REGULATOR IS VERY CONSTANT WITH SOURCE PRESSURE VARIATIONS REDUCED IN THE RATIO OF 1:100 OR MORE.

LAB 023
LABORATORY SET-UP

700-23 MANIFOLD WITH MODEL NO. 1500 AND MODEL NO. 1600 PRESSURE EXTRACTORS



MODEL NO. 700-23 MANIFOLD, INCORPORATING:

(1) #760G1 AIR FILTER (FORMERLY #12-005-013)
(2) #766P0250 REGULATOR (FORMERLY 11-002-017)
(3) #766P0125 REGULATOR, TWO (FORMERLY 11-002-013)
(4) #780P0300 0-300 PSI TEST GAUGE (FORMERLY 1082-A)
(5) #780P0060 0-60 PSI TEST GAUGE (FORMERLY 1082-A)
(6) #765 NULLMATIC REGULATOR (FORMERLY 40-50)
ALL NECESSARY VALVES AND FITTINGS

LABORATORY SETUP FOR MODEL NO. 1500, 15 BAR CERAMIC PLATE EXTRACTOR AND MODEL NO. 1600, 5 BAR EXTRACTOR USING MODEL NO. 1290 PRESSURE PLATE CELL OR MODEL NO. 1690, 3 BAR CERAMIC PLATE CELL, OPERATING INDEPENDENTLY WITH THE MODEL NO. 500 COMPRESSOR AS A PRESSURE SOURCE.

AT THE END OF A RUN WHEN IT IS DESIRED TO EXHAUST THE AIR FROM THE EXTRACTOR, IT IS ONLY NECESSARY TO CLOSE EITHER OF THE REGULATORS BEING USED BY TURNING IN A COUNTERCLOCKWISE DIRECTION. AS THIS IS DONE, THE AIR FROM THE EXTRACTOR WILL EXHAUST THROUGH THE REGULATOR. THIS IS A FEATURE OF "RELIEVING TYPE" REGULATORS AND IT ELIMINATES THE NECESSITY OF HAVING A SEPARATE EXHAUST VALVE. ON A RELIEVING TYPE REGULATOR, ANY PRESSURE ON THE OUTPUT SIDE OF THE REGULATOR WHICH IS IN EXCESS OF THE PRESSURE VALUE SET BY THE REGULATOR WILL AUTOMATICALLY EXHAUST THROUGH THE REGULATOR MECHANISM.

THE 700-3 STATION CONSISTS OF A CAT. NO. 11-002-013, 0 TO 125 PSI, PRESSURE REGULATOR; A NULLMATIC PRESSURE REGULATOR, 0 TO 60 PSI; CAT. NO. 1082-A, 0 TO 60 PSI TEST GAUGE; AND ALL NECESSARY VALVES AND FITTINGS. IN MAKING PRESSURE SETTINGS AT THIS STATION, IT IS IMPORTANT TO CAREFULLY ADJUST BOTH OF THE PRESSURE REGULATORS. THE NULLMATIC REGULATOR CONTINUOUSLY EXHAUSTS A CERTAIN AMOUNT OF AIR WHEN IT IS USED ON "DEAD END SERVICE" SUCH AS IS THE CASE WITH OUR EXTRACTORS. THE AMOUNT OF AIR EXHAUSTED IS PROPORTIONAL TO THE PRESSURE DIFFERENCE BETWEEN THE SUPPLY AIR AND THE PRESSURE SETTING OF THE DELIVERED AIR. FOR NORMAL USE AND FOR MAXIMUM CONSERVATION OF COMPRESSED AIR FROM THE TANK, THE CAT. NO. 11-002-013 REGULATOR SHOULD BE SET AT A PRESSURE 2-3 PSI HIGHER THAN THE EQUILIBRIUM PRESSURE THAT YOU WISH TO DELIVER FROM THE NULLMATIC REGULATOR. WHEN THIS PROCEDURE IS FOLLOWED, THE AMOUNT OF AIR ESCAPING FROM THE NULLMATIC REGULATOR IS IN THE ORDER OF 2/100 CU.FT. OF AIR PER MINUTE. THIS AMOUNT OF AIR IS VERY EASILY BUILT UP BY THE COMPRESSOR PUMP IN THE COURSE OF ITS PUMPING CYCLE.

WHEN SETTING THE PRESSURE FOR A RUN, THE PROCEDURE IS AS FOLLOWS, REFERENCE THE ATTACHED ENGINEERING SKETCH. THE VALVE AT THE END OF THE CONNECTING HOSE IS FIRST CLOSED. THE NULLMATIC REGULATOR IS THEN OPENED A NUMBER OF TURNS SO THAT YOU ARE SURE IT IS SET AT A PRESSURE CONSIDERABLY ABOVE THAT WHICH YOU PLAN TO USE. THEN, THE CAT. NO. 11-002-013 REGULATOR IS OPENED SO THAT THE PRESSURE CAN FLOW THROUGH THE NULLMATIC REGULATOR AND REGISTER ON THE TEST GAUGE. THE CAT. NO. 11-002-013 REGULATOR IS ADJUSTED SO THAT THE PRESSURE GAUGE READS, SAY, 3 PSI HIGHER THAN THE EQUILIBRIUM PRESSURE YOU PLAN TO USE IN THE EXTRACTOR. THE NULLMATIC REGULATOR IS NOW CLOSED UNTIL THE EXCESS AIR IS EXHAUSTED UP TO THE PRESSURE VALUE YOU DESIRE IN THE EXTRACTOR AND WHICH WILL NOW BE REGISTERED ON THE TEST GAUGE. THE VALVE TO THE PRESSURE EXTRACTOR CAN NOW BE OPENED AND THE REGULATORS WILL MAINTAIN THE PRESSURE IN THE EXTRACTOR AT THE VALUE SET.

IN ORDER TO EXHAUST THE AIR FROM AN EXTRACTOR AFTER A RUN, EITHER OF THE PRESSURE REGULATORS ARE SIMPLY CLOSED IN A COUNTERCLOCKWISE DIRECTION AND, SINCE THESE ARE BOTH RELIEVING TYPE REGULATORS, THE AIR FROM THE EXTRACTOR WILL EXHAUST THROUGH THE REGULATOR.

EQUIPMENT LIST WITH NORMAL ACCESSORIES:

1	1500	15 BAR CERAMIC PLATE EXTRACTOR
1	1080	PM HINGE
1	1081	ADAPTER PLATE
1	1091	CONNECTING HOSE
6	1093	SOIL SAMPLE RETAINING RINGS, per dz.
1	1600G1	5 BAR PRESSURE PLATE EXTRACTOR WITH 4 EA. MODEL NO. 1290 1 BAR PRESSURE PLATE CELLS
2	1690	3 BAR PRESSURE PLATE CELL
1	1293	CONNECTING HOSE, 40" long
1	700-23	MANIFOLD
1	710	CONNECTING HOSE COMBINATION, 60" LONG
1	500	PM COMPRESSOR - SEE ORDERING INFO BELOW

ORDERING INFORMATION:

When ordering, please specify one of the Model No.'s below

LAB 023	Laboratory Set-up less Compressor
LAB 023G1	Laboratory Set-up w/110V, 60CY. Compressor
LAB 023G2	Laboratory Set-up w/230V, 60CY. Compressor
LAB 023G3	Laboratory Set-up w/110V, 50CY. Compressor
LAB 023G4	Laboratory Set-up w/230V, 50CY. Compressor

ADDRESS ORDERS TO:

SOILMOISTURE EQUIPMENT CORP.
P.O. Box 30025
Santa Barbara, CA 93105 U.S.A.

Telephone:
Area Code 805 964-3525

Cable Address:
Soilcorp

Plant and Office Location:
801 South Kellogg Ave.
G[illegible] CA 93117

All prices are in U.S. Dollars, F.O.B. Santa Barbara, California, U.S.A. — Subject to change without notice.

On Export Orders we are prepared to handle details of export packing and forwarding and will submit Proforma Invoices covering all costs delivered, upon receipt of detailed requirements.

SOILMOISTURE

Appendix B-19 – Lab Procedures for Chelates: TVA HPLC Method

Chelate Method is under development and will be added at a later date.

Appendix B-20 – Lab Procedures for Total Metals: Scanning Electron Microscope for Plants

Scanning Electron Microscope Method is under development and will be added at a later date.

APPENDIX C

SAMPLING AND EXCAVATION PLANS

Appendix C-1 – Soil Sampling Plan for Lead Contaminated Soil at the Sunflower AAP, Desoto, Kansas

SOIL SAMPLING PLAN
For
LEAD CONTAMINATED SOIL
at the
SUNFLOWER AAP, DESOTO, KANSAS

Prepared for the
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401
and the
U.S. ARMY CORPS OF ENGINEERS
Kansas City District

Prepared by
Tennessee Valley Authority
Environmental Research Center
Muscle Shoals, Alabama 35660-1010

September, 1996
TVA Contract No. RG-99712V

NOTICE

This Soil Sampling Plan for Lead Contaminated Soil at the Sunflower AAP, Desoto, Kansas, was prepared by employees of the Tennessee Valley Authority (TVA) loaned to the U.S. Army Environmental Center (USAEC) at Aberdeen Proving Grounds, Maryland, 21010-5401, pursuant to the provisions of TVA Contract RG-99712V and Military Interdepartmental Purchase Order Request (MIPR) MIPR 9526.

Under that agreement and MIPR, TVA provided the services mutually agreed upon as loaned employees. In regard to the services provided by the TVA employees, sections d and e of the contract and MIPR state as follows:

d. TVA will provide the services of mutually agreed upon loaned employees for purposes of the MIPR. It is expressly understood and agreed that services of such loaned employees will be made available, at TVA's discretion, when the schedule for such services is consistent with TVA's requirements and that TVA does not guarantee the availability of such loaned employees' services at any time during the term of this agreement.

e. It is expressly understood that for all purposes under this MIPR the TVA employees will be acting as loaned employees and will be under the complete supervision and control of the Army at all times and that TVA shall not and cannot supervise or control such employees during the time that they are providing services to the Army. It is further understood and agreed that neither TVA nor any of the loaned employees warrant or guarantee the advice under this agreement and that the Army is solely responsible for determining the suitability and acceptability of such advice and consultations for any purpose. Neither TVA, its agents and employees, nor the loaned employees assume any liability, or responsibility to the Army, its agents, employees, or contractors, or any third party for any costs, charges, damages, (either direct or consequential), demands, claims, or causes of action for any personal injuries (including death) or damage to property, real or personal, or delays arising out of or resulting from any such action or failures to act on the part of such loaned employees whose services are provided under this MIPR.

As provided above, this report was prepared by the TVA loaned employees under direct supervision and control of the U.S. Army. The U.S. Army is solely responsible for its content and use and not TVA, its employees or agents. Wherever it appears in this report, the term "TVA" shall mean TVA loaned employees which are subject to sections d and e quoted.

SECTION 1.0

INTRODUCTION

Disposal and burning of scrap ammunition and powder and similar activities have resulted in contamination of soils by lead (Pb) and other heavy metals at a number of Department of Defense installations. Lead has been identified under CERCLA as a priority element for remediation in contaminated soils, prompting the need for effective procedures for lead removal.

As part of the Department of Defense (DoD) program to evaluate treatment technologies, the U.S. Army Environmental Center (USAEC) has funded a project to assess the effectiveness of phytoremediation procedures for extraction of lead from contaminated soil. In phytoremediation, plants are used to extract lead from the soil and translocate the lead to the plant shoots for removal by harvesting. Soil amendments are used to enhance plant uptake and translocation. This project has been executed under an agreement among the:

- U.S. Army Environmental Center (USAEC)
- Tennessee Valley Authority Environmental (TVAE)

The USAEC and the U.S. Army Corp of Engineers (USACE) Kansas City District are providing contaminated soil from the Sunflower Army Ammunition Plant at Desoto, Kansas. TVAE is providing technical expertise in plant lead uptake, application of soil amendments, and metals analysis for soil and plant samples.

Part of this project consists of screening sources of contaminated soil, collecting samples of this soil, and analyzing the soil to determine the degree of heavy metal contamination. In a later phases of the project, samples of the soil will be excavated and shipped to TVAE's facilities in Muscle Shoals, Alabama , for use in greenhouse experiments.

This sampling plan outlines the methods to be used for collecting lead contaminated soil samples at SFAAP for the purpose of characterizing and mapping selected soil at two sites for soil type and degree and location of lead and other heavy metal contamination. After this procedure is complete TVAE will return to SFAAP for the purpose of excavating the soil to be used in the experiments at Muscle Shoals.

The plan presented here is limited to the soil sampling phase of this project and does not include methods to be used during the soil excavation phase. Sampling procedures for soil excavation will be issued at a later date.

SECTION 2.0

SAMPLING PLAN

2.1 Overview of Sampling Operations

The purpose of the sampling operations will be to characterize and map the soil and the sampling sites for soil type and degree and location of lead and other heavy metal contamination. Sampling will be conducted by collecting multiple soil cores taken at various depths from two contaminated sites.

2.2 Sample Collection and Analytical Procedures

2.2.1 Soil Sampling Procedures (Initial Characterization)

The sampling will be conducted on an explosives burning ground located at the Sunflower Army Ammunition Plant in Desoto, Kansas. The explosives burning ground consists of five approximately 1 acre "cells" plus additional outlying areas of approximately 7-10 acres. Lead contamination in the burning grounds originated from the burning of N-5 propellant, a mixture of organic and Lead-organic compounds. The range of Lead contamination over the burning area is 10-15,800 mg/kg. Other heavy metals are also present in varying concentrations.

Two sites have been selected for soil sampling, one site will be located in Cell 1 and the other in Cell 7 (Figure 2-1). Soil physical analysis shows the soil in Cell 1 to be an alluvial silty clay (50% silt, 50% clay); the soil in Cell 7 is an alluvial silt loam (60% silt, 25% sand, and 15% clay). Cell 7 is within 850 feet of a flowing creek, while Cell 1 is approximately 1500 feet distant. Both cells are located on a sloping, grassy meadow. The soil in this area is classified as alluvial, or that which resulted from water deposition. There is sufficient distance between cells that there is a distinct difference in textural

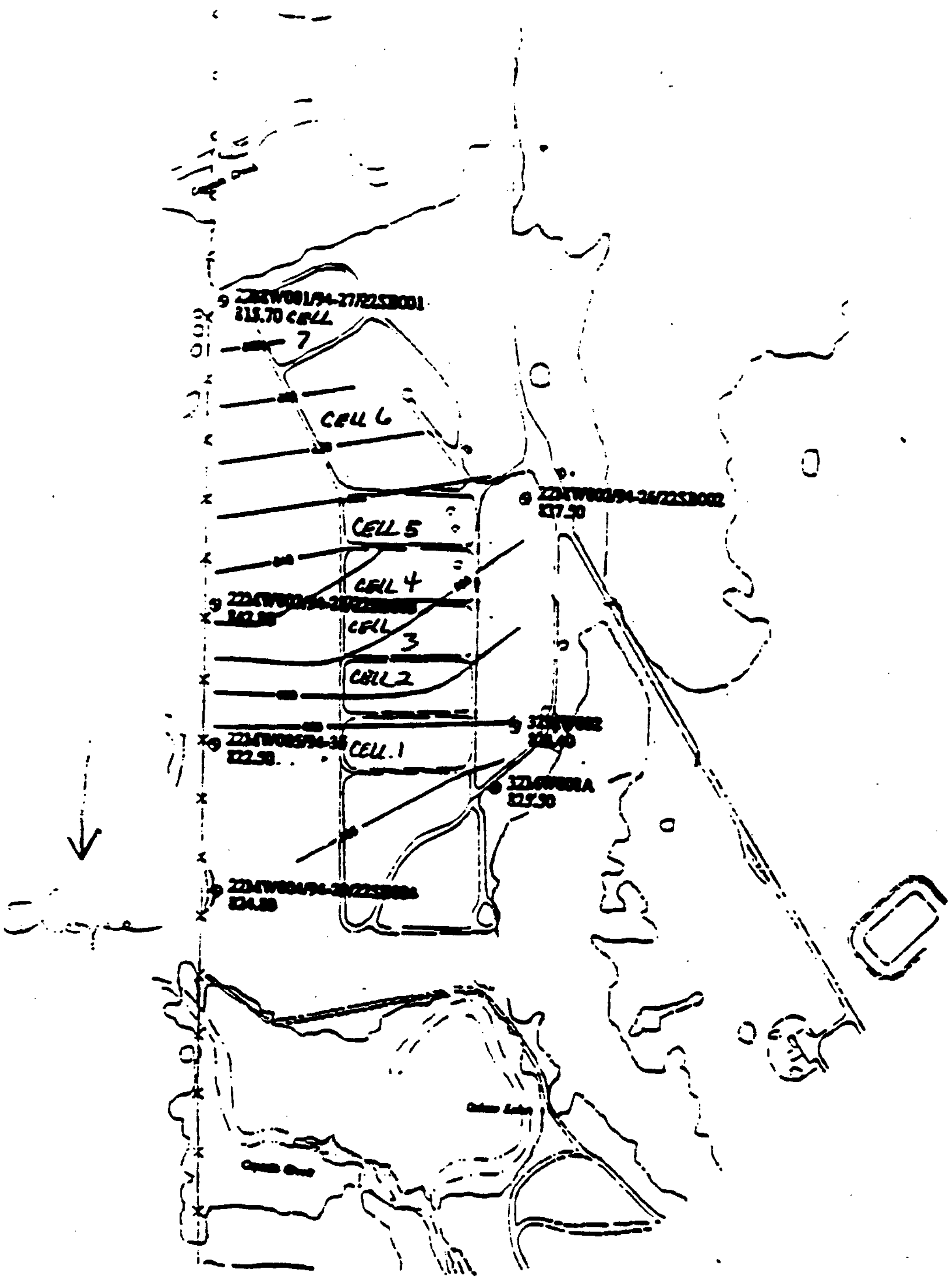


Figure 2-1 : Location of Cells 1 and 7 at the SFAAP in Desoto, Kansas classification in the soil, and thus for the purpose of this project, the soil may be considered as being of two distinct types.

Soil sampling will be performed by TVA personnel. Safety precautions and site controls to be used during the sampling procedure are outlined in the Health and Safety plan. The sampling procedure will be as follows:

1. TVA personnel will select and mark one 90 feet x 90 feet area within each of Cell 1 and Cell 7.

2. TVA personnel will then subdivide the area into 36 fifteen foot square grids (1 grid every 6 feet).

3. TVA personnel will further subdivide each fifteen foot grid into four seven and one-half foot squares.

4. Then using a hand held soil probe, TVA personnel will take one soil core to a depth of 12 inches from each 7.5 foot square and subdivide this core by depth into two portions (0-6, and 6-12 inches). TVA personnel will then composite the cores taken from the four 7.5 foot squares, according to depth, into one sample for each depth and place into an appropriately identified and labeled plastic zip-loc bags.

5. Package samples for shipment to ERC and transfer to the TVAE's Environmental Applications Analytical Laboratories (EAAL) in Muscle Shoals, AL, in accordance with TVAE's chain of custody procedures (EAAL procedure SP-0001, "Sample Chain of Custody").

A total of 144 samples will be taken (36 grids/site x 2 depths/sample core x 2 sites = 144). Upon leaving the sampling site all TVA personnel involved in the sampling procedure will undergo decontamination as per the Heath and Safety plan.

Appendix C-2 – Soil Excavation Plan for Lead Contaminated Soil at the Sunflower AAP, Desoto, Kansas

DRAFT SOIL EXCAVATION PLAN
For
LEAD CONTAMINATED SOIL
at the
SUNFLOWER AAP, DESOTO, KANSAS

Prepared for the
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401
and the
U.S. ARMY CORPS OF ENGINEERS
Kansas City District

Prepared by
Tennessee Valley Authority
Environmental Research Center
Muscle Shoals, Alabama 35660-1010

October, 1996
TVA Contract No. RG-99712V

NOTICE

This Soil Excavation Plan for Lead Contaminated Soil at the Sunflower AAP, Desoto, Kansas, was prepared by employees of the Tennessee Valley Authority (TVA) loaned to the U.S. Army Environmental Center (USAEC) at Aberdeen Proving Grounds, Maryland, 21010-5401, pursuant to the provisions of TVA Contract RG-99712V and Military Interdepartmental Purchase Order Request (MIPR) MIPR 9526.

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>
> e. It is expressly understood that for all purposes under this MIPR the TVA employees will be acting as loaned employees and will be under the complete supervision and control of the Army at all times and that TVA shall not and cannot supervise or control such employees during the time that they are providing services to the Army. It is further understood and agreed that neither TVA nor any of the loaned employees warrant or guarantee the advice under this agreement and that the Army is solely responsible for determining the suitability and acceptability of such advice and consultations for any purpose. Neither TVA, its agents and employees, nor the loaned employees assume any liability, or responsibility to the Army, its agents, employees, or contractors, or any third party for any costs, charges, damages, (either direct or consequential), demands, claims, or causes of action for any personal injuries (including death) or damage to property, real or personal, or delays arising out of or resulting from any such action or failures to act on the part of such loaned employees whose services are provided under this MIPR.

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SECTION 1.0

INTRODUCTION

Disposal and burning of scrap ammunition and powder and similar activities have resulted in contamination of soils by lead (Pb) and other heavy metals at a number of Department of Defense installations. Lead has been identified under CERCLA as a priority element for remediation in contaminated soils, prompting the need for effective procedures for lead removal.

As part of the Department of Defense (DoD) program to evaluate treatment technologies, the U.S. Army Environmental Center (USAEC) has funded a project to assess the effectiveness of phytoremediation procedures for extraction of lead from contaminated soil. In phytoremediation, plants are used to extract lead from the soil and translocate the lead to the plant shoots for removal by harvesting. Soil amendments are used to enhance plant uptake and translocation. This project has been executed under an agreement among the:

- U.S. Army Environmental Center (USAEC)
- Tennessee Valley Authority Environmental (TVAE)

The USAEC and the U.S. Army Corp of Engineers (USACE) Kansas City District are providing contaminated soil from the Sunflower Army Ammunition Plant at Desoto, Kansas. TVAE is providing technical expertise in plant lead uptake, application of soil amendments, and metals analysis for soil and plant samples.

Part of this project consists of screening sources of contaminated soil, collecting samples of this soil, and analyzing the soil to determine the degree of heavy metal contamination. In a later phases of the project, samples of the soil will be excavated and shipped to TVAE's facilities in Muscle Shoals, Alabama , for use in greenhouse experiments.

This sampling plan outlines the methods to be used for excavating lead contaminated soil at SFAAP for the purpose of using the soil in the experiments at Muscle Shoals. The plan presented here is limited to the soil excavation phase of this project.

SECTION 2.0

SAMPLING PLAN

2.1 Background

During the week of September 23, 1996, TVAE personnel sampled soil at the explosives burning ground located at the Sunflower Army Ammunition Plant in Desoto, Kansas. The explosives burning ground consists of five approximately 1 acre "cells" plus additional outlying areas of approximately 7-10 acres. The soil in the area is generally classified as a Kennebec alluvial silt loam, although there are distinct textural differences ranging from the silt loam to a silty clay. Soil core samples were taken from an area in Cell 1 and from an area in the northern-most outlying area (wherein is located soil drilling site 22MW001/94-27 - see accompanying map, Figure2-1). For the purposes of this plan the northern-most area is designated as Cell 7. Soil physical analysis shows the soil in Cell 1 to be alluvial silty clay; the soil in the Cell 7 is an alluvial silt loam (60% silt, 25% sand, and 15% clay). The Cell 7 is within 850 feet of the northern-most arm of a flowing creek (Captain Creek), while Cell 1 is approximately 1500 feet distant to the south. Both areas are located on a sloping, grassy meadow. There is sufficient distance between cells that there is a distinct difference in textural classification in the soil, and thus for the purpose of this project, the soil may be considered as being of two distinct types.

Lead contamination in the cells originated from the burning of N-5 propellant, a mixture of organic and Pb-organic compounds. The range of Pb contamination over the burning area is 10-15,800 mg/kg. Other heavy metals are also present in varying concentrations

The purpose of the current operation will be to excavate soil from the two sites sampled during TVAE's September visit.

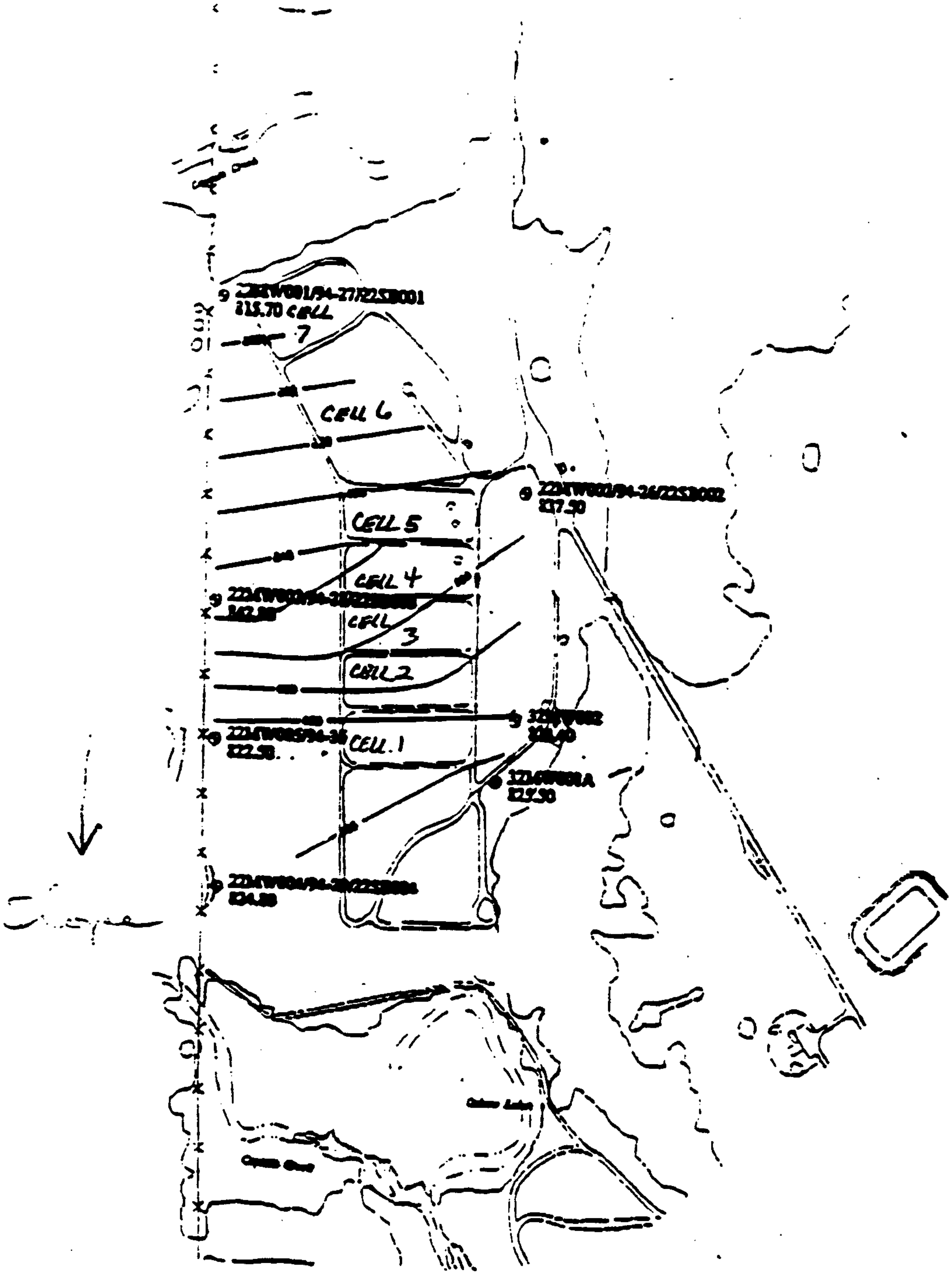

Figure 2-1 : Location of Cells 1 and 7 at the SFAAP in Desoto, Kansas

2.2 Soil Excavation Procedures

During the present operation, soil will be excavated from the two sites sampled during the soil sampling phase of this project. The soil excavation will be performed by TVAE personnel. Safety precautions and site controls to be used during the soil excavation procedure are outlined in the Health and Safety plan. The soil excavation procedure will be as follows:

Based on the criteria of soil texture and total Pb content, bulk quantities of soil will be collected by TVAE personnel from the two sites identified during the sampling phase of this project. 1,000 kg of soil is to be collected from each site. The soil will be collected with hand tools by shoveling the soil into 55 gallon steel drums lined with a heavy duty plastic barrel liner. The soil will be collected to a depth of twelve inches, and there will be a total of about ten drums. The soil in each drum will be labeled appropriately for identification and for DOT regulatory requirements for hazardous waste shipment, and shipped by best available method to ERC, Muscle Shoals, AL.

The procedure will be as follows:

1. Soil will be collected from the previously marked and flagged sampling areas designated in Section 4.2.2.

2. Pre-determine and record the empty weight of the 55 gallon steel drums.

3. Determine the approximate weight of soil excavated by one shovel blade in order to keep a running estimate of the cumulative weight of the soil as it is collected.

4. Collect one shovel full of soil down to 12" depth from each of the four quadrants within each fifteen foot grid and place into plastic-lined 55 gallon steel drums.

5. Load drums onto a suitable vehicle for transport to a weighing station on the Sunflower Plant site and determine the total weight of soil collected by subtracting the pre-recorded weight of each drum from the total weight of soil and drum.

6. Upon leaving the sampling site all TVA personnel involved in the sampling procedure will undergo decontamination as per the Heath and Safety plan.

7. Prepare the appropriate chain of custody documents and ship the containers of soil by motor freight to ERC, Muscle Shoals, AL.