State of Michigan
Department of Environmental Quality

LABORATORY

TRAINING MANUAL FOR WASTEWATER TREATMENT PLANT OPERATORS

2008

Prepared by:

Operator Training and Certification Unit
Environmental Science and Services Division

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INTRODUCTION

The role of the Wastewater Treatment Plant Operator has become very important in the prevention of environmental degradation here in Michigan. The operator is expected to optimize treatment to obtain the highest quality effluent possible as well as to demonstrate that the effluent is indeed within the set standards. The laboratory, of course, is essential to the operator in providing the data to meet these two goals. This manual was prepared by the MDEQ Operator Training and Certification Unit as a training tool to be used by those attending laboratory training courses presented by this unit. Included are procedures that meet governmental regulations for effluent monitoring as well as procedures to provide reliable data that can be used to make decisions in the day-to-day control of the treatment facility. Also included are a number of analytical methods that may be used to monitor influent levels of contaminants that would have a negative impact on treatment and the environment.

This manual is not intended to replace other more extensive methods manuals but to be a clarification of many of the procedures specifically applicable to the wastewater treatment field. The procedures are presented in a step by step "cookbook" style. This provides an easy to follow and understandable format but any unusual conditions or applications may require reference to the other more general manuals.

Many of the procedures meet the requirements of the National Pollutant Discharge Elimination System Program (NPDES) and may be used to demonstrate that effluent discharges meet applicable pollutant discharge limitations. Those that do meet these requirements are identified at the beginning of the procedure.

Discussions of the significance as well as the means of treatment of several of the parameters are included in the manual. These are intended to help the operator understand the vital link between laboratory data and plant operations and control.

Also included are several general discussions such as lab safety, sample handling and preservation, and laboratory quality assurance. Users of this manual are encouraged to read through these and follow the suggestions given to help ensure the highest possible reliability of the data generated in the lab.

LABORATORY SAFETY

One of the major concerns of any worker is to be safe while on the job. Both from the aspect of personal safety and from the aspect of liability for employees, the concern is justified. Many areas of wastewater treatment involve certain potential hazards and the analytical laboratory is certainly no exception. The key to job safety in spite of this is the recognition of the hazards involved in laboratory work, an understanding of what can be done to reduce the risk of having an accident, and knowing the proper responses to accidents that may occur.

The following list contains seven of the most common hazards and types of dangerous materials associated with working in a laboratory which handles wastewater samples, and includes some suggestions for steps which may be taken to help prevent an accident from occurring. The list does not include all possible situations which may arise; many types of hazards may be specific to a particular facility or type of process.

Infectious Materials:

Even a small amount of wastewater or sludge contains millions of microorganisms; some of them may be disease causing. Diseases such as tetanus, typhoid, dysentery, and hepatitis may be contracted through improper handling of wastewater samples.

The table below lists examples of the numerous diseases associated with wastewater contaminated environments.

DISEASES ASSOCIATED WITH WASTEWATER CONTAMINATED ENVIRONMENTS

Disease	Organism	Mode Of Transmission
Bacillary dysentery	Shigella spp.	Ingestion[b]
Asiatic cholera	Vibrio cholerae	Ingestion
Typhoid fever	Salmonella Typhi	Ingestion
Tuberculosis	Mycobacterium tuberculosis	Inhalation[c]
Tetanus	Clostridium tetani	Wound contact
Infectious hepatitis	Hepatitis A virus	Ingestion
Poliomyelitis	Poliovirus	Ingestion
Common cold[a]	Echovirus	Inhalation
Hookworm disease	Necator americanus Ancylostoma duodenale	Skin contact
Histoplasmosis	Histoplasma capsulatum	Inhalation

[a]The common cold is usually associated with various rhinovirus types, several coronaviruses, and some unknown viruses.
[b]Inhalation is by way of mouth and nose and taken through the lungs and into the bloodstream.
[c]Ingestion is by way of mouth or nose and taken in through the stomach and intestine and into the bloodstream.

An important means of protection against infection is to receive the appropriate inoculations. Your local health officials and personal physician should be consulted as to what inoculation may be needed.

Probably the most obvious means of protection is to observe good personal hygiene, including thorough washing of hands and face, changing clothes before leaving work, and the use of protective clothing such as gloves and aprons where warranted. The analyst must always use a pipet bulb in the wastewater lab, and make the assumption that all glassware and samples are contaminated. Special precautions should be taken when working with cuts or scrapes on the hands. There should be no eating, drinking, or smoking in the lab area, and food and drink should never be stored

in the same area as samples and reagents.

Poisons:

Many of the chemicals commonly used in the lab are deadly poisons. Some of these such as carbon tetrachloride or mercury can be absorbed through the skin and may build up over a long period of time to dangerous levels. Others such as cyanide may take on a gaseous form that is extremely dangerous when breathed in.

Warning labels on chemicals used should be read and understood. If the chemical being used is poisonous, special care should be taken to assure that the material will not be ingested or absorbed through the skin. All such reagents should be clearly labeled as to its poisonous nature.

Explosive Materials:

Almost all labs use acetone, azide compounds, and many other explosive chemicals. Other chemicals which may not be explosive alone may form explosive compounds with other non-explosive chemicals. Heat, an electric spark, sudden shock, pressure, or even contact with air may trigger an explosion from some compounds.

Whenever explosive solvents such as ether or acetone are being used in the lab, open flames must not be used. Procedures using these chemicals should be carried out in the fume hood, if possible, with the fan on. If sample digestions involve the use of perchloric acid, an explosion proof fume hood rated for perchloric acid must be used. Analytical procedures must be followed exactly as written using the chemicals specified. Substitutions of chemicals or alterations in the procedures may cause dangerous reactions to occur.

Electrical Shock:

This usually occurs due to improper grounding of instrumentation or improper contact between the analyst, electricity, and water.

Make sure that any instrument is grounded before use. Avoid using electrical

instrumentation near sinks or other sources of water. Do not operate electrical instruments while standing in water. If an instrument does get wet do not use it until it has been dried out and has been determined safe for use. Do not have electrical outlets placed near sinks. All permanent wiring should be installed by a qualified electrician. Do not overload electrical circuits in the lab. Know the location of circuit breaker boxes that control circuits in the lab and have the breakers clearly marked.

Toxic Fumes:

These are generated as part of many routine procedures. An example of this is the generation of sulfur trioxide fumes during the analysis for total Kjeldahl nitrogen. This becomes dangerous when a properly operating fume hood is not used.

Observe precautions printed on all reagent bottles. If the analyst uses a chemical which emits toxic fumes the work must be done in a fume hood. The fume hood must be inspected at least once each year to assure an adequate air displacement and to check for leaks in the duct work. Spills of such materials, such as mercury, must be cleaned up immediately using appropriate procedures.

Corrosive Materials:

Most labs use concentrated acids and bases for a wide variety of purposes. These not only are corrosive to laboratory equipment and instrumentation, but can also damage clothing and cause severe burns. This is especially critical when these materials come in contact with the eyes.

Concentrations of acids and bases should always be specified on the label. When making up dilutions of acids always add the acid to the water or violent splashing or explosion will occur. Make such solutions cautiously and slowly, expecting the solution to get very hot. Quantities of these materials of one gallon or more are best stored in unbreakable containers. Put together a kit to handle spills of acids and bases and keep this in a handy location. Always wear eye protection, apron, and gloves when

handling concentrated acids or bases.

Fire:

Fires are usually caused by improper handling of chemicals or from overloaded or improper electrical conditions.

Follow proper storage procedures for all reagents. Dispose of chemicals in a safe manner. Observe shelf lives of any reagents which are so dated. Use common sense when using open flames. Know the service capacity of the electrical circuits in the lab to avoid creating an overloaded condition. Label all circuit breakers according to major equipment operated on each circuit.

General Lab Considerations:

Cylinders of compressed gases are extremely dangerous and require special precautions for moving and storage. If the valve is knocked off accidentally the cylinder may be propelled with rocket force, damaging almost anything in its path. When moving cylinders the valve protection cap must be installed and the cylinder should be strapped to a trussed handcart. For storage and for use, the cylinders should be chained or strapped securely to prevent them from being knocked over.

Chemicals should be stored in an adequate storeroom. Heavy items should be stored as near as possible to the floor. All chemicals should be clearly labeled and dated. A discussion on proper labeling procedures follows this discussion. The storage room should be properly ventilated to prevent a possible buildup of vapors or heat. Care should be taken to assure that incompatible materials are not stored together. The list on the pages following this discussion list some of the chemicals commonly used in wastewater analysis and indicate which materials may not be safely stored with them.

The lab must have at least one emergency eye wash and shower. These must be inspected and flushed at least once per month. The location of fire extinguishers,

fire alarms, and telephones must be clearly visible. An emergency telephone number list should be developed and posted near the telephone. A first aid kit must be readily accessible in case of emergency.

Proper disposal procedures should be followed for any outdated or spent reagents. The local fire department may offer information or assistance in disposing of hazardous chemicals. Broken glass and glass containers should be disposed of in a container designated for only this type of waste.

Response to Emergencies:

In many types of emergencies quick response may mean the difference between having a close call or having a disaster. One of the best ways to assure a quick response to emergencies is to make sure that laboratory personnel are adequately trained in the use of safety equipment and in first aid procedures. Safety equipment training should include the use of fire extinguishers, emergency shower and eyewash, respirators, and all other safety equipment which would be appropriate for the particular facility. First aid training should include basic first aid as well as a course in cardiopulmonary resuscitation.

In summary, the best way to prevent accidents from happening is to know the procedures and materials that must be used, to understand the hazards associated with them, and to use the proper safety precautions and equipment. The best way to prepare laboratory personnel to react to an emergency situation is by providing the necessary safety equipment and by providing training in their use.

CHEMICAL STORAGE

THESE CHEMICALS:	SHOULD NOT BE STORED WITH:
Acetic acid	Chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, perchloric acid, peroxides, permanganates.
Acetylene	Chlorine, bromine, copper, fluorine, silver.
Ammonium nitrate	Acids, powered metals, flammable liquids, chlorates, nitrites, sulfur, finely divided organic or combustible materials.
Carbon, activated	Calcium hypochlorite, all oxidizing agents.
Chlorates	Ammonium salts, acids, powdered metals, sulfur, finely divided organic or combustible materials.
Chromic acid	Acetic acid, naphthalene, camphor, glycerine, turpentine, alcohol, flammable liquids in general.
Chlorine	Ammonia, acetylene, butadiene, butane, methane, propane (or other petroleum gases), hydrogen, sodium carbide, turpentine, benzene, finely divided metals.
Copper	Acetylene, hydrogen peroxide.
Flammable liquids	Ammonium nitrate, chromic acid, hydrogen peroxide, nitric acid, sodium peroxide, the halogens.
Hydrocarbons	Fluorine, chlorine, bromine, chromic acid, sodium peroxide.
Hydrofluoric acid, anhydrous	Ammonia, aqueous or anhydrous.
Hydrogen peroxide	Copper, chromium, iron, most metals or their salts, alcohols, acetone, organic materials, aniline, nitromethane, flammable liquids, combustible materials.
Hydrogen sulfide	Fuming nitric acid, oxidizing gases.
Mercury	Acetylene, fulminic acid, ammonia, oxalic acid.
Nitric acid, concentrated	Acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids, flammable gases.
Oxalic acid	Silver, mercury.
Potassium permanganate	Glycerin, ethylene glycol, benzaldehyde, sulfuric acid.
Silver	Acetylene, oxalic acid, tartaric acid, ammonium compounds.
Sulfuric acid	Potassium chlorate, potassium perchlorate, potassium permanganate, or similar compounds with light metals.

LABELING

Adequate labeling of containers of laboratory reagents is essential to providing a safe working environment in any laboratory. Federal (40CFR Part 1910) and State (R325.70101) laws both specify that "Identity labels, showing contents of containers (including waste receptacles) and associated hazards" are required. These also state that "employers shall ensure that labels on incoming containers of hazardous chemicals are not removed or defaced."

It is recommended that labels on containers of chemicals acquired by the laboratory should include the following information:

a) A product name, trade name, chemical name or generic name if the product or trade name is used,

b) A signal word to draw attention and designate the degree of hazard such as:

   1) DANGER shall mean most serious hazard

   2) WARNING shall mean a lesser hazard

   3) CAUTION shall mean the least hazard,

c) A statement as to hazards that are present with customary use or handling of the substance, for example "causes burns" or "vapor hazardous".

d) Date of preparation and / or expiration.

The label for a 1 Normal Sulfuric Acid solution would be as follows:

Sulfuric Acid H_2SO_4 1N Danger – Causes Burns Prepared 12/3/2007

Several labeling tools are available, and each has its place in the laboratory. Most beakers and flasks will have a hexagonal space of ground glass which can be written on to identify it. A lead pencil should be used for this type of marking. Grease pencils are primarily used for temporary labeling. It should be noted that the grease pencil marking will readily rub off. Commercially available labeling tape is especially useful in many situations. It may be purchased in several different colors, and may be blank or imprinted with a form which may be filled out to provide the necessary information.

High temperature markers are available for marking on surfaces that are exposed to extreme high temperature environments, such as Gooch crucibles. The marks become permanent after heat is applied.

Whatever labeling techniques you use, be consistent, and remember that the label is intended not only for convenience but also for safety.

PERIODIC TABLE OF THE ELEMENTS

PERIOD	IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII			IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	
1	1 H 1.00797																	2 He 4.0026	2
2	3 Li 6.939	4 Be 9.0122											5 B 10.811	6 C 12.01115	7 N 14.0067	8 O 15.9994	9 F 18.9884	10 Ne 20.179	2 8
3	11 Na 22.9898	12 Mg 24.305											13 Al 26.9815	14 Si 28.086	15 P 30.9738	16 S 32.064	17 Cl 35.453	18 Ar 39.948	2 8 8
4	19 K 39.102	20 Ca 40.08	21 Sc 44.956	22 Ti 47.90	23 V 50.942	24 Cr 51.996	25 Mn 54.938	26 Fe 55.847	27 Co 58.933	28 Ni 58.71	29 Cu 63.54	30 Zn 65.37	31 Ga 69.72	32 Ge 72.59	33 As 74.922	34 Se 78.96	35 Br 79.909	36 Kr 83.80	2 8 18 8
5	37 Rb 85.47	38 Sr 87.62	39 Y 88.905	40 Zr 91.22	41 Nb 92.906	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.905	46 Pd 106.4	47 Ag 107.868	48 Cd 112.40	49 In 114.82	50 Sn 118.69	51 Sb 121.75	52 Te 127.60	53 I 126.904	54 Xe 131.30	2 8 18 18 8
6	55 Cs 132.905	56 Ba 137.34	★ 57 La 138.91	72 Hf 178.49	73 Ta 180.948	74 W 183.85	75 Re 186.2	76 Os 190.2	77 Ir 192.2	78 Pt 195.09	79 Au 196.967	80 Hg 200.59	81 Tl 204.37	82 Pb 207.19	83 Bi 208.980	84 Po (210)	85 At (210)	86 Rn (222)	2 8 18 32 18 8
7	87 Fr (223)	88 Ra (226)	★★ 89 Ac (227)	104															

★	58 Ce 140.12	59 Pr 140.907	60 Nd 144.24	61 Pm (145)	62 Sm 150.35	63 Eu 151.96	64 Gd 157.25	65 Tb 158.924	66 Dy 162.50	67 Ho 164.930	68 Er 167.26	69 Tm 168.934	70 Yb 173.04	71 Lu 174.97
★★	90 Th 232.038	91 Pa (231)	92 U 238.03	93 Np (237)	94 Pu (242)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (249)	99 Es (254)	100 Fm (253)	101 Md (256)	102 No (254)	103 Lw (257)

From Science Related Materials, Inc.
Janesville, Wisconsin

ATOMIC WEIGHTS OF THE ELEMENTS
IN ALPHABETICAL ORDER

Name	Symbol	Atomic Number	Atomic Weight
Actinium	Ac	89	227.0278
Aluminum	Al	13	26.9815
Americium	Am	95	241.0568
Antimony	Sb	51	121.75
Argon	Ar	18	39.948
Arsenic	As	33	74.9216
Astatine	At	85	209.9871
Barium	Ba	56	137.33
Berkelium	Bk	97	247.0703
Beryllium	Be	4	9.01218
Bismuth	Bi	83	208.9804
Boron	B	5	10.81
Bromine	Br	35	79.904
Cadmium	Cd	48	112.41
Calcium	Ca	20	40.08
Californium	Cf	98	249.0748
Carbon	C	6	12.011
Cerium	Ce	58	140.12
Cesium	Cs	55	132.9054
Chlorine	Cl	17	35.453
Chromium	Cr	24	51.996
Cobalt	Co	27	58.9332
Copper	Cu	29	63.546
Curium	Cm	96	243.0614
Dysprosium	Dy	66	162.50
Einsteinium	Es	99	252.083
Erbium	Er	68	167.26
Europium	Eu	63	151.96
Fermium	Fm	100	257.0951
Fluorine	F	9	18.998403
Francium	Fr	87	223.0197
Gadolinium	Gd	64	157.25
Gallium	Ga	31	69.72
Germanium	Ge	32	72.59
Gold	Au	79	196.9665
Hafnium	Hf	72	178.49
Helium	He	2	4.00260
Holmium	Ho	67	164.9304
Hydrogen	H	1	1.0079
Indium	In	49	114.82
Iodine	I	53	126.9045
Iridium	Ir	77	192.22
Iron	Fe	26	55.847
Krypton	Kr	36	83.80
Lanthanum	La	57	138.9055
Lawrencium	Lr	103	262.11
Lead	Pb	82	207.2
Lithium	Li	3	6.941
Lutetium	Lu	71	174.967
Magnesium	Mg	12	24.305
Manganese	Mn	25	54.9380
Mendelevium	Md	101	256.094
Mercury	Hg	80	200.59

Name	Symbol	Atomic Number	Atomic Weight
Molybdenum	Mo	42	95.94
Neodymium	Nd	60	144.24
Neon	Ne	10	20.179
Neptunium	Np	93	237.0482
Nickel	Ni	28	58.70
Niobium	Nb	41	92.9064
Nitrogen	N	7	14.0067
Nobelium	No	102	259.1009
Osmium	Os	76	190.2
Oxygen	O	8	15.9994
Palladium	Pd	46	106.4
Phosphorus	P	15	30.97376
Platinum	Pt	78	195.09
Plutonium	Pu	94	238.0496
Polonium	Po	84	208.9824
Potassium	K	19	39.0983
Praseodymium	Pr	59	140.9077
Promethium	Pm	61	144.9127
Protactinium	Pa	91	231.0359
Radium	Ra	88	226.0254
Radon	Rn	86	210.9906
Rhenium	Re	75	186.207
Rhodium	Rh	45	102.9055
Rubidium	Rb	37	85.4678
Ruthenium	Ru	44	101.07
Samarium	Sm	62	150.4
Scandium	Sc	21	44.9559
Selenium	Se	34	78.96
Silicon	Si	14	28.0855
Silver	Ag	47	107.868
Sodium	Na	11	22.98977
Strontium	Sr	38	87.62
Sulfur	S	16	32.06
Tantalum	Ta	73	180.9479
Technetium	Tc	43	96.9064
Tellurium	Te	52	127.60
Terbium	Tb	65	158.9254
Thallium	Tl	81	204.37
Thorium	Th	90	232.0381
Thulium	Tm	69	168.9342
Tin	Sn	50	118.69
Titanium	Ti	22	47.90
Tungsten	W	74	183.85
Unnilhexium	Unh	106	263.118
Unnilpentium	Unp	105	262114
Unnilquadium	Unq	104	261.11
Uranium	U	92	238.029
Vanadium	V	23	50.9415
Xenon	Xe	54	131.30
Ytterbium	Yb	70	173.04
Yttrium	Y	39	88.9059
Zinc	Zn	30	65.38
Zirconium	Zr	40	91.22

Names and Formulas of Compounds

Compounds are pure substances that are composed of two or more elements. Elements may be referred to as the basic building blocks of all substances. The current number of known elements is around 112. The ones up through atomic number 92 (uranium) are naturally occurring, whereas the "transuranic" elements are synthesized in experiments wherein heavy nuclei are made to interact with each other.

Each element has a particular symbol. The symbol is an abbreviation for that element. The symbols that are used to represent the elements are also used to represent compounds. For example the compound NaCl represents the combination of sodium (Na #11) and chlorine (Cl #17) and its name is sodium chloride.

In several of the chemical formulas, you will note that subscripts are used. The subscript tells how many atoms of that element are contained in the compound. In water (H_2O) there are two atoms of hydrogen and one atom of oxygen. The subscripts help to differentiate one compound from another. The compound hydrogen peroxide (H_2O_2) although similar to water is obviously not the same since there are 2 atoms of oxygen in the peroxide and only 1 atom in the water.

In choosing the proper chemical for an analysis, it cannot be over emphasized that the name and formula that occur on the label of the chemical must match the name and formula in the procedure that has been given. Several names may appear to be correct because of similarities in spelling such as:

sodium sulfate Na_2SO_4 and sodium sulfite Na_2SO_3

These are not the same. The sulfate compound has one more oxygen atom than the sulfite. Another minor spelling variation would be potassium nitrate KNO_3 and potassium nitrite KNO_2. What is the difference here?

Another variation and, in fact, a very important property of compounds, is the addition of the word anhydrous to the name. This means without water. The chemical has been prepared (by the manufacturer) without water. If the chemical does have water in it, it

will be referred to as a hydrate. Example:

Sodium Thiosulfate Pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)

This means that the compound has 5 water molecules associated with it. Note that the prefixes to the word hydrate are mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca referring to the numbers 1 through 10 respectively.

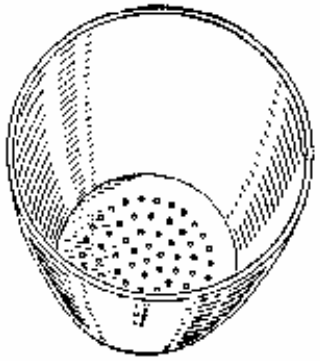
Calcium Chloride, Anhydrous ($CaCl_2$)

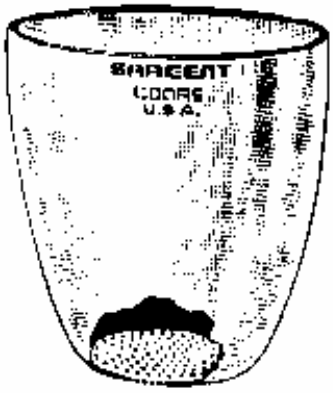
This means that the compound contains no water.

When choosing a chemical for a particular analysis, the stock chemical bottle must be considered very carefully. It contains a label that gives the name of the compound as well as the formula. It also contains cautions such as explosive, toxic (poisonous). The hazards presented by these chemicals are not evident from appearance, smell, or everyday knowledge. Hazards must be foreseen and avoided. It is safest to assume that all chemicals, even water if not safely handled, can be hazardous. Read the label completely and follow the warnings that are indicated. The label will also mention any additional storage requirements that might be necessary for a particular reagent such as "Store at 25 degrees C". The purity of the chemical is also indicated. Analytical or Reagent Grade is the highest purity. The amounts of impurities are shown on the label. The word ACS (American Chemical Society) also might be shown. This also means reagent grade. A lower grade of chemical would be laboratory or practical grade. Usually, amounts of impurities would not be listed on this label. A sample label is shown.

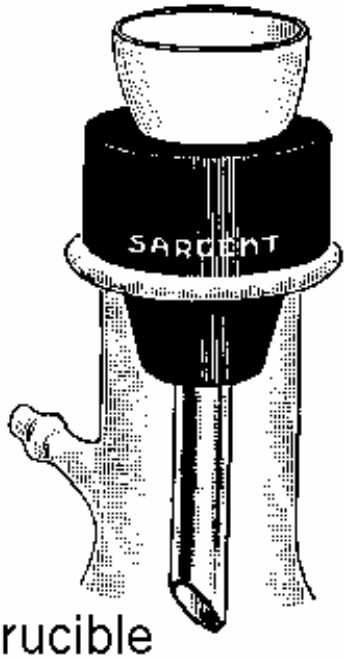
$Na_2S_2O_3 \cdot 5H_2O$	500 Grams	CAUTION
SODIUM THIOSULFATE (crystals)		Emits Toxic Fumes When Heated Keep container tightly closed. Do not take internally.
Reagent, A.C.S.		

ILLUSTRATIONS OF LABORATORY APPARATUS

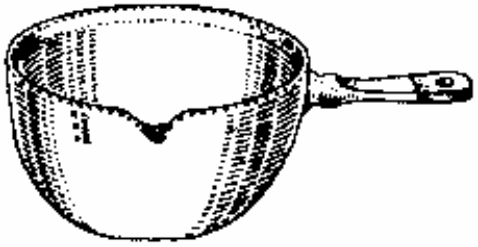




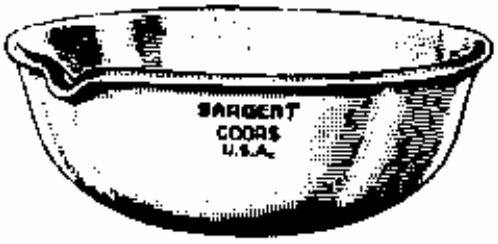
Gooch Crucible



Crucible Holder

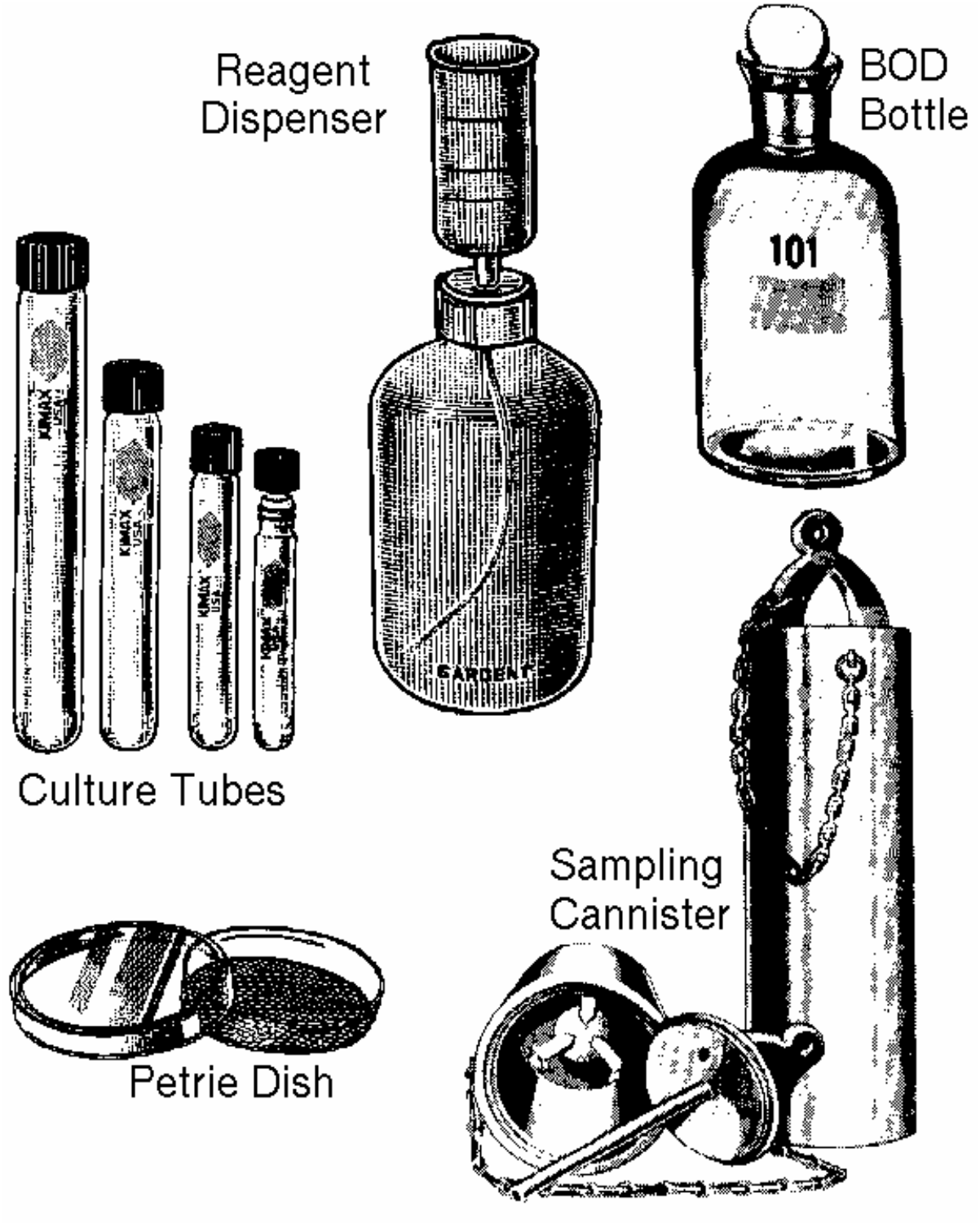


Casserole



Evaporating Dish

Desiccator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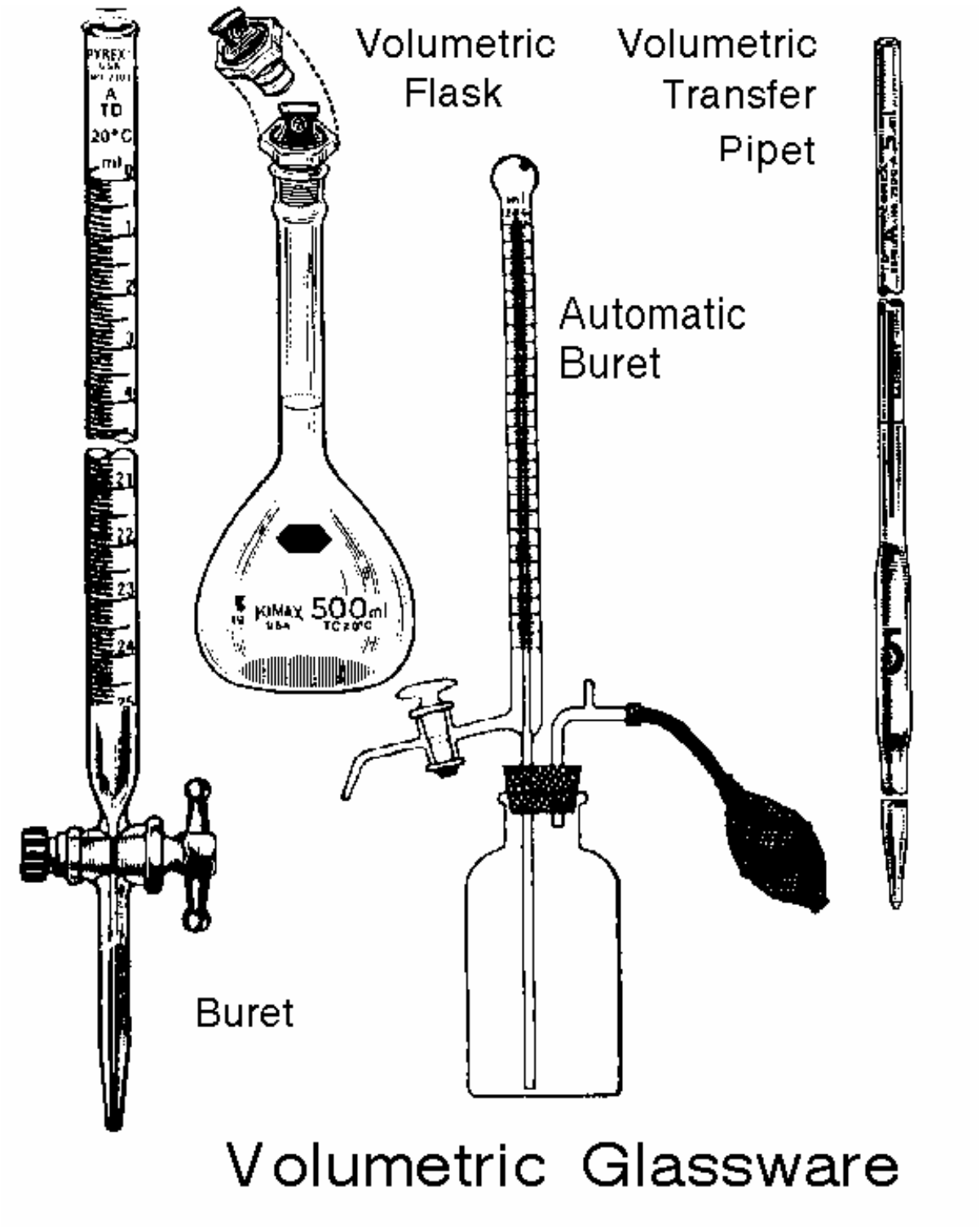
Dispenser
BOD
Bottle
101
SARGENT
Culture Tubes
Sampling
Cannister
Petrie Dish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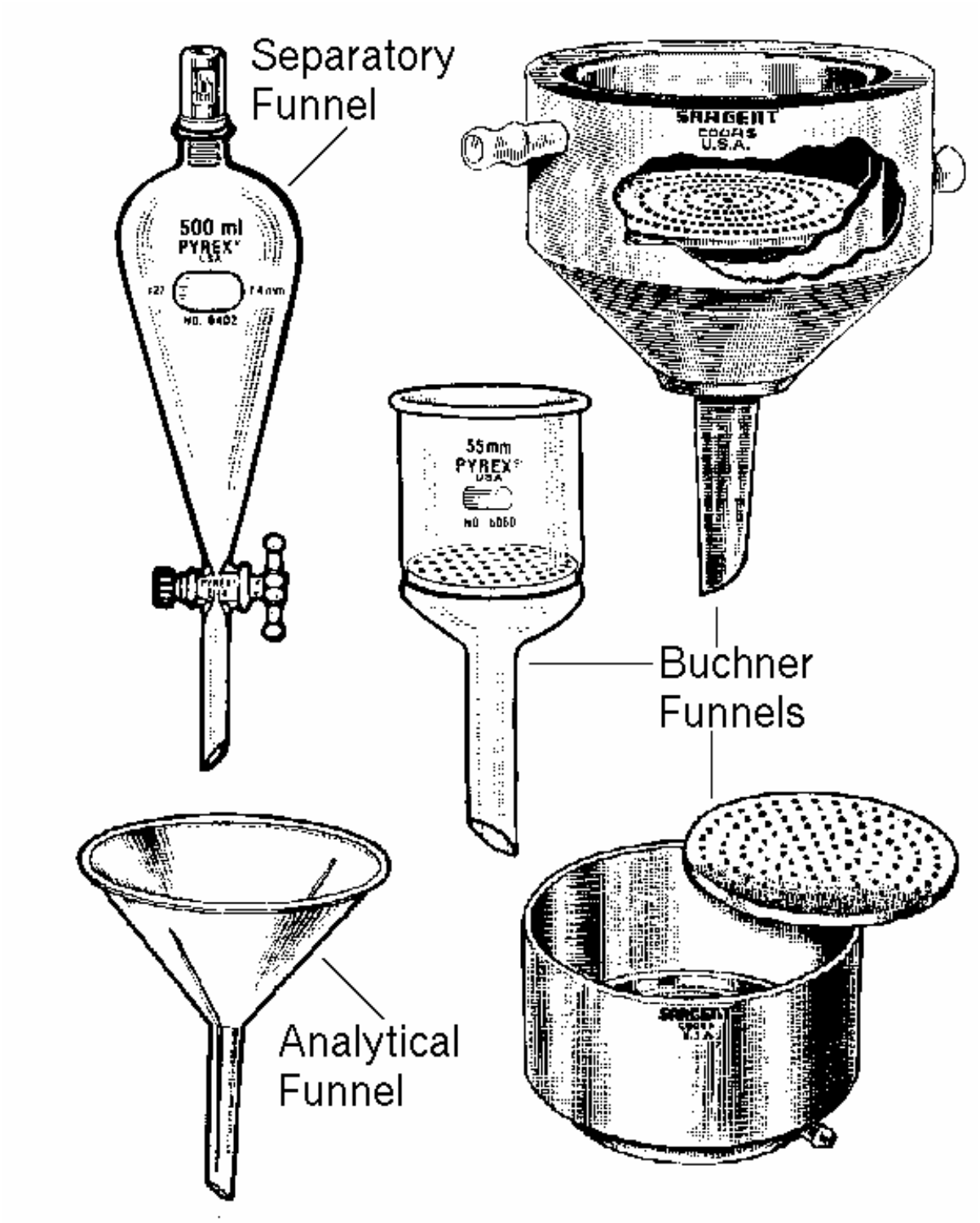
Volumetric Transfer Pipet
Automatic Buret
Buret
Volumetric Glassware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PYREX
SARGENT
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U.S.A.
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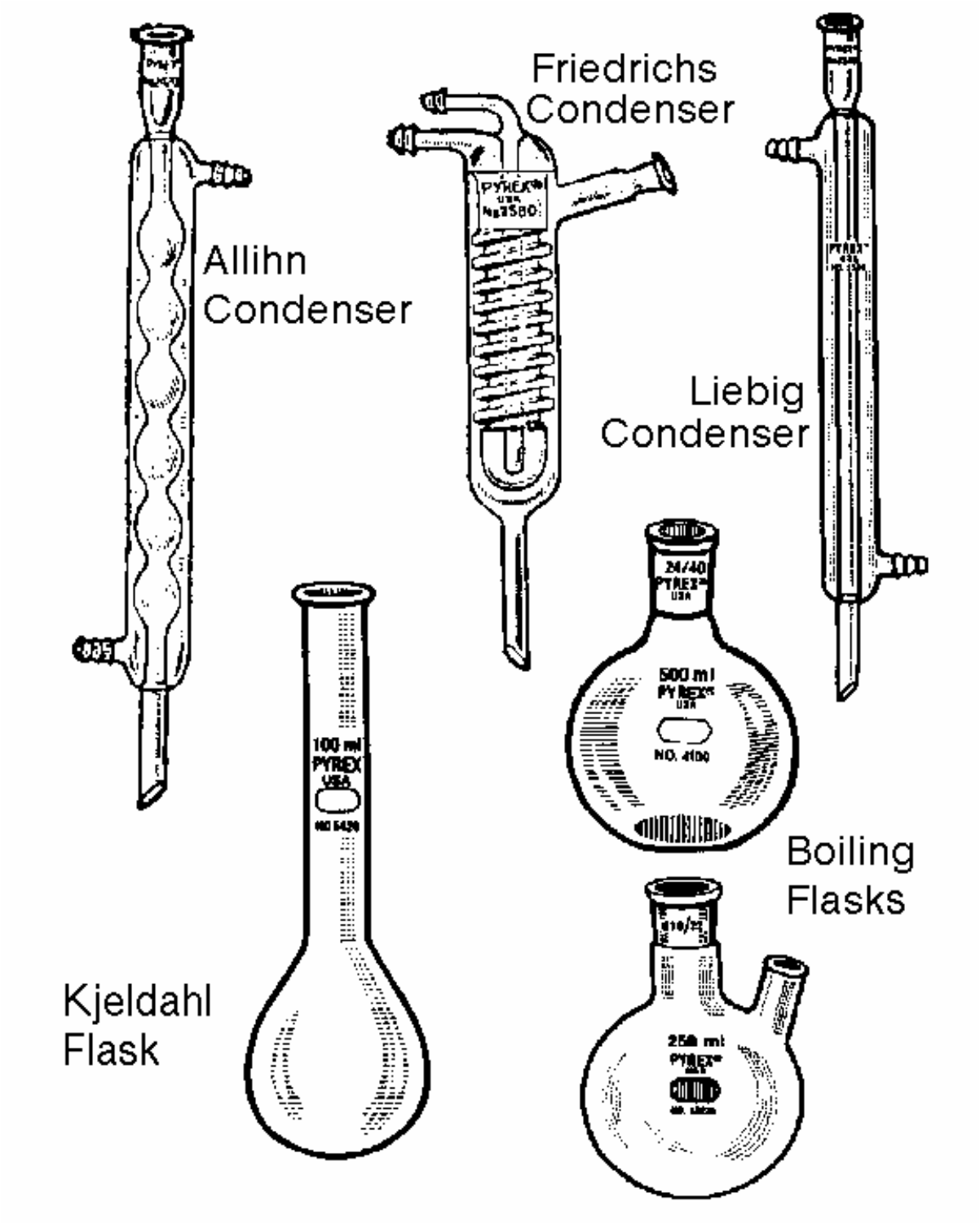
PYREX
Buchner
Funnels
Analytical
Funnel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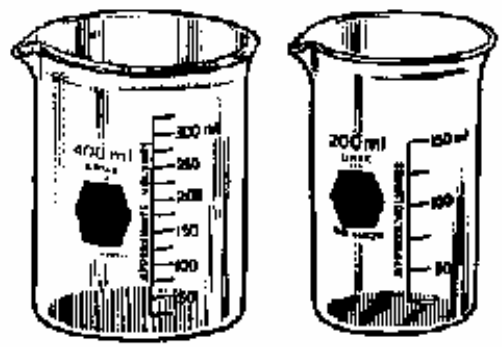
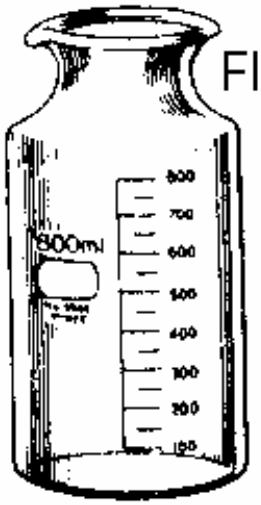
Condenser
Allihn
Condenser
Liebig
Condenser
Boiling
Flasks
Kjeldahl
Flask

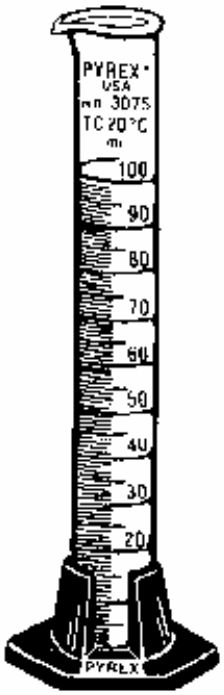


Griffin Beaker

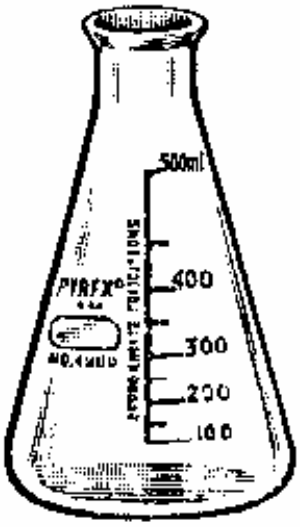
Tall form Beaker



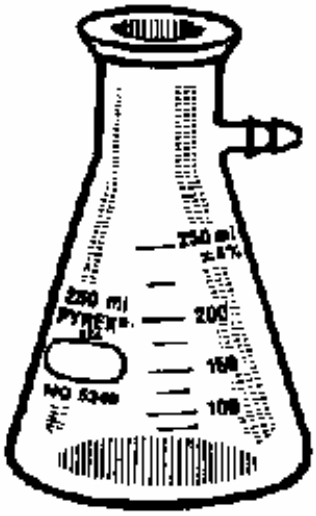
Fleaker



Graduated Cylinder

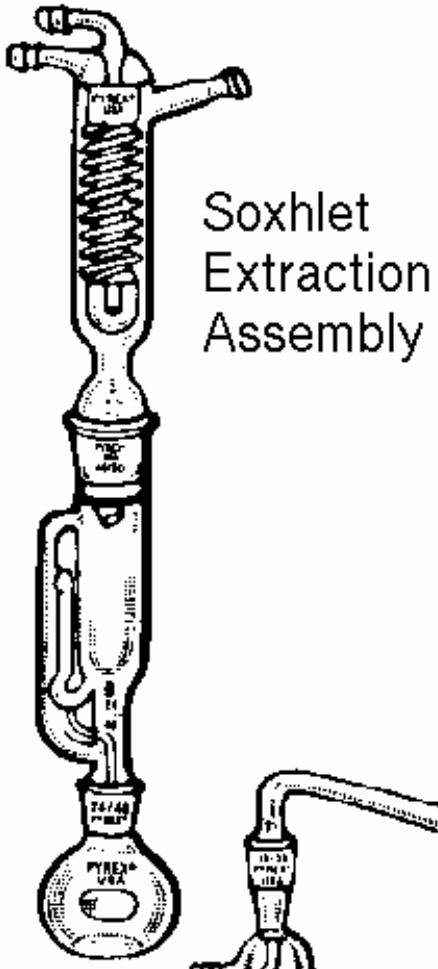


Erlenmeyer Flask

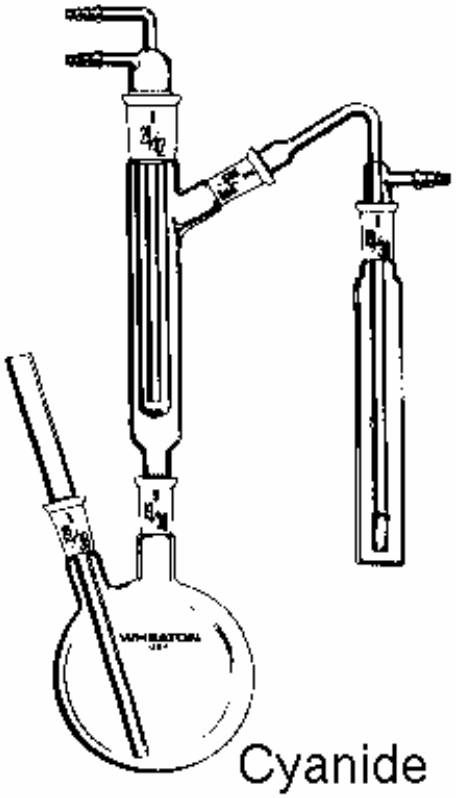


Filtration Flask

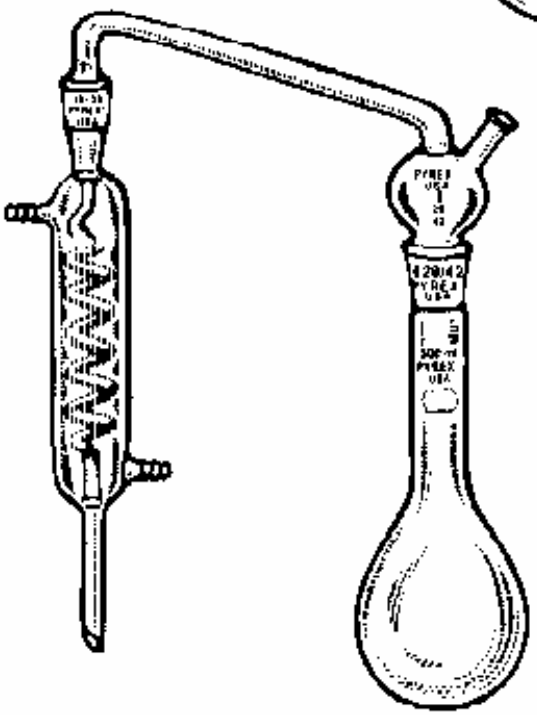
Graduated Pipet



Soxhlet Extraction Assembly



Cyanide Distillation



Ammonia Distillation

OPERATION OF THE ANALYTICAL BALANCE

The analytical balance is one of the most important pieces of equipment in the wastewater treatment plant laboratory, since the accuracy of almost every analytical procedure depends on being able to make accurate weighings. In gravimetric procedures the component being analyzed is usually weighed directly, while in titrimetric or colorimetric procedures, standards and reagents are often prepared by weighing. Thus, the efficient operation of the laboratory is dependent on the analytical balance in many ways. Unfortunately, many times laboratory personnel do not realize the limitations of the equipment and the sources of possible error.

There are three general sources of error that are commonly encountered by the analyst in using an analytical balance. The first source of error is the balance itself. It is impossible to develop instrumentation which can consistently make perfect measurements, even after the elimination of all other sources of error; a tolerance for error is always specified by the manufacturer. With continued use, the balance may also introduce errors due to worn parts, or being out of adjustment. Laboratories will usually contract with a service company to provide annual calibration and maintenance of the balance to minimize these types of errors.

The second type of errors are those imposed by the environment in which the balance is used. Excessive humidity, vibration, drafts, temperature changes, and dust are all environmental factors which may contribute to weighing errors. These errors may be minimized to a large extent by proper placement of the balance within the laboratory, and assuring proper preparation and handling of the materials being weighed. The following pages which have been provided by the Mettler Instrument Corporation discuss these factors, their effects, and possible solutions to problems.

The third source of weighing errors is the analyst. This is probably the greatest source of error since it is variable from day to day and from person to person. While other

sources of errors may be minimized because they would be expected to be somewhat consistent, operational errors occur very inconsistently. Errors may occur in handling the material to be weighed, in operating the balance, or even in reading the weight which is properly displayed by the equipment. The only way to minimize these errors is for the analyst to make a conscientious effort toward knowing the instrument, following correct procedure, and taking time to double check results.

There are several manufacturers of analytical balances which are being used in wastewater treatment plant laboratories, and many models to choose from. In addition, the age of the equipment being used includes a fairly wide range. This makes it impossible to include an operating procedure in this manual. The analyst should become familiar with operating manuals provided by the manufacturer and should consult these when problems arise, keeping in mind the more general information included here.

Mettler

Weighing the right way

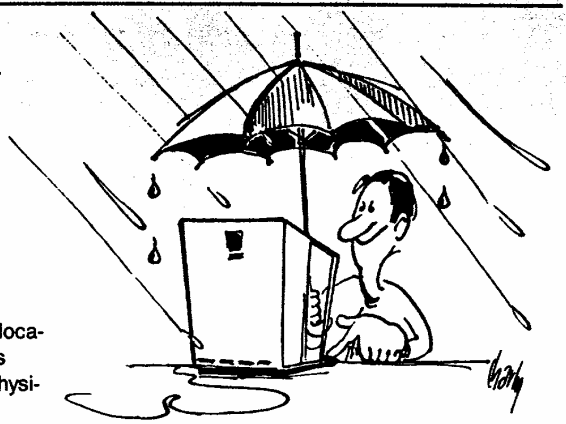
Part 1 of 2

Correct operation of electronic analytical, semimicro and microbalances

The electronic balances available today are much easier to use than were their mechanical forerunners. In addition, they are capable of "overcoming" certain environmental influences during operation. Perhaps as a consequence of these facts, some users of electronic micro-, semimicro- or analytical balances tend to be careless about sound weighing procedures.

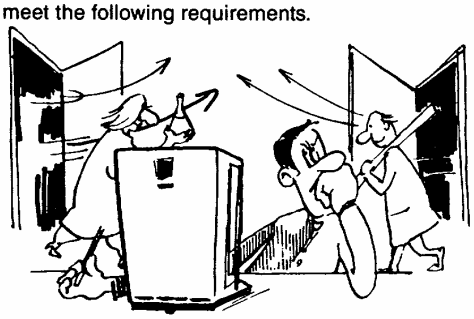
In this two-part series, we will touch on the most important points to be considered when working with micro-, semimicro- or analytical balances.

This installment will cover selection of the balance location and the weighing table. Next time, we will discuss balance operation and weighing errors traceable to physical phenomena.

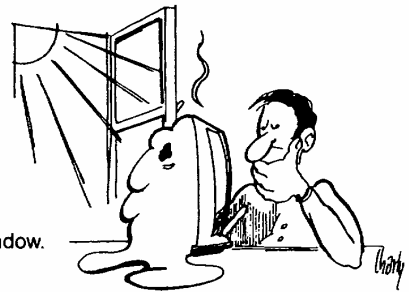


Selection of the balance location

The accuracy and reliability of weighing results depends not only on precise measuring performance, but also very much on the location of the balance. An optimal weighing station must meet the following requirements.



Whenever possible, the room in which the balance is located should have only one entrance so that the room is not used as a passageway. The corners of the room are especially suitable as work locations because they are the most rigid locations in the building and are subject to the least amount of vibration.

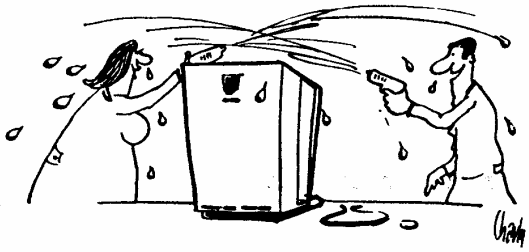


Never set up the balance near a window. Exposure to direct sunlight can cause the balance to warm up unevenly. Also keep clear of heating devices. They not only generate heat radiation, but also produce rather strong convective air currents that can interfere with the balance's operation.

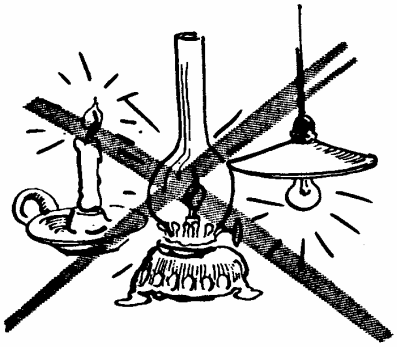
The balance should not be set up near air conditioners or fans because they generate a great deal of air turbulence.

Temperature drift: If the temperature of a room changes, the temperature in the balance will also change shortly thereafter. This can be seen from the "drift" of the balance display. When working with semimicro- or microbalances, room

temperature should be held constant by using a thermostat. With micro- and ultramicro balances, the temperature should be held constant within 1° C.



Relative humidity: The higher the readability of a balance, the stronger the effect a change in relative humidity will be on weight. For this reason, humidity should be monitored and held constant. Recommended relative humidity: 45%–60%.



Lighting: Artificial light—preferably fluorescent—in a room without windows is best for accurate weighing results. Light fixtures must be sufficiently far from the weighing table to prevent disturbing thermal radiation. High wattage lamps should not be installed.

Weighing table

Weighing results from micro-, semimicro- or analytical balances are as good—or as bad—as the weighing table. For this reason, the following points should be kept in mind when selecting the weighing table.

The weighing table should:

— Not transmit oscillations and vibrations. (Oscillations and vibrations can be caused by machinery running in the same building, or on roads or rail lines in the vicinity of the building, etc.)

— Have no deflection when it is worked on.
— Be made of antimagnetic materials.
— Be protected against static charges.

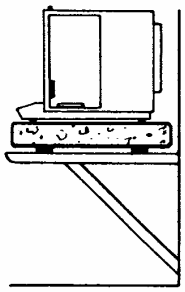


Figure A

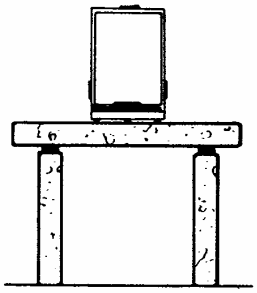


Figure B

Weighing tables made of solid stone plates are recommended. They can be placed on consoles which are fastened to the wall (Figure A), or placed on two solid floor supports (Figure B). It is recommended that the plate not be fastened to both the wall and the floor because vibrations will be transmitted through both media. The weighing table should not be used for storage of heavy objects. To prevent weighing errors, the weighing table should not be covered with plastic, glass or metal plates.

Mettler
Weighing the right way

Part 2 of 2

Correct operation of electronic analytical, semimicro and microbalances

Balance operation

When weighing with micro-, semimicro- and analytical balances, the following points always apply:

> Proceed carefully, taking into consideration the requirements of the object being weighed. It is important, however, to proceed quickly.

These somewhat contradictory statements are based on the fact that the longer it takes to make a weight determination, the greater the risk that environmental influences such as vibrations, air disturbances, temperature fluctuations, humidity, reactions of the object being weighed, etc., can cause the result to be off.

Specifically, the following points should be taken into consideration:

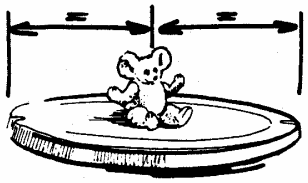
Switch the balance on at least 30 minutes prior to making the first weighing. If possible, leave the balance switched on all the time.
Reason: Any possible zero point error and sensitivity drift caused by the balance warmup are eliminated.
Note: The balance warmup time is eliminated when the instrument is equipped with a standby circuit (Mettler AE balances, for instance), as long as it is not disconnected from the power source.

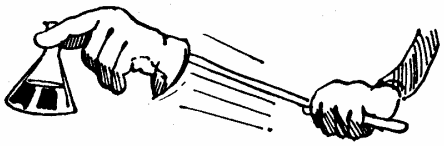
Before opening the weighing chamber, check to make sure the display indicates zero.
Reason: Any zero point error will be counted in with your weighing result.

Do not touch the tare container or the object to be weighed with your fingers; use long tweezers or tongs.
Reason: Physical contact can cause the temperature and humidity of the tare container and object to change.

Open the weighing chamber only to place the tare container or object on the pan, and only for as long as it takes to assure that the object is squarely on the pan.
Reason: Every time the weighing chamber is opened, temperature changes and air drafts can influence the result.

Place the object in the middle of the pan.
Reason: This will help to avoid a cornerload error.





Never place your hand into the weighing chamber; use long tweezers or tongs.
Reason: You can change both the temperature and humidity of the weighing chamber.

Close the weighing chamber immediately after placing the tare container or object on the pan.
Reason: The longer the weighing chamber stays open, the greater the risk that temperature/humidity changes and drafts influence the result.

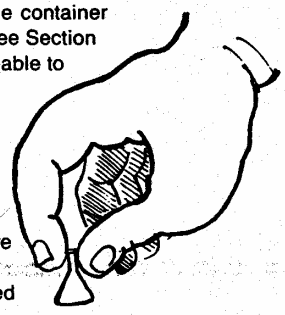
Once the display stabilizes, read the result.
Reason: The object being weighed can release or absorb humidity, which means the result can change.

As soon as the weighing is completed, remove the object from the weighing chamber—using tweezers or tongs—and close the weighing chamber.
Reason: The object being weighed can change the temperature and humidity within the weighing chamber.

Keep the weighing chamber and pan clean. When weighing, use only clean tare containers.
Reason: The weight of any dirt or impurities on the pan can cause incorrect results.

Do not use tare containers made of plastic. And don't use glass containers when relative humidity is below 30%–40%.

Reason: There is a risk that the container will electrostatically charge. See Section entitled "Weighing errors traceable to physical phenomena."



Always use the smallest possible container.

Be aware of the possible influence of any surface moisture films. To avoid this, weigh the object only when it has reached ambient temperature.

Reason: Every object has a surface moisture film, and the thickness of the film depends on relative humidity. It also depends on the temperature of the object relative to the temperature of the ambient atmosphere. For instance, the lower the temperature of the object relative to the environment, the thicker the surface moisture film. Thus, a cold object appears to be heavier than it really is, and a warmer object appears to be lighter than it really is.

Also be aware of any possible influence of absorbed moisture on the weighed object. Hygroscopic substances should first be dried in a desiccator and cooled in a sealed container. Take steps to prevent the substance from reabsorbing atmospheric moisture when it is placed on the weighing pan.

Reason: If you weigh a moist substance, the liquid portion is also counted in with the result—causing the substance to appear heavier than it actually is. Hygroscopic substances can also change their weight while they are being weighed. This can be seen on the unstable balance display.

Please be aware that an electronic micro-, semimicro- or analytical balance is a force compensating instrument that must be calibrated at the location where it is being used. Leave the instrument on for at least one hour (warmup time) before calibrating it. Calibration should be repeated periodically.

Reason: The force required to compensate a given mass is very much dependent on both geographic location and altitude above sea level. If such a precision instrument is not calibrated, or is incorrectly calibrated, at the location where it is used, serious sensitivity errors can occur.

Weighing errors traceable to physical phenomena

Electrostatic influences

Materials with high electrical insulation properties (glass and plastic, for example) can charge electrostatically. For the most part, this charging results from friction that occurs during treatment or shipment of materials (especially powders and granules).

If an electrostatically charged material is weighed, serious weighing errors can result from the electrostatic forces between the material and its surrounding (e.g. balance housing, weighing chamber, etc.)

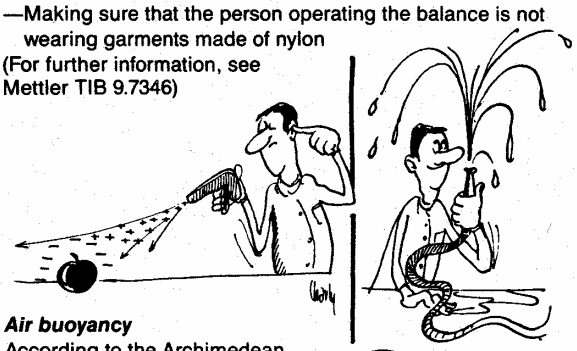
If the material and its surrounding have the same charge, the charges repel each other. However, if the charges are different, there is attraction between the material and its surrounding. For this reason, it is purely random whether the weight of an electrostatically charged material appears to be too light or too heavy.

Correction:

It is possible to minimize the effects of electrostatic forces on a weighing result to a large extent by:

—Bombarding the material with an antistatic pistol
—Grounding the weighing pan
—Increasing the relative humidity
—Making sure that the person operating the balance is not wearing garments made of nylon

(For further information, see Mettler TIB 9.7346)

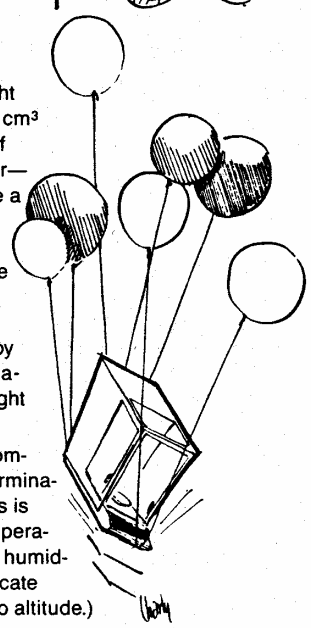


Air buoyancy

According to the Archimedean principle, a body immersed in a fluid undergoes an apparent loss in weight equal to the weight of the fluid it displaces. Since 1 cm^3 of air corresponds to a weight of 1.2 mg at 20°C, errors can occur—especially with bodies that have a low specific weight.

Correction:

After weighing, the weight of the displaced air is added to the weighing result. The weight of the displaced air is calculated by multiplying the volume of the material being weighed by the weight of the air (approx. 1.2 mg/cm^3).



With higher weights, it is recommended that a more exact determination of air density be made. This is dependent on air pressure, temperature, and, to a lesser extent, on humidity. (Note: most barometers indicate atmospheric pressure relative to altitude.)

Magnetic influences

Electronic micro-, semimicro- and analytical balances are high resolution instruments. For this reason, weighing magnetic parts is always problematic. This is because ferromagnetic parts in the immediate vicinity of the pan (balance housing, table mounts, etc.) produce magnetic forces that influence the weighing result.

Correction:

Demagnetization of the part, or completely enclosing the part in a ferrous metal shield.

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LABORATORY WATER

Water used in the laboratory to prepare solutions, or to rinse glassware in preparation for analytical work must be of adequate quality. It is obvious that water used to prepare standardizing solutions or other reagents used to analyze for a particular parameter must not contain a detectable amount of that parameter. It is equally important that laboratory water not contain materials which interfere with the analyses that are being performed. Some contaminants may cause a positive interference, making it appear that the sample contains more of the analyte than is actually present. Or contaminants may cause a negative interference, reducing the analyzed value of the sample.

Although potable (drinking) water may be thought of as being “pure” from a health standpoint, it is understood that this water probably contains many materials which would cause positive or negative interferences in analytical work. Potable water often contains significant amounts of several of the parameters regulated in wastewater discharge permits, and analyzed for in wastewater laboratories. Some common water contaminants are listed below:

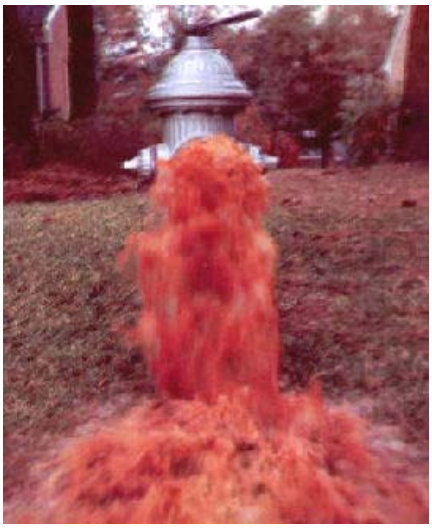
Water Contaminants

Particulates

- Silt
- Plumbing Pipe Debris
- Colloidal Material
- Iron Particles

Dissolved Inorganics

- Calcium and Magnesium
- Silicates
- Iron and Other Metals
- Chloride and Fluoride
- Phosphate
- Nitrate



Gases

Carbon Dioxide

Chlorine

Ammonia

Dissolved Organics

Pesticides

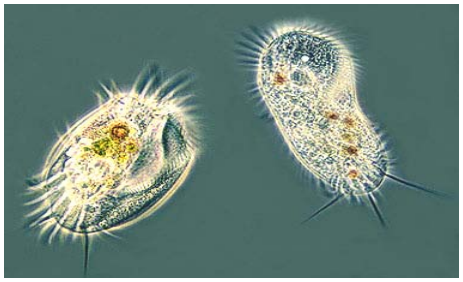
Herbicides

Hydrocarbons

Decayed Plant and Animal Tissue

Plasticizers from Piping, Plumbing Fixtures, and Plastic Storage Tanks

Microorganisms



Bacteria

Protozoa

Algae

Pyrogens

Bacterial Cell Wall Fragments

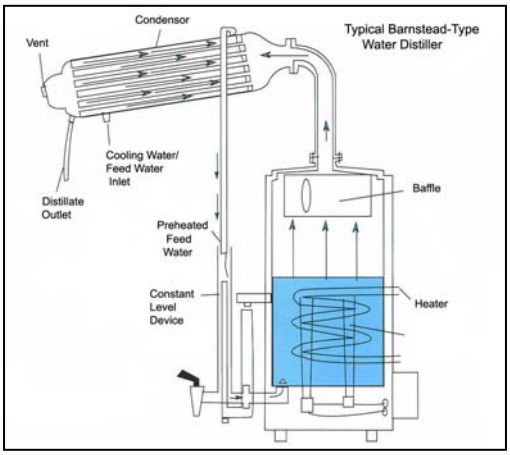
(Lipopolysaccharides)

While it is necessary to remove any material which would interfere with an analysis, it would be impractical (and impossible) to remove all of the materials listed above to a zero concentration. It is important to prepare water of sufficient quality for the analyses that are required. In other words, the required lab water quality depends on the intended use. There are many processes available for preparation of laboratory water; each with advantages and disadvantages, and each having the ability to remove certain types of contamination to various levels of quality. It should be noted that oftentimes a process used to remove one set of contaminants may introduce another set to the water. The most commonly used methods of lab water preparation are discussed below:

I. Preparing Reagent Water

A. Distillation

1. Water is heated to produce steam, and the steam is condensed. Most contaminates are not carried over with the steam.
2. Will effectively remove all ionized solids (hardness, salts), organics with boiling point greater than 212 deg. F, bacteria and pyrogens (bacterial byproducts)
3. Without further treatment, distilled water may contain dissolved gases (NH_3, Cl_2), and materials leached from storage containers and piping. Volatile organics may distill over, and non-volatiles may be carried over by steam.



4. Often stills (especially the older ones) are constructed of copper, brass, or bronze, coated with tin. This may be a concern if testing for metals, or for biological testing if metal contamination occurs.
5. Many newer units are made entirely of glass to eliminate the possibility of metal contamination.
6. Feed water to the still may be pretreated to improve quality of distillate and to prevent scale formation in boiler
   a. Soften hard water to remove Mg and Ca which form scale in boiler
   b. Carbon filtration ahead of still removes many organics which may distill over
   c. Mixed-bed ion exchanger ahead of still removes trace ions. This may be an expensive operation, since the ion exchanger will quickly

become spent if the feed water is hard or contains high concentrations of ionized materials.

7. Cleaning of still must be done often enough to keep efficiency high; must be done carefully, especially in metal still to avoid scratching or chipping tin plating, and with glass care must be taken to avoid breakage.

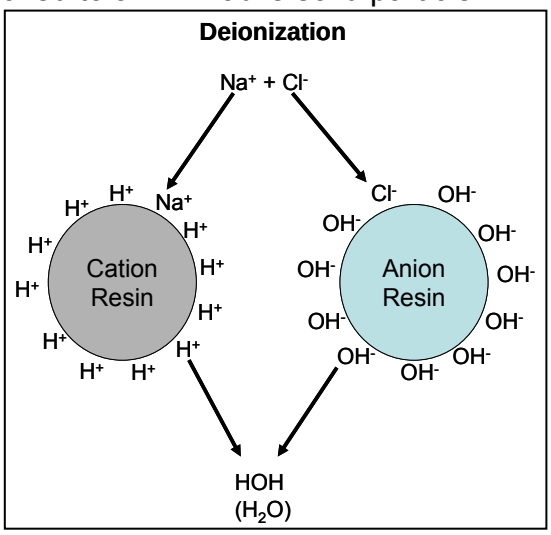
8. Stills are usually rated in gal/hr. or liters/hr. distillate produced. Larger units produce about 6 - 8 liters/hr. Since distillate not produced on demand, a storage container is required.

B. Ion exchange

1. How it works

   a. Chemical reaction where an ion from the solution is exchanged for a similarly charged ion attached to an immobile solid particle.

   b. Synthetic organic resins (polymers) most often used as immobile particle because they can be tailored to specific applications.



   c. In water deionization systems, the resins exchange either H^+ ions for the cations in the water (metals, NH_4) or exchange OH^- ions for the anions in the water (Cl^-, SO_4^{-2})

2. Classification of resins

   a. Strong acid cation resins

      (1) Chemical behavior similar to strong acids - highly ionized.

      (2) Can convert metal salts to corresponding acid (i.e. resin-SO_3H + $NiCl_2$ -----> resin-Ni + HCl

(3) Useful over entire pH range

(4) H^+ exchange resin used for deionization, Na^+ exchange resin used for softening (resin is same, recharge solution is different)

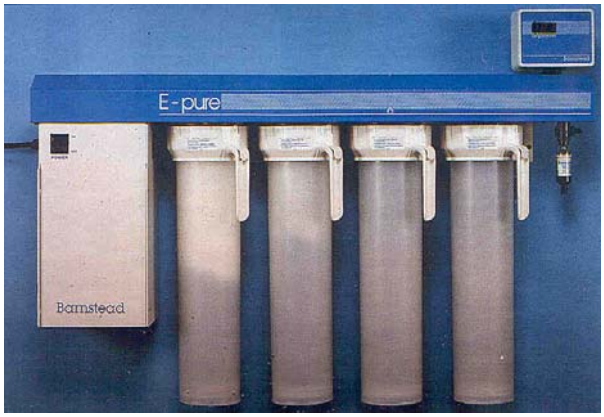
b. Strong base anion resins

(1) Highly ionized, behave like strong base, useful over entire pH range

(2) In water deionization, used in the hydroxide form; exchanges anions in solution with OH^-

3. Mixed bed of strong cation and strong anion resins most often used in lab water purification systems

4. Where cationic and anionic resins are used separately, use anionic resin downstream from cationic resins.



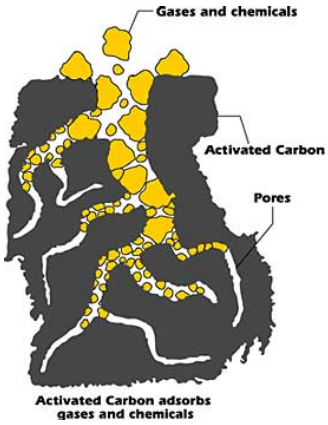
5. Use of ion exchange columns often adds some organic material to the water, especially when the resin is fresh. This may be a significant source of contamination in such analyses as BOD, COD, TOC, etc. Bacteria may also grow on the media.

6. Ion exchange is sometimes used as a means of pretreating water to be distilled. While water softening is encouraged for this purpose, the use of smaller columns will be expensive due to their limited capacity and the high amount of hardness typically found in many feed water sources.

7. Many units are capable of supplying sufficient purified water on demand such that storage is not necessary

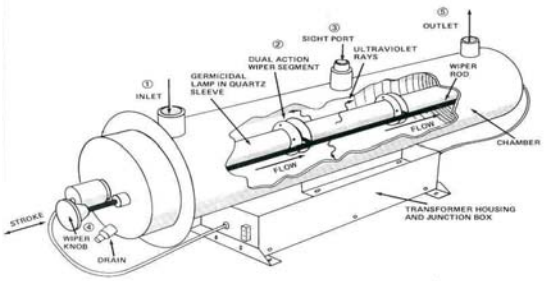
C. Carbon adsorption

Activated carbon is a granular, inorganic form of carbon which is very porous, giving it a very high surface area. Many organic, and some inorganic chemicals will adsorb onto the carbon.



1. Generally used to remove chlorine and organic impurities
2. May be used before or after distillation process
3. Columns may be purchased with carbon/ion exchange resin mixture
4. Will not remove bacteria

D. Ultraviolet Oxidation



1. Oxidizes Organic Contaminants
2. 185 nM UV Lamp
3. Can Remove Organics to Less Than 20 ppb

E. Membrane filtration

1. Removes particulate matter
2. Removes bacteria
3. May contribute some organics to water (some filters contain as much as 8% soluble mass)

F. Reverse Osmosis

1. First developed in 1958. Uses include several areas:
   a. Reclamation of precious metals
   b. Reclamation of chemicals

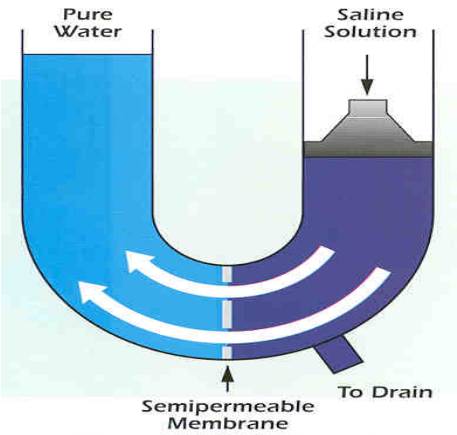
c. Food processing (maple syrup)

d. Lab water purification

2. Basic principles

a. Osmosis - water flows from less concentrated solution to more concentrated solution thru a semi-permeable membrane.

b. Reverse Osmosis - uses pressure to reverse normal osmotic flow; water flows under pressure from more concentrated solution to less concentrated solution thru semi-permeable membrane. Pressure pushes the purified water through the membrane, leaving contaminates behind.



c. Combination of reactions is involved, both physical and chemical

d. Salts are rejected by membrane, only water passes thru. Large organic molecules, bacteria, and pyrogens also may be removed.

3. Since RO removes a only a percentage of the feedwater contaminants, this process is usually used as a pretreatment system for other water purification processes.

4. RO generally removes:

95% of hardness

85% of salts

100% of bacteria and particulates

G. Storage of Lab Water

1. Purified water will leach soluble materials from glass

2. Organic plasticizers may be leached from plastic storage containers

3. Rubber stoppers contain organics which may leach into water, and concentrations of zinc that will cause significant contamination of water

4. Highly purified water must be used immediately after preparation; storage will degrade the quality of the water. See discussion in quality guidelines below.

H. Piping Systems

1. Lab water may be stored in tank or may be distributed throughout lab by plumbing system
2. Plumbing may be tin, tin-lined brass, stainless steel, plastic, or glass (tin is best but very expensive)
3. Plastic or glass plumbing with Teflon O-rings is usually satisfactory; use glass if concerned with organics
4. For delivery tubes use glass if possible. Vinyl tubing may leach some organics; latex tubing should be avoided.

II. Determining Laboratory Water Quality

A. Quality Indicators

1. Specific Conductance, micromho per centimeter, µmho/cm
   a. Measures the amount of current that the solution will carry
   b. Increases with increasing ionic concentration
   c. Does not reflect most organics, particulates, bacteria or very low concentrations of metals

2. Resistivity, Megohm centimeters, Mohm-cm
   a. Measures the amount of resistance to carrying a current
   b. Decreases with increasing ionic concentration
   c. Reciprical µmho/cm
   d. Does not reflect most organics, particulates, bacteria or very low concentrations of metals
   e. Usually in-line instrument monitors water quality

3. Total Organic Carbon, TOC, µg/L
4. Sodium, µg/L
5. Chloride, µg/L
6. Silica, µg/L
7. Bacteria, CFU/100 mL
8. BOD blank depletion
9. Phosphorus blanks
10. Split samples, reference samples

B. Quality Guidelines

1. Standard Methods (21st Edition)

a. High Quality

1. < 0.1 µmhos/cm, or >10 megohm-cm at 25°C
2. SiO_2 < 0.05 mg/L
3. Typically prepared by distillation, deionization, or reverse osmosis treatment of feedwater, followed by polishing with a mixed-bed deionizer and 0.2 µm pore membrane filtration. Alternatively, prepare by reverse osmosis followed by carbon adsorption and deionization.
4. High quality water cannot be stored without significant degradation; produce it continuously and use it immediately.

b. Medium Quality

1. <1 µmhos/cm, or >1 megohm-cm at 25°C
2. SiO_2 < 0.1 mg/L
3. Produced by distillation or deionization.
4. May be stored for limited time in material that will protect it from contamination, such as TFE or glass for organics

analysis, or plastics for metals.

5. Adequate for most general analytical work

c. Low Quality

1. 10 µmhos/cm, or 0.1 megohm-cm at 25°C

2. SiO_2 <1 mg/L

3. Use for glassware washing, preliminary rinsing, etc.

4. Use as feed water in production of higher grade water

2. American Society for Testing and Materials

American Society for Testing and Materials (ASTM) D1193-91

Standard specification for Reagent Grade Water

This specification covers requirements for water suitable for use in methods of chemical analysis and physical testing, the choice of one of the various grades being designated by the method or the investigator.

	Type I*	Type II **	Type III***	Type IV
Electrical Conductivity Max. (µS/cm @ 25°C)	0.056	1.0	0.25	5.0
Electrical Resistivity Min. (MΩ-cm @ 25°C)	18.0	1.0	4.0	0.2
pH @ 25°C	-	-	-	5.0 - 8.0
TOC max. (µ g/L)	100	50	200	No limit
Sodium max (µg/L)	1	5	10	50
Silica max. (µg/L)	3	3	500	No limit
Chloride max. (µg/L)	1	5	10	50

Key:

*Requires the use of 0.2µm membrane filter
** Prepared by distillation
*** Requires the use of a 0.45µm membrane filter

When bacterial levels need to be controlled, reagent grade types should be further classified as follows:

	Type A	Type B	Type C
Total Bacterial Count max. CFU/100 ml	1	10	1000
Endotoxin max. IU/ml	0.03	0.25	-

3. **International Organization for Standardization specification for water for laboratory use ISO 3696: 1987**

This standard covers three grades of water as follows:

a. Grade 1

Essentially free from dissolved or colloidal ionic and organic contaminants. It is suitable for the most stringent analytical requirements including those of high performance liquid chromatography (HPLC). It should be produced by further treatment of grade 2 water for example by reverse osmosis or ion exchange followed by filtration through a membrane filter of pore size 0.2µm to remove particle matter or re-distillation from a fused silica apparatus.

b. Grade 2

Very low inorganic, organic or colloidal contaminants and suitable for sensitive analytical purposes including atomic absorption spectrometry (AAS) and the determination of constituents in trace quantities. Can be produced by multiple distillation, ion exchange or reverse osmosis followed by distillation.

c. Grade 3

Suitable for most laboratory wet chemistry work and preparation of reagent solutions. Can be produced by single distillation, by ion exchange, or by reverse osmosis. Unless otherwise specified, it should be used for ordinary analytical work.

International Organization for Standardization specification for water for laboratory use ISO 3696: 1987

Parameter	Grade 1	Grade 2	Grade 3
pH value at 25°C inclusive range	N/A	N/A	5.0 to 7.5
Electrical conductivity µS/cm 25°C, max.	0.1	1.0	5.0
Oxidizable matter Oxygen (O_2) content mg/L max.	N/A	0.08	0.4
Absorbance at 254 nm and 1 cm optical path length, absorbance units, max.	0.001	0.01	Not specified
Residue after evaporation on heating at 110°C mg/kg, max.	N/A	1	2
Silica (SiO_2) content mg/L, max.	0.01	0.02	Not specified

METRIC SYSTEM

It is necessary to be familiar with the metric system in order to work effectively in a laboratory. Most measurements used in the laboratory are in the metric system. The metric system is convenient and easy to work with because all values are based on multiples or divisions of ten. Here are some of the most common values of the metric system used in wastewater treatment plant laboratories. An English system equivalent is given at the end of each category.

WEIGHTS	1 gram (g)	=	1000 milligrams (mg)
	1 gram	=	.001 kilograms (kg)
	1 kilogram	=	1000 grams
	1 milligram	=	1000 micrograms (µg)
	1 microgram	=	1000 nanograms (ng)
	1 pound	=	453.6 grams
VOLUMES	1 liter (l)	=	1000 milliliters (ml)
	1 gallon	=	3.785 liters
LINEAR	1 meter (m)	=	1000 millimeters (mm)
	1 meter	=	100 centimeters (cm)
	1 centimeter	=	10 millimeters
	1 millimeter	=	1000 microns (µ)
	1 micron	=	1000 nanometers (nm)
	1 inch	=	2.54 centimeters

CONCENTRATION
And
CONCENTRATION - VOLUME RELATIONSHIPS

The concentration, or strength, of a solution can be defined as the amount of a substance in a specified amount of solution. The amount of the substance is described (quantified) by the weight of the substance and may be expressed in terms of grams, ounces, or any other weight measurement. The amount of a solution is described (quantified) by volume and may be expressed in terms of gallons, liters, etc. Concentration, then, would be expressed as the ratio of the weight of the substance to one unit of volume of the solution, such as ounces per gallon, or grams per liter. The concentration of solutions used in wastewater laboratories is very commonly expressed in terms of milligrams per liter (mg/L). For example if 100 mg of phosphorus were dissolved in water and brought to a volume of 1 liter, the concentration of the solution is 100 mg/L.

Using the above definition of concentration, it can be seen that the weight of material in a given volume of liquid may be determined as follows:

The weight per unit volume times the number of unit volumes equals the weight of the substance in that volume of solution:

OR

Concentration X Volume = Weight

In the example of the phosphorus (P) solution, the amount (weight) of P in a solution may be determined if the concentration and the volume of the solution are known. For example: Calculate the amount of Phosphorus that would be in 50 mL of a 100 mg/L solution.

First convert the 50 mL to the equivalent volume in Liters:

$$50 \text{ mL} \times \frac{1 \text{ Liter}}{1000 \text{ mL}} = 0.05 \text{ L}$$

Then using the formula Concentration X Volume = Weigh:

$$100 \text{ mg/L} \times 0.05 \text{ L} = 5.0 \text{ mg}$$

Now consider the result of adding enough distilled water to bring 50 mL of this solution to 1 Liter. Since the weight of phosphorus in the total mixture remained unchanged even after the addition of water, the resulting concentration would be 5.0 mg in one Liter, or 5.0 mg/L.

This discussion demonstrates the principle behind the very important and useful lab calculation involved in making dilutions of solutions, both standards and samples.

When making a dilution there are two solutions involved, one before adding the water (Initial) and one after adding the water (Final). Because this relationship between volume and concentration, (Concentration X Volume = Weight), is true for both solutions, and since the amount (weight) of the substance is the same in both solutions, the relationship between the solutions can be given by:

Initial Concentration x Initial Volume = Final Concentration x Final Volume

By abbreviating concentrations with the letter C, the volumes with the letter V, and denoting the Initial solution by subscript 1 and the Final solution by subscript 2, the above equation becomes:

Initial Concentration (C_1) x Initial Volume (V_1) = Final Concentration (C_2) x Final Volume (V_2)

OR

$$C_1 \times V_1 = C_2 \times V_2$$

Where:

C_1 is the concentration of the solution before dilution

V_1 is the volume of the solution before dilution

C_2 is the concentration of the solution after dilution

V_2 is the volume of the solution after dilution

As stated above, this equation is very useful for many applications in the lab where dilution of a solution is involved.

Example One: Calculate the final concentration of a solution made by diluting 50 mL of a 100 mg/L phosphorus solution to one Liter.

C_1 = Initial Concentration = 100 mg/L
V_1 = Initial Volume = 50 mL
C_2 = Final Concentration = ?
V_2 = Final volume = one Liter = 1000 mL

$$C_1 \text{ X } V_1 = C_2 \text{ X } V_2$$
$$100 \text{ mg/L X } 50 \text{ mL} = C_2 \text{ X } 1000 \text{ mL}$$

Rearranging the relationship to solve for C_2:

$$\frac{C_1 \text{ X } V_1}{V_2} = C_2 \qquad \frac{100 \text{ mg/L X } 50 \text{ mL}}{1000 \text{ mL}} = 5.0 \text{ mg/L}$$

Therefore, 50 mL of a 100 mg/L solution diluted to one Liter gives a 5 mg/L solution.

Example Two: Calculate the volume (mL) of a 50 mg/L phosphorus needed to make 50 mL of a 2.0 mg/L phosphorus solution.

C_1 = Initial Concentration = 50 mg/L
V_1 = Initial Volume = ? mL
C_2 = Final Concentration = 2.0 mg/L
V_2 = Final volume = 50 mL

$$C_1 \text{ X } V_1 = C_2 \text{ X } V_2$$
$$50 \text{ mg/L X ? mL} = 2.0 \text{ mg/L X } 50 \text{ mL}$$

Rearranging the relationship to solve for V_1:

$$V_1 = \frac{C_2 \text{ X } V_2}{C_1} \qquad \frac{2.0 \text{ mg/L X } 50 \text{ mL}}{50 \text{ mg/L}} = 2.0 \text{ mL}$$

Therefore, 2.0 mL of a 50 mg/L solution is needed to make 50 mL of a 2.0 mg/L solution.

There are several other ways of indicating the concentration of a solution. Normality (N) is defined as the number of equivalent weights of material dissolved per liter of solution. Molarity is another expression of the concentration of chemical solutions often used in laboratories, and is defined as the number of moles of a substance dissolved in one liter of the solution. It is not necessary to be concerned with the technical understanding of equivalent weights or moles at this point, but to realize that normality and molarity describe concentration, and may be used in concentration – volume relationship calculations.

For instance, since normality is a concentration term, it can be substituted for concentration in the formula. We how have:

$$\text{Normality } (N_1) \times \text{Volume } (V_1) = \text{Normality } (N_2) \times \text{Volume } (V_2)$$

As an example problem, consider the following situation. Suppose we have a 5.0 normal solution of sodium hydroxide, NaOH and want to dilute that to end up with 500 mL of 0.40N NaOH. Calculate the mL of 5.0N NaOH that must be diluted to 500 mL to get the 0.40N solution required.

Initial Concentration, N_1 = 5.0 N

Volume required, V_1 = Unknown

Final Concentration, N_2 = 0.40 N

Final Volume, V_2 = 500 mL

$$N_1 \times V_1 = N_2 \times V_2$$

$$5.0\text{ N} \times V_1 = 0.40\text{N} \times 500\text{ mL}$$

$$\frac{5.0\text{ N} \times V_1}{5.0\text{ N}} = \frac{0.40\text{ N} \times 500\text{ mL}}{5.0\text{ N}}$$

$$V_1 = \frac{0.40\text{ N} \times 500\text{ mL}}{5.0\text{ N}}$$

$$V_1 = 40\text{ mL}$$

Therefore, if 40 mL of a 5.0N NaOH solution are diluted to 500 mL, the resulting solution will have a concentration of 0.40N.

Federal Register

Monday,
March 12, 2007

Part III

Environmental Protection Agency

40 CFR Part 122, 136, et al.

Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; National Primary Drinking Water Regulations; and National Secondary Drinking Water Regulations; Analysis and Sampling Procedures; Final Rule

■ 5. Section 122.44 is amended by revising paragraph (i)(1)(iv) to read as follows:

§ 122.44 Establishing limitations, standards, and other permit conditions (applicable to State NPDES programs; see § 123.25).

* * * * *

(i) * * *

(1) * * *

(iv) According to test procedures approved under 40 CFR Part 136 for the analyses of pollutants or another method is required under 40 CFR subchapters N or O. In the case of pollutants for which there are no approved methods under 40 CFR Part 136 or otherwise required under 40 CFR subchapters N or O, monitoring must be conducted according to a test procedure specified in the permit for such pollutants.

* * * * *

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

■ 6. The authority citation for Part 136 continues to read as follows:

Authority: Secs. 301, 304(h), 307, and 501(a) Pub. L. 95–217, 91 Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977.)

■ 7. Section 136.3 is amended as follows:

■ a. In paragraph (a) by revising the introductory text and Tables IA, IB, IC, ID, and IE.

■ b. In paragraph (a) by adding Table IG after the notes of Table IF.

■ c. In paragraph (b) by revising references 6, 10, and 17, and adding references 63 through 69.

■ d. By revising paragraphs (c), (d), and (e).

§ 136.3 Identification of test procedures.

(a) Parameters or pollutants, for which methods are approved, are listed together with test procedure descriptions and references in Tables IA, IB, IC, ID, IE, IF, and IG. In the event of a conflict between the reporting requirements of 40 CFR Parts 122 and 125 and any reporting requirements associated with the methods listed in these tables, the provisions of 40 CFR Parts 122 and 125 are controlling and will determine a permittee's reporting requirements. The full text of the referenced test procedures are incorporated by reference into Tables IA, IB, IC, ID, IE, IF, and IG. The incorporation by reference of these documents, as specified in paragraph (b) of this section, was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51. Copies of the documents may be obtained from the sources listed in paragraph (b) of this section. You can get information about obtaining these documents from the EPA Office of Water Engineering and Analysis Division at 202–566–1000. Documents may be inspected at EPA's Water Docket, EPA West, 1301 Constitution Avenue, NW., Washington, DC (Telephone: 202–566–2426); or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*

These test procedures are incorporated as they exist on the day of approval and a notice of any change in these test procedures will be published in the **Federal Register**. The discharge parameter values for which reports are required must be determined by one of the standard analytical test procedures incorporated by reference and described in Tables IA, IB, IC, IE, IF, and IG or by any alternate test procedure which has been approved by the Administrator under the provisions of paragraph (d) of this section and §§ 136.4 and 136.5. Under certain circumstances (paragraph (b) or (c) of this section or 40 CFR 401.13) other test procedures may be used if such other test procedures have been previously approved by the Regional Administrator of the Region in which the discharge will occur, and the Director of the State in which such discharge will occur does not object to the use of an additional or alternate test procedure.

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th Ed.	Standard methods online	AOAC, ASTM, USGS	Other
Bacteria:						
1. Coliform (fecal), number per 100 mL.	Most Probable Number (MPN), 5 tube 3 dilution, or	p. 132 [3]	9221C or E	9221C or E–99.		
	Membrane filter (MF) [2], single step.	p. 124 [3]	9222D	9222D–97	B–0050–85 [5].	
2. Coliform (fecal) in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 132 [3]	9221C or E	9221C or E–99.		
	MF, single step [6]	p. 124 [3]	9222D	9222D–97.		
3. Coliform (total), number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 114 [3]	9221B	9221B–99.		
	MF [2], single step or two step.	p. 108 [3]	9222B	9222B–97	B–0025–85 [5].	
4. Coliform (total), in presence of chlorine, number per 100 mL.	MPN, 5 tube, 3 dilution, or	p. 114 [3]	9221B	9221B–99.		
	MF [2] with enrichment	p. 111 [3]	9222 (B+B.5c)	9222 (B+B.5c)–97.		
5. E. coli, number per 100 mL [28].	MPN [7, 9, 15], multiple tube,		9221B.1/ 9221F [12 14].	9221B.1–99/ 9221F [12 14].		

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th Ed.	Standard methods online	AOAC, ASTM, USGS	Other
	multiple tube/multiple well,	.....	9223B [13]	9223B–97 [13]	991.15 [11]	Colilert® [13, 17], Colilert-18® [13, 16, 17]
	MF two step, or	1103.1 [20]	9222B/9222G [19], 9213D.	9222B–97/ 9222G [19].	D5392–93 [10].	
	single step	1603 [21], 1604 [22]	.....	.....	.....	mColiBlue-24® [18]
6. Fecal streptococci, number per 100 mL.	MPN, 5 tube, 3 dilution,	p. 139 [3]	9230B	9230B–93.		
	MF [2], or	p. 136 [3]	9230C	9230C–93	B–0055–85 [5].	
	Plate count	p. 143 [3].				
7. Enterococci, number per 100 mL [28].	MPN [7, 9] multiple tube, ..	.....	9230B	9230B–93.		
	multiple tube/multiple well.	.....	.....	.....	D6503–99 [10].	Entero-lert® [13, 23]
	MF [2, 6, 7, 8, 9], two step ...	1106.1 [24]	9230C	9230C–93	D5259–92 [10].	
	single step, or	1600 [25].				
	Plate count	p. 143 [3].				
Protozoa:						
8. *Cryptosporidium* [28].	Filtration/IMS/FA	1622 [26], 1623 [27].				
9. *Giardia* [28]	Filtration/IMS/FA	1623 [27].				
Aquatic Toxicity:						
10. Toxicity, acute, fresh water organisms, LC_{50}, percent effluent.	*Ceriodaphnia dubia* acute.	2002.0 [29].				
	Daphnia puplex and *Daphnia magna* acute.	2021.0 [29].				
	Fathead Minnow, *Pimephales promelas*, and Bannerfin shiner, *Cyprinella leedsi*, acute.	2000.0 [29].				
	Rainbow Trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, acute.	2019.0 [29].				
11. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC_{50}, percent effluent.	Mysid, *Mysidopsis bahia*, acute.	2007.0 [29].				
	Sheepshead Minnow, *Cyprinodon variegatus*, acute.	2004.0 [29].				
	Silverside, *Menidia beryllina*, *Menidia menidia*, and *Menidia peninsulae*, acute.	2006.0 [29].				
12. Toxicity, chronic, fresh water organisms, NOEC or IC_{25}, percent effluent.	Fathead minnow, *Pimephales promelas*, larval survival and growth.	1000.0 [30].				
	Fathead minnow, *Pimephales promelas*, embryo-larval survival and teratogenicity.	1001.0 [30].				
	Daphnia, *Ceriodaphnia dubia*, survival and reproduction.	1002.0 [30].				

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS—Continued

Parameter and units	Method [1]	EPA	Standard methods 18th, 19th, 20th Ed.	Standard methods online	AOAC, ASTM, USGS	Other
13. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC_{25}, percent effluent.	Green alga, *Selenastrum capricornutum*, growth.	1003.0 [30].				
	Sheepshead minnow, *Cyprinodon variegatus*, larval survival and growth.	1004.0 [31].				
	Sheepshead minnow, *Cyprinodon variegatus*, embryo-larval survival and teratogenicity.	1005.0 [31].				
	Inland silverside, *Menidia beryllina*, larval survival and growth.	1006.0 [31].				
	Mysid, *Mysidopsis bahia*, survival, growth, and fecundity.	1007.0 [31].				
	Sea urchin, *Arbacia punctulata*, fertilization.	1008.0 [31].				

[1] The method must be specified when results are reported.

[2] A 0.45-μ membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

[3] USEPA. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, EPA/600/8–78/017.

[4] [Reserved]

[5] USGS. 1989. U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, U.S. Geological Survey, U.S. Department of Interior, Reston, VA.

[6] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

[7] Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

[8] When the MF method has not been used previously to test ambient waters with high turbidity, large number of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

[9] To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current *Standard Methods for the Examination of Water and Wastewater* or EPA alternate test procedure (ATP) guidelines.

[10] ASTM. 2000, 1999, 1996. *Annual Book of ASTM Standards—Water and Environmental Technology*. Section 11.02. ASTM International. 100 Barr Harbor Drive, West Conshohocken, PA 19428.

[11] AOAC. 1995. *Official Methods of Analysis of AOAC International*, 16th Edition, Volume I, Chapter 17. Association of Official Analytical Chemists International. 481 North Frederick Avenue, Suite 500, Gaithersburg, MD 20877–2417.

[12] The multiple-tube fermentation test is used in 9221B.1. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

[13] These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by *E. coli*.

[14] After prior enrichment in a presumptive medium for total coliform using 9221B.1, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F. Commercially available EC–MUG media or EC media supplemented in the laboratory with 50 μg/mL of MUG may be used.

[15] Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® or Quanti-Tray® 2000, and the MPN calculated from the table provided by the manufacturer.

[16] Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35 °C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

[17] Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

[18] A description of the mColiBlue24® test, Total Coliforms and *E. coli*, is available from Hach Company, 100 Dayton Ave., Ames, IA 50010.

[19] Subject total coliform positive samples determined by 9222B or other membrane filter procedure to 9222G using NA MUG media.

[20] USEPA. 2002. Method 1103.1: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC, EPA–821–R–02–020.

[21] USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington, DC, EPA–821–R–02–023.

[22] Preparation and use of MI agar with a standard membrane filter procedure is set forth in the article, Brenner *et al.* 1993. "New Medium for the Simultaneous Detection of Total Coliform and *Escherichia coli* in Water." Appl. Environ. Microbiol. 59:3534–3544 and in USEPA. 2002. Method 1604: Total Coliforms and *Escherichia coli* (*E. coli*) in Water by Membrane Filtration by Using a Simultaneous Detection Technique (MI Medium). U.S. Environmental Protection Agency, Office of Water, Washington, DC, EPA 821–R–02–024.

[23] A description of the Enterolert® test may be obtained from IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092.

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology [58]	Reference (method number or page)					
		EPA [35, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM	USGS/AOAC/other
1. Acidity, as $CaCO_3$, mg/L	Electrometric endpoint or phenolphthalein endpoint.		2310 B(4a)	2310 B(4a)	2310 B(4a)–97	D1067–92, 02	I–1020–85 [2]
2. Alkalinity, as $CaCO_3$, mg/L	Electrometric or Colorimetric titration to pH 4.5, manual, or		2320 B	2320 B	2320 B–97	D1067–92, 02	973.43 [3], I–1030–85 [2]
	automatic	310.2 (Rev. 1974) [1].					I–2030–85 [2]
3. Aluminum—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration [36]		3111 D		3111 D–99		I–3051–85 [2]
	AA furnace		3113 B		3113 B–99.		
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES [36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–9750
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14 [3]
	Direct Current Plasma (DCP) [36]					D4190–94, 99	See footnote [34]
	Colorimetric (Eriochrome cyanine R)		3500–Al D	3500–Al B	3500–Al B–01.		
4. Ammonia (as N), mg/L	Manual, distillation (at pH 9.5) [6] followed by:	350.1, Rev. 2.0 (1993).	4500–NH B_3	4500–NH_3 B	4500–NH_3 B–97		973.49 [3]
	Nesslerization		4500–NH_3 C (18th only).			D1426–98, 03 (A)	973.49 [3], I–3520–85 [2]
	Titration		4500–NH_3 C (19th) and 4500–NH_3 E (18th).	4500–NH_3 C	4500–NH_3 C–97.		
	Electrode		4500–NH_3 D or E (19th) and 4500–NH_3 F or G (18th).	4500–NH_3 D or E	4500–NH_3 D or E–97.	D1426–98, 03 (B).	
	Automated phenate, or	350.1 [60], Rev. 2.0 (1993).	4500–NH_3 G (19th) and 4500–NH_3 H (18th).	4500–NH_3 G	4500–NH_3 G–97		I–4523–85 [2]
	Automated electrode						See footnote 7
	Ion Chromatography					D6919–03.	
5. Antimony—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration [36]		3111 B		3111 B–99.		
	AA furnace		3113 B		3113 B–99.		
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES [36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14 [3]
6. Arsenic—Total, [4] mg/L	Digestion [4] followed by	206.5 (Issued 1978) [1].					
	AA gaseous hydride		3114 B 4.d		3114 B 4.d–97	D2972–97, 03 (B)	I–3062–85 [2]
	AA furnace		3113 B		3113 B–99	D2972–97, 03 (C)	I–4063–98 [49]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES [36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14 [3]
	Colorimetric (SDDC)		3500–As C	3500–As B	3500–As B–97	D2972–97, 03 (A)	I–3060–85
7. Barium—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration [36]		3111 D		3111 D–99		I–3084–85 [2]
	AA furnace		3113 B		3113 B–99	D4382–95, 02.	

	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP[36]						See footnote[34]
8. Beryllium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 D		3111 D–99	D3645–93 (88), 03 (A).	I–3095–85[2]
	AA furnace		3113 B		3113 B–99	D3645–93 (88), 03 (B).	
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP, or					D4190–94, 99	See footnote[34]
	Colorimetric (aluminon)		3500–Be D.				
9. Biochemical oxygen demand (BOD_5), mg/L.	Dissolved Oxygen Depletion		5210 B	5210 B	5210 B–01		973.44,[3] p. 17.[9], I–1578–78[8]
10. Boron—Total,[37] mg/L	Colorimetric (curcumin)		4500–B B	4500–B B	4500–B B–00		I–3112–85[2]
	ICP/AES, or	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B99		I–4471–97[50]
	DCP					D4190–94, 99	See footnote 34
11. Bromide, mg/L	Titrimetric					D1246–95, 99 (C)	p. S44.[10]
							I–1125–85[2]
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30[3]
	CIE/UV						D6508, Rev. 2[54]
12. Cadmium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B or C		3111 B or C–99	D3557–95, 02 (A or B).	974.27,[3] p. 37.[9], I–3135–85[2] or I–3136–85[2]
	AA furnace		3113 B		3113 B–99	D3557–95, 02 (D)	I–4138–89[51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–1472–85[2] or I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP[36]					D4190–94, 99	See footnote[34]
	Voltametry[11], or					D3557–95, 02 (C).	
	Colorimetric (Dithizone)		3500–Cd D.				
13. Calcium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99	D511–93, 03(B)	I–3152–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	DCP, or						See footnote[34]
	Titrimetric (EDTA)		3500–Ca D	3500–Ca B	3500–Ca B–97	D511–93, 03(A).	
	Ion Chromatography					D6919–03.	
14. Carbonaceous biochemical oxygen demand ($CBOD_5$), mg/L[12].	Dissolved Oxygen Depletion with nitrification inhibitor.		5210 B	5210 B	5210 B–01.		
15. Chemical oxygen demand (COD), mg/L	Titrimetric	410.3 (Rev. 1978)[1].	5220 C	5220 C	5220 C–97	D1252–95, 00 (A)	973.46[3], p. 17[9] I–3560–85[2]
	Spectrophotometric, manual or automatic.	410.4, Rev. 2.0 (1993).	5220 D	5220 D	5220 D–97	D1252–95, 00 (B)	See footnotes[13, 14]. I–3561–85[2]
16. Chloride, mg/L	Titrimetric: (silver nitrate) or		4500–Cl–B	4500–Cl–B	4500–Cl–B–97	D512–89(99) (B)	I–1183–85[2]
	(Mercuric nitrate)		4500–Cl–C	4500–Cl–C	4500–Cl–C–97	D512–89 (99) (A)	973.51[3], I–1184–85[2]
	Colorimetric: manual or						I–1187–85[2]
	Automated (Ferricyanide)		4500–Cl–E	4500–Cl–E	4500–Cl–E–97		I–2187–85[2]
	Potentiometric Titration		4500–Cl–D	4500–Cl–D	4500–Cl–D–97.		
	Ion Selective Electrode					D512–89(99)(C).	

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology[58]	Reference (method number or page)					
		EPA[35, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM	USGS/AOAC/other
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30[3]
	CIE/UV						D6508, Rev. 2[54]
17. Chlorine—Total residual, mg/L; Titrimetric	Amperometric direct, or		4500–Cl D	4500–Cl D	4500–Cl D–00	D1253–86 (96), 03.	
	Amperometric direct (low level)		4500–Cl E	4500–Cl E	4500–Cl E–00.		
	Iodometric direct		4500–Cl B	4500–Cl B	4500–Cl B–00.		
	Back titration ether end–point[15] or		4500–Cl C	4500–Cl C	4500–Cl C–00.		
	DPD–FAS		4500–Cl F	4500–Cl F	4500–Cl F–00.		
	Spectrophotometric, DPD or		4500–Cl G	4500–Cl G	4500–Cl G–00.		
	Electrode						See footnote[16]
18. Chromium VI dissolved, mg/L	0.45–micron Filtration followed by:						
	AA chelation–extraction or		3111 C		3111 C–99		I–1232–85
	Ion Chromatography	218.6, Rev. 3.3 (1994).	3500–Cr E	3500–Cr C	3500–Cr C–01	D5257–97	993.23
	Colorimetric (Diphenyl–carbazide)		3500–Cr D	3500–Cr B	3500–Cr B–01	D1687–92, 02 (A)	I–1230–85
19. Chromium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B		3111 B–99	D1687–92, 02 (B)	974.27[3], I–3236–85[2]
	AA chelation–extraction		3111 C		3111 C–99.		
	AA furnace		3113 B		3113 B–99	D1687–92, 02 (C)	I–3233–93[46]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP,[36] or					D4190–94, 99	See footnote[34]
	Colorimetric (Diphenyl–carbazide)		3500–Cr D	3500–Cr B	3500–Cr B–01.		
20. Cobalt—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B or C		3111 B or C–99	D3558–94, 03 (A or B).	p. 37[9], I–3239–85[2]
	AA furnace		3113 B		3113 B–99	D3558–94, 03 (C)	I–4243–89[51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP					D4190–94, 99	See footnote[34]
21. Color, platinum cobalt units or dominant wavelength, hue, luminance purity.	Colorimetric (ADMI), or		2120 E	2120 E			See footnote[18]
	(Platinum cobalt), or		2120 B	2120 B	2120 B–01		I–1250–85[2]
	Spectrophotometric		2120 C	2120 C.			
22. Copper—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B or C		3111 B or C–99	D1688–95, 02 (A or B).	974.27[3] p. 37[9] I–3270–85[2] or I–3271–85[2]
	AA furnace		3113 B		3113 B–99	D1688–95, 02 (C)	I–4274–89[51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP[36] or					D4190–94, 99	See footnote[34]
	Colorimetric (Neocuproine) or		3500–Cu D	3500–Cu B	3500–Cu B–99.		
	(Bicinchoninate)		3500–Cu E	3500–Cu C	3500–Cu C–99		See footnote[19]

23. Cyanide—Total, mg/L	Automated Distillation and Colorimetry, or.						Kelada–01 [55]
	Manual distillation with $MgCl_2$ followed by:	335.4, Rev. 1.0 (1993) [57].	4500–CN–C	4500–CN–C		D2036–98(A)	10–204–00–1–X [56]
	Titrimetric or		4500–CN–D	4500–CN–D	4500–CN–D–99		p. 22 [9]
	Spectrophotometric, manual or		4500–CN–E	4500–CN–E	4500–CN–E–99	D2036–98(A)	I–3300–85
	Automated [20] or	335.4, Rev. 1.0 (1993) [57].					10–204–00–1–X [56], I–4302–85 [2]
	Ion Selective Electrode		4500–CN–F	4500–CN–F	4500–CN–F–99	D2036–98(A).	
24. Available Cyanide, mg/L	Cyanide Amenable to Chlorination (CATC); Manual distillation with $MgCl_2$ followed by Titrimetric or Spectrophotometric.		4500–CN–G	4500–CN–G	4500–CN–G–99	D2036–98(B).	
	Flow injection and ligand exchange, followed by amperometry [61].					D6888–04	OIA–1677 [44]
	Automated Distillation and Colorimetry.						Kelada–01 [55]
25. Fluoride—Total, mg/L	Manual distillation [6] followed by:		4500–F–B	4500–F–B	4500–F–B–97.		
	Electrode, manual or		4500–F–B	4500–F–B	4500–F–C–97	D1179–93, 99 (B).	
	Automated						I–4327–85 [2]
	Colorimetric, (SPADNS) or		4500–F–D	4500–F–D	4500–F–D–97	D1179–93, 99 (A).	
	Automated complexone		4500–F–E	4500–F–E	4500–F–E–97.		
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97,03	993.30 [3]
	CIE/UV						D6508, Rev. 2 [54]
26. Gold—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration, or		3111 B		3111 B–99.		
	AA furnace, or	231.2 (Rev. 1978) [1].					
	DCP						See footnote [34]
27. Hardness—Total, as $CaCO_3$, mg/L	Automated colorimetric,	130.1 (Issued 1971) [1].					
	Titrimetric (EDTA) or		2340 B or C	2340 B or C	2340 B or C–97	D1126–86(92), 02	973.5 2B [3], I–1338–85 [2]
	Ca plus Mg as their carbonates, by inductively coupled plasma or AA direct aspiration. (See Parameters 13 and 33)..						
28. Hydrogen ion (pH), pH units	Electrometric measurement or		4500–H^+ B	4500–H^+ B	4500–H^+ B–00	D1293–84 (90), 99 (A or B).	973.41. [3], I–1586–85 [2]
	Automated electrode	150.2 (Dec. 1982) [1].					See footnote [21], I–2587–85 [2]
29. Iridium—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration or		3111 B		3111 B–99.		
	AA furnace	235.2 (Issued 1978) [1].					
30. Iron—Total,[4] mg/L	Digestion [4] followed by:						
	AA direct aspiration [36]		3111 B or C		3111 B or C–99	D1068–96, 03 (A or B).	974.27 [3], I–3381–85 [2]
	AA furnace		3113 B		3113 B–99	D1068–96, 03 (C).	
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES [36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97 [50]
	DCP [36] or					D4190–94, 99	See footnote [34]
	Colorimetric (Phenanthroline)		3500–Fe D	3500–Fe B	3500–Fe B–97	D1068–96, 03 (D)	See footnote [22]
31. Kjeldahl Nitrogen [5]—Total, (as N), mg/L	Digestion and distillation followed by: [20]		4500–N_{org} B or C and 4500–NH_3 B.	4500–N_{org} B or C and 4500–NH_3 B.	4500–N_{org} B or C–97 and 4500–NH_3 B–97.	D3590–89, 02 (A).	
	Titration or		4500–NH_3 C (19th) and 4500–NH_3 E (18th).	4500–NH_3 C	4500–NH_3 C–97	D3590–89, 02 (A)	973.48 [3]

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology[58]	Reference (method number or page)					
		EPA[35, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM	USGS/AOAC/other
	Nesslerization or		4500–NH_3 C (18th Only).			D3590–89, 02 (A).	
	Electrode		4500–NH_3 F or G (18th) and 4500–NH_3 D or E (19th).	4500–NH_3 D or E	4500–NH_3 D or E–97.		
	Automated phenate colorimetric	351.1 (Rev. 1978)[1].					I–4551–78[8]
	Semi-automated block digestor colorimetric.	351.2, Rev. 2.0 (1993).				D3590–89, 02 (B)	I–4515–91[45]
	Manual or block digestor potentiometric.					D3590–89, 02 (A).	
	Block digester, followed by Auto distillation and Titration, or.						See footnote[39]
	Nesslerization, or						See footnote[40]
	Flow injection gas diffusion						See footnote[41]
32. Lead—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B or C		3111 B or C–99	D3559–96, 03 (A or B).	974.27[3], I–3399–85[2]
	AA furnace		3113 B		3113 B–99	D3559–96, 03 (D)	I–4403–89[51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP[36]					D4190–94, 99	See footnote[34]
	Voltametry[11] or					D3559–96, 03 (C).	
	Colorimetric (Dithizone)		3500–Pb D	3500–Pb B	3500–Pb B–97.		
33. Magnesium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99	D511–93, 03(B)	974.27[3], I–3447–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	DCP or						See footnote[34]
	Gravimetric		3500–Mg D.				
	Ion Chromatography					D6919–03.	
34. Manganese—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B		3111 B–99	D858–95, 02 (A or B).	974.27[3], I–3454–85[2]
	AA furnace		3113 B		3113 B–99	D858–95, 02 (C).	
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP36, or					D4190–94, 99	See footnote[34]
	Colorimetric (Persulfate), or		3500—Mn D	3500–Mn B	3500–Mn B–99		920.203[3]
	(Periodate)						See footnote[23]
35. Mercury—Total[4], mg/L	Cold vapor, manual or	245.1, Rev. 3.0 (1994).	3112 B		3112 B–99	D3223–97, 02	977.22[3], I–3462–85[2]
	Automated	245.2 (Issued 1974).					
	Cold vapor atomic fluorescence spectrometry (CVAFS).	245.7 Rev. 2.0 (2005)[59].					
	Purge and Trap CVAFS	1631E[43].					
36. Molybdenum—Total[4], mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 D		3111 D–99		I–3490–85[2]

	AA furnace		3113 B		3113 B–99		I–3492–96 [47]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97 [50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14 [3]
	DCP						See footnote [34]
37. Nickel—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration [36]		3111 B or C		3111 B or C–99	D1886–90, 94 (98) (A or B).	I–3499–85 [2]
	AA furnace		3113 B		3113 B–99	D1886–90, 94 (98) (C).	I–4503–89 [51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES [36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97 [50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14 [3]
	DCP [36], or					D4190–94, 99	See footnote [34]
	Colorimetric (heptoxime)		3500–Ni D (17th Edition).				
38. Nitrate (as N), mg/L	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30 [3]
	CIE/UV						D6508, Rev. 2 [54]
	Ion Selective Electrode		4500–NO_3^-–D	4500–NO_3^-–D	4500–NO_3^-–D–00.		
	Colorimetric (Brucine sulfate), or	352.1 [1]					973.50 [3], 419D [1,7], p. 28 [9]
	Nitrate-nitrite N minus Nitrite N (See parameters 39 and 40)..						
39. Nitrate-nitrite (as N), mg/L	Cadmium reduction, manual or		4500–NO_3^-–E	4500–NO_3^-–E	4500–NO3–E–00	D3867–99(B).	
	Automated, or	353.2, Rev. 2.0 (1993).	4500–NO_3^-–F	4500–NO_3^-–F	4500–NO_3^-–F–00	D3867–99(A)	I–4545–85 [2]
	Automated hydrazine		4500–NO_3^-–H	4500–NO_3^-–H	4500–NO_3^-–H–00.		
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97	993.30 [3]
	CIE/UV						D6508, Rev. 2 [54]
40. Nitrite (as N), mg/L	Spectrophotometric: Manual or		4500–NO_2^-–B	4500–NO_2^-–B	4500–NO_2^-–B–00		See footnote [25]
	Automated (Diazotization)						I–4540–85 [2]
	Automated (*bypass cadmium reduction).	353.2, Rev. 2.0 (1993).	4500–NO_3^-–F	4500–NO_3^-–F	4500–NO_3^-–F–00	D3867–99(A)	I–4545–85 [2]
	Manual (*bypass cadmium reduction)		4500–NO_3^-–E	4500–NO_3^-–E	4500–NO_3^-–E–00	D3867–99(B).	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30 [3]
	CIE/UV						D6508, Rev.2 [54]
41. Oil and grease—Total recoverable, mg/L	Hexane extractable material (HEM): n–Hexane extraction and gravimetry.	1664A [42]		5520 B [38]	5520 B–01 [38].		
	Silica gel treated HEM (SGT–HEM): Silica gel treatment and gravimetry..	1664A [42].					
42. Organic carbon—Total (TOC), mg/L	Combustion or oxidation		5310 B, C, or D	5310 B, C, or D	5310 B, C, or D–00	D2579–93 (A or B)	973.47,[3] p. 14 [24]
43. Organic nitrogen (as N), mg/L	Total Kjeldahl N (Parameter 31) minus ammonia N (Parameter 4).						
44. Orthophosphate (as P), mg/L	Ascorbic acid method:.						
	Automated, or	365.1, Rev. 2.0 (1993).	4500–P F	4500–P F			973.56 [3], I–4601–85 [2]
	Manual single reagent		4500–P E	4500–P E		D515–88(A)	973.55 [3]
	Manual two reagent	365.3 (Issued 1978)[1].					

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology[58]	Reference (method number or page)					
		EPA[35, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM	USGS/AOAC/other
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30[3]
	CIE/UV						D6508, Rev. 2[54]
45. Osmium—Total[4], mg/L	Digestion[4] followed by:						
	AA direct aspiration, or		3111 D		3111 D–99.		
	AA furnace	252.2 (Issued 1978)[1].					
46. Oxygen, dissolved, mg/L	Winkler (Azide modification), or		4500–O C	4500–O C	4500–O C–01	D888–92, 03 (A)	973.4 5B[3], I–1575–78[8]
	Electrode		4500–O G	4500–O G	4500–O G–01	D888–92, 03 (B)	I–1576–78[8]
47. Palladium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration, or		3111 B		3111 B–99		p. S27[10]
	AA furnace	253.2[1] (Issued 1978).					p. S28[10]
	DCP						See footnote[34]
48. Phenols, mg/L	Manual distillation[26] Followed by:	420.1[1] (Rev. 1978).					See footnote[27]
	Colorimetric (4AAP) manual, or	420.1[1] (Rev. 1978).					See footnote[27]
	Automated	420.4 Rev. 1.0 (1993).					
49. Phosphorus (elemental), mg/L	Gas–liquid chromatography						See footnote[28]
50. Phosphorus—Total, mg/L	Persulfate digestion followed by:[20]		4500–P B.5	4500–P B.5			973.55[3]
	Manual or	365.3[1] (Issued 1978).	4500–P E	4500–P E		D515–88(A).	
	Automated ascorbic acid reduction	365.1 Rev. 2.0 (1993).	4500–P F	4500–P F			973.56[3], I–4600–85[2]
	Semi–automated block digestor	365.4[1] (Issued 1974).				D515–88(B)	I–4610–91[48]
51. Platinum—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99.		
	AA furnace	255.2[1].					
	DCP						See footnote[34]
52. Potassium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99		973.53[3], I–3630–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	Flame photometric, or		3500–K D	3500–K B	3500–K B–97.		
	Colorimetric						317 B[17]
	Ion Chromatography					D6919–03.	
53. Residue—Total, mg/L	Gravimetric, 103–105°		2540 B	2540 B	2540 B–97		I–3750–85[2]
54. Residue—filterable, mg/L	Gravimetric, 180°		2540 C	2540 C	2540 C–97		I–1750–85[2]
55. Residue—non–filterable (TSS), mg/L	Gravimetric, 103–105 °C post washing of residue.		2540 D	2540 D	2540 D–97		I–3765–85[2]
56. Residue—settleable, mg/L	Volumetric, (Imhoff cone), or gravimetric.		2540 F	2540 F	2540 F–97.		
57. Residue—Volatile, mg/L	Gravimetric, 550 °C	160.4[1]					I–3753–85[2]
58. Rhodium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration, or		3111 B		3111 B–99.		
	AA furnace	265.2[1].					
59. Ruthenium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration, or		3111 B		3111 B–99.		
	AA furnace	267.2[1].					
60. Selenium—Total,[4] mg/L	Digestion[4] followed by:						
	AA furnace		3113 B		3113 B–99	D3859–98, 03 (B)	I–4668–98[49]

	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
61. Silica—Dissolved,[37] mg/L	AA gaseous hydride		3114 B		3114 B–97	D3859–98, 03 (A)	I–3667–85[2]
	0.45 micron filtration followed by:						
	Colorimetric, Manual or		4500–Si D	4500–SiO_2 C	4500–SiO_2C–97	D859–94, 00	I–1700–85[2]
	Automated (Molybdosilicate), or						I–2700–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
62. Silver—Total,[4, 31] mg/L	Digestion[4, 29] followed by:						
	AA direct aspiration		3111 B or C		3111 B or C–99		974.27[3], p. 37[9], I–3720–85[2]
	AA furnace		3113 B		3113 B–99		I–4724–89[51]
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP						See footnote[34]
63. Sodium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99		973.54[3], I–3735–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	DCP, or						See footnote[34]
	Flame photometric		3500–Na D	3500–Na B	3500–Na B–97.		
	Ion Chromatography					D 6919–03.	
64. Specific conductance, micromhos/cm at 25°C.	Wheatstone bridge	120.1[1] (Rev. 1982).	2510 B	2510 B	2510 B–97	D1125–95 (99) (A)	973.40[3], I–2781–85[2]
65. Sulfate (as SO_4), mg/L	Automated colorimetric	375.2, Rev. 2.0 (1993).					
	Gravimetric		4500–SO_4^{2-}C or D	4500–SO_4^{2-}C or D			925.54[3]
	Turbidimetric					D516–90, 02	426C[30]
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997).	4110 B	4110 B	4110 B–00	D4327–97, 03	993.30[3]
	CIE/UV						D6508, Rev. 2[54]
66. Sulfide (as S), mg/L	Titrimetric (iodine), or		4500–S^{2-}F (19th) 4500–S^{2-}E (18th).	4500–S^{2-}F	4500–S^{2-}F–00		I–3840–85[2]
	Colorimetric (methylene blue)		4500–S^{2-}D	4500–S^{2-}D	4500–S^{2-}D–00.		
	Ion Selective Electrode		4500–S^{2-}G	4500–S^{2-}G	4500–S^{2-}G–00	D4658–03.	
67. Sulfite (as SO_3), mg/L	Titrimetric (iodine-iodate)		4500–SO_3^{2-}B	4500–SO_3^{2-}B	4500–SO_3^{2-}B–00.		
68. Surfactants, mg/L	Colorimetric (methylene blue)		5540 C	5540 C	5540 C–00	D2330–88, 02.	
69. Temperature, °C	Thermometric		2550 B	2550 B	2550 B–00		See footnote[32]
70. Thallium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99.		
	AA furnace	279.2[1] (Issued 1978).					
	STGFAA	200.9, Rev. 2.2 (1994).					
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99.		
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
71. Tin—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 B		3111 B–99		I–3850–78[8]
	AA furnace, or		3113 B		3113 B–99.		
	STGFAA	200.9, Rev. 2.2 (1994).					

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES—Continued

Parameter	Methodology[58]	Reference (method number or page)					
		EPA[35, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM	USGS/AOAC/other
	ICP/AES	200.7, Rev. 4.4 (1994).					
72. Titanium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 D		3111 D–99.		
	AA furnace	283.2[1] (Issued 1978).					
	DCP						See footnote[34]
73. Turbidity, NTU[53]	Nephelometric	180.1, Rev. 2.0 (1993).	2130 B	2130 B	2130 B–01	D1889–94, 00	I–3860–85[2]
74. Vanadium—Total,[4] mg/L	Digestion[4] followed by:						
	AA direct aspiration		3111 D		3111 D–99.		
	AA furnace					D3373–93, 03.	
	ICP/AES	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP, or					D4190–94, 99	See footnote[34]
	Colorimetric (Gallic Acid)		3500–V D	3500–V B	3500–V B–97.		
75. Zinc –Total[4], mg/L	Digestion[4] followed by:						
	AA direct aspiration[36]		3111 B or C		3111 B or C–99	D1691–95, 02 (A or B).	974.27[3], p. 37[9], I–3900–85[2]
	AA furnace	289.2[1] (Issued 1978).					
	ICP/AES[36]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99[59]		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03	993.14[3]
	DCP,[36] or					D4190–94, 99	See footnote[34]
	Colorimetric (Dithizone) or		3500–Zn E.				
	(Zincon)		3500–Zn F	3500–Zn B	3500–Zn B–97		See footnote[33]

Table 1B Notes:

[1] "Methods for Chemical Analysis of Water and Wastes," Environmental Protection Agency, Environmental Monitoring Systems Laboratory–Cincinnati (EMSL–CI), EPA–600/4–79–020 (NTIS PB 84–128677), Revised March 1983 and 1979 where applicable.

[2] Fishman, M. J., *et al.* "Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments," U.S. Department of the Interior, Techniques of Water-Resource Investigations of the U.S. Geological Survey, Denver, CO, Revised 1989, unless otherwise stated.

[3] "Official Methods of Analysis of the Association of Official Analytical Chemists," Methods Manual, Sixteenth Edition, 4th Revision, 1998.

[4] For the determination of total metals (which are equivalent to total recoverable metals) the sample is not filtered before processing. A digestion procedure is required to solubilize analytes in suspended material and to break down organic-metal complexes (to convert the analyte to a detectable form for colorimetric analysis). For non–platform graphite furnace atomic absorption determinations a digestion using nitric acid (as specified in Section 4.1.3 of Methods for the Chemical Analysis of Water and Wastes) is required prior to analysis. The procedure used should subject the sample to gentle, acid refluxing and at no time should the sample be taken to dryness. For direct aspiration flame atomic absorption determinations (FLAA) a combination acid (nitric and hydrochloric acids) digestion is preferred prior to analysis. The approved total recoverable digestion is described as Method 200.2 in Supplement I of "Methods for the Determination of Metals in Environmental Samples" EPA/600R–94/111, May, 1994, and is reproduced in EPA Methods 200.7, 200.8, and 200.9 from the same Supplement. However, when using the gaseous hydride technique or for the determination of certain elements such as antimony, arsenic, selenium, silver, and tin by non–EPA graphite furnace atomic absorption methods, mercury by cold vapor atomic absorption, the noble metals and titanium by FLAA, a specific or modified sample digestion procedure may be required and in all cases the referenced method write–up should be consulted for specific instruction and/or cautions. For analyses using inductively coupled plasma-atomic emission spectrometry (ICP–AES), the direct current plasma (DCP) technique or the EPA spectrochemical techniques (platform furnace AA, ICP–AES, and ICP–MS) use EPA Method 200.2 or an approved alternate procedure (*e.g.*, CEM microwave digestion, which may be used with certain analytes as indicated in Table IB); the total recoverable digestion procedures in EPA Methods 200.7, 200.8, and 200.9 may be used for those respective methods. Regardless of the digestion procedure, the results of the analysis after digestion procedure are reported as "total" metals.

[5] Copper sulfate may be used in place of mercuric sulfate.

[6] Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary: however, manual distillation will be required to resolve any controversies.

[7] Ammonia, Automated Electrode Method, Industrial Method Number 379–75 WE, dated February 19, 1976, Bran & Luebbe (Technicon) Auto Analyzer II, Bran & Luebbe Analyzing Technologies, Inc., Elmsford, NY 10523.

[8] The approved method is that cited in "Methods for Determination of Inorganic Substances in Water and Fluvial Sediments", USGS TWRI, Book 5, Chapter A1 (1979).

[9] American National Standard on Photographic Processing Effluents, April 2, 1975. Available from ANSI, 25 West 43rd st., New York, NY 10036.

[10] "Selected Analytical Methods Approved and Cited by the United States Environmental Protection Agency," Supplement to the Fifteenth Edition of *Standard Methods for the Examination of Water and Wastewater* (1981).

[11] The use of normal and differential pulse voltage ramps to increase sensitivity and resolution is acceptable.

[12] Carbonaceous biochemical oxygen demand ($CBOD_5$) must not be confused with the traditional BOD_5 test method which measures "total BOD." The addition of the nitrification inhibitor is not a procedural option, but must be included to report the $CBOD_5$ parameter. A discharger whose permit requires reporting the traditional BOD_5 may not use a nitrification inhibitor in the procedure for reporting the results. Only when a discharger's permit specifically states $CBOD_5$ is required can the permittee report data using a nitrification inhibitor.

[13] OIC Chemical Oxygen Demand Method, Oceanography International Corporation, 1978, 512 West Loop, P.O. Box 2980, College Station, TX 77840.

[14] Chemical Oxygen Demand, Method 8000, Hach Handbook of Water Analysis, 1979, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

[15] The back titration method will be used to resolve controversy.

[16] Orion Research Instruction Manual, Residual Chlorine Electrode Model 97–70, 1977, Orion Research Incorporated, 840 Memorial Drive, Cambridge, MA 02138. The calibration graph for the Orion residual chlorine method must be derived using a reagent blank and three standard solutions, containing 0.2, 1.0, and 5.0 mL 0.00281 N potassium iodate/100 mL solution, respectively.

[17] The approved method is that cited in *Standard Methods for the Examination of Water and Wastewater,* 14th Edition, 1976.

[18] National Council of the Paper Industry for Air and Stream Improvement, Inc., Technical Bulletin 253, December 1971.

[19] Copper, Biocinchoinate Method, Method 8506, Hach Handbook of Water Analysis, 1979, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

[20] When using a method with block digestion, this treatment is not required.

[21] Hydrogen ion (pH) Automated Electrode Method, Industrial Method Number 378–75WA, October 1976, Bran & Luebbe (Technicon) Autoanalyzer II. Bran & Luebbe Analyzing Technologies, Inc., Elmsford, NY 10523.

[22] Iron, 1,10-Phenanthroline Method, Method 8008, 1980, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

[23] Manganese, Periodate Oxidation Method, Method 8034, Hach Handbook of Wastewater Analysis, 1979, pages 2–113 and 2–117, Hach Chemical Company, Loveland, CO 80537.

[24] Wershaw, R. L.,*et al.,* "Methods for Analysis of Organic Substances in Water," Techniques of Water-Resources Investigation of the U.S. Geological Survey, Book 5, Chapter A3, (1972 Revised 1987) p. 14.

[25] Nitrogen, Nitrite, Method 8507, Hach Chemical Company, P.O. Box 389, Loveland, CO 80537.

[26] Just prior to distillation, adjust the sulfuric-acid-preserved sample to pH 4 with 1 + 9 NaOH.

[27] The approved method is cited in *Standard Methods for the Examination of Water and Wastewater,* 14th Edition. The colorimetric reaction is conducted at a pH of 10.0±0.2. The approved methods are given on pp 576–81 of the 14th Edition: Method 510A for distillation, Method 510B for the manual colorimetric procedure, or Method 510C for the manual spectrometric procedure.

[28] R.F. Addison and R. G. Ackman, "Direct Determination of Elemental Phosphorus by Gas–Liquid Chromatography," *Journal of Chromatography,* Vol. 47, No.3, pp. 421–426, 1970.

[29] Approved methods for the analysis of silver in industrial wastewaters at concentrations of 1 mg/L and above are inadequate where silver exists as an inorganic halide. Silver halides such as the bromide and chloride are relatively insoluble in reagents such as nitric acid but are readily soluble in an aqueous buffer of sodium thiosulfate and sodium hydroxide to pH of 12. Therefore, for levels of silver above 1 mg/L, 20 mL of sample should be diluted to 100 mL by adding 40 mL each of 2 M $Na_2S_2O_3$ and NaOH. Standards should be prepared in the same manner. For levels of silver below 1 mg/L the approved method is satisfactory.

[30] The approved method is that cited in *Standard Methods for the Examination of Water and Wastewater,* 15th Edition.

[31] For samples known or suspected to contain high levels of silver (*e.g.*, in excess of 4 mg/L), cyanogen iodide should be used to keep the silver in solution for analysis. Prepare a cyanogen iodide solution by adding 4.0 mL of concentrated NH_4OH, 6.5 g of KCN, and 5.0 mL of a 1.0 N solution of I_2 to 50 mL of reagent water in a volumetric flask and dilute to 100.0 mL. After digestion of the sample, adjust the pH of the digestate to >7 to prevent the formation of HCN under acidic conditions. Add 1 mL of the cyanogen iodide solution to the sample digestate and adjust the volume to 100 mL with reagent water (NOT acid). If cyanogen iodide is added to sample digestates, then silver standards must be prepared that contain cyanogen iodide as well. Prepare working standards by diluting a small volume of a silver stock solution with water and adjusting the pH>7 with NH_4OH. Add 1 mL of the cyanogen iodide solution and let stand 1 hour. Transfer to a 100-mL volumetric flask and dilute to volume with water.

[32] Stevens, H.H., Ficke, J. F., and Smoot, G. F., "Water Temperature—Influential Factors, Field Measurement and Data Presentation," Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, Chapter D1, 1975.

[33] Zinc, Zincon Method, Method 8009, Hach Handbook of Water Analysis, 1979, pages 2–231 and 2–333, Hach Chemical Company, Loveland, CO 80537.

[34] "Direct Current Plasma (DCP) Optical Emission Spectrometric Method for Trace Elemental Analysis of Water and Wastes, Method AES0029," 1986—Revised 1991, Thermo Jarrell Ash Corporation, 27 Forge Parkway, Franklin, MA 02038

[35] Precision and recovery statements for the atomic absorption direct aspiration and graphite furnace methods, and for the spectrophotometric SDDC method for arsenic are provided in Appendix D of this part titled, "Precision and Recovery Statements for Methods for Measuring Metals."

[36] Microwave-assisted digestion may be employed for this metal, when analyzed by this methodology. "Closed Vessel Microwave Digestion of Wastewater Samples for Determination of Metals", CEM Corporation, P.O. Box 200, Matthews, NC 28106–0200, April 16, 1992. Available from the CEM Corporation.

[37] When determining boron and silica, only plastic, PTFE, or quartz laboratory ware may be used from start until completion of analysis.

[38] Only use n-hexane extraction solvent when determining Oil and Grease parameters—Hexane Extractable Material (HEM), or Silica Gel Treated HEM (analogous to EPA Method 1664A). Use of other extraction solvents (*e.g.*, those in the 18th and 19th editions) is prohibited.

[39] Nitrogen, Total Kjeldahl, Method PAI-DK01 (Block Digestion, Steam Distillation, Titrimetric Detection), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[40] Nitrogen, Total Kjeldahl, Method PAI–DK02 (Block Digestion, Steam Distillation, Colorimetric Detection), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[41] Nitrogen, Total Kjeldahl, Method PAI–DK03 (Block Digestion, Automated FIA Gas Diffusion), revised 12/22/94, OI Analytical/ALPKEM, P.O. Box 9010, College Station, TX 77842.

[42] Method 1664, Revision A "*n*-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated *n*-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry" EPA–821–R–98–002, February 1999. Available at NTIS, PB–121949, U.S. Department of Commerce, 5285 Port Royal, Springfield, VA 22161.

[43] USEPA. 2001. Method 1631, Revision E, "Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry" September 2002, Office of Water, U.S. Environmental Protection Agency (EPA–821–R–02–024). The application of clean techniques described in EPA's draft Method 1669: *Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels* (EPA–821–R–96–011) are recommended to preclude contamination at low-level, trace metal determinations.

[44] Available Cyanide, Method OIA–1677, "Available Cyanide by Flow Injection, Ligand Exchange, and Amperometry," ALPKEM, A Division of OI Analytical, P.O. Box 9010, College Station, TX 77842–9010.

[45] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Ammonia Plus Organic Nitrogen by a Kjeldahl Digestion Method," Open File Report (OFR) 00–170.

[46] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Chromium in Water by Graphite Furnace Atomic Absorption Spectrophotometry," Open File Report (OFR) 93–449.

[47] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Molybdenum by Graphite Furnace Atomic Absorption Spectrophotometry," Open File Report (OFR) 97–198.

[48] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Total Phosphorus by Kjeldahl Digestion Method and an Automated Colorimetric Finish That Includes Dialysis" Open File Report (OFR) 92–146.

[49] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Arsenic and Selenium in Water and Sediment by Graphite Furnace-Atomic Absorption Spectrometry" Open File Report (OFR) 98–639.

[50] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Elements in Whole-water Digests Using Inductively Coupled Plasma-Optical Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry," Open File Report (OFR) 98-165.

[51] "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Inorganic and Organic Constituents in Water and Fluvial Sediment," Open File Report (OFR) 93–125.

[52] All EPA methods, excluding EPA Method 300.1, are published in "Methods for the Determination of Metals in Environmental Samples," Supplement I, National Exposure Risk Laboratory-Cincinnati (NERL–CI), EPA/600/R–94/111, May 1994; and "Methods for the Determination of Inorganic Substances in Environmental Samples," NERL–CI, EPA/600/R–93/100, August, 1993. EPA Method 300.1 is available from *http://www.epa.gov/safewater/methods/pdfs/met300.pdf.*

[53] Styrene divinyl benzene beads (*e.g.* AMCO–AEPA–1 or equivalent) and stabilized formazin (e.g., Hach StablCal™ or equivalent) are acceptable substitutes for formazin.

[54] Method D6508, Rev. 2, "Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte," available from Waters Corp, 34 Maple St., Milford, MA, 01757, Telephone: 508/482–2131, Fax: 508/482–3625.

[55] Kelada-01, "Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate," EPA 821–B–01–009, Revision 1.2, August 2001, National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161 [Order Number PB 2001–108275]. The toll free telephone number is: 800–553–6847. Note: A 450–W UV lamp may be used in this method instead of the 550–W lamp specified if it provides performance within the quality control (QC) acceptance criteria of the method in a given instrument. Similarly, modified flow cell configurations and flow conditions may be used in the method, provided that the QC acceptance criteria are met.

[56] QuikChem Method 10–204–00–1–X, "Digestion and Distillation of Total Cyanide in Drinking and Wastewaters using MICRO DIST and Determination of Cyanide by Flow Injection Analysis" is available from Lachat Instruments 6645 W. Mill Road, Milwaukee, WI 53218, Telephone: 414–358–4200.

[57] When using sulfide removal test procedures described in Method 335.4, reconstitute particulate that is filtered with the sample prior to distillation.

[58] Unless otherwise stated, if the language of this table specifies a sample digestion and/or distillation "followed by" analysis with a method, approved digestion and/or distillation are required prior to analysis.

[59] Method 245.7, Rev. 2.0, "Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry," February 2005, EPA–821–R–05–001, available from the U.S. EPA Sample Control Center (operated by CSC), 6101 Stevenson Avenue, Alexandria, VA 22304, Telephone: 703–461–2100, Fax: 703–461–8056.

SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, either domestic sewage, industrial wastes or natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source.

The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may change valence states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations, such as iron and lead, may also adsorb onto surfaces (glass, plastic, quartz, etc.) Biological changes taking place in a sample may change the state of an element or a radical to a different state. Soluble constituents may be converted to organically bound material in cell structures, or cell lysis may result in release of cellular materials into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Required containers, preservation techniques and holding times have been designated by the EPA for parameters required under the NPDES permit. These are listed in the following table taken from 40 CFR Part 136 dated March 12, 2007. Please note that this table does not include preservation and holding information for the analysis of organics that are included in the original document. If that information is needed, please refer to the original document referenced above.

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

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Parameter number/name	Container [1]	Preservation [2,3]	Maximum holding time [4]
Table IA—Bacterial Tests:			
1–5. Coliform, total, fecal, and *E. coli*	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5].	6 hours
6. Fecal streptococci	PA, G	Cool, <10°C, 0.0008% $Na_2S_2O_3$ [5].	6 hours
7. Enterococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$ [5].	6 hours
Table IA—Protozoan Tests:			
8. *Cryptosporidium*	LDPE; field filtration	0–8 °C	96 hours [21]
9. *Giardia*	LDPE; field filtration	0–8 °C	96 hours [21]
Table IA—Aquatic Toxicity Tests:			
10–13. Toxicity, acute and chronic	P, FP, G	Cool, ≤6 °C [16]	36 hours
Table IB—Inorganic Tests:			
1. Acidity	P, FP, G	Cool, ≤6 °C [18]	14 days
2. Alkalinity	P, FP, G	Cool, ≤6 °C [18]	14 days
4. Ammonia	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
9. Biochemical oxygen demand	P, FP, G	Cool, ≤6 °C [18]	48 hours
10. Boron	P, FP, or Quartz	HNO_3 to pH<2	6 months
11. Bromide	P, FP, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, FP G	Cool, ≤6 °C [18]	48 hours
15. Chemical oxygen demand	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
16. Chloride	P, FP, G	None required	28 days
17. Chlorine, total residual	P, G	None required	Analyze within 15 min
21. Color	P, FP, G	Cool, ≤6 °C [18]	48 hours
23–24. Cyanide, total or available (or CATC)	P, FP, G	Cool, ≤6 °C [18], NaOH to pH>12 [6], reducing agent [5].	14 days
25. Fluoride	P	None required	28 days
27. Hardness	P, FP, G	HNO_3 or H_2SO_4 to pH<2.	6 months
28. Hydrogen ion (pH)	P, FP, G	None required	Analyze within 15 min
31, 43. Kjeldahl and organic N	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
Table IB—Metals: [7]			
18. Chromium VI	P, FP, G	Cool, ≤6 °C [18], pH = 9.3–9.7 [20].	28 days
35. Mercury (CVAA)	P, FP, G	HNO_3 to pH<2	28 days
35. Mercury (CVAFS)	FP, G; and FP- lined cap [17].	5 mL/L 12N HCl or 5 mL/ L BrCl [17].	90 days [17]
3, 5–8, 12, 13, 19, 20, 22, 26, 29, 30, 32–34, 36, 37, 45, 47, 51, 52, 58–60, 62, 63, 70–72, 74, 75. Metals, except boron, chromium VI, and mercury.	P, FP, G	HNO_3 to pH<2, or at least 24 hours prior to analysis [19].	6 months

TABLE II.—REQUIRED CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES

Parameter number/name	Container [1]	Preservation [2,3]	Maximum holding time [4]
38. Nitrate	P, FP, G	Cool, ≤6 °C [18]	48 hours
39. Nitrate-nitrite	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
40. Nitrite	P, FP, G	Cool, ≤6 °C [18]	48 hours
41. Oil and grease	G	Cool to ≤6 °C[18], HCl or H_2SO_4 to pH<2.	28 days
42. Organic Carbon	P, FP, G	Cool to ≤6 °C[18], HCl, H_2SO_4, or H_3PO_4 to pH<2	28 days
44. Orthophosphate	P, FP, G	Cool, ≤6 °C [18]	Filter within 15 min Analyze within 48 hr
46. Oxygen, Dissolved Probe	G, Bottle and top	None required	Analyze within 15 min
47. Winkler	G, Bottle and top	Fix on site and store in dark.	8 hours
48. Phenols	G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
49. Phosphorous (elemental)	G	Cool, ≤6 °C [18]	48 hours
50. Phosphorous, total	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH<2.	28 days
53. Residue, total	P, FP, G	Cool, ≤6 °C [18]	7 days
54. Residue, Filterable	P, FP, G	Cool, ≤6 °C [18]	7 days
55. Residue, Nonfilterable (TSS)	P, FP, G	Cool, ≤6 °C [18]	7 days
56. Residue, Settleable	P, FP, G	Cool, ≤6 °C [18]	48 hours
57. Residue, Volatile	P, FP, G	Cool, ≤6 °C [18]	7 days
61. Silica	P or Quartz	Cool, ≤6 °C [18]	28 days
64. Specific conductance	P, FP, G	Cool, ≤6 °C [18]	28 days
65. Sulfate	P, FP, G	Cool, ≤6 °C [18]	28 days
66. Sulfide	P, FP, G	Cool, ≤6 °C [18], add zinc acetate plus sodium hydroxide to pH>9.	7 days
67. Sulfite	P, FP, G	None required	Analyze within 15 min
68. Surfactants	P, FP, G	Cool, ≤6 °C [18]	48 hours
69. Temperature	P, FP, G	None required	Analyze
73. Turbidity	P, FP, G	Cool, ≤6 °C [18]	48 hours

1 ‘‘P’’ is polyethylene; ‘‘FP’’ is fluoropolymer (polytetrafluoroethylene (PTFE; Teflon□), or other fluoropolymer, unless stated otherwise in this Table II; ‘‘G’’ is glass; ‘‘PA’’ is any plastic that is made of a sterlizable material (polypropylene or other autoclavable plastic); ‘‘LDPE’’ is low density polyethylene.

2 Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), refrigerate the sample at ≤6 °C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤6 °C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed. Add the preservative to the sample container prior to sample collection when the preservative will not compromise the integrity of a grab sample, a composite sample, or an aliquot split from a composite sample ; otherwise, preserve the grab sample, composite sample, or aliquot split from a composite sample with in 15 minutes of collection. If a composite measurement is required but a composite sample would compromise sample integrity, individual grab samples must be collected at prescribed time intervals (e.g., 4 samples over the course of a day, at 6-hour intervals). Grab samples must be analyzed separately and the concentrations averaged. Alternatively, grab samples may be collected in the field and composited in the laboratory if the compositing procedure produces results equivalent to results produced by arithmetic averaging of the results of analysis of individual grab samples. For examples of laboratory compositing procedures, see EPA Method 1664A (oil and grease) and the procedures at 40 CFR 141.34(f)(14)(iv) and (v) (volatile organics).

3 When any sample is to be shipped by common carrier or sent via the U.S. Postal Service, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR Part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H2SO4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

4 Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of analysis and still be considered valid (e.g., samples analyzed for fecal coliforms may be held up to 6 hours prior to commencing analysis). Samples may be held for longer periods only if the permittee or monitoring laboratory has data on file to show that, for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under § 136.3(e). For a grab sample, the holding time begins at the time of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), the holding time begins at the time of the end of collection of the composite sample. For a set of grab samples composited in the field or laboratory, the holding time begins at the time of collection of the last grab sample in the set. Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if it knows that a shorter time is necessary to maintain sample stability. See § 136.3(e) for details. The date and time of collection of an individual grab sample is the date and time at which the sample is collected. For a set of grab samples to be composited, and that are all collected on the same calendar date, the date of collection is the date on which the samples are collected. For a set of grab samples to be composited, and that are collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15. For a composite sample collected automatically on a given date, the date of collection is the date on which the sample is

collected. For a composite sample collected automatically, and that is collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14–15.

5 Add a reducing agent only if an oxidant (e.g., chlorine) is present. Reducing agents shown to be effective are sodium thiosulfate (Na2S2O3), ascorbic acid, sodium arsenite (NaAsO2), or sodium borohydride (NaBH4). However, some of these agents have been shown to produce a positive or negative cyanide bias, depending on other substances in the sample and the analytical method used. Therefore, do not add an excess of reducing agent. Methods recommending ascorbic acid (e.g., EPA Method 335.4) specify adding ascorbic acid crystals, 0.1—0.6 g, until a drop of sample produces no color on potassium iodide (KI) starch paper, then adding 0.06 g (60 mg) for each liter of sample volume. If NaBH4 or NaAsO2 is used, 25 mg/L NaBH4 or 100 mg/L NaAsO2 will reduce more than 50 mg/L of chlorine (see method (Kelada-01'' and/or Standard Method 4500-CN¥ for more information). After adding reducing agent, test the sample using KI paper, a test strip (e.g. for chlorine, SenSafeTM Total Chlorine Water Check 480010) moistened with acetate buffer solution (see Standard Method 4500-Cl.C.3e), or a chlorine/oxidant test method (e.g., EPA Method 330.4 or 330.5), to make sure all oxidant is removed. If oxidant remains, add more reducing agent. Whatever agent is used, it should be tested to assure that cyanide results are not affected adversely.

6 Sample collection and preservation: Collect a volume of sample appropriate to the analytical method in a bottle of the material specified. If the sample can be analyzed within 48 hours and sulfide is not present, adjust the pH to >12 with sodium hydroxide solution (e.g., 5 % w/v), refrigerate as specified, and analyze within 48 hours. Otherwise, to extend the holding time to 14 days and mitigate interferences, treat the sample immediately using any or all of the following techniques, as necessary, followed by adjustment of the sample pH to >12 and refrigeration as specified. There may be interferences that are not mitigated by approved procedures. Any procedure for removal or suppression of an interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide. Particulate cyanide (e.g., ferric ferrocyanide) or a strong cyanide complex (e.g., cobalt cyanide) are more accurately measured if the laboratory holds the sample at room temperature and pH >12 for a minimum of 4 hours prior to analysis, and performs UV digestion or dissolution under alkaline (pH=12) conditions, if necessary.

(1) Sulfur: To remove elemental sulfur (S8), filter the sample immediately. If the filtration time will exceed 15 minutes, use a larger filter or a method that requires a smaller sample volume (e.g., EPA Method 335.4 or Lachat Method 01). Adjust the pH of the filtrate to >12 with NaOH, refrigerate the filter and filtrate, and ship or transport to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in µg or mg), and divide by the original sample volume to obtain the cyanide concentration.

(2) Sulfide: If the sample contains sulfide as determined by lead acetate paper, or if sulfide is known or suspected to be present, immediately conduct one of the volatilization treatments or the precipitation treatment as follows: Volatilization—Headspace expelling. In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a 4.4-L collapsible container (e.g., CubitainerTM). Acidify with concentrated hydrochloric acid to pH <2. Cap the container and shake vigorously for 30 seconds. Remove the cap and expel the headspace into the fume hood or open area by collapsing the container without expelling the sample. Refill the headspace by expanding the container. Repeat expelling a total of five headspace volumes. Adjust the pH to >12, refrigerate, and ship or transport to the laboratory. Scaling to a smaller

or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (> 10%). Dynamic stripping: In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a container of the material specified and acidify with concentrated hydrochloric acid to pH <2. Using a calibrated air sampling pump or flowmeter, purge the acidified sample into the fume hood or open area through a fritted glass aerator at a flow rate of 2.25 L/min for 4 minutes. Adjust the pH to >12, refrigerate, and ship or transport to the laboratory. Scaling to a smaller or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (>10%). Precipitation: If the sample contains particulate matter that would be removed by filtration, filter the sample prior to treatment to assure that cyanide associated with the particulate matter is included in the measurement. Ship or transport the filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in µg or mg), and divide by the original sample volume to obtain the cyanide concentration. For removal of sulfide by precipitation, raise the pH of the sample to >12 with NaOH solution, then add approximately 1 mg of powdered cadmium chloride for each mL of sample. For example, add approximately 500 mg to a 500-mL sample. Cap and shake the container to mix. Allow the precipitate to settle and test the sample with lead acetate paper. If necessary, add cadmium chloride but avoid adding an excess. Finally, filter through 0.45 micron filter. Cool the sample as specified and ship or transport the filtrate and filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate form the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in (g or mg), and divide by the original sample volume to obtain the cyanide concentration. If a ligand-exchange method is used (e.g., ASTM D6888), it may be necessary to increase the ligand-exchange reagent to offset any excess of cadmium chloride.

(3) Sulfite, thiosulfate, or thiocyanate: If sulfite, thiosulfate, or thiocyanate is known or suspected to be present, use UV digestion with a glass coil (Method Kelada-01) or ligand exchange (Method OIA–1677) to preclude cyanide loss or positive interference.

(4) Aldehyde: If formaldehyde, acetaldehyde, or another water-soluble aldehyde is known or suspected to be present, treat the sample with 20 mL of 3.5% ethylenediamine solution per liter of sample.

(5) Carbonate: Carbonate interference is evidenced by noticeable effervescence upon acidification in the distillation flask, a reduction in the pH of the absorber solution, and incomplete cyanide spike recovery. When significant carbonate is present, adjust the pH to ≥ 12 using calcium hydroxide instead of sodium hydroxide. Allow the precipitate to settle and decant or filter the sample prior to analysis (also see Standard Method 4500-CN.B.3.d).

(6) Chlorine, hypochlorite, or other oxidant: Treat a sample known or suspected to contain chlorine, hypochlorite, or other oxidant as directed in footnote 5.

7 For dissolved metals, filter grab samples within 15 minutes of collection and before adding preservatives. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), filter the sample within 15 minutes after completion of collection and before adding preservatives. If it is known or suspected that dissolved sample integrity will be compromised during collection of a composite sample collected automatically over time (e.g., by interchange of a metal between dissolved and suspended forms), collect and filter grab samples to be composited (footnote 2) in place of a composite sample collected automatically.

8 Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

9 If the sample is not adjusted to pH 2, then the sample must be analyzed within seven days of sampling.

10 The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within 3 days of sampling.

11 When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity (i.e., use all necessary preservatives and hold for the shortest time listed). When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to ≤6 °C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6-9; samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (regarding the requirement for thiosulfate reduction), and footnotes 12, 13 (regarding the analysis of benzidine).

12 If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

13 Extracts may be stored up to 30 days at <0 °C.

14 For the analysis of diphenylnitrosamine, add 0.008% $Na_2S_2O_3$ and adjust pH to 7–10 with NaOH within 24 hours of sampling.

15 The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% $Na_2S_2O_3$.

16 Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the preservation temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

17 Samples collected for the determination of trace level mercury (<100 ng/L) using EPA Method 1631 must be collected in tightly-capped fluoropolymer or glass bottles and preserved with BrCl or HCl solution within 48 hours of sample collection. The time to preservation may be extended to 28 days if a sample is oxidized in the sample bottle. A sample collected for dissolved trace level mercury should be filtered in the laboratory within 24 hours of the time of collection. However, if circumstances preclude overnight shipment, the sample should be filtered in a designated clean area in the field in accordance with procedures given in Method 1669. If sample integrity will not be maintained by shipment to and filtration in the laboratory, the sample must be filtered in a designated clean area in the field within the time period necessary to maintain sample integrity. A sample that has been collected for determination of total or dissolved trace level mercury must be analyzed within 90 days of sample collection.

18 Aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. Also, for purposes of NPDES monitoring, the specification of ‘‘≤ °C’’ is used in place of the ‘‘4 °C’’ and ‘‘<4 °C’’ sample temperature requirements listed in some methods. It is not necessary to measure the sample temperature to three significant figures (1/100th of 1 degree); rather, three significant figures are specified so that rounding down to 6 °C may not be used to meet the ≤6 °C requirement. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

19 An aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately (see footnote 2). Soil and sediment samples do not need to be preserved with acid. The allowances in this footnote supersede the preservation and holding time requirements in the approved metals methods.

20 To achieve the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. The allowance in this footnote supersedes preservation and holding time requirements in the approved hexavalent chromium methods, unless this supersession would compromise the measurement, in which case requirements in the method must be followed. 21 Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field

LABORATORY QUALITY ASSURANCE

The National Pollutant Discharge Elimination System (NPDES) has been initiated by the Federal Congress through the enactment of "The Federal Water Pollution Control Act Amendments of 1972" (United States Public Law 92-500). Implementation of a portion of the Act is being carried out by issuance of permits for wastewater discharges to surface waters. One of the "conditions applicable to all permits" as stated in the Code of Federal Regulations (40 CFR Section 122.41 (e)) requires the permit holder to "operate and maintain all facilities and systems of control..." It further states "proper operation and maintenance also includes adequate laboratory controls and appropriate quality assurance procedures." The State of Michigan has passed similar legislation that regulates discharges to groundwater and surface water.

In order to meet these requirements the permit holder must look at all aspects of the laboratory operations, check into the reliability of the data generated, change areas where problems are found, and document that these things were done. In other words, a laboratory quality assurance program must be developed. The following discussion will give a general idea of what a QA program is and what aspects should be considered.

PURPOSE OF QUALITY ASSURANCE PROGRAM

The purpose of a laboratory is to provide data to be used in decision making. The decisions may be as limited as the adjustment of a single valve, to as far-reaching as whether millions of dollars should be spent to improve the facility. These decisions rely on data that is assumed to be accurate. In many cases, an approximate answer or incorrect results is worse than no answer at all, because it will lead to faulty interpretations. Therefore, it is just as important for the laboratory to assure that the data is reliable as it is to provide that data.

This then is the purpose of the Laboratory Quality Assurance program, to provide confidence, or assure, that the data reliably describes the characteristics or the concentration of constituents in the samples submitted to the laboratory. This assurance must extend not only to the data being compiled at this time, but also to the data of the past and the data that will be compiled in the future. The program then, must be an on-going project that continually monitors and judges the reliability of the results of all analyses, records the checks made, and works to assure that future analyses can and will be done to give reliable results.

The quality assurance program should be developed to meet two primary functions. First, the program should act to control the quality of data generated in order to meet the requirements for reliability. To do this, the program must be set up to assure that the analyses used are acceptable and that these analyses are carried out using proper equipment and laboratory techniques. The second function is to monitor the reliability or truth of the results reported. This basically amounts to checking to see if the controls developed in the first part of the program are working. Just as each analytical method has a rigid protocol, so the quality control associated with that test must also involve definite required steps to monitor and assure that the results are correct. Ideally, all of the variables which can affect the final answer should be considered, evaluated, and controlled.

Laboratory Facilities and Equipment

Laboratory Services

The quality of laboratory services available to the analyst will affect the reliability of the generated data. The following items should be provided:

- An adequate supply of distilled water, free from interferences and other undesirable contaminants. Routine water quality checks should be conducted and documented;
- Adequate bench, instrumentation, storage, and recordkeeping space;
- Adequate lighting and ventilation;
- Dry, uncontaminated air when required;
- Efficient fume hood systems;
- Hot plate, refrigerator for samples, pH meter, thermometer, balance;
- Electrical power for routine laboratory use and, if appropriate, voltage-regulated sources for delicate electronic instruments; and
- Emergency equipment, fire extinguishers, eye wash station, shower, first aid kit, gloves, goggles.

Supplies

The reliability of data generated by a laboratory is directly affected by the reagents used in the analysis. The quality assurance program must address the special precautions required to insure proper selection, preparation, and storage of the reagents. The following items should be considered:

- The required reagent purity for specific analytical method is met;
- Standard reagents and solvents are stored according to the manufacturer's directions;
- Working standards are checked frequently to determine changes in concentration or composition;

- Concentrations of stock solutions are verified before being used to prepare new working standards;
- Laboratory supplies with limited shelf life are dated upon receipt and shelf life recommendations, including the discard date on the container and the storage requirements, are observed;
- Reagents are prepared and standardized against reliable primary standards; and
- Standards and reagents are properly labeled.

Glassware

Every laboratory analysis involves the use of glassware. Whether this is used merely to hold the sample, to measure a volume of reagent, or is a complicated apparatus used for digestion, it must be cleaned and used in a proper manner. The following items should be considered:

- Standard and specific procedures for cleaning glassware and containers are followed;
- The proper grade and type of glass (or plastic is used);
- Volumetric glassware must be used for measurement of solutions when a high degree of accuracy and precision is required.

Instruments and Equipment

The modern analytical laboratory depends very heavily upon instrumentation. The operation and maintenance of these devices ought to be a primary consideration in production of satisfactory data. The analyst should not only be very familiar with the manufacturer's suggested method of operation, but also have a fundamental understanding of the instrument design. This will assist in the correct use of the instruments, realizing its limitations, and in some cases, in detecting instrumental failures. Other items to consider include the following:

- Written requirements for daily operation of instruments and equipment are provided and followed;
- Standards are available to perform standard calibration procedures;
- Written trouble-shooting procedures are available;
- Written schedules for required or recommended replacement, cleaning, checking, and/or adjustment by service personnel are both available and followed.

Maintenance

Since laboratory data is dependent upon the equipment used to produce the data, improperly operating equipment may adversely affect the quality of the data, and equipment which is not operable will cause an interruption of data production. It can be seen therefore, that a maintenance program is as important to a laboratory as it is for the operation of a complex plant. Laboratory equipment and instrumentation need to be checked and serviced periodically to assure continued performance. Scheduling is very important in this program to make sure that equipment is not taken out of service during times when it is needed for use. The following items are suggested for consideration:

- Analytical balances should be scheduled for cleaning and calibration at least annually;
- Water distillers should be cleaned periodically depending on the quality of the feed water;
- Equipment such as ovens, furnaces, vacuum pumps, refrigerators, and incubators should be inspected and cleaned frequently;
- Safety equipment such as fire extinguishers, fume hoods, eyewash, and emergency shower must be included for inspection and cleaning;
- An annual schedule should be prepared which reserves a time for maintenance for each piece of equipment. This schedule should be reviewed periodically to plan the day on which the maintenance is to be done.

Sampling and Sample Handling

The reliability of numbers generated by any analytical procedure relies to a great extent upon the sampling used. If the sample collected does not represent the flow or process sampled, the laboratory results will be almost meaningless. The NPDES permit for each wastewater treatment facility will dictate how most types of samples are to be taken, whether by individual grab or by compositing samples over a specified period of time. Sampling devices and containers must be kept clean, and automatic composite samplers must have a flow velocity great enough to prevent solids from settling out in the sampling changer or sample collection line.

If samples are to be stored for a period of time before analysis it is important that proper storage and preservation procedures are implemented. These procedures usually include refrigeration of the sample both during (in the case of composite samples) and after collection, to slow biological activity. Preservation procedures for some types of samples also include the addition of an acid. This slows biological activity in the sample and also prevents the deposition of components onto the surface of the container. Proper procedures for storage and preservation of samples collected for NPDES reporting have been dictated by the EPA and may be found in the Sample Preservation Unit of this manual.

Depending upon the intended purpose of samples collected and the legal liability of the laboratory for these samples, it may be necessary for the Quality Assurance Program to include a "chain of custody" procedure. This will involve the use of a log book in which all phases of sample collection, preservation, transportation, and storage are recorded.

Also, depending on the legal liability for these samples, the use of a locking sample storage area may be required to which only authorized personnel have access.

Recordkeeping

A necessary part of the Quality Assurance Program is an adequate recordkeeping system. An important consideration for laboratories whose results are submitted on NPDES monitoring reports is that this becomes public information. In the event of a court action this information may be called upon by any of the parties concerned. It is essential that the information is accurate, understandable and complete. This includes records of any analysis as well as the quality assurance work done. Records of data will also be of benefit to the laboratory by providing a means of recognizing trends in data, possibly pointing out systematic errors in analytical procedures or techniques. Wastewater treatment plants depend upon the recordkeeping system in preparing financial reports, fine-tuning operations, and in troubleshooting problems that occur. Facilities which are required to monitor and report under the NPDES are required to keep all records for a minimum of three years. The kinds of laboratory records that must be kept depend somewhat upon the size and complexity of the facility, but the following list may be used as a general guideline.

- A listing of all analytical procedures used by the lab should be prepared. In the case of data required for NPDES reporting, a reference to the procedure in the EPA "Chemical Methods" manual or in "Standard Methods" should be sufficient. If there are any deviations from the approved method, however, the deviations must be listed and explained.
- All lab data should be initially recorded on a bench sheet prepared by the lab. The bench sheet should provide space for recording "raw" data as it is taken from the laboratory instruments, etc., and should show how these numbers are used to calculate reported data. The analyst should assure that numbers recorded on the bench sheet have been properly rounded off, and that proper use of significant figures is observed. Sampling dates and times should also be recorded on this sheet. The analyst responsible for the data must initial and date the bench sheet.

- Temperatures of all equipment which are used to maintain a specified temperature must be recorded daily. This would include equipment such as the drying oven, sample refrigerator, BOD incubator, muffle furnace, incubator for bacterial testing, and the autoclave. The person recording the temperature should also record the time and date, and should initial the log. In the smaller facilities it is acceptable to record this information directly on the bench sheet rather than to keep a separate temperature log.
- Log sheets should be kept for each piece of laboratory equipment and should provide recording space for the serial number, model number, the manufacture's name and address, and the person to call when service is necessary. The date and nature of each service call should be recorded as well as the recommended date of the next service call.
- An equipment inventory should be maintained, listing each piece of laboratory equipment and the model and serial numbers. This information is usually required by insurance companies and is also beneficial in large organizations for budgeting purposes and for maintaining spare parts inventories. The equipment inventory may also be used to keep records of glassware, filter papers, and other equipment which must be ordered on a periodic basis. This will help assure that the necessary supplies are on hand at the time that they are needed.
- The chemical inventory is another useful record which should be kept by the lab. It should contain a listing of each chemical used, the quantity on hand, and the shelf life, if appropriate. Records of chemical orders should be kept for future reference, and chemical containers should be dated as they are received and logged into the inventory. The chemical inventory will help prevent using chemicals past their shelf life, will identify which chemicals on

hand are not used and should be disposed of, and will prevent running out of a particular chemical when need for it is critical.

- Written procedures should be developed for hazard response methods, chemical spill cleanup, and disposal of spent or outdated reagents.

Precision and Accuracy of Laboratory Data

The purpose of laboratory control procedures is to ensure high quality sampling and analyses by the use of control samples, control charts, reference materials, and instrument calibrations. It is essential that controls are initiated and maintained throughout the analysis of samples. Each testing batch must contain at least one blank, and a schedule for including analysis of duplicate and spiked samples must be developed.

The precision of laboratory findings refers to the reproducibility of replicate observations. In a laboratory quality assurance program, precision is determined by the use of actual samples that cover a range of concentrations and the variety of interfering materials usually encountered by the analyst. Accuracy refers to the degree of difference between observed values and known or actual values. The accuracy of a procedure may be determined by recovery analysis. This is done by analyzing separate portions of a sample, one of which has been spiked with a known quantity of reference standard. Both portions of sample are taken through the entire analytical procedure and the percent recovery of the spike is calculated as follows:

$$\% \text{ Recovery} = \frac{(\text{conc sample} + \text{spike}) - (\text{conc sample})}{(\text{conc spike})} \times 100\%$$

To evaluate the precision and accuracy of the analytical procedures, the following steps should be taken:

. Control samples are introduced into the train of actual samples to monitor the performance of the analytical system. These control samples include duplicates, spikes, and reference samples.

. Duplicate analyses are performed to determine precision.

. Spiked and reference samples are used to monitor accuracy.

. Precision and accuracy control charts are prepared and used.

- limits are usually based on the standard deviation of determinations made on at least 15 - 20 control samples. All these need not be obtained on the same day; in fact, it is best if they are accumulated as part of a day-to-day operation.
- standard deviation(s) is calculated as follows:

$$s = \sqrt{\frac{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}{n-1}}$$

- warning limits are established at two times the standard deviation. If the results of subsequent control samples fall outside this range, possible sources of error should be investigated.
- control limits are established at three times the standard deviation. If the results of subsequent control samples fall outside this range, the procedure is said to be out of control and results of analysis of unknown samples cannot be considered reliable. These values should not be used for NPDES reporting or for making operational decisions.
- warning and control limits for a precision control chart may also be calculated using what are known as the D_4 factors. These are statistical values which eliminate the need to calculate the standard deviation. For duplicate analysis the control limit factor is 3.27 and the warning limit

factor is 2.51. An example of the use of this method will be given later in this discussion.

- out-of-control data and corrective actions taken should be fully documented.
- for examples of the calculation of standard deviation, warning and control limits, and preparation of precision and accuracy control charts see the following pages of this manual.

. After control charts have been developed, analysis of control samples should be done on a frequency which depends upon several factors. These factors include size and complexity of facility, number of samples analyzed, impact on environment, legal liability, and degree of analytical control required. These factors should be considered by each facility in determining how frequently control samples should be analyzed. The EPA suggests that for NPDES reporting, 10 - 20 percent of all samples analyzed should be control samples.

CALCULATION OF STANDARD DEVIATION

1. LIST RESULTS → x
2. COUNT NUMBER OF RESULTS → n
3. FIND SUM OF RESULTS → Σx
4. SQUARE SUM OF RESULTS → $\Sigma (x)^2$
5. SQUARE EACH RESULT → x^2
6. FIND SUM OF SQUARED RESULTS → Σx^2
7. DETERMINE STANDARD DEVIATION(S) USING FORMULA:

$$s = \sqrt{\frac{\Sigma (x^2) - \frac{(\Sigma x)^2}{n}}{n-1}}$$

CALCULATION OF CONTROL LIMITS

1. AVERAGE OF RESULTS → $\bar{x} = \frac{\Sigma x}{n}$
2. UPPER WARNING LIMIT = AVERAGE + (2 X STANDARD DEVIATION)
3. LOWER WARNING LIMIT = AVERAGE - (2 X STANDARD DEVIATION)
4. UPPER CONTROL LIMIT = AVERAGE + (3 X STANDARD DEVIATION)
5. LOWER CONTROL LIMIT = AVERAGE - (3 X STANDARD DEVIATION)

EXAMPLE OF CALCULATIONS FOR DEVELOPING AN

ACCURACY CONTROL CHART*

SAMPLE	DATE	SAMPLE RESULTS mg/L	SPIKED RESULTS mg/L (1.00 mg/L added)	PERCENT RECOVERY
1	3/10	1.02	1.93	91.0%
2	3/11	0.23	1.21	98.0
3	3/16	0.03	0.96	93.0
4	3/17	0.64	1.61	97.0
5	3/25	2.01	3.01	100.0
6	3/30	0.07	1.06	99.0
7	4/01	0.03	1.01	98.0
8	4/11	2.01	2.96	95.0
9	4/13	1.01	1.93	92.0
10 = n	4/15	0.04	1.05	101.0

* For simplification only 10 data points are given instead of the recommended minimum of 20.

To Calculate Standard Deviation Using Outline Above

1. x = 91.0%, 98.0, 93.0, 97.0, 100.0, 99.0, 98.0, 95.0, 92.0, 101.0

2. $n = 10$

3. $\Sigma x = 964$

4. $(\Sigma x)^2 = 964 \times 964 = 929296$

5. x^2 = 8281, 9604, 8649, 9409, 10000, 9801, 9604, 9025, 8464, 10201

6. $\Sigma x^2 = 93038$

7. $$s = \sqrt{\frac{93038 - \frac{929296}{10}}{10-1}} = \sqrt{\frac{93038 - 92929.6}{9}}$$

$$= \sqrt{\frac{108.4}{9}} = \sqrt{12.04} = 3.47$$

CALCULATION OF CONTROL LIMITS
FOR EXAMPLE DATA

1. AVERAGE OF RESULTS $\longrightarrow$ $x = \frac{\Sigma x}{n} = \frac{964}{10} = 96.4$

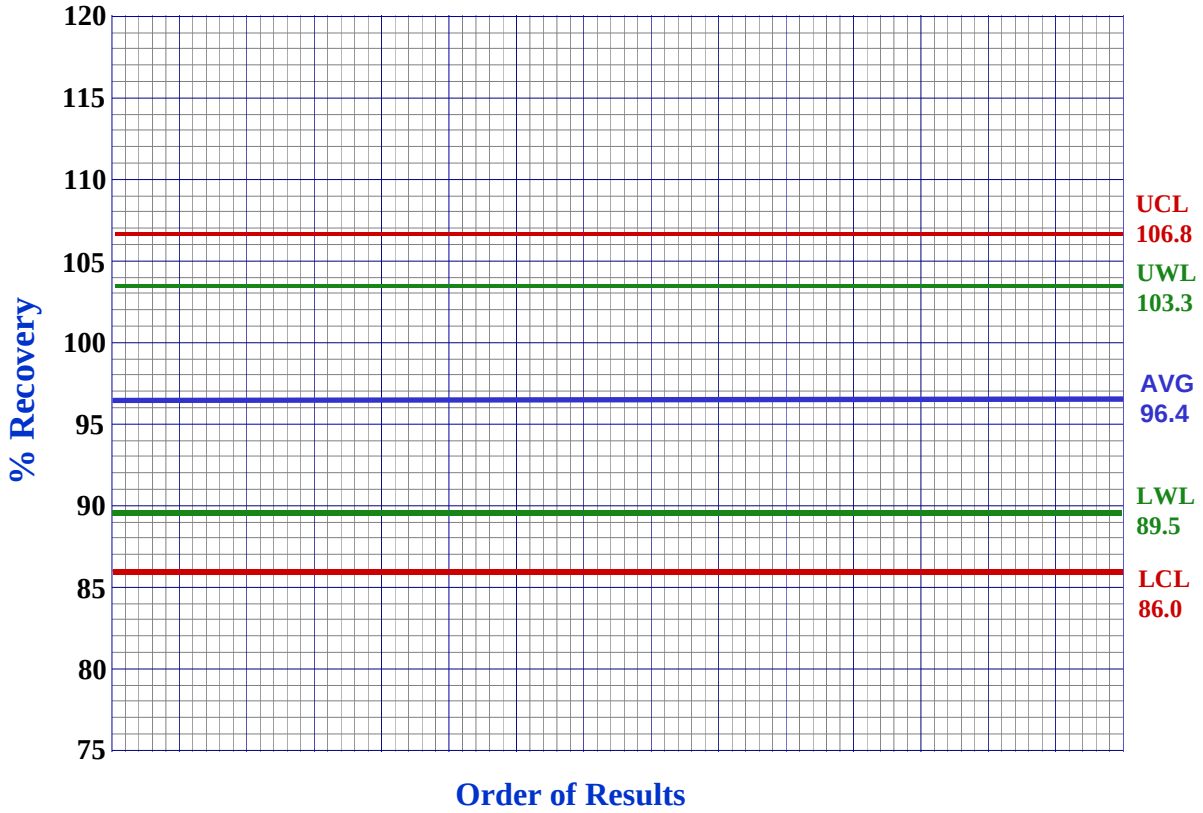
2. Upper Warning Limits = Average + (2 X Standard Deviation)
   = 96.4 + (2 X 3.47)
   = 96.4 + 6.94 = <u>103.34</u>

3. Lower Warning Limits = Average - (2 X Standard Deviation)
   = 96.4 - (2 X 3.47)
   = 96.4 - 6.94 = <u>89.46</u>

4. Upper Control Limits = Average + (3 X Standard Deviation)
   = 96.4 + (3 X 3.47)
   = 96.4 + 10.41
   = <u>106.81</u>

5. Lower Control Limits = Average - (3 X Standard Deviation)
   = 96.4 - (3 X 3.47)
   = 96.4 - 10.41
   = <u>85.99</u>

Example Accuracy Control Chart



EXAMPLE
PRECISION CONTROL CHART

DUPLICATE SUSPENDED SOLIDS ANALYSES OF EFFLUENT SAMPLES*

DUPLICATE #1	DUPLICATE #2	DIFFERENCE di
17 mg/L	15 mg/L	2 mg/L
18	15	3
19	14	5
14	15	1
20	18	2
22	14	8
13	12	1
15	10	5
10	11	1
16	19	3

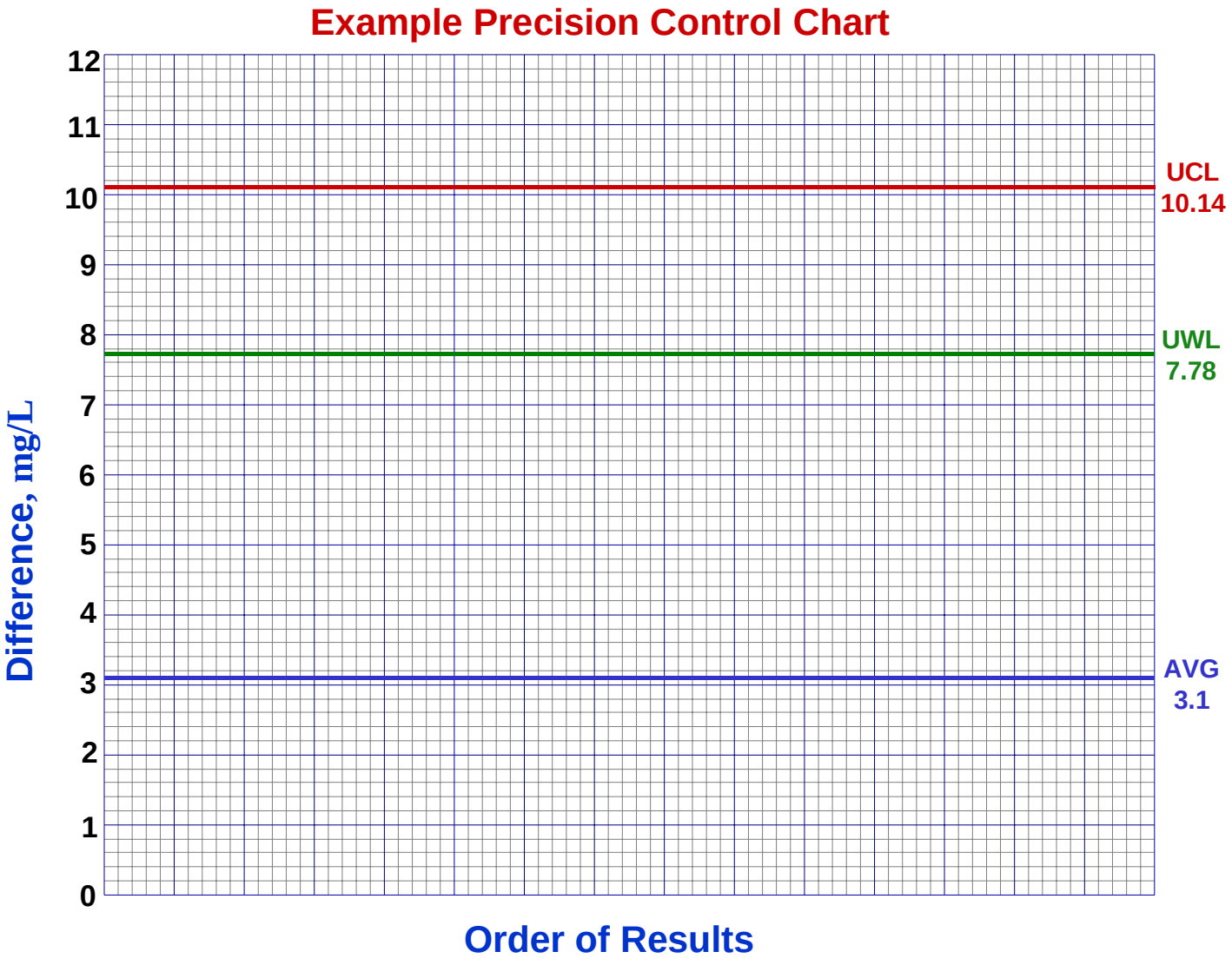
$$\Sigma\ di = 31$$

$$\bar{d} = \frac{\Sigma\ di}{n} = 3.1$$

WARNING LIMIT $= 2.51 \times \bar{d}$
$= 2.51 \times 3.1$
$= 7.78$

CONTROL LIMIT $= 3.27 \times \bar{d}$
$= 3.27 \times 3.1$
$= 10.14$

* FOR SIMPLIFICATION ONLY TEN DATA POINTS ARE GIVEN INSTEAD OF THE RECOMMENDED MINIMUM OF TWENTY.


Example Precision Control Chart
12
11
10
9
8
7
6
5
4
3
2
1
0
Difference, mg/L
UCL
10.14
UWL
7.78
AVG
3.1
Order of Results

DISSOLVED OXYGEN

Dissolved oxygen (DO) is one of the most often used and important analyses in the field of wastewater treatment. One of the most common uses is in the analysis for biochemical oxygen demand. The DO test is also used to determine the amount of oxygen present in secondary wastewater treatment processes. It is important, for example, that activated sludge systems have adequate oxygen for use by the bacteria and other microorganisms which live in the sludge. The microorganisms need this oxygen for respiration to metabolize the biodegradable matter present in the wastewater, as shown below:

$$CH_2O + O_2 \longrightarrow CO_2 + H_2O$$

Thus, the organic pollutants are changed by bacterial action to relatively relatively harmless carbon dioxide and water. This is the basic principle of biological treatment; in activated sludge, trickling filters, or any other aerobic biological treatment systems.

Other biodegradable matter in the wastewater is also acted upon by oxygen consuming bacteria. For example, ammonia nitrogen (NH_3-N) is oxidized to nitrate nitrogen (NO_3-N) in the process called nitrification. Since NO_3 exerts no oxygen demand, the oxygen depletion in the receiving water is reduced.

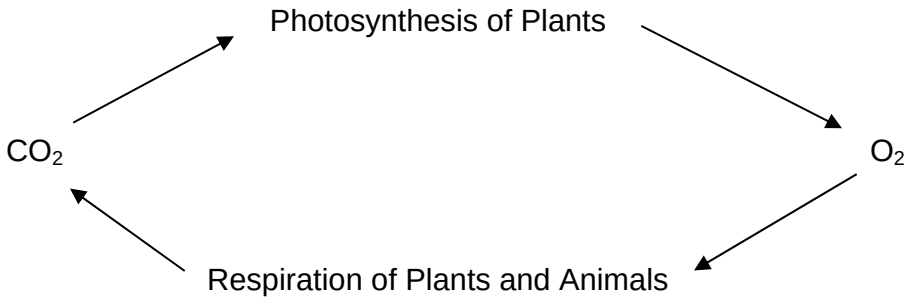
Oxygen measurements are also taken on plant effluents and in receiving streams to determine if there is enough oxygen in the effluent to prevent substantial reduction of the oxygen concentration in the stream.

Oxygen will dissolve in water from the atmosphere to a certain extent depending upon the temperature of the water. Below is the maximum concentration of oxygen which can be present in water at various temperatures:

TEMP DEG. C	DO mg/L	TEMP DEG. C	DO mg/L
0	14.6	16	10.0
1	14.2	17	9.7
2	13.8	18	9.5
3	13.5	19	9.4
4	13.1	20	9.2
5	12.8	21	9.0
6	12.5	22	8.8
7	12.2	23	8.7
8	11.9	24	8.5
9	11.6	25	8.4
10	11.3	26	8.2
11	11.1	27	8.1
12	10.8	28	7.9
13	10.6	29	7.8
14	10.4	30	7.6
15	10.2		

For example, given enough exposure to air, water at a temperature of 20 degrees C would dissolve up to 9.2 mg/L of oxygen.

Another way that oxygen can be transferred to water is directly from plants or algae which are growing in the water. This is the principal source of oxygen in wastewater stabilization lagoons. Plants produce oxygen by a process called photosynthesis. The chemical chlorophyll, with energy derived from sunlight, produces the oxygen and uses up CO_2. Thus, there is a continuous cycle in which oxygen is consumed and produced. The following is a simplified diagram of this:



SAMPLING

It is necessary to be very careful when collecting samples so as not to introduce additional oxygen from the air into the sample, since air is 21% oxygen. Samplers may be constructed or purchased which are designed to fill from the bottom, avoiding this problem.

When sampling a large body of water, it is necessary to collect many samples from various locations since DO can vary significantly in different locations and depths. Analysis must be done immediately following sample collection.

MEASUREMENT

Dissolved oxygen can be measured either by the iodometric method (chemically) or electrometrically (using a DO meter). Both procedures are included in this manual.

Iodometric Method

This method, also known as the Winkler Method, involves reaction of the dissolved oxygen in the sample to release iodine that can be measured by titration. The first part of this conversion involves the addition of a manganous sulfate solution and an alkali-iodide-azide solution to the sample in a standard 300 mL BOD bottle. The manganous sulfate and the alkali-iodide-azide reagent should be added at the surface of the water to minimize the reaction with atmospheric oxygen.

The reaction of the manganous sulfate with the potassium hydroxide in the alkali-iodide-azide reagent added to the B.O.D. bottle forms manganous hydroxide and potassium sulfate.

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4$$

The manganous hydroxide then combines with the dissolved oxygen in the water to form oxygenated manganic hydroxide.

$$2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$$

The cap should then be replaced and the bottles inverted rapidly until the resulting floc is mixed throughout. The floc should be allowed to settle half-way, and then mixed and allowed to settle again.

Next, concentrated sulfuric acid is added, the cap is replaced, and the bottle is inverted until the floc is dissolved. The sulfuric acid reacts with the oxygenated manganic hydroxide to form manganic sulfate plus water. The manganic sulfate then reacts with the

potassium iodide which was added with the alkali-iodide-azide reagent to form elemental iodine, manganous sulfate and potassium sulfate.

$$MnO(OH)_2 + 2H_2SO_4 \longrightarrow Mn(SO_4)_2 + 3H_2O$$

$$Mn(SO_4)_2 + 2KI \longrightarrow MnSO_4 + K_2SO_4 + I_2$$

One molecule of iodine is produced for each molecule of free oxygen originally present in the sample. This iodine may be measured by titration with sodium thiosulfate, thus determining the concentration of DO in the sample.

$$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$

The azide modification is required to be used for wastewater analyses. The azide prevents interference due to nitrites which are common in effluents from biological treatment processes and in incubated B.O.D. samples.

Electrode Method

The DO meter operates on the following principles: Oxygen which is dissolved in the sample diffuses through a teflon or polyethylene membrane on the DO probe. The oxygen is chemically reduced (accepts electrons), producing an electrical current between the anode and cathode in the probe. The amount of current is proportional to the concentration of DO. Following proper calibration, the meter relates this current to the concentration of DO.

There are several advantages to measuring DO with the dissolved oxygen meter. Some of these are: 1) a large number of samples may be analyzed in a shorter period of time, 2) reagent preparation is minimized, 3) DO may be continuously monitored by connecting a recorder to the meter, 4) field measurements may be made, since most DO meters are portable, and 5) the possibility of chemical interference is reduced.

NPDES
APPROVED
METHOD

DISSOLVED OXYGEN
Iodometric (Winkler) Method with the Azide Modification

DISCUSSION: This method involves reaction of the dissolved oxygen in the sample to release iodine that can be measured by titration. The azide modification is used for most wastewaters and surface waters. The addition of azide prevents interference due to nitrites which are common in effluents from biological treatment processes and in incubated B.O.D. samples.
This procedure makes use of a 0.0125 N sodium thiosulfate solution so that a direct dissolved oxygen reading may be obtained by titration of a 100 mL sample. This also allows for 100 mL of sample to be used for additional titrations should an error in technique arise. If a 0.025 N sodium thiosulfate solution is preferred, then a 200 mL sample should be used for titration so that a direct reading may be obtained

REFFERENCE: This conforms to the following EPA-approved procedure:

Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 4500-O C.

SAMPLING - The sample should be collected in completely filled 300 mL BOD bottle. Special precautions are required to avoid entrainment or dissolution of atmospheric oxygen (air bubbles). Do not let sample remain in contact with air or be agitated, because either condition may cause a change in oxygen concentration. Samples should not be preserved and there should be no delay in the determination of D.O.

1. REAGENTS

1.1 Sulfuric acid, H_2SO_4, Concentrated.

1.2 Manganous sulfate solution. Dissolve 240 g of manganous sulfate, $MnSO_4 \cdot 4\ H_2O$, 200 g $MnSO_4 \cdot 2\ H_2O$, or 182 g $MnSO_4\ H_2O$ in 250 mL distilled water.

1.21 Filter this solution through #42 Whatman filter paper in a Buchner funnel.

1.22 After filtering, dilute to 500 mL in a graduated cylinder.

1.3 Alkali-iodide-azide reagent.

1.31 Dissolve 250 g sodium hydroxide, NaOH (or 350 g potassium hydroxide KOH), and 67.5 g sodium iodide, NaI (or 75 g potassium iodide, KI), in distilled water and dilute to 500 mL in a graduated cylinder.

1.32 Dissolve 5 g sodium azide, NaN_3 in 20 mL distilled water. Add this to alkali-iodide solution and mix well.

1.4 Starch solution.

1.41 Dissolve 2.0 gram laboratory-grade soluble starch in100 mL hot distilled water.

1.42 Preserve with 0.2 g salicylic acid.

1.5 Sodium thiosulfate solution. This solution is approximately equal to 0.0125 N and should be standardized as in Section 2.

1.51 Dissolve 3.103 g sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ in distilled water in a 1000 mL in a volumetric flask.

1.52 Add 0.4 g of sodium hydroxide, NaOH.

1.53 Dilute to volume.

1.54 This solution should not be stored for more than 6 months, and discarded sooner if biological growth appears in the solution. It is recommended that the solution be re-standardize at least every month.

1.6 Standard potassium bi-iodate solution, 0.0125 N.

1.61 Dissolve exactly 0.4062 g potassium bi-iodate, $KH(IO_3)_2$ in distilled water and dilute to 1000 mL in a volumetric flask.

2. STANDARDIZATION OF 0.0125 N SODIUM THIOSULFATE SOLUTION

2.1 Titration.

2.11 Dissolve approximately 2 g potassium iodide, KI in approximately 150 mL of distilled water using a 250 mL Erlenmeyer flask.

2.12 Add a few drops of concentrated sulfuric acid, H_2SO_4.

2.13 Using a volumetric pipet, add exactly 10.0 mL of potassium bi-iodate solution (Section 1.6).

2.14 From the 1000 mL volumetric flask, carefully measure out 50 mL of sodium thiosulfate to be used for titration.

2.15 Titrate the iodine solution with thiosulfate adding starch toward the end of the titration, when a pale straw color is reached.

2.16 If between 9.8 and 10.2 mL of thiosulfate are titrated, the solution may be used as a standard.

2.17 Should the thiosulfate used be greater than 10.2 mL, the solution is too weak and should be thrown out.

2.18 Less than 9.8 mL of thiosulfate used in the titration would indicate that the solution is too strong and should be diluted.

2.2 Dilution correction.

2.21 When a solution of unknown normality is titrated against one of known normality a relationship exists that can be expressed as:

$V_1 \times N_1 = V_2 \times N_2$

V_1 = Volume of solution of unknown normality

N_1 = Unknown normality of V_1

V_2 = Volume of solution of known normality

N_2 = Known normality of V_2

Example: $V_1 \times N_1 = V_2 \times N_2$

V_1 = 9.6 mL of prepared thiosulfate used in titration

N_1 = Unknown normality of thiosulfate prepared

V_2 = 10 mL of 0.0125 N bi-iodate

N_2 = 0.0125 N bi-iodate

Formula rearranged $= \frac{V_2 \times N_2}{V_1} = N_1$

$\frac{10 \times 0.0125}{9.6} = \frac{0.125}{9.6} = 0.0130$ N thiosulfate

The solution prepared is 0.0130 N

2.22 Determine amount of distilled water needed to dilute the above solution to 0.0125 N.

Solution I (solution prepared)	Solution II (solution desired)
V_1 = 950 mL	V_2 = unknown
N_1 = 0.0130 N	N_2 = 0.0125 N

Formula rearranged: $\frac{V_1 \times N_1}{N_2} = V_2$

$\frac{950 \times 0.013}{0.0125} = 988$ mL

Therefore the Final volume needed = 988 mL of 0.0125 N thiosulfate.

Add distilled water to the 950 mL of 0.013 N thiosulfate to bring the volume up to 988 mL to dilute it to a 0.0125 N.

Use the formula:

Final volume - Original volume = Volume to be added

Example: 988 mL - 950 mL = 38 mL to be added

Add 38 mL of distilled water to the 950 mL of 0.0130 N to make 988 mL of 0.0125 N.

2.221 Measure the distilled water as accurately as possible.

2.222 Mix the final solution thoroughly.

2.23 Recheck the strength of this solution by repeating 2.11 through 2.18.

2.24 The final solution should be stored in a reagent bottle.

3. PROCEDURE - (See Illustration following)

3.1 Sample treatment.

3.11 By holding the tip of a graduated pipet at the surface of the liquid, add 1 mL manganous sulfate solution and 1 mL alkaline azide solution.

3.12 Stopper the bottle, taking care not to trap any air, mix well by gentle inversion and allow floc to settle.

3.13 Repeat mixing after floc has settled halfway and allow floc to settle again.

3.14 Remove stopper and by holding the tip of a graduated pipet at the surface of the liquid, add 1 mL of conc. sulfuric acid, H_2SO_4. re-stopper and mix by inverting several times until floc is dissolved.

3.2 Titration.

3.21 Using a graduated cylinder, carefully measure out 100 mL of the treated sample.

3.22 Pour this 100 mL into a 250 mL Erlenmeyer flask.

3.23 Titrate with the standardized 0.0125 N sodium thiosulfate solution.

3.24 When the solution reaches a pale yellow, add a few drops of starch solution.

3.25 Continue titration carefully until blue color just disappears.

3.26 Disregard any return of the blue color and record mL of thiosulfate used.

3.27 When over-titration occurs, repeat the titration with another 100 mLs of sample.

4. CALCULATIONS

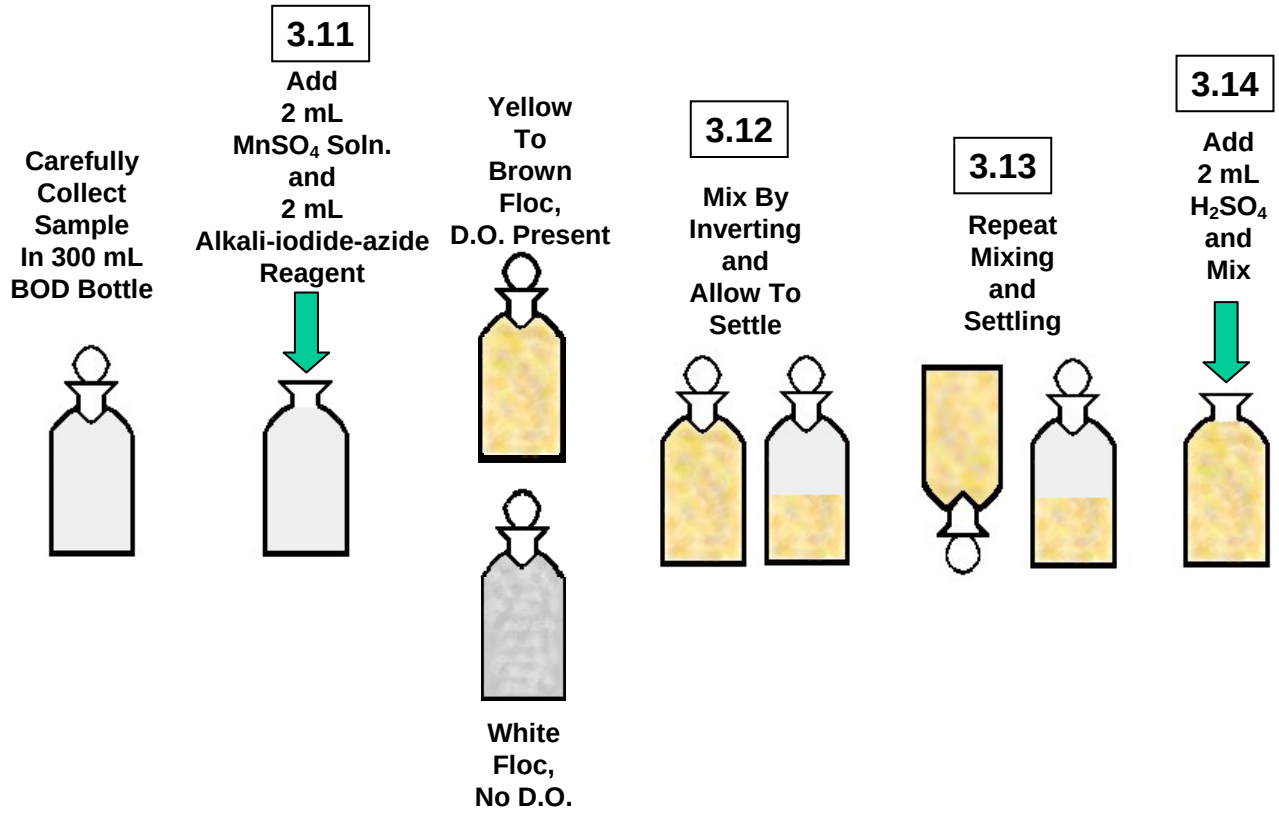
$$\text{mg/L D.O.} = \frac{\text{mL } Na_2S_2O_3 \text{ x Normality } Na_2S_2O_3 \text{ x 8 x 1000}}{\text{mL sample}}$$

If: Normality $Na_2S_2O_3$ = 0.0125 N

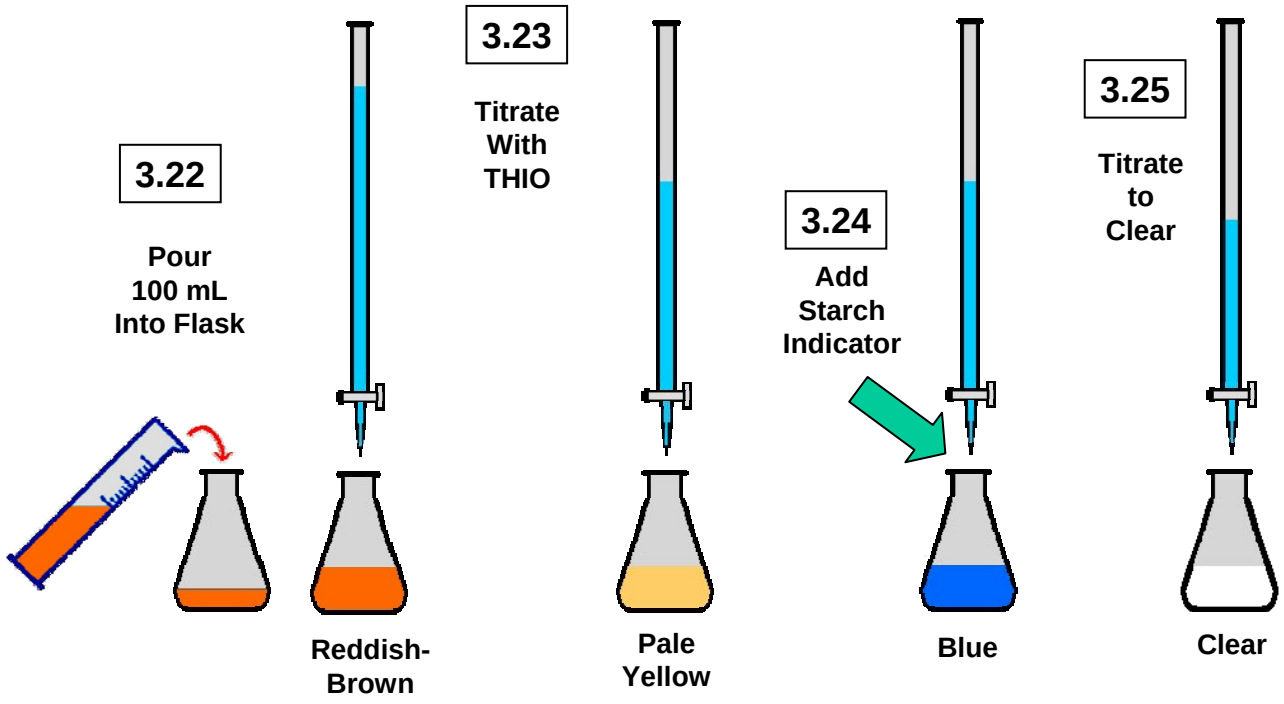
mL Sample = 100 mL

Then: mg/L D.O. = mL thiosulfate used in titration.

Outline Of Winkler Dissolved Oxygen Procedure



Titration of Iodine Solution



NPDES
APPROVED
METHOD

DISSOLVED OXYGEN

Membrane Electrode Method

DISCUSSION: The membrane electrode is composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a gas permeable membrane. Oxygen dissolved in the sample diffuses through the membrane on the DO probe and is chemically reduced (accepts electrons), producing an electrical current between the anode and cathode in the probe. The amount of current is proportional to the concentration of DO. Following proper calibration, the meter relates this current to the concentration of DO.

This outline is to be used in conjunction with the manufacturer's recommended procedures for calibration and operation of the equipment. Refer to the instrument manual for specific instructions.

Two means of calibration of the meter are in wide use: Comparison with the Winkler titration; and air calibration. Either method is acceptable.

REFERENCE: This conforms to the following EPA-approved procedure: Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 4500-O G.

SAMPLING – When ever possible the analysis should done directly in the body of water being tested. If sampling is required, use the same precautions suggested for the iodometric method. Samples should not be preserved and there should be no delay in the determination of D.O.

1. CALIBRATION

1.1 Comparison with Winkler Titration

1.11 Fill two BOD bottles completely full of BOD dilution water, being very careful not to introduce air into either bottle.

1.12 Analyze one bottle for D.O. using the Winkler titration.

1.13 Insert the electrode into the second bottle, turn on the stirring mechanism, and wait for the reading to stabilize.

1.14 Calibrate the meter to the D.O. value obtained in the titration.

1.15 The meter is now ready for sample analysis.

1.2 Air Calibration - This procedure varies considerably among the various instrument models available. Therefore, the procedure must be obtained from the instrument manual, but the following points should be noted.

1.21 Where possible with the specific equipment being used, compensation should be made during calibration for both ambient temperature and local atmospheric pressure. This pressure should be determined using a reliable onsite barometer. The oxygen solubility table following this procedure may be used.

1.22 Carefully blot any water droplets from the membrane using a soft tissue.

1.23 During calibration, be sure the membrane is exposed to fresh air. Laying the electrode on the bench for calibration is usually adequate.

1.24 Complete the calibration as soon as possible before the electrode membrane begins to dry.

1.25 The temperature registered on the meter should be checked against a trusted thermometer often.

1.3 Daily calibration of the D.O. meter is required. Calibration should also be verified after every five or six sample measurements.

1.4 Assure sufficient sample flow across membrane surface during analysis to overcome erratic response.

2. MAINTENANCE

2.1 Check the electrode membrane before each use to assure that there are

no significant air bubbles and that the membrane is not wrinkled.

2.2 Refill the electrode with filling solution and replace the membrane when experiencing excessive drift or inability to calibrate.

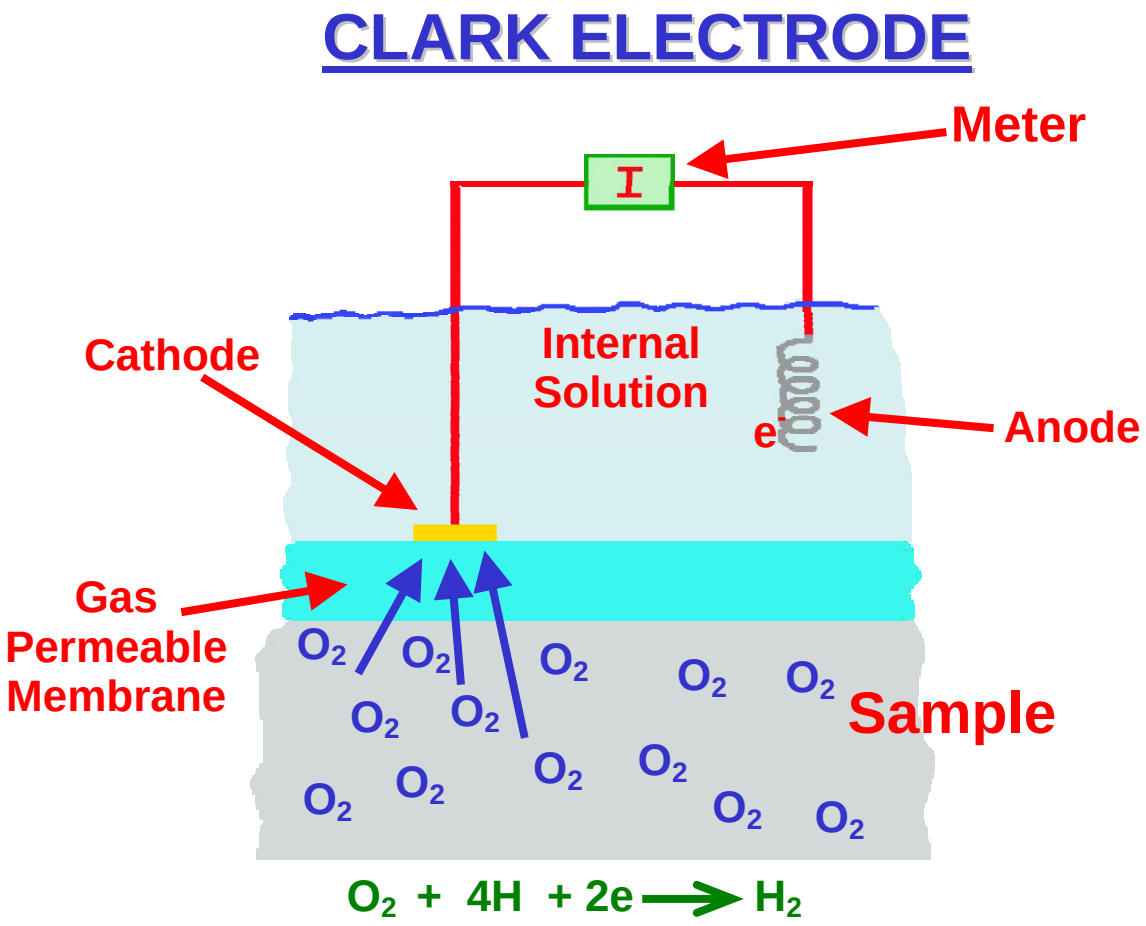
2.3 Batteries may require recharging or replacement if calibration is not possible.

3. INTERFERENCES

3.1 Chlorine, hydrogen sulfide, and sulfur dioxide may interfere. Consult manufacturer's manual for specific information.

4. STORAGE

4.1 For storage of the electrode between uses, it is usually recommended that the electrode be inserted into a BOD bottle which contains about one half inch of water, assuring that the membrane is not submerged.



D.O. - Electrode

Solubility of Oxygen in Fresh Water (mg/L) by Temperature and Onsite Pressure Reading
For Use in Calibration of DO Meters in Wastewater Laboratories

	Onsite Barometric Pressure														
	Atm:	0.970	0.975	0.980	0.985	0.990	0.995	1.000	1.005	1.010	1.015	1.020	1.030	1.040	1.050
	mm Hg:	737	741	745	749	752	756	760	764	768	771	775	783	790	798
	Inch Hg:	29.02	29.17	29.32	29.47	29.62	29.77	29.92	30.07	30.22	30.37	30.52	30.82	31.12	31.42
Temperature° Celsius	15.00	9.78	9.83	9.88	9.93	9.98	10.03	10.08	10.13	10.19	10.24	10.29	10.39	10.49	10.60
	15.50	9.67	9.72	9.77	9.82	9.88	9.93	9.98	10.03	10.08	10.13	10.18	10.28	10.38	10.48
	16.00	9.57	9.62	9.67	9.72	9.77	9.82	9.87	9.92	9.97	10.02	10.07	10.17	10.27	10.37
	16.50	9.47	9.52	9.57	9.62	9.67	9.72	9.77	9.82	9.87	9.92	9.97	10.07	10.16	10.26
	17.00	9.37	9.42	9.47	9.52	9.57	9.62	9.66	9.71	9.76	9.81	9.86	9.96	10.06	10.16
	17.50	9.27	9.32	9.37	9.42	9.47	9.52	9.57	9.61	9.66	9.71	9.76	9.86	9.96	10.05
	18.00	9.18	9.23	9.27	9.32	9.37	9.42	9.47	9.51	9.56	9.61	9.66	9.76	9.85	9.95
	18.50	9.08	9.13	9.18	9.23	9.28	9.32	9.37	9.42	9.47	9.51	9.56	9.66	9.75	9.85
	19.00	8.99	9.04	9.09	9.13	9.18	9.23	9.28	9.32	9.37	9.42	9.47	9.56	9.65	9.75
	19.50	8.90	8.95	9.00	9.04	9.09	9.14	9.18	9.23	9.28	9.32	9.37	9.47	9.56	9.65
	20.00	8.81	8.86	8.91	8.95	9.00	9.05	9.09	9.14	9.18	9.23	9.28	9.37	9.45	9.56
	20.50	8.72	8.77	8.82	8.86	8.91	8.96	9.00	9.05	9.10	9.14	9.19	9.28	9.37	9.46
	21.00	8.64	8.68	8.73	8.78	8.82	8.87	8.91	8.96	9.01	9.05	9.10	9.19	9.28	9.37
	21.50	8.56	8.60	8.65	8.69	8.74	8.78	8.83	8.87	8.92	8.96	9.01	9.10	9.19	9.28
	22.00	8.47	8.52	8.56	8.61	8.65	8.70	8.74	8.79	8.83	8.88	8.92	9.01	9.10	9.19
	22.50	8.39	8.44	8.48	8.53	8.57	8.62	8.66	8.70	8.75	8.79	8.84	8.93	9.02	9.10
	23.00	8.31	8.36	8.40	8.44	8.49	8.53	8.58	8.62	8.67	8.71	8.75	8.84	8.93	9.02
	23.50	8.23	8.28	8.32	8.37	8.41	8.45	8.50	8.54	8.58	8.63	8.67	8.76	8.85	8.93
	24.00	8.16	8.20	8.24	8.29	8.33	8.37	8.42	8.46	8.50	8.55	8.59	8.68	8.76	8.85
	24.50	8.08	8.12	8.17	8.21	8.25	8.30	8.34	8.38	8.43	8.47	8.51	8.60	8.68	8.77
	25.00	8.01	8.05	8.09	8.13	8.18	8.22	8.26	8.31	8.35	8.39	8.43	8.52	8.60	8.69
	25.50	7.93	7.97	8.02	8.06	8.10	8.15	8.19	8.23	8.27	8.31	8.36	8.44	8.53	8.61
	26.00	7.86	7.90	7.94	7.99	8.03	8.07	8.11	8.15	8.20	8.24	8.28	8.36	8.45	8.53
	26.50	7.79	7.83	7.87	7.91	7.96	8.00	8.04	8.08	8.12	8.16	8.21	8.29	8.37	8.46
	27.00	7.72	7.76	7.80	7.84	7.88	7.93	7.97	8.01	8.05	8.09	8.13	8.22	8.30	8.38
	27.50	7.65	7.69	7.73	7.77	7.81	7.86	7.90	7.94	7.98	8.02	8.06	8.14	8.22	8.31
	28.00	7.58	7.62	7.66	7.70	7.75	7.79	7.83	7.87	7.91	7.95	7.99	8.07	8.15	8.23
	28.50	7.52	7.56	7.60	7.64	7.68	7.72	7.76	7.80	7.84	7.88	7.92	8.00	8.08	8.16
	29.00	7.45	7.49	7.53	7.57	7.61	7.65	7.69	7.73	7.77	7.81	7.85	7.93	8.01	8.09
	29.50	7.38	7.42	7.46	7.50	7.54	7.58	7.62	7.66	7.70	7.74	7.78	7.86	7.94	8.02
	30.00	7.32	7.36	7.40	7.44	7.48	7.52	7.56	7.60	7.64	7.68	7.72	7.79	7.87	7.95

NOTE: The first three lines are different units for the same pressure measurement.

BIOCHEMICAL OXYGEN DEMAND

The B.O.D. test is one of the most commonly used indicators of water pollution. It gives an indication of the amount of oxygen used up, or demanded, by the waste being tested. Microorganisms use up this oxygen as they feed on the carbonaceous material in the waste. This is important because wastes which have a high oxygen demand will deplete the oxygen in the receiving water. This oxygen depletion may have adverse effects on the quality of life in that water. As the oxygen level decreases, the number of higher life forms in the stream decreases. If the oxygen level decreases too far, the only surviving organisms will be those which are normally considered to be nuisances, and the usefulness of the water will be greatly diminished. This is why it is necessary to reduce the B.O.D. of the waste as much as possible before discharge. The amount of B.O.D. which may be discharged by each wastewater treatment plant is limited by the State. This is based on the amount of flow being discharged and the size, type, and uses of the receiving water. Streams with little flow or low velocity cannot support high B.O.D. loading and therefore B.O.D. discharge limitations will be more stringent.

Material which exerts B.O.D. may be either soluble or insoluble. In a wastewater treatment plant, much of the insoluble B.O.D. is removed in the primary tanks by the settling process. Most of the remaining insoluble B.O.D. and the soluble B.O.D. is removed in the secondary process, where the microorganisms which feed on carbonaceous material in the wastes being received are concentrated and provided with air so that B.O.D. will be removed. Soluble B.O.D. will be absorbed directly into the cell by the microorganisms, while insoluble B.O.D. will stick to the outer cell wall until the cell excretes enzymes which solubilize the material and it is absorbed. The maintenance of a healthy biological population and good settling conditions will help assure efficient B.O.D. removal in the wastewater treatment plant.

B.O.D.

The B.O.D. test is an attempt to simulate what happens when a waste enters the receiving waters. The test normally specifies a five day incubation period. During the five days the waste is oxidized by the bacteria normally present in the waste and the dissolved oxygen in the bottle is therefore depleted. In the receiving water the bacteria oxidize wastes in a similar manner, thus using the dissolved oxygen. Five days is an arbitrary time period selected for the test. This time period works out very well since a large percentage of the total oxygen demand is met in five days.

Good technique is very important for all B.O.D. testing but especially at those plants which have stringent B.O.D. limits. When a sample is collected for the B.O.D. test, it should be taken at a place where it will represent the flow being sampled as well as possible. If the sample is not going to be analyzed immediately (such as in composite samples), it should be refrigerated at $\leq 6^{o}C$ until the time of analysis.

Accuracy in the B.O.D. test is dependent on several factors; preparing proper dilutions of the sample, correctly measuring the dissolved oxygen before and after incubation, and proper incubation conditions. It is also necessary that sufficient numbers of microorganisms are present in the B.O.D. bottle to feed on the waste being tested. These microorganisms are normally present in domestic wastes being received by the wastewater treatment plant, but there are some instances where this may not be the case. Many industrial wastes do not contain sufficient numbers of the organisms, therefore no oxygen would be demanded in the B.O.D. test in spite of the presence of organic materials in the waste. This would also be the case in effluents which have been disinfected.

The necessary organisms may be added to the B.O.D. bottle in a procedure called "seeding". The waste would first be treated to remove the disinfecting agent, if present, and a quantity of domestic sewage is added to the B.O.D. bottle containing the sample. Typically, 1 mL of primary effluent or settled sewage has been used as seed for industrial samples and de-chlorinated wastewater treatment plant effluent samples. Since the seed

material will also exert some oxygen demand due to organics in the material used as seed, this oxygen depletion must be subtracted out in the calculation for B.O.D. of the sample. This calculation is addressed in the CALCULATION section of the B.O.D. procedure.

While the B.O.D. test was originally designed to measure the oxygen depletion due to carbonaceous compounds in the waste, ammonia may also exert an oxygen demand if nitrifying bacteria are present in sufficient quantities. These bacteria use oxygen to convert ammonia to nitrates in the process called nitrification. Since many secondary wastewater treatment plants are now designed to encourage the growth of nitrifying bacteria, B.O.D. analysis of effluent samples from these plants may be misleading. A plant which has a lower B.O.D. result and no nitrification may actually have a higher carbonaceous B.O.D. than a plant with a higher B.O.D. reading which is largely or completely nitrogenous B.O.D.

Many discharge permits issued by the State now require the analysis of carbonaceous B.O.D. (CBOD). This may be determined by adding a nitrification inhibitor to the B.O.D. bottle. The chemical which is currently approved for this purpose is 2-chloro-6-(trichloromethyl) pyridine (TCMP), and is available from Hach Chemical Company in a form which is quite easily dissolved. The use of the nitrification inhibitor in the CBOD test is addressed in the B.O.D. procedure.

NPDES
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METHOD

BIOCHEMICAL OXYGEN DEMAND
5-Day BOD Test

DISCUSSION: A well mixed sample is diluted as necessary and incubated in an airtight bottle at a specified time and temperature. The Dissolved Oxygen (DO) concentration is measured initially and after incubation. The Biochemical Oxygen Demand (BOD) is calculated from the difference between the initial and final DO measurements.

REFFERENCE: This conforms to the following EPA-approved procedure:

Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 5210 B.

1. APPARATUS

   1.1 Incubation bottles - 300 mL capacity, with ground glass stoppers and flared mouth for water seal. Clean bottles with detergent, rinse thoroughly, and drain before use.

   1.2 Air incubator - thermostatically controlled at 20 ± 1°C.

2. REAGENTS

   2.1 Dilution water. Water used for reagents and preparation of dilution water must be free of toxic materials such as copper and chlorine, and also must not contain oxygen-demanding substances such as organic compounds. It is suggested that demineralized water not be used, since the resins used in such columns seem to contribute contaminants to the water.

   NOTE: Prepare reagents in advance but discard if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable and different stock concentrations may be used if doses are adjusted proportionally. Biological growth will be inhibited if the reagents are stored in the dark.

2.2 Phosphate buffer solution. Dissolve 4.25 g potassium dihydrogen phosphate, KH_2PO_4, 10.9 g dipotassium hydrogen phosphate, K_2HPO_4, 16.7 g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$, and 0.85 g ammonium chloride, NH_4Cl in about 250 mL distilled water and dilute to 500 mL in a graduated cylinder.

2.3 Magnesium sulfate solution. Dissolve 11.25 g magnesium sulfate, $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 500 mL in a graduated cylinder.

2.4 Calcium chloride solution. Dissolve 13.75 g anhydrous calcium chloride, $CaCl_2$ in distilled water and dilute to 500 mL in a graduated cylinder.

2.5 Ferric chloride solution. Dissolve 0.125 g ferric chloride, $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 500 mL in a graduated cylinder.

2.6 Glucose-glutamic acid solution. (See page 124-1 for procedure.)

2.7 If carbonaceous B.O.D. (CBOD) is to be determined, nitrification inhibitor will be required; 2-chloro-6-(trichloro methyl) pyridine (TCMP). (Hach Chemical Formula 2533 or equivalent)

2.8 If samples are to be neutralized (pH greater than 8.5 or less than 6.0), prepare the following solution(s) as required:

2.81 Alkali - Sodium hydroxide solution, 1N. Dissolve 40 g of sodium hydroxide, NaOH, in distilled water. Dilute to 1 Liter.

2.82 Acid - Sulfuric acid, 1N. Slowly and while stirring, add 28 mL concentrated sulfuric acid, H_2SO_4, to distilled water. Dilute to 1 Liter.

2.9 If samples are to be de-chlorinated, prepare the following solutions:

2.91 Sodium sulfite solution, 0.025N. Dissolve 0.16 g of sodium sulfite, Na_2SO_3, in 100 mL distilled water. Prepare solution daily.

2.92 Sulfuric acid, 0.7 N. Carefully add 10 mL concentrated sulfuric acid,

H_2SO_4, to 500 mL distilled water.

2.93 Potassium iodide, 10%. Dissolve 10 g potassium iodide, KI, in distilled water and bring to 100 mL.

2.94 Starch indicator. Dissolve 2 g soluble starch in 100 mL hot distilled water.

3. SAMPLE PRETREATMENT

3.1 pH adjustment – For samples with pH greater than 8.5 or less than 6.0, neutralize to pH 6.5 to 7.5 with 1N sulfuric acid or sodium hydroxide solution (step 2.8). This step must not dilute the sample by more than 0.5%. Always seed samples that have been pH-adjusted.

3.2 De-chlorination - Samples which contain residual chlorine must be de-chlorinated following the procedure below. If the sample has been de-chlorinated, or if it has been disinfected but no chlorine residual is present, the procedure for seeding (step 4.4) must be followed.

3.21 Add 1 mL of 0.7 N sulfuric acid and 1 mL of 10% potassium iodide to a 100 mL portion of the sample.

3.22 Add about 1 mL of starch indicator and titrate with 0.025 N sodium sulfite to obtain a change from blue to clear. Record volume used.

3.23 Measure out another portion of the sample and add a proportionate amount of sodium sulfite and mix. After 10 to 20 minutes check sample for chlorine residual.

3.3 Supersaturated dissolved oxygen - If dissolved oxygen in samples is above 9 mg/L at 20°C, bring the sample to 20°C in a partially filled bottle and agitate vigorously or aerate with clean, filtered compressed air to reduce DO to saturation.

3.4 Temperature adjustment - Bring samples to 20 ± 1°C before making dilutions.

4. PROCEDURE

4.1 Preparation of dilution water.

4.11 Measure the desired volume of distilled water into a suitable bottle.

4.12 For each liter of dilution water to be prepared, add 1 mL of magnesium sulfate solution, 1 mL of calcium chloride solution, 1 mL of ferric chloride solution, and 1 mL of the phosphate buffer solution

4.13 Saturate the solution with oxygen by shaking it in a partially filled bottle or by drawing air through it with a vacuum pump.

4.14 Store the dilution water in the B.O.D. incubator until use. Do not store prepared dilution water for more than 24 hours after adding nutrients, minerals, and buffer unless dilution water blanks consistently meet quality control limits (step 4.33)

4.15 Protect water quality by using clean glassware, tubing, and bottles.

4.2 Sample dilution.

4.21 Prepare at least two dilutions of each sample using the dilution water, such that at least 2 mg/L of dissolved oxygen will be used up (depletion) during the incubation time and at least 1 mg/L of dissolved oxygen remains (residual). For samples where the approximate B.O.D. is unknown, several dilutions may be necessary.

4.22 If the B.O.D. is expected to be less than approximately 500 mg/L, the dilution may be made directly in the B.O.D. bottle. For dilutions greater than 1:100 (3 mL in 300 mL bottle) make a primary dilution in a graduated cylinder before making final dilution in the bottle. The table below may be helpful in determining the appropriate sample

volumes to use when diluting directly in the B.O.D. bottle.

Expected BOD_5	Sample Volume For 300 mL B.O.D. Bottle
2 - 20 mg/L	300 - 75 mL
20 - 100 mg/L	75 - 15 mL
100 - 500 mg/L	15 - 3 mL

NOTE: - When using more than 200 mL of sample, low nutrient concentrations may limit biological activity. In such samples, add 0.33 mL of each of the nutrient, mineral, and buffer solutions directly to the BOD bottles or use commercially available products prepared for single bottle use.

4.23 Thoroughly mix the sample to be analyzed and using a wide-tip graduated pipet, transfer the volume of sample into a B.O.D. bottle according to the dilution desired. (For sample volumes over 25 mL, an appropriate graduated cylinder may be used).

4.3 Carbonaceous BOD: - If analysis for carbonaceous BOD (CBOD) is required, nitrification inhibitor should be added at this point.

4.31 Add 3 mg of 2-chloro-6-(trichloro methyl) pyridine (TCMP) to each 300 mL bottle where CBOD is to be determined.

Note: If using the Hach Chemical Company nitrification inhibitor and dispenser bottles, add two "shots" of inhibitor (0.10 g total) to each bottle.

4.32 TCMP may float on the top of the sample and may dissolve slowly. If necessary, gently shake the bottle to get the TCMP below the liquid surface before filling the bottle.

4.33 Nitrification inhibitor should only be used in samples where nitrifying organisms may be present and where CBOD is the required parameter.

4.34 Indicate use of nitrogen inhibitor in reporting results.

4.4 Seeded BOD: - If samples are to be seeded, the seed material should be added at this time.

NOTE: Seeding is only necessary for samples that do not have an adequate population of microorganisms. Examples include QA/QC reference samples, certain industrial discharges, and some disinfected effluents. Parallel analysis of seeded and un-seeded samples may be used to determine if seeding is necessary.

4.41 Pipet an appropriate volume of seed into the bottle using a wide-tip graduated pipet.

4.42 One mL of primary effluent is often used as seed but fresh settled sewage may also be used.

4.43 The DO uptake due to the seed added to each bottle should be between 0.6 and 1.0 mg/L, but the amount added should be adjusted from this range to that required to provide glucose-glutamic acid check results in the range of 198 ± 30.5 mg/L.

4.44 The B.O.D. of the seed material should be determined separately as for any other sample. This is the seed control.

4.5 Dissolved Oxygen (DO) measurement and incubation

4.51 Fill the bottle with dilution water, such that when the stopper is placed in the bottle a water seal is formed around the stopper. Don't add so much that liquid is lost from the bottle.

4.52 Determine the initial DO on each dilution prepared immediately after

filling the BOD bottle. The time period between preparing the dilution and measuring initial DO should not be more than 30 minutes.

4.521 If using the DO probe, measure concentration directly in each bottle before incubation.

4.522 If using the Winkler titration to determine DO, set up a duplicate of each dilution prepared, being very careful not to introduce air during addition of sample or dilution water. Determine initial DO on one duplicate using the titration and incubate the other.

4.53 Place the ground glass stoppers in the B.O.D. bottles, making sure that no air bubbles have been trapped.

4.54 If un-dissolved nitrification inhibitor is present, mix by inverting the B.O.D. bottle until dissolved.

4.55 Add distilled water to form a seal on the top of the stoppers. Prevent this seal from evaporating during incubation by inverting a paper cup over each stopper, or use plastic caps which have been manufactured for this purpose.

4.56 Incubate the bottles in the dark at 20 ± 1°C for 5 days.

4.57 Determine the final DO using either the probe or the titration on all incubated bottles.

4.6 Dilution water blank:

4.61 With each set of B.O.D. samples incubated, a dilution water blank must also be incubated as a check on dilution water quality.

4.62 Fill a B.O.D. bottle with dilution water only.

4.63 Determine DO in the bottle, either directly using the probe, or on a duplicate using the titration, again being very careful not to introduce air into either bottle.

4.64 If after 5 days of incubation the DO has been depleted more than 0.2 mg/L, the quality of the dilution water as well as sources of possible contamination should be investigated. (NOTE: This blank depletion is not subtracted from sample depletions.)

4.7 Glucose-glutamic acid check:

4.71 Periodically run a BOD measurement on a “standard” check solution of Glucose-glutamic acid using the procedure on page 124-1. This should be done at least monthly or more often if more stringent quality control is required.

5. CALCULATIONS

5.1 Calculate the BOD concentration using the data for the dilution which resulted in a DO depletion of at least 2 mg/L and a residual DO of at least 1 mg/L. If more than one dilution resulted in a DO depletion and residual in the proper range, the BOD for each should be calculated and the average value reported.

5.2 Non-seeded BOD

$$\text{BOD, mg/L} = \frac{\text{DO Depletion, mg/L}}{\text{mL Sample}} \times 300 \text{ mL}$$

DO Depletion, mg/L = Initial DO, mg/L - Residual DO, mg/L

EXAMPLE: Calculate BOD for a sample using the data below:

Volume of sample used	3 mL	6 mL	10 mL
initial DO, mg/L	8.4	8.4	8.4
residual DO, mg/L	7.9	4.2	0.6
DO depletion, mg/L	0.5	4.2	7.8

Since the dilution using 3 mL of sample did not deplete at least 2 mg/L DO, it is not valid. The dilution using 10 mL of sample did not have a DO residual of at least 1 mg/L, so it also is not valid. The calculation for BOD would be

based on the dilution using 6 mL of sample.

$$\text{BOD, mg/L} = \frac{\text{DO Depletion, mg/L}}{\text{mL Sample}} \times 300 \text{ mL}$$

$$= \frac{4.2 \text{ mg/L}}{6 \text{ mL}} \times 300 \text{ mL} = 210 \text{ mg/L}$$

5.3 Seeded BOD

$$\text{BOD, mg/L} = \frac{D_1 - D_2}{\text{mL Sample}} \times 300 \text{ mL}$$

Where: D_1 = DO depletion due to sample and seed
D_2 = DO depletion due to seed

EXAMPLE:

A seeded BOD is set up on a de-chlorinated effluent sample using 150 mL of sample and 1 mL of primary effluent as seed. A seed control was also set up on the primary effluent (PE) using 9 mL of sample. Calculate the BOD of the effluent using the data below:

DO depletion for 150 mL sample + 1 mL seed = 4.2 mg/L

9 mL PE DO Depletion = 3.2 mg/L

1. Calculate DO depletion in the effluent sample bottle due to the 1 mL of seed which was added (D_2)

In the PE BOD determination, 9 mL of sample depleted 3.2 mg/L of DO

Therefore, 1 mL of PE would deplete

$$\frac{3.2 \text{ mg/L}}{9 \text{ mL}} = 0.36 \text{ mg/L}$$

2. Calculate the BOD of the effluent sample

$$\text{BOD, mg/L} = \frac{D_1 - D_2}{\text{mL Sample}} \times 300 \text{ mL}$$

$$\text{BOD, mg/L} = \frac{4.2 \text{ mg/L} - 0.36 \text{ mg/L}}{150 \text{ mL}} \times 300 \text{ mL}$$

BOD, mg/L = 7.7 mg/L

TROUBLESHOOTING EXCESSIVE BOD BLANK DEPLETION

Possible Causes:

- Slime growth in delivery tubing
- Tubing used is constructed of oxygen-demand leaching material
- Poor water quality / contaminated lab water
- Poorly cleaned BOD bottles or dilution water storage bottle
- Contaminated nutrient solutions
- Contamination during aeration
- Improperly calibrated or malfunctioning DO Meter / Probe

Possible Solutions:

Use a glass bottle for storage of the dilution water.

Use only glass or latex delivery tubing. Tygon and black rubber tubing may leach organic materials into the water, causing an oxygen demand.

Clean delivery tube weekly with either bleach (25 mL bleach / L water) or a dilute solution of Hydrochloric Acid (100 mL HCl / L water)

> **NOTE**:
> 1. DO NOT mix acid with bleach! Chlorine gas is produced in this reaction. Even in small quantities, exposure to chlorine gas can be hazardous.
> 2. Use reinforced nylon tape around larger glass bottles for safety
> 3. Nothing should contact the water except Teflon or glass

Aging dilution water may help to reduce dilution water quality problems. Do not add the phosphate buffer solution until the day that the dilution water is used. Store the water at room temperature or in the BOD incubator.

“Grocery store" distilled water may or may not be of sufficient quality. Many facilities have not experienced problems using purchased water, while others have attributed problems to this. If in doubt, test against water of known quality.

Always discard water if growth is observed in the dilution water container.

Follow manufacturer’s recommendations for cleaning stills, etc.

Distilled (not deionized) water is generally best for dilution water. Use a water softener ahead of the distillation unit to reduce scale in the distillation boiler.

Aeration:

Aeration is best done by pulling filtered air through the bottle using vacuum rather than blowing compressed air into the bottle. Compressed air may contaminate the water with dust and oil.

Never use an air stone (aquarium bubbler) to aerate dilution water.

Never put "fish tank" (Tygon) tubing directly in dilution water.

Don't leave dilution water open to the air.

Small quantities (one gallon or less) may be sufficiently aerated by shaking a partially filled bottle.

Glassware Cleaning:

Clean BOD bottles and dilution water bottle after each use.

Use a good lab-grade, non-phosphate detergent and a bottle brush to thoroughly clean bottles. It may be helpful to follow this by rinsing with tap water and then with bleach or dilute HCl solution (be sure to rinse this out completely).
Rinse thoroughly with tap water followed by distilled water.

Allow glassware to dry before storing.

Always cover glassware and store in a clean, dry place.

D.O. Meter Calibration:

Improper meter calibration may give the appearance of a dilution water problem even though the water quality may be fine.

Follow manufacturer's meter calibration instructions and be consistent.
If air calibration or air-saturated water calibration is used, always account for both temperature and barometric pressure in the calibration. Be sure to use a good quality barometer in the laboratory.

PROCEDURE FOR USE OF GLUCOSE-GLUTAMIC ACID AS A QUALITY CONTROL CHECK OF THE BOD_5 TEST

DISCUSSION: Because the BOD test is a bioassay, its results can be greatly influenced by the presence of toxic materials or by use of poor seeding material. Distilled waters may be contaminated with copper; some sewage seeds are relatively inactive. Low results are always obtained in these situations. This procedure should be used periodically to check dilution water quality, seed effectiveness, and analytical technique.

REFFERENCE: This is adapted from:

Standard Methods for the Examination of Water and Wastewater, 20th Edition,

Method 5210 B 4c&d.

1. PROCEDURE

1.1 Dry about 200 milligrams each of reagent grade glucose (also called dextrose) and glutamic acid at 103 ^{o}C for 1 hour. Add 150 mg of each into a 1 liter volumetric flask, dissolve and bring to volume in distilled water. Prepare fresh immediately before use.

1.2 Pipet 6.0 mL of this solution into a 300 mL BOD bottle, add 1 mL of suitable seed (usually settled sewage) and fill with dilution water. Adjust commercial mixtures to give 3 mg/L glucose and 3 mg/L glutamic acid in the BOD bottle.

1.3 Set up a BOD on the sample used as seed. Set dilutions to obtain a minimum depletion of 2.0 mg/L and a minimum residual of 1.0 mg/L.

1.4 Measure and record initial D.O. in each bottle.

1.5 Incubate these bottles at 20 ^{o}C for 5 days.

1.6 Determine the final D.O. in the bottles and subtract the depletion due to the seed from the depletion of the glucose-glutamic acid mixture plus seed. (See example calculation.)

1.7 Calculate BOD_5 for the glucose-glutamic acid mixture using the seeded BOD calculation. The BOD_5 should be 198 ± 30.5 mg/L. If it is outside this range check for errors.

EXAMPLE CALCULATION:

6.0 mL of glucose-glutamic acid solution were pipetted into a BOD bottle and 1 mL of settled sewage added as seed. A BOD was set up in a separate bottle using 5 mL of settled sewage. The following data is obtained:

	Glucose-Glutamic Acid + Seed	Settled Sewage
Initial D.O.	8.1 mg/L	8.0 mg/L
(-) Final D.O.	3.6 mg/L	5.0 mg/L
(=) Depletion	4.5 mg/L	3.0 mg/L

Since the DO depletion in the bottle containing 5 mL of settled sewage was 3.0 mg/L, the amount of depletion in glucose-glutamic acid mixture due to the 1 mL of seed added can be calculated as follows:

$$= \frac{3.0 \text{ mg/L}}{5.0 \text{ mL}} = 0.60 \text{ mg/L}$$

BOD_5 of glucose - glutamic acid sample =

$$\frac{(\text{Depletion of Sample + Seed}) - (\text{Depletion Due to Seed})}{\text{mL Sample}} \times 300 \text{ mL}$$

$$= \frac{4.5 \text{ mg/L} - 0.60 \text{ mg/L}}{6 \text{ mL}} \times 300 \text{ mL} = 195 \text{ mg/L}$$

SOLIDS DETERMINATIONS

The determination of solids may be important for several different reasons. Discharges of wastewater into the environment are monitored for solids content because of their impact on aquatic life and usefulness of the water being affected. Solids are also monitored at various locations in wastewater treatment plants so that process control may be optimized and efficiency determined.

Solids are classified into two general types; the particulate material and the material that is dissolved in the liquid. The particulate material is called "Suspended Solids" and may be defined as solids which will not pass through a filter of specific pore size and is not volatilized at 103° - 105° C. The second type is called "Dissolved Solids" and may be defined as those solids which are in solution and will therefore pass through the filter. The sum of these two are called "Total Solids" and may be defined as all of the solids present whether suspended or dissolved.

It is often of interest to determine the organic content of the solids. This can be approximated by igniting the dried solids at 550°C in a muffle furnace. The weight loss on ignition is called "Volatile Solids" and is taken to be the organic portion. "Fixed Solids" is the term applied to the solids left (ash) after this ignition and is considered to be inorganic. Thus there are sub-categories called "Volatile Suspended Solids", "Volatile Dissolved Solids", and "Total Volatile Solids". The term "Total Suspended Solids" (TSS) therefore refers to all of the suspended solids present whether volatile or not. These should not be confused with "Total Solids" which, as defined above, refers to all of the solids present or the sum of the "TSS" and the "TDS".

Another term that is often used in the wastewater field is "Settleable Solids". This refers to material that will settle out of suspension in a specified time period. Much of the material that will not settle consists of very fine particles that may or may not be separated

out by the filters used in the suspended solids procedure. These are called "Colloidal Solids" and may be seen as turbidity or cloudiness in a sample.

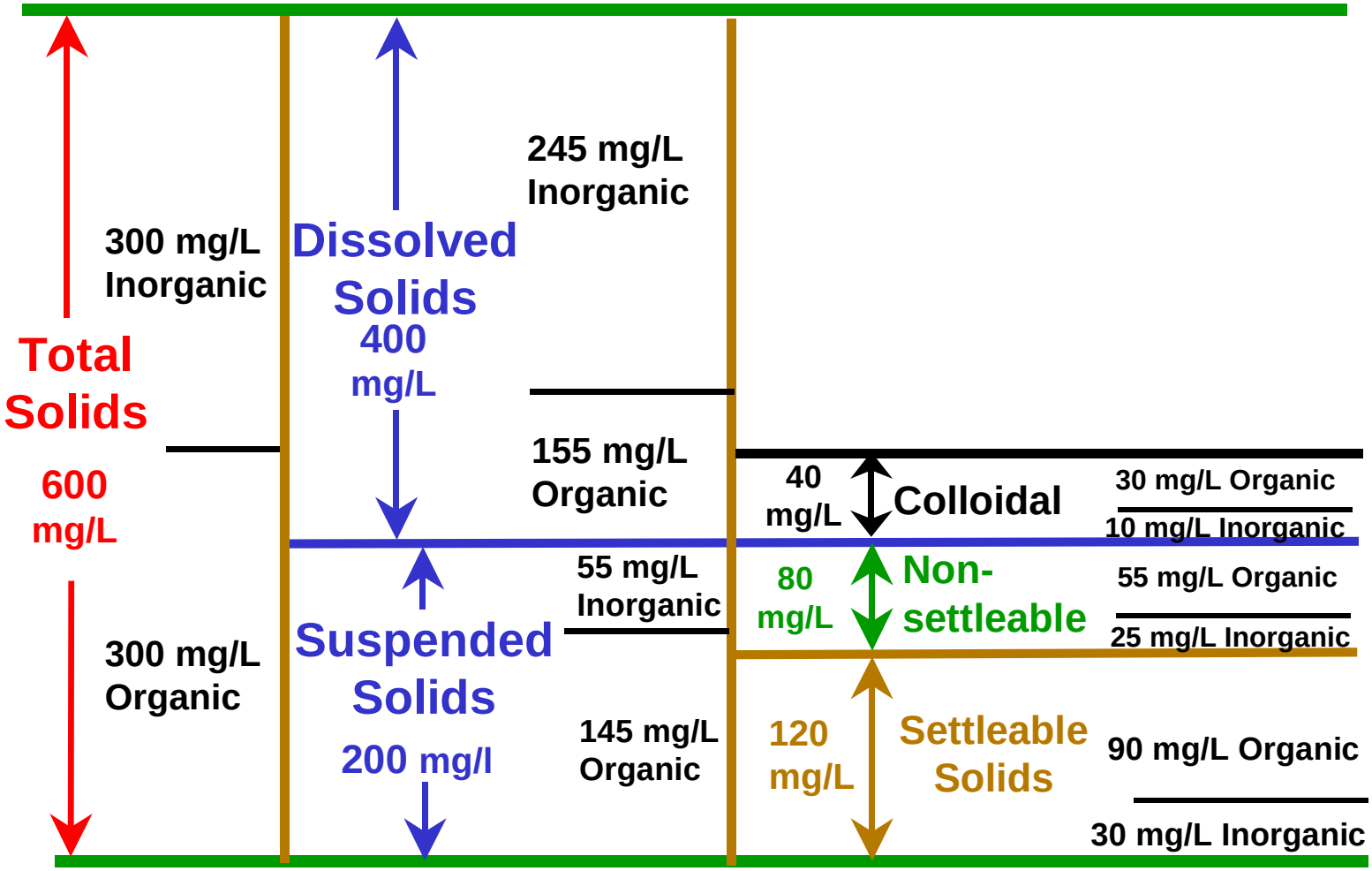
Solids are removed throughout the wastewater treatment plant using several different processes depending on the type of solids present. The first solids removal usually occurs at the bar screens where large objects are screened out to prevent damage to pumps and other down-stream equipment. After this the heavier non-organic materials, such as sand, are removed by settling in the grit chamber. The flow then continues on to the primary clarifiers where settleable material is removed. The solids which remain in the wastewater after the primary clarifiers include about 50% of the influent suspended solids and almost all of the dissolved solids, much of which is organic. Biological secondary treatment processes remove the soluble solids as they are absorbed into the cells of microorganisms. These microorganisms also remove suspended solids by first adsorbing the solids onto the outside of the cell. An enzyme is then secreted which breaks the solids down into soluble matter which can be absorbed by the cell. Secondary clarifiers then settle out the microorganisms and the solids which they have removed from the wastewater. Some wastewater treatment plants make use of tertiary treatment systems to remove any solids which escape from the secondary system. This often includes filtration of the flow through screens or sand filters. Such plants typically exceed 90% removal of influent suspended solids.

Solids which are removed in the wastewater treatment plant are treated to reduce the volume that must be disposed of, to provide a material which will not readily undergo further biological decomposition, and to destroy pathogenic bacteria. Sludge treatment might include aerobic digestion, anaerobic digestion, or less commonly, lime stabilization. The solids are then dewatered and disposed of in the environment, often on agricultural land to take advantage of the nutrient value of the sludge.

Solids

Suspended and volatile suspended solids concentrations are generally determined in the wastewater treatment plant on the influent flow, primary effluent, secondary effluent, tertiary effluent, and activated sludge and return sludge samples. Total and total volatile solids are usually determined on raw, digesting and digested sludge, digester supernatant, and dewatered sludge. Methods for suspended, dissolved, and total solids are included in this manual.

Composition of Solids in Average Domestic Sewage



TOTAL SUSPENDED AND VOLATILE SUSPENDED SOLIDS

DISCUSSION: A well mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter represents the total suspended solids. The residue is then ignited to a constant weight at 550°C. The remaining solids represent the fixed suspended solids while the weight loss on ignition is the volatile solids.

REFFERENCE: This conforms to the following EPA-approved procedures: Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 2540 D and Method 2540 E.

1. APPARATUS

1.1 Glass fiber filters, Whatman 934 AH, Gelman type A/E, Millipore type AP 40, or other products that give demonstrably equivalent results.

1.2 Filtration apparatus: One of the following, suitable for the filter disk selected:

1) Membrane filter funnel.

2) Gooch crucible, 25 mL to 40 mL capacity, with Gooch crucible adapter.

3) Filtration apparatus with reservoir and course (40- to 60-µm) fritted disk as filter support.

1.3 Aluminum weighing dishes (if using Membrane filter funnel apparatus).

1.4 Vacuum pump.

1.5 Vacuum flask of sufficient capacity for sample size selected.

1.6 Drying oven, capable of maintaining a temperature of 103°C to 105°C.

1.7 Muffle furnace, capable of maintaining a temperature of 550°C ± 50°C.

1.8 Desiccator, cabinet or jar type, with indicating desiccant.

1.9 Analytical balance, capable of weighing to 0.1 mg

2. PROCEDURE

2.1 Filter preparation

2.11 Place a glass fiber filter, wrinkled side up, in filtration apparatus.

2.12 Wet the filter with three successive 20 mL portions of distilled water while applying a gentle vacuum. Continue suction to remove all traces of water.

2.13 Remove the filter from the filtration apparatus and transfer to an inert aluminum weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Place in the oven until dry and then in the muffle furnace at 550°C for 15 minutes.

NOTE: From this point through the analysis, use tongs to handle the filter and dish, or crucible and filter.

2.14 Remove the filter and weighing dish, or the crucible and filter, from the furnace and place in the drying oven for partial cooling, then in the desiccator for cooling to room temperature.

NOTE: If these are not to be used immediately they should be stored in the drying oven, then cooled and weighed just before use.

2.15 Determine the weight on an analytical balance and record on a bench sheet.

2.16 Repeat cycle of igniting, cooling, desiccating, and weighing until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

2.2 Sample analysis

2.21 Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to one liter.

2.22 Thoroughly mix sample to obtain a representative portion for analysis. With the sample mixing, measure the appropriate volume using a graduated cylinder and record the volume on the bench sheet.

2.23 Place a prepared and weighed filter, or crucible with filter, on the vacuum flask, turn on the vacuum, and wet filter with a small volume of distilled water to seat it.

2.24 Add the measured volume of sample to the filtering apparatus and allow to filter through.

2.25 Rinse the graduated cylinder with three successive 10 mL volumes of distilled water, adding each to the filtering apparatus, allowing complete drainage between washings. Continue suction for about three minutes after filtration is complete. If complete filtration takes more than 10 minutes, increase filter diameter or decrease sample volume.

2.26 Remove the filter from the filtration apparatus and transfer to an inert aluminum weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in an oven at 103 to 105°C for 1 hour.

2.27 Place in the desiccator for cooling to room temperature.

2.28 Determine the weight of the filter and dish, or crucible and filter,

containing the dried solids on an analytical balance and record on a bench sheet.

2.29 Repeat cycle of drying, desiccating, and weighing until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

2.3 Volatile suspended solids analysis

2.31 After recording the weight from step 2.28, place the filter and weighing dish, or the crucible and filter, containing the dry solids in the muffle furnace at 550°C for 15 minutes. Longer time may be necessary if igniting more than one sample.

2.32 Place in the drying oven to allow it to partially cool, and then in the desiccator for cooling to room temperature.

2.33 Determine the weight of the filter and weighing dish, or the crucible and filter, containing the ash on an analytical balance and record on a bench sheet.

2.34 Repeat cycle of igniting, cooling, desiccating, and weighing until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

3. CALCULATIONS

3.1 Suspended Solids:

A. Subtract weight determined in step 2.15 (filter) from weight determined in step 2.28 (filter and dry solids) to get weight of dry solids.

B. Suspended Solids, mg/L =

$$\frac{\text{weight of dry solids (gram)}}{\text{volume of sample filtered (mL)}} \times \frac{1000\ \text{mL}}{\text{liter}} \times \frac{1000\ \text{mg}}{\text{gram}}$$

--OR--

Suspended Solids, mg/L =

$$\frac{\text{grams dry solids}}{\text{mL sample filtered}} \times 1{,}000{,}000$$

3.2 Volatile Suspended Solids

A. Subtract weight determined in step 2.33 (filter and ash) from weight determined in step 2.28 (filter and dry solids) to obtain weight of volatile solids.

B. Vol. Sus. Sol., mg/L = $\frac{\text{grams volatile solids}}{\text{mL sample filtered}} \times 1{,}000{,}000$

3.3 Example

Calculate the concentration of suspended and volatile suspended solids from the data below:

Volume of sample filtered	100 mL
Wt. crucible	15.5817 g
Wt. crucible with dry solids	15.5999 g
Wt. crucible with ash	15.5869 g

Suspended Solids (mg/L)

A.

Wt. crucible + dry	15.5999 g
- Wt. crucible	- 15.5817 g
Wt. dry solids	0.0182 g

B. $\text{Sus. Sol. mg/L} = \frac{0.0182\ \text{g}}{100\ \text{mL}} \times 1{,}000{,}000$

Suspended Solids = 182 mg/L

Volatile Suspended Solids (mg/L)

A.

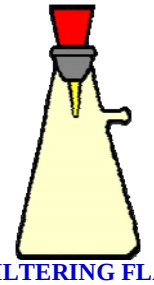
Wt. crucible + dry	15.5999 g
- Wt. crucible + ash	- 15.5869 g
Wt. volatile solids	0.0130 g

B. $\text{Vol. Sus. Sol.} = \frac{0.0130\ \text{g}}{100\ \text{mL}} \times 1{,}000{,}000$

Volatile Suspended Solids = 130 mg/L

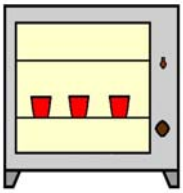
TOTAL SUSPENDED
AND
VOLATILE SUSPENDED SOLIDS
PROCEDURE

1. Insert glass fiber filter

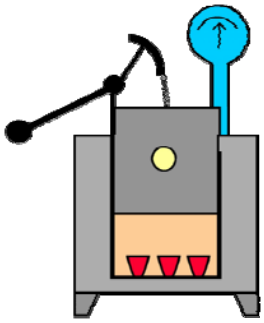


FILTERING FLASK

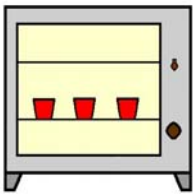
2. Seat filter



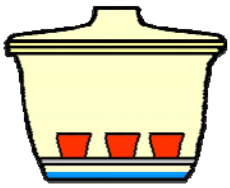
3. Dry briefly 103 deg. C



4. Ignite in muffle furnace 550 deg. C for 15 minutes

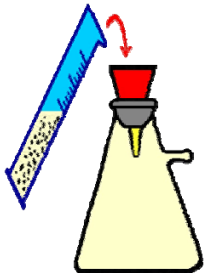


5. Cool in drying oven briefly



6. Cool in desiccator to room temperature

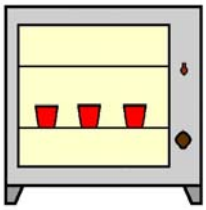
7. Weigh crucible



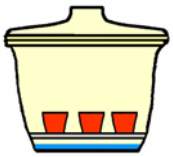
8. Pour measured volume of sample in Gooch crucible.

9. Filter with vacuum.

10. Wash graduate, crucible, and filter with distilled water.

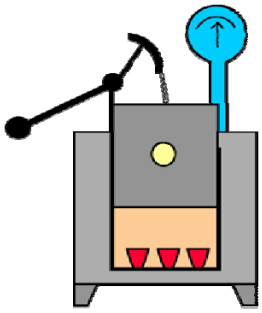


11. Dry crucibles plus solids for one hour at 103 º C.

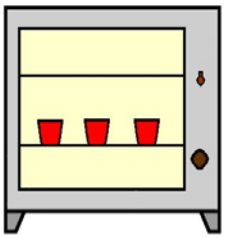


12. Cool in desiccator to room temperature

13. Weigh crucible plus suspended solids.



14. Ignite in muffle furnace at 550 deg. C for 15 minutes



15. Cool in drying oven briefly

16. Cool in desiccator to room temperature

17. Weigh crucible plus ash.

TOTAL AND VOLATILE SLUDGE SOLIDS

DISCUSSION: A well mixed sample is evaporated in a weighed dish and dried to a constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents total solids. The residue is then ignited to a constant weight at 550°C. The remaining solids represent the fixed solids (ash) while the weight loss on ignition is the volatile solids. This method is applicable for solid and semisolids samples such as sludges separated from wastewater treatment processes and sludge cakes from dewatering processes.

REFFERENCE: This conforms to the following EPA-approved procedure:

Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 2540 G.

1. APPARATUS

1.1 Evaporating dish: 100 mL capacity made of porcelain, platinum, or high-silica glass.

1.2 Steam bath

1.3 Drying oven, capable of maintaining a temperature of 103°C to 105°C

1.4 Muffle furnace, capable of maintaining a temperature of 550°C ± 50°C

1.5 Balance, accurate to 0.01 gram

1.6 Desiccator and indicating desiccant

2. PROCEDURE

2.1 Preparation of evaporating dishes

2.11 Ignite a clean evaporating dish for 1 hour at a temperature of 550°C ± 50°C.

2.12 Allow to cool in a drying oven and then transfer to a desiccator until cooled to room temperature.

2.13 Immediately before use, weigh the dish to the nearest 0.01 g and record on a bench sheet.

2.14 Dishes which are not to be used immediately should be stored in the drying oven following step 2.11

2.2 Sample analysis - Fluid samples (sludge)

2.21 If sample contains enough moisture to flow, mix well by stirring or shaking then pour a portion of the sample into the prepared evaporating dish until it is about half full (25 to 50 grams).

2.22 Immediately, to avoid loss of moisture, weigh to the nearest 0.01 g and record weight (dish and sample).

2.23 Evaporate to dryness on a steam bath.

2.24 Dry at 103 to 105°C for one hour.

2.25 Cool to room temperature in a desiccator, weigh and record weight (dish and dry solids).

2.26 Repeat heating, cooling, desiccating, and weighing steps until weight change is less than 4% or 50 mg, whichever is less.

2.27 Place dried sample in muffle furnace at 550°C for 1 hour.

2.28 Remove the dish from the furnace and, after partial cooling in the drying oven, place it in a desiccator until it is at room temperature.

2.29 Weigh and record results (dish and ash).

2.30 Repeat igniting (30 min.), cooling, desiccating, and weighing steps until weight change is less than 4% or 50 mg, whichever is less.

2.3 Sample analysis - Dewatered sludge (cake)

2.31 Break up cake into small pieces and place 25 to 50 grams into the prepared evaporating dish.

2.32 Immediately, to avoid loss of moisture, weigh to the nearest 0.01 g and record weight (dish and sample).

2.33 Dry at 103 to 105°C for 16 hours (overnight).

2.34 Cool to room temperature in a desiccator, weigh and record weight (dish and dry).

2.35 Repeat heating, cooling, desiccating, and weighing steps until weight change is less than 4% or 50 mg, whichever is less.

2.36 Place sample in muffle furnace at 550°C for 1 hour.

2.37 Remove the dish from the furnace and after partial cooling in the drying oven, place it in the desiccator until at room temperature.

2.38 Weigh and record results (dish and ash).

2.39 Repeat igniting (30 min.), cooling, desiccating, and weighing steps until weight change is less than 4% or 50 mg, whichever is less.

3. CALCULATIONS

3.1 % Total Solids

A. Subtract the weight of the dish (step 2.13) from the weight of dish and sample (step 2.22 or 2.32) to determine grams of sample analyzed (wet).
B. Subtract the weight of the dish (step 2.13) from the dish and dry weight (step 2.25 or 2.34) to determine grams of dry solids

$$\% \text{ Total Solids} = \frac{\text{Weight of Solids (Dry)}}{\text{Weight of Sample (Wet)}} \times 100\%$$

3.2 % Volatile Solids

A. Subtract the weight of the dish and ash (step 2.29 or 2.38) from the weight of dish and dry solids (step 2.25or 2.34) to determine grams of weight loss on ignition (volatile).
B. Subtract the weight of the dish (step 2.13) from the dish and dry weight (step 2.25 or 2.34) to determine grams of dry solids

$$\% \text{ Volatile Solids} = \frac{\text{Weight of Volatile Solids}}{\text{Weight of Dry Solids}} \times 100\%$$

--OR--

$$\% \text{ Volatile Solids} = \frac{(\text{Weight of Dry Solids - Weight of Ash})}{\text{Weight of Dry Solids}} \times 100\%$$

(Example Next Page)

3.3 Example Calculations

Calculate the Percent Total Solids and Percent Volatile Solids of a sludge sample given the following data:

Wt. of Dish	= 104.55 grams
Wt. of Dish and Wet Sludge	= 199.95 grams
Wt. of Dish and Dry Sludge	= 108.34 grams
Wt. of Dish and Ash	= 106.37 grams

$$\% \text{ Total Solids} = \frac{\text{Weight of Solids (Dry)}}{\text{Weight of Sample (Wet)}} \times 100\%$$

A. Wt. of Dish and Dry Sludge - Wt. of Dish = Weight of Solids (Dry)
108.34 grams - 104.55 grams = 3.79 gram

B. Wt. of Dish and Wet Sludge - Wt. of Dish = Weight of Sample (Wet)
199.95 grams - 104.55 grams = 95.40 gram

$$\% \text{ Total Solids} = \frac{3.79 \text{ gram}}{95.40 \text{ gram}} \times 100\%$$

$$= 0.40 \times 100\%$$

$$= 4.0\%$$

$$\% \text{ Volatile Solids} = \frac{\text{Weight of Volatile Solids}}{\text{Weight of Dry Solids}} \times 100\%$$

A. Wt. of Dish and Dry Sludge - Wt. of Dish and Ash = Weight of Volatile
108.34 grams - 106.37 grams = 1.97 gram

B. Wt. of Dish and Dry Sludge - Wt. of Dish = Weight of Solids (Dry)
108.34 grams - 104.55 grams = 3.79 gram

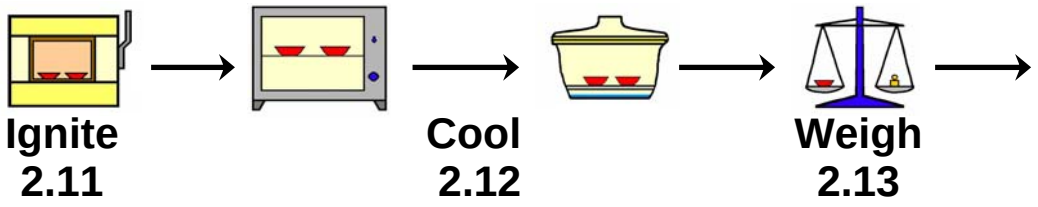
$$\% \text{ Volatile Solids} = \frac{1.97 \text{gram}}{3.79 \text{ gram}} \times 100\%$$

$$= 0.52 \times 100\%$$

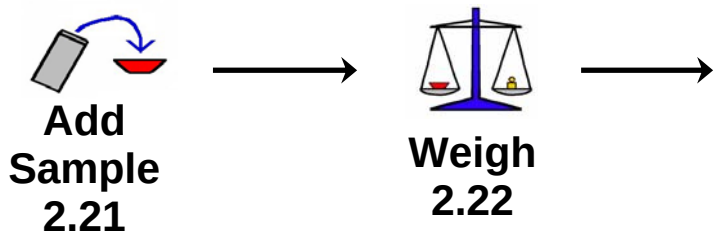
$$= 52.0\%$$

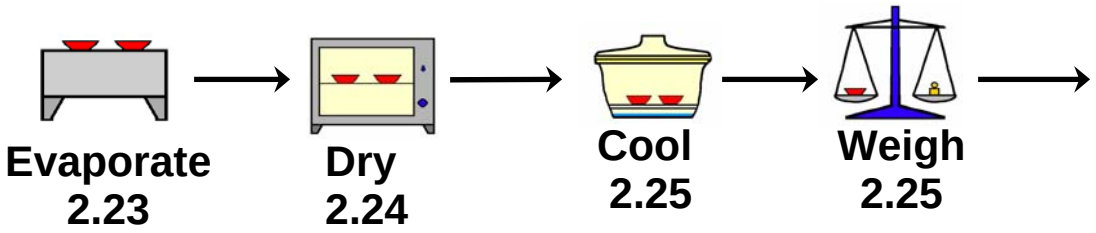
SLUDGE TOTAL SOLIDS PROCEDURE

Evaporating Dish Preparation

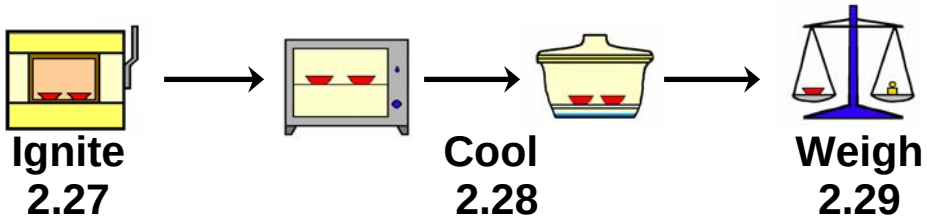


Total Solids Analysis





Volatile Solids Analysis



TOTAL DISSOLVED SOLIDS
Gravimetric, 180°C

DISCUSSION: A well mixed sample is filtered through a weighed standard glass-fiber filter and the filtrate is evaporated to dryness in a weighed dish and dried to a constant weight at 180°C. The increase in weight represents total dissolved solids

REFFERENCE: This conforms to the following EPA-approved procedures: Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 2540 C.

1. APPARATUS

1.1 Glass fiber filters, Whatman 934 AH, Gelman type A/E, Millipore type AP 40, or other products that give demonstrably equivalent results.

1.2 Filtration apparatus: One of the following, suitable for the filter disk selected:

1) Membrane filter funnel.

2) Gooch crucible, 25 mL to 40 mL capacity, with Gooch crucible adapter.

3) Filtration apparatus with reservoir and course (40 to 60 µm) fritted disk as filter support.

1.3 Evaporating dish: 100 mL capacity made of porcelain, platinum, or high-silica glass.

1.4 Vacuum pump

1.5 Vacuum flask of sufficient capacity for sample size selected.

1.6 Steam bath or drying oven for operation at 103 to 105°C.

1.7 Drying oven, for operation at 180 ± 2°C.

1.8 Desiccator, cabinet or jar type, with indicating desiccant

1.9 Analytical balance, capable of weighing to 0.1 mg

2. PROCEDURE

2.1 Preparation of glass-fiber filter.

2.11 Place a glass fiber filter disk, wrinkled side up, in filtration apparatus and place the apparatus on a clean vacuum flask.

2.12 Apply vacuum and wash the filter disk with three successive 20 mL portions of distilled water while applying a gentle vacuum.

2.13 Continue suction to remove all traces of water. Discard washings.

2.2 Preparation of evaporating dish.

2.21 Heat clean dish to 180°C ± 2°C in an oven.

2.22 Allow to cool to room temperature in the desiccator, determine the weight on an analytical balance and record on a bench sheet.

2.3 Sample analysis

2.31 Choose sample volume to yield between 2.5 and 200 mg dried residue. If complete filtration takes more than 10 minutes, increase filter diameter or decrease sample volume.

2.32 Thoroughly mix sample to obtain a representative portion for analysis. With the sample mixing, measure the appropriate volume using a graduated cylinder and record the volume on the bench sheet.

2.33 Place a prepared filter, or crucible with filter, on the vacuum flask, turn on the vacuum, and wet filter with a small volume of distilled water to seat it.

2.34 Add the measured volume of sample to the filtering apparatus and allow to filter through.

2.35 Rinse the graduated cylinder with three successive 10 mL volumes of distilled water, adding each to the filtering apparatus, allowing complete drainage between washings. Continue suction for about three minutes after filtration is complete.

2.36 Transfer the total volume of filtrate, including washings, to a weighed evaporating dish.

2.37 Evaporate to dryness on a steam bath or in a drying oven. If necessary, add successive portions to the same dish after evaporation.

2.38 Dry evaporated sample for a least 1 hour in an oven at 180°C.

2.39 Place in the desiccator for cooling to room temperature.

2.40 Using tongs to handle, determine the weight of the dish containing the dried solids on an analytical balance and record on a bench sheet.

2.41 Repeat cycle of drying, desiccating, and weighing until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

3. CALCULATIONS

3.1 Total Dissolved Solids:

A. Subtract weight determined in step 2.22 (dish) from weight determined in step 2.40 (dish and dry solids) to get weight of dry solids.

B. Total Dissolved Solids, mg/L =

$$\frac{\text{weight of dry solids (gram)}}{\text{volume of sample (mL)}} \times \frac{1000\ \text{mL}}{\text{liter}} \times \frac{1000\ \text{mg}}{\text{gram}}$$

--OR--

Total Dissolved Solids, mg/L =

$$\frac{\text{grams dry solids}}{\text{mL sample}} \times 1{,}000{,}000$$

3.2 Example

Calculate the total dissolved solids from the data below:

Volume of sample filtered	75 mL
Weight of dish	105.5817 gram
Weight of dish with dry solids	105.5952 gram

Total Dissolved Solids (mg/L)

A.

Wt. dish + dry	105.5952 g
- Wt. dish	- 105.5817 g
Wt. dry solids	0.0135 g

B. Sus. Sol. mg/L = $\frac{0.0135\ \text{g}}{75\ \text{mL}} \times 1{,}000{,}000$

Total Dissolved Solids = 180 mg/L

pH

DISCUSSION: The pH of a solution gives an indication of the intensity of the acidity or alkalinity of the solution. Pure water exists in a partially ionized state as indicated by the equation:

$$H_2O \rightleftharpoons H^+ + (OH)^-$$

Since an acid may be defined as a substance which produces hydrogen ions and a base may be defined as a substance which produces hydroxyl ions, water may be thought of as being both an acid and a base. Since these ions are present in equal quantities pure water is said to be neutral.

It has been found, experimentally, that in pure water the concentration of H^+ is 0.0000001 Molar, which may also be written 1×10^{-7}M. This means that pure water contains 1 molecular weight (1.008 grams) of hydrogen ions for every 10 million liters. Since there is an equivalent amount of hydroxyl ions its concentration is also 1×10^{-7}M.

As a means of making these concentrations easier to work with the term "pH" was developed. pH is defined as the negative logarithm of the hydrogen ion concentration. The log of 1×10^{-7} is -7 and the negative of this number is 7, so that the pH of a 1×10^{-7}M H^+ solution would be 7. As the concentration of H^+ increases, the pH value decreases. For example, if the H^+ concentration of a solution is 1×10^{-3} we can see that the pH value would be 3, since the negative log of 1×10^{-3} equals 3.

The pH of a neutral solution is 7. When a solution has a higher concentration of H^+ than a neutral solution the pH is below 7 and we say that it is acidic. When the H^+ concentration of a solution is less than that of a neutral solution the pH is above 7 and we say that the solution is basic or alkaline. The following diagram helps to illustrate this

pH

relationship.

pH SCALE

1	2	3	4	5	6	7	8	9	10	11	12	13	14

ACIDIC NEUTRAL BASIC

The same type of notation can be used for the $(OH)^-$ concentration. The p(OH) of a neutral solution would also be 7 since the concentrations of H^+ and $(OH)^-$ are equal.

In water we know that the concentration of H^+ times the concentration of $(OH)^-$ gives the constant value 1×10^{-14}. Since adding the logs of numbers is the same as multiplying the numbers we can say that pH + p(OH) = 14. This makes it possible for us to know the p(OH) for a solution by measuring pH and subtracting this value from 14. This also explains why the entire range of possible pH values in water is from 0 to 14.

Acids and bases which ionize almost completely in water are called "strong" acids or bases. Those which do not ionize to this extent are called "weak" acids or bases. Examples of strong acids are HCl, H_2SO_4, and HNO_3, whereas H_2S and H_2CO_3 are weak acids. An example of a strong base is NaOH because it ionizes into Na^+ and $(OH)^-$ almost entirely, but since $Ca(OH)_2$ only partially ionizes into Ca^{+2} and $(OH)^-$ it is considered a weak base.

pH is very important in the wastewater field for several reasons. Most microorganisms are sensitive to changes in pH and wide fluctuations may cause problems in wastewater treatment plants that rely on biological processes. One example of this is the anaerobic digester where pH must be maintained between specific limits for the bacteria to stabilize the sludge. Precipitation reactions such as the removal of phosphorus or heavy

metals by addition of lime also depend on close control of pH. Corrosion control is very dependent upon close control of pH levels. The discharge of acid into a wastewater collection system will usually corrode the piping and may produce toxic gases such as H_2S. Many types of laboratory analyses require samples and reagents to be held at specific pH levels. In many of these analyses failure to adjust pH to the proper level will cause the results to be completely unreliable.

pH levels are measured electrometrically using an electrode which has a pH sensitive glass tip. When the glass electrode is placed in a solution which differs in pH from the solution inside the electrode an electrical potential is generated between the glass electrode and a reference electrode. This potential, which is proportional to the pH difference, is measured and the output is related to the pH of the sample solution in the electronics of the meter.

Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by making sure that the calibrating buffers and the samples are at the same temperature. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled. On very critical work pH measurements should, therefore, be accompanied by the temperatures at which the measurements were made.

pH VALUE PROCEDURE
Electrometric Method

REFERENCE - This procedure conforms to the EPA-approved method as found in Standard Methods, 20th edition, 4500-H^+ B, Electrometric Method. Note that this method differs from Standard Methods in that commercially prepared standards are recommended rather than laboratory preparation from solid salts.

1. APPARATUS

   1.1 pH metering system consisting of potentiometer, sensing electrode, reference electrode, and temperature compensating device, capable of accuracy to at least 0.1 pH unit. A combination electrode may be used.

   1.2 Buffer solutions of pH 4.0, 7.0, and 10.0, stored at room temperature.

   1.3 Beakers, preferably polyethylene or Teflon.

   1.4 Magnetic stirrer with Teflon coated stir bar.

2. CALIBRATION

   2.1 Follow the manufacturer's instructions regarding the operation of the meter, and storage and maintenance of the electrode.

   2.2 Place pH 7.0 buffer solution in a clean beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar. Stir at slow speed; try to maintain the same stirring speed for all standards and samples.

   2.3 Remove electrodes from storage solution and rinse with distilled or deionized water. Blot electrode dry with a soft cloth or tissue.

   2.4 Immerse the electrode in the buffer, and wait for a stable reading. Calibrate the instrument to pH 7.0.

pH Value

2.5 Rinse and blot the electrode, and immerse in either the pH 4.0 or 10.0 buffer solution, bracketing the expected sample pH. Calibrate the meter to this value after a stable reading is obtained.

2.6 Rinse and blot the electrode, and place it back into the pH 7.0 buffer. The reading should be within 0.1 pH unit. If it is not, the electrode and/or buffers must be evaluated to determine the cause of the problem, and correction made.

2.7 When only occasional pH measurements are made, calibrate the instrument before each sample measurement.

3. PROCEDURE

3.1 Collect sample in either plastic or glass container. Analyze samples for NPDES reporting within 15 minutes of collection.

3.2 Bring the sample to the temperature of the calibration buffers if possible. Use automatic temperature compensation. Record sample temperature at time of measurement.

3.3 Place the sample in a clean beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar. Stir slowly.

3.4 After rinsing and blotting the electrode, immerse it in the sample solution, wait for a stable reading, and record the value to 0.1 pH unit.

4. ELECTRODE MAINTENANCE

4.1 Monitor the performance of the electrode by recording the slope of the pH

electrode at least weekly. The theoretical slope of a perfect electrode is -59.16 mV/pH unit at 25°C. Digital pH meters report slope either as percent of theoretical slope (i.e. 98%), or as mV/pH unit (i.e. 57.2 mV/pH). The minimum acceptable slope value is usually given by the electrode manufacturer; typical values are 95%, or 55mV/pH. Slopes below that value indicate that electrode maintenance is required or that a new electrode must be purchased.

4.2 Assure that the reference electrode filling solution is maintained at an appropriate level, typically at least 2 cm above the surface of the liquid that is being measured. Refill according to manufacturer's direction; usually this filling solution is 3M KCl saturated with AgCl, but consult electrode instructions for proper solution. Make sure that the electrolyte fill hole is not covered during electrode use.

4.3 Electrodes which cannot be successfully calibrated, or whose slope is below the minimum may sometimes be rejuvenated. A typical cleaning procedure follows:

4.3.1 Soak in 0.1M HCl or 0.1M HNO_3 for half an hour.

4.3.2 Drain and refill the electrode filling solution.

4.3.3 Soak the electrode in filling or storage solution for 1hour.

4.3.4 Further steps may be required to restore reference electrode electrolyte flow, or to remove oil and grease and other contaminants. Consult electrode instructions for details.

5. QUALITY CONTROL

5.1 Determine and record electrode slope.

5.2 Obtain and analyze reference standards from an outside source, the frequency depending on the number of samples analyzed, and the sensitivity/importance of the data.

BUFFERS

A buffer is a combination of substances which, when dissolved in water, resists a pH change in the water, as might be caused by the addition of an acid or base by accepting or donating hydrogen ions to the solution.

Buffer solutions usually are composed of mixtures of weak acids and their salts or weak bases and their salts. An example of a buffer formed by a weak acid and its salt is the solution of acetic acid and sodium acetate in water. Ionization of these two compounds occurs as in the equations below:

$$HC_2H_3O_2 \rightleftharpoons H^+ + (C_2H_3O_2)^-$$

Acetic Acid $\rightleftharpoons$ Hydrogen Ion + Acetate Ion

$$NaC_2H_3O_2 \rightleftharpoons Na^+ + (C_2H_3O_2)^-$$

Sodium Acetate $\rightleftharpoons$ Sodium Ion + Acetate Ion

Since acetic acid is a weak acid, ionization does not occur to a large extent. The sodium acetate, however, ionizes almost completely. Making a solution of these two chemicals results in a large excess of the acetate ion in the solution.

When an acid (H^+) is added, the H^+ reacts with the excess acetate ion to form acetic acid, leaving the H^+ concentration almost unchanged, thus the pH of the solution remains almost unchanged.

When a base (OH^-) is added, the OH^- reacts with the H^+ to form water, but the acetic acid ionizes more to donate more H^+. Again, the H^+ concentration changes very little and as a result the pH also remains almost unchanged.

It is possible to prepare buffer solutions which have the ability to buffer within various pH ranges by using different acid and salt or base and salt pairs. Listed below are a few chemicals which, when combined in the proper proportions, will tend to maintain the pH to within the indicated range.

Buffers

CHEMICALS	pH RANGE
Acetic Acid + Sodium Acetate	3.7 - 5.6
Sodium Dihydrogen Phosphate + Disodium Hydrogen Phosphate	5.8 - 8.0
Boric Acid + Borax	6.8 - 9.2

It must be realized that the buffering capacity of any buffering solution can be exceeded. For example, in a buffering solution prepared with acetic acid and sodium acetate, the buffer will work as long as there is still enough acetic acid present to supply H^+ ions and enough sodium acetate to supply the acetate ions. If enough base is added to the solution to deplete the acetic acid, the buffering capacity of the solution will be exceeded and the pH of the solution will increase rapidly. Also, if enough acid is added to the solution to deplete the sodium acetate the buffering capacity will be exceeded and the pH of the solution will decrease rapidly.

ALKALINITY

DISCUSSION: Alkalinity is defined as the capacity of a solution to react with an acid as measured to a predetermined pH value. The alkalinity of water is mainly due to the presence of salts of weak acids and strong bases. These act as buffers to resist a drop in pH resulting from the addition of an acid. Therefore, alkalinity is a measure of the buffering capacity of water.

Bicarbonates are the major form of alkalinity in natural water due to the reaction of carbon dioxide (CO_2) in the air with basic materials in soil. When CO_2 dissolves in water carbonic acid is formed. When this solution comes in contact with calcium carbonate and magnesium carbonate in soil the acid is neutralized and calcium bicarbonate and magnesium bicarbonates are formed.

CO_2 Carbon Dioxide + H_2O Water $\rightleftharpoons$ H_2CO_3 Carbonic Acid

H_2CO_3 Carbonic Acid + $CaCO_3$ Calcium Carbonate $\rightleftharpoons$ $Ca(HCO_3)_2$ Calcium Bicarbonate

Hydroxides and carbonates also contribute to the alkalinity of water, and under some conditions may be present in natural waters. This situation normally occurs in surface waters where algae are flourishing, such is the case in stabilization lagoons during the warm months of the year. The algae remove CO_2 from the water, and since this is an acidic gas the pH value of the water increases. As the pH increases, the alkalinity present in the water changes from bicarbonates to carbonates and CO_2. As this CO_2 is used by the algae, the pH rises again and the carbonates are converted to hydroxide and CO_2. These reactions occur as in the following equations:

$2HCO_3$ Bicarbonates $\rightleftharpoons$ $(CO_3)^{-2}$ Carbonates + H_2O Water + CO_2 Carbon Dioxide

$(CO_2)^{-2}$ Carbonates + H_2O Water $\rightleftharpoons$ $2(OH)^-$ Hydroxide + CO_2 Carbon Dioxide

Alkalinity

Algae can continue this extraction of CO_2 until the pH is high enough to be inhibitory to the organisms. Such pH values may range as high as pH 10 to pH 11.

A process similar to this occurs in boiler waters. Since CO_2 is not soluble in boiling water, it is removed with the steam. This increases the pH of the water, and the bicarbonates present change into carbonates and the carbonates into hydroxide. Under these conditions pH in the boiler water may get as high as 11.

The measurement of alkalinity is especially important in the wastewater field in the operation of anaerobic sludge digesters. When used in conjunction with a measurement of the volatile acids in the digesting sludge, the ratio of volatile acids to alkalinity provides a means of determining whether or not the digester is functioning normally. A complete discussion of this analysis may be found in the Volatile Acids/Alkalinity unit of this manual.

Alkalinity of water and wastewater samples is measured by titration with 0.02 N sulfuric acid and is reported in terms of equivalent $CaCO_3$. If the sample pH is above 8.3, the titration is done in two steps. In the first step, the acid is titrated into the sample until the pH is lowered to 8.3. This pH value corresponds to the point at which all of the hydroxide and one-half of the carbonate alkalinity has been converted to bicarbonate and is also the pH at which phenolphthalein color indicator changes from pink to clear. Because of this, the alkalinity measured to pH 8.3 is often referred to as the "phenolphthalein alkalinity."

In the second step, the titration is continued until the sample pH has been reduced to about 4.5. This pH value corresponds to the point at which the carbonates and bicarbonates have been converted to carbonic acid. Since the methyl orange end point is also at this pH, alkalinity measured to pH 4.5 is often referred to as "methyl orange alkalinity."

If the first titration is eliminated and the sample is titrated directly to pH 4.5 the alkalinity is called "total alkalinity." Measurements of both the phenolphthalein and total alkalinity permit the calculation of the quantity of each species of alkalinity present in the sample.

Sample Collection and Handling

Make certain the sample taken for analysis is representative of the medium being analyzed. When samples such as digester supernatant are drawn from a pipe, the sample should be taken after sufficient flow has flushed out the line. Samples should be collected in polyethylene or borosilicate glass containers (such as Pyrex). Avoid excessive agitation and exposure of sample to air. The sample container should be filled completely, tightly capped, and refrigerated at ≤ 6°C until analysis. The allowed holding time is 14 days.

ALKALINITY DETERMINATION
POTENTIOMETRIC METHOD

This procedure may be used to determine total and / or bicarbonate alkalinity. The minimum concentration reportable using this method is 20 mg/L. The sample is titrated at room temperature, to a pH 4.5 endpoint using a pH meter and electrode. Results are reported in terms of mg/L of $CaCO_3$.

REFERENCE

This procedure conforms to the EPA approved procedure referenced in the 20th Edition of Standard Methods, Method 2320B.

1. REAGENTS

   1.1 Carbon dioxide free deionized water for dilution of samples and preparation of reagents and standards.

   1.2 Standard Sulfuric Acid Titrant, 0.02N

      1.21 Dilute 2.8 mL of concentrated H_2SO_4 to 1 liter with deionized water.

      1.22 Dilute 200 mL of this solution to 1 liter with deionized water.

   1.3 Sodium carbonate, 0.02 N - Oven dry about 2 grams anhydrous sodium carbonate, Na_2CO_3, at 250^0C for 4 hours and cool in a desiccator. Dissolve 1.060 grams of the dried reagent in distilled water and make up to 1 liter in a volumetric flask.

2. STANDARDIZATION OF 0.02N SULFURIC ACID

   2.1 Using a volumetric pipet, place 25.0 mL of 0.02 N Sodium Carbonate, Na_2CO_3, in a 125 mL Erlenmeyer flask.

   2.2 Titrate with 0.02N sulfuric acid until pH reaches 4.5, using a calibrated pH meter to detect the endpoint.

2.3 Calculate normality of the acid using the following formula:

$$\text{Normality of } H_2SO_4 = \frac{25 \times 0.02}{\text{mL of } H_2SO_4 \text{ titrated}}$$

3. PROCEDURE FOR TOTAL ALKALINITY

3.1 Place 100 mL of sample or a portion of sample diluted to 100 mL in a beaker or flask.

3.2 Place on magnetic stirrer and insert pH probe(s) into solution.

3.3 Titrate with 0.02N sulfuric acid while stirring until pH 4.5 is reached.

3.4 Calculate total alkalinity using the equation below:

$$\text{mg/L total alkalinity} = \frac{(A) \times (N) \times (50{,}000)}{\text{mL of sample titrated}}$$

Where: A = mL of H_2SO_4 used

N = normality of H_2SO_4

4. PROCEDURE FOR BICARBONATE ALKALINITY

4.1 Place 100 mL of sample or a portion of sample diluted to 100 mL in a beaker or flask.

4.2 Place on magnetic stirrer and insert calibrated pH probe(s) into sample solution.

4.3 Determine sample pH and record this value. If pH of sample is below pH 8.3 then the bicarbonate alkalinity is equal to the total alkalinity as determined above.

4.4 If the pH of the sample is above 8.3, continue through the following procedure.

4.5 Titrate with 0.02N Sulfuric Acid, H_2SO_4 while stirring to a pH of 8.3 and record the volume of sulfuric acid used.

4.6 Continue the titration to a pH of 4.5 and again record the volume of acid used.

4.7 If the first volume of acid recorded is equal to or greater than one-half the total volume of acid titrated to reach pH 4.5, then the bicarbonate alkalinity = 0.

If the first volume of acid is less than one-half the total volume of acid titrated, then use the formula below to calculate the bicarbonate alkalinity.

$$\text{Bicarbonate Alkalinity, mg/L} = \frac{(T - 2P) \times N \times 50{,}000}{\text{mL sample titrated}}$$

Where: T = total volume of acid titrated

P = Volume of acid titrated to reach pH 8.3

N = Normality of H_2SO_4

VOLATILE ACIDS AND TOTAL ALKALINITY
TITRATION METHOD

DISCUSSION: This is a rapid method for determining both volatile acids and alkalinity in sludge from an anaerobic digester. This method gives accurate results which will enable the operator to monitor the digester precisely and frequently. The volatile acids/alkalinity ratio is important in providing the operator with information which enables him to start up a new digester and to maintain a properly functioning digester in a healthy condition. The first measurable changes in a digester on the way toward upset will be reflected in the volatile acids/alkalinity ratio. Normally, ratios up to 0.5 are not inhibitory to digester performance. Ratios increasing beyond 0.5 warn of undesirable changes, which if unchecked will result in diminished gas quality and quantity and a depression in pH.

The sludge sample for this determination should be taken from the primary digester at a point where the sample will be fresh and well mixed. The usefulness of this analysis will depend on obtaining a sample which will be representative of the actual conditions in the digester.

1. APPARATUS

    1.1 Erlenmeyer flask, 250 mL

    1.2 Burets, two, 50 mL, with stands

    1.3 pH meter

    1.4 Hot plate

    1.5 Beaker, 100 mL

    1.6 Magnetic stirrer

2. REAGENTS

    2.1 pH buffer solutions, 4.0 and 7.0

    2.2 Sulfuric Acid, H_2SO_4, 0.10N

2.21 Add 14 mL concentrated sulfuric acid to approx. 500 mL distilled water and dilute to 1 liter.

2.22 Add 200 mL of this solution to a 1 liter volumetric flask and dilute to volume with distilled water.

2.3 Sodium carbonate solution, 0.10N

2.31 Dry approx. 7 grams of anhydrous sodium bicarbonate, Na_2CO_3 in an oven at 140°C.

2.32 Dissolve 5.3 grams of the dried reagent in distilled water and dilute to 1 liter in a volumetric flask.

2.4 Sodium hydroxide, 0.05N. Dissolve 2 g of sodium hydroxide, NaOH, in freshly distilled water and dilute to 1 liter in a volumetric flask.

2.5 Phenolphthalein indicator solution. Dissolve 0.5 g phenolphthalein in 50 mL of ethyl alcohol (95%) or isopropyl alcohol and dilute to 100 mL with distilled water.

2.6 Methyl orange indicator. Dissolve 0.5 g of methyl orange powder in distilled water and dilute to 1 liter.

3. STANDARDIZATION OF 0.10N SULFURIC ACID

3.1 Pipet 25.0 mL of 0.10N sodium carbonate solution into a 250 mL Erlenmeyer flask and add about 50 mL distilled water.

3.2 Add 2 - 3 drops methyl orange indicator.

3.3 Titrate with 0.10N sulfuric acid until the solution turns from orange to pink.

3.4 Calculate the normality of the acid solution as follows:

$$\text{Normality of } H_2SO_4 = \frac{25 \text{ mL} \times 0.10\text{N}}{\text{mL } H_2SO_4 \text{ titrated}}$$

4. STANDARDIZATION OF 0.05 N SODIUM HYDROXIDE

4.1 Dispense 10.0 mL of 0.10N sulfuric acid from the buret into a 250 mL Erlenmeyer flask and add about 50 mL distilled water.

4.2 Add 2 - 3 drops phenolphthalein indicator solution.

4.3 Titrate with 0.05 N sodium hydroxide to a faint pink endpoint.

4.4 Calculate normality of the NaOH as follows:

$$\text{Normality of NaOH} = \frac{10\text{ mL} \times \text{Normality of } H_2SO_4}{\text{mL NaOH titrated}}$$

5. PROCEDURE

5.1 Properly calibrate pH meter using pH 7.0 and 4.0 buffer solutions.

5.2 Allow a sample of digesting sludge to settle until supernatant is relatively free of solids.

5.3 Measure 50 mL supernatant into a 100 mL beaker and place on a magnetic stirrer.

5.4 Record temperature of the sample.

5.5 Record pH of sample.

5.6 Record initial buret reading and titrate with 0.10N H_2SO_4 to a pH of 4.0; record mL of acid used.

5.7 Continue to add acid to a pH of 3.3 (volume of acid used in this step not used in calculations.)

5.8 LIGHTLY boil sample for 3 min., being careful not to lose any sample.

5.9 Cool sample to original temperature.

5.10 Titrate sample back to pH 4.0 with 0.05N NaOH (volume of NaOH in this step not used in calculations.)

5.11 Record buret reading and titrate to pH 7.0; record mL NaOH used in this step of titration.

CALCULATIONS

$$\text{Total Alkalinity mg/L} = \frac{\text{Normality of } H_2SO_4 \text{ x mL } H_2SO_4 \text{ x } 50{,}000}{\text{mL of sample used}}$$

$$\text{Volatile Acid Alkalinity} = \frac{\text{Normality of NaOH x mL NaOH x } 50{,}000}{\text{mL of sample used}}$$

Total Alkalinity - Volatile Acid Alkalinity = HCO_3 Alkalinity

Volatile Acids = Volatile Acid Alkalinity (when this value is less than 180 mg/L)

Volatile Acids = 1.5 x Volatile Acid Alkalinity (when this value is greater than 180 mg/L)

$$\frac{\text{Volatile Acids mg/L}}{\text{Total Alkalinity}} = \text{Volatile Acid/Alkalinity Ratio}$$

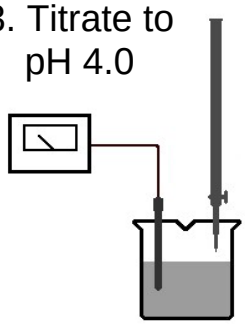
VOLATILE ACIDS AND TOTAL ALKALINITY

Outline of Procedure

1. Separate Solids

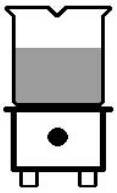
2. Measure 50 mL

3. Titrate to pH 4.0



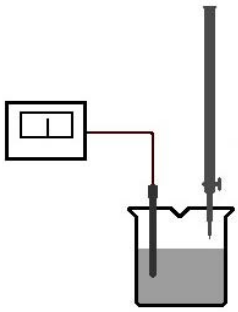
4. Record mL used, Then Titrate to pH 3.3

5. Lightly Boil Sample 3 Min.



6. Cool in Water Bath

7. Titrate from pH 4.0 to 7.0



CARBON DIOXIDE, CO_2 IN DIGESTER GAS

DISCUSSION: If the methane-producing bacteria in an anaerobic digester are inhibited or killed off because of improper volatile acid/alkalinity ratio or other causes, methane gas production will decrease and CO_2 gas percentage will increase. To determine if the CO_2 gas in the digester is at a high range an analysis for CO_2 gas can be performed. The CO_2 content of a properly operating digester will range from 30% to 35% by volume. If the percent of CO_2 gas is above 44% the methane gas will not burn.

A graduated cylinder containing a gas sample is inverted into a potassium hydroxide solution. The carbon dioxide in the gas sample is absorbed by the potassium hydroxide. As the carbon dioxide is absorbed, water displaces the volume of the graduate formerly occupied by the CO_2 gas.

1. APPARATUS

   1.1 Plastic tubing

   1.2 100 mL graduated cylinder

   1.3 250 mL beaker

2. REAGENTS

   2.1 Potassium hydroxide solution. Dissolve 500 g of potassium hydroxide, KOH into 1 liter of distilled water.

3. PROCEDURE

   3.1 Measure total volume of a 100 mL graduate by filling it to the top with water (approx. 125 mL). Record this volume.

   3.2 Pour approx. 125 mL of potassium hydroxide, KOH in a 250 mL beaker. <u>CAUTION:</u> DO NOT GET ANY OF THIS CHEMICAL ON YOUR SKIN OR CLOTHES. WASH IMMEDIATELY WITH RUNNING WATER UNTIL

CO_2

SLIPPERY FEELING IS GONE OR SEVERE BURNS CAN OCCUR.

3.3 Collect a representative sample of gas from the gas dome on the digester, a hot water heater using digester gas to heat the sludge or any other gas outlet.

3.4 With gas running through the hose from a gas sampling outlet, place hose inside inverted calibrated graduated cylinder and allow digester gas to displace air in graduate. Turn off gas.

CAUTION: THE PROPER MIXTURE OF DIGESTER GAS AND AIR IS EXPLOSIVE WHEN EXPOSED TO A FLAME!

3.5 Place graduate full of digester gas upside down in beaker containing carbon dioxide, CO_2 absorbent.

3.6 Insert gas hose inside upside down graduate.

3.7 Turn on gas, but DO NOT BLOW OUT LIQUID. Run gas for at least 60 seconds.

3.8 Carefully remove hose from graduate with gas still running.

3.9 IMMEDIATELY TURN OFF GAS.

3.10 Wait for ten minutes and shake gently. If liquid continues to rise, wait until it stops.

3.11 Read gas remaining in graduate to nearest mL.

4. EXAMPLE

Total Volume of graduate = 126 mL

Gas Remaining in graduate = 80 mL

5. CALCULATION

$$\% CO_2 = \frac{(\text{Total Volume, mL} - \text{Gas Remaining, mL})}{\text{Total Volume, mL}} \times 100\%$$

$$= \frac{(126 \text{ mL} - 80 \text{ mL})}{126 \text{ mL}} \times 100\%$$

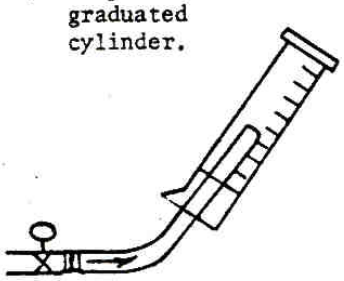
$$= \frac{46}{126} \times 100\%$$

$$= 37\%$$

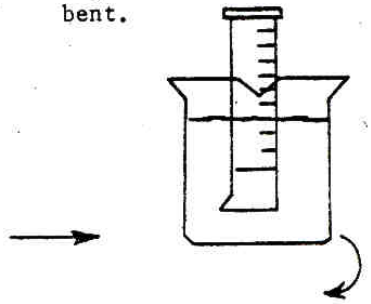
CO_2

OUTLINE OF CO_2 PROCEDURE

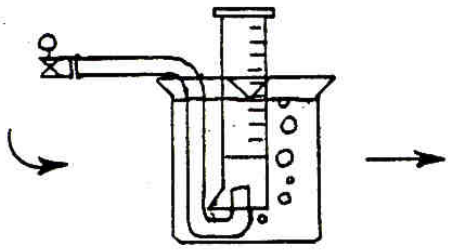
1. Displace air in graduated cylinder.



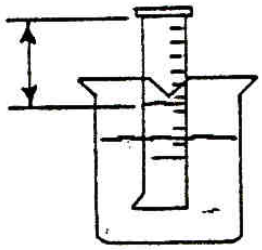
2. Place graduate upside down in beaker containing CO_2 absorbent.



3. Insert hose in graduate and run gas for 60 seconds.



4. Remove hose from graduate and then turn off gas. Wait 10 minutes.



5. Read volume of gas remaining to nearest ml.

PRECAUTIONS

1. Avoid any open flames near the digester.
2. Work in a well ventilated area to avoid the formation of explosive mixtures of methane gas.
3. If your gas sampling outlet is on top of your digester, turn on outlet and vent the gas to the atmosphere for several minutes to clear the line of old gas. Start with step 1, displace air in graduated cylinder. NEVER ALLOW ANY SMOKING OR FLAMES NEAR THE DIGESTER AT ANY TIME.

BACTERIAL MONITORING

DISCUSSION: Wastes from the bodies of warm-blooded animals, including humans, contain many different species of bacteria. Some of these bacteria may be pathogenic, meaning that they cause diseases in humans, and some are harmless. In determining water quality it is important know whether pathogenic bacteria may be present. This information is especially important when wastewater which is known to have contained human wastes is discharged to surface or ground water. There are some problems with trying to quantify the number of pathogenic bacteria in a sample, however. These problems include having to work with dangerous bacteria, procedures for isolating these are difficult and costly, and there are a very large number of such bacteria that would have to be included in an analysis. The situation is simplified by analyzing for bacteria that are not pathogenic, but would be expected to be present whenever pathogenic bacteria are present.

The coliform bacteria group includes many different species of bacteria. Members of this group may be found throughout the environment, including human and animal wastes. Since some members of the coliform group are found in the intestines of warm-blooded animals, coliform analysis is used as an indication as to whether pathogenic bacteria may be present in wastewater. For this reason coliform bacteria are referred to as "indicator organisms". The effectiveness of disinfection may also be determined by coliform analysis. If an adequate reduction in the number of coliform bacteria occurs, then it is assumed that a corresponding reduction of pathogenic organisms has also taken place. Formerly the “total coliform” group was used as an indication of pollution, but because this group may include bacteria from several sources the test was not very specific. Since fecal coliform are a group of coliform bacteria which reside in the intestines of warm-blooded animals, the presence of this bacteria in a sample is a better indicator of whether pollution from animal waste, and

possibly human waste is present. The limit for fecal coliform in wastewater discharged to surface water in Michigan has been set at 200 in a 100 milliliter sample for a 30 day geometric mean, and 400 / 100 mL for a mean of the worst 7 day period.

A member of the fecal coliform group which is being increasingly used as an indicator organism, especially in recreational water is Escherichia coli (E. coli). Studies have shown a more direct relationship between the density of *E. coli* and the risk of gastrointestinal illness associated with swimming in the water.

There are two EPA approved methods included in this manual which may be used to analyze for fecal coliform bacteria, each having advantages and disadvantages. The multiple tube method, also called the most probable number (MPN) method, has been in use for many years in bacteria analysis. It is based on the principle that members of the coliform group will ferment lactose, producing gas in a culture media at a suitable temperature. The analyst sets up a series of dilutions of a sample in the culture media and incubates the tubes at a specific temperature. The presence or absence of gas in the tube after a period of time is used to determine statistically the probable concentration of bacteria in the sample. The MPN test using A-1 medium included in this manual replaces the older version of this procedure. This modification reduces time to completion, requiring 24 hours of incubation rather than up to 72 hours, and requiring less reagents and glassware. The analyst should be aware that although the MPN method is generally more time consuming than the membrane filtration method, the MPN method is required when significant turbidity or solids are present in the sample.

In the membrane filter method, a portion of the sample is filtered through a membrane which typically has a pore size of 0.45 microns. The bacteria are trapped on the filter which is then placed in a Petri dish containing a nutrient rich media. After a suitable incubation time

and temperature the colonies which have developed are counted, each having formed from an individual bacterium. Since this test only requires 24 hours for completion and requires much less glassware, it has replaced the MPN method in most wastewater treatment plant laboratories. The test for E. coli included in the manual is also a membrane filtration procedure, although other options are available as noted in that discussion.

Some specialized laboratory equipment is required for the determination of coliform bacteria. Since all glassware, equipment, and reagents to be used in this test must be free of bacterial contamination, a sterilizer is required. The most commonly used means of sterilization in wastewater laboratories is the autoclave. This is a device in which glassware, small equipment, and solutions may be subjected to steam heat under pressure. A dry heat sterilizer may be used for glassware and other equipment, but may not be used for liquids. A means of incubating the MPN tubes or Petri dishes at a very consistent temperature is required. There are three types of incubators commonly used, including water bath, air type, and aluminum block. Each type has its own advantages and the type used normally depends on whether the MPN or filtration method is used, ability to maintain the desired temperature, the number of samples that must be incubated, bench space available, and cost of the equipment. The water bath incubator is probably the most often used, mainly because it has the ability to maintain a set temperature within a very close tolerance. If the number of samples is not large, the newer aluminum block incubators may be an advantage due to lower cost and less bench spaced used. It should be noted that the membrane filtration procedure for E. coli requires two incubators set at different temperatures.

SAMPLING. Sampling containers may be either glass or plastic, as long as they are able to withstand sterilizing conditions and do not release toxic compounds when sterilized.

Bacteria Analysis

Wide mouth bottles with either screw-on or ground glass fittings should be used and should have a capacity of at least 125 milliliters to provide adequate sample size and mixing capability. Containers which are chipped, cracked, or etched should not be used. Sampling containers may be cleaned by washing with hot water and detergent, followed by hot water rinse, and rinsing three times with distilled water. The container must then be sterilized.

Samples collected for bacteria analysis should not be composited, but should be analyzed as grab samples. If samples are taken of chlorinated flows, 0.1 mL of a 10% sodium thiosulfate solution must be added to the container prior to sterilization. This is sufficient to neutralize 15 mg/L of residual chlorine in a 100 mL sample.

The first step in actually sampling a flow is to remove the top from the sampling container, protecting it from possible contamination. The bottle is then plunged 6 - 12 inches below the surface, being careful to avoid introduction of surface scum. The mouth of the bottle should be positioned into the flow, away from the hand, tipping the bottle slightly so as to allow air to escape. Then remove the bottle from the stream, quickly pour out a small portion to allow for mixing, and replace the top. The sample should be analyzed immediately. If this is not possible, approved sample preservation and holding times must be observed.

Sample analysis should begin immediately, preferably within 2 hours of collection. Samples not analyzed within 15 minutes of collection must be preserved by cooling to $<10^{o}C$. The maximum transport time to the laboratory is 6 hours, and samples must be processed within 2 hours of receipt at the laboratory.

FECAL COLIFORM
Membrane Filter Method

The Membrane Filter (MF) procedure uses an enriched lactose medium (M-FC Broth) and incubation temperature of 44.5 ± 0.2^{o}C to differentiate between coliforms found in warm blooded animals and those from other environments. Because incubation temperature is critical, submerge waterproofed Petri dishes in a warm water bath for incubation, or use an accurate solid heat sink incubator.

REFERENCE

This procedure conforms to the EPA approved procedure referenced as Standard Methods 20th Edition, Method 9222 D.

1. APPARATUS

   1.1 Sample bottles - sterilizable, plastic or glass, at least 125 mL capacity.

   1.2 Erlenmeyer flask - 125 mL, screw top, for culture medium

   1.3 Pipets - graduated, pre-sterilized disposable or serological, with large tip opening, volumes 1 mL and 10 mL graduated in 1/10 mL divisions.

   1.4 Graduated cylinder - sterilized, 100 mL

   1.5 Pipet canister - stainless steel or aluminum

   1.6 Petri dishes – plastic disposable, 50 x 12 mm, for 47 mm membrane filters, pre-sterilized.

   1.7 Membrane filter holder and funnel - plastic, glass, or stainless steel.

   1.8 Absorbent pads, 47 mm diameter, pre-sterilized.

   1.9 Membrane filters - pre-sterilized, 0.45 micron pore size, 47 mm diameter, with grid.

1.10 Forceps - round tipped without corrugations on inner side of tips.

1.11 Incubator - water bath or solid heat sink, capable of maintaining 44.5^{o}C ± 0.2^{o}C.

1.12 Microscope (optional) - dissecting, magnification 10X - 15X with fluorescent light source.

1.13 Vacuum pump

1.14 Vacuum flask, at least 1 liter capacity.

2. WASHING AND STERILIZATION

2.1 All equipment should be washed with hot tap water and detergent, then rinsed with hot water, and rinsed 3 times with distilled water.

2.2 Sterilization

2.21 Autoclave - sterilize equipment and reagents at 15 psi (121°C) for 15 minutes.

2.22 Dry heat - sterilize equipment (no liquids) at 170°C for at least 60 min.

2.23 All glassware should be capped or the opening covered with aluminum foil. Pipets should be sterilized in a pipet canister.

3. PREPARATION OF CULTURE MEDIA AND REAGENTS

3.1 Sodium hydroxide, 1N - Dissolve 40 g of sodium hydroxide, NaOH, in 500 mL of distilled water. Dilute to 1 liter in a graduated cylinder.

3.2 Sodium thiosulfate, 10% - Dissolve 10 g of sodium thiosulfate in 100 mL of distilled water. This solution is only needed if samples contain chlorine.

3.3 Buffered Dilution Water

3.31 Stock phosphate buffer solution

3.311 Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in

500 mL distilled water.

3.312 Using a pH meter adjust this solution to pH 7.2 with 1N NaOH.

3.313 Dilute to 1 liter using a graduated cylinder.

3.32 Magnesium Chloride solution

3.321 Dissolve 81.1 g magnesium chloride hexahydrate, $MgCl_2 \cdot 6H_2O$, in distilled water and dilute to 1 liter.

3.33 Buffered dilution water. Add 1.25 mL of stock phosphate buffer solution and 5 mL of magnesium chloride solution to 1 liter of distilled water. This solution should be dispensed into milk dilution bottles and stoppered and must be sterilized before use.

3.4 Sodium hydroxide, NaOH 0.2 N. Dissolve 8 g of sodium hydroxide in 500 mL of distilled water; dilute to 1 liter with distilled water.

3.5 Rosolic Acid Solution, 1% - This solution is added to re-hydrated broth when background growth causes interference. If background growth is not a problem, broth may be used without the addition of rosolic acid.

3.51 Weigh 1 g of dehydrated rosolic acid and place in screw capped 250 mL Erlenmeyer flask containing 50 mL of 0.2 N sodium hydroxide solution; swirl to mix.

3.52 Add an additional 50 mL of 0.2 N sodium hydroxide solution and swirl again.

3.53 NOTE: Do not sterilize this solution. Refrigerate in the dark and discard after 2 weeks or sooner if color changes from dark red to muddy brown.

3.6 M-FC Broth – This may be prepared from dehydrated media as outlined below, or may be purchased in sterilized ampoules ready for use. Media in ampoules may be purchased with or without rosolic acid.

3.61 Place 3.7 g of dehydrated broth into a125 mL screw top Erlenmeyer flask containing 50 mL distilled water and swirl.

3.62 Add an additional 50 mL distilled water, rinsing the sides of the flask; mix by swirling.

3.63 Pipet 1 mL of the 1% rosolic acid solution into the flask and swirl.

3.64 Place the flask loosely covered into a boiling water bath, heat for ten minutes, remove and cool.

3.65 This media should be stored in a refrigerator and must be discarded after 96 hours.

4. SAMPLE COLLECTION

4.1 Appropriate containers and sampling procedures are outlined in the Bacterial Monitoring discussion of this manual.

5. PROCEDURE

5.1 Disinfect the lab bench surface by pouring a small amount of bleach on the bench and wiping with a damp sponge or cloth.

5.2 Set out 3 Petri dishes for each sample to be analyzed and label each according to origin of sample and the sample volume to be filtered.

(3 different volumes of each sample will be filtered, so that at least one of the volumes will result in a colony count on the dish which is within the accurate counting range. For fecal coliform, that range is 20 - 60. Sample volumes of 1 mL, 10 mL, and 100 mL are recommended for samples of secondary

effluent following disinfection, but sample volumes may be adjusted as necessary.)

5.3 Place a sterile absorbent pad in each Petri dish using sterile forceps.

5.31 The forceps are sterilized by storing the tip in about 1 inch of alcohol. The alcohol must be burned off the forceps before use by passing the tip through a flame.

5.4 Deliver approximately 2 mL of the broth solution onto the absorbent pad in each dish. The pad should be saturated, but should not have more than 1 drop in excess.

5.5 Assemble the sterilized filtration apparatus and place on the vacuum flask.

5.6 Connect the vacuum flask to vacuum pump.

5.7 Using the sterilized forceps, carefully place a membrane filter on the filter holder, grid-side up, centered over the porous part of the filter support plate. Place the funnel on the base.

5.8 With the vacuum off, pour about 20 mL of sterilized dilution water into the funnel.

5.9 Thoroughly mix the sample by shaking it vigorously about 25 times.

5.10 Dispense the appropriate volume of sample into the funnel.

5.101 Sample volumes of 1 - 20 mL may be pipetted using sterilized graduated pipets. Larger volumes may be measured using a sterilized 100 mL graduated cylinder.

5.102 Volumes less than 1 mL may be filtered by first diluting the sample with an appropriate amount of sterilized dilution water and taking a portion of this for analysis.

5.11 Swirl the contents of the funnel, turn on the vacuum, and filter the sample through the membrane.

5.12 After all of the sample has passed through the membrane, rinse the sides of the funnel with at least 20 mL of dilution water, swirling the funnel as the water passes through the filter. Repeat the rinse two more times.

5.13 Turn the vacuum off and remove the funnel from the filter base; place the funnel, inverted, on a sterile area.

5.14 Using the sterilized forceps, very carefully remove the membrane from the filter base and place it on the absorbent pad in the appropriate Petri dish. Be sure that no air bubbles have been trapped between the membrane and the absorbent pad.

5.15 Repeat steps 5.7 through 5.14 for each sample volume to be filtered.

5.16 Place the Petri dishes in an inverted position into the incubator within 15 minutes from the time of filtration.

5.161 If a water bath incubator is used, seal the Petri dishes into water-tight plastic bags, submerge in an inverted position, and anchor below water level.

5.17 Incubate the Petri dishes at a temperature of 44.5^{o}C $\pm$ 0.2^{o}C for 24 ($\pm$ 2) hours.

6. COUNTING COLONIES

6.1 After the 24 hour incubation time, the colonies on each Petri dish are counted. Only the blue colonies should be included in the count; a small number of cream colored colonies may be present, but these are not fecal coliform and should not be counted.

6.2 Although the colonies are large enough to be counted with the naked eye, the use of a low power binocular dissecting microscope with a fluorescent light source may be beneficial.

6.3 Record on the bench sheet the number of colonies counted for each Petri dish along with the volumes filtered.

7. CALCULATIONS

7.1 The calculated coliform density is reported in terms of fecal coliforms per 100 mL, determined by the number of blue colonies counted and sample volumes filtered.

7.2 Use Petri dishes with colony counts between 20 and 60 to calculate the reported value.

7.21 Calculate Colony Forming Units (CFU) per 100 mL sample.

$$\text{CFU/100 mL} = \frac{\text{\# colonies counted x 100}}{\text{sample volume filtered, mL}}$$

EXAMPLE:

1 mL	5 colonies
10 mL	36 colonies
100 mL	Too Numerous To Count (TNTC)

Since only the 10 mL portion resulted in a colony count between 20 and 60, the reported result would be calculated as:

$$\text{CFU/100 mL} = \frac{\text{36 colonies x 100}}{\text{10 mL}} = 360 / 100 \text{ mL}$$

7.22 If more than one Petri dish results in a count of between 20 and 60, calculate CFU per 100 mL for each and report the average of the results.

7.23 If none of the dishes are in the counting range, use the rules outlined in Chapter 233 of this manual, Bacti Counting and Reporting, to determine the value to report.

Counting and Reporting Bacterial Colonies*
Membrane Filtration Methods

Determine the total number of colonies on each Petri dish and record these on the laboratory bench sheet. Bacterial quantities are reported in terms of Colony Forming Units (CFU) per 100 mL sample. This value is calculated by considering the number of colonies counted on a Petri dish and the mL of sample filtered. Petri dishes with colony counts in the acceptable range should be used to determine the reported value. The acceptable range of colonies that are countable on a membrane is a function of the method. The acceptable counting range for fecal coliform is 20 to 60; the acceptable counting range for E. coli is 20 to 80. All of the examples presented here assume that the acceptable range of counts is 20 to 60 colonies per membrane. Instruction is also given in determining reported values when no Petri dish has the acceptable colony count.

Calculation of Results

Select the membrane filter with the number of colonies in the acceptable range and calculate Colony Forming Units (CFU) per 100 mL according to the general formula:

$$\text{CFU per 100 mL} = \frac{\text{No. of colonies counted}}{\text{mL sample filtered}} \times 100$$

Counts With-in the Acceptable Range

Example: Assume that filtration of volumes of 1 mL, 10 mL, and 100 mL produced colony counts of 5, 57, and 125, respectively.

Since only the 10 mL sample volume resulted in a count within the acceptable range, only that result is used in determining the reported value.

$$\text{CFU per 100 mL} = \frac{\text{57 colonies}}{\text{10 mL sample}} \times 100 = \text{570 CFU/100 mL}$$

More Than One Acceptable Count

If more than one sample volume yields membranes within the acceptable range of counts, independently carry counts to final reporting units, and take the average to determine the final reported value.

Example: Volumes of 1, 10, and 50 mL produced colony counts of 1, 20, and 59, respectively. Two volumes, 10 mL and 50 mL, produced colonies in the acceptable counting range.

Independently carry each MF count to CFU per 100 mL:

(20 / 10) × 100 = 200 CFU /100 mL and (59 / 50) × 100 = 118 CFU /100 mL

Calculate the arithmetic mean:

(200 CFU/100 mL + 118 CFU/100 mL) / 2 = 159 CFU/100 mL

Report this as 159 CFU/100 mL.

All Counts Below Acceptable Range, At Least One Has Countable Colonies

If all counts are below the lower acceptable count limit, select the most nearly acceptable count.

Example: Sample volumes of 1, 10, and 100 mL produced colony counts of 0, 1 and 17, respectively.

No colony count falls within recommended limits. Calculate on the basis of the most nearly acceptable plate count, 17, and report as 17 CFU/100 mL.

Note that in this case, because no calculations were done (i.e. this is the count for 100 mL), the count is recorded as 17 CFU/100 mL rather than an "estimated count of 17 CFU/100 mL." Report as 17 CFU / 100 mL on the daily Discharge Monitoring Report (DMR).

Second Example: Assume a count in which sample volumes of 1 and 10 mL produced colony counts of 0 and 18, respectively.

No colony count falls within recommended limits. Calculate on the basis of the most nearly acceptable plate count.

(18 / 10) × 100 = 180 CFU /100 mL

Record this as an "estimated" count of 180 CFU/100 mL on the bench sheet because a calculation was involved in determining the final value. Report as 180 CFU / 100 mL on the daily Discharge Monitoring Report (DMR).

Counts From All Membranes Are Zero

If counts from all membranes are zero, calculate using count from largest filtration volume.

Example: Sample volumes of 2, 10, and 25 mL produced colony counts of 0, 0, and 0. Calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume. In this example, the largest volume filtered was 25 mL and thus the calculation would be:

(1 / 25) × 100 = 4 CFU /100 mL

Report this as < (less than) 4 CFU/100 mL on the bench sheet and on the Daily DMR. Use 4 CFU/100 mL in calculating the 7-day and monthly geometric means for the Monthly DMR.

Counts From All Membranes Are Above the Upper Acceptable Limit, But At Least One Membrane Is Countable

If all membrane counts are above the upper acceptable limit, calculate count using the smallest volume filtered.

Example: Assume that the volumes 1, 10, and 100 mL produced colony counts of 110, 150, and Too Numerous To Count (TNTC), respectively. Since all colony counts are above the acceptable limit, use the colony count from the smallest sample volume filtered and estimate the count as:

(110 / 1) × 100 = 11,000 CFU /100 mL

Record this as “estimated” count 11,000 CFU/100 mL on the bench sheet, and report as 11,000 CFU/100 mL on the daily DMR.

Counts From All Membranes are Too Numerous To Count

If colonies on all membranes are too numerous to count (TNTC), use upper limit count with smallest filtration volume.

Example: Assume that the volumes 1, 10, and 100 mL all resulted in TNTC.

Use the upper acceptable count for the method (60 colonies in this example) as the basis of calculation with the smallest filtration volume and estimate the count as:

(60 / 1) × 100 = 6000 CFU /100 mL

Record as “TNTC” on the laboratory bench sheet. Report as > (greater than) 6000 CFU/100 mL on the Daily DMR, and use 6000 CFU/100 mL in calculating 7-day and monthly geometric means on the Monthly DMR.

Colonies Both Above And Below Acceptable Counting Limits

If colonies are both above and below the upper and lower acceptable limits (i.e., no counts are within the acceptable limits), select the most nearly acceptable count.

Example: Sample volumes of 1, 10 and 100 mL produced colony counts of 0, 8 and 64, respectively.

Here, no colony count falls within recommended limits. Calculate on the basis of the most nearly acceptable plate count, 64, and report as 64 CFU/100 mL.

Note that in this case, because no calculations were done (i.e. this is the count for 100 mL), the count is recorded and reported as 64 CFU/100 mL rather than an “estimated” count of 64 CFU/100 mL

Second Example: Assume a count in which sample volumes of 1, 10 and 100 mL produced colony counts of 0, 18, and 98, respectively.

No colony count falls within acceptable limits. Calculate on the basis of the most nearly acceptable plate count, 18.

$(18 / 10) \times 100 = 180$ CFU /100 mL

Record this on the bench sheet as “estimated” count 180 CFU/100 mL because a calculation was involved, and report as 180 CFU/100 mL on the daily DMR.

*This counting method was adapted from EPA Method 1603 for the determination of E. coli in ambient waters and disinfected wastewater, summarizing the counting rules given in EPA publication "Microbiological Methods for Monitoring the Environment" EPA-600/8-78-017, December 1978.

Determination of Fecal Coliform in Biosolids

The analysis of fecal coliform may be used to demonstrate pathogen reduction in biosolids that are to be land applied. Federal and State law classify biosolids as either Class A or Class B with respect to pathogen reduction. Class A biosolids must not exceed 1000 fecal coliform per gram of dry solids, while Class B requires that the geometric mean of 7 samples must not exceed 2 million fecal coliform per gram of dry solids.

Although procedures are available for preparation of solid samples, this procedure assumes that liquid samples will be analyzed. According to EPA Method 1681, liquid samples are generally defined as samples containing ≤ 7% total solids.

Samples of class B biosolids may be analyzed using either the membrane filtration method or the multiple tube fermentation method, both of which are included in this manual. Class A biosolids must be analyzed using the multiple tube fermentation method. The information presented here describes the sample preparation steps, and gives examples of the calculations involved prior to analysis by filtration or fermentation. The analyst must refer to either the filtration or fermentation procedure for detailed information regarding those procedures.

1. DETERMINATION OF TOTAL SOLIDS

Since results are reported in terms of Colony Forming Units (CFU) per gram of dry solids, or Most Probable Number (MPN) per gram of dry solids, the sample must be analyzed for percent total solids. This analysis may be performed using the Total and Volatile Sludge Solids procedure in this manual.

2. SAMPLE PREPARATION:

2.1 Use a sterile graduated cylinder to transfer 30.0 mL of well mixed sample to a sterile blender jar. Use 270 mL of sterile buffered dilution water to rinse any remaining sample from the cylinder into the blender. Cover and blend for two minutes on high speed. 1.0 mL of this mixture is equivalent to 0.1 mL of the original sample.

2.2 **Dilution A** - Use a sterile pipet to transfer 11.0 mL of the blended sample mixture to 99 mL of sterile buffered dilution water in a sterile screw cap bottle and mix by vigorously shaking the bottle a minimum of 25 times. This is dilution "A". 1.0 mL of this mixture is 0.010 mL of the original sample.

2.3 **Dilution B** - Use a sterile pipet to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "B". 1.0 mL of this mixture is 0.00010 mL of the original sample.

2.4 **Dilution C** - Use a sterile pipet to transfer 1.0 mL of dilution "B" to a sterile screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "C". 1.0 mL of this mixture is 0.0000010 mL of the original sample.

2.5 Although other dilution and inoculation schemes may be used, the first transfer from the "homogenized" sample should always be 11 mL of homogenized sample to 99 mL dilution water or 10 mL of homogenized sample to 90 mL dilution water. This will ensure that a sufficient amount of the original biosolids sample is transferred at the beginning of the dilution scheme.

3. **Membrane Filtration Procedure**
Chapter 235, Fecal Coliform by Membrane Filtration

3.1 At least three portions of each sample should be filtered. Typically this includes 10.0 mL of dilution C, corresponding to 0.000010 mL of original sample, 1.0 mL of dilution B, corresponding to 0.00010 mL of original sample, and 10 mL of dilution B, corresponding to 0.0010 mL of original sample.

3.2 Incubate samples and count the number of colonies as directed in the procedure.

3.3 This dilution scheme may be modified as needed to obtain filters that yield between 20 and 60 Colony Forming Units (CFU)

3.4 Calculate the reported results from filters with counts in the 20 – 60 range.

$$\text{CFU / gram} = \frac{\text{colonies counted} \times 100}{\text{mL sample filtered} \times \text{\% dry solids}}$$

3.5 EXAMPLE:

A biosolids sample was analyzed for fecal coliform with the following results:

% Solids	mL Sample	Colonies Counted
3.8	0.000010 mL	0
	0.00010 mL	1
	0.0010 mL	23

$$\frac{23 \text{ colonies} \times 100}{0.0010 \text{ mL} \times 3.8} = 600{,}000 \text{ CFU per 100 mL}$$

3.6 If no Petri dishes result in a count between 20 and 60, refer to the Bacti Counting and Reporting chapter of this manual.

3.7 Report the geometric mean of at least seven samples analyzed.

4. Multiple Tube Fermentation Procedure

Chapter 237, Fecal Coliform by Multiple Tube Fermentation, A-1 medium

4.1 Four series of five tubes are inoculated with the prepared sample.

4.11 Inoculate the first series of 5 tubes each with 10.0 mL of dilution B. This is equivalent to 0.0010 mL of the original sample.

4.12 Inoculate the second series of 5 tubes each with 1.0 mL of dilution B. This is equivalent to 0.00010 mL of the original sample.

4.13 Inoculate the third series of 5 tubes each with 10.0 mL of dilution C. This is equivalent to 0.000010 mL of original sample.

4.14 Inoculate the fourth series of 5 tubes each with 1.0 mL of dilution C. This is equivalent to 0.000001.0 mL of original sample.

4.2 Incubate the tubes as required in the procedure. Check for gas production after a total of 24 hour incubation.

4.3 Calculate the Most Probable Number (MPN) per gram dry solids.

4.31 Only 3 of the 4 series of tubes inoculated will be used to determine MPN. Choose the highest dilution (lowest sample volume) that gives positive results in all five tubes, and the next two higher dilutions to determine MPN.

4.32 Calculate MPN / gram using the following equation:

$$\text{MPN / gram} = \frac{\text{10 X MPN Index / 100 mL}}{\text{Largest volume planted X \% dry solids}}$$

4.33 EXAMPLE:

Four series of 5 tubes was inoculated with a sample of biosolids. The solids concentration of the original sample was determined to be 4.0 %. The following results were obtained:

mL	Number of Positive Tubes of the 5 Planted
0.0010	5
0.00010	5
0.000010	3
0.0000010	0

Since the highest dilution resulting in all positive tubes was 0.00010 mL, use the combination 5,3,0 to determine the MPN index. The MPN table on page 237-5 indicates an MPN index of 79 for that combination.

$$\text{MPN / gram} = \frac{10 \times 80}{0.00010 \times 4.0} = 2{,}000{,}000$$

Report 2,000,000 MPN / gram for that sample

FECAL COLIFORM

Multiple Tube Fermentation Method
Direct Test, A-1 Medium

DISCUSSION: The multiple-tube fermentation, or most probable number (MPN) method, determines the presence and number of coliform bacteria through the planting of a series of measured sample portions into tubes containing favorable culture media. The A-1 medium may be used for the direct isolation of fecal coliforms from water. Prior enrichment in a presumptive medium is not required. The MPN value is determined by referring to a table of Most Probable Numbers.

Wastewater testing for reporting purposes involves the planting of five 10 mL portions, five 1 mL portions and five 0.1 mL portions; this provides an analytical range of 2 to 1600 fecal coliform bacteria per 100 mL. This range may be extended by diluting the sample and planting five 1.0 mL, five 0.1 mL, and five 0.01 mL sample portions.

Quantitative results can be achieved only when the sample-planting volumes are selected so that positive results are obtained from some sample portions and negative results are obtained from others in a series of tubes of culture medium planted with measured sample volumes.

REFERENCE:

This procedure conforms to the EPA approved procedure included in the 20th Edition of Standard Methods, 9221 C E.

1. APPARATUS

1.1 Autoclave.

1.2 Incubator maintained at 35 ± 0.5°C.

1.3 Water bath incubator maintained at 44.5 ± 0.2°C.

1.4 Microbiological pipets - pre-sterilized disposable, graduated with cotton mouth plug or glass serological pipets with large tip opening (volumes 10 mL and 1 mL subdivided to 1/10 mL).

1.5 Culture tubes containing inverted fermentation vials, 20 x 150 mm tubes with 10 x 75 mm vials to contain 10 mL portions of culture media, with metal or heat resistant plastic caps.

2. CULTURE MEDIA AND SOLUTIONS

2.1 DIFCO, Merck, Cat. No. A-1 Medium, 1823 or equivalent

2.11 Directions for preparation from dehydrated product

2.111. Suspend 31.5 g of the powder in 1 L of distilled or deionized water. Mix thoroughly.

2.112. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

2.113. Dispense into tubes containing inverted fermentation vials. Add enough broth to cover inverted vials after sterilization (typically 10 mL of broth is adequate). Place caps on tubes.

2.114. Autoclave at 121°C for 10 minutes. Assure that inverted vials are completely filled with media after autoclaving.

2.115 Store tubes in the dark at room temperature for not longer than 7 days. Ignore the formation of precipitate.

2.12 For 10 mL samples, prepare double-strength (31.5 g / 500 mL) medium to ensure ingredient concentrations are not reduced below those of the standard medium.

3. PROCEDURE

3.1 Inoculate tubes of A-1 Medium with sample.

3.11 Mix sample by shaking at least 25 times before sample portion is withdrawn.

3.12 Using a sterilized graduated pipet, transfer the appropriate amount of sample into each tube. Add 10 mL sample to each of 5 tubes, 1 mL sample to each of 5 tubes, and 0.1 mL sample to each of 5 tubes. Be sure the keep the sample well mixed during this step.

3.2 Incubate the tubes at 35 ± 0.5°C for 3 hours.

3.3 Transfer the tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hours. Maintain water level in bath above level of liquid in inoculated tubes.

3.4 Remove the tubes from the incubator and check for gas production. Gas that collects in the inverted vial, or dissolved gas that forms fine bubbles when slightly agitated, is a positive reaction indicating the presence of fecal coliforms.

3.5 Record results as number of positive 10 mL tubes, positive 1 mL tubes, and positive 0.1 mL tubes.

3.6 Calculate fecal coliform densities using MPN tables on the following pages.

4. CALCULATIONS:

4.1 The MPN table lists MPN values for combinations of positive and negative results when five 10 mL, five 1.0 mL, and five 0.1 mL sample portions are tested.

4.2 If the sample volumes used are those found in the table, report the value corresponding to the MPN / 100 mL.

4.3 If the series of sample volumes tested is different than the MPN table, select the MPN value from the table and calculate using the following formula:

$$\text{MPN / 100 mL} = \frac{\text{Table MPN X 10}}{\text{Largest sample volume used, mL}}$$

Example: Suppose five 10 mL, five 1.0 mL and five 0.1 mL portions of a sample were analyzed for fecal coliform. Three of the 10 mL portions, two of the 1.0 mL portions, and one of the 0.1 mL portions resulted in positive tests. Calculate the reported result.

Solution: Express the number of positive confirmed tubes as a series, beginning with the highest volume used; in this case 3, 2, 1.

Find the MPN Index that corresponds with this series in the MPN table, in this case the MPN index is 17. This value is reported as 17 bacteria per 100 mL of sample.

4.4 Not all possible combinations are included in the MPN table. If a combination is encountered that is not included in the table, the “Thomas Simple Formula” may be used to calculate the MPN Index:

$$\textbf{MPNi} = \frac{\textbf{No. Positive Tubes X 100}}{\sqrt{\textbf{mL in Neg Tubes X mL in All Tubes}}}$$

MPN for Five 10-mL, Five 1-mL, and Five 0.1-mL Tubes Planted

No. of Tubes Giving Positive Reaction out of:			
Five 10-mL Portions	Five 1-mL Portions	Five 0.1-mL Portions	MPN Index
0	0	1	2
0	0	2	4
0	1	0	2
0	1	1	4
0	1	2	6
0	2	0	4
0	2	1	6
0	3	0	6
1	0	0	2
1	0	1	4
1	0	2	6
1	0	3	8
1	1	0	4
1	1	1	6
1	1	2	8
1	2	0	6
1	2	1	8
1	2	2	10
1	3	0	8
1	3	1	10
1	4	0	11
2	0	0	5
2	0	1	7
2	0	2	9
2	0	3	12
2	1	0	7
2	1	1	9
2	1	2	12
2	2	0	9
2	2	1	12
2	2	2	14
2	3	0	12
2	3	1	14
2	4	0	15
3	0	0	8
3	0	1	11
3	0	2	13
3	1	0	11
3	1	1	14
3	1	2	17
3	1	3	20
3	2	0	14
3	2	1	17
3	2	2	20
3	3	0	17
3	3	1	21
3	4	0	21
3	4	1	24
3	5	0	25

No. of Tubes Giving Positive Reaction out of:			
Five 10-mL Portions	Five 1-mL Portions	Five 0.1-mL Portions	MPN Index
4	0	0	13
4	0	1	17
4	0	2	21
4	0	3	25
4	1	0	17
4	1	1	21
4	1	2	26
4	2	0	22
4	2	1	26
4	2	2	32
4	3	0	27
4	3	1	33
4	3	2	39
4	4	0	34
4	4	1	40
4	5	0	41
4	5	1	48
5	0	0	23
5	0	1	31
5	0	2	43
5	0	3	58
5	0	4	76
5	1	0	33
5	1	1	46
5	1	2	63
5	1	3	84
5	2	0	49
5	2	1	70
5	2	2	94
5	2	3	120
5	2	4	148
5	2	5	177
5	3	0	79
5	3	1	110
5	3	2	140
5	3	3	180
5	3	4	212
5	3	5	253
5	4	0	130
5	4	1	170
5	4	2	220
5	4	3	280
5	4	4	345
5	4	5	426
5	5	0	240
5	5	1	350
5	5	2	540
5	5	3	920
5	5	4	1600
5	5	5	>1600

ANALYSIS OF WASTEWATER AND AMBIENT WATER FOR *E. coli*

*Escherichia coli (E. col*i) is a bacterium that is a natural inhabitant only of the intestinal tract of warm-blooded animals. Because of this, its presence in water samples is an indication of fecal pollution and the possible presence of enteric (intestinal) pathogens.

Tests for *E. coli* are often used as a measure of ambient (recreational) water quality. The significance of finding *E. coli* in recreational water samples is the relationship that has been demonstrated between the density of *E. coli* and the risk of gastrointestinal illness associated with swimming in the water.

The USEPA has approved several methods for the determination of E. coli in ambient waters; some of those have also been approved for testing wastewater and biosolids. This includes both MPN as well as membrane filtration methods. Those developed by EPA include membrane filtration methods 1603 and 1103.1, both approved for testing ambient water, while 1603 may also be used for testing wastewater and biosolids. It should be recognized that these methods developed by EPA are extensive, including over 40 pages for each. Much of that information deals with quality assurance practices that must be adhered to in order to use those methods.

The EPA has also approved E. coli methods patented by private companies. Hach Chemical Company has received approval for the mColiBlue-24® membrane filtration method for testing ambient water, wastewater, and biosolids. IDEXX Laboratories, Inc. has received EPA approval for testing E. coli in ambient water, wastewater, and biosolids using their Colilert ®, Colilert-18 ®, and Quant-Tray® multi-well system. The analyst would be wise to consider these options when deciding upon a method for E. coli determination.

The method included in this manual is an adaptation of Standard Methods 9213 D, a membrane filtration method using MTEC media and Urea substrate and two step incubation. This method is EPA approved for testing ambient waters, but not wastewater or biosolids.

EPA Approved E. coli Methods for Ambient Water

Standard Methods	
9221 B.1/9221 F	multiple tube fermentation, presumptive followed by confirmed test using EC-MUG Medium
9222 B / 9222 G	membrane filtration, total or fecal coliform followed by confirmation using EC-MUG or Nutrient Agar-MUG.
9213 D	membrane filtration, mTEC agar, followed by Urea Substrate (2 step incubation)
USEPA	www.epa.gov/waterscience/methods/method/biological/
1103.1	membrane filtration, mTEC agar, followed by Urea Substrate (2 step incubation)
1603	membrane filtration, modified mTEC (2 step incubation)
1604	membrane filtration, MI agar or MI broth and TSA
Hach Chemical Co.	http://www.hach.com/
mColiBlue-24®	membrane filtration, incubation at 35°C for 24 hrs
IDEXX Laboratories, Inc.	http://www.idexx.com/water/
Colilert®	mpn, multi-well, 24 hour incubation, MUG fluorescent
Colilert-18®	mpn, multi-well, 18 hour incubation, MUG fluorescent

EPA Approved E. coli Methods for Wastewater and Biosolids

USEPA	www.epa.gov/waterscience/methods/method/biological/
1603	membrane filtration, modified mTEC (2 step incubation)
Hach Chemical Co.	http://www.hach.com/
mColiBlue-24®	membrane filtration, incubation at 35°C for 24 hrs
IDEXX Laboratories, Inc.	http://www.idexx.com/water/
Colilert®	MPN, multi-well, 24 hour incubation, MUG fluorescent
Colilert-18®	MPN, multi-well, 18 hour incubation, MUG fluorescent

MEMBRANE FILTER METHOD FOR *E. coli*

This method describes a membrane filter (MF) procedure for the detection and enumeration of *Escherichia coli (E. col*i) in ambient waters. Since a wide range of sample volumes or dilutions thereof can be analyzed by the MF technique, a wide range of *E. coli* levels in water can be detected and enumerated.

The MF method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter. A water sample is filtered through the membrane which retains the bacteria. After filtration, the membrane containing the bacterial cells is placed on a selective medium, m-TEC, incubated at 35 °C for 2 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5 °C for 22 hours. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 min, yellow or yellow-brown colonies are counted with the aid of a fluorescent lamp and a magnifying lens. In this method, *E. coli* are those bacteria which produce yellow or yellow-brown colonies on a filter pad saturated with urea substrate broth after primary culturing on m-TEC medium.

REFERENCE

This method conforms to the EPA approved method for analysis of E. coli in ambient water in Standard Methods, 20th edition, 9213 D.

1. Apparatus and Equipment

1.1 Pipet container, stainless steel, aluminum, or borosilicate glass, for glass pipets.

1.2 Pipets, 10 mL, sterile, bacteriological or Mohr, glass or plastic.

1.3 Graduated cylinders, 100 mL, covered with aluminum foil or kraft paper and sterilized.

1.4 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel, wrapped with aluminum foil or kraft paper and sterilized.

1.5 Vacuum source.

1.6 Flask, filter vacuum, usually 1 L, with appropriate tubing. A filter manifold to hold a number of filter bases is optional.

1.7 Forceps, straight or curved, with smooth tips to handle filters without damage.

1.8 Ethanol, methanol or isopropanol in a small, wide-mouth container, for flame-sterilizing forceps.

1.9 Bunsen Burner, for sterilizing forceps.

1.10 Petri dishes, sterile, plastic, 50 × 12 mm, with tight-fitting lids.

1.11 Membrane filters, sterile, white grid marked, 47 mm diameter, with 0.45 ± 0.02 µm pore size.

1.12 Absorbent pads, sterile, 47 mm diameter.

1.13 Incubator maintained at 35 ± 0.5 °C.

1.14 Water bath incubator maintained at 44.5 ± 0.2 °C.

2. Reagents and Materials

2.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. The agar used in preparation of culture media must be of microbiological grade.

2.2 Purity of Water: Reagent water conforming to ASTM Type II water.

2.3 Buffered Dilution Water

2.31 Stock phosphate buffer solution

2.311 Dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 mL distilled water.

2.312 Using a pH meter adjust this solution to pH 7.2 with 1N NaOH.

2.313 Dilute to 1 liter using a graduated cylinder.

2.32 Magnesium Chloride solution

3.321 Dissolve 81.1 g magnesium chloride hexahydrate, $MgCl_2 \cdot 6H_2O$, in distilled water and dilute to 1 liter.

2.33 Buffered dilution water. Add 1.25 mL of stock phosphate buffer solution and 5 mL of magnesium chloride solution to 1 liter of distilled water. This solution should be dispensed into milk dilution bottles and stoppered and must be sterilized before use.

2.4 m-TEC Agar (Difco 0334-15-0)

2.41 Add 45.26 g of M-TEC medium to 1 L of reagent water in a flask and heat to boiling, until ingredients dissolve.

2.42 Autoclave at 121° C (15 psi) for 15 minutes and cool in a 44 -46 °C water bath.

2.43 Pour the medium into each 50 × 10 mm culture dish to a 4-5 mm depth (approximately 4-6 mL) and allow to solidify. Final pH should be 7.3 ± 0.2. Store in a refrigerator.

2.5 Urea Substrate

2.51 Add 2.0 grams Urea and 0.01 grams Phenol Red to 100 mL reagent grade water and stir to dissolve.

2.52 Adjust to pH between 3 and 4 with a few drops of 1N HCl. The substrate solution should be a straw-yellow color at this pH.

2.53 Store in refrigerator at 2 to 8 ^{o}C. Use within 1 week.

3. Sample Preservation and Holding Times

Adherence to sample preservation procedures and holding time limits is critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

3.1 Storage Temperature and Handling Conditions

Ice or refrigerate water samples at a temperature < 10 ° C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water during transit or storage.

3.2 Holding Time Limitations

Sample analysis should begin immediately, preferably within 2 hours of collection. The maximum transport time to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory.

4. Calibration and Standardization

4.1 Check temperatures in incubators daily to insure operation within stated limits.

4.2 Check thermometers at least annually against an NIST certified thermometer or one traceable to NIST. Check mercury columns for breaks.

5. Procedure

5.1 Set out 3 petri dishes containing the m-TEC agar for each sample to be analyzed.

5.2 Mark the petri dishes and report forms with sample identification and sample volumes to be filtered.

5.3 Place a sterile membrane filter on the filter base, grid-side up and attach the funnel to the base; the membrane filter is now held between the funnel and the base.

5.4 Before filtering sample volumes less than 10 mL, add approximately 20-30 mL dilution water to the filtration funnel.

5.5 Shake the sample bottle vigorously about 25 times to distribute the bacteria uniformly, and measure the desired volume of sample or dilution into the funnel.

5.6 Select sample volumes based on previous knowledge of pollution level, to produce 20-80 *E. coli* colonies on the membranes. Sample volumes of 1 mL, 10 mL and 100 mL are normally tested. Smaller sample size or sample dilutions can be used to minimize the interference of turbidity or high bacterial densities. Multiple volumes of the same sample dilution may

be filtered and the results combined.

5.7 Swirl the contents of the filtration funnel and filter the sample. Add another 20-30 mL portion of rinse water, swirl, and filter the contents of the funnel. Repeat the rinse step a second time.

5.8 Turn off the vacuum and remove the funnel from the filter base. Use sterile forceps to aseptically remove the membrane filter from the filter base and roll it onto the M-TEC agar to avoid the formation of bubbles between the membrane and the agar surface. Reseat the membrane, if bubbles occur. Close the dish, invert, and incubate at 35° C for 2 h.

5.9 After 2 h incubation at 35° C, transfer the plates to Whirl-Pak bags, seal, and place inverted in a 44.5° C water bath for 22-24 h.

5.10 After 22-24 h, remove the dishes from the water bath. Place absorbent pads in new petri dishes or the lids of the same petri dishes, and saturate with urea broth. Aseptically transfer the membranes to absorbent pads saturated with urea substrate and hold at room temperature.

5.11 After 15-20 min. incubation on the urea substrate at room temperature, count and record the number of yellow or yellow-brown colonies on those membrane filters ideally containing 20-80 colonies.

6. Calculation and Reporting of Results

6.1 Select the membrane filter with the number of colonies within the acceptable range (20-80) and calculate the count per 100 mL according to the general formula:

$$\text{E. coli/100 mL} = \frac{\text{No. E. coli Colonies Counted} \times 100\text{ mL}}{\text{Volume in mL of Sample Filtered}}$$

6.2 Report the results as *E. coli* per 100 mL of sample.

6.3 Refer to Chapter 233 of this manual, Bacti Counting and Reporting, for rules regarding reporting sample results when membrane filters do not produce colony counts in the 20 to 80 range.

7. Verification Procedure

7.1 Verify a portion of the yellow and yellow-brown colonies with a commercial multi-test system (fermentation of lactose with gas production).

GEOMETRIC MEAN

Results of daily coliform analyses for the monthly operating reports are required to be reported based on a geometric mean, rather than simply taking the average. This is true of the monthly average as well as the 7 day average. This is done because of the possibility of data which may vary over a wide range, and actually will be to the benefit of the reporting facility.

The geometric mean may be most easily found with the use of a calculator which includes log functions, but may also be calculated using a logarithm table. In either case, the same three basic steps are involved:

1. Find the logarithm of the #/100 ml recorded for each day.
2. Calculate the average log by totaling the individual logs and dividing by the number of entries.
3. Find the anti-log of the average. This number is the geometric mean for that data.

Included below is a brief review of how to use a log table to find the geometric mean of a series of number.

STEP 1: Find the log of each number.

The log of a number is composed of two parts. The first part of the log, called the characteristic, is found by taking the number of digits to the left of the decimal point and subtracting 1.

EXAMPLE:

Data	# Digits Left Of Decimal	Characteristic
120	3	2
1520	4	3
5	1	0

The second part of the log is called the **mantissa**, and is found in the log table. The attached log table is a 3-place table, meaning that only the first 3 digits of the number will be used in determining the mantissa. Since the mantissa for a number is not affected by the location of the decimal point, we can use this log table by changing our data to 3-digit numbers (round off or add zeros if necessary). Then find the first two digits of the number in the left hand column of the table and follow that row to the right to the column headed by the third digit of the number. This will be the mantissa and always follows a decimal point in the log.

EXAMPLE:

Data	3 Digit Number	Mantissa
120	120	.0792
1520	152	.1818
5	500	.6990

The log for each number is the characteristic plus the mantissa.

EXAMPLE:

Data	Characteristic	Mantissa	Log
120	2	.0792	2.0792
1520	3	.1818	3.1818
5	0	.6990	0.6990

STEP 2: Average the Logs.

EXAMPLE:

$$\begin{array}{r} 2.0792 \\ 3.1818 \\ \underline{0.6990} \\ 5.9600 \end{array} \qquad \frac{5.9600}{3} = 1.9867$$

This average is the logarithm of the geometric mean for that data.

STEP 3: Finding the anti-log of the average.

Now we must determine what number corresponds to the average log by taking the anti-log. The anti-log is simply the log process in reverse. Search the mantissas in the log table to find the one that most closely matches the mantissa of the average log, and determine what 3 digit number corresponds to that mantissa. Add 1 to the characteristic of the average log and place the decimal point such that there are this number of digits to the left (add zeros if necessary).

EXAMPLE:	Avg. Log	Corr. 3 Digit #	Anti-Log
	1.9867	970	97.0

Therefore, 97.0 is the geometric mean for the data.

TABLE OF LOGARITHMS

No.	0	1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396
No.	0	1	2	3	4	5	6	7	8	9

No.	0	1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996
No.	0	1	2	3	4	5	6	7	8	9

CHLORINE RESIDUAL

Disinfection of wastewater treatment plant effluent is necessary to protect drinking water supplies, as well as to assure the safety of recreational waters, and to protect aquatic organisms. Microorganisms are present in large numbers in wastewater treatment plant effluents, and waterborne disease outbreaks have been associated with sewage-contaminated water supplies or recreational waters.

Although the use of ultraviolet radiation has increased in recent years, chlorination is still the most common method of wastewater disinfection, and has been used worldwide for over a century. Chlorine is known to be effective in destroying a variety of bacteria, viruses and protozoa, including Salmonella, Shigella and Vibrio cholera. Chlorine is a powerful oxidizing agent, and destroys target organisms by oxidizing cellular material.

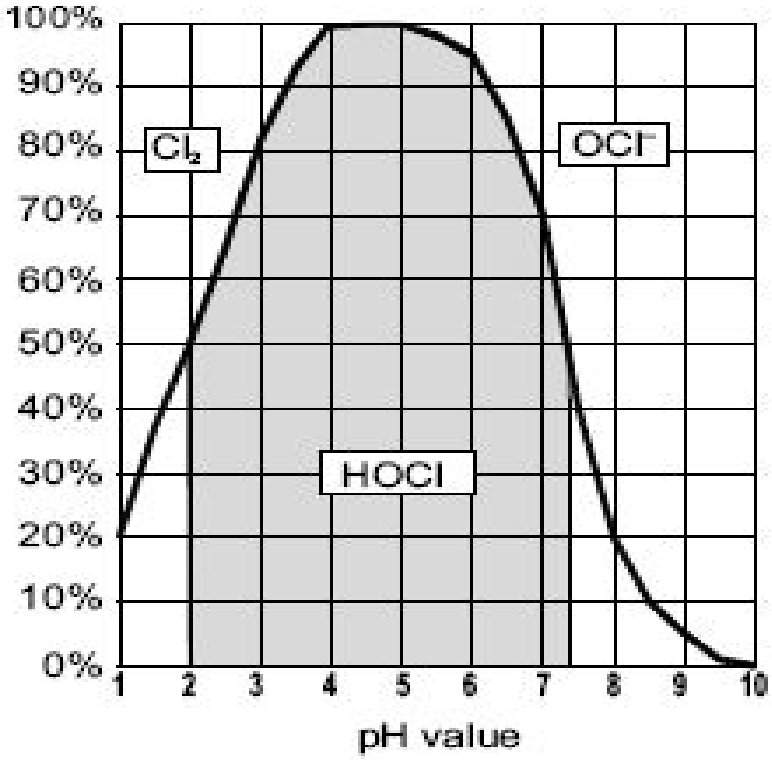
Chlorine can be used in wastewater disinfection as a gas, liquid sodium hypochlorite solution or solid calcium hypochlorite. Where a large amount of chlorine is needed for disinfection, chlorine gas is preferred over sodium hypochlorite due to cost considerations. Mainly due to safety concerns however, the use of sodium hypochlorite solution is increasing, especially in small to mid-size facilities. Solid calcium hypochlorite is usually limited to small flow situations, where close control of the chlorination process is not required.

Regardless the form of chlorine used, the operator must have an understanding of the dangers of using chlorine, and the safety precautions that are necessary to protect workers in the facility, the public and the environment. Although a thorough discussion of chlorine safety is not included here, a wealth of information may be obtained by searching the internet. Also, several publications are available that outline

Cl_2

handling precautions, recommendations and safety practices. These include the Chlorine Institute's "The Chlorine Manual" and the American Water Works Association's "Safety Practice for Water Utilities."

When chlorine is added to water it takes on various forms depending on the pH of the wastewater. It is important to understand the forms of chlorine which are present because each has a different disinfecting capability. Sodium hypochlorite or chlorine gas added to the effluent produces hypochlorus acid (HOCl), hypochlorite ion (OCl^-), and if gas is used, a small amount of dissolved gas (Cl_2). A measurement of the free available chlorine includes the total of these three chemical species. The pH of the water determines the relative amounts of hypochlorus acid and hypochlorite ion that are formed.



Cl_2

At a pH of 7.3 there are roughly equal amounts of HOCl and OCL^-. At pH less than 7.3, HOCl is favored; at pH greater than that, OCl^- is favored. The importance being that HOCl is about 100 times more powerful as an oxidant and disinfectant than is the hypochlorite ion. Consequently, free chlorine is most effective when the pH of the effluent is below 7 where HOCl is the predominant form.

$$Cl_2 + H_2O \longrightarrow HOCl + HCl$$

$$HOCl \longleftrightarrow H^+ + OCl^-$$

Free chlorine reacts readily with ammonia in wastewater to form chloramines. One of three types of chloramines will be formed in this reaction, depending on the pH, temperature, and reaction time. Monochloramine and dichloramine are formed in the pH range of 4.5 to 8.5, however monochloramine is most common when the pH is above 8, and is the most effective disinfectant of the chloramines. When the pH of the wastewater is below 4.5, the most common form of chloramine is trichloramine, a less effective disinfectant. The equations for the formation of the different chloramines are as follows:

Monochloramine: $NH_3 + HOCl \longrightarrow NH_2Cl + H_2O$

Dichloramine: $NH_2Cl + 2HOCl \longrightarrow NHCl_2 + 2H_2O$

Trichloramine: $NHCl_2 + 3HOCl \longrightarrow NHCl_3 + 3H_2O$

These compounds are available for disinfection and are termed combined available chlorine. Chloramines are weaker disinfectants than free chlorine but are more stable.

Cl_2

The germicidal strength of different forms of chlorine in water is ranked as follows:

HOCl > OCl^- > inorganic chloramines > organic chloramines

The amount of chlorine added for disinfection is referred to as the **chlorine dosage,** usually expressed as a concentration in milligrams per liter (mg/L). Because of its reactive nature, free chlorine as well as combined available chlorine, will react with a number of reduced compounds in wastewater, including sulfide, ferrous iron, and organic matter. These reactions result in the formation of many compounds such as chloro-organics, and chloride, which are not effective as disinfectants. The amount of chlorine that is used up through these competing reactions in the wastewater, and is no longer available for disinfection; is termed the **chlorine demand**. The amount of chlorine remaining after the demand has been satisfied, and is available for disinfection is called the **chlorine residual**. This relationship is represented as:

Chlorine Dosage – Chlorine Demand = Chlorine Residual

Total available residual chlorine is a combination of the free and combined available residual chlorine; this is the type of chlorine most commonly tested for in wastewater plant effluents. So chlorine that remains after the demand has been satisfied can be classified into three types (1) **free available residual chlorine** (hypochlorus acid or hypochlorite ion), (2) c**ombined available residual chlorine** (chloramines) and (3) **total available residual chlorine** (the sum of 1 & 2).

The total available residual chlorine (TRC), therefore, includes all forms of chlorine that are available for disinfection. So while the TRC analyzed value can remain the same, the ratio of all the chlorine compounds that make up this value can vary

Cl_2

widely, depending largely on pH. If the effluent pH changes as a result of a process change or from an industrial discharge, the disinfection ability of chlorination can change even though the TRC concentration hasn't changed. This helps to explain why fecal coliform analyses may indicate a change in the effectiveness of disinfection, even though the measured TRC has remained the same.

The required degree of disinfection can be achieved by applying a sufficient chlorine dosage, providing that adequate contact time, and good distribution of the disinfectant is available. The contact chamber should be designed to prevent dead flow areas and be baffled to minimize short-circuiting. Appropriate design allows for adequate contact time between the microorganisms to be destroyed and a minimal chlorine concentration for a specific period of time.

The chlorine dosage required will vary based on chlorine demand, wastewater characteristics, and discharge requirements. The required dosage usually ranges from 5 to 20 mg/L, depending largely on the degree of treatment that the wastewater has received prior to disinfection.

While the level of chlorination must be adequate to kill the pathogenic bacteria, the operator must realize that chlorine is also toxic to fish and other aquatic life. Concentrations of less than 1.0 mg/L of free chlorine and of less than 0.1 mg/L of chloramines for time periods as short as one hour have been found to kill trout and other game fish. Because of this, wastewater treatment facilities using chlorine for disinfection usually have a discharge permit limit for chlorine, and are therefore required to dechlorinate following the disinfection process. Dechlorination is the process of

Cl_2

removing the free and combined chlorine residuals to reduce toxicity after chlorination and before discharge. Sulfur dioxide gas, and sodium bisulfite or sodium metabisulfite solutions are commonly used dechlorinating chemicals. Activated carbon has also been used in a few facilities. Through dechlorination, the total chlorine residual can be reduced to a level that is not toxic to aquatic life.

Several methods are approved by the EPA for analysis of chlorine residual in wastewater effluents. These include the iodometric, amperometric, DPD-FAS, DPD spectrophotometric, and the electrode methods. Only the electrode method is included in this manual; that is the method most often used in Michigan wastewater treatment plant laboratories. It is fairly rapid, and is able to analyze at the low concentrations required in NPDES permits.

CHLORINE RESIDUAL PROCEDURE
ION SELECTIVE ELECTRODE METHOD

The procedure described below may be used with a direct-reading selective ion meter or with an expanded-scale pH / millivolt meter with 0.1 mV readability. This procedure assumes that the meter will be calibrated to indicate concentration directly. Alternately, a calibration curve may be prepared by plotting the millivolt readings versus concentration for standards on semi-logarithmic paper. Consult the manufacturer's literature for specific information dealing with calibration and operation of these meters.

REFERENCE

This procedure conforms to the EPA approved method referenced as Orion Research Instruction Manual, Residual Chlorine Electrode Model 97-70, 1977, Orion Research Incorporated, 840 Memorial Drive, Cambridge, MA 02138.

1. APPARATUS

   1.1 Specific ion meter – Use either an expanded-scale pH / millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

   1.2 Electrodes – Either a combination electrode consisting of a platinum electrode and an iodide ion selective electrode, or two individual electrodes may be used.

   1.3 Storage bottles - five, 4-oz. amber glass, wide mouth with screw-on caps.

   1.4 Volumetric flasks – five, 100 mL flasks with stoppers.

2. REAGENTS

   2.1 Chlorine-demand-free water

      2.11 Prepare chlorine-demand-free water from good quality distilled or

deionized water by adding sufficient chlorine to give 5 mg/L free chlorine. After standing 2 days this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better quality water.

2.12 Remove remaining free chlorine by placing container in sunlight or irradiating with an ultraviolet lamp. After several hours take a portion of the water, add KI, and measure total chlorine with a colorimetric method using a nessler tube to increase sensitivity. Do not use before the last trace of free and combined chlorine has been removed.

2.2 Residual chlorine standard, (Orion 977007 or equivalent)

2.21 Dissolve 0.1002 g potassium iodate, KIO_3, in chlorine-demand-free distilled water and dilute to 1000 ml. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1 mg/L as Cl_2.

2.3 Acid reagent, pH 4.0 (Orion 977011 or equivalent)

2.31 Dissolve 146 g anhydrous $NaC_2H_3O_2$ or 243 g $NaC_2H_3O_2 \bullet 3H_2O$ in 400 mL distilled water.

2.32 Add 480 g conc. acetic acid, and dilute to 1000 mL with chlorine-demand-free water.

2.4 Iodide reagent (Orion 977010 or equivalent)

2.41 Dissolve 42 g KI and 0.2 g Na_2CO_3 in 500 mL chlorine-demand-free distilled water. Store in a dark bottle.

3. PROCEDURE

3.1 Preparation of 0.2 mg/L, 1.0 mg/L, and 5.0 mg/L standard solutions.

3.1.1 Pipet 0.2 mL, 1.0 mL, and 5.0 mL of the chlorine standard into three

100 mL volumetric flasks.

3.1.2 Pipet 1.0 mL potassium iodide reagent and 1 mL acid reagent into each 100 mL volumetric flask. Do not add water. Swirl to mix and let stand for 2 minutes.

3.1.3 Dilute each to 100 mL with distilled water, mix, and pour into an amber glass bottle.

3.1.4 These standard solutions must be made fresh daily and the bottles tightly capped between uses.

3.2 Preparation of reagent blank (for sample measurements below 0.2 mg/L)

3.2.1 Pipet 1.0 mL potassium iodide reagent and 1 mL acid reagent into a 100 mL volumetric flask. Do not add water. Swirl to mix and let stand for 2 minutes.

3.2.2 Dilute to100 mL with distilled water, mix, and pour into an amber glass bottle.

3.2.3 The reagent blank must be made fresh daily and the bottle tightly capped between uses.

3.3 Meter Calibration

3.3.1 Place the electrode into the amber bottle containing the 0.2 mg/L standard, wait for a stable reading, and calibrate the meter to 0.2 mg/L. Do not stir during calibration or sample measurement.

3.3.2 Remove the electrode from the standard solution, rinse with distilled water, and blot dry.

3.3.3 Place the electrode into the amber bottle containing the 1.0 mg/l

standard, wait for a stable reading and calibrate the meter to 1.0 mg/L.

3.3.4 Place the electrode into the amber bottle containing the 5.0 mg/l standard, wait for a stable reading and calibrate the meter to 5.0 mg/L.

3.3.5 Record the slope of the calibration determined by the meter. The slope of the electrode should be about + 29 mV. If the reading is below + 26 mV see electrode manual for troubleshooting procedures.

3.3.6 Place the electrode into the amber bottle containing the reagent blank, and wait for a stable reading. Record the reagent blank concentration in mg/L.

3.3.7 The meter is now ready for sample analysis. Recalibrate using the prepared standards every two hours when used throughout the day.

3.4 Sample Analysis

3.41 If sample concentrations are greater than 20 mg/L, dilute the sample to between 0.2 and 20 mg/L. Record the dilution factor.

3.42 Transfer 100 mL of sample to a clean amber glass bottle. Add 1.0 mL of iodide reagent and 1 mL of acid reagent. Cap tightly and mix. Let stand until sample is at same temperature as standards (or at least two minutes).

3.43 Rinse the electrode with distilled water, and blot dry. Place electrode in sample, making sure that the reference element on the electrode is submerged. Wait for a stable reading and read residual chlorine concentration in mg/L.

3.44 Rinse electrode, store dry in air, or as indicated by manufacturer.

4. CALCULATION

4.1 Undiluted Samples less than 0.2 mg/L:

Chlorine Residual, mg/L = Sample Value, mg/L - Blank Value, mg/L

4.2 Undiluted Samples with concentration between 0.2 mg/L and 20 mg/L

Chlorine Residual, mg/L = Meter Reading for Sample (no blank correction)

4.3 Diluted Samples, initial concentration greater than 20 mg/L

Chlorine Residual, mg/L = Meter Reading for Sample X Dilution Factor

CHLORIDE

Since many types of solid and liquid wastes, including human wastes, contain high concentrations of the chloride ion, chloride analysis can be effective in detecting contamination from these wastes in water. As a general rule, chloride salts are very soluble and are not removed from the environment by natural biological action or by conventional wastewater treatment methods. Because of this, chlorides that dissolve in water may be carried from waste disposal sites into surface and ground waters. Chloride analysis is usually performed on ground water samples taken from monitoring wells located around such areas as wastewater treatment lagoons, groundwater discharge sites, and landfills. Although the chloride content is usually not considered a health threat, a significant increase in the background chloride concentration might indicate that contamination of the groundwater has occurred. Further investigation into the cause and extent of the contamination would then be required.

There are other times in which high chloride concentrations become a concern. Chlorides are corrosive to metallic pipes in high concentrations and are often monitored in boiler feed water for steam generation plants. Also, large amounts of chloride are harmful to growing plants and may be monitored in agricultural irrigation water.

Several procedures have been EPA approved for the determination of chloride in water. The methods included in this manual are probably the most straightforward of those. No sample preservation is necessary, and samples may be held for up to 28 days before analysis.

NPDES
APPROVED
METHOD

ARGENTOMETRIC METHOD FOR CHLORIDE

DISCUSSION: When a chloride solution is titrated with silver nitrate, silver chloride is produced. When potassium chromate is present, red silver chromate is formed after all of the chlorides have combined with the silver. Therefore, the endpoint for the titration is a change from yellow to pinkish yellow. Color and colloidal solids will make the endpoint harder to detect. The procedure is applicable to a minimum concentration of 1.5 mg/L when 100 mL sample are titrated.

REFERENCE

This procedure conforms to the EPA approved method referenced as Standard Methods 20th edition, 4500-Cl^- B.

1. REAGENTS

1.1 Chloride-free distilled water.

1.2 Silver nitrate titrant, 0.0141N. Dissolve 2.395 grams of silver nitrate, $AgNO_3$, in distilled water and dilute to 1 liter.

1.3 Potassium chromate indicator solution.

1.31 Dissolve 50 grams of potassium chromate, K_2CrO_4, in a small amount of distilled water.

1.32 Add silver nitrate solution until a definite red precipitate is formed.

1.33 Allow to stand 12 hours and then filter.

1.34 Dilute the filtrate to 1 liter with distilled water.

1.4 Sodium Chloride Standard, 0.0141N. Dissolve 0.8241 grams of sodium chloride, NaCl, (dried at 140°C) in distilled water and dilute to 1 liter. This solution has a chloride concentration of 500 mg/L.

2. STANDARDIZATION OF 0.0141N SILVER NITRATE

2.1 Place 25.0 mL of 0.0141N standard sodium chloride in a flask.

2.2 Add 1.0 mL of potassium chromate indicator.

2.3 Titrate with the silver nitrate to a pinkish yellow endpoint.

2.4 If 25 mL $\pm$ 1 ml of silver nitrate is used to reach the endpoint, the normality is 0.0141N. If the volume used is outside this range, the solution may be remade, adjusted, or the actual normality may be calculated as follows:

$$\text{Normality of } AgNO_3 = \frac{0.0141 \times 25}{\text{mL } AgNO_3 \text{ used}}$$

3. PROCEDURE

3.1 Determination of blank.

3.11 Place 100 mL of distilled water into a 250 mL Erlenmeyer flask.

3.12 Add 1.0 mL of potassium chromate indicator solution.

3.13 Titrate with standard silver nitrate titrant to a pinkish yellow end point. A white background under the flask will aid in detecting the endpoint.

3.14. Record the number of mL titrant used. This is the blank correction value. A blank value of 0.2 to 0.3 mL is typical.

3.15 Place this titrated blank to the side to use as a reference in detecting sample endpoint.

3.2 Sample Titration.

3.21 Place 100 mL sample into a 250 mL Erlenmeyer flask.

3.22 If the sample is not in the pH range of 7 to 10 adjust it to this range with 0.1 N sulfuric acid or 0.1N sodium hydroxide.

3.23 Add 1.0 mL of potassium chromate indicator solution.

3.24 Titrate with standard silver nitrate titrant to a pinkish yellow end point, using the titrated blank as a color reference. A white background under the flask will aid in detecting the endpoint.

4. CALCULATION

4.1 $$\text{mg/L } Cl^- = \frac{(A - B) \times N \times 35{,}450}{\text{mL sample titrated}}$$

Where: A = mL $AgNO_3$ titrated for sample

B = mL $AgNO_3$ titrated for blank

N = normality of $AgNO_3$ solution

4.2 If N equals 0.0141 and 100 mL of sample were titrated, then

$$\text{mg/L } Cl^- = (A - B) \times 5$$

5. QUALITY ASSURANCE

5.1 Precision may be determined by analyzing duplicate samples.

5.2 Accuracy may be determined by analyzing spiked samples as outlined in the procedure on the following page.

% Recovery for Chloride Titration

1. Take 2 portions of sample, each being 100 mL.
2. Titrate the first portion to determine the Cl^- content of the sample.
3. Spike the second portion with 10 mL of the 0.0141N NaCl standard solution.
    - Each mL of standard contains 0.50 mg Cl
        - 0.0141 eq/L X 35.45 G/eq = 0.50 G/L = 0.50 mg/mL
    - This spikes the 100 mL sample with 50 mg/L Cl.
        - 0.5 mg/100 mL = 5 mg/L for each mL spiked
4. Titrate the spiked portion with 0.0141N $AgNO_3$ titrant, determine mg/L chloride in the spiked sample.
5. Calculate the % Recovery:

$$\% R = \frac{(\text{mg/L Sample + Spike}) - (\text{mg/L Sample})}{(\text{mL Spike Used}) \times 5 \text{ mg/L}} \times 100\ \%$$

Notes:

- In a titration, do not consider the added volume due to the spike in the calculation.
- The volume of the standard that is spiked may be varied as needed. Adjust the calculation to reflect the mL of spike added.

ION SELECTIVE ELECTRODE METHOD FOR CHLORIDE

DISCUSSION: The ion-selective electrode method is an EPA-approved test procedure to determine chloride in wastewater. This is a relatively fast and simple procedure. Interferences to the chloride measurement are minimized by addition of CISA reagent. This method is applicable to a minimum concentration of 2 mg/L.

REFERENCE

This conforms to the EPA-approved procedure referenced as ASTM. D512-04, (C). Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Volume 11.01, 2005.

1. EQUIPMENT

   1.1 Ion Selective Electrode Meter

   1.2 Chloride Ion Selective Combination Electrode, (Orion 9617BNWP)

   Note: Not all chloride ion-selective electrodes are suitable for this test method, since the ionic strength adjuster is incompatible with some membranes. In particular, silver chloride/silver sulfide membranes are inappropriate, since the sulfide can be oxidized by the ionic strength adjuster.

   1.3 Magnetic stirrer

2. REAGENTS

2.1 Chloride-free distilled / deionized (DI) water.

2.2 Chloride Solution, 1000 mg/L, (Orion 941708) Dissolve 1.648 g of sodium chloride, NaCl, (dried for 1 hour at 600°C) in reagent water and dilute to 1000 mL in a volumetric flask.

2.3 Chloride Ionic Strength Adjuster (CISA), (Orion 940011), Dissolve 15.1 g of sodium bromate in 800 mL of water. Add 75 mL of concentrated nitric acid, and stir well. Dilute to 1 L, and store in a polyethylene or glass container. Note: Sodium bromate is a strong oxidant and should be handled appropriately. Preparation and dilutions of CISA should be made in a well-ventilated area, preferably a fume hood.

2.4 Electrode Filling Solution (Orion 900017)

3. PREPARATION OF STANDARDS

3.1 100 mg/L chloride standard: pipette 10mL of 1000 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with DI water.

3.2 20 mg/L chloride standard: pipette 20 mL of 100 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with DI water.

3.3 2 mg/L chloride standard: pipette 20 mL of 10 mg/L standard into a 100 mL volumetric flask. Dilute to the mark with DI water.

4. CALIBRATION

Calibrate the meter using chloride standards which are at room temperature, and which bracket the expected sample concentration.

4.1 Pipet 10.0 mL of each standard and 10.0 mL of CISA into 50 mL beakers; stir thoroughly for 1 to 2 minutes before analysis.

4.2 Place the electrode into each standard and after the meter reading has stabilized, enter that concentration as a calibration point. Rinse the electrode with distilled or deionized water between standards.

4.3 After calibration, the electrode slope should be above 54 mV per decade.

4.4 Analyze a mid-range standard to verify the calibration. If reading is not acceptable, see the troubleshooting section of electrode manual.

5. SAMPLE ANALYSIS

5.1 Allow samples to reach room temperature before analysis.

5.2 Pipet 10.0 mL of sample and 10.0 mL of CISA into a 50 mL beaker; stir thoroughly for 1 to 2 minutes. CISA must be added to all standards and samples. A larger sample size can be used if desired as long as CISA is added in a 1:1 ratio.

5.3 Place the electrode in the prepared sample. When the meter reading has stabilized, record the concentration of chloride in the sample in milligrams per liter.

6. ELECTRODE PERFORMANCE CHECK

6.1 Check and record electrode slope each day that it is used.

6.2 Drift may be checked by comparing a 1 minute to a 2 minute reading. See troubleshooting section of manual if slope or drift problems develop.

7. Electrode Storage

See electrode manual for storage 1) between measurements, 2) overnight, and 3) for long periods of time.

8. QUALITY CONTROL (QC)

Recommended QC procedures include analysis of matrix spikes (percent recovery), sample duplicates, and independent reference materials.

DETERMINATION OF PERCENT RECOVERY

MATRIX SPIKE

Percent recovery of chloride from a matrix spike is determined by adding a known amount of the 1000 mg/L chloride standard to a sample that has been analyzed. The use of a micro-pipet is encouraged, since these do not add a significant amount of volume during the spiking procedure, allowing for easier calculation of percent recovery. Disregard the volume of CISA in the calculation, since this is added equally to samples and standards.

1. Analyze a sample for chloride.
2. Using a micro-pipet, add a suitable amount of the 1000 mg/L chloride standard to the analyzed sample. As shown below, each microliter (µL) of the standard spikes the 10 mL sample with 0.10 mg/L Cl^-. (Disregard the volume of CISA in the calculation)

   0.001 mL X 1000 mg/1000 mL = 0.001 mg Cl^- added for each µL added.

   $$\frac{0.001 \text{ mg } Cl^-}{10 \text{ mL Sample}} = \frac{0.1 \text{ mg } Cl^-}{1000 \text{ mL}} = 0.1 \text{ mg/L}$$

3. Determine percent recovery as follows:

$$\% \text{ Recovery} = \frac{(\text{Sample + Spike, mg/L}) - (\text{Sample, mg/L})}{\text{Spike, mg/L}} \times 100\ \%$$

mg/L Cl^- spiked into 10 mL sample = µL of standard added x 0.1

Example: 200 µL of the 1000 mg/L chloride standard are added to10 mL of sample. The sample concentration had been determined to be 43.2 mg/L, and the sample with the spike in it was analyzed at 64.4 mg/L.

Sample = 43.2 mg/L Cl^-

Sample + Spike = 64.4 mg/L

mg/L spiked into sample = 200 µL x 0.1 = 20.0 mg/L

$$\% \text{ Recovery} = \frac{64.4 \text{ mg/L} - 43.2 \text{ mg/L}}{20.0 \text{ mg/L}} \times 100\ \%$$

$$= \frac{21.2 \text{ mg/L}}{20.0 \text{ mg/L}} \times 100\ \%$$

$$= 106\%$$

Note 1: While 100% is perfect recovery, 90-110% is generally considered acceptable; outside this range check for possible errors in procedure or technique.

Note 2: The volume of standard used for the spike should be varied frequently.

HARDNESS
(EDTA Titrimetric Method)

DISCUSSION: A dye such as Eriochrome Black T in an aqueous solution containing hardness (calcium and magnesium ions), at a pH of 10, will produce a wine red color. When this solution is titrated with EDTA, the EDTA complexes the calcium and magnesium causing the solution to turn blue.

REFERENCE:

This procedure conforms to the EPA approved procedure referenced as Standard Methods, 20th edition, 2340 C.

1. REAGENTS

1.1 Buffer solution - Dissolve 16.9 g of ammonium chloride, NH_4Cl in 143 mL conc. ammonium hydroxide, NH_4OH, add 1.25 g of magnesium salt of EDTA (magnesium disodium ethylenediamine tetraacetate) and dilute to 250 mL with distilled water. Keep the solution in a plastic or borosilicate glass container. Stopper tightly to prevent loss of NH_3 or absorption of CO_2. Do not store more than a month's supply in a frequently opened container. Dispense the buffer solution by means of a bulb-operated pipet. Discard the buffer where 1 or 2 mL added to the sample fails to produce a pH of 10.0 $\pm$ 0.1 at the end point of the titration.

1.2 Indicator - Dissolve 0.5 g of Eriochrome Black T in 100 g triethanolamine. Add 2 drops per 50 mL solution to be titrated. This indicator tends to deteriorate. If the end-point color change is not sharp the indicator could need to be remade. If the end-point is not sharp using fresh indicator, an inhibitor may be necessary for that particular sample, (see current edition of "Standard Methods for the Examination of Water and Wastewater.")

1.3 Standard EDTA titrant, 0.01 M. Weigh 3.723 g analytical reagent-grade disodium ethylenediamine tetraacetate dihydrate (disodium salt EDTA) $Na_2H_2C_{10}H_{12}O_8N_2 \cdot 2\ H_2O$, dissolve in distilled water and dilute to 1000 mL

1.4 Methyl red solution. Dissolve 0.1 g of methyl red sodium salt and dilute to 100 mL with distilled water.

1.5 Ammonium hydroxide 3 N. Dilute 20 mL of concentrated ammonium hydroxide, NH_4OH to 100 mL with distilled water.

1.6 Standard calcium solution - 1000 mg/L

1.61 Weigh 1.000 g of anhydrous calcium carbonate, $CaCO_3$, powder (primary standard or special reagent low in heavy metals, alkalies, and magnesium) into a 500 mL erlenmeyer flask.

1.62 Place a funnel in the neck of the flask.

1.63 Add -- a little at a time – 1+1 HCl until all the calcium carbonate, $CaCO_3$ has dissolved.

1.64 Add 200 mL of distilled water and boil a few minutes to expel carbon dioxide, CO_2.

1.65 Cool, add a few drops of methyl red indicator solution.

1.66 Adjust to the intermediate orange color by adding ammonium hydroxide, NH_4OH, 3 N or 1+1 hydrochloric acid, HCl as required.

1.67 Transfer all of the solution to a 1 liter volumetric flask, rinse erlenmeyer flask. Add to volumetric flask and fill to mark with distilled water. 1 mL = 1.00 mg $CaCO_3$.

2. STANDARDIZATION OF EDTA

2.1 With a volumetric pipet add 10.0 mL of 1000 mg/L calcium solution (10 mg Ca) to an erlenmeyer flask. Add about 50 mL of distilled water.

2.2 Add 1 to 2 mL of buffer solution to adjust pH to 10.0 - 10.1.

2.3 Add 1 to 2 drops of fresh indicator solution.

2.4 For a sharp end point, titrate in daylight or under a daylight fluorescent lamp.

2.5 Titrate with EDTA slowly, but within 5 minutes, with continuous stirring until the last reddish tinge disappears, adding the last few drops at 3 to 5 second intervals.

2.6 Record the number of mL of EDTA used and calculate how many mg of $CaCO_3$ are equivalent to 1.00 mL of EDTA titrant (10 mg Ca / mL EDTA).

3. PROCEDURE

3.1 Dilute 25 mL of sample to about 50 mL with distilled water.

3.2 Add 1 to 2 mL of buffer solution to adjust pH to 10.0 - 10.1.

3.3 Add 1 to 2 drops of indicator solution.

3.4 Titrate with EDTA solution slowly, but within 5 minutes, with continuous stirring until the last reddish tinge disappears, add the last few drops at 3 to 5 second intervals.

3.5 For a sharp end point do the titration in daylight or under a daylight fluorescent lamp.

3.6 If more than 15 mL of EDTA is used, dilute sample and repeat the titration.

4. CALCULATION

$$\text{Hardness, as mg/L } CaCO_3 = \frac{A \times B \times 1000}{\text{mL of original sample}}$$

A = mL EDTA titrant used

B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant

SPECIFIC CONDUCTANCE

DISCUSSION: Conductivity is defined as the capacity of water to conduct an electric current. Ions in the solution are the agents of this conductance, and the amount of current that is carried is proportional to the concentration of the conducting ions. Therefore, by measuring the conductance of a solution, we get an indication of the amount of material that is dissolved in it. This information is often useful in detecting the presence of contaminants in surface and ground waters. Conductivity is measured by placing a pair of electrodes in the solution, applying a voltage to the electrodes, and measuring the current across them.

In practice, the conductivity exhibited by a solution depends on several factors besides the concentration of ions, including surface area of the electrodes, distance between the electrodes, and the temperature of the solution. In order to make conductivity measurements consistent it has become standard practice to relate measurements to a cell in which the electrodes are 1 centimeter apart and have a surface area of 1 square centimeter. Because temperature is also critical, standards and samples must either be adjusted to 25°C, or the temperature of the standards and samples must be the same. Under these conditions we call the measured conductivity "specific conductance".

The unit of measurement for specific conductance is the mho per centimeter (mho/cm). Since the specific conductance of most samples is much lower than this range, the unit most often used is the micromhos per centimeter or (µmho/cm). In the International System of Units (SI), conductivity is reported in terms of millisiemens per meter (mS/m). To report results in SI units of mS/m divide µmho/cm by 10.

It is not often practical to use a conductivity cell that measures exactly 1 cm^3, but it is convenient to use cells which vary in size and configuration. We can use these various

cells and still obtain specific conductance by calibrating the meter to a known standard. Also, because measurements are seldom made at precisely 25° C, this effect must be accounted for. Conductivity probes commonly contain a temperature sensor which will allow the meter to correct the conductivity reading to that at 25°C.

Conductivities of samples vary widely according to the amount and type of material dissolved. The conductivity of distilled water usually ranges from 0.5 - 4.0 µmho/cm, while the conductivity of groundwater samples may vary from 300 - 1000 µmho/cm. Since conductivity in a solution is dependent upon the ions dissolved, substances which ionize to a large extent when dissolved will conduct more current than those which do not. For example, solutions which contain strong acids and bases which ionize almost entirely exhibit high conductivities, but solutions which contain sugar or other materials which do not ionize to a large degree exhibit a lesser amount of conductivity.

The measurement of conductance may be used for many different purposes. It is commonly used as a means of detecting groundwater contamination by analyzing groundwater samples taken from monitoring wells located at wastewater treatment lagoons, landfills, and wastewater sludge disposal sites. A change in the conductance of the groundwater may be an indication that contamination has occurred and would warrant further investigation into the cause and extent of the contamination. The conductivity of laboratory water is typically monitored to assure adequate quality.

SPECIFIC CONDUCTANCE

The process of meter calibration and temperature compensation varies with manufacturer.

Follow the manufacturer's instructions for calibration and operation of the meter.

REFERENCE:

This procedure conforms to the EPA approved method referenced as Standard Methods, 20th edition, 2510 B.

1. APPARATUS

   1.1 Conductivity meter – Use an instrument capable of measuring conductivity with an error not exceeding of 1% or 1 µmho/cm, whichever is greater.

   1.2 Thermometer - capable of being read to the nearest 0.1°C.

   1.3 Conductivity cell - platinum electrode type.

2. REAGENTS

   2.1 Standard potassium chloride solution, KCl, 0.0100 M

   Dissolve 745.6 mg anhydrous KCl in distilled/deionized water and dilute to 1000 mL in a volumetric flask. This is the standard reference solution, and has a conductivity of 1412 µmho/cm at 25°C. It is satisfactory for most samples when the cell constant is between 1 and 2 cm^{-1}. Stronger or weaker standards may be prepared as needed.

3. PROCEDURE (Meter with Temperature Compensation)

   3.1 Rinse conductivity probe three times with 0.0100 M KCL.

   3.2 Adjust temperature compensation dial to 0.0191 C^{-1}.

   3.3 With probe in standard KCL solution, calibrate meter to read 1412 µmho/cm.

   3.4 Rinse the conductivity probe with a portion of the sample to be analyzed.

   3.5 Adjust temperature of the sample to about 25°C.

3.6 Measure the conductivity of the sample and note the temperature to ± 0.1°C.

3.7 Report temperature compensated conductivity measurements as "µmho/cm @ 25.0°C".

4. CALCULATIONS (Meter Without Temperature Compensation)

4.1 If sample conductivity is measured without internal temperature compensation, the conductivity measurement can be mathematically adjusted to 25°C using the following equation:

$$\text{Conductivity, µmho/cm} = \frac{k_m}{1 + 0.0191(t - 25)}$$

k_m = measured conductivity

t = actual temperature of sample when measurement was obtained

4.2 Example:

A sample was analyzed for conductivity using a meter without temperature compensation. The meter reading for the sample was 450 µmho/cm and the temperature of the sample was 23.5°C.

$$\text{Conductivity, µmho/cm} = \frac{450\ \text{µmho/cm}}{1 + 0.0191(23.5 - 25)} = \frac{450\ \text{µmho/cm}}{1 + 0.0191(-1.5)}$$

$$\frac{450\ \text{µmho/cm}}{1 + (-0.02865)} = \frac{450\ \text{µmho/cm}}{0.97135} = 463\ \text{µmho/cm}$$

OIL AND GREASE
HEXANE EXTRACTION METHOD

DISCUSSION: Hexane is used to extract dissolved or emulsified oil and grease from water. The hexane is then distilled off, and the amount of oil and grease is determined by weighing. The method is suitable for biological lipids and well as mineral hydrocarbons. The method is not applicable to measurement of low boiling fractions that volatilize at temperatures below 85°C.

REFERENCE:

This method conforms to the EPA approved procedure referenced as Standard Methods, 20th edition, 5520 B. Liquid–Liquid, Partition-Gravimetric Method.

1. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

   The sample must be collected in a wide mouth glass bottle that has been washed with soap, rinsed with water, and rinsed with solvent to remove any residue. Use PTFE lined caps for sample bottles. Do not overfill the sample container, and do not subdivide the sample in the laboratory. Typically, one liter of wastewater sample is collected, unless the oil and grease concentration is expected to be greater than 1000 mg/L. If analysis will not occur within 2 hours after sample collection, acidify to pH 2 or lower with either 1:1 HCl or 1:1 H_2SO_4 and refrigerate to ≤ 6°C. Maximum holding time of preserved samples is 28 days.

2. APPARATUS

   1.1 Separatory funnel – 2 liter capacity, with teflon stopcock.

   1.2 Distilling Flask, 125 mL, flat bottom.

1.3 Liquid funnel, glass.

1.4 Filter paper, 11 cm diameter, Whatman No. 40 or equivalent.

1.5 Centrifuge, capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm or more.

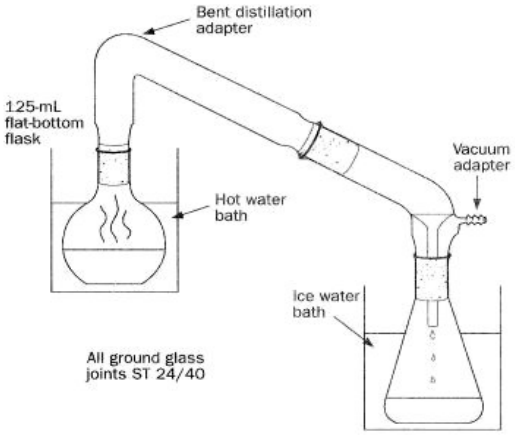
1.6 Centrifuge Tubes, 100 mL, glass.

1.7 Water Bath, capable of maintaining 85°C.

1.8 Vacuum Pump or other source of vacuum.

1.9 Distilling Adapter with drip tip, see diagram.

2.0 Ice Bath.



2. REAGENTS

2.1 Sulfuric acid, H_2SO_4 1 + 1, or HCl 1+1.

2.11 Mix equal volumes of concentrated acid with distilled water. Be sure to add the acid to the water, not the reverse.

2.2 n-Hexane, 85% minimum purity. Caution: n-Hexane is a narcotic agent; an irritant to the eyes, upper respiratory tract, and skin; and a neurotoxin. It is classified as a severe fire hazard.

2.3 Acetone.

2.4 Sodium sulfate, Na_2SO_4 anhydrous crystals.

2.5 Hexadecane, 98% minimum purity (A major component in diesel fuel).

2.6 Stearic Acid, 98% minimum purity (A major component in animal fat).

2.7 Standard Mixture, hexadecane/stearic acid 1:1 w/w, in acetone at 2mg/mL each. Purchase commercially prepared standard, or prepare as follows:

2.71 Place 200 ± 2 mg stearic acid, and 200 ± 2 mg hexadecane in a 100 mL volumetric flask and fill to mark with acetone. The solution may require warming to completely dissolve the stearic acid (be careful, acetone is

flammable).

2.72 Transfer solution to a 100 to 150 mL vial with TFE lined cap. Mark the solution level on the side of the container and store in the dark at room temperature.

2.73 Immediately before use, verify the level of liquid in the vial, and bring back to volume with acetone if needed. Warm to re-dissolve any visible precipitated material.

3. PROCEDURE

3.1 Prepare a distilling flask for each sample and standard by adding a few boiling chips to a clean distilling flask, dry in an oven at 103°C, cool in a desiccator, and weigh to the nearest 0.1 mg.

3.2 Mark sample bottle at the water meniscus, or weigh the bottle for later determination of sample volume.

3.3 If the sample has not already been acidified, acidify with either 1:1 sulfuric acid, or 1:1 Hydrochloric Acid. Five mL of either should be sufficient for a 1 L sample.

3.4 Using a liquid funnel, transfer the sample to the separatory funnel.

3.5 Rinse sample bottle with 30 mL hexane, and add washings to the separatory funnel.

3.6 Shake vigorously for 2 minutes.

3.7 Allow the layers to separate.

3.8 Drain the water layer (at the bottom of the separatory funnel), and a small amount of the solvent layer into the original sample container.

3.9 Prepare a funnel by adding filter paper and 10 g Na_2SO_4. Rinse this with hexane into a waste container.

4.0 Transfer the hexane layer through the funnel containing Na_2SO_4 into the prepared distilling flask.

4.01 If the solvent layer is not clear, and more than 5 mL of emulsion has formed:

4.011 Drain emulsion and solvent layer into a glass centrifuge tube, and centrifuge for 5 min at approximately 2400 rpm.

4.012 Transfer centrifuged material to separatory funnel, and drain solvent layer through the prepared funnel containing Na_2SO_4 into the distilling flask.

4.013 Combine the aqueous layers, along with any remaining emulsion or solids into the separatory funnel.

4.02 For samples with less than 5 mL of emulsion:

4.021 Drain only the clear solvent through the funnel containing Na_2SO_4

4.022 Recombine all aqueous layers, along with any remaining emulsion or solids into the separatory funnel.

4.1 Twice more, rinse original sample container with 30 mL hexane, add to separatory funnel, shake, allow layers to separate, drain water layer into sample container, and drain hexane layer through funnel into distilling flask.

4.2 Rinse filter paper and funnel with 10-20 mL of hexane, adding this rinse to the distilling flask.

4.3 Distill the hexane from the distilling flask in a water bath at 85°C, capturing the distillate in the ice bath cooled receiver.

4.4 When all visible hexane has been distilled from the flask, disconnect the bent distillation adapter, and draw air through the flask by means of an applied

vacuum for the final minute.

4.5 Remove the distilling flask from the water bath, and wipe outside of flask to remove moisture.

4.6 Cool in a desiccator until a constant weight is obtained.

4.7 To determine initial sample volume, fill the sample bottle to the mark and transfer to a 1L graduated cylinder.

4. CALCULATIONS

$$\text{mg/L Oil \& Grease} = \frac{\text{(wt of flask and residue, g)} - \text{(tare wt of flask, g)}}{\text{Initial Sample Volume, mL}} \times 1{,}000{,}000$$

Example:

Sample Volume	980 mL
wt. of flask & residue	121.8936 g
wt. of flask	121.8821 g

$$\frac{121.8936\text{ g} - 121.8821\text{ g}}{980\text{ mL}} \times 1{,}000{,}000 = \frac{0.0115\text{ g}}{980\text{ mL}} \times 1{,}000{,}000 = 11.7\text{ mg/L}$$

Oil and Grease

Hexane Extraction

Determination of Percent Recovery – Spiked Matrix

1. At the time of sample collection, collect a duplicate sample for the matrix spike. Care must be taken to assure consistency between these two samples; any variability between the samples will increase the amount of error in the recovery analysis.
2. One sample is analyzed to determine the actual concentration of oil and grease.
3. The second sample is spiked with the hexadecane/stearic acid mixture prepared in step 2.7 of the Procedure.
   - 3.1 The spike should increase the concentration of the sample by 1 to 5 times.
   - 3.2 Each mL of the standard contains 4 mg of oil and grease (200 mg hexadecane + 200 mg stearic acid per 100 mL). In 1000 mL sample, each mL of the standard spiked will increase the concentration by 4 mg/L.
4. Both samples are processed through the analytical procedure, and mg/L oil and grease are determined for each.
5. Determine percent recovery as follows:

$$\frac{\text{Sample with Spike, mg/L} - \text{Sample}}{\text{Concentration Spiked, mg/L}} \times 100\ \% = \text{Percent Recovery}$$

6. Example:

Duplicate 1000 mL samples of wastewater were obtained. To one sample, 20 mL of the hexadecane/stearic acid standard were added. The following results were obtained upon analysis of each.

Sample 62 mg/L
Sample with Spike 147 mg/L
Conc. Spiked = 20 mL X 4 mg oil / mL standard = 80 mg in 1000 mL Sample

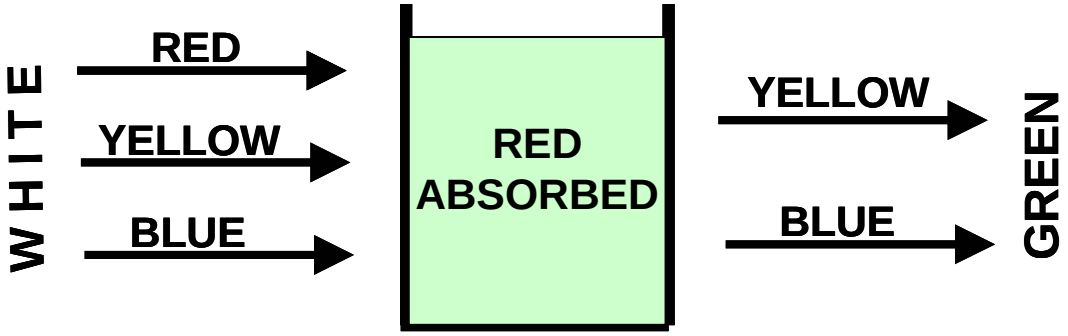
$$\%\ R = \frac{147\ \text{mg/L} - 62\ \text{mg/L}}{80\ \text{mg/L}} \times 100\ \% = \frac{85\ \text{mg/L}}{80\ \text{mg/L}} \times 100\ \% = 106\ \%\ R$$

COLORIMETRY

PRINCIPLES

The identification or determination of constituents by methods of analytical chemistry may be made by taking advantage of physical properties of the constituent. Useful physical properties we may measure include solubility, volatility, odor, and similar attributes which serve for qualitative identification, and mass, volume, density, color and various other properties serve for quantitative measurements.

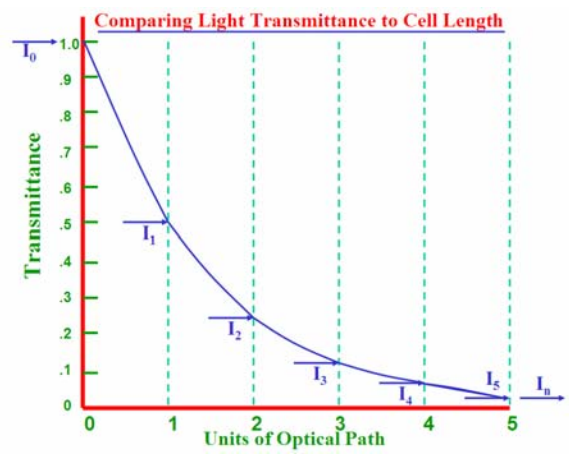
Color measurement, or colorimetry, makes use of the interaction between light and matter dissolved in a solution that results in absorption of some of the light by the matter. As light travels through a solution, some of the energy of the light may be transferred to the elements or compounds in the water. The light is absorbed due to the utilization of the light energy by the atoms or molecules to cause position shifts in certain of the electrons within the atoms. Color results when more light of one range of wavelengths is absorbed more than others. For example, if white light (light made up of all wavelengths) enters a solution that contains a material that absorbs the red wavelengths, the solution would appear green because the yellow and blue wavelengths will be transmitted through the solution.



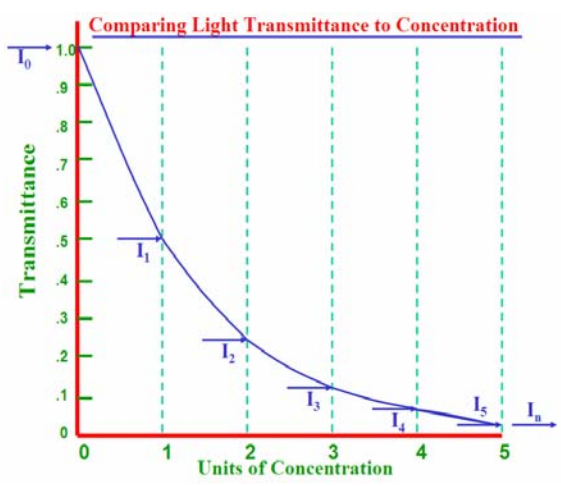
By measuring the intensity of the light entering a solution (I_o) and the intensity of the light transmitted through the solution (I), we can determine the amount of light that is absorbed. The calculated relationship between these two intensities, or the ratio of I_o to I, is called the Transmittance.

$$\textbf{Transmittance (T)} = \frac{I}{I_o}$$

Two principles described by Lambert and Beer are relied upon in using color for quantitative analysis. Lambert's law states that each layer of equal thickness will absorb the same fraction of light which passes through it. Thus, while the <u>thickness</u> of the solution increases in arithmetical progression, transmitted light intensity decreases in geometrical progression.



Beer's law states that the fraction of light absorbed on passing through a solution is directly proportional to the <u>concentration</u> of the absorbing material. As the concentration increases in arithmetical progression, the transmitted light decreases in geometrical progression.



We use this principle of light absorption to analyze for a particular material by adding reagents that react with the specific element of interest to form a compound that will develop a measurable color. The amount of light that is absorbed by this solution is related to the chemistry involved, the length that the light has to travel through

the solution (Lambert's Law), and the amount (concentration) of the absorbing material (Beer's Law). Since these relationships are geometric, the relationship is expressed mathematically as:

$$\textbf{Transmittance (T)} = \frac{I}{I_o} = 10^{-abc}$$

Where:

a = a constant for the particular solution
b = light path length
c = concentration of the absorbing material

(Since greater values for a, b, and c result in less transmittance, or a smaller value for T, this is an inverse relationship and is indicated in the mathematical relation by the negative sign.)

Because the relationship between the transmittance of light and the concentration of the absorbing material is geometric (logarithmic), the term Absorbance was introduced. Absorbance is defined as the negative logarithm of Transmittance. Although this appears to be very complicated (and we will not get into the details of the math involved), this does simplify the relationship between light absorbed and concentration as seen below:

$$\text{Absorbance (A)} = -\log T$$

$$\text{Since: } T = 10^{-abc}$$

$$A = -\log(10^{-abc})$$

Therefore (skipping a few mathematical steps):

$$\text{Absorbance (A)} = abc$$

Where:

a = a constant for the particular solution

b = light path length

c = concentration of the absorbing material

What this means then, is that for a particular analytical procedure (a held constant), using a specific size of sample container (b held constant), the concentration (c) of an absorbing material can be determined by measuring the absorbance (A) of light. This relationship of concentration and light absorbance is utilized in all colorimetric measuring systems.

It is important to note that the relationship between light absorption and concentration requires ideal conditions both for the beam of light, which must be monochromatic (light of a single wave length), and for the solution, in which the absence of any action on the absorbing material not due to the beam of light is assumed.

We use this principle of light absorption for analysis by adding reagents that react with the specific element of interest to form a compound that will develop a measurable color. We must either select a reagent addition which will develop a color only with the desired constituent, or the interfering compounds must be removed prior to color development.

Electronic instruments, called spectrophotometers or colorimeters, utilize photoelectric cells to determine the intensity of light transmitted through a portion of sample that has been chemically treated to produce color. The concentration is then determined by comparing this reading to a previously prepared “calibration curve” obtained from the readings of a series of standards of known concentrations of the constituent of interest. Electronic instruments can measure intensities of narrow light wavelength bands (approaching monochromatic light) but cannot recognize the presence of turbidity or differentiate between light transmission reduction due to absorbance or scatter by turbidity from color absorbance.

The analyst must have an understanding of these basic principles of colorimetry to be sure to account for the requirements and limitations of the analysis. The important considerations of colorimetry are summarized below:

CONCENTRATION CAN BE COLORIMETRICALLY DETERMINED IF:

1. ABLE TO CHEMICALLY DEVELOP A COLOR WITH THAT SUBSTANCE AND ONLY THAT SUBSTANCE

2. THE DEVELOPED COLOR OBEYS (FOLLOWS) BEER'S LAW OVER A REASONABLE RANGE OF CONCENTRATIONS

3. THE DEVELOPED COLOR MUST BE <u>STABLE</u> FOR REASONABLE LENGTH OF TIME, <u>REPRODUCIBLE</u>, AND <u>SENSITIVE</u> TO SMALL CHANGES IN CONCENTRATION

4. ALL LOSS OF TRANSMITTED LIGHT MUST BE FROM ABSORBANCE BY SUBSTANCE MEASURED (DEVELOPED COLOR)

5. ALL OF SUBSTANCE PRESENT IN SAMPLE MUST BE AVAILABLE FOR REACTION WITH COLOR DEVELOPING AGENT

6. ABLE TO MEASURE AMOUNT OF LIGHT ABSORBED

SAMPLE COLLECTION

Samples for determination by colorimetry must be collected with the same care which is necessary for any other analytical system. Sample containers must be carefully cleaned to prevent contamination. For example, containers for samples which will be analyzed for metal ions may have to be acid rinsed using a specific acid. For phosphorus analysis, sample bottles should be washed with phosphate free cleaning agent, rinsed with a 10% hydrochloric acid solution, and then rinsed with de-mineralized or distilled water.

SAMPLE PREPARATION

One or more of the following sample preparation procedures are necessary prior to color development:

1. Dilution

Each colorimetric test procedure has a limited range of sample concentration which will result in a color absorbance which a detector can accurately measure. With higher concentration of the unknown constituent the original sample must be diluted with distilled water, free of the constituent of interest. Dilution must be made to a definite ratio and this ratio used in calculating the unknown concentration.

2. Filtration

Spectrophotometers will indicate suspended solids and turbidity as additional color (apparent absorption of light) resulting in a higher than actual indicated concentration. With low turbidity and high dilution ratios the effects of turbidity may be reduced sufficiently that it may be ignored. If turbidity remains noticeable after dilution, or when dilution is not required, the turbidity must be removed. Several methods such as coagulation or centrifuging are available, however the sensitivity of most analyses requires filtering if any noticeable solids are present in the sample.

3. pH Adjustment

The chemistry involved, as well as the complex compounds that are formed, are often pH sensitive. Colorimetric procedures must be carefully followed in regard to pH. The adjustment of pH may be made necessary by sample preparations (such as digestion) or to correct original sample pH. With adequate care, small quantities of relatively concentrated acids and bases should be used, to minimize changes in sample volume and constituent concentration.

4. Digestion

Organic matter found in wastewaters often will react with many of the reagents used for color development resulting in low measurement readings. It may also contribute to erroneous results by absorbing light, resulting in high readings. The constituent that is being analyzed may be combined with other compounds in the wastewater and would not be available to react with color developing reagents. Also, many constituents in wastewater can exist in more that one chemically reactive (valence) state, some of which may not react with the color developing reagents. For example, phosphorus in wastewater may be bound in organic material, in the combined form found in detergents (poly-phosphates), or in the form called ortho-phosphorus. Of these, only the ortho-phosphorus reacts with the color developing reagents used for analysis. To measure all of the phosphorus in a wastewater sample (Total Phosphorus), the sample must be digested with strong chemicals at an elevated temperature.

The digestion of samples is intended to destroy organic material, release the combined constituent, and, where necessary, change the chemical form (valence) of the constituent being analyzed to make it available for reaction with the color forming reagents.

COLOR DEVELOPMENT

The color to be measured will be most desirable if it is stable for a reasonable length of time, reproducible, and sensitive to small changes in concentration of the desired constituent. In addition to dilution to place test sample concentration within suitable ranges, there are some additional important considerations that must be controlled in any colorimetric analysis.

Hydrogen ion concentration (pH) often affects both the speed and/or intensity of color development. The control of pH also affects the tolerance to certain diverse ions by preventing hydrolysis or precipitation. The pH adjustments given in the analytical procedures must be carefully followed.

The various developed colors have differing time requirements to reach maximum intensity and the color persistence also is variable. The time increments stated in the procedure must be closely adhered to in order to obtain reliable results. These same comments are equally appropriate regarding temperature of sample and reagents to achieve reproducible color development.

Some compounds, when present in sufficient concentration, may consume so much of the color development reagent, even though the complexes formed may be colorless, that there is insufficient reagent to completely react with the desired constituent. Although the purpose and importance of some analytical steps may not be obvious, they are, none the less, critical to obtaining reliable results.

It is also important in most colorimetric analyses that standards that are used for instrument calibration or for various QA/QC procedures are taken through the identical analytical steps that are required for the sample.

COLOR MEASUREMENT

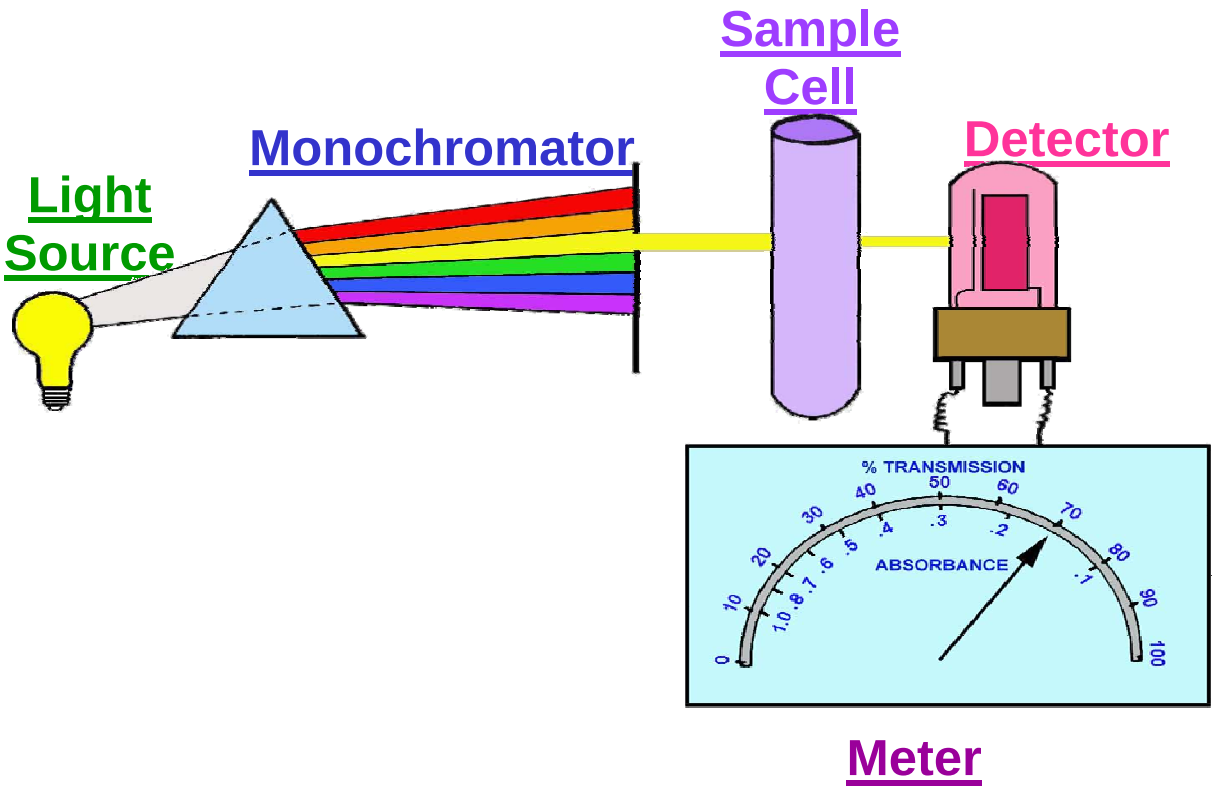
As explained earlier, colorimetry is an analytical method used to determine the concentration of a substance in a solution using the relationship of the concentration to the absorbance of light in the visible range of radiation. The absorbance of light results in a change in the intensity of the color of a chemically prepared solution. The color developed in a sample is compared to known

standards to determine the concentration in the sample. This can be done visually (using the human eye) and there are a number of manufacturers that have produced “color comparators” for this purpose. These comparators have obvious limitations in the reliability and sensitivity of the results obtained. Although these comparators may have use for routine monitoring or controlling some treatment processes, they are not accurate enough for detailed monitoring and are not acceptable for reporting to regulatory agencies such as the US EPA or the Michigan DEQ.

To obtain the most reliable (and reportable) results, an electronic instrument must be used. These instruments, called spectrophotometers (measure light intensity) or specifically colorimeters (measure light intensity in the visible range), utilize photoelectric cells to determine the intensity of light transmitted through a portion of sample that has been chemically treated to produce color. The instrument is constructed to permit regulation of a constant intensity light source and a system to duplicate this intensity for subsequent analysis. The colorimeter is standardized or calibrated initially by measuring the color developed in a series of standards with known concentrations. These meter readings are used to develop a "calibration curve". This calibration curve may be used for many determinations over a period of several weeks. At least one standard should be included with each group of samples analyzed, as a check on calibration (including instrument, reagents, and procedure). If the standard results in a meter reading are essentially equal to that obtained when the calibration curve was made, it can be assumed that the calibration curve remains valid. If a diverse value is obtained a new curve must be developed.

COLORIMETRIC INSTRUMENTS

Colorimeters are generally very easy to use but there are many factors that impact the reliability of the results obtained. It is very important for the analyst to have a basic knowledge of how these instruments work to be able to recognize the importance of careful operation and calibration as well as the limitations of the instrument. To help understand these limitations, five major components of colorimeters will be discussed. These components, shown in the figure below, are the light source, monochromator, sample cell, photo detector, and the indicating meter.



Light Source

Each instrument must have a light source which will emit a beam of light which has a constant intensity and color distribution. Incandescent lamps are used in the visible light range. These lamps emit a stable beam when excited by a constant voltage power supply. Variations in voltage cause irregularity in both light

intensity and color distribution. These variations can be avoided by using a voltage regulator to adjust line voltage. Voltage regulation is usually built into the instrument and is usually adequate, but in some wastewater treatment laboratories the line voltage fluctuations are significant and the use of an additional voltage regulator may be required.

The light intensity must be controllable so that full scale instrument readings can be attained at all wavelengths and when using various solvents with differing optical characteristics. The light intensity may be varied by the use of an iris diaphragm of the type used in cameras or by voltage adjustment in the lamp circuit.

The lamps used for light sources are subject to fatigue over an extended period of time. If full scale readings on the meter cannot be attained when manipulating the light intensity control, lamp fatigue is a probably cause. Due to the varying intensity of the several colors of light in a white beam from the lamp, fatigue may be limiting at some wavelengths earlier than for other wavelengths.

Monochromator

The light coming from the light source consists of radiation of the full visible spectrum of wavelengths (white light). To be able to obtain the high degree of sensitivity required for analysis, the colorimeter must limit the range of wavelengths of light passing through the sample to a narrow band of one color (monochromatic). The device in the colorimeter that accomplishes this is called a monochromator and consists of two parts, a diffraction grating and a narrow slit or aperture. The white light from the source is directed to the diffraction grating which functions in the manner of a series of small prisms and spreads the light into a rainbow spectrum. This spectrum is cast upon a narrow opening (or aperture) which will pass only a limited color band. Narrow wavelength bands are attainable if a strong source, wide spectrum dispersion system, and a narrow slit or aperture are used.

The monochromator is adjusted to different wavelength ranges for various analyses by rotating the diffraction grating, allowing a different portion of the spectrum though the aperture. The analyst must be very careful in setting the monochromator, especially when the instrument is used for more than one type of analysis, because the calibration of the instrument is affected by the wavelength setting.

Sample Cell

The colored liquid samples are placed in the light path within the colorimeter using a sample cell called a cuvette. These frequently are shaped similar to round test tubes, however, square shapes are sometimes used. The cuvettes should always be placed in the instrument with the same side or area facing the front of the instrument. Circular cuvettes should be inserted with the reference mark aligned with an index mark on the cuvette holder. Some instruments will accommodate cuvettes of varying dimension or light path length. The useful concentration range of the individual procedures can be extended with correct cell path length selections.

The instrument's light detector is not capable of differentiating the light absorbed due to the sample, from that due to the cuvette. It is essential that the cuvettes are free of fingerprints, scratches, dried deposits from previous use, or any such situation that may affect the transmittance of light through the cell. The analyst must be very careful in handling the cuvette during analysis as well as being sure of thorough cleaning following the analysis.

If more than one cuvette is used the cuvettes must have duplicate absorption and reflection properties. Although matched sets of cuvettes may be purchased for some instruments, these should be checked to verify consistent results. Cuvettes may be checked for similarity of properties by comparing them while containing distilled water. To be valid, this comparison must be made with the light wavelength used in the individual determinations. It is suggested, however, that only one cuvette is used for each analysis and that the cuvette is thoroughly rinsed with the prepared sample between each reading.

Light Detector

Photometers make use of a receptor which converts light energy transmitted through the sample being analyzed to an electrical current. When light strikes the photoelectric tube, electrons are released at the sensitized surface, resulting in an electrical potential or voltage sufficient to produce a measurable current in an

external circuit or meter. The magnitude of the current is proportional to the light received. A variety of electrical circuits or amplifiers are utilized to improve indication on the meters. The photoelectric cells have differing electrical response to the several light colors or wavelengths. This differing response necessitates variation in the light source intensity to attain full scale reading at each color or wavelength within the instrument range. Some instruments use single receptors throughout their wavelength range; whereas others use two photocells each selected for best response within a best power range. With the latter system a filter may be required with one of the photocells. The inconvenience of changing receptor tubes (and possible filters) is compensated for by improved performance of the instrument.

In each instrument the detector must be protected from any stray light which has not passed through the monochromator and the sample. The instrument cuvette holders include covers which exclude room illumination from the light path when closed. Generally the cover should be closed both while adjusting the meter zero indication and making sample readings. Light detector tubes are subject to fatigue which causes reduced effectiveness prior to failing to function.

Indicating Meter

The electric current resulting from light activation of the detector is measured with an ammeter and the reading displayed on a digital meter. Most colorimeters allow for the out-put to be read in either Transmittance (T) or in Absorbance (A). Although either scale may be used, the absorbance scale is almost always used as readings in Transmittance must be plotted on semi-log graph paper to account for the logarithmic aspect of Beer's law.

Some instruments include a microprocessor in its circuitry that allows for read-out directly in concentration. Instruments may also have built-in calibration curves. The analyst must use these with caution to be sure that readings are not used that are outside of the linear range of calibration and that the internal calibration is regularly verified using current standards and reagents.

Optical System

In addition to the five major components of colorimeters discussed above, the instrument will also include an optical system to direct the light beam. The optical system in each instrument utilizes various combinations of lenses, mirrors, apertures and occluders. These are used to focus and control the light from the lamp so that it will pass through the sample and to the detector. The optics must be protected from dust, corrosive fumes, and from any shock that may disrupt alignment. The compartments of the instrument containing these units should be kept tightly closed and any cleaning or maintenance should be done only by qualified technicians.

INSTRUMENT OPERATION

Only careful observation of instrument warm-up, operation and maintenance instructions will enable the analyst to obtain reliable results. The following general instructions are intended to supplement or emphasize the instructions in the instrument operation manual.

The meter readings are unreliable until the instrument has been turned on long enough to come to a constant temperature and the electronic components become stabilized. The necessary instrument warm up period is ordinarily listed in the instruction material for the specific instrument

When setting the monochromator to the desired wavelength, the adjustment knob should consistently be rotated in the same direction when approaching the set point. This will minimize variation in light color due to any slack in monochromator linkage.

Following warm-up of the instrument, the unit must be adjusted to indicate complete absorbance (no light reaching the detector). The light path is blocked in some instruments by simply removing the cuvette from the sample holder while other instruments use other devices. Next a cuvette is filled with a reagent blank and placed in the colorimeter. This blank is prepared using distilled water that has been taken through the color development steps that are required for samples. With the reagent blank in the light path, the lamp intensity is adjusted to give a

read-out of zero absorbance. Adjustment of one end of the scale will affect the other in some instruments requiring multiple adjustments to attain both indicated values simultaneously. The instrument is then ready for reading the absorbance of prepared standards and samples.

Good spectrophotometric techniques generally consider only those absorbance readings that fall between 0.100 and 0.700 to be reliable. Some specific analyses may be more restrictive than this general statement. For example, analysis of phosphorus using the ascorbic acid method has been found to be linear (acceptable) up to an absorbance reading of about 0.4 with many colorimeters. The most reliable readings are those that are between the lowest and highest reading obtained for the standards used in calibrating the colorimeter.

Whenever the appearance of the sample, following color development, varies from the normal it is probable that the variation is the result of an interfering substance in the sample or from deterioration of one or more of the reagents used in the analysis. The instrument is only capable of determining the apparent absorption of light and, in this situation, is probably providing incorrect readings. It is very important that the analyst remain alert to recognize any color development irregularities and then take steps to determine and eliminate the cause of the irregularities.

COLORIMETER STANDARDIZATION OR CALIBRATION

As discussed earlier, the colorimeter is standardized or calibrated initially by measuring the color developed in a series of standards with known concentrations. These meter readings are used to prepare a "calibration curve" that allows for the determination of sample concentrations by comparing sample absorbance readings to the readings from the standards. The comparison of absorbance readings may be done using a computer spreadsheet (like Excel), using an instrument with an internal microprocessor, or by “plotting” the absorbance verses concentration on graph paper. The calibration will take into consideration the individual characteristics or variations in the instrument, the reagents, the laboratory, and the analyst. It will be unusual if duplicate calibration curves are obtained following significant changes in any one of these components. Following development of a

calibration curve it should be verified frequently using standards of known concentration taken through the sample preparation and color development process. Generally, the analyzed results for a standard should be within 10% of the true value, although the acceptable range may be based on quality control parameters for specific analyses. The most reliable results are obtained when this verification is done each time samples are analyzed.

Alterations including aging of reagents or the mixing of new color reagents, replacement of an instrument lamp or detector tube, or any other change in the color development or measuring system will make it necessary to repeat the calibration procedure. Also, the analyst should determine a specified period of time to repeat the standardization. A minimum of six months is suggested, however a shorter interval may be necessary for some colorimetric analyses. A third consideration in determining the need for re-standardization is when the analysis of the standards used for calibration curve verification becomes questionable.

The necessary calibration steps include:

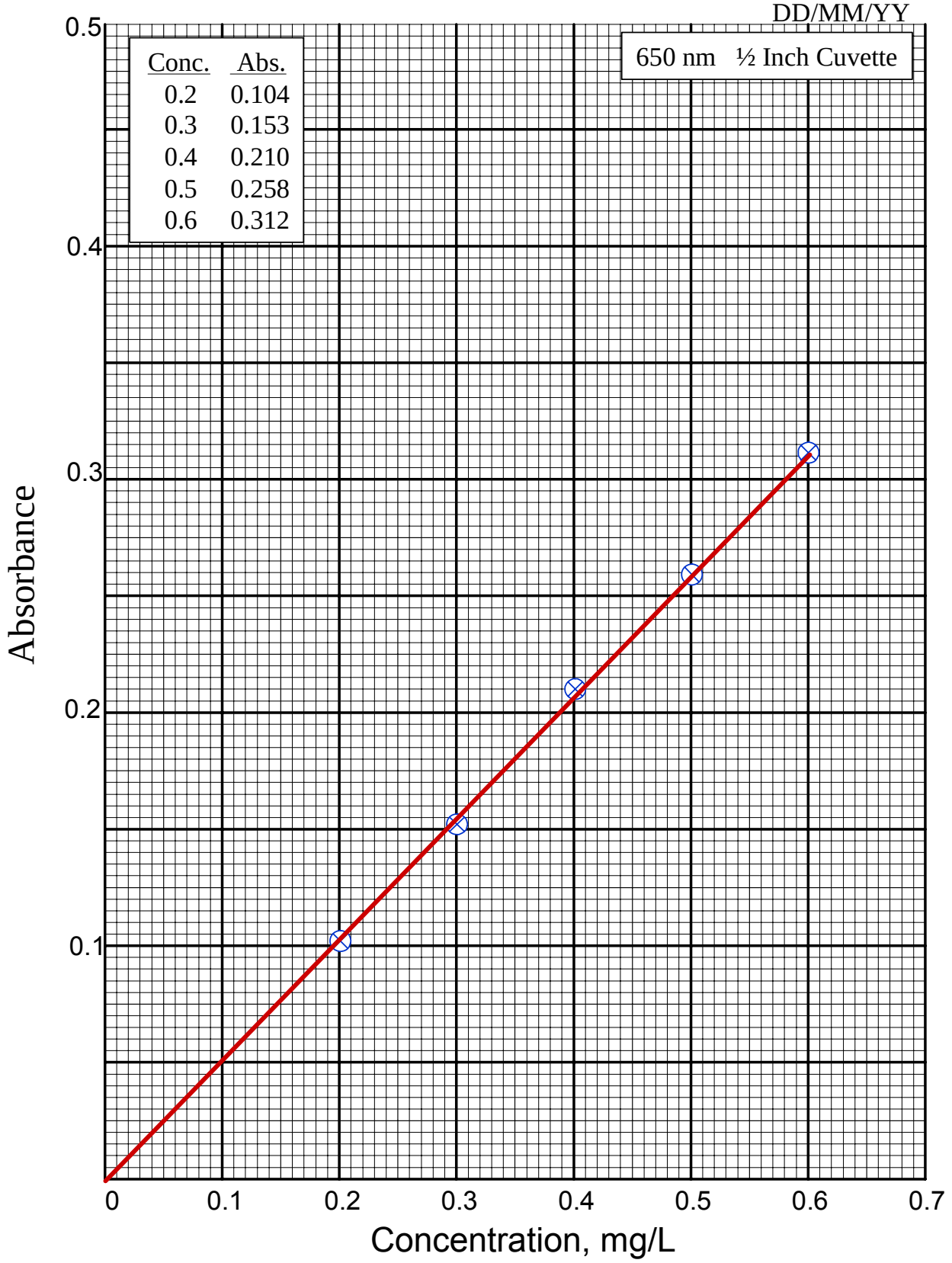
1. Prepare a stock solution of accurately-known concentration of the constituent.
2. Prepare six or more dilutions from the stock solution covering the full range of useful meter readings (generally 0.1 to 0.7 absorbance).
3. Perform the same sample preparation steps that are anticipated for use on the unknown samples.
4. Develop the color in the same manner as will be used for unknown samples. All chemical and reagent concentrations should be essentially equal to those in the unknown.
5. Measure the absorbance within the recommended time intervals and record this data.
6. Using the concentration and instrument reading data prepare a calibration curve.

After the instrument reading has been obtained for an unknown sample, the concentration value is obtained by referring to the calibration curve. The value from the calibration curve must be corrected to account for any dilution made during sample preparation.

(See example calibration curve next page)

Total Phosphorus Ascorbic Acid – Two Reagent Method

DD/MM/YY



Conc.	Abs.
0.2	0.104
0.3	0.153
0.4	0.210
0.5	0.258
0.6	0.312

NUTRIENT CONTROL - REMOVAL OF PHOSPHORUS
PROCESS AND FACILITIES

Phosphorus is considered to be the key nutrient to accelerated eutrophication. If we can control the amount of phosphorus entering a stream or body of water we thereby, can control the rate of eutrophication of that stream or body of water.

Conventional treatment methods of wastewater are oriented toward the stabilization of organic carbonaceous matter and are not efficient in phosphorus removal. The percentage removal of phosphorus for the various types of conventional treatment are as indicated in Table I.

TABLE I

TYPE	PERCENT PHOSPHORUS REMOVED
Primary Sedimentation	5 - 15
Primary and Trickling Filter	20 - 30
Primary and Activated Sludge	30 - 50

It can readily be seen that to achieve the current water quality objective of removal of at least 80% of the phosphorus from wastewater discharges, an additional process or modification to existing processes is needed.

METHODS OF REMOVAL

Several methods for removal of phosphorus from municipal wastewaters have been studied. A number of biological removal methods have proven to be quite reliable and economical. At this time, the method that is most widely used is the chemical treatment method for phosphorus removal.

The chemical treatment method involves the addition of metal salts to the primary, secondary, or tertiary steps with or without the addition of a polyelectrolyte (polymer). A modification of the chemical treatment method is the chemical-biological method which employs direct dosing of the metal salt to the aerator of an activated sludge plant. Soluble

phosphorus reacts with the metal in solution to form insoluble compounds. These insoluble compounds are then flocculated to allow separation by sedimentation. The chemically-bound precipitated phosphorus is removed with the sludge and is not resolubilized during digestion or sludge disposal unless the pH is substantially lowered. Effluent phosphorus concentrations of 1 - 2 mg/L can be achieved if the precipitation is accomplished in the primary or secondary portions of the plant. Addition of a polymer to aid in coagulation of the precipitate may be necessary to obtain concentrations below 1.0 mg/L.

Factors affecting choice of chemical and point of addition are influent phosphorus level, effluent discharge standard, wastewater characteristics (such as alkalinity), plant size, chemical costs including transportation, sludge handling facilities, ultimate sludge disposal alternatives, and other processes utilized.

CHEMICALS

The chemicals presently appearing most useful for the formation of a precipitate in wastewaters have been long used in the treatment of water for public water supply systems. These chemicals include calcium, iron and aluminum ions in several forms. Lime is customarily fed as the hydrated oxide $Ca(OH)_2$. It may be purchased and stored as a dry material in this form, usually at smaller plants. Reduced shipping costs result if calcium oxide (CaO) is purchased and stored. This requires the use of a feeder slaker combination to convert dry lime, CaO, to hydrated lime, $Ca(OH)_2$, and is customarily used at larger water treatment plants.

Iron may be fed in either the ferrous or the ferric state in combination with chloride or sulfate anions. Each of these forms may be purchased as a dry chemical. In some locations waste iron solutions (waste pickling liquor) are available from metal finishing

preparation. Aqueous solutions of iron have a low pH, (pickle liquor usually also contains free acid) and must be stored and handled in corrosion resistant materials. Rubber and plastics have been widely used in the water supply industry for handling iron solutions.

Aluminum may be fed as alum (aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$ or as sodium aluminate ($Na_2Al_2O_4$). Alum may be purchased as a dry chemical or in a liquid solution. Sodium aluminate has not been as widely used for water treatment. It is available as a dry chemical or solution. Aluminum solutions like iron, must be stored and handled in corrosion resistant materials.

Coagulant aids have been found to be necessary in some of the studies of chemical removal of phosphorus. These materials are complex organic polymers which are available in many formulations from several suppliers. They must be carefully selected to suit individual wastewater character. Laboratory trials of various polymers are needed to select the most useful material.

POINTS FOR CHEMICAL APPLICATION

There are a variety of points in the conventional biological treatment plant where chemicals can be applied to develop a phosphorus precipitate which can be removed, utilizing conventional settling units. The chemical treatment process can be integrated into the primary clarification facilities with improvement in the effectiveness of removal of both BOD and suspended solids. In an aeration system the chemicals can be added to the aeration tank. In the case of a trickling filter, the chemicals can be added to the influent of the filter. The chemical precipitation process may be integrated into the secondary settling system of either a trickling filter or an activated sludge treatment process. Phosphorus removal with chemicals can also be accomplished subsequent to conventional final settling.

Use of chemicals in the final settling process will normally result in minimum turbidity (suspended or colloidal solids) in the plant effluent, an item of considerable importance in some locations.

CHEMICAL PROCESS REQUIREMENTS

The chemical removal of phosphorus from wastewaters utilize the same principles of chemistry and physics as have long been used and developed to a fine art (both facility and operation) in the treatment of water for domestic water supplies.

The chemical treatment of water to remove phosphorus consists of the addition of a chemical or chemicals which will react with the phosphorus to produce a slightly soluble precipitate. This precipitate will usually consist of very fine particles which do not settle quickly in our clarification units until they have been flocculated or agglomerated into larger particles with improved settling rates. Like water supply treatment, filtration following settling will further reduce residual phosphorus concentrations.

The chemical precipitation process, whether for the softening of water or the removal of phosphorus, has several requirements necessary to achieve success. These requirements will be discussed in the order of their occurrence in the process.

Optimum chemical dosage must be applied to the water. This will be the sum of the treatment chemical needed to react with the phosphorus in the water, the excess of chemical required to drive the chemical reaction to the desired state of completion plus any surplus required due to inefficiencies in mixing or dispersion of the added chemical. Knowledge of the exact influent phosphorus concentration supplies only part of the information needed to predict optimum dosage. The optimum dosage can best be selected by performing laboratory testing which should be repeated whenever there are significant

changes in the chemical characteristics in the water. Jar tests utilizing a varying concentration of the treatment chemicals in beakers of the wastewater are performed. (See Chapter 325). The lowest chemical dosage achieving desired results is then translated into a plant scale dosage.

Present experience indicates the need, in many cases, of more than one chemical to accomplish our objectives. These chemicals must be added in a proper sequence to obtain beneficial results.

Each chemical added to the wastewater must be rapidly and uniformly mixed if it is to effectively react with the phosphorus in the water. This will require multiple mixing operations if the proper addition sequence prevents multiple chemical addition at one point. The use and complete mixing of recycled previously formed precipitates has been found useful in water treatment. These solids are introduced to the wastewater along with the first chemical.

Following the formation of the precipitate in the form of many extremely fine particles that will not settle adequately we must flocculate or agglomerate these tiny particles. This process proceeds if gentle stirring is applied. This gentle motion imparted to the water promotes opportunity for the particles to join together. The coagulant aids or so-called polyelectrolytes assist in this agglomeration process when applied at extremely low dosage rates (less than 1 mg/L).

The motion imparted to the water must promote the merging of particles, and at the same time prevent the deposition of solids in the flocculation compartment (unless solids removal equipment is provided). Flow velocities and turbulence between flocculators and settling tanks must not be great enough to damage the floc.

Phos. Removal

The chemical treatment process will utilize one or more chemical reactions. There must be adequate reaction time allowed for each of these chemical reactions. These reactions may coincide in time or it may be necessary that they occur in sequence.

The deposition of solids in the mixing and flocculation equipment must be prevented. This has been necessary in the water treatment field where solids are normally chemically stable and will be of greater importance with wastewaters. Accumulation of solids on moving equipment can cause damaging imbalance of rotating elements on overload drive components. Deposition in the corners of mix or flocculation units will reduce reaction or flocculation time. The organic or volatile solids that may be present in chemical sludges will be subject to bacterial decomposition. If this bacterial action results in anaerobic conditions an unsatisfactory situation will result.

After the precipitates have formed and have been flocculated they must be separated from the wastewater stream. The normal settling tanks and mechanisms can be used for solids separation or the solids contact or upflow clarifiers developed for water treatment may be used. After the solids have settled they must be removed from the settling unit promptly to prevent excessive bacterial action upon the volatile components. The phosphorus precipitate sludges will have the same need for thickening as ordinary sludges from the same point in the plant flow stream.

TOTAL PHOSPHORUS

Ascorbic Acid Method - Single Reagent

DISCUSSION - Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to from a complex that is reduced to intensely colored molybdenum blue by ascorbic acid. This single reagent method is preferred over the two reagent method when analyzing for low levels of phosphorus (< about 0.2 mg/L) or in situations where a high level of sensitivity is desired.

REFFERENCE - This conforms to the following EPA-approved procedures.

Standard Methods for Examination of Water and Wastewater, 20th Edition,

Method 4500 - P B.5 (digestion) and Method 4500-P E.

SAMPLING - Sample bottles should be washed with a phosphate free cleaning agent and rinsed with a 10% hydrochloric acid solution. Samples may be stored up to 28 days if refrigerated to ≤ 6° C and acidified with H_2SO_4 to pH <2.

1. APPARATUS

   1.1 Spectrophotometer - for use at 880 nm, providing a light path of 2.5 cm or longer.

   1.2 Acid-washed glassware: All glassware, including sample containers, should be cleaned in warm water containing phosphate free detergent, rinsed with a 10% hydrochloric acid solution, and then rinsed thoroughly with distilled water. Reserve this glassware for only phosphorous analysis.

2. INTERFERENCES

   2.1 Concentrations as low as 0.10 mg/L arsenic interfere.

   2.2 Hexavalent chromium and nitrite interfere to give results about 3% low at concentrations of 1.0 mg/L and 10-15% low at concentrations of 10 mg/L chromium and nitrite.

3. REAGENTS

3.1 Stock phosphorus solution, 50 mg/L as P. Dissolve 0.2195 g of potassium phosphate monobasic, KH_2PO_4 in distilled water and dilute to 1 liter in a volumetric flask.

3.2 Strong acid solution. Carefully add 300 mL of concentrated sulfuric acid, H_2SO_4 to approximately 600 mL of distilled water and dilute to 1 liter with distilled water.

Note: Be sure to add the ACID TO THE WATER.

3.3 Ammonium persulfate, $(NH_4)_2S_2O_8$, or potassium persulfate, $K_2S_2O_8$. Used as a solid (store in cool, dry location out of direct sunlight).

3.4 Ammonium molybdate solution. Dissolve 20 g of ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL of distilled water.

3.41 Store in glass-stoppered bottle.

3.5 Sulfuric acid solution 5 N. Dilute 70 mL conc. sulfuric acid, H_2SO_4. to 500 mL with distilled water.

3.6 Antimony potassium tartrate. Dissolve 1.3715 g antimony potassium tartrate, $K(SbO)C_4H_4O_6 \cdot \frac{1}{2} H_2O$ (sometimes listed as $C_8H_4K_2Sb_2O_{12} \cdot 3H_2O$) in 400 mL distilled water in a 500 mL volumetric flask and dilute to volume.

3.61 Store in glass-stoppered bottle.

3.7 Ascorbic acid 0.1M. Dissolve 1.76 g ascorbic acid in 100 mL distilled water.

3.71 This solution is stable for about 1 week if refrigerated.

3.8 Sodium hydroxide, 5 N. Dissolve 200 g of sodium hydroxide, NaOH in 600 mL of distilled water.

3.81 Cool and dilute to 1 liter.

3.9 Combined reagent. Mix the above reagents in the following proportions for 100 mL combined reagent.

IMPORTANT: Mix after addition of each reagent. Allow all reagents to reach room temperature before they are mixed, and mix in the order given. If turbidity forms in the combined reagent after the addition of antimony potassium tartrate or ammonium molybdate, shake the combined reagent and let it stand for a few minutes until the turbidity disappears before proceeding. The reagent is stable for 4 hours.

3.91 50 mL 5 N sulfuric acid solution.

3.92 5 mL antimony potassium tartrate solution.

3.93 15 mL ammonium molybdate solution.

3.94 30 mL ascorbic acid solution.

3.10 Phenolphthalein indicator. Dissolve 0.5 g of phenolphthalein in a solution of 50 mL of ethyl or isopropyl alcohol and add 50 mL of distilled water.

4. STANDARDIZATION OF COLORIMETER

4.1 Prepare a 5.0 mg/L phosphorus standard solution using a volumetric pipet to deliver 100 mL of stock phosphorus solution (50 mg/L) to a 1000 mL volumetric flask and dilute to mark. This solution is stable for about six weeks if refrigerated.

4.2 Using volumetric pipets, deliver the following volumes of the standard phosphorus solution (5.0 mg/L) into separate 125 mL Erlenmeyer flasks

Flask No.	mL of 5.0 mg/L Standard	Conc. mg/L as P when diluted to 50 mL
1	0.0	0.00 (Blank)
2	2.0	0.2
3	3.0	0.3
4	4.0	0.4
5	5.0	0.5
6	6.0	0.6

Note: these standards are applicable to most wastewater samples. Lower concentrations may be used in situations requiring low level analysis.

4.3 Fill all flasks (1-6) to approximately 50 mL with distilled water.

4.4 To each flask add the following:

4.41 1 mL strong acid.

4.42 0.4 g of ammonium persulfate or 0.5 g potassium persulfate.

4.43 boiling chip(s).

4.5 Place the flasks on a preheated hot plate and boil gently for 30 to 40 minutes. Do not boil below 10 mL.

4.6 Cool and dilute to about 30 mL with distilled water.

4.7 Add 1 drop of phenolphthalein indicator solution and neutralize to a faint pink color with 5 N sodium hydroxide, added drop-wise. Mix well after each addition of hydroxide solution.

4.8 Add 5 N sulfuric acid drop-wise to just discharge the pink color.

4.9 Transfer to 50 mL volumetric flask. Rinse boiling flask with distilled water and add to 50 mL volumetric flask, being careful to not exceed 40 mL.

4.10 Add 8.0 mL of combined reagent to each flask using a volumetric pipet. Dilute to volume with distilled water, cap, and mix thoroughly.

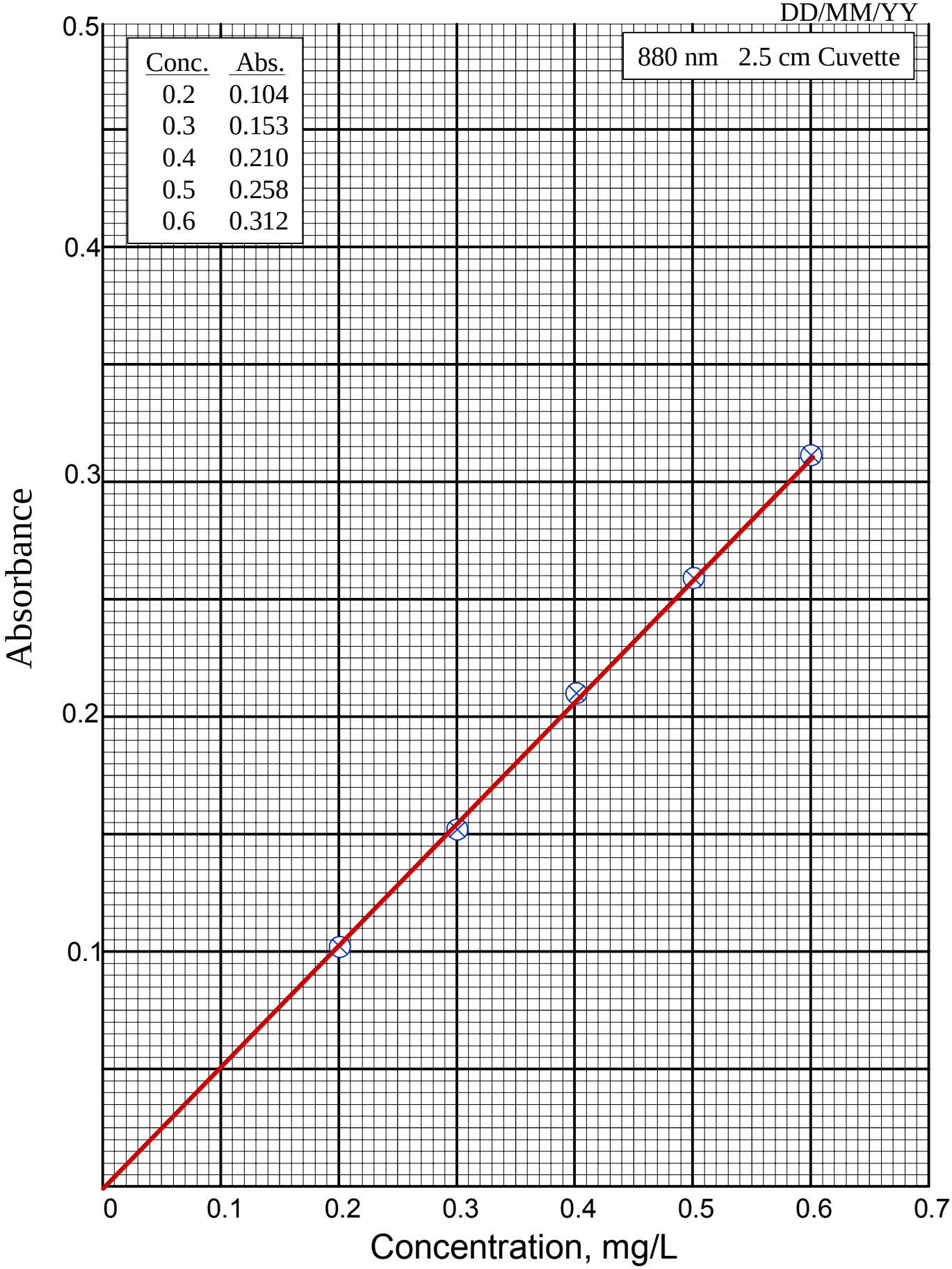
4.11 Allow at least 10 minutes but no more than 30 minutes for color development.

4.12 Using the reagent blank to zero the instrument, determine the absorbance for each standard at 880 nm.

4.13 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations. (See example standard curve on next page.)

Total Phosphorus Ascorbic Acid – Single Reagent Method

DD/MM/YY



Conc.	Abs.
0.2	0.104
0.3	0.153
0.4	0.210
0.5	0.258
0.6	0.312

5. PROCEDURE

5.1 Prepare a reagent blank and one standard following the steps given in Section 4 "Standardization of Colorimeter".

5.2 Being sure to mix each sample well, carefully measure 100 mL of each sample into separate 100 mL graduated cylinders.

5.3 Pour each sample into separate 250 mL Erlenmeyer flasks and rinse the graduated cylinder with distilled water, adding the rinse to the flask.

5.4 To each flask add the following:

5.41 2 mL strong acid.

5.42 0.8 g of ammonium persulfate or 1.0 g potassium persulfate.

5.43 boiling chip(s).

5.5 Place the flasks on a preheated hot plate and gently boil each sample 30 to 40 minutes and until the total volume has been reduced to 75 mL.

5.6 Filter each sample that is turbid or contains visible solids.

5.61 Use filter paper that has been rinsed with distilled water.

5.62 Filter into the 100 mL graduated cylinder that was used for sample measurement, being sure to rinse each flask to remove all solids.

5.63 Following sample filtration, rinse filter paper with distilled water into the graduated cylinder.

5.7 Return each sample to the Erlenmeyer flask used for digestion.

5.8 Add 1 drop phenolphthalein indicator solution to each flask and neutralize to a faint pink color with 5 N sodium hydroxide solution, added drop-wise. Mix well after each addition of hydroxide solution.

5.9 Add 5 N sulfuric acid solution, H_2SO_4 drop-wise to just discharge the pink color.

5.10 Carefully bring the volume of each sample back to 100 mL with distilled water in

the graduated cylinder.

5.11 Return each sample to the digestion flask and mix well by swirling.

5.12 Deliver two different volumes of each sample with volumetric pipets into separate 50 mL volumetric flasks.

5.121 Use volumes such that at least one dilution gives an absorbance reading on the linear portion of the standard curve. (See step 5.161)

5.122 Volumes selected should not exceed 42 mL since volume is needed for 8.0 mL of combined reagent.

5.123 Dilution factor $= \dfrac{50 \text{ mL}}{\text{Volume of wastewater sample put into 50 mL vol. flask}}$

5.124 Record volumes used and dilution factors on bench sheet.

5.13 Add 8.0 mL of combined reagent to each flask using a volumetric pipet. Dilute to volume with distilled water, cap, and mix thoroughly.

5.14 Allow at least 10 minutes but no more that 30 minutes for color development.

5.15 Using the reagent blank to zero the instrument, determine the absorbance of each sample and standard at 880 nm.

5.16 Obtain concentration results by referring absorbance readings to the previously constructed standard curve.

5.161 Use only absorbance readings that fall between the absorbance readings for lowest and highest standard concentrations used in preparing the calibration curve.

5.162 Use the results for the standard to verify the standard curve. It is recommended that action is taken immediately to determine and correct the source of variances greater than 10%.

5.163 Multiply sample results taken from standard curve by appropriate dilution factor.

5.164 Results are mg/L Total Phosphorus as P.

QA/QC Recommendations for Total Phosphorus Analysis

1. Vary the concentration of the standard used to verify the standard curve so that the entire concentration range will be covered.

2. Periodically run recovery analysis on each type of sample analyzed (see following procedure).

3. Periodically run duplicate analysis on each type of sample analyzed.

4. Analyze a reference sample obtained from an outside source once or twice each year.

5. Split sample with another lab once or twice each year.

6. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.

7. Control charts should be prepared for each type of QA/QC analysis done.

PROCEDURE FOR DETERMINATION OF PERCENT RECOVERY OF PHOSPHORUS ANALYSIS

1. When preparing regular samples for phosphorus analysis measure out an additional 100 mL sample in a 100 mL graduated cylinder that duplicates a sample already set up.
2. Using a volumetric pipet, add 1.0 to 4.0 mL of a 50 mg/L phosphorus standard to the sample. This spikes the sample with an additional 0.5 - 2.0 mg/L of phosphorus, respectively.
3. Take the spiked sample through the same digestion and analysis procedures as the other samples and determine the total concentration of phosphorus. (Note: bring the sample up to 100 mL after digestion.)
4. Determine the percent of phosphorus that was recovered of the amount that was added using the following formulas:

mg/L spiked into sample = mL of standard added x 0.5

$$\% R = \frac{\text{conc. of sample with spike - conc. of sample}}{\text{mg/L spiked into sample}} \times 100\%$$

Note 1: While 100% is perfect recovery, 90-110% is generally acceptable; outside this range check for possible errors in procedure or technique.

Note 2: The volume of standard used for the spike and the source of the sample (influent, effluent, etc.) should be varied frequently.

Example: If 4.0 mL of 50 mg/L standard is added to 100 mL of influent and the following results are obtained, percent recovery is calculated as shown.

Influent sample = 4.0 mg/L

Influent sample + spike = 6.2 mg/L

mg/L spiked into sample = 4.0 mL x 0.5 = 2.0 mg/L

$$\% \text{ Recovery} = \frac{6.2 \text{ mg/L} - 4.0 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= \frac{2.2 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= 110\%$$

TOTAL PHOSPHORUS

Ascorbic Acid Method - Two Reagent

DISCUSSION - Ammonium molybdate and antimony potassium tartrate react in acid medium with dilute solutions of phosphorus to from a complex that is reduced to intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration. This two reagent method is acceptable for most wastewater samples, however the single reagent method (page 331-1) is preferred when analyzing for low levels of phosphorus (< about 0.2 mg/L) or in situations where a high level of sensitivity is desired.

REFFERENCE - This conforms to the following EPA-approved procedure.

Methods for Chemical Analysis of Water and Wastes, US Environmental Protection Agency, EPA-600/4-79-020, Revised March 1983, Method 365.3

SAMPLING - Sample bottles should be washed with a phosphate free cleaning agent and rinsed with a 10% hydrochloric acid solution. Samples may be stored up to 28 days if refrigerated to ≤ 6° C and acidified with H_2SO_4 to pH <2.

1. APPARATUS

   1.1 Spectrophotometer - for use at 650 or 880 nm, providing a light path of 1 cm or longer.

   1.2 Acid-washed glassware: All glassware, including sample containers, should be cleaned in warm water containing phosphate free detergent, rinsed with a 10% hydrochloric acid solution, and then rinsed thoroughly with distilled water. Reserve this glassware for only phosphorous analysis.

2. INTERFERENCES

   2.1 Arsenate is determined similarly to phosphorus and should be considered when present.

   2.2 When high concentrations of iron are present low recovery of phosphorus will

be obtained because it will use some of the reducing agent.

3. REAGENTS

3.1 Ammonium molybdate- antimony potassium tartrate solution: Dissolve 8 g of ammonium molybdate and 0.2 g antimony potassium tartrate in 800 mL of distilled water and dilute to 1 liter.

3.2 Ascorbic acid solution: Dissolve 30 g ascorbic acid in 400 mL distilled water and dilute to 500 mL. Add 1 mL acetone.

3.21 This solution is stable for about 2 weeks.

3.3 Sulfuric acid, 11 N: Slowly add 310 mL of concentrated sulfuric acid, H_2SO_4 to approximately 600 mL of distilled water. Cool and dilute to 1 liter.

Note: Be sure to add the ACID TO THE WATER.

3.4 Ammonium persulfate, $(NH_4)_2S_2O_8$. (Used as a solid)

3.5 Stock phosphorus solution, 100 mg/L as P. Dissolve 0.4393 g of pre-dried (105°C for one hour) potassium phosphate monobasic, KH_2PO_4 in distilled water and dilute to 1 liter in a volumetric flask.

3.6 Standard phosphorus solution, 5.0 mg/L as P. Using a volumetric pipet, deliver 50 mL of the stock phosphorus solution (100 mg/L) to a 1000 mL volumetric flask and dilute to mark.

4. STANDARDIZATION OF COLORIMETER

4.1 Using volumetric pipets, deliver the following volumes of the standard phosphorus solution (5.0 mg/L) into separate 50 mL volumetric flasks.

Flask No.	mL of 5.0 mg/L Standard	Conc. mg/L as P when diluted to 50 mL
1	0.0	0.00 (Blank)
2	2.0	0.2
3	3.0	0.3
4	4.0	0.4
5	5.0	0.5
6	6.0	0.6

Note: these standards are applicable to most wastewater samples. Other concentrations may be used in the linear range of analysis.

4.2 Fill all flasks (1-6) to volume with distilled water.

4.3 Add 1 mL of 11 N sulfuric acid (Step 3.3).

4.4 Add 4 mL of ammonium molybdate- antimony potassium tartrate solution (Step 3.1), cap, and mix.

4.5 Add 2 mL of ascorbic acid solution (Step 3.2), cap, and mix thoroughly.

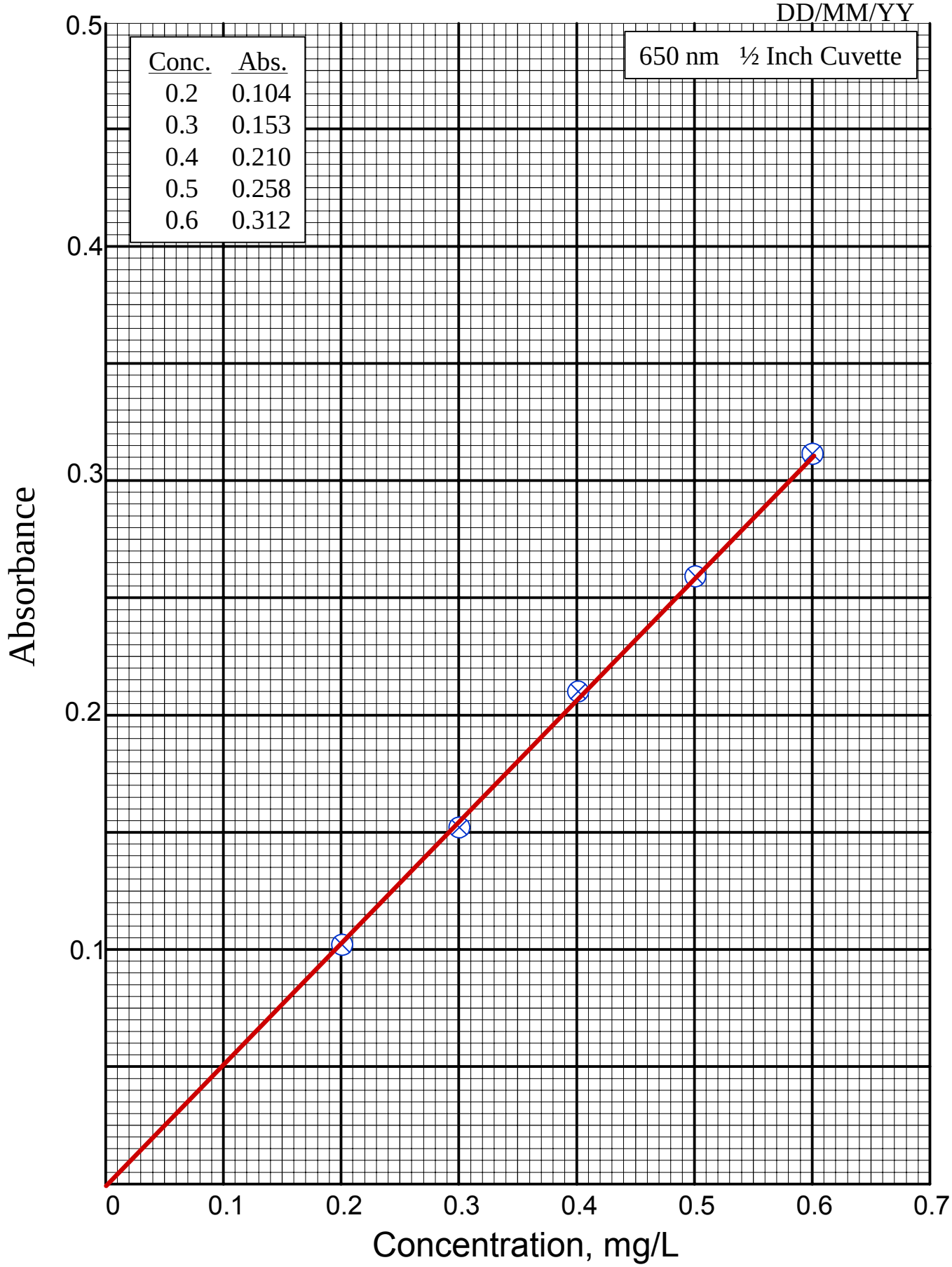
4.6 Allow at least 5 minutes but no more than 1 hour for color development.

4.7 Using the reagent blank to zero the instrument, determine the absorbance for each standard at 650 nm.

4.8 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phosphorus concentrations. (See example standard curve on next page.)

Total Phosphorus Ascorbic Acid – Two Reagent Method

DD/MM/YY



Conc.	Abs.
0.2	0.104
0.3	0.153
0.4	0.210
0.5	0.258
0.6	0.312

5. PROCEDURE

5.1 Prepare a reagent blank and one standard following the steps given in Section 4 "Standardization of Colorimeter".

5.2 Being sure to mix each sample well, carefully measure 100 mL of each sample into separate 100 mL graduated cylinders.

5.3 Pour each sample into separate 250 mL Erlenmeyer flasks and rinse the graduated cylinder with distilled water, adding the rinse to the flask.

5.4 To each flask add the following:

5.41 2 mL 11 N sulfuric acid (Step 3.3).

5.42 0.8 g of ammonium persulfate.

5.43 boiling chip(s).

5.5 Place the flasks on a preheated hot plate and gently boil each sample 30 to 40 minutes and until the total volume has been reduced to 75 mL.

5.6 Filter each sample that is turbid or contains visible solids.

5.61 Use filter paper that has been rinsed with distilled water.

5.62 Filter into the 100 mL graduated cylinder that was used for sample measurement, being sure to rinse each flask to remove all solids.

5.63 Following sample filtration, rinse filter paper with distilled water into the graduated cylinder.

5.10 Carefully bring the volume of each sample back to 100 mL with distilled water in the graduated cylinder.

5.11 Return each sample to the digestion flask and mix well by swirling.

5.12 Deliver two different volumes of each sample with volumetric pipets into separate 50 mL volumetric flasks.

5.121 Use volumes such that at least one dilution gives an absorbance reading

on the linear portion of the standard curve.

5.122 Dilution factor = $\frac{50 \text{ mL}}{\text{Volume of wastewater sample put into 50 mL vol. flask}}$

5.123 Record volumes used and dilution factors on bench sheet.

5.13 Dilute to volume with distilled water,

5.14 Add 4 mL of ammonium molybdate- antimony potassium tartrate solution (Step 3.1), cap and mix.

4.10 Add 2 mL of ascorbic acid solution (Step 3.2), cap, and mix thoroughly.

5.14 Allow at least 5 minutes but no more that 1 hour for color development.

5.15 Using the reagent blank to zero the instrument, determine the absorbance of each sample and standard at 650 nm.

5.16 Obtain concentration results by referring absorbance readings to the previously constructed standard curve.

5.161 Use only absorbance readings that fall between the absorbance readings for lowest and highest standard concentrations used in preparing the calibration curve.

5.162 Use the results for the standard to verify the standard curve. It is recommended that action is taken immediately to determine and correct the source of variances greater than 10%.

5.163 Multiply sample results taken from standard curve by appropriate dilution factor.

5.164 Results are mg/L Total Phosphorus as P.

QA/QC Recommendations for Total Phosphorus Analysis

1. Vary the concentration of the standard used to verify the standard curve so that the entire concentration range will be covered.

2. Periodically run recovery analysis on each type of sample analyzed (see following procedure).

3. Periodically run duplicate analysis on each type of sample analyzed.

4. Analyze a reference sample obtained from an outside source once or twice each year.

5. Split sample with another lab once or twice each year.

6. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.

7. Control charts should be prepared for each type of QA/QC analysis done.

PROCEDURE FOR DETERMINATION OF PERCENT RECOVERY OF PHOSPHORUS ANALYSIS

1. When preparing regular samples for phosphorus analysis measure out an additional 100 mL sample in a 100 mL graduated cylinder that duplicates a sample already set up.
2. Using a volumetric pipet, add 1.0 to 4.0 mL of a 50 mg/L phosphorus standard to the sample. This spikes the sample with an additional 0.5 - 2.0 mg/L of phosphorus, respectively.
3. Take the spiked sample through the same digestion and analysis procedures as the other samples and determine the total concentration of phosphorus. (Note: bring the sample up to 100 mL after digestion.)
4. Determine the percent of phosphorus that was recovered of the amount that was added using the following formulas:

mg/L spiked into sample = mL of standard added x 0.5

$$\% R = \frac{\text{conc. of sample with spike - conc. of sample}}{\text{mg/L spiked into sample}} \times 100\%$$

Note 1: While 100% is perfect recovery, 90-110% is generally acceptable; outside this range check for possible errors in procedure or technique.

Note 2: The volume of standard used for the spike and the source of the sample (influent, effluent, etc.) should be varied frequently.

Example: If 4.0 mL of 50 mg/L standard is added to 100 mL of influent and the following results are obtained, percent recovery is calculated as shown.

Influent sample = 4.0 mg/L

Influent sample + spike = 6.2 mg/L

mg/L spiked into sample = 4.0 mL x 0.5 = 2.0 mg/L

$$\% \text{ Recovery} = \frac{6.2 \text{ mg/L} - 4.0 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= \frac{2.2 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= 110\%$$

AMMONIA NITROGEN

Two procedures are included in this manual for the analysis of ammonia nitrogen, the titrimetric method and the ion selective electrode (ISE) method. Both of these methods are EPA approved for NPDES reporting purposes, provided that samples have been distilled prior to the analytical procedure. The procedure for the distillation step has also been included in the manual.

While the titration method is to be used only on samples that have been distilled, the EPA will allow the distillation step to be omitted in the ISE method if data is on file which shows that the distillation step is not necessary. A **suggested** procedure for making this determination is given.

There are other EPA approved methods for ammonia analysis that are not included in this manual. The nesslerization method is a colorimetric method, useful down to 0.02 mg/L. While the nesslerization method has been in use for many years in the analysis of wastewater, it does require the use of some hazardous reagents. Also, since mercury is one component of the color reagent, the method by which spent reagents will be disposed must be considered. Considering the hazards involved, the use of mercury, and the time required to distill standards and samples, the analyst would be wise to consider either the electrode or titration methods.

The method chosen for analysis of ammonia nitrogen depends on several factors; these may include initial cost of setup, time requirements, safety hazards, spent reagent disposal, and detection limits. Some aspects of each method are listed below.

The titrimetric method is probably the least costly to set up but cannot be used for samples with ammonia nitrogen concentrations less than 1 mg/L. As stated above, the distillation step is required.

Ammonia Nitrogen

.The ion selective electrode method is probably the most often used method for ammonia nitrogen analysis in Michigan. Although it requires the purchase of a specific ion meter and ammonia electrode, there may be several advantages; the procedure is fairly simple, requires a minimal amount of time, and can be used with a wide variety of sample types and concentrations. According to Standard Methods, the method is useful from 0.03 to 1400 mg/L. Although Standard Methods also states that "sample distillation is unnecessary", the EPA requires that data be on file that shows this to be true.

Sample Handling

Discharge permits typically require that ammonia be analyzed in composite samples taken before disinfection. If necessary, destroy any residual chlorine immediately after sample collection to prevent its reaction with ammonia. If prompt analysis is impossible, preserve sample with H_2SO_4 to pH <2, and store at ≤ 6°C. Samples which have been preserved in this manner may be stored for up to 28 days. It is important that samples and standards be at room temperature before analysis.

AMMONIA NITROGEN
DISTILLATION PROCEDURE

DISCUSSION: The distillation of samples prior to analysis for ammonia nitrogen removes the ammonia from components of the sample which would present interferences. A borate buffer solution is added to the sample before distillation which buffers at a pH of 9.5. This minimizes the hydrolysis of cyanates and organic nitrogen compounds which would increase the ammonia concentration of the sample. Ammonia distilled out of the sample is absorbed into either boric acid or sulfuric acid. Boric acid must be the absorbing solution if the titration method will be used to determine ammonia nitrogen concentration; sulfuric acid must be the absorbing solution if the ion selective electrode (ISE) will be used.

While prior distillation is a requirement for the titrimetric procedure, it may be omitted under certain conditions for the ISE method. For purposes of N.P.D.E.S. reporting, the EPA requires distillation of all samples unless data on representative effluent samples are on file that show that comparable results are obtained without distillation.

1. APPARATUS

   1.1 Distillation apparatus - a borosilicate flask of 800-2000 mL capacity attached to a vertical condenser, so that the delivery tip may be submerged in the receiving solution (see diagram below).

   1.2 pH meter.

2. REAGENTS

   2.1 Ammonia-free distilled or deionized water for dilution of samples and preparation of reagents and standards.

   2.2 Borate buffer solution, pH 9.5 - Dissolve 9.5 grams Sodium Tetraborate Decahydrate, $Na_2B_4O_7 \cdot 10\ H_2O$ in distilled water and dilute to 1 liter. To

500 mL of this solution, add 88 mL of 0.1 N Sodium Hydroxide, NaOH and dilute to 1 liter.

2.3 Absorbing Solution. For the ISE method use 0.04 N sulfuric acid; for the titrimetric method use indicating boric acid.

2.31 Sulfuric Acid, 0.04 N - Dilute 1.0 mL concentrated Sulfuric Acid, H_2SO_4 to 1 liter.

2.32 Indicating Boric Acid Solution – Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

2.321 Mixed indicator solution - Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare fresh monthly.

2.4 Sodium Hydroxide, 1 N. Dissolve 40 g of sodium hydroxide, NaOH in distilled water and dilute to 1 liter.

2.5 Sodium Hydroxide, 0.1 N. Dissolve 4 g of sodium hydroxide, NaOH in distilled water and dilute to 1 liter.

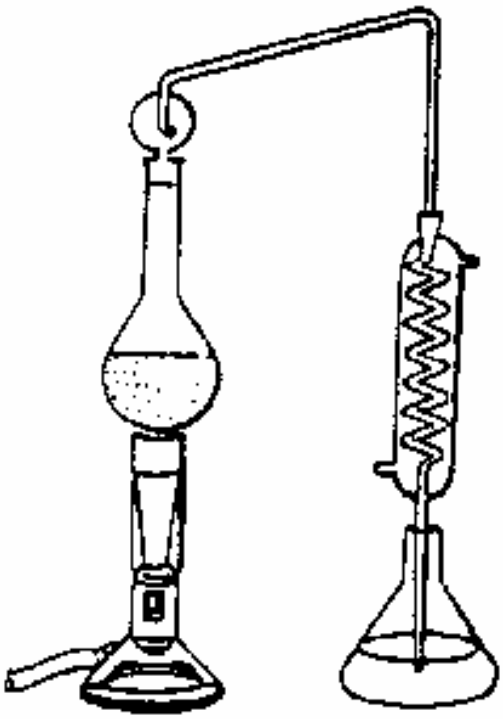
2.6 Dechlorinating agent, use only if sample contains chlorine residual.

2.61 Sodium Thiosulfate, 0.014 N. Dissolve 3.5 g of Sodium Thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$, in distilled water and dilute to 1 liter. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500 mL sample.

3. PROCEDURE

3.1 If more than 4 hours have elapsed since the last use of the distillation apparatus, add 500 mL of distilled water, 25 mL of borate buffer solution, and a few boiling chips to the distillation flask. Steam out the distillation apparatus until at least 300 mL of distillate has been collected.

3.2 Measure out 500 mL of sample or an aliquot diluted to 500 mL. If the ammonia nitrogen concentration of the sample is expected to be less than 0.1 mg/L, use a sample volume of 1000 mL. If the sample has a chlorine residual, dechlorinate using the appropriate amount of 0.014 N Sodium Thiosulfate (1 mL of 0.014 N solution removes 1 mg/L residual chlorine in 500 mL sample).



3.3 Add 25 mL of borate buffer to the sample, and adjust the pH to 9.5 with 1 N Sodium Hydroxide.

3.4 Remove the water from the steamed out flask and pour in treated sample.

3.5 Distill at a rate of 6-10 mL/min, with the tip of the delivery tube submerged in 50 mL of the absorbing solution in a 500 mL Erlenmeyer receiving flask.

3.6 Collect at least 200 mL of distillate.

3.7 Lower the receiving flask so that the end of the delivery tube no longer contacts

the liquid in the flask, and continue distilling for a couple of minutes to clean out the apparatus.

3.8 Dilute the distillate to 500 mL with ammonia-free distilled water and mix well. (NOTE: If the **titrimetric** method is to be used, it is not necessary to dilute the distillate to 500 mL; the volume of distillate collected may be titrated directly.)

AMMONIA NITROGEN
TITRIMETRIC METHOD

DISCUSSION: The concentration of ammonia-nitrogen, NH_3-N, can be determined by the following titrimetric procedure for sample concentrations above 1 mg/L. Sample dilution is necessary for concentrations over about 25 mg/L. This method may be used only on samples that have been carried through the preliminary distillation step using boric acid as the absorbing solution. The distillation step provides two essential functions:

1. It separates the ammonia from interfering substances.
2. It accomplishes the reaction between the ammonia and boric acid.

The boric acid combines with the ammonia to form ammonium and borate ions, as shown in the following equation:

$$NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$$

This reaction causes the pH to increase slightly but the use of excess boric acid holds the pH in an acceptable range for absorption of ammonia. The borate ions formed are then back titrated with acid as follows:

$$H_2BO_3^- + H \longrightarrow H_3BO_3$$

When the pH of the boric acid solution has been decreased to its original value, indicated by a color change from green to lavender, the amount of ammonia absorbed and the concentration of the ammonia in the original sample can be calculated.

Ammonia-Titration

REFERENCE

This method conforms to the EPA approved procedure referenced as Standard Methods, 20th Edition, 4500-NH_3 C. Titrimetric Method

1. APPARATUS

1.1 The distillation apparatus as listed in the ammonia distillation chapter of this manual.

1.2 pH meter.

1.3 Buret, 50 mL.

2. REAGENTS

2.1 Ammonia-free deionized water for dilution of samples and preparation of reagents and standards.

2.2 All of the reagents needed for ammonia distillation will be required.

2.3 Mixed indicator solution - Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare fresh monthly.

2.4 Indicating Boric Acid Solution – Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

2.5 Sodium carbonate, 0.02 N - Oven dry about 2 grams anhydrous sodium carbonate, Na_2CO_3, at 250^0C for 4 hours and cool in a desiccator. Dissolve 1.060 grams of the dried reagent in distilled water and make up to 1 liter in a volumetric flask.

2.6 Standard Sulfuric Acid Titrant, 0.02N

2.61 Dilute 2.8 mL of concentrated H_2SO_4 to 1 liter with deionized water.

2.62 Dilute 200 mL of this solution to 1 liter with deionized water.

2.7 Ammonia-Nitrogen Standard Solution, 1000 mg/L - Dry about 5 g anhydrous ammonium chloride, NH_4Cl, at 100^oC. Dissolve 3.819 g in deionized water and dilute to 1000 mL. 1.00 mL = 1.00 mg NH_3-N.

3. STANDARDIZATION OF 0.02N SULFURIC ACID

3.1 Using a volumetric pipet, place 25.0 mL of 0.02 N Sodium Carbonate, Na_2CO_3, in a 125 mL Erlenmeyer flask.

3.2 Titrate with 0.02N sulfuric acid until pH reaches 4.5, using a calibrated pH meter to detect the endpoint.

3.4 If 25 mL, plus or minus 1 mL, of sulfuric acid is used in the standardization the acid may be used for the titration of ammonia nitrogen. If it is outside this range the actual normality of H_2SO_4 is used in the final calculation.

3.5 To calculate normality of the sulfuric acid, use the following formula:

$$\text{Normality of sulfuric acid} = \frac{25 \times 0.02}{\text{mL of } H_2SO_4 \text{ titrated}}$$

4. PROCEDURE

4.1 Follow the procedure for distillation of ammonia samples included in this manual, using the indicating boric acid solution to absorb the distillate.

4.2 NOTE: it is not necessary to dilute the distillate up to 500 mL; the titration may be done in the erlenmeyer flask using the volume of distillate collected.

4.3 Titrate the distillate with standard 0.02N Sulfuric Acid titrant until the indicator turns from green to pale lavender.

4.4 Carry a blank through all steps of the procedure and apply the necessary correction to the results. Match the end point of each sample to the titrated blank.

5. CALCULATIONS

5.1 Using sulfuric acid in acceptable standardization range:

$$NH_3\text{-N, mg/L} = \frac{(A - B) \times 280}{\text{mL sample distilled}}$$

5.2 Using actual normality of sulfuric acid:

$$NH_3\text{-N, mg/L} = \frac{(A - B) \times (\text{normality of } H_2SO_4) \times (14000)}{\text{mL sample distilled}}$$

Where: A = mL H_2SO_4 used for Sample titration

B = mL H_2SO_4 used for Blank titration

QA/QC Recommendations for Ammonia-Nitrogen Analysis by Titration

1. Periodically run recovery analysis on each type of sample analyzed (see procedure below).
2. Periodically run duplicate analysis on each type of sample analyzed.
3. Analyze a reference sample obtained from an outside source at least once or twice each year.
4. Split sample with another lab once or twice each year.
5. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.
6. Quality Control Charts should be prepared for each type of QA/QC analysis.

Procedure for Determination of Percent Recovery of Ammonia Analysis by Titration

1. When preparing regular samples for ammonia analysis measure out an additional 500 mL sample that duplicates a sample already set up.
2. Using a volumetric pipet, add a volume of standard solution that will approximately double the ammonia-nitrogen concentration in the sample. This can be determined as follows:
   - 2.1 For sample concentrations of 1 to 20 mg/L, add 0.5 to 10.0 mL of a 1000 mg/L ammonia-nitrogen standard.
   - 2.2 For sample concentrations over 20 mg/L, dilute sample to within the 1 to 20 mg/L range and treat as above. Be sure to consider this dilution in calculating the final concentration.
3. Take the spiked sample through the same distillation and analysis procedures as

the other samples and determine the total ammonia-nitrogen concentration.

4. Determine the percent ammonia-nitrogen that was recovered of the amount that was added using the following formulas:

$$\text{mg/L spiked into sample} = \frac{\text{conc. of stnd. added x mL of stnd. added}}{\text{500 mL}}$$

$$\text{percent recovery} = \frac{\text{total conc. sample with spike - conc. sample}}{\text{mg/L spiked into sample}} \times 100\%$$

NOTE 1: While 100% is perfect recovery, 90-110% is generally considered to be acceptable; outside this range check for possible errors in procedure or technique. Specific control limits calculations are explained in the QA/QC discussion of this manual.

NOTE 2: The volume of standard used for the spike and the source of the sample (influent, effluent, etc.) should be varied frequently.

Example: If 5.0 mL of 1000 mg/L standard is added to 500 mL of influent and the following results are obtained, the percent recovery is calculated as shown.

Influent Sample = 9.6 mg/L

Influent Sample & Spike = 19.4 mg/L

$$\text{mg/L Spiked into Sample} = \frac{\text{1000 mg/L x 5.0 mL}}{\text{500 mL}} = \text{10.0 mg/L}$$

$$\text{Percent Recovery} = \frac{\text{19.4 mg/L - 9.6 mg/L}}{\text{10.0 mg/L}} \times 100\%$$

$$= \frac{\text{9.8 mg/L}}{\text{10.0 mg/L}} \times 100\%$$

$$= 98.0\%$$

AMMONIA-NITROGEN
ION SELECTIVE ELECTRODE

The ammonia nitrogen ion selective electrode is a gas sensing electrode. In the procedure, NaOH is added to samples to bring to pH up to at least 11. This causes a release of ammonia gas from the solution. The ammonia gas diffuses through the electrode membrane and causes a change in the pH of the electrode filling solution. This change in pH is detected by the electrode and is related to the concentration of ammonia nitrogen in the sample.

The procedure described below may be used to determine ammonia nitrogen within a sample concentration range of 0.03 mg/l to 1400 mg/l. Distillation of samples before measurement is required by the EPA unless the analyst has data on file to prove that the distillation step is unnecessary. When distilling samples which will be analyzed by this method, 0.04N H_2SO_4 should be used to trap the distillate.

As with all electrode methods, temperature is an important factor in making accurate determinations. Temperature compensation (automatic or manual) must not be used. Instead, assure that standards and samples are at the same temperature before analysis.

Consult the manufacturer's literature for information dealing with calibration and operation of the specific ion meter and electrode.

REFERENCE:

This conforms to the EPA approved procedure referenced as Standard Methods, 20th Edition, 4500-NH_3 D. Ammonia-Selective Electrode Method.

NH_3-N Electrode

1. APPARATUS

1.1 Specific ion meter.

1.2 Ammonia-selective electrode, Orion Model 95-12, EIL Model 8002-2, Beckman Model 39565, or equivalent.

1.3 Magnetic stirrer, thermally insulated, with TFE- coated stirring bar.

2. REAGENTS

2.1 Ammonia Stock Solution, 1000 mg/L as N (Orion 951007). Dissolve 3.819 g anhydrous NH_4Cl, dried at 100°C, in distilled water, and dilute to 1000 mL.

2.2 Ammonia Standard Solution, 100 mg/L as N. Pipet 10.0 mL of the 1000 mg/L stock ammonia solution into a 100 mL volumetric flask and dilute to volume with distilled water. Prepare fresh at least monthly.

2.3 Ammonia pH adjusting solution, 10 N NaOH (Orion 951211). Dissolve 400 g NaOH in 800 mL distilled water. Cool and dilute to 1000 mL with distilled water.

3. PROCEDURE

3.1 Prepare two (or more) standards that will provide accurate calibration for the expected range of sample concentrations. The concentrations of the standards used should differ by a factor of ten.

3.11 Although “Standard Methods” describes the preparation of five standards, it is widely accepted to use fewer when the approximate sample concentrations are known. Generally standards of 1.0 mg/L and 10.0 mg/L would be used for most wastewater samples. According to the electrode manufacturers, this provides accurate calibration for sample concentrations between 0.1 mg/L and 100 mg/L.

3.12 Prepare the 1.0 mg/L standard by pipeting 1.0 mL of the 100 mg/L standard into a 100 mL volumetric flask and diluting to volume with distilled water.

3.13 Prepare the 10 mg/L standard by pipeting 10.0 mL of 100 mg/L standard into a 100 mL volumetric flask and diluting to volume with distilled water.

3.2 Deliver 100 mL of the lowest standard to be used into a 150 mL beaker, add a stir bar, place on the magnetic stirrer, and insert the electrode.

3.3 Using the graduated pipet, add 1 mL of 10 N sodium hydroxide, NaOH, while slowly mixing with magnetic stirrer (pH should be above 11).

3.31 The NaOH solution must not be added to standards or samples until the electrode is in the solution to be measured.

3.4 When a stable reading is obtained, calibrate the meter to the concentration of the first standard.

3.5 Rinse electrode and immerse in 100 mL of the next standard.

3.6. Add 1 mL of 10 N sodium hydroxide, NaOH, while slowly mixing.

3.7 When a stable reading is obtained, calibrate the meter to the concentration of the second standard.

3.71 If it is difficult to obtain a stable reading during calibration, it is sometimes helpful to immediately go through the calibration procedure a second time.

3.8 Repeat steps 3.5 through 3.7 for each additional standard used.

3.9 Display electrode slope and record this value. Assure that slope is within manufacturer's guidelines.

3.10 Rinse electrode and immerse in 100 mL of sample.

3.11 Add 1 mL of 10 N sodium hydroxide while stirring.

3.12 When a stable reading is obtained, record the concentration of the sample.

3.13 Repeat steps 3.10, 3.11, and 3.12 for each sample.

3.14 Consult manufacturer's literature for instructions concerning short term storage of the electrode. For long term storage, the electrode should be disassembled, rinsed with distilled water, dried, and reassembled without the filling solution or membrane.

Ammonia-Nitrogen Recovery Analysis

Determination of percent recovery should be performed on ammonia-nitrogen analyses as part of the laboratory quality control program. This procedure outlines the steps required in making that determination.

A. PROCEDURE

1. Analyze a sample for ammonia as described.
2. Without removing the electrode from the beaker containing the sample, use a volumetric pipet to add a suitable volume of the 100 mg/L standard into the beaker. Use 10-15 mL for influent samples, and 1-5 mL for effluent samples.
3. Do not add more sodium hydroxide after the addition of standard.
4. Record the meter reading when it is stable.
5. Determine the percent recovery using the formula below.

B. CALCULATION

In this analysis, the addition of standard will dilute the sample to some extent. The formula given here will account for this added volume.

$$\frac{(C_{ms} \times V_{ms}) - (C_m \times V_m)}{C_s \times V_s} \times 100\% = \text{Percent Recovery}$$

Where:

C_{ms}	=	mg/L of NH_3-N after spiking
V_{ms}	=	mL sample + mL standard
C_m	=	mg/L NH_3-N before spiking
V_m	=	mL sample before spiking
C_s	=	mg/L of standard used (100 mg/L in this case)
V_s	=	mL standard added to sample

Alternate Procedure Using a Micropipet

The use of a micro-pipet is encouraged, since these do not add a significant amount of volume during the spiking procedure, allowing for easier calculation of percent recovery. Disregard the volume of sodium hydroxide solution in the calculation, since this is added equally to samples and standards.

1. Analyze a sample for ammonia as described.
2. With the electrode still in the sample, use a micro-pipet to add a suitable amount of the 1000 mg/L ammonia standard to the analyzed sample. When a stable reading is obtained, record the concentration of the sample and spike.
3. As shown below, each 1.0 microliter (µL) of the standard spikes the 100 mL sample with 0.01 mg/L NH_3.

   0.001 mL X 1000 mg/1000 mL = 0.001 mg NH_3 added for each µL added.

   $$\frac{0.001 \text{ mg } NH_3}{100 \text{ mL Sample}} = \frac{0.01 \text{ mg } NH_3}{1000 \text{ mL}} = 0.01 \text{ mg/L}$$

4. Determine percent recovery as follows:

   Amount Spiked into 100 mL sample = µL of 1000 mg/L standard added x 0.01

   $$\% \text{ Recovery} = \frac{(\text{Sample + Spike, mg/L}) - (\text{Sample, mg/L})}{\text{Amount Spiked, mg/L}} \times 100\ \%$$

Example: 200 µL of the 1000 mg/L NH_3 standard are added to 100 mL of sample. The sample concentration had been determined to be 2.24 mg/L, and the sample with the spike in it was analyzed at 4.36 mg/L.

Sample = 2.24 mg/L NH_3
Sample + Spike = 4.36 mg/L

Amount Spiked into sample = 200 µL x 0.01 = 2.0 mg/L

$$\% \text{ Recovery} = \frac{4.36 \text{ mg/L} - 2.24 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= \frac{2.12 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= 106\% \text{ Recovery}$$

Note 1: While 100% is perfect recovery, 90-110% is generally considered acceptable; outside this range check for possible errors in procedure or technique.

Note 2: The volume of standard used for the spike should be varied frequently.

AMMONIA-NITROGEN
ION SELECTIVE ELECTRODE
Known Addition Method

This method uses the process of known addition of one standard, rather than determining a calibration curve with a series of standards. Accurate measurement using this method requires that sample concentration at least double as a result of the standard addition, so sample concentration must be known to within a factor of 3.

This procedure may be used to determine ammonia nitrogen to a lower limit of 0.8 mg/l. Distillation of samples before measurement is required by the EPA unless the analyst has data on file to prove that the distillation step is unnecessary. When distilling samples which will be analyzed by this method, 0.04N H_2SO_4 should be used to trap the distillate.

As with all electrode methods, temperature is an important factor in making accurate determinations. Temperature compensation (automatic or manual) must not be used. Instead, assure that standards and samples are at the same temperature before analysis.

Consult the manufacturer's literature for information dealing with calibration and operation of the specific ion meter and electrode.

REFERENCE:

This conforms to the EPA approved procedure referenced as Standard Methods, 20th Edition, 4500-NH_3 E. Ammonia-Selective Electrode Using Known Addition.

1. APPARATUS

1.1 Specific ion meter with ammonia selective electrode.

1.2 Beakers - plastic or glass, 150 mL, one for each sample.

1.3 Volumetric flasks, one 1000 mL, one 100 mL.

1.4 Volumetric pipet, 10 mL.

1.5 Graduated pipet, 5 mL.

1.6 Magnetic stirrer with stir bars.

2. REAGENTS

2.1 Ammonia Stock Solution, 1000 mg/L as N (ThermoOrion 951007 or equivalent).

2.11 Dissolve 3.819 g anhydrous NH_4Cl, dried at 100^oC, in distilled water, and dilute to 1000 mL. 1.00 mL = 1.00 mg N. Prepare fresh at least every six months.

2.2 Ammonia Standard Solution, 100 mg/L as N

2.21 Pipet 10.0 mL of the 1000 mg/L stock ammonia solution into a 100 mL volumetric flask and dilute to volume with distilled water. 1.00 mL = 0.10 mg N. Prepare fresh at least weekly.

2.3 Ammonia pH adjusting solution, 10 N NaOH (ThermoOrion 951211 or equivalent).

2.31 Dissolve 400 g NaOH in 800 mL distilled water.

2.32 Add 45.2 g ethylenediaminetetracetic acid, tetrasodium salt, tetrahydrate ($Na_4EDTA{\cdot}4H_20$) and stir to dissolve. Cool and dilute to 1000 mL.

3. PROCEDURE

3.1 Determine the slope of the electrode at least every two weeks using the procedure at the end of this method.

3.2 Assure that samples and standard(s) are at room temperature.

3.3 Measure 100 mL sample using a graduated cylinder and place in a 150 mL beaker. Add a stir bar and stir continuously on a magnetic stirrer.

3.4 Add 1 mL of the pH adjusting solution and immediately immerse the electrode.

3.5 Set the meter to read millivolts. When the meter reading stabilizes, record the millivolt reading as E_1. Leave the electrode in this sample.

3.6 Pipet 10.0 mL of 100 mg/L standard solution into the sample, and record the new millivolt reading when it stabilizes. Record this as E_2.

3.7 Note: Many modern digital ISE meters have the capability of performing the known addition calculations, and report the sample concentration directly in mg/L.

4. CALCULATION

4.1 Determine the difference between E_1 and E_2.

$$\Delta E = E_1 - E_2$$

4.2 From the Known Additions Table at the end of this discussion (or the equation following the table), find the concentration ratio, Q, that corresponds to change in potential ΔE, for the slope of the electrode being used (choose the slope from the table closest to actual slope).

4.3 Determine the concentration of the original sample by multiplying Q by the concentration of the added standard:

$$C_o = Q \times C_s$$

C_o = Original Sample Concentration

Q = Concentration Ratio from Table

C_s = Concentration of Standard Added

Example:

The millivolt reading for a sample is -221.2. After addition of 10 mL of 100 mg/L standard, the mV reading is -255.3 mV. Calculate the concentration of the sample, given an electrode slope of -59.2 mV/decade.

$\Delta E = E_1 - E_2$ ΔE = 255.3 mV – 221.2 mV = 34.1 mV

Concentration ratio, Q, for 34.1 mV at 59.2 mV/decade slope = 0.0319

Sample Concentration, mg/L N = Q X C_s = 0.0319 X 100 mg/L = 3.19 mg/L

Determination of Electrode Slope

1. Place 100 mL distilled water into a 150 mL beaker, and add 2 mL pH-adjusting ISA. Stir continuously on a magnetic stirrer. Set the meter to the mV mode.
2. Rinse electrode with distilled water and place in the solution prepared above.
3. Pipet 1.0 mL of the 1000 mg/L ammonium-N standard into the beaker. When a stable reading is displayed, record the electrode potential in millivolts.
4. Pipet 10.0 mL of the same standard into the same beaker. When a stable reading is displayed, record the electrode potential in millivolts.
5. Subtract the first potential reading from the second one. The difference gives the electrode slope. This should be in the range of -54 to -60 mV/decade when the solution temperature is between 20-25 °C. If the potential is not within this range, refer to the troubleshooting section of the electrode manual.

Known Addition Table for an added volume one-tenth the sample volume. Slopes (in the column headings) are in units of mV/decade.

ΔE	Q, Concentration Ratio			
Monovalent	**(-57.2)**	**(-58.2)**	**(-59.2)**	**(-60.1)**
5.0	0.2894	0.2933	0.2972	0.3011
5.2	0.2806	0.2844	0.2883	0.2921
5.4	0.2722	0.2760	0.2798	0.2835
5.6	0.2642	0.2680	0.2717	0.2754
5.8	0.2567	0.2604	0.2640	0.2677
6.0	0.2495	0.2531	0.2567	0.2603
6.2	0.2436	0.2462	0.2498	0.2533
6.4	0.2361	0.2396	0.2431	0.2466
6.6	0.2298	0.2333	0.2368	0.2402
6.8	0.2239	0.2273	0.2307	0.2341
7.0	0.2181	0.2215	0.2249	0.2282
7.2	0.2127	0.2160	0.2193	0.2226
7.4	0.2074	0.2107	0.2140	0.2172
7.6	0.2024	02.056	0.2088	0.2120
7.8	0.1975	0.2007	0.2039	0.2023
8.0	0.1929	0.1961	0.1992	0.2023
8.2	0.1884	0.1915	0.1946	0.1977
8.4	0.1841	0.1872	0.1902	0.1933
8.6	0.1800	0.1830	0.1860	0.1890
8.8	0.1760	0.1790	0.1820	0.1849
9.0	0.1722	0.1751	0.1780	0.1809
9.2	0.1685	0.1714	0.1742	0.1771
9.4	0.1649	0.1677	0.1706	0.1734
9.6	0.1614	0.1642	0.1671	0.1698
9.8	0.1581	0.1609	0.1636	0.1664
10.0	0.1548	0.1576	0.1603	0.1631
10.2	0.1517	0.1544	0.1571	0.1598
10.4	0.1487	0.1514	0.1540	0.1567
10.6	0.1458	0.1484	0.1510	0.1537
10.8	0.1429	0.1455	0.1481	0.1507
11.0	0.1402	0.1427	0.1453	0.1479
11.2	0.1375	0.1400	0.1426	0.1451
11.4	0.1349	0.1374	0.1399	0.1424
11.6	0.1324	0.1349	0.1373	0.1398
11.8	0.1299	0.1324	0.1348	0.1373
12.0	0.1276	0.1300	0.1324	0.1348
12.2	0.1253	0.1277	0.1301	0.1324
12.4	0.1230	0.1254	0.1278	0.1301
12.6	0.1208	0.1232	0.1255	0.1278
12.8	0.1187	0.1210	0.1233	0.1256
13.0	0.1167	0.1189	0.1212	0.1235
13.2	0.1146	0.1169	0.1192	0.1214
13.4	0.1127	0.1149	0.1172	0.1194

ΔE	Q, Concentration Ratio			
Monovalent	**(-57.2)**	**(-58.2)**	**(-59.2)**	**(-60.1)**
13.6	0.1108	0.1130	0.1152	0.1174
13.8	0.1089	0.1111	0.1133	0.1155
14.0	0.1071	0.1093	0.1114	0.1136
14.2	0.1053	0.1075	0.1096	0.1118
14.4	0.1036	0.1057	0.1079	0.1100
14.6	0.1019	0.1040	0.1061	0.1082
14.8	0.1003	0.1024	0.1045	0.1065
15.0	0.0987	0.1008	0.1028	0.1048
15.5	0.0949	0.0969	0.0989	0.1009
16.0	0.0913	0.0932	0.0951	0.0971
16.5	0.0878	0.0897	0.0916	0.0935
17.0	0.0846	0.0865	0.0883	0.0901
17.5	0.0815	0.0833	0.0852	0.0870
18.0	0.0786	0.0804	0.0822	0.0839
18.5	0.0759	0.0776	0.0793	0.0810
19.0	0.0733	0.0749	0.0766	0.0783
19.5	0.0708	0.0724	0.0740	0.0757
20.0	0.0684	0.0700	0.0716	0.0732
20.5	0.0661	0.0677	0.0693	0.0708
21.0	0.0640	0.0655	0.0670	0.0686
21.5	0.0619	0.0634	0.0649	0.0664
22.0	0.0599	0.0614	0.0629	0.0643
22.5	0.0580	0.0595	0.0609	0.0624
23.0	0.0562	0.0576	0.0590	0.0605
23.5	0.0545	0.0559	0.0573	0.0586
24.0	0.0528	0.0542	0.0555	0.0569
24.5	0.0512	0.0526	0.0539	0.0550
25.0	0.0497	0.0510	0.0523	0.0536
25.5	0.0482	0.0495	0.0508	0.0521
26.0	0.0468	0.0481	0.0493	0.0506
26.5	0.0455	0.0467	0.0479	0.0491
27.0	0.0442	0.0454	0.0466	0.0478
27.5	0.0429	0.0441	0.0453	0.0464
28.0	0.0417	0.0428	0.0440	0.0452
28.5	0.0405	0.0417	0.0428	0.0439
29.0	0.0394	0.0405	0.0416	0.0427
29.5	0.0383	0.0394	0.0405	0.0416
30.0	0.0373	0.0383	0.0394	0.0405
31.0	0.0353	0.0363	0.0373	0.0384
32.0	0.0334	0.0344	0.0354	0.0364
33.0	0.0317	0.0326	0.0336	0.0346
34.0	0.0300	0.0310	0.0319	0.0328
35.0	0.0285	0.0294	0.0303	0.0312
36.0	0.0271	0.0280	0.0288	0.0297
37.0	0.0257	0.0266	0.0274	0.0283
38.0	0.0245	0.0253	0.0261	0.0269

ΔE	Q, Concentration Ratio			
Monovalent	**(-57.2)**	**(-58.2)**	**(-59.2)**	**(-60.1)**
39.0	0.0233	0.0241	0.0249	0.0257
40.0	0.0222	0.0229	0.0237	0.0245
41.0	0.0211	0.0218	0.0226	0.0233
42.0	0.0201	0.0208	0.0215	0.0223
43.0	0.0192	0.0199	0.0205	0.0212
44.0	0.0183	0.0189	0.0196	0.0203
45.0	0.0174	0.0181	0.0187	0.0194
46.0	0.0166	0.0172	0.0179	0.0185
47.0	0.0159	0.0165	0.0171	0.0177
48.0	0.0151	0.0157	0.0163	0.0169
49.0	0.0145	0.0150	0.0156	0.0162
50.0	0.0138	0.0144	0.0149	0.0155
51.0	0.0132	0.0137	0.0143	0.0148
52.0	0.0126	0.0131	0.0136	0.0142
53.0	0.0120	0.0125	0.0131	0.0136
54.0	0.0115	0.0120	0.0125	0.0130
55.0	0.0110	0.0115	0.0120	0.0124
56.0	0.0105	0.0110	0.0115	0.0119
57.0	0.0101	0.0105	0.0110	0.0114
58.0	0.0096	0.0101	0.0105	0.0109
59.0	0.0092	0.0096	0.0101	0.0105
60.0	0.0088	0.0092	0.0096	0.0101

The equation for the calculation of Q for different slopes and volume changes is given below:

$$Q = \frac{p}{(1 + p)10^{\Delta E/S} - 1}$$

where:

Q = concentration ratio

$\Delta E = E_2 - E_1$

S = slope of the electrode

$$p = \frac{\text{volume of standard}}{\text{volume of sample}}$$

SUGGESTED METHOD FOR DEMONSTRATING COMPARABILITY OF AMMONIA ANALYSIS BY ISE WITH AND WITHOUT DISTILLATION

1. Each sample is divided into three portions.

   Portion A to be analyzed after distillation.

   Portion B to be analyzed after distillation.

   Portion C to be analyzed without distillation.

2. Determine the standard deviation (SD) of the differences of Portion A and Portion B on twenty different samples.

3. Determine the average value of Portion A and Portion B for each sample.

4. Compare the average value of Portions A and B to the value for Portion C. If the value for Portion C is consistently within three standard deviations of the average of Portions A and B, then distillation would not be required.

SEE EXAMPLE

EXAMPLE OF DATA USED FOR COMPARABILITY DETERMINATION

DATE	PORTION A	PORTION B	DIFF. OF A & B	AVE. OF A & B
July 6	1.52	1.55	0.03	1.535
July 7	2.03	2.01	0.02	2.02
July 8	1.95	1.93	0.02	1.94
July 9	1.88	1.90	0.02	1.89
July 10	1.09	1.08	0.01	1.085
July 13	2.23	2.26	0.03	2.245
July 14	1.86	1.88	0.02	1.87
July 15	1.62	1.58	0.04	1.60
July 16	1.77	1.80	0.03	1.785
July 17	1.28	1.27	0.01	1.275
			SD 0.0095	

DATE	AVE A & B - 3 X SD (AVE - 0.0285)	PORTION C	AVE A & B + 3 X SD (AVE + 0.0285)
July 6	1.5065	1.56	1.5635
July 7	1.9915	2.02	2.0485
July 8	1.9115	1.95	1.9685
July 9	1.8615	1.91	1.9185
July 10	1.0565	1.07	1.1135
July 13	2.2165	2.23	2.2735
July 14	1.8415	1.88	1.8985
July 15	1.5715	1.61	1.6285
July 16	1.7565	1.80	1.8135
July 17	1.2465	1.29	1.3035

RESULTS OF COMPARABILITY OF AMMONIA ANALYSIS
ION SELECTIVE ELECTRODE WITH AND WITHOUT DISTILLATION

#	Date	Dist. A	Dist. B	Diff. A & B	Ave. A & B	Ave. - 3 X SD	Non-Dist. (C)	Ave. + 3 X SD	Comments	Analyst
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										

Standard Deviation	
3 X Standard Deviation	
Sum of Diff A & B	
Average of Diff A & B	

Laboratory

NPDES
APPROVED
METHOD

TOTAL KJELDAHL NITROGEN
Macro-Kjeldahl Method

DISCUSSION: This method applies to the determination of total Kjeldahl nitrogen in drinking, surface, and saline waters, domestic and industrial wastes. Samples are digested to convert nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia. Following this, the sample is distilled and the distillate analyzed for ammonia nitrogen by titration, or ion selective electrode. The results of this analysis yield the total kjeldahl nitrogen content of the sample.

Total kjeldahl nitrogen (TKN) is defined as the sum of ammonia nitrogen and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below. Ammonia nitrogen and organic nitrogen may be determined separately by distillation and analysis of the ammonia before sample digestion. The sample would be distilled again following digestion and the distillate analyzed to indicate the organic nitrogen fraction.

REFERENCE:

This method conforms to the EPA approved method referenced as Standard Methods, 20th Edition, 4500-N_{org}-B.

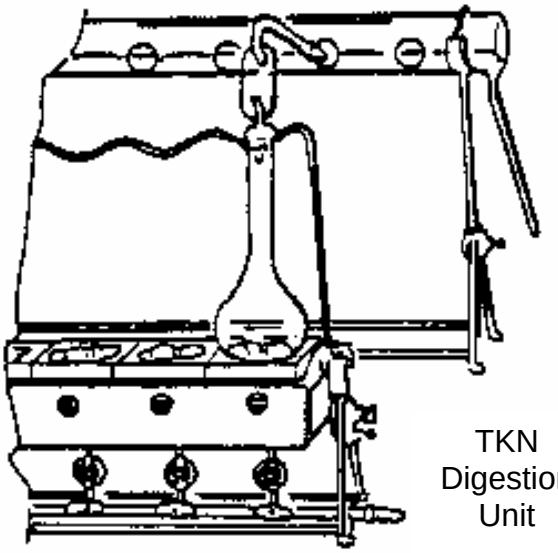
1. APPARATUS

   1.1 Kjeldahl digestion apparatus, with 800 mL flasks, heating mantles capable of heating 250 mL of distilled water to boiling in 5 minutes, and suction outlet for removal of fumes (see diagram below).

1.2 Kjeldahl distillation apparatus.

1.3 pH meter.

1.4 Equipment for measurement of ammonia nitrogen by ion selective electrode or titration.



TKN Digestion Unit

2. REAGENTS

2.1 Ammonia-free distilled and deionized water.

2.11 Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin.

2.2 Dechlorinating agent. 0.014 N. Prepare if needed. One mL of either of the following in 500 mL of sample will remove 1 mg/L of residual chlorine.

2.21 Sodium sulfite. Dissolve 0.9 g of sodium sulfite, Na_2SO_3 in ammonia-free distilled water and dilute to 1 liter. Prepare fresh daily.

2.22 Sodium thiosulfate. Dissolve 3.5 g sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ in ammonia-free distilled water and dilute to 1 liter. Prepare fresh daily.

2.3 Digestion reagent. Dissolve 134 g of potassium sulfate, K_2SO_4, and 7.3 g Copper Sulfate, $CuSO_4$ in about 800 mL deionized water. Carefully add 134 mL of concentrated sulfuric acid, H_2SO_4 . Allow the solution to cool to

room temperature and dilute to 1 liter with deionized water and mix well. Keep temperature close to 20°C to prevent crystallization.

2.4 Sodium hydroxide-sodium thiosulfate reagent. Dissolve 500 g of sodium hydroxide, NaOH and 25 g of sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ in deionized water and dilute to 1 liter.

2.5 Sodium hydroxide, 6 N. Dissolve 240 g of sodium hydroxide, NaOH in distilled water and dilute to 1 liter.

2.6 Absorbing Solution. For the ISE method use 0.04 N sulfuric acid, for the titrimetric method use indicating boric acid.

2.61 Sulfuric Acid, 0.04 N - Dilute 1.0 mL concentrated Sulfuric Acid, H_2SO_4 to 1 liter.

2.62 Indicating Boric Acid Solution – Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

2.61 Mixed indicator solution - Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare fresh monthly.

2.7 Reagents necessary for the determination of ammonia nitrogen by titration or ion selective electrode will be required.

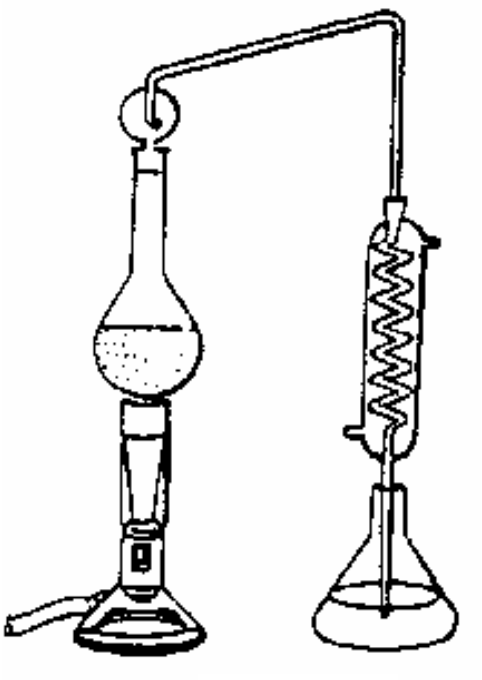
3. PROCEDURE

3.1 Preparation of distillation apparatus. If more than 4 hours has elapsed since last use of the distillation apparatus, follow the procedure below to eliminate all traces of ammonia.

3.11 Adjust the pH of 500 mL ammonia-free water to 9.5 with 6 N NaOH

and deliver this to the distillation flask.

3.12 Add boiling chips to the flask and distill until about 300 mL distillate has been collected. Discard this distillate.



Distillation Unit

3.2 If sample has a chlorine residual, dechlorinate by adding 0.014 N dechlorinating agent in an amount equivalent to the chlorine residual present.

3.3 Place a measured volume of sample, determined by the Total Kjeldahl ammonia nitrogen concentration expected, into the distillation flask. The following table may serve as a guide in selecting sample size.

Total Kjeldahl Nitrogen in Sample	Sample Size
0 - 5 mg/L	500 mL
5 - 10 mg/L	250 mL
0 - 20 mg/L	100 mL
0 – 50 mg/L	50 mL

3.31 If less than 300 mL of sample is used, dilute the sample to 300 mL with deionized water. Record mL of original sample used for later calculation of ammonia content.

3.4 Prepare a reagent blank by adding 300 mL of deionized water to a Kjeldahl distillation flask to be analyzed with the sample.

3.5 Add 50 mL of digestion reagent and a few glass beads to sample flasks and reagent blank flask.

3.6 Mix the contents of the flask, and heat until fumes of sulfur trioxides, SO_3 are observed. CAUTION: THESE FUMES ARE TOXIC. The SO_3 fumes are very dense white fumes. Assure that these fumes will be carried away by suitable ejection equipment.

3.7 Continue to boil briskly until the volume is reduced to 25 to 50 mL, then digest for an additional 30 minutes. The solution will become transparent and pale green.

3.8 Allow flask and contents to cool and dilute to 300 mL with deionized water. CAUTION: BE SURE TO COOL UNDER EJECTION APPARATUS SINCE SO_3 FUMES WILL STILL BE PRESENT.

3.9 Make the contents of the digestion flask alkaline by careful addition of 50 mL of sodium hydroxide-thiosulfate solution without mixing (assure that flask is pointed away from personnel). NOTE: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause the solution to underlay the aqueous sulfuric acid solution without loss of free-ammonia. Do not mix flask contents until the flask has been connected to the distillation apparatus.

3.10 Connect the flask to the distillation apparatus and mix the contents completely by carefully swirling the flask.

3.11 Distill and collect 200 mL of distillate, using 50 mL indicating boric acid as the absorbing solution if ammonia nitrogen will be determined by titration, or 50 mL 0.04N H_2SO_4 if using the ion selective electrode.

3.12 Following distillation, bring the distillate back to original sample volume with deionized water (if the titration method will be used, this step is not necessary). If the original sample volume digested is less than the amount of distillate collected, dilute distillate to a known volume and consider this dilution in calculation of the concentration.

3.13 Determine ammonia nitrogen in the distillate by the Titrimetric, or Ion Selective Methods. Report as Total Kjeldahl Nitrogen.

TOTAL KJELDAHL NITROGEN
SEMI-MICRO METHOD

DISCUSSION: The semi-micro total kjeldahl nitrogen method is especially well suited to wastewater and sludge samples which are highly concentrated in ammonia and organic nitrogen. Equipment often used in this procedure includes a digestion rack and a steam distillation apparatus.

Advantages to the micro TKN procedure include the ability to analyze smaller sample sizes, the use of smaller reagent quantities, less time required for analysis, and less lab space occupied by the equipment.

As with the macro TKN method, samples are digested to convert nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia. Following this, the sample is distilled and the distillate analyzed for ammonia nitrogen by titration, or ion selective electrode. The results of this analysis yield the total kjeldahl nitrogen (ammonia plus organic N) content of the sample.

REFERENCE:

This procedure conforms to the EPA approved method referenced as Standard Methods, 20th Edition, 4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. APPARATUS

   1.1 Kjeldahl digestion apparatus; 100 mL flasks, digestion rack with fume ejector for removal of fumes.

   1.2 Semi-micro Kjeldahl steam distillation apparatus.

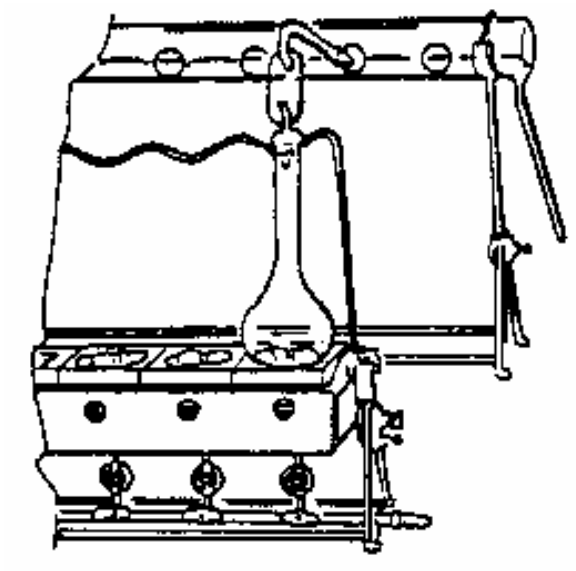
   1.3 pH meter.

   1.4 Equipment for measurement of ammonia nitrogen by ion selective

electrode or titration.

2. REAGENTS

2.1 Ammonia-free distilled and deionized water.



2.11 Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin.

2.2 Dechlorinating agent. 0.014 N.

Prepare if needed. One mL of either of the following in 500 mL of sample will remove 1 mg/L of residual chlorine.

2.21 Sodium sulfite. Dissolve 0.9 g of sodium sulfite, Na_2SO_3 in deionized water and dilute to 1 liter. Prepare fresh daily.

2.22 Sodium thiosulfate. Dissolve 3.5 g sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ in deionized water and dilute to 1 liter. Prepare fresh daily.

2.3 Digestion reagent. Dissolve 134 g of potassium sulfate, K_2SO_4, and 7.3 g Copper Sulfate, $CuSO_4$ in about 800 mL deionized water. Carefully add 134 mL of concentrated sulfuric acid, H_2SO_4 . Allow the solution to cool to room temperature and dilute to 1 liter with deionized water and mix well. Keep temperature close to 20°C to prevent crystallization.

2.4 Sodium hydroxide-sodium thiosulfate reagent. Dissolve 500 g of sodium hydroxide, NaOH and 25 g of sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$ in deionized water and dilute to 1 liter.

2.5 Sodium hydroxide, 6 N. Dissolve 240 g of sodium hydroxide, NaOH in deionized water and dilute to 1 liter.

2.6 Absorbing Solution. For the ISE method use 0.04 N sulfuric acid, for the titrimetric method use indicating boric acid.

2.61 Sulfuric Acid, 0.04 N - Dilute 1.0 mL concentrated Sulfuric Acid, H_2SO_4 to 1 liter.

2.62 Indicating Boric Acid Solution – Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

2.61 Mixed indicator solution - Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare fresh monthly.

2.7 Reagents necessary for the determination of ammonia nitrogen by titration or ion selective electrode will be required.

3. PROCEDURE

3.1 Preparation of steam distillation apparatus. If more than 4 hours has elapsed since last use of the distillation apparatus, follow the procedure below to eliminate all traces of ammonia.

3.11 Add a sufficient supply of deionized water to the steam generation flask and adjust heat to produce steam.

3.12 Adjust the pH of 50 mL deionized water to 9.5 with 6 N NaOH and

deliver this to a 100 mL distillation flask.

3.13 Add 5 or 6 glass boiling beads to the distillation flask and distill over about 30 mL of distillate. Discard this distillate.

3.2 Wastewater Samples

3.21 If sample has a chlorine residual, dechlorinate by adding 0.014 N dechlorinating agent in an amount equivalent to the chlorine residual present.

3.22 Place 50 mL of sample or an aliquot of sample, determined by the Total Kjeldahl ammonia nitrogen concentration expected, into a micro kjeldahl digestion flask. The following table may serve as a guide in selecting sample size.

Total Kjeldahl Nitrogen in Sample	Sample Size
4 - 40 mg/L	50 mL
8 - 80 mg/L	25 mL
20 - 200 mg/L	10 mL
40 - 400 mg/L	5 mL

3.23 If less than 50 mL of sample is used, dilute the sample to 50 mL with deionized water. Record mL of original sample used for later calculation of ammonia content.

3.3 Sludge Samples

3.31 Accurately weigh out about 1 gram of liquid sludge (to 4 decimal places) in a 100 mL beaker.

3.32 Transfer the weighed sludge to the micro kjeldahl digestion flask; rinse the beaker with deionized water and add this to the flask (don't exceed a total volume of about 50 mL in the flask).

3.33 Determine percent total solids on a portion of the original sample.

3.4 Prepare a reagent blank by adding 50 mL of deionized water to a micro Kjeldahl digestion flask to be analyzed with the sample.

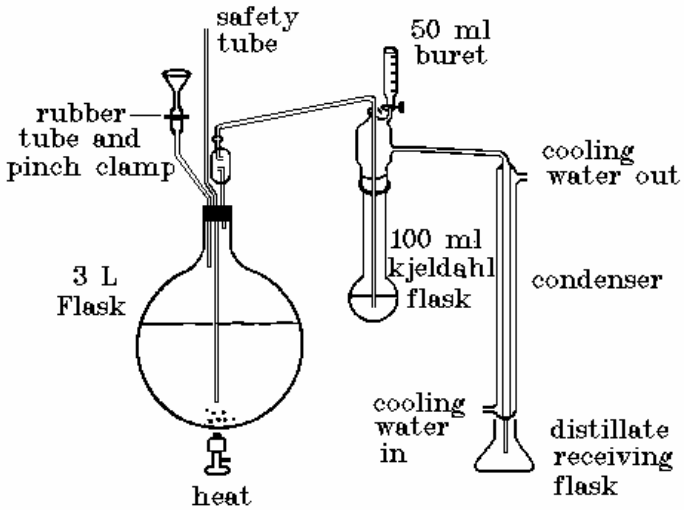
3.5 Add 10 mL of digestion reagent and 5 or 6 glass boiling beads to sample flasks and reagent flask.

3.6 Mix the contents of each flask, and heat until fumes of sulfur trioxides, SO_3 are observed.

<u>CAUTION:</u> THESE FUMES ARE TOXIC. The SO_3 fumes are very dense, white fumes. Assure that these fumes will be carried away by suitable ejection equipment.

3.7 Continue to boil briskly until the solution turns colorless or pale yellow, then digest for an additional 30 minutes.

3.8 Allow flask and contents to cool and transfer the contents to a 100 mL distillation flask. Rinse the digestion flask and add the rinse to the distillation flask.



3.9 Connect the flask to the steam distillation apparatus.

3.10 Add 10 mL of sodium hydroxide-thiosulfate solution to the buret and

dispense into the distillation flask.

3.11 Distill, collecting 30 to 40 mL of distillate, below the surface of 10 mL absorbing solution in a 125 mL erlenmeyer flask. Use indicating boric acid as the absorbing solution if ammonia nitrogen will be determined by titration, or 0.04N H_2SO_4 if using the ion selective electrode.

3.12 During the final minute of distillation, lower the receiving flask so that the delivery tube is not in contact with the distillate.

3.13 Transfer the distillate to a 100 mL volumetric flask. Rinse the receiving flask, add the rinse water to the volumetric flask, and bring up to volume with deionized water (if the titration method will be used, it is not necessary to dilute to 100 mL).

3.14 Determine ammonia nitrogen concentration in the distillate by the Titrimetric, or Ion Selective Methods. Report calculated value as Total Kjeldahl Nitrogen.

4. CALCULATIONS

4.1 Wastewater Samples.

4.11 Titrimetric Method.

$$\text{mg/L TKN} = \frac{\text{mL Acid Titrated X Normality of Acid X 14,000}}{\text{mL Sample}}$$

4.12 Ion Selective Electrode Method

$$\text{mg/L TKN} = \frac{\text{mg/L X Final Vol, mL}}{\text{Sample Vol, mL}}$$

4.2 Sludge Samples

4.21 Titrimetric Method

$$\text{mg/Kg TKN} = \frac{\text{mL Acid Titrated X Normality of Acid X 14}}{\text{Kg Wet Sludge X (\% Solids/100)}}$$

4.22 Ion Selective Electrode Method

$$\text{mg/Kg TKN} = \frac{\text{mg/L X Final Vol, mL / 1000}}{\text{Kg Wet Sludge X (\% Solids/100)}}$$

NITRITE-NITROGEN
Colorimetric Method

DISCUSSION: The diazonium compound formed by diazotization of sulfanilamide by nitrite in water under acid conditions is coupled with N - (1 - naphthyl) - ethylenediamine to produce a reddish-purple color which is read in a spectrophotometer at 543 nm.

REFFERENCE - This conforms to the following EPA-approved procedure.

Standard Methods for Examination of Water and Wastewater, 20th Edition,

Method 4500-NO_2 B.

SAMPLING - Samples should be analyzed as soon as possible after collection, but may be stored up to 48 hours if refrigerated at ≤ 6°C. Do not use acid preservation.

1. APPARATUS

   1.1 Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.

2. REAGENTS

   (Use nitrite-free water in making all reagents and dilutions.)

   2.1 Nitrite-free water. If it is not known that the distilled or demineralized water is free from nitrite, use the following procedure to prepare nitrite-free water.

      2.11 Add 1 mL conc. sulfuric acid, H_2SO_4 and 0.2 mL manganous sulfate solution (36.4 g $MnSO_4 \cong H_2O$/100 mL distilled water) to each 1 liter distilled water, and make pink with 1 to 3 mL potassium permanganate solution (400 mg $KMnO_4$/L distilled water). Redistill in an all borosilicate glass apparatus and discard the first 50 ml of distillate. Collect the distillate fraction that is free of permanganate.

2.2 Color reagent. To 800 mL of distilled water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N - (1 - napthyl) - ethylenediamine dihydrochloride. Mix to dissolve. Then dilute to 1 L with distilled water. This solution is stable for about a month when stored in a dark bottle in refrigerator

2.3 Nitrite-nitrogen stock solution - 100 mg/L NO_2-N. (The procedure for preparation of this solution is not included in this manual because of the difficulties of obtaining and standardizing an accurate Nitrite solution. The procedures are available in "Standard Methods", however it is suggested that this solution should be purchased from a reputable supplier.)

2.4 Nitrite nitrogen standard solution - 1 mg/L NO_2-N. Dilute 1.0 mL of the stock solution to 100 mL in a volumetric flask. Prepare fresh daily.

3. STANDARDIZATION OF COLORIMETER

3.1 Prepare a series of standards in 50 mL volumetric flasks as follows:

Flask No.	mL of Standard Solution 1 mg/L NO_2-N	Conc. when diluted to 50 mL mg/L of NO_2-N
1	0.0	0.00 (Blank)
2	2.0	0.04
3	3.0	0.06
4	5.0	0.10
5	7.0	0.14
6	10.0	0.20

3.2 Fill each flask to the 50 mL mark with distilled water.

3.3 Using a volumetric pipet, add 2.0 mL of color reagent to each flask.

3.4 Mix well and allow color to develop for between 10 minutes and 2 hours.

3.5 Set the spectrophotometer at 543 nm and adjust to 0.00 absorbance using the prepared blank.

3.6 Read and record the absorbance of all standards.

3.7 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding nitrite concentrations. (See example standard

curve on page following the procedure.)

4. PROCEDURE

4.1 Prepare a reagent blank and one standard following the steps given in Section 3 "Standardization of Colorimeter".

4.2 If the sample contains suspended solids, filter the sample through a 0.45 µm pore size filter using the first portion of filtrate to rinse the filter flask.

4.3 If the sample pH is not between 5 and 9, adjust to that range with 1 N hydrochloric acid, HCl, or ammonium hydroxide, NaOH, as required.

4.4 To 50.0 mL of sample, or to a portion diluted to 50.0 mL, add 2.0 mL of color reagent.

4.5 Mix well and allow color to develop for between 10 minutes and 2 hours.

4.6 Set the spectrophotometer at 543 nm and adjust to 0.00 absorbance using the prepared blank.

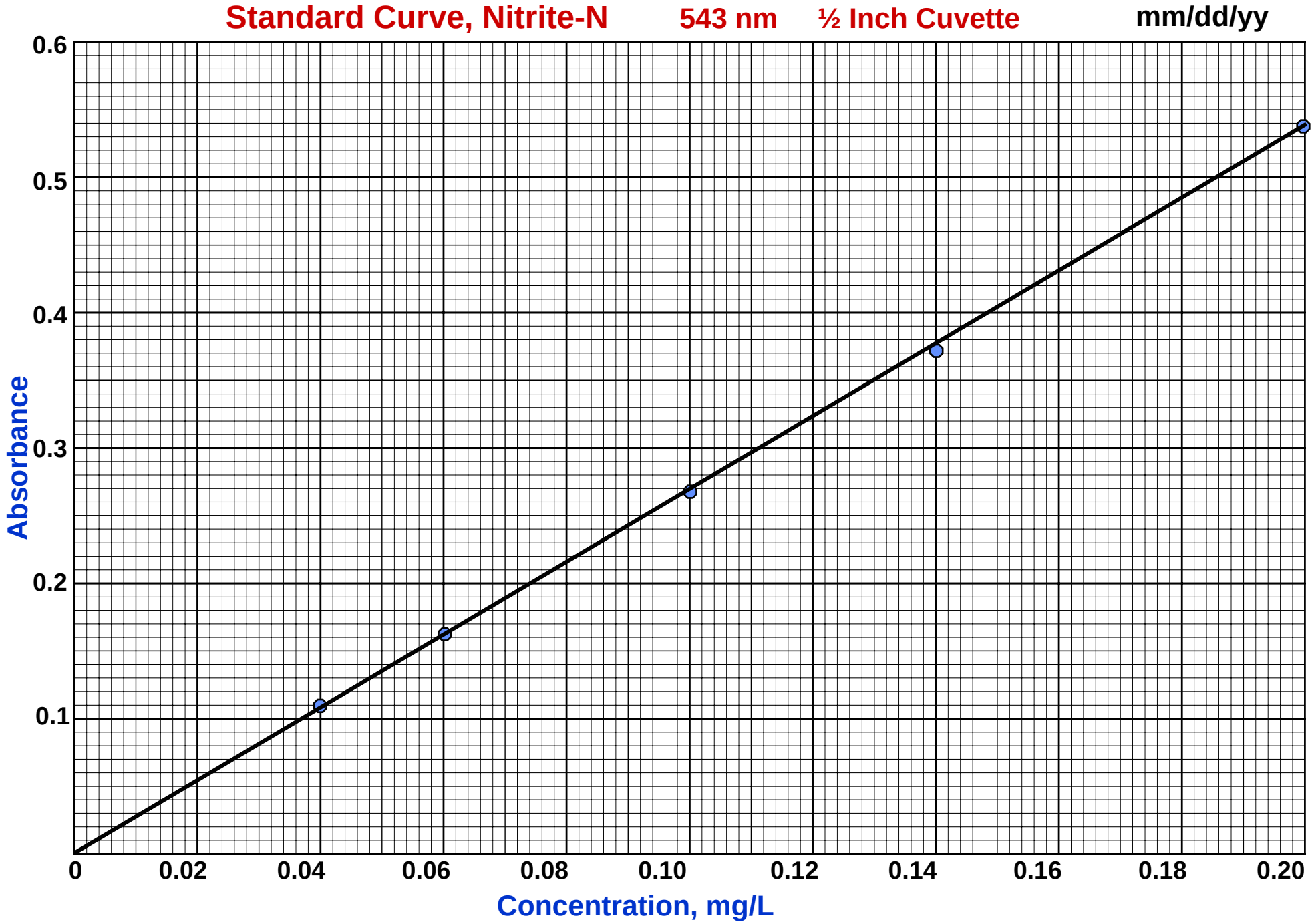
4.7 Read and record the absorbance of all standards and samples.

4.8 Obtain concentration results by referring absorbance readings to the previously constructed standard curve.

4.81 Use the results for the standard to verify the standard curve. It is recommended that action is taken immediately to determine and correct the source of variances greater than 10%.

4.82 If less than 50.0 mL of sample was used, calculate sample concentration as follows:

$$NO_2\text{-N mg/L} = \frac{\text{mg/L from std. curve x 50}}{\text{mL sample used}}$$


Standard Curve, Nitrite-N
543 nm
½ Inch Cuvette
mm/dd/yy
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
Concentration, mg/L

QA/QC Recommendations for Nitrite-Nitrogen Analysis

1. Vary the concentration of the standard used to verify the standard curve so that the entire concentration range will be covered.
2. Periodically run recovery analysis on each type of sample analyzed (see procedure following).
3. Periodically run duplicate analysis on each type of sample analyzed.
4. Analyze a reference sample obtained from an outside source once or twice each year.
5. Split sample with another lab once or twice each year.
6. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.
7. Quality Control Charts should be prepared for each type of QA/QC analysis done.

Procedure for Determination of Percent Recovery for Nitrite-Nitrogen Analysis

1. Using a volumetric pipet, add 2.0 to 10.0 mL of the 1.0 mg/L standard solution to a 50 mL volumetric flask. Record the exact amount of standard used.

2. Fill the volumetric flask to the mark with sample that has been pH adjusted, filtered and diluted as necessary (see steps 4.1, 4.2 and 4.3 of nitrite procedure).

3. Carry this spiked sample through the same color development as the other samples and standards, and determine the total concentration of NO_2-N.

4. Determine the percent of nitrite spike recovered using the following equation:

$$\text{Percent Recovery} = \frac{C_t V_t - C_m V_m}{C_s V_s} \times 100\%$$

Where:

C_t = measured concentration of total NO_2-N in the solution of sample and spike (mg/L).

V_t = 50 mL

C_m = measured concentration of NO_2-N in flask containing sample (mg/L)

V_m = Volume in mL of sample mixed with spike = 50 mL - volume (mL) of spike

C_s = concentration of spiking solution (mg/L)

V_s = volume (mL) of spiking solution added.

Example: A 50 mL volumetric flask was filled to the mark with a filtered final effluent sample. 4.0 mL of a 1.0 mg/L standard solution was added to a second 50 mL volumetric flask. This flask was then filled to the mark with another portion of the filtered effluent sample. The nitrite-nitrogen concentration in flask containing only sample was found to be 0.11 mg/L and in the flask with the spiked sample it was 0.18 mg/L. The percent recovery of NO_2-N in the spiked sample is calculated as follows:

$$\text{Percent Recovery} = \frac{(0.18\ \text{mg/L})(50\ \text{mL}) - (0.11\ \text{mg/L})(46\ \text{mL})}{(1.0\ \text{mg/L})(4.0\ \text{mL})} \times 100\%$$

$$= \frac{9 - 5.06}{4} \times 100\%$$

$$= \frac{3.94}{4} \times 100\%$$

$$= 98.5\%$$

Alternate Percent Recovery Procedure Using a Micropipet

1. Fill two 50 mL volumetric flasks to the mark with sample that has been pH adjusted, filtered, and diluted as necessary (see steps 4.1, etc.).

2. To one of these add 20 to 100 µl of a 100 mg/L standard NO_2-N solution. Record exact amount used.

3. Carry both through the entire analysis procedure.
4. Calculate the percent recovery of spike using the following formula:

$$\text{Percent Recovery} = \frac{C_t - C_m}{C_s} \times 100\%$$

Where:

C_t = Measured concentration of NO_2-N in the flask containing sample and spike (mg/L)

C_m = Measured concentration of NO_2-N in flask containing sample (mg/L)

C_s = 0.002 x (µL of 100 mg/L standard solution used for spike)

Example: Two flasks were filled to the mark with an effluent sample that was filtered. To one of the flasks, 50.0 µL of a 100 mg/L standard solution was added using a micro-pipet. Both flasks were then carried through the analysis procedure.
The concentration of NO_2-N in the flask with sample and spike was found to be 0.170 mg/L. The concentration of NO_2-N in the flask containing sample was found to be 0.074 mg/L. The percent recovery of NO_2-N in the spiked sample is calculated as follows:

C_t = 0.170 mg/L

C_m = 0.074 mg/L

C_s = 0.002 x 50.0 = 0.10 mg/L

$$\text{Percent Recovery} = \frac{0.170\ \text{mg/L} - 0.074\ \text{mg/L}}{0.100\ \text{mg/L}} \times 100\%$$

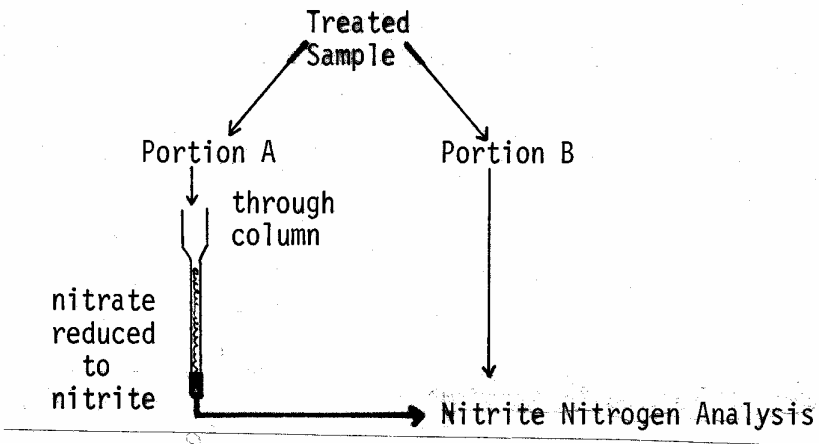
$$= \frac{0.096}{0.100} \times 100\%$$

$$= 96.0\%$$

NITRATE-NITRITE NITROGEN
CADMIUM REDUCTION METHOD

DISCUSSION: This method can be used to determine the concentration of nitrite singly, or nitrite and nitrate combined. The nitrate concentration can be calculated using these two values.

The method employs the use of amalgamated cadmium (Cd) granules to quantitatively reduce nitrates, NO_3^- to nitrites, NO_2^-. A filtered sample is passed through a specially prepared column containing the granules where the reaction takes place. The reduced sample is then analyzed by the diazotization method for nitrite nitrogen. Since nitrites originally present in the sample are also measured, this procedure in effect measures the sum of nitrate plus nitrite nitrogen. In order to distinguish between the nitrate and nitrite concentrations, a separate portion of the sample is treated identically except that it is not passed through the column. Analysis of that portion gives the nitrite nitrogen concentration in the original sample. This can then be subtracted from the nitrite-nitrate value determined using the cadmium column. The result of this subtraction gives the nitrate nitrogen concentration of the sample.



Nitrate + Nitrite Nitrogen, mg/L = Results from Portion A
Nitrite Nitrogen, mg/L = Results from Portion B
Nitrate Nitrogen, mg/L = (Results A) - (Results B)

Because of the sensitivity of the diazotization method for nitrites, this procedure is applicable in the range of 0.01 to 1.0 mg/L nitrate-nitrite nitrogen. The range may be extended by diluting samples prior to passage through the cadmium column.

Buildup of suspended solids in the reduction column will restrict sample flow. Since the nitrite and nitrate nitrogen forms are soluble, the sample may be pre-filtered through a glass fiber filter or a 0.45 µm membrane filter.

Concentrations of iron, copper, or other metals above several milligrams per liter will lower the efficiency of nitrate reduction. EDTA is added to the samples to eliminate this interference.

Samples that contain high concentrations of oil and grease will coat the surface of the cadmium granules. This problem may be eliminated by pre-extracting the sample with an organic solvent. (See Step 5.2)

Residual chlorine may interfere by oxidizing the Cd column, reducing its efficiency. Remove residual chlorine by adding sodium thiosulfate solution. (See Step 5.4)

REFFERENCE - This conforms to the following EPA-approved procedure.
Standard Methods for Examination of Water and Wastewater, 20th Edition,
Method 4500-NO_3 E.

Sample Handling and Preservation - Analysis should be made as soon as possible after collection. If reporting the combination of Nitrite and Nitrate, samples may be stored for up to 28 days if they are preserved with sulfuric acid to pH <2 and refrigerated at ≤ 6 °C. If reporting Nitrite or Nitrate concentration separately, samples must not be acidified, but may be stored for up to 48 hours if they are refrigerated at ≤ 6 °C.

1. APPARATUS

1.1 Spectrophotometer for use at 543 nm, providing a light path of 1 cm or longer.

1.2 Reduction column: Purchase (Tudor scientific Glass Co., Cat. TP1730, or equivalent) or construct. The column in Figure 1 was constructed from a 100 mL pipet by removing the top portion. This column may also be constructed from two pieces of tubing joined end to end. A 10 mm length of 3 cm I.D. tubing is joined to a 25 cm length of 3.5 mm I.D. tubing.

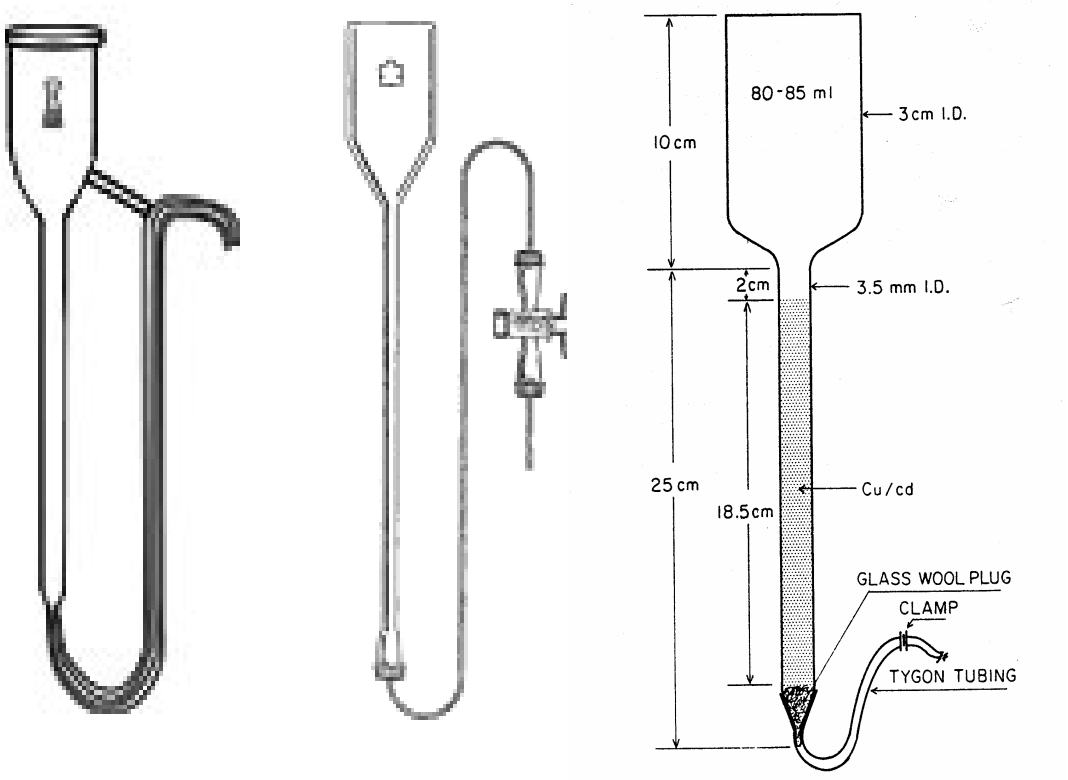


FIGURE 1. REDUCTION COLUMN

2. REAGENTS

2.1 Granulated cadmium: 20-100 mesh granules.

2.2 Copper-Cadmium granules: The cadmium granules (new or used) are cleaned with dilute HCl and copperized with 2% solution of copper sulfate in the following manner.

2.21 Wash the cadmium with dilute HCl (6 N) and rinse with distilled water. The color of the cadmium should be silver.

2.22 Swirl 25 g cadmium in 100 mL portions of a 2% solution of copper sulfate for 5 minutes or until blue color partially fades. Decant and repeat with fresh copper sulfate until a brown colloidal precipitate deigns to develop.

2.23 Gently flush the copper-cadmium with distilled water to remove all the precipitated copper. The color of the cadmium so treated should be black.

2.3 Ammonium chloride - EDTA solution. Dissolve 13 g ammonium chloride, NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL of distilled water. Adjust the pH to 8.5 with conc. ammonium hydroxide and dilute to 1 liter.

2.4 Dilute ammonium chloride-EDTA solution. Dilute 300 mL of ammonium chloride-EDTA solution to 500 mL with distilled water.

2.5 Color reagent. To 800 mL of distilled water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 gram of N - (1 - napthyl) - ethylenediamine dihydrochloride. Mix to dissolve. Then dilute to 1 L with distilled water. This solution is stable for about a month when stored in a dark bottle in refrigerator.

2.6 Dilute sodium hydroxide solution, 6N - Dissolve 240 g NaOH in 500 mL distilled water, cool and dilute to 1 liter.

2.7 Dilute hydrochloric acid, 6N - Dilute 50 mL of conc. HCl to 100 mL with distilled water.

2.8 Copper sulfate solution, 2% - Dissolve 20 g of Copper Sulfate, $CuSO_4 \cdot 5H_2O$ in 500 mL of distilled water and dilute to 1 liter.

2.9 Dechlorinating reagent – Dissolve 3.5 gram sodium thiosulfate , $Na_2S_2O_3 \cdot 5H_2O$, in distilled water and dilute to 1 Liter. Prepare fresh weekly.

2.10 Stock nitrate nitrogen solution, 100 mg/L:

2.101 Dry about 1 gram potassium nitrate (KNO_3) in an oven at 105°C for 24 hours.

2.102 Dissolve 0.7218 g KNO_3 in nitrate-free water and dilute to 1000mL.

2.103 Preserve with 2 mL chloroform.

2.104 This solution is stable for at least 6 months.

2.11 Intermediate nitrate nitrogen solution, 10.0 mg/L - Dilute 100 mL of nitrate stock solution to 1000 mL with distilled water. Preserve with 2 mL chloroform. This solution is stable for 6 months.

2.12 Nitrite-nitrogen stock solution,

The procedure for preparation of this solution is not included in this manual because of the difficulties of obtaining and standardizing an accurate Nitrite solution. The procedures are available in “Standard Methods”, however it is suggested that this solution should be purchased from a reputable supplier.

NOTE: Any dilutions of this solution must be prepared daily.

3. PREPARATION OF REDUCTION COLUMN

3.1 Insert a glass wool plug loosely into the bottom of the reduction column. Refer to Figure 1.

3.2 Close tubing clamp and fill column with distilled water.

3.3 Add sufficient copper-cadmium granules to produce a column 18.5 cm in length. Gently tap side of column while filling to ensure even filling and compaction.

NOTE: Always maintain a level of liquid above the copper-cadmium granules to eliminate entrapment of air. If air entrapment occurs, liquid flow through the column will be impeded and column will have to be repacked.

3.4 Flush the column with 200 mL of dilute ammonium chloride solution.

3.5 The column is then activated by flushing the column with 100 mL of a solution composed of 25 mL of a 1.0 mg/L NO_3-N standard and 75 mL of ammonium chloride-EDTA solution. Use a flow rate between 7 and 10 mL per minute.

3.6 If column is not to be used within several hours, flush the column with 50 mL of dilute ammonium chloride-EDTA solution. Store copper-cadmium column in this solution and never allow liquid level to go below top of granules.

4. PREPARATION OF STANDARDS

4.1 To develop a curve for standardization of colorimeter, prepare a series of nitrate nitrogen, NO_3^--N, standards using the 10.0 mg/L NO_3^--N standard in 100 mL volumetric flasks. Use volumetric pipets.

Conc. NO_3^--N mg/L	mL of 10.0 mg/L NO_3^--N standard/100 mL
0.00 (Blank)	0.0
0.05	0.5
0.10	1.0
0.25	2.5
0.50	5.0
1.00	10.0

This should be done whenever new color reagent is made.

4.2 When using a previously developed standard curve, prepare a blank and at least one NO_3^--N standard to verify the curve.

4.3 Using volumetric glassware, prepare at least one nitrite nitrogen, NO_2^--N, standard at the same concentration as one of the nitrate standards used. This is used to verify the reduction column efficiency.

5. SAMPLE PREPARATION

5.1 Remove turbidity and suspend matter from sample by filtering through a glass fiber filter or a 0.45 µm pore diameter membrane filter.

5.2 If necessary remove oil and grease from sample as follows:

5.21 Adjust the pH of 100 mL of filtered sample to 2 by addition of concentrated HCl.

5.22 Transfer the sample to a separatory funnel and add 25 mL of an organic solvent (such as hexane).

5.23 Shake vigorously for 2 minutes, releasing built-up gas pressure often.

5.24 Allow solvent to separate. Drain and discard solvent.

5.25 Repeat extraction with a second 25 mL portion of solvent.

5.3 Adjust pH of sample to between 5 and 9 with either concentrated HCl or concentrated NH_4OH. This is done to insure a sample pH of 8.5 after adding ammonium chloride-EDTA solution.

5.4 Chlorine residual can interfere by oxidizing the Cd column, reducing its efficiency. Use 1 mL of dechlorinating reagent (Step 2.9) to remove 1 mg/l residual chlorine in 500-mL sample.

6. PROCEDURE

6.1 Using a volumetric pipet place 25.0 mL of blank and each standard in separate labeled 125 mL Erlenmeyer flasks.

6.2 Pipet two separate 25.0 mL portions of sample into separate 125 mL Erlenmeyer flasks. Label one "A" and the other "B".

6.3 Add 75 mL of ammonium-chloride-EDTA solution to each flask containing blank, standards, and sample. Mix well by swirling. (This is not to be considered sample dilution and does not enter into final concentration calculations).

6.4 Pipet 50.0 mL from the flasks containing nitrite standard(s) and sample portion "B" into separate, labeled 100 mL beakers. Using a volumetric pipet, add 2.0 mL of color reagent to each of these beakers and mix by swirling. Set these aside for color development and later reading of absorbance on spectrophotometers. Readings must be made between 10 minutes and 2 hours from addition of color reagent.

6.5 Carefully drain liquid level in reduction column to within 1 cm of top of granules. Do not allow liquid level to go below top of granules.

6.6 Pour approximately 25 mL from the flask containing the blank solution into the reduction column.

6.7 Allow this to pass through the column and discard. Do not allow the liquid level to go below the top of the granules.

6.8 Pour the rest of the blank solution into the reduction column.

6.9 Allow this portion to pass through the column and collect in the Erlenmeyer flask at a rate of 7-10 mL per minute. Stop flow when liquid level is just over granules.

6.10 Pour about 25 mL of first standard solution into reduction column. Allow this to pass through the column and discard.

6.11 Pour the rest of the standard solution into the reduction column. Allow this to pass through the column and collect in the Erlenmeyer flask at a rate of 7-10 mL per minute.

6.12 While the second solution is passing through the column, pipet 50.0 mL of the reduced blank solution into a labeled 100 mL beaker. Add 2.0 mL of color reagent to this and set aside for color development.

6.13 Continue this process (Steps 6.6 through 6.12) until all remaining standards and sample(s) have been passed through the column, collected, and the color development started. Keep in mind the following points:

6.131 Never allow the liquid level in the reduction column to drop below the top of the granules.

6.132 The flow rate through the column should be between 7 and 10 mL per minute.

6.133 Reduced standards and samples should not be allowed to stand longer than 15 minutes before addition of color reagent.

6.134 Absorbance must be read between 10 minutes and 2 hours from addition of color reagent.

6.135 Use volumetric pipets for all measurements of standards, samples, and color reagent.

(NOTE: See Step 3.6 for column storage after use.)

6.14 Set the spectrophotometer at 543 nm and adjust to 0.000 absorbance using the prepared blank.

6.15 Read and record the absorbance of all standards and samples.

NOTE: If the absorbance of sample exceeds the upper range of the calibration curve, the remainder of the reduced sample may be used to make an appropriate dilution.

6.16 To prepare calibration curve, plot concentration of nitrate nitrogen standards run through this procedure against absorbance on 10 x 10 graph paper. Verify reliability of previously prepared calibration curves with standard(s) prepared in Step 4.1.

6.17 Verify reduction column efficiency by comparing unreduced nitrite standard reading to reduced nitrate standard. Reactivate copper-cadmium granules as described in Step 2.2 when efficiency of reduction falls below about 75%.

6.18 Determine concentration values of samples directly from curve. (Be sure to include dilution factors as necessary).

Results from sample portion A = Nitrate + Nitrite Nitrogen, mg/L

Results from sample portion B = Nitrite Nitrogen, mg/L

Nitrate Nitrogen, mg/L = (Results A) - (Results B)

QA/QC Recommendations for Nitrate-Nitrite Nitrogen Analysis

1. Vary the concentration of the standards used to verify the calibration curve so that the entire concentration range will be covered.
2. Periodically run recovery analysis on each type of sample analyzed (see procedure following).
3. Periodically run duplicate analysis on each type of sample analyzed.
4. Analyze a reference sample obtained from an outside source once or twice each year.
5. Split sample with another lab once or twice each year.
6. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.
7. Quality Control Charts should be prepared for each type of QA/QC analysis done.

Procedure for Determination of Percent Recovery of Nitrate-Nitrite Nitrogen Analysis

1. When preparing regular samples for nitrate-nitrite analysis, measure out an additional 25.0 mL sample in a 125 mL Erlenmeyer flask that duplicates a sample already set up.
2. Using a volumetric pipet, add 1.0 or 2.0 mLs of a 10 mg/L nitrate nitrogen standard to the sample. This spikes the sample with an additional 0.4 and 0.8 mg/L of nitrate nitrogen respectively.
3. In step 6.3 of the procedure when adding the ammonium chloride-EDTA solution add only 74 mL when using 1.0 mL of spike solution and only 73 mL when using 2.0 mL of spike solution, instead of the full 75 mL of ammonium chloride-EDTA solution.
4. Take the spiked sample through the same reduction and analysis procedures as the other samples and determine the total concentration of nitrate-nitrite nitrogen.
5. Determine the percent of nitrate-nitrite that was recovered of the amount that was added using the following formulas:

   mg/L spiked into sample = mL of standard added x 0.40

   $$\text{Percent recovery} = \frac{\text{conc of sample with spike - conc of sample}}{\text{mg/L spiked into sample}} \times 100\%$$

   NOTE 1: While 100% is perfect recovery, 90-110% is generally acceptable; outside this range check for possible errors in procedure or technique. Refer to the QA/QC unit of this manual for specific calculations of control limits.

   NOTE 2: The volume of standard used for the spike and the source of the sample (influent, effluent, etc.) should be varied frequently.

Example: If 2.0 mL of 10 mg/L standard is added to 25.0 mL of influent and the following results are obtained, percent recovery is calculated as shown:

Influent sample = 0.65 mg/L nitrate-nitrite nitrogen

Influent sample + spike = 1.42 mg/L nitrate-nitrite nitrogen

mg/L spiked into sample = 2.0 mL x 0.40 = 0.80 mg/L

$$\% \text{ Recovery} = \frac{1.42 \text{ mg/L} - 0.65 \text{ mg/L}}{0.80 \text{ mg/L}} \times 100\%$$

$$= \frac{0.77 \text{ mg/L}}{0.80 \text{ mg/L}} \times 100\%$$

$$= 96.25\%$$

NITRATE - ION SELECTIVE ELECTRODE METHOD

DISCUSSION: The Nitrate ion (NO_3^-) electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that is proportional to the concentration of nitrate in sample. Although there are several interferences to the nitrate measurement, these are minimized by addition of the buffer solution. This method is applicable to concentrations between about 0.14 to 1400 mg/L of nitrate nitrogen (NO_3-N).

REFERENCE

This conforms to the following EPA-approved procedure.

Standard Methods for Examination of Water and Wastewater, 20th Edition, Method 4500 – NO_3^- D.

1. EQUIPMENT

   1.1 Ion Selective Electrode Meter or a pH/mV meter capable of 0.1 mV resolution.

   1.2 Nitrate ion electrode, Orion Model 93-07, Corning Model 476134, or equivalent.

   1.3 Double-junction reference electrode, Orion Model 93-02, or equivalent. (Not needed if using Ion Selective Combination Electrode)

   1.4 Magnetic stirrer and TFE-coated stirring bar.

2. REAGENTS

2.1 Nitrate-free water: Use redistilled or distilled, deionized water of highest purity to prepare all solutions and dilutions.

2.2 Stock nitrate solution, 100 mg/L:

2.21 Dry about 1 gram potassium nitrate (KNO_3) in an oven at 105°C for 24 hours.

2.22 Dissolve 0.7218 g KNO_3 in nitrate-free water and dilute to 1000mL.

2.23 Preserve with 2 mL chloroform.

2.24 This solution is stable for at least 6 months.

2.3 Buffer solution: Prepare as follows or use commercially available Nitrate Interference Suppressor Solution (Orion 930710).

2.31 Dissolve the following in about 800 mL water.

2.311 17.32 g aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$)

2.312 3.43 g silver sulfate (Ag_2SO_4)

2.313 1.28 g boric acid (H_3BO_3)

2.314 2.52 g sulfamic acid (H_2NSO_3H)

2.32 Adjust to pH 3.0 by slowly adding 0.10 N sodium hydroxide (NaOH). [4 g NaOH to 1 liter water]

2.33 Dilute to 1000 mL and store in dark glass bottle.

2.4 Reference electrode filling solution. Prepare as follows or use commercially available solution specific for nitrate analysis. (Orion 900002)

2.41 Dissolve 0.53 g ammonium sulfate $(NH_4)_2SO_4$ in water and dilute to 100 mL.

NOTE: This solution is required as part of the nitrate measuring system and is specific for nitrate analysis. Do not use the filling solution that generally comes with separate reference electrodes.

3. STANDARD SOLUTIONS PREPARATION

3.1 1.0 mg/L NO_3-N standard: pipette 1.0mL of 100 mg/L standard into 100 mL volumetric flask. Dilute to the mark.

3.2 10 mg/L NO_3-N standard: pipette 10 mL of 100 mg/L standard into 100 mL volumetric flask. Dilute to the mark.

3.3 50 mg/L NO_3-N standard: pipette 50 mL of 100 mg/L standard into 100 mL volumetric flask. Dilute to the mark.

4. CALIBRATION

Perform three point meter calibration using 1, 10, and 50 mg/L nitrate standards which are at room temperature.

4.1 Pipet 10 mL of each standard and 10 mL of buffer solution into 50 mL beakers.

4.2 While stirring continuously, place the electrode into each standard and record the meter reading when stable (after about 1 min.). Rinse the electrode with distilled or deionized water between standards.

4.3 When using an ISE meter, enter each standard concentration as a calibration point. When using a pH/mv meter, plot meter readings against

nitrate concentrations on semilogarithmic graph paper with millivolt reading on the linear axis.

4.4 An electrode slope of +57 ± 3 mV per decade should result.

4.5 Calibration should be performed daily. If analysis is to be repeated later in the day, verify the calibration using the 10 mg/L standard and adjust using the calibration control as necessary.

5. SAMPLE ANALYSIS

5.1 Allow samples to reach room temperature before analysis.

5.2 Pipet 10 mL of sample and 10 mL of buffer solution into a 50 mL beaker and stir for about 1 minute. The buffer must be added to all standards and samples. A larger sample size can be used if desired as long as the buffer is added in a 1:1 ratio.

5.3 Place the electrode in the prepared sample. When the meter reading has stabilized, record the concentration of chloride in the sample in milligrams per liter from the meter or calibration curve.

6. IMPORTANT ISE MEASUREMENT CONSIDERATIONS

6.1 Check reference electrode fill solution daily before use. The filling solution should be no lower than 1 inch from the fill hole and must be above the reference junction.

6.2 Do not submerge electrodes too far into solutions. (see specific electrode instruction manual) Submerge the reference and sensing electrodes to same depth.

6.3 Reference electrode fill hole must be uncovered during measurement.

6.4 Check and record electrode slope each day that it is used.

6.5 Always use fresh standards.

6.6 Care should be taken to ensure all standards and samples are stirred at the same rate. Insulate between stir plate and sample beaker to prevent temperature change of sample.

6.7 Rinse the electrode with distilled or deionized water between measurements. Shake electrode after rinsing to prevent solution carryover, then blot dry. Do not wipe or rub sensing membrane which may result in damage or contamination.

7. ELECTRODE STORAGE

See specific electrode manuals for electrode storage requirements for between measurements, and for short or long term storage.

QA/QC Recommendations for Nitrate - ISE Analysis

1. Periodically run recovery analysis on each type of sample analyzed (see following procedure).

2. Periodically run duplicate analysis on each type of sample analyzed.

3. Analyze a reference sample obtained from an outside source once or twice each year.

4. Split sample with another lab once or twice each year.

5. The number of QA/QC analyses is determined by a number of factors discussed in the QA/QC unit of this manual. As a general rule, a QA/QC analysis should be run for every 5 to 10 samples.

7. Control charts should be prepared for each type of QA/QC analysis done. Recommended QC procedures include analysis of matrix spikes (percent recovery), sample duplicates, and independent reference materials.

DETERMINATION OF PERCENT RECOVERY

Percent recovery of nitrate is determined by adding a known amount of the 100 mg/L nitrate standard to a sample that has been analyzed. The use of a micro-pipet is encouraged, since these do not add a significant amount of volume during the spiking procedure, allowing for easier calculation of percent recovery. Disregard the volume of buffer solution in the calculation, since this is added equally to samples and standards.

1. Analyze a sample for nitrate.
2. With the electrode still in the sample, use a micro-pipet to add a suitable amount of the 100 mg/L nitrate standard to the analyzed sample. When a stable reading is obtained, record the concentration of the sample and spike.
3. As shown below, each 1 microliter (µL) of the standard spikes the 10 mL sample with 0.01 mg/L NO_3^-. (Disregard the volume of buffer solution in the calculation)

   0.001 mL X 100 mg/1000 mL = 0.0001 mg NO_3^- added for each µL added.

   $$\frac{0.0001 \text{ mg } NO_3^-}{10 \text{ mL Sample}} = \frac{0.01 \text{ mg } NO_3^-}{1000 \text{ mL}} = 0.01 \text{ mg/L}$$

4. Determine percent recovery as follows:

   $$\% \text{ Recovery} = \frac{(\text{Sample + Spike, mg/L}) - (\text{Sample, mg/L})}{\text{Amount Spiked, mg/L}} \times 100\ \%$$

   Amount Spiked into 10 mL sample = µL of 100 mg/L standard added x 0.01

Example: 200 µL of the 100 mg/L NO_3^- standard are added to10 mL of sample. The sample concentration had been determined to be 3.22 mg/L, and the sample with the spike in it was analyzed at 5.38 mg/L.

Sample = 3.22 mg/L NO_3^-
Sample + Spike = 5.38 mg/L

Amount Spiked into sample = 200 µL x 0.01 = 2.0 mg/L

$$\% \text{ Recovery} = \frac{5.38 \text{ mg/L} - 3.22 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

$$= \frac{2.16 \text{ mg/L}}{2.0 \text{ mg/L}} \times 100\ \%$$

= 108% Recovery

Note 1: While 100% is perfect recovery, 90-110% is generally considered acceptable; outside this range check for possible errors in procedure or technique.

Note 2: The volume of standard used for the spike should be varied frequently.

APPENDIX

CONVERSION FACTORS AND USEFUL INFORMATION

International Metric System - Le Systeme International d'Unites (SI Units)

Base Units of the International Metric System (SI)

Quantity	Name of the Unit	Symbol
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Temperature	Kelvin	K
Electric Current	Ampere	A
Luminous Intensity	Candela	cd
Amount of Substance	Mole	mol

Recommended Decimal Multiples and Submultiples and the Corresponding Prefixes and Names

Factor	Prefix	Symbol	Meaning
10^{12}	tera	T	One trillion times
10^{9}	giga	G	One billion times
10^{6}	mega	M	One million times
10^{3}	kilo	K	One thousand times
10^{2}	hecto	H	One hundred times
10	deca	D	Ten times
10^{-1}	deci	d	One tenth of
10^{-2}	centi	c	One hundredth of
10^{-3}	milli	m	One thousandth of
10^{-6}	micro	u	One millionth of
10^{-9}	nano	n	One billionth of
10^{-12}	pico	p	One trillionth of
10^{-15}	femto	f	One quadrillionth of
10^{-18}	atto	a	One quintillionth of

EXAMPLES

LENGTH	=	One kilometer	=	1,000 meters
		One meter (m)	=	1.0 meter
		One decimeter (dm)	=	0.1 meter
		One centimeter (cm)	=	0.01 meter
		One millimeter (mm)	=	0.001 meter

WEIGHT	=	One kilogram	=	1,000 grams
		One gram (g)	=	1.0 gram
		One decigram (dg)	=	0.1 gram
		One centigram (cg)	=	0.01 gram
		One milligram	=	0.001 gram

VOLUME	=	One kiloliter	=	1,000 liters
		One liter (L)	=	1.0 liter
		One deciliter (dL)	=	0.1 liter
		One centiliter (cL)	=	0.01 liter
		One milliliter (mL)	=	0.001 liter

AREA = One sq. kilometer (Km^2) = 1,000 square meters

LENGTH CONVERSION FACTORS

1 inch (in)	=	2.54 centimeters	=	25.4 millimeters
1 foot (ft.)	=	12 inches	=	0.305 meters
1 yards(yd.)	=	3 feet	=	0.914 meters
1 mile (mi.)	=	5,280 feet	=	1,760 yards
1 meter (m.)	=	39.37 inches	=	3.261 feet
1 kilometer	=	0.62 miles	=	1,000 meters

AREA CONVERSION FACTORS

A square foot (ft.^{2})	=	144 square inches (inch^2)
1 square yard (yd.^{2})	=	9 square feet (ft.^{2})
1 acre	=	43,560 square feet (ft.^{2})
1 square mile (mi.^{2})	=	640 acres or 1 section
1 square meter (m.^{2})	=	10.8 square feet (ft.^{2})
1 square meter (m.^{2})	=	10,000 square centimeters
1 hectare	=	2.5 acres

VOLUME CONVERSION FACTORS

1 cubic foot (ft.^{3})	=	1,728 cubic inches (inch^3)
1 cubic foot (ft.^{3})	=	7.48 gallons
1 cubic yard (yd.^{3})	=	27 cubic feet (ft.^{3})
1 acre foot	=	43,560 cubic feet (ft.^{3})
1 acre foot	=	325,851 gallons
1 gallon (gal.)	=	231 cubic inches (inch^3)
1 gallon (gal.)	=	4 quarts
1 cubic meter (m.^{3})	=	35.3 cubic feet (ft.^{3})
1 cubic meter (m.^{3})	=	1.3 cubic yards (yd.^{3})
1 liter	=	1.06 quarts
1 liter	=	1,000 milliliters

WEIGHT CONVERSION FACTORS

1 gallon	=	8.34 pounds (lbs.) of water
1 cubic foot	=	62.4 pounds (lbs.) of water
1 foot of water	=	0.434 PSI (pounds per square inch)
1 pound (lb)	=	0.454 kilograms (Kgs.)
1 kilogram (Kg)	=	2.2 pounds (lbs.)
1 kilogram (Kg)	=	1,000 grams
1 PSI	=	2.31 feet of water

TEMPERATURE

1. Convert Fahrenheit to Celsius $^{o}C = \frac{5\,(^{o}F - 32)}{9}$

   $0^{o}F = -17.8^{o}C$

2. Convert Celsius to Fahrenheit $^{o}F = \frac{^{o}C \times 9}{5} + 32$

   $0^{o}C = 32^{o}F$
   $100^{o}C = 212^{o}F$

   Remember: 100° between Ice/Steam = Celsius
   180° between Ice/Steam = Fahr.

1. Convert Fahrenheit to Celsius:

   $^{o}C = (^{o}F + 40) \times 5/9 - 40$

2. Convert Celsius to Fahrenheit:

   $^{o}F = (^{o}C + 40) \times 9/5 - 40$

   Quick Approximation:

   $(^{o}C \times 2) + 30 = {}^{o}F$ (about)

CONVERSION FACTORS FOR OPERATORS

MULTIPLY	BY	TO OBTAIN
Acres	43,560	Square feet
Acre-feet	43,560	Cubic feet
Acre-feet	325,851	Gallons
Centimeters	0.3937	Inches
Cubic feet	1728	Cubic inches
Cubic feet	7.48052	Gallons
Cubic feet	28.32	Liters
Cubic feet/second	448.831	Gal./min
Cubic feet/second	0.646317	Million gal/day
Cubic yards	27	Cubic feet
Degrees (angle)	60	Minutes
Feet	30.48	Centimeters
Feet	12	Inches
Feet	0.3048	Meters
Feet	1/3	Yards
Feet of water	0.4335	lbs/square in.
Gallons	0.1337	Cubic feet
Gallons	3.785	Liters
Gallons	4	Quarts (liquid)
Gallons, Imperial	1.20095	U.S. gallons
Gallons U.S.	0.83267	Imperial gallons
Gallons water	8.3453	Pounds of water
Gallons/min.	2.228×10^{-3}	Cubic ft/sec.
Gallons/min.	8.0208/area (sq.ft.)	Overflow rate (ft/hr)
Grains/U.S. gal.	17.118	Milligrams/liter
Grains/U.S. gal.	142.86	lbs/million gal.
Grams	0.3527	Ounces
Grams	2.205×10^{-3}	Pounds
Grams/liter	58.417	Grains/gal.
Grams/liter	1000	Milligrams/liter
Horse-power	33,000	foot-lbs./min.
Horse-power	0.7457	Kilowatts
Horse-power	745.7	Watts
Inches	2.540	Centimeters
Inches of mercury	1.133	Feet of water
Inches of mercury	0.4912	lbs/sq. inch
Inches of water	0.07355	In. of mercury
Inches of water	0.03613	lbs/sq. inch
Kilowatt-hours	1.341	Horse-power hrs.

MULTIPLY	BY	TO OBTAIN
Liters	0.03531	Cubic feet
Liters	0.2642	Gallons
Liters	1.057	Quarts (liquid)
Width(in) x Thickness(in) / 12	Length (ft.)	Board feet
Meters	3.281	Feet
Meters	39.37	Inches
Miles	5280	Feet
Miles	1760	Yards
Milligrams/liter	1	Parts/million
Million gals./day	1.54723	Cubic ft/sec
Ounces	0.0625	Pounds
Ounces	28.349527	Grams
Overflow rate (ft/hr)	0.12468 x area (sq.ft.)	Gal/min
Milligrams/liter	0.0584	Grains U.S. gal.
Milligrams/liter	8.345	lbs/million gal.
Pounds	16	Ounces
Pounds	7000	Grains
Pounds	453.5924	Grams
Pounds of water	0.01602	Cubic feet
Pounds of water	0.1198	Gallons
Pounds/sq. inch	2.307	Feet of water
Pounds/sq. inch	2.036	In. of mercury
Revolutions	360	Degrees
Square feet	2.29×10^{-5}	Acres
Square feet	144	Square inches
Square feet	1/9	Square yards
Square inches	6.452	Square centimeters
Square meters	10.76	Square feet
Square miles	640	Acres
Square yards	9	Square feet
Temp. EC + 17.78	1.8	Temp. EF
Temp. EF - 32	5/9	Temp. EC
Watts	1.34×10^{-3}	Horse-power
Yards	3	Feet
Yards	36	Inches
Yards	0.9144	Meters

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