FACULTY OF ENGINEERING

ENVIRONMENTAL ENGINEERING DEPARTMENT

CEV207 ENVIRONMENTAL CHEMISTRY LABORATORY MANUAL

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Faculty of Engineering
Environmental Engineering Department

CEV207 ENVIRONMENTAL CHEMISTRY LABORATORY

Laboratory Manual
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Preface

The Environmental Chemistry Laboratory Manual is written to describe the experiments in Environmental Chemistry Lab course (CEV 207). Each experiment procedure is explained thoroughly along with related background. Environmental chemistry laboratory is designed to introduce the student to common procedures for analyzing water, air, and soil samples. As part of these analyses, the student will gain familiarity with important properties that environmental chemists use to characterize such samples. The student will learn the laboratory skills needed to design, safely conduct and interpret chemical research.

To acquire broad knowledge of the field of Environmental Chemistry including development of methods for ultra-trace analysis of pollutants in air, water, soil and biological matrices; understanding of sources, chemodynamics and fate of environmental pollutants in ecosystems can be listed as one of objectives of Environmental Chemistry Laboratory. Also, the student will interpret and critically analyze the data on environmental chemical analysis; conduct research independently and be able to perform basic statistical analysis of data generated from laboratory or field studies.

The authors devoted considerable attention to laboratory report development and associated technical writing. These issues are one of the important objectives of CEV 207. Laboratory report must be written in accordance with laboratory report format described in the laboratory manual. Furthermore, the student will be expected to demonstrate student’s understanding of the experimental design. The artifact of student’s understanding will be in the form of a Pre-lab Flowchart. The format of flowchart can be in diagram or outline form.

Part of this manual is developed based on information obtained from books referenced at the last section of the manual. A sincere appreciation and credit should be given to authors of these books. Students are encouraged to check these resources for more information or interest in any topic.
# SCHEDULE

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1. COURSE REQUIREMENTS AND POLICIES

1.1. Laboratory Guidelines

In order to receive points for any given lab, the following conditions must be met:

- The class will be divided into 4-5 students that will be working together in the lab and also, each one of group members will individually write the laboratory reports.
- Always bring lab manual, calculator and lab note book.
- You must attend lab.
- Prior to attending any given laboratory period you must have completed all of the reading assignments.
- You must prepare a prelab in your lab notebook. The prelab may be a series of discussion questions to be answered in groups or may be something you have to prepare in advance of attending lab. Your prelab must include as a minimum, what is asked for in the laboratory manual at the beginning of the experiment.
- You must **arrive to lab on time**. In general, the first 10-15 minutes of every laboratory period are dedicated to a safety discussion, which is an important part of the experiment. Therefore, if you show up late you will not be allowed to participate in lab for that day.
- You must wear protective clothing and eyewear during the laboratory period. Your instructor can ask you to leave the lab for the day if you are not wearing such clothing or eyewear.
- You must record detailed observations about the experiment. Do not just make a checklist of what you are supposed to do and then check off the procedures as you carry them out without making observations as to what actually happened. All observations must be written in your lab notebook during, not after, the laboratory period.
- You must record all expected data during, not after, the laboratory period. This includes the mass of chemical weighed, the volume used in titration, etc.
- You must complete the report for the experiment.
- All lab reports will be **due on the day of lab**.
- You must turn in the lab report at the beginning of the lab period it is due (the next lab period after the experiment was completed). Late lab reports will not be
accepted. Lab reports cannot be submitted to the instructor using e-mail or any other type of electronic format.

- Any questions you have regarding a lab report grade must be resolved with your instructor within one week of having received the graded lab report. All regrades are subject to final approval by the course instructor.

1.2. Grading

If you do not complete all of the conditions in the laboratory guidelines for any given lab, you will receive a 0 for that experiment. The consequences of a 0 are as follows:

- If you receive one zero during the semester, this will be your dropped lab score.
- If you receive two zeros during the semester, you not only will lose the 10 points associated with that experiment, but your course grade will also be dropped by one third of a grade. For example, if you earn enough points to get a B3 in the class, but you have two zero’s, you will receive a B1.
- If you receive three zeros you will receive a failing grade in the course.

All lab reports will be graded in 100 points.

- **Midterm Exam : 20 %**
- **Final Exam : 40 %**
- **Laboratory portion: 40 %**
  - Lab quizzes & prelab: 20 %
  - Reports: 20 %

1.3. Attendance

All experiments will require a formal lab write up and are mandatory. If you miss one of the experiments assigned to formal lab write ups due to illness, death in the family, or other unavoidable circumstances, you must discuss your absence with your instructor responsible for the course. She/He will help you to schedule a make up lab in the remaining sessions for the missed lab experiment. Please provide paper documentation of illness or family crisis. Attendance is compulsory in all labs. If a student’s attendance is less than 75 %, the student will be awarded one grade less than the actual grade that he/she has earned. All reports should be submitted within one week of absence.
1.4. Collaboration, Cheating and Plagiarism

You will work in groups on most lab experiments. We encourage you to work together on the preliminary work for lab reports as well. However, anything you hand in for a grade must be your own work. Work together and discuss the concepts and calculations but then write your final lab report alone. This will help you to ensure that your own thoughts are captured. If your instructor suspects you have copied a report of another student, or if sections of lab reports are exactly the same, the students involved will lose all the credit for that experiment. If you do not you are in danger of committing plagiarism. The course instructor will further review any suspect lab reports and submit a form called FACULTY DISPOSITION FOR ACADEMIC DISHONESTY to the Official of Student Affairs. The findings may result in your dismissal from the course and possible further action by the Academic Senate.
2. LABORATORY SAFETY RULES AND REGULATIONS

Safety is our number one chemistry laboratory concern. Please read and follow all safety instructions in this manual. Your laboratory instructor will explain safe working procedures throughout the semester. Don’t hesitate to ask at any time if you do not understand a safety guideline or procedure. The rules and regulations that follow are universal for the laboratories. In addition to becoming familiar with these, take note of safety warnings given with each specific experiment.

2.1. Completion of Safety Checklist

In your second lab period, your lab instructor will provide you with and help you complete a safety checklist. You are required to initial each item and sign the checklist.

2.2. General Safety Guidelines

- SAFETY GLASSES are provided as part of the locker equipment. They must be worn at all times whenever anyone in the lab room is working on an experiment.
- CONTACT LENSES are prohibited in the lab.
- ALWAYS WORK NEATLY, plan ahead and clean up after yourself. Most laboratory accidents are a result of poor planning, clutter or spilled chemicals. Clean up spills immediately; ask your lab instructor for help. Your dried acid spill can seriously burn someone later.
- GLOVES are provided in the stock room for handling corrosive chemicals.
- FUME HOODS will be used to contain the more hazardous chemicals. Your lab instructor will explain their use.
- GLASSWARE, pipettes, stirring rods, thermometers, etc., can cut, burn, and puncture. Use care when handling glassware. Most injuries in our labs are cuts caused by broken glass. Your instructor will demonstrate good technique.
- When mixing concentrated acid with water, **always add the acid to water.** Adding water to concentrated acid can cause to splash out of the container.
- Never pipette anything by mouth.
- Use only as much of any reagent as you need-collecting and treating leftover chemicals is both expensive and environmentally unfriendly.
• When heating a solution in a test tube, do it by placing the tube in a beaker of boiling water, rather than by heating it directly over the burner flame.

• Follow carefully the indicated instructions for the collection of excess chemical solids and solutions. Never return unused reagents to their original bottles. When in doubt about proper collection procedures, consult your laboratory instructor.

**General Rules:**

No person may work alone in the lab, supervision is needed all time. No work outside regular lab hours is permitted without specific permission. Visitors are not allowed in the labs.

**Clothing:**

Shorts and skirts should not be worn to the lab. Avoid wearing expensive clothes. Sandals or open–toe shoes are not acceptable. Confine long hair, or any loose clothing or accessories.

**Eye Protection:**

Safety glasses are required item to be worn in all areas of the laboratories. The wearing of contact lenses in the laboratory is strongly discouraged, even when eye protection is worn. There is a distinct possibility that chemicals may violate the eye protection policy are subject to dismissal from the lab.

**Housekeeping:**

All designated experimentation areas should be left in a neat orderly state at the conclusion of an experiment. The following items should be checked:

• All excess water should be removed from the floor.
• All loose paper should be picked up and deposited in trashcans.
• All working surfaces (tables, chairs, etc.) should be cleaned if needed.
• All miscellaneous items should be returned to their proper initial locations.
• All glassware should be washed prior to returning to the cabinet.
• All scales should have weights removed and scale arms locked.
• All manholes (sewers) should have their lids closed.
• All drums or containers used should be checked.
• Check all valves and electrical units. Turn off what is required.

**Chemicals:**

In several of the experiments, chemicals are required to perform the experiment. Students should check with their instructor as to where to get these chemicals and what safety precautions, if any, are to be taken in conjunction with the use of these chemicals.

• Do not use mouth suction to fill pipettes.
• Label all containers to avoid errors and read labels carefully.
• Never remove shared chemicals from their original locations, others will need them.
• Waste chemicals are placed in receivers and are not discharged in the drain, unless told otherwise.

**Electrical:**

In many instances electrical extension cords are required for the operation of auxiliary equipment. Special precautions should be taken when using these cords. When an electrical extension cord is checked out, be sure to examine its condition. If you find frayed or broken wires, insulation broken, prongs bent, no ground, etc., do not use but return to the stockroom. When using extension cords, be sure they do not lie on the floor, in particular, when the floor is wet, but are safety supported in such a fashion that they are not a bodily hazard. When making electrical connections, be sure the area you are standing in is dry.

**Accidents:**

If an accident happens, inform your instructor immediately. In case of a serious accident, do not attempt first aid if you are not familiar with the proper technique, but do attempt to make the person comfortable until aid arrives. Whenever your skin (hands, arms, face...) comes into contact with laboratory chemicals, wash it quickly and thoroughly with soap and warm water.
**Unauthorized Areas:**

Do not touch unauthorized equipment, chemicals or experiments.

**Food or Drink:**

Food and drink are forbidden in laboratories, that includes chewing gum and applying makeup. DO NOT taste chemicals, if instructed to smell chemicals do so by carefully fanning the top of test tube or bottle so that a little of the vapor is directed towards your nose.

**Smoking:**

Smoking is not permitted.

**Ventilation:**

Be sure that hoods are functioning, and that your work areas are properly ventilated.

**Obligation:**

Each student has a professional obligation to contribute a full and honest effort in the group execution of experiments and reports. Consistent failure to observe this rule is considered unprofessional behavior, and will be penalized.
**Agreement**

Sign and date this as a reminder of procedures you will be practicing during environmental chemistry lab.

- I have read and agree to follow all of the safety rules set forth in this contract. I realize that I must obey these rules to insure my own safety, and that of my fellow students and instructors. I will cooperate to the fullest extent with my instructor and fellow students to maintain a safe lab environment. I will also closely follow the oral and written instructions provided by the instructor. I am aware that any violation of this safety contract that results in unsafe conduct in the laboratory or misbehavior on my part may result in being removed from the laboratory.

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**Bring:**
- Notebook
- Safety Glasses
- Calculator
- Lab Coat
- Lab Manual
- Pen
3. LAB EQUIPMENT

The measurement of trace constituents in water demands methods capable of maximum sensitivity. In addition to sensitive methods, however, there are other areas that require special consideration. One such area is that of the cleanliness of laboratory glassware. Obviously, the very sensitive analytical systems are more sensitive to errors resulting from the improper use or choice of apparatus, as well as to contamination effects due to an improper method of cleaning the apparatus.

Types of Glassware

Laboratory vessels serve three functions:

1. Storage of reagents
2. Measurement of solution volumes
3. Confinement of reactions

For special purposes, vessels made from materials such as porcelain, nickel, iron, aluminum, platinum, stainless steel, and plastic may be employed to advantage. Glass, however, is the most widely used material of construction.

There are many grades and types of glassware from which to choose, ranging from student grade to others possessing specific properties such as super strength, low boron content, and resistance to thermal shock or alkali. The mainstay of the modern analytical laboratory is a highly resistant borosilicate glass, such as that manufactured by Corning Glass Works under the name “Pyrex”.

The use of plastic vessels, containers, and other apparatus made of Teflon, polyethylene, polystyrene, and polypropylene has increased markedly. Some of these materials, such as Teflon, are quite expensive. However, Teflon stopcock plugs have practically replaced glass plugs in burettes, separatory funnels, etc., because lubrication to avoid sticking or “freezing” is not required. Polypropylene, a methylpentene polymer, is available as laboratory bottles, graduates, beakers, and even volumetric flasks. It is crystal clear, shatterproof, autoclavable, and chemically resistant.
The following are some points to consider in choosing glassware or plasticware:

a. Unless instructed otherwise, borosilicate or polyethylene bottles may be used for the storage of reagents and standard solutions.

b. Dilute metal solutions are prone to plate out on container walls over long periods of storage. Thus, dilute metal standard solutions must be prepared fresh at the time of analysis.

c. For some operations, disposable glassware is entirely satisfactory.

d. Plastic bottles of polyethylene and Teflon have been found satisfactory for the shipment of water samples. Strong mineral acids (such as sulfuric acid) and organic solvents will readily attack polyethylene and are to be avoided.

e. Borosilicate glassware is not completely inert, particularly to alkalies. Therefore; standard solutions of silica, boron, and the alkali metals are usually stored in polyethylene bottles.
Volumetric Analyses
By common usage, accurately calibrated glassware for precise measurements of volume has become known as volumetric glassware. This group includes volumetric flasks, volumetric pipettes, and accurately calibrated burettes. Less accurate types of glassware including graduated cylinders and serological and measuring pipettes also have specific uses in the analytical laboratory when exact volumes are unnecessary.

The precision of volumetric work depends in part upon the accuracy with which volumes of solutions can be measured. There are certain sources of error that must be carefully considered. The volumetric apparatus must be read correctly; that is, the bottom of the meniscus should be tangent to the calibration mark.

There are other sources of error, however, such as changes in temperature, which result in changes in the actual capacity of glass apparatus and in the volume of the solutions.

The capacity of an ordinary glass flask of 1000-ml volume increases 0.025 ml/deg with rise in temperature, but if the flask is made of borosilicate glass, the increase is much less. One thousand milliliters of water or of most 0.1N solutions increases in volume by approximately 0.20 ml/deg increase at room temperature. Thus solutions must be measured at the temperature at which the apparatus was calibrated. This temperature (usually \(20^\circ\text{C}\)) will be indicated on all volumetric ware.
There may also be errors of calibration of the apparatus; that is, the volume marked on
the apparatus may not be the true volume. Such errors can be eliminated only by recal-
ibrating the apparatus or by replacing it.

**Volumetric** apparatus is calibrated to contain or to deliver a definite volume of liquid.
This will be indicated on the apparatus with the letters “TC” (TO CONTAIN) or “TD”
(TO DELIVER). Volumetric flasks are calibrated to contain a given volume and are
available in various shapes and sizes.

**A.** Volumetric pipettes are calibrated to deliver a fixed volume. The usual capacities
are 1 through 100 ml although micropipette are also available.

**B.** Measuring and serological pipettes should also be held in a vertical position for dis-
pensing liquids; however, the tip of the pipette is only touched to the wet surface of the
receiving vessel after the outflow has ceased. For those pipettes where the small amount
of liquid remaining in the tip is to be blown out and added, indication is made by a
frosted band near the top.
C. Burettes are used to deliver definite volumes. The more common types are usually of 25- or 50-ml capacity, graduated to tenths of a milliliter, and are provided with stopcocks. For precise analytical methods in microchemistry, microburettes are also used.

Automatic burettes with reservoirs are also available ranging in capacity from 10 to 100 ml. Reservoir capacity ranges from 100 to 4,000 mL.

**General Rules**

regarding the manipulation of a burette:

**i.** Do not attempt to dry a burette that has been cleaned for use, but rinse it two or three times with a small volume of the solution with which it is to be filled.

**ii.** Do not allow alkaline solutions to stand in a burette because the glass will be attacked, and the stopcock, unless made of Teflon, will tend to freeze.

**iii.** A 50-ml burette should not be emptied faster than 0.7 ml/s, otherwise too much liquid will adhere to the walls and as the solution drains down, the meniscus will gradually rise, giving a high false reading.

**iv.** In the case of all apparatus for delivering liquids, the glass must be absolutely clean so that the film of liquid never breaks at any point. Careful attention must be paid to this fact or the required amount of solution will not be delivered.

*It should be emphasized that improper use or reading of burettes can result in serious calculation errors.*
Cleaning of Glass and Porcelain

The method of cleaning should be adapted to both the substances that are to be removed, and the determination to be performed.

Water-soluble substances are simply washed out with hot or cold water, and the vessel is finally rinsed with successive small amounts of distilled water. Other substances more difficult to remove may require the use of a detergent, organic solvent, dichromate cleaning solution, nitric acid, or AQUA REGIA (25 percent by volume concentrated HNO₃ in concentrated HCl).

In all cases it is good practice to rinse a vessel with tap water as soon as possible after use. Material allowed to dry on glassware is much more difficult to remove.

Dichromate cleaning solution (chromic acid) is a powerful cleaning agent; however, because of its destructive nature upon clothing and upon laboratory furniture, extreme care must be taken when using this mixture. If any of the solution is spilled, it must be cleaned up immediately.

A persistent greasy layer or spot may be removed by acetone or by allowing a warm solution of sodium hydroxide, about 1 g per 50 ml of water, to stand in the vessel for 10 to 15 min; after rinsing with water, dilute hydrochloric acid, and water again, the vessel is usually clean.

Special Cleaning Requirements

Absorption cells, used in spectrophotometers, should be kept scrupulously clean, free of scratches, fingerprints, smudges, and evaporated film residues.

Glassware to be used for phosphate determinations should not be washed with detergents containing phosphates. This glassware must be thoroughly rinsed with tap water and distilled water.

Bottles to be used for the collection of samples for organic analyses should be rinsed successively with chromic acid cleaning solution, tap water, distilled water, and, finally, several times with a redistilled solvent such as acetone. Caps are washed with detergent, rinsed with tap water, distilled water, and solvent. Liners are treated in the same way as the bottles and are stored in a sealed container.
Alcoholic potassium hydroxide is also effective in removing grease. To dry glass apparatus, rinse with acetone and blow or draw air through it. Glassware may be dried for immediate use by rinsing with redistilled acetone. Otherwise glassware may be oven dried or drip dried. Glassware should be stored immediately after drying to prevent any accumulation of dust and stored inverted or with mouth of glassware covered with foil.

**Disposable Glassware**

When the risk of washing a pipette for reuse becomes too great, as in the case of use with toxic materials, or when the cost of washing glassware becomes prohibitive, disposable vessels may be the answer, provided they meet the necessary specification. Various types are available including bacteriological, serological, and microdilution pipettes. Disposable glassware generally is made of soft glass although plastic vessels and pipettes are also available.

**Sterilizing Contaminated Glassware**

Autoclave glassware or sterilize it in large steam ovens or similar apparatus. If viruses or spore-bearing bacteria are present, autoclaving is absolutely necessary.

**Handling and Storing**

Protect clean glassware from dust. This is done best by plugging with cotton, corking, taping a heavy piece of paper over the mouth or placing the glassware in a dust-free cabinet. Store glassware in specially designed racks. Avoid breakage by keeping pieces separated.

**Glassware Proper Usage:**

- **Graduated cylinders** are used to measure small volume of liquids and solution for experiments.
- **Pipettes** (moher/ graduated) are used to deliver any precise volume within its range. A detailed way of using the pipette is described next:
  
  a. Prepare the pipette: clean the pipette with soap solution; rinse with several portion of tap water then with deionized water. No water droplets should adhere to the inner wall of the pipette. Transfer the liquid or solution that you intend to
pipette from the reagent bottle to a beaker. Dry the pipette tip with a clean, dust free towel or tissue. Rinse through the pipette tip into a waste beaker. Using the pipette pump (never use your OWN MOUTH) draw 2-3 ml volumes into the pipette as rinse. Roll each rinse around in the pipette so that the solution washes the entire surface of the inner wall. Deliver each rinse through the pipette tip into a waste beaker.

b. Fill and operate the pipette: to fill the pipette, place the tip well below the surface of the solution in the beaker. Then using the pipette pump, draw the solution into the pipette until its level is 3-5 mm above the mark.

c. Deliver the solution: remove the tip from the solution, dry the tip with a dust free towel, and holding the pipette in a vertical position over a waste beaker, control the delivery of the excess solution from the pipette by lightly pressing the release bottom until the meniscus is at the mark. Remove any suspended droplet on the pipette tip by touching the inside wall of the waste beaker. Deliver the solution to the receiving vessel; keep the tip above the level of liquid and against the wall of the receiving vessel. Do not blow or shake out the Last bit of solution that remains in the tip; this liquid has been included in the calibration of the pipette.

d. Clean the pipette: once the use of pipette is complete, rinse the pipette several times with deionized water. Roll each rinse to flush the inner wall of the pipette and drain through the tip.
- **Burettes**: It may be used when samples of various sizes must be dispensed or measured precisely. The burette consists of a narrow calibrated glass tube, fitted at the bottom with a valve for controlling the flow of liquid. The valve is commonly called stopcock. A burette must be cleaned before use. If the burette is not completely clean, the level of precision is not attained.

  a. Clean the burette with soap and water, using a special long handed burette brush to scrub the interior of the glass. Then rinse the burette with tap water.

  b. Do not attempt to admit water directly to the burette from the water tap. Fill a beaker with tap water, and pour from the beaker into the burette.

  c. Finally, rinse the burette several times with distilled water. Before use, the burette should again rinse with distilled water.

*Many of the reagent solutions used in burette may attack the glass of the burette if they are not removed. This would destroy the calibration.*
A common mistake made by junior students is to fill the burette with the reagents solution to be dispensed to exactly the 0.00 mark. This is not necessary or desirable in most cases and is a waste of time. The burette should be filled to a level that is comfortable for you to read (based on your height). The precise initial liquid level reading of the burette should be taken before the solution is dispensed and again after the liquid is dispensed. The reading should be made to the exact and precise ml. The volume of the liquid dispensed is then obtained by simple subtraction of the two volume readings.
4. SAMPLING

The analytical results of a sample are only as accurate as the quality of the sample taken. If your technique for collecting samples is poor, then no matter how accurate your lab procedures are, the results will be poor. By sampling according to set procedures, you reduce the chance of error and increase the accuracy of your sample results.

This draft will cover the proper methods of sampling, sample preparation, documentation and sampler cleaning.

4.1. Sample Types

There are mainly three types of Water/Wastewater samples:

1. **Grab samples**: Grab sample shows the characteristics of the water at the time of sampling only and should not exceed a sampling time of 15 minutes. Grab sampling is done for such procedures as batch discharge, constant waste stream characteristics and when the parameter tested deteriorates rapidly such as cyanides, volatile organic compounds and phenols.

2. **Composite Samples**: These are individual samples taken and deposited in the same collection bottle. There are two methods that are most common to collecting composite samples.

   a. **Time paced** is when samples are collected at set increments of time.
b. **Flow paced samples**: which are taken when a measured volume of water flows over the sensor of a flow meter, which is more preferred; since it gives the most representative sample. Metals, Base/Neutral/Acid Organics, BOD and TSS samples may be collected by this method.

3. **Integrated samples**: Those are combination of grab samples collected at the same time but at different locations. Integrated samples are required when the knowledge of the volume, movement, and composition of the various parts of the water being sampled usually is required. Collecting integrated samples is a complicated and specialized process that must be described adequately in a sampling plan for each test.

4.2. **Sampling Equipment**

Typical sampling equipment when taking water samples it's important to:

- Use the correct sampling equipment
- Use the correct personal protective equipment (PPE)
- Record the necessary information correctly
- Check all equipment before carrying out sampling.
There are many different types of equipment used for sampling water. Groundwater sampling is conducted using groundwater bores and low flow pumps. Using this kind of equipment requires advanced knowledge. You may have an opportunity to observe this sort of water sampling.

Equipment typically used when sampling surface water includes:
1. New, prepared plastic and glass sampling bottles, clearly labelled with a marker pen.
2. Sterile disposable gloves to avoid contamination.
3. A cooler for storing and transporting filled sample bottles.
4. Ice or dry ice to maintain samples at the correct temperature.
5. Any equipment needed for taking on-site tests (for example: thermometer, conductivity meter, pH meter)
6. Communication equipment (for example: mobile phone, walkie-talkie)
7. Any maps needed
4.3. Sample Labelling

Correct labelling of samples is essential. They need to be easily identified at all times. Without proper labelling, all samples can look alike and mistakes can happen.

Water sample labels must include:
- a) A unique identifying code for cross-referencing
- b) Date of sampling.
- c) They can also include:
- d) The location and name of the sampling site
- e) The name of the sampler
- f) The time of sampling
- g) The type of sample
- h) Any observations that might affect test results.

4.4. Sampling Methods

a. **Manual sampling:** Manual sampling involves minimal equipment but may be un- duly costly and time-consuming for routine or large-scale sampling programs. It re- quires trained field technicians and is often necessary for regulatory and research in- vestigations; for which critical appraisal of field conditions and complex sample col- lection techniques are essential. Manually collect certain samples, such as waters con- taining oil and grease.

b. **Automatic sampling:** Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling, and are used increasingly

4.5. Sample Storage and Preservation

Complete and unequivocal preservation of samples, whether domestic wastewater, in- dustrial wastes, or natural waters, is a practical impossibility because complete stability
for every constituent never can be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection.

**Sample Storage before Analysis**

a. Nature of sample changes: Some determinations are more affected by sample storage than others.

- Certain cations are subject to loss by adsorption on, or ion exchange with, the walls of glass containers. These include aluminum, cadmium, chromium, copper, iron, lead, manganese, silver, and zinc, which are best collected in a separate clean bottle and acidified with nitric acid to a pH below 2.0; to minimize precipitation and adsorption on container walls.

- Temperature changes quickly; pH may change significantly in a matter of minutes; dissolved gases (oxygen, carbon dioxide) may be lost. Because changes in such basic water quality properties may occur so quickly, determine temperature, reduction-oxidation potential, and dissolved gases in situ and pH, specific conductance, turbidity, and alkalinity immediately after sample collection. Many organic compounds are sensitive to changes in pH and/or temperature resulting in reduced concentrations during storage.

- Changes in the pH-alkalinity-carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values for calcium and total hardness. Microbiological activity may affect the BOD concentration. Color, odor, and turbidity may increase, decrease, or change in quality.

- Zero head-space is important in preservation of samples with volatile organic compounds and radon. After capping or sealing bottle, check for air bubbles by inverting and gently tapping it; if one or more air bubbles are observed then, if practical, discard the sample and repeat refilling bottle with new sample until no air bubbles are observed (this cannot be done if bottle contained preservatives before it was filled).
Preservation Techniques

To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment.

- Avoid using dry ice because it will freeze samples and may cause glass containers to break.
- Dry ice also may effect a pH change in samples.
- Keep composite samples cool with ice or a refrigeration system set at 4°C during compositing.
- Analyze samples as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, preferably store at 4°C.

- No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations to be made.
- Use chemical preservatives only when they do not interfere with the analysis being made.
<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTAINERS</th>
<th>SAMPLE VOLUME (mL)</th>
<th>PRESERVATION</th>
<th>MAXIMUM HOLDING TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WATER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ROUTINE WATER SAMPLE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Cubitainer or Glass</td>
<td>100</td>
<td>Cool to 4 °C, dark</td>
<td>14 days</td>
</tr>
<tr>
<td>Total Suspended Solids/Suspended Solids</td>
<td>Cubitainer or Glass</td>
<td>400</td>
<td>Cool to 4 °C, dark</td>
<td>7 days</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
<td>Cubitainer or Glass</td>
<td>100</td>
<td>None required</td>
<td>28 days</td>
</tr>
<tr>
<td>Sulfate (SO₄)</td>
<td>Cubitainer or Glass</td>
<td>100</td>
<td>Cool to 4 °C, dark</td>
<td>28 days</td>
</tr>
<tr>
<td>Orthophosphate (PO₄)</td>
<td>Cubitainer or Glass</td>
<td>150</td>
<td>Filter ASAP, Cool to 4 °C, dark</td>
<td>48 hours</td>
</tr>
<tr>
<td>Nitrate + Nitrite (NO₃ + NO₂)</td>
<td>Cubitainer or Glass</td>
<td>150</td>
<td>1-2 mL conc. H₂SO₄ to pH &lt;2, and Cool to 4 °C, dark</td>
<td>28 days</td>
</tr>
<tr>
<td>Ammonia (NH₃)</td>
<td>Cubitainer or Glass</td>
<td>150</td>
<td>1-2 mL conc. H₂SO₄ to pH &lt;2, and Cool to 4 °C, dark</td>
<td>28 days</td>
</tr>
<tr>
<td>Total Phosphorus (TPO₄)</td>
<td>Cubitainer or Glass</td>
<td>150</td>
<td>1-2 mL conc. H₂SO₄ to pH &lt;2, and Cool to 4 °C, dark</td>
<td>28 days</td>
</tr>
<tr>
<td>Total Organic Carbon (TOC)</td>
<td>Cubitainer or Glass</td>
<td>100</td>
<td>1-2 mL conc. H₂SO₄ to pH &lt;2, and Cool to 4 °C, dark</td>
<td>28 days</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>Quart cubitainer</td>
<td>1,000</td>
<td>Cool to 4 °C, dark</td>
<td>Filter 48 hours Filters may be stored frozen up to 30 days</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Quart cubitainer</td>
<td>50</td>
<td>Cool to 4 °C, dark</td>
<td>48 hours</td>
</tr>
<tr>
<td>Total Dissolved Solids</td>
<td>Quart cubitainer</td>
<td>250</td>
<td>Cool to 4 °C, dark</td>
<td>7 days</td>
</tr>
<tr>
<td>Hardness</td>
<td>Quart cubitainer</td>
<td>250</td>
<td>2 mL conc. HNO₃ to pH&lt;2; Cool to 4 °C, dark</td>
<td>6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 mL conc. H₂SO₄ to pH &lt;2; Cool to 4 °C, dark</td>
<td></td>
</tr>
</tbody>
</table>

**ROUTINE WATER SAMPLE COLLECTION PROCEDURE**

- Label container before collection with a unique sample identifier number, Station Location, Date and Sample Type
- Place an X on the container lid to identify the acidified sample.
- Open containers by pulling apart. Pre-rinsing cubitainers with ambient water is not necessary.
- Fill each container with ambient water by submerging container approximately one foot below the surface mid-stream until filled.
- Place sample on ice immediately. Acidify the X container as soon as possible.
- Place on ice and ship as soon as possible.
5. TECHNIQUES IN PREPARATION OF SOLUTIONS

There are five units of concentration that are particularly useful to chemists. The first three: Molality, Molarity and Normality are dependent upon the mole unit. The last two: percent by volume and percent by weight have nothing to do with mole, only weight or volume of the solute or substance to be diluted, versus the weight or volume of the solvent or substance in which the solute is diluted. Percentages can also be determined for solids within solids.

5.1. Molarity "M"

The molar unit is probably the most commonly used chemical unit of measurement. Molarity is the number of moles of a solute dissolved in a liter of solution. A molar solution of sodium chloride is made by placing 1 mole of a solute into a 1-liter volumetric flask.

\[ M = \frac{\text{moles of solute}}{1 \text{ L of solvent}} \]

5.2. Molality "m"

The molal unit is not used nearly as frequently as the molar unit. A molality is the number of moles of solute dissolved in one kilogram of solvent. Be careful not to confuse molality and molarity. Note that the solvent must be weighed unless it is water.

\[ m = \frac{\text{moles of solute}}{1 \text{ Kg of solvent}} \]

5.3. Normality "N"

A measure of concentration that is equal to the gram equivalent weight per liter of solution. Gram equivalent weight is a measure of the reactive capacity of a molecule.

\[ N = \frac{\text{eq.wt.}}{1 \text{ L of solvent}} \]
5.4. **Percent by weight**: To make up a solution based on percentage by weight, one would simply determine what percentage was desired. For example, a 20% by weight aqueous solution of sodium chloride, and the total quantity to be prepared.

If the total quantity needed is 1 kg, then it would simply be a matter of calculating 20% of 1 kg which, of course is:

\[0.20 \text{ NaCl} \times 1000 \text{ g/kg} = 200 \text{ g NaCl/kg}.\]

In order to bring the total quantity to 1 kg, it would be necessary to add 800g water.

5.5. **Percent by volume**: Solutions based on percent by volume are calculated the same as for percent by weight, except that calculations are based on volume. Thus one would simply determine what percentage was desired (for example, a 20% by volume aqueous solution of sodium chloride) and the total quantity to be prepared.

If the total quantity needed is 1 liter, then it would simply be a matter of calculating 20% of 1 liter which, of course is:

\[0.20 \text{ NaCl} \times 1000 \text{ ml/l} = 200 \text{ ml NaCl/l}.\]

Percentages are used more in the technological fields of chemistry (such as environmental technologies) than they are in pure chemistry.

**Dilution**

When preparing a dilution, decide the volume and molar concentration of the resulting solution you require. Use the following equation to determine how much of the concentrated reagent is needed to prepare the diluted solution,

\[
\text{No. of moles (reagent)} = \text{No. of moles (dilution)}
\]

\[
\text{MReagent} \times \text{VReagent} = \text{MDilution} \times \text{Vdilution}
\]
Where $M$ is molarity and $V$ is volume.

Slowly add the calculated volume of concentrated reagent to the proper-size volumetric flask half filled with distilled or de-ionized water and swirl the flask to mix. Once the solution is at room temperature, dilute to the mark with water and invert the flask several times to mix.

**Dilution Factor (DF)**

Dilution: is the mixing of a small accurately measured sample with a large volume of sterile water or normal saline called (diluents or dilution blank)

\[
\text{Dilution} = \frac{\text{Volume of Sample}}{\text{Total volume of (sample+diluent)}}
\]

\[
\text{Dilution Factor } DF = \frac{\text{Total Volume (sample+diluent)}}{\text{Volume of sample}}
\]
6. LABORATORY REPORT FORMAT

6.1. Objective of Lab Reports:

The primary objective of an experiment report is to inform instructors about testing procedure and the results being collected. The report should be well organized and written so that someone who is not familiar with the particular experiment or test set-up can understand the following:

- The objective and aim of the experiment.
- What procedures were followed and assumption being made.
- What data was collected and type of materials tested.
- What analysis was completed along with the necessary calculations?
- Which conclusions were made and recommendations established.

6.2. Laboratory Report Sections:

The format of each section of the laboratory report will be described next. **The format should be followed exactly as described below to avoid losing points.**

6.2.1. Cover Page

The cover page should include the name of the experiment, group number, group members, course number, date of the lab and date of report submittal and other relative information. A sample of required cover page with specific format is presented in next page.
HACETEPE UNİVERSİTY
Faculty of Engineering
Department of Environmental Engineering
CEV207 – Environmental Chemistry Laboratory
Instructor Name

Experiment No: Name of the Experiment as Listed in the Manual

Group Number
Student Name / Student Number
Date Submitted: MM/DD/YY

1. (Middle -15 pt- Times New Roman)
2. (Middle – Bold-16 pt-Arial)
3. (Middle -13 pt- Times New Roman)
4. (Middle – Bold-14 pt-Arial)
5. (Middle -12 pt- Times New Roman)

This page order is required
6.2.2. Abstract

The abstract is a brief summary of the full report. It should include any essential background information (e.g., the purpose of the experiment), a brief description of procedures or testing method used, a concise quantitative statement of results and important conclusions. *The purpose of the abstract is to allow the reader to determine whether or not it will be worth the while to read the entire paper.* The abstract should be on a separate page (the 2nd of the report). The title of the Abstract should be centered at the top of the page and **TYPED IN BOLD WITH UPPER-CASE LETTERS.**

Notes:

- Note how the Abstract begins with a statement of what was done and why. The style of an abstract is formal, with more frequent use of passive voice than you might employ in other sections of the report.
- Note also the mention of *specific results* and conclusions.

Points of Weakness:

- Omits critical findings.
- Refers reader to figures or tables in the report.
- Relies on vague language.
- Write introduction context and detail theoretical background.
- Omits the experiment summary or brief description.
6.2.3. Grading Table

The following is a grading table presents required sections in the lab report related points assigned for each one. This **table should be listed after the Abstract** and can be used as a check list when finish lab report.

<table>
<thead>
<tr>
<th>Item</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Organization &amp; Readability – 10%</td>
<td></td>
</tr>
<tr>
<td>Technical Writing and Adherence to lab report format – 5%</td>
<td></td>
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<tr>
<td>Abstract – 5%</td>
<td></td>
</tr>
<tr>
<td>Introduction &amp; Objective – 5%</td>
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<tr>
<td>Theoretical Background – 10%</td>
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<tr>
<td>Materials and Apparatus – 5%</td>
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<td>Procedure – 15%</td>
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<td>Calculations – 15%</td>
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<tr>
<td>Results and Discussion- 20%</td>
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<tr>
<td>Conclusions – 10%</td>
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</tr>
<tr>
<td>Total (100 Points)</td>
<td></td>
</tr>
</tbody>
</table>

6.2.4. Introduction & Objective

The ‘Introduction’ identifies the experiment, the physical phenomena that is being investigated, the objectives of the experiment, the importance of the experiment, and overall background for understanding the experiment. The objectives of the experiment are important to state because these objectives are usually analyzed in the conclusion to determine whether the experiment succeeded. The background often includes theoretical predictions for what the results should be.

**Points of Weakness:**

- Fails to clearly define the problem and the relevance of the experiment.
6.2.5. Theoretical Background

The theory behind the experiment should be clearly explained, including the key components of the theory, equations used in calculations, and any assumptions that are being considered during experimental work. Equations relevant to the experiment should be numbered. All referenced material should be properly footnoted.

6.2.6. Materials and Apparatus

All laboratory apparatus used in the investigation, along with a detailed diagram to illustrate the configuration of the apparatus, should be included in this section. The variables to be measured should be clearly pictured

Points of Weakness:

- Includes unnecessary detail and makes repeated use of ‘then’.
- Fails to use lists or diagrams when those would be helpful.

6.2.7. Procedure

This is where the experimental methods are undesired in details. A very detailed description of procedures include the information necessary to allow someone to repeat what you did. This section should identify and name all experimental variables and briefly describe how the independent variables are controlled. Someone who was not present during the lab should be able to understand how the experiment was performed by reading your procedure.

Points of Weakness:

- Includes unnecessary detail and makes repeated use of ‘then’.

6.2.8. Calculations & Evaluation of Data

This section should include all graphs, analysis of graphs, and post laboratory calculations (hand calculations). State each formula, and if necessary, identify the symbols used in the formula.
6.2.9. Results & Discussion

In discussing the results, you should not only analyze the results, but also discuss the implications of those results. Moreover, pay attention to the errors that existed in the experiment, both where they originated and what their significance is for interpreting the reliability of conclusions. One important way to present numerical results is to show them in graphs.

6.2.10. Conclusion

In longer laboratory reports, a ‘Conclusion’ section often appears. Whereas the ‘Results and Discussion’ section has discussed the results individually, the ‘Conclusion’ section discusses the results in the context of the entire experiment. Usually, the objectives mentioned in the ‘Introduction’ are examined to determine whether the experiment succeeded. If the objectives were not met, you should analyze why the results were not as predicted. Note that in shorter reports or in reports where ‘Discussion’ is a separate section from ‘Results’, you often do not have a ‘Conclusion’ section.

6.3. Why This Format?

By the 1970s, nearly all academic journals required this standard for scientific experimental reporting. The basic outline is shown below. An example lab report format is shown next pages.
6.4. Important Notes

- Avoid using personal pronouns (e.g. I, we, our, you, me, my...etc) in lab report.
- Make sure to write clearly. Ask when you read it loud to yourself or a friend, does it make sense? Don’t forget to use the spell-checker in your word processor.
- Do not use reports from previous semesters.
- **Past tense** should be used to describe what you did in lab. **Present tense** should be used for statements of fact and chemical properties. For example: ‘The melting point of unknown sample was measured to be 109 °C. The melting point of acetanilide is 114 °C.
- **Avoid using the first person** and any statements of how you ‘felt’ about an experiment, whether it was easy, or the supposition that you ‘learned a lot’ from the lab.
- This document follows standard academic formatting guidelines. These include 12 pt Font, 1 margins and headings which subdivide the information into manageable sections.

6.5. Laboratory Report Grading Criteria

The Lab Reports will be graded using the following guidelines:

1. Overall Organization & Readability – 10%
2. Technical writing and adherence to lab report format – 5%
3. Abstract – 5%
4. Introduction and Objective – 5%
5. Theoretical Background - 10%
6. Materials and Apparatus – 5%
7. Procedures – 15%
8. Calculations – 15%
9. Results and Discussion – 20%
10. Conclusions – 10%
HACETEPE UNIVERSITY

Faculty of Engineering

Department of Environmental Engineering

CEV207 – Environmental Chemistry Laboratory

Instructor Name

Experiment No: Name of the Experiment as Listed in the Manual

Group Number

Student Name / Student Number

Date Submitted: MM/DD/YY

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This page order is required
Grading Table

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</table>
1. Introduction & Objective (Left - Bold, 12 pt-Times New Roman)

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2. Theoretical Background (Left - Bold, 12 pt-Times New Roman)

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3. Materials and Apparatus (Left - Bold, 12 pt-Times New Roman)

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4. Procedure (Left - Bold, 12 pt-Times New Roman)

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5. Calculations (Left - Bold, 12 pt-Times New Roman)

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6. Results and Discussion (Left - Bold, 12 pt-Times New Roman)

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7. Conclusion (Left - Bold, 12 pt-Times New Roman)

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8. References (Left - Bold, 12 pt-Times New Roman)

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<th>Signature</th>
<th>Date</th>
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</table>

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### 7. EXPERIMENTS

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Introduction to the laboratory, lab safety, solution preparation, sampling and sample preservation</td>
</tr>
<tr>
<td>Week 2</td>
<td>Alkalinity and carbonate system</td>
</tr>
<tr>
<td>Week 3</td>
<td>Determination of hardness and conductivity</td>
</tr>
<tr>
<td>Week 4</td>
<td>Determination of solids (TS, VS, TSS, VSS, DS, etc.)</td>
</tr>
<tr>
<td>Week 5</td>
<td>Dissolved oxygen (DO) and Biochemical oxygen demand (BOD analysis)</td>
</tr>
<tr>
<td>Week 6</td>
<td>Chemical oxygen demand (COD analysis)</td>
</tr>
<tr>
<td>Week 7</td>
<td>Midterm Exam</td>
</tr>
<tr>
<td>Week 8</td>
<td>Khejdahl method (Determination of total organic nitrogen)</td>
</tr>
<tr>
<td>Week 9</td>
<td>Determination of total phosphorus</td>
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<tr>
<td>Week 10</td>
<td>Color, turbidity and oil-grease determination</td>
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<tr>
<td>Week 11</td>
<td>Determination of chloride and sulphate</td>
</tr>
<tr>
<td>Week 12</td>
<td>Quantitative analysis of metals (Fe-Mn)</td>
</tr>
<tr>
<td>Week 13</td>
<td>Make-up</td>
</tr>
<tr>
<td>Week 14</td>
<td>Make-up</td>
</tr>
<tr>
<td>Week 15</td>
<td>Make-up</td>
</tr>
<tr>
<td>Week 16</td>
<td>Final Exam</td>
</tr>
</tbody>
</table>
7.1. EXPERIMENT 1
ALKALINITY and CARBONATE SYSTEM

A. PURPOSE

To determine carbonate, bicarbonate, hydroxide and total alkalinity of a water sample.

B. THEORY

The alkalinity of a water sample is its acid-neutralizing capacity. It is significant in many uses and treatments of natural and wastewaters. Since alkalinity of many surface waters is primarily a function of carbonate, bicarbonate and hydroxide content, the alkalinity is taken as an indication of concentration of these constituents.

Carbonate, bicarbonate, hydroxide and total alkalinity are titrated with standard sulfuric or hydrochloric acid solutions. Depending on the nature of the sample and the specific intent of the examination, the procedure employed and the data produced vary from direct titration using a color indicator endpoint to construction of a titration curve.

Routine examination of water which is fairly clear and colorless may be performed by direct titration to a color indicator endpoint. Where the color change is obscured by turbid or highly colored waters a calibrated pH meter must be used to detect the endpoint.

The most accurate determination of the endpoint is found by construction of a titration curve, plotting the observed pH reading against volume of titrant added. Points of inflection in the curve indicate the pH of appropriate endpoints and serve as a guide to approximation of the various species of alkalinity. Samples containing free available residual chlorine and low alkalinity require special handling or pretreatment prior to examination.
C. APPARATUS AND MATERIALS

1- pH meter  
2- titration stand  
3- 250 mL erlenmeyer flask  
4- 100 mL beaker  
5- magnetic stirrer  
6- hot plate  
7- 50mL/25mL burets  
8- 25 mL volumetric flask  
9- 10 mL pipette

D. REAGENTS

Distilled water: Remove carbon dioxide by boiling to room temperature just prior to use.

Sodium carbonate solution (0.05N): Dry 3 to 5 g primary standard grade anhydrous sodium carbonate (Na$_2$CO$_3$) at 250 °C for 4 hours and cool in a desiccator. Dissolve 2.5 ± 0.2 g sodium carbonate in distilled water and dilute to 1000 mL.

Standard sulfuric or hydrochloric acid titrant (0.1N): Dilute 3.0 mL conc. H$_2$SO$_4$ or 8.3 mL conc. HCl to 1000 mL with distilled water. Standardize against 40 mL 0.05 N Na$_2$CO$_3$ solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH of about 5. Lift out electrodes, rinse into the same beaker, and boil gently for 3-5 min under a watch glass cover. Cool to room temperature, rinse the cover glass into the beaker, and finish the titration to the pH infection point. Calculate the normality according to

\[
\text{Normality, } N = \frac{AxB}{DxC}
\]

A: g Na$_2$CO$_3$ weighed into 1000 mL  
B: mL Na$_2$CO$_3$ solution taken for titration  
C: mL acid used  
D: equivalent weight of Na$_2$CO$_3$, 53 g
Standard sulfuric or hydrochloric acid titrant (0.02 N): Dilute 200 mL 0.1000 N standard acid to 1000 mL. Standardize the 0.02 N solution by the same method as used for the 0.1 N titrant, but titrate only 15 mL of the 0.05 N Na₂CO₃.

Mixed bromcresol green-methyl red indicator solution: Dissolve 20 mg methyl red sodium salt and 100 mg bromcresol green in 100 mL 95 % ethyl alcohol or isopropyl alcohol.

Methyl orange indicator solution: 0.05 % in distilled water.

Phenolphthalein indicator solution: 0.1 % in ethyl alcohol.

Sodium thiosulfate solution (0.1 N): Dissolve 25 g of sodium thiosulfate (Na₂S₂O₃.5H₂O) into a 1-liter volumetric flask and dilute to volume with distilled water.

E. PROCEDURE

1. Selection of sample size and normality of titrant
   - Samples having concentration of 500 mg/L alkalinity or higher, use 50-100 mL of sample and 0.1 N titrant.
   - Samples having alkalinity of 150 mL or below use 0.02 N titrant.
   - Samples which have low alkalinity of 20 mg/L or below may be reported only if a 100-200 mL sample is titrated with 0.02 N titrant using a 10 mL micro burette.
   - If the nature and concentration of the sample is totally unknown use a 100 mL sample portion.
   - Roughly titrate to the phenolphthalein end point then add mixed indicator solution and titrate to the light pink end point at approximately 4.5-4.6 pH.
   - End points: The following pH values are suggested as equivalence point for various concentrations of alkalinity.
<table>
<thead>
<tr>
<th>Concentration mg CaCO₃/L</th>
<th>Endpoint pH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Alkalinity</td>
<td>Phenolphthalein Alkalinity</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>5.1</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>4.8</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>4.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>silicate, phosphate</td>
<td>4.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>industrial waste or complex system</td>
<td>3.7</td>
<td>8.3</td>
<td></td>
</tr>
</tbody>
</table>

2. Color change titration

- Adjust sample to room temperature if it has been cooled for storage. Do not shake or agitate sample; otherwise, dissolved gases may be lost, thus affecting results. Pipet an appropriate volume of sample in to a 250 mL volumetric flask.
- If free available residual chlorine is present or suspected to be present add 1 drop of 0.1 N sodium thiosulfate solution.
- Add 2 drops of phenolphthalein indicator solution and if below 100 mL, bring sample volume up to approximately 100 mL with distilled water. Titrate to the color-change end point, using the appropriate strength of the titrant. Record the volume of titrant used. Color change is from reddish pink to the first colorless stage at pH 8.3.
- Add 2 drops of the mixed indicator solution and titrate to the appropriate color-change end point for total alkalinity. The color changes for the mixed indicator are approximately as follows: above 5.2 pH, greenish blue; pH 5.0 light blue with lavender gray cast; pH 4.8 light pink-gray with bluish cast; and pH 4.6 light pink. Record the volume of titrant used and pH of the color change end point used. For color change below pH=4.6, use methyl orange indicator solution.

3. Potentiometric titration

- Adjust sample to room temperature if it has been cooled for storage. Do not shake or agitate sample; otherwise, dissolved gases may be lost, thus affecting results. Pipet an appropriate volume of sample in to a 250 mL volumetric flask.
• Measure the pH of sample. Add standard acid increments of 0.5 mL or less, such that a change of less than 0.2 pH units occurs per increment. After each addition, mix thoroughly but gently with a magnetic stirrer.

• Record the pH when a constant reading is obtained. Continue adding titrant and measure pH until pH 3.7 is reached.

• Construct the titration curve by plotting the observed pH values against the cumulative mL titrant added.

F. CALCULATION

Report results as alkalinity in mg CaCO₃/L at the selected pH endpoint.

Alkalinity, mg CaCO₃/L = \frac{A \times N \times D}{mL \text{ of sample}} \times 1000

Where,

A: mL of titrant used to neutralize alkalinity to specific pH endpoint.

N: normality of titrant.

D: equivalent weight of CaCO₃, 50 g

G. REFERENCES

The results obtained from the phenolphthalein and total alkalinity determination offer a means for stoichiometric classification of the three principal forms of alkalinity present in many waters. The classification ascribes the entire alkalinity to bicarbonate, carbonate, and hydroxide, and assumes the absence of other (weak) inorganic or organic acids, such as silicic, phosphoric, and boric acids.

<table>
<thead>
<tr>
<th>RESULTS of TITRATION</th>
<th>HYDROXIDE ALKALINITY</th>
<th>CARBONATE ALKALINITY</th>
<th>BICARBONATE ALKALINITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolphthalein Alkalinity = 0</td>
<td>0</td>
<td>0</td>
<td>Equal to total</td>
</tr>
<tr>
<td>Phenolphthalein Alkalinity less than one half of total alkalinity</td>
<td>0</td>
<td>Two times the Phenolphthalein Alkalinity</td>
<td>Total Alkalinity minus two times Phenolphthalein Alkalinity</td>
</tr>
<tr>
<td>Phenolphthalein Alkalinity equal to one half of total alkalinity</td>
<td>0</td>
<td>Two times the Phenolphthalein Alkalinity</td>
<td>0</td>
</tr>
<tr>
<td>Phenolphthalein Alkalinity greater than one half of total alkalinity</td>
<td>Two times the Phenolphthalein minus Total Alkalinity</td>
<td>Two times the difference between Total and Phenolphthalein Alkalinity</td>
<td>0</td>
</tr>
<tr>
<td>Phenolphthalein Alkalinity equal to total alkalinity</td>
<td>Equal to the Total Alkalinity</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
ALKALINITY

FLOWCHART OF THE EXPERIMENT
### Data Obtained From Direct Titration Method

<table>
<thead>
<tr>
<th>Titrant =</th>
<th>Normality of Titrant =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Volume of Sample (mL)</td>
</tr>
<tr>
<td>Blank (DW)</td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td></td>
</tr>
</tbody>
</table>

### Data Obtained From Potentiometric Method

<table>
<thead>
<tr>
<th>Titrant =</th>
<th>Normality of Titrant =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative Titrant Added (mL)</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>
ALKALINITY
7.2. EXPERIMENT 2

Part 1: DETERMINATION OF THE TOTAL HARDNESS OF WATER

A. PURPOSE

To determine the total hardness of a given water sample by EDTA method before and after softening operations with a synthetic zeolite.

B. THEORY

The hardness of water is characterized by the presence of divalent metallic cations such as Ca$^{2+}$, Mg$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Sr$^{2+}$, Zn$^{2+}$… The hardness of water is also understood to be a measure of the capacity of the water for precipitating soap by the commonly present ions, Ca$^{2+}$ and Mg$^{2+}$. Hardness is defined as total concentration of Ca and Mg ions expressed as calcium carbonate.

Hardness in water is mainly due to the contact of water (rain water) with the soil and the consequent dissolution of basic materials, especially limestone formations. Thus, the hardness of water changes from place to place depending on the geological formation, but also from source to source, so that surface waters are softer than ground waters. Total hardness value of a water sample can be evaluated by multiplying the concentration of each hardness-producing cation by the proper factor to obtain equivalent calcium carbonate concentrations. To obtain the CaCO$_3$ equivalent of the following cations, multiply the concentration found by the factor given in the table:

<table>
<thead>
<tr>
<th>Cation</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>2.497</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>4.116</td>
</tr>
<tr>
<td>Sr$^{2+}$</td>
<td>1.142</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>1.192</td>
</tr>
<tr>
<td>Al$^{3+}$</td>
<td>5.564</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>1.531</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>1.822</td>
</tr>
</tbody>
</table>
For example:

Hardness: $2.497 \text{Ca}^{2+} + 4.116 \text{Mg}^{2+} + 1.192 \text{Fe}^{2+} + \ldots (\text{mg/L CaCO}_3)$

Hardness of water is generally determined with the EDTA titrimetric method. Ethylenediaminetetraacetic acid (EDTA) and its disodium salts are able to form extremely stable chelated soluble complexes when added to solutions containing metal cations like calcium and magnesium ions.

$$M^{2+} + \text{EDTA} \rightarrow [M \cdot \text{EDTA}]_{\text{complex}}$$

During the hardness determination a dye, usually Eriochrome Black T, is added to the sample as an indicator solution. At a pH of about 10, Eriochrome Black T, having a blue color, combines with the $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ ions to form a weak complex ion which is wine red in color.

$$M^{2+} + \text{Eriochrome Black T} \rightarrow [M \cdot \text{Eriochrome Black T}]_{\text{complex}}$$

blue $\rightarrow$ wine-red

When EDTA is added as titrant, all hardness ions are complexed as represented in the first equation, releasing the Eriochrome Black T and thus turning the red color to a distinct blue color. This indicates the END POINT of titration.

When reporting hardness, the ions determined should be stated as: hardness (Ca, Mg); hardness (Ca, Mg, Sr, Fe, etc.) and EDTA hardness. Generally, hardness of a water is an important consideration in the determining the suitability of a water for domestic and industrial uses.

A water sample can be classified as follows:

<table>
<thead>
<tr>
<th>CaCO$_3$, mg/L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0-75</td>
<td>Soft</td>
</tr>
<tr>
<td>75-150</td>
<td>Medium</td>
</tr>
<tr>
<td>150-300</td>
<td>Hard</td>
</tr>
<tr>
<td>&gt;300</td>
<td>Very Hard</td>
</tr>
</tbody>
</table>
C. APPARATUS AND MATERIALS

Erlenmeyer flasks, burettes, funnels.

D. REAGENTS

1. Buffer solution (pH 10 ± 0.1): Dissolve 6.74 g ammonium chloride (NH₄Cl) in 57 mL conc. ammonium hydroxide (NH₄OH). Add 0.5 g magnesium salt of EDTA and dilute to 100 mL with distilled water. If magnesium salt of EDTA is not available, dissolve 0.47 g disodium salt of ethylenediamine tetraacetic acid dihydrate and 310.91 mg magnesium sulfate (MgSO₄·7H₂O) or 256.7 mg magnesium chloride (MgCl₂·6H₂O) in 20 mL distilled water and add this solution to 6.74 g NH₄Cl and 57 mL conc. NH₄OH while mixing. Dilute to 100 mL with distilled water. The pH should be equal to 10.

2. Indicator solution: Dissolve 0.5 g Eriochrome Black T and 4.5 g hydroxylamine hydrochloride (NH₂OH.HCl) in 100 mL 95% ethyl or isopropyl alcohol.

3. Standard EDTA titrant, 0.01 M: Dissolve 3.723 g analytical grade disodium EDTA in distilled water and dilute to 1000 mL. Standardize with CaCO₃ solution as follows;
   - Place 25 mL of standard CaCO₃ solution into a 250 mL Erlenmeyer flask.
   - Add 2 mL buffer solution and 2 drops of indicator solution.
   - Titrate slowly and carefully with EDTA titrant. The end point is when the color changes from red to blue.

Calculate the molarity of standard EDTA titrant. Calculate the mg CaCO₃ equivalent to 1 mL EDTA titrant.

4. Standard CaCO₃ solution: Weigh 0.25 g of anhydrous CaCO₃, powder into a 500 mL Erlenmeyer flask, add 1+1 HCl dropwise until all the CaCO₃ has dissolved. Add 100 mL of distilled water and boil for a few minutes to expel CO₂. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3 N NH₄OH or 1+1 HCL, as required.
CALCULATION

**Factor:** \( \frac{a}{b} \)

Where;

a: mL of CaCO\(_3\) standard solution

b: mL of 0.01 M EDTA used in the titration

E. PROCEDURE

For the procedure given below work with parallel samples.

1. Titration of sample:

   - Place 25 mL of the sample in an Erlenmeyer flask and dilute to 50 mL with distilled water.
   - Add 1-2 mL buffer solution (do not extend duration of titration beyond 5 minutes measured from the time of buffer addition).
   - Add 2 drops of indicator solution.
   - Titrate slowly and carefully with disodium EDTA solution until the color changes from red to blue.

2. Titration of softened water sample:

   - Take 25 mL sample
   - Follow the steps given above for sample, making corrections to the volume of the reagent to be taken.
   - Follow the same steps for a 25 mL tap water sample.
   - Subtract the volume of EDTA used for blank from the volume of EDTA used for sample.

F. CALCULATIONS

Hardness (CaCO\(_3\), mg/L) = \( \frac{A \times B}{\text{mL sample}} \times 1000 \)
Where;

A: mL of EDTA titrant used for sample

b: mg CaCO₃ equivalent to 1.00 mL EDTA titrant
HARDNESS

FLOWCHART OF THE EXPERIMENT
DETERMINATION OF THE TOTAL HARDNESS OF WATER

Preparation of Standard EDTA

EDTA Purity Factor
Mass EDTA Used
Molarity of EDTA

Hardness Titration

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Sample Volume</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Volume of EDTA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hardness (ppm)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
DETERMINATION OF THE TOTAL HARDNESS OF WATER
7.2. EXPERIMENT 2  
Part 2: CONDUCTIVITY

A. PURPOSE

To determine the ability of a water sample to carry electrical current.

B. THEORY

Conductivity is a numerical expression of the ability of a water sample to carry electrical current. This number depends on the total concentration of the ionized substances dissolved in the water and the temperature at which the measurement is made. The mobility of each of the various dissolved ions, their valences, and their actual and relative concentrations affect conductivity.

An aqueous system containing ions will conduct electrical current. In a direct current field the positive ions migrate toward the negative electrode, while the negatively charged ions migrate toward positive electrode. Most inorganic acids, bases and salts (such as hydrochloric acid, sodium carbonate and sodium chloride) are relatively good conductors. Conversely, molecules of such organic compounds as sucrose and benzene that do not dissociate in aqueous solution conduct a current very poorly, if at all.

Freshly distilled water has a conductivity of 0.5 to 2 µmhos/cm, increasing after a few weeks of storage to 2 to 4 µmhos/cm. This increase is caused mainly by absorption of atmospheric carbon dioxide, and, to a lesser extent, ammonia.

The conductivity of potable waters ranges generally from 50 to 1500 µmhos/cm. The conductivity of domestic wastewater reflects to a degree the characteristic of the water supply serving the district. Some industrial wastes may have conductivities well in excess of 10000 µmhos/cm. Conductivity determination in laboratory is usually measured in resistance units (ohms or megaohms). The reciprocal of resistance is conductance, which is converted to the specific conductivity with the help of cell constant.
C. APPARATUS

Conductivity Salinity Meter (WTW LF 320/SET Microprocessor Conductivity Meter)

D. PROCEDURE

1. Switch on the Conductivity Salinity Meter
2. Select conductivity measurement from scroll mode
3. If cell constant, C, temperature function TC and reference temperature are set correctly, rinse probe and immerse into sample and read conductivity as µS/cm (1mS/m = 10 µmhos/cm)
4. If cell constant, C, temperature function TC and reference temperature are not set correctly, set them as described in the instruction manual of WTW LF 320/SET and measure conductivity.

E. REFERENCES

2. Instruction Manual of WTW LF 320/SET.
CONDUCTIVITY

FLOW CHART OF THE EXPERIMENT
Conductivity Data for Water Samples

<table>
<thead>
<tr>
<th>Water Sample</th>
<th>Conductivity (µS/cm)/(µhmhos/cm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
CONDUCTIVITY
7.3. EXPERIMENT 3
SOLIDS DETERMINATION

A. PURPOSE

To familiarize the students with various analytical operations such as weighing, filtration, evaporation and combustion which are commonly encountered in gravimetric analysis. To demonstrate the separation and categorization of different kinds of solids.

B. THEORY

Gravimetric analysis is based on the determination of constituents or categories of materials by measurement of their weight. In this experiment, filtration, evaporation and combustion operations are illustrated in addition to weighing.

Filtration is used to separate the suspended solids from the dissolved portion of polluted water. Glass-fiber filters are used for this purpose.

Evaporation is used to separate water from dissolved or suspended solids. Evaporation of water and wastewaters is usually done at 103-105 °C at which decomposition and volatilization of carbonates and chlorides are not seen.

Combustion is used to determine the organic portion of dissolved or suspended solids. The standard procedure is to conduct ignitions at 550 ± 50 °C. This is about the lowest temperature at which organic matter is converted to carbon dioxide and water. Also, at 600 °C inorganic salts are relatively stable, with the exception of magnesium carbonate, as shown below:

\[ 350 \, ^\circ C \]

MgCO\(_3\) \rightleftharpoons MgO + CO\(_2\)
C. APPARATUS AND MATERIALS

1. Analytical balance
2. Drying oven, 103 °C
3. Muffle furnace, 550±50 °C
4. Glass fiber filter discs, 4.7 cm, without organic binder.
5. Filter holder, membrane filter funnel, suction flask.
6. Forceps
7. Evaporating dishes, porcelain, 100 mL volume.
8. Steam-bath
9. Desiccator
10. Imhoff Cones
11. Crucibles

D. REAGENTS

Sample
1. Lactose 2.0 g/L
2. NaCl, 0.3 g/L
3. NaCO₃, 0.7 g/L
4. Kaolinite, 1.0 g/L

E. PROCEDURE

1. Total Solids

- If volatile residue is to be measured, heat at 550 ± 50 °C for one hour in a muffle furnace. If only total solids are to be measured, heat the clean evaporating dish to 103-105 °C for one hour. Cool, desiccate, weigh and store in desiccator.
- Transfer a measured aliquot of sample to the pre-weighed dish and evaporate to dryness on a steam bath or in a drying oven.
- Dry the evaporated sample for at least one hour at 103-105 °C. Cool in a desiccator and weigh until a constant weight is obtained.
2. Total Suspended Solids

- Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 mL volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus and dry in an oven at 103-105 °C for one hour. Keep in the desiccator until needed. Weigh immediately before use. After weighing handle the filter with forceps only.
- Under vacuum, filter 50 mL well mixed sample.
- Carefully remove the filter from the funnel, and transfer to an aluminum or stainless steel planchet as a support.
- Dry for one hour at 103-105 °C. Cool in a desiccator and weigh until a constant weight is obtained.

3. Total Dissolved Solids

- Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 mL volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard the washings.
- Preparation of evaporating dishes: If volatile residue is also to be measured, heat the clean dish at 550 ± 50 °C for one hour in a muffle furnace. If only total dissolved solids are to be measured, heat the clean evaporating dish at 103-105 °C for one hour. Cool, desiccate, weigh and store in desiccator.
- Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 50 mL to the funnel by means of a 50 mL graduated cylinder.
- Filter the sample through glass fiber filter, rinse with three 10 mL portions of distilled water and continue to apply vacuum for about 3 min. after filtration is complete to remove as much water as possible.
- Transfer 50 mL of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.
- Dry the evaporated sample for at least one hour at 103-105 °C. Cool in a desiccator and weigh until a constant weight is obtained.
4. Total Volatile and Fixed Solids

- Ignite residues produced from Total Solids determination to constant weight in a muffle furnace at 550 ± 50 °C. (Usually 15-20 min. ignition is required).
- Allow the dish to cool partially in air until most of the heat has been dissipated and transfer to a desiccator for final cooling in a dry atmosphere. Weigh the dish as soon as it has cooled completely. Repeat weighing until a constant weight is obtained.

5. Volatile Suspended Solids

- Ignite residue on filter produced from Total Suspended Solids determination at 550 ± 50 °C to a constant weight. (Usually 15-20 min. ignition are required)
- Cool in desiccator and reweigh until a constant weight is obtained.

6. Volatile Dissolved Solids

- Ignite residues from Total Dissolved Solids determination for 30 min at 550 ± 50 °C.
- Cool in desiccator and reweigh until a constant weight is obtained.

7. Settleable Solids

- Transfer 1 liter samples to Imhoff Cones.
- Read volume of sludge at 10 min intervals for 1 hour.
- Report as total mL/L/h and mL/10 min.

F. CALCULATIONS

1. Write down the data obtained in the table form.
2. Indicate results in mg/L.
Bringing Materials to Constant Weight:

I- Weight of Filter Paper

First Weighing  
Second Weighing  
Average Weighing

II- Weight of Crucible

First Weighing  
Second Weighing  
Average Weighing

III- Weight of Evaporating Dish (used for Dissolved Solids Determination)

First Weighing  
Second Weighing  
Average Weighing

IV- Weight of Evaporating Dish (used for Total Solids Determination)

First Weighing  
Second Weighing  
Average Weighing
### Suspended Solids and Volatile Suspended Solids

Sample Volume = mL

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tare mass of filter paper</td>
<td></td>
</tr>
<tr>
<td>Weight of filter paper and residue remained (after 105 °C)</td>
<td></td>
</tr>
<tr>
<td>Tare mass of crucible</td>
<td></td>
</tr>
<tr>
<td>Weight of filter paper + crucible + filter residue (after 600 °C)</td>
<td></td>
</tr>
</tbody>
</table>

### Total Dissolved Solids and Total Volatile Suspended Solids

Sample Volume = mL

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tare mass of evaporating dish</td>
<td></td>
</tr>
<tr>
<td>Weight of dish + sample (after 105 °C)</td>
<td></td>
</tr>
<tr>
<td>Weight of dish + sample (after 600 °C)</td>
<td></td>
</tr>
</tbody>
</table>

### Total Solids and Total Volatile Solids

Sample Volume = mL

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tare mass of evaporating dish</td>
<td></td>
</tr>
<tr>
<td>Weight of dish + sample (after 105 °C)</td>
<td></td>
</tr>
<tr>
<td>Weight of dish + sample (after 600 °C)</td>
<td></td>
</tr>
</tbody>
</table>

### Settling Test Data (in an Imhoff Cone for 1 hour)

Sample Volume = 1 L

<table>
<thead>
<tr>
<th>t (min)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>mL/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SOLIDS DETERMINATION
7.4. EXPERIMENT 4
DISSOLVED OXYGEN ANALYSIS and BIOCHEMICAL OXYGEN DEMAND

A. PURPOSE
To determine the BOD\(_5\) value that is used as a criterion for the biodegradable pollutants present in water and the resulting load on the oxygen balance of the same sample by using the dilution method.

B. THEORY
The biochemical oxygen demand (BOD) is a measure of the amount of oxygen required by bacteria to oxidize waste aerobically to simple end-products such as carbon dioxide, water and ammonia. It is used for estimating the water pollution potential of a given amount of waste.

The oxidation can be shown as:

Microorganisms + organic matter + O\(_2\) $\rightarrow$ more microorganisms + CO\(_2\) + H\(_2\)O + NH\(_3\)

BOD\(_5\) determination is made by the addition of a known and sufficient quantity of elemental oxygen-saturated dilution water such that at the end of the contact time a residual amount of at least 0.5 mg/L oxygen is left. BOD\(_5\) determination requires the presence of bacterial flora capable of degrading the organic matter present in the sample.

Theoretically, an infinite time is required for complete biological oxidation but for practical purposes a period of 5 days is found satisfactory.

The rate of oxidation has been approximated by a first order equation:

$$ y = L \left[ 1 - e^{-kt} \right] $$

$$ y = \text{BOD at any time} $$

$$ L = \text{Total or ultimate BOD} $$

$$ k = \text{First order rate constant} $$

$$ t = \text{Time, days} $$

The time dependent equation enables us to find the BOD value at any given time.
BOD determination are carried out via DO measurements according to Alsterberg Azide Modification of the Winkler Method. The reactions involved are as follows:

\[ 2 \text{Mn}^{2+} + 4 \text{OH}^- + \text{O}_2 \rightarrow 2\text{MnO}_2 + 2 \text{H}_2\text{O} \]

brown

\[ \text{MnO}_2 + 4\text{H}^+ + 2\text{I}^- \rightarrow \text{I}_2 + \text{Mn}^{2+} + 2\text{H}_2\text{O} \]

brown solution

\[ \text{I}_2 + 2\text{S}_2\text{O}_3^{2-} \rightarrow \text{S}_4\text{O}_6^{2-} + 2\text{I}^- \]

DO measurements can be performed using DO probes, if available.

Interference caused by nitrites can be eliminated by the addition of sodium azide.

\[ 6\text{N}_3^- + 2\text{NO}_2^- + 8\text{H}^+ \rightarrow 10 \text{N}_2 + 4\text{H}_2\text{O} \]

The azide modification method for DO determination can be used if samples contain more than 50 mg/L nitrite nitrogen and not more than 1 mg/L ferrous iron or other reducing or oxidizing materials should be absent.

C. APPARATUS AND MATERIALS

1. Air incubation or water bath, thermostatically controlled at 20±1 °C (in the dark).
2. Incubation bottles, 300 mL capacity.

D. REAGENTS

1. Distilled water, high quality.
2. Phosphate buffer solution, pH=7.2.

Dissolve 0.850 g KH\(_2\)PO\(_4\)

\[ 2.175 \text{ g K}_2\text{HPO}_4 \]

\[ 3.340 \text{ g Na}_2\text{HPO}_4.7\text{H}_2\text{O} \]

0.17 g NH\(_4\)Cl in 50 mL distilled water and dilute to 100 mL. The pH should be 7.2 without further adjustment.
3. Magnesium sulfate solution

Dissolve 2.25 g MgSO$_4$. 7H$_2$O in distilled water and dilute to 100 mL.

4. Calcium chloride solution

Dissolve 2.75 g anhydrous CaCl$_2$ in distilled water and dilute to 100 mL.

5. Ferric chloride solution

Dissolve 0.25 g FeCl$_3$.6H$_2$O in distilled water and dilute to 100 mL. Take 10 mL from this solution dilute to 100 mL.

6. Manganese sulfate solution

Dissolve 48.0 g MnSO$_4$.4H$_2$O, 40.0 g MnSO$_4$.2H$_2$O or 36.4 g MnSO$_4$.H$_2$O in distilled water, filter and dilute to 100 mL.

7. Alkali-iodide-azide reagent

Dissolve 50 g NaOH or 70 g KOH and 13.5 g NaI or 15 g KI in distilled water and dilute to 100 mL. To this solution add 1 g sodium azide, NaN$_3$, dissolved in 4 mL distilled water.

8. Concentrated sulfuric acid

9. Starch solution

Dissolve 2 g laboratory-grade soluble starch on a 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

10. Standard sodium thiosulfate titrant, 0.025 N

Dissolve 6.205 g NaS$_2$O$_3$.5H$_2$O in freshly boiled and cooled distilled water, add 1.5 mL 6 N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. (1 mL 0.025 N thiosulfate is equivalent to 0.2 mg DO). Standardize against the standard potassium dichromate solution.

11. Standard potassium dichromate solution, 0.025 N

Dissolve 1.226 g K$_2$Cr$_2$O$_7$ (Dried for 2 h at 103 °C) in distilled water and dilute to 1000 mL.
E. PROCEDURE

1. Standardization of sodium thiosulfate solution
Dissolve 2 g KI in 100 mL distilled water in a 500 mL Erlenmeyer.
Add 10 mL 1+3 H$_2$SO$_4$.
Add 20 mL standard dichromate solution
Dilute to 200 mL.

Titrate liberated iodine with the thiosulfate titrant; add 2 mL starch solution toward the end of the titration when a pale straw color is reached. Calculate the normality of the thiosulfate solution.

2. Preparation of dilution water
Store the distilled water in cotton-plugged bottles long enough to become saturated with DO; use distilled water at 20 ± 1 °C.
Add for 1 L of dilution water:
- 1 mL MgSO$_4$ solution
- 1 mL CaCl$_2$ solution
- 1 mL FeCl$_3$ solution, and
- 1 mL phosphate buffer solution (on the day of use)

3. Preliminary steps:
- Take clean 9 BOD bottles and measure exact capacity, (300 mL)
- Prepare 4 L dilution water
- Use 6 BOD bottles for sample and the rest for blank solution
- Add suitable amount of sample to make 2% mixture in BOD bottle
- Stopper and water seal BOD bottles
- Incubate at 20 °C
- 2 sample bottles + 1 blank bottle for 0$^{th}$ day
- 2 sample bottles + 1 blank bottle for 3$^{rd}$ day
- 2 sample bottles + 1 blank bottle for 5$^{th}$ day
- Determine initial DO values for (2 sample + 1 blank) bottles, 15 min after preparation.
4. Determination of DO:

4.1. Titrimetric Method: Alkali-azide modification of Winkler Method:

To the bottles;

- Add 2 mL MnSO$_4$ solution
- 2 mL alkali-iodide-azide reagent well below the surface of the liquid.
- Stopper carefully to exclude air bubbles and mix by inverting the bottle at least 15 times.
- When precipitate settles, leaving a clear supernatant above the MnO$_2$ floc, shake again.
- After at least 2 min settling has produced at least 100 mL of clear supernatant, carefully add 2 mL conc. H$_2$SO$_4$ by allowing the acid to run down the neck of the bottle, restopper, and mix by inversion until dissolution is complete.
- Take 203 mL solution (200x300/(300-4)=203) to a clean erlenmeyer flask, titrate with 0.0250 N standard sodium thiosulfate titrant to a pale straw color.
- Add 1-2 mL starch solution. Continue the titration to the disappearance of the blue color. Note the volume of titrant used.

4.2. Electrode Method:

In this method, Portable Dissolved Oxygen Meter is used.

Operating Procedure:

- Prepare probe and connect to instrument
- Perform battery check
- Set MODE switch to OPERATE
- Zero instrument
- Take temperature measurement and determine calibration value for appropriate atmospheric pressure
- Select desired range and adjust instrument to calibration value
- Place probe in sample solution and read mg/L DO.

F. CALCULATIONS

Calculate DO value (mg/L)

BOD (mg/L) = (D$_1$ − D$_2$) − (B$_1$ − B$_2$) f / P
D₁ = DO of diluted sample immediately after preparation, mg/L

D₂ = DO of diluted sample after incubation, mg/L

P = Decimal volumetric fraction of sample used

B₁ = DO of seed control before incubation, mg/L

B₂ = DO of seed control after incubation, mg/L

f = fraction of seed in diluted sample to seed in seed control

a. Draw the rate curve as BOD (mg/L) vs. time (days) and find the value of ultimate value (L) of BOD.

b. Draw log L/L₄ versus t and find the value of the rate constant (k).

G. REFERENCES

DISSOLVED OXYGEN ANALYSIS and BIOCHEMICAL OXYGEN DEMAND
FLOWCHART OF THE EXPERIMENT
**DISSOLVED OXYGEN ANALYSIS and BIOCHEMICAL OXYGEN DEMAND**

Experimental Data of DO Measurements by Electrode Method

<table>
<thead>
<tr>
<th>Day</th>
<th>DO in Blank</th>
<th>DO in Seeded Blank</th>
<th>DO in Sample 1</th>
<th>DO in Sample 2</th>
<th>DO (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2\textsuperscript{nd} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experimental Data of Titrmetric Method for DO Determination

<table>
<thead>
<tr>
<th>Day</th>
<th>Volume of Titrant Used (mL)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td>Seeded Blank</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Average</td>
</tr>
<tr>
<td>0\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2\textsuperscript{nd} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5\textsuperscript{th} day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISSOLVED OXYGEN ANALYSIS and BIOCHEMICAL OXYGEN DEMAND
7.5. EXPERIMENT 5
CHEMICAL OXYGEN DEMAND

I. OPEN REFLUX, Titrimetric Method

A. PURPOSE

To determine the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of oxidizing agent, temperature and time.

B. THEORY

The chemical oxygen demand (COD) determination is a measure of the pollutional strength of a waste in terms of the total quantity of oxygen required for oxidation of organic compounds. The oxidation action $K_2Cr_2O_7$ in the presence of $Ag_2SO_4$ is effective enough to degrade all substances to $CO_2$ and $H_2O$.

Most types of organic matter are destroyed by a boiling mixture of chromic and sulfuric acids. The principle of this method is that a sample is refluxed with known amounts of $K_2Cr_2O_7$ and $H_2SO_4$ and the excess dichromate is back titrated with ferrous ammonium sulfate using ferroin indicator. The amount of oxidizable organic matter is proportional to the amount of potassium dichromate consumed. The reactions involved may be represented as follows:

$C_nH_aO_b + cCr_2O_7^{2-} + 8cH^+ \rightarrow nCO_2 + (a+8c)/2 H_2O + 2cCr^{3+}$

$c = (2/3) + a/6 - b/3$

$6/Fe^{2+} \rightarrow Fe^{3+} + e^-$

$Cr_2O_7^{2-} + 14H^+ +6e^- \rightarrow 2Cr^{3+} +7H_2O$

$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 6Fe^{3+} + 2Cr^{3+} + 7H_2O$
Interference:
If chloride ion is present in wastewaters, high result in COD test is expected due to oxidation of chloride itself. This can be eliminated by addition of mercuric sulfate before refluxing.

\[6\text{Cl}^- + \text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ \rightarrow 3\text{Cl}_2 \uparrow + 2\text{Cr}^{3+} + 7\text{H}_2\text{O}\]

Volatile materials may be lost when the sample temperature rises during the sulfuric acid addition step. To minimize this lost the flask should be cooled during addition of the sulfuric acid solution.
Trace of organic material either from the glassware or atmospheric may cause a gross positive error.
Some types of aromatic organic compounds are not oxidized in the COD test, even with \(\text{Ag}^+\). When aromatics are present the measured COD is lower than the true value.

Application:
This method can be applied to domestic and industrial waste samples having an organic carbon concentration greater than 50 mg/L.

C. APPARATUS AND MATERIALS
Reflux apparatus, burette 25 mL, and heater.

D. REAGENTS

1. Standard potassium dichromate solution, 0.25 N: Dissolve 12.259 g \(\text{K}_2\text{Cr}_2\text{O}_7\), primary standard grade (previously dried at 103 °C for 2 hr) in distilled water and dilute to 1000 mL.
   2. Sulfuric acid reagent: Add \(\text{Ag}_2\text{SO}_4\), reagent or technical grade, crystals or powder, to conc. \(\text{H}_2\text{SO}_4\) at the rate of 5.5 g \(\text{Ag}_2\text{SO}_4/\text{kgH}_2\text{SO}_4\). Let stand 1 to 2 days to dissolve \(\text{Ag}_2\text{SO}_4\).
3. Standard ferrous ammonium sulfate titrant, 0.25 N:
Dissolve 98 g Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O in distilled water, add 20 mL conc. H$_2$SO$_4$, cool and dilute to 1000 mL. Standardize daily against the standard K$_2$Cr$_2$O$_7$ solution as follows:

Dilute 25 mL standard K$_2$Cr$_2$O$_7$ solution to about 100 mL. Add 30 mL conc. H$_2$SO$_4$ and cool.
Titrate with ferrous ammonium sulfate titrant using 2-3 drops ferroin indicator.

Normality of FAS = \( \frac{A \times B}{C} \)

A: mL of standard K$_2$Cr$_2$O$_7$
B: Normality of standard K$_2$Cr$_2$O$_7$ solution
C: mL of Fe(NH$_4$)$_2$(SO$_4$)$_2$ titrant used

4. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate together with 695 mg FeSO$_4$.7H$_2$O in water and dilute to 100 mL.
5. Mercuric sulfate: HgSO$_4$ crystals.
6. Sulfamic Acid: Required only if the interference of nitrites is to be eliminated. To eliminate a significant interference due to NO$_2^-$, add 10 mg sulfamic acid for each mg NO$_2$-N present in the sample volume used; add the same amount of sulfamic acid to the reflux vessel containing the distilled water blank.
7. Potassium hydrogen phthalate (KHP) standard: Lightly crush and then dry KHP (HOOC$_6$H$_4$COOK) to constant weight at 120 °C. Dissolve 425 mg in distilled water and dilute to 1000 mL. This solution has a theoretical COD of 500 µg O$_2$/mL. This solution is stable when refrigerated for up to 3 months in the absence of visible biological growth.
E. PROCEDURE

- Prepare reflux apparatus, follow the order of reagents:

<table>
<thead>
<tr>
<th>Flask No</th>
<th>Sample Type</th>
<th>Sample Volume (mL)</th>
<th>HgSO₄ g/flask</th>
<th>K₂Cr₂O₇ solution</th>
<th>H₂SO₄ Reagent vol.(mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Sample</td>
<td>25</td>
<td>0.5</td>
<td>13</td>
<td>38</td>
</tr>
<tr>
<td>3-4</td>
<td>Blank(DW)</td>
<td>25</td>
<td>0.5</td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

- Add 0.5 g HgSO₄
- Add several boiling chips and 5.0 mL H₂SO₄, cool while mixing to avoid possible less of volatile materials in the sample.
- Add 13.0 mL 0.25 N K₂Cr₂O₇ solution, mix.
- Attach the flask to the condenser start the cooling water.
- Add the remaining H₂SO₄ (33 mL) through the open end of the condenser, continue swirling and mixing while the acid is being added, mix thoroughly.
- Reflux for 2 hr. Turn off heat and cool down the flask to room temperature.
- Wash condenser with about 25 mL distilled water.
- Transfer the contents to 500 mL conical flask and dilute to about twice its volume with distilled water.
- Titrate the excess dichromate with standard ferrous ammonium sulfate using 2-3 drops ferroin indicator to the reddish brown end point.

F. CALCULATIONS

COD results are reported in terms of milligrams of oxygen.

Calculate COD mg/L

\[
\text{COD (mg/L)} = \frac{(V₁ - V₂) \times N \times A \times 1000}{mL\text{sample}}
\]

\[V₁ = mL \text{Fe(NH₄)₂(SO₄)₃} \text{ used for blank}\]

\[V₂ = mL \text{Fe(NH₄)₂(SO₄)₃} \text{ used for sample}\]

\[N = \text{Normality of the Fe(NH₄)₂(SO₄)₃ solution}\]

\[A = \text{Equivalent weight of oxygen}\]

Report the experimental according to the report guide.
II. CLOSED REFLUX, Colorimetric and Titrmetric Method

A. APPARATUS AND MATERIALS

COD reactor (heater), digestion vessels (screw capped tubes), and spectrophotometer

B. REAGENTS

1. Digestion Solution:
Add to about 500 mL distilled water 10.216 g K$_2$Cr$_2$O$_7$, primary standard grade, previously dried at 103 °C for 2 hr, 167 mL concentrated H$_2$SO$_4$, and 33.3 g HgSO$_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

2. Sulfuric Acid Reagent: Same as in open reflux


4. Standard Ferrous Ammonium Sulfate Titrant (0.2 M):
Dissolve 78.4 g Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O in distilled water, add 40 mL concentrated H$_2$SO$_4$, cool and dilute to 1000 mL, standardize daily against the standard K$_2$Cr$_2$O$_7$ solution as follows:
Add 2.5 mL distilled water, 1.5 mL digestion solution and 3.5 mL sulfuric acid reagent to a tube. Cool to room temperature and add 1 or 2 drops of Ferroin indicator and titrate with FAS titrant.

\[
\text{Normality of FAS solution} = \frac{\text{mL of } K_2Cr_2O_7 \text{ solution} \times \text{Normality of } K_2Cr_2O_7(0.21 \text{ N})}{\text{mL FAS used in titration}}
\]

5. Ferroin Indicator Solution: Same as in open reflux.
C. PROCEDURE

Treatment of samples:

- Place 2.5 mL sample in tube, add 1.5 mL digestion solution. Carefully run 3.5 mL sulfuric acid reagent down inside of tube, so an acid layer is formed under the sample-digestion solution layer. Tightly cap tubes and invert each several times to mix completely. Do the same for blank, putting distilled water instead of sample this time.
- CAUTION: Protect hands from heat produced when contents of tubes are mixed.
- Place tubes in block digester preheated to 150 °C and reflux for 2 h. Cool to room temperature and place tubes in test tube rack.

Cool to room temperature and place tubes in test tube rack.

**Measurement of Dichromate reduction:**

Colorimetric Method:

- Invert cooled samples, blank and standards several times and allow solids to settle before measuring absorbance. Insert unopened tube into light path of spectrophotometer set at 600 nm. Read absorbance and compare to calibration curve.

Titrimetric Method:

- Transfer contents of tubes to a larger container. Add 1 or 2 drops of Ferroin indicator and stir rapidly while titrating with 0.2 M FAS. The endpoint is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes.

D. CALCULATIONS:

*Colorimetric method (spectrophotometric method):*

\[
\text{COD as mg O}_2/\text{L} = \frac{\text{mg O}_2 \text{ in final volume} \times 1000}{\text{mL of sample}}
\]

*Titrimetric method:*
For the titrimetric method calculate COD as follows:

\[
\text{COD as mg O}_2/\text{L} = \frac{(A-B) \times 8.0 \text{(equivalent weight of O}_2) \times 1000}{\text{ml of sample}} 
\]

Where;
A= mL FAS used for blank
B= mL FAS used for the sample
N= Normality of FAS
COD

FLOWCHART OF THE EXPERIMENT
COD

I – Colorimetric Method:

Experimental Data of Calibration Curve for COD Determination

<table>
<thead>
<tr>
<th>Standard Calibration Solutions (Total Volume 50 mL)</th>
<th>COD (mg/L)</th>
<th>Absorbance ($\lambda = 600$ nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel 1</td>
<td>Parallel 2</td>
</tr>
<tr>
<td>5 mL Standard KHP solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mL Standard KHP solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mL Standard KHP solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mL Standard KHP solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mL Standard KHP solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Blank solutions are used for zeroing the spectrophotometry. The average absorbance values should be considered for calculations!

II – Titrimetric Method:

Experimental Data of Titrimetric Method for COD Determination

<table>
<thead>
<tr>
<th>Solution Titrated</th>
<th>Volume of FAS used (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel 1</td>
</tr>
<tr>
<td>Blank</td>
<td></td>
</tr>
<tr>
<td>Unknown Sample</td>
<td></td>
</tr>
</tbody>
</table>
Titrimetric Method

Normality of $K_2Cr_2O_7 = $

Determination of FAS Normality =

Normality of FAS =

$$\frac{\text{mL of } K_2Cr_2O_7 \text{ solution} \times \text{Normality of } K_2Cr_2O_7(0.21 \text{ N})}{\text{mL FAS used in titration}}$$
COD
7.6. EXPERIMENT 6
TOTAL KJELDAHL NITROGEN

A. PURPOSE

To determine organic nitrogen in domestic and industrial wastes by Kjeldahl Method.

B. THEORY

Domestic and industrial wastes contain nitrogen in many forms and in various oxidation states. In a digestion step, organic nitrogen (present in many organic materials) and ammonia are converted to ammonium sulfate. The digestion step involves heating the sample in the presence of sulfuric acid, potassium sulfate and mercuric sulfate.

Heating is continued until a clear, slightly yellow solution remains, and white SO₃ fumes begin to evolve, indicating that the digestion of all organic materials is complete. Following digestion, cooling and dilution of the sample, sodium hydroxide is added to decompose the mercuric ammonia complex and convert the ammonium ion to ammonia.

The ammonia is liberated by distillation from the alkaline medium and absorbed by passing the distillate into a boric acid solution in the receiver. The ammonia is then titrated with standard sulfuric acid solution.

This result is the total Kjeldahl nitrogen. If the ammonia nitrogen concentration of the sample has been determined by the electrode method, subtract the ammonia nitrogen from the total nitrogen to determine the organic nitrogen concentration.

To determine organic nitrogen, the ammonia present in the sample must be removed by distillation in a pretreatment step prior to digestion. Borate buffer and sodium hydroxide are added to adjust the pH to 9.5, and then the ammonia is distilled off. The remaining sample is digested, distilled and examined for the organic nitrogen. The difference between the total Kjeldahl nitrogen and the organic nitrogen is the ammonia nitrogen.
C. APPARATUS AND MATERIAL

Kjeldahl digestion apparatus, Kjeldahl distillation apparatus, titration stand, 50 mL burette, rheostat heater.

D. REAGENTS AND SOLUTIONS

1. Ammonia free distilled water
2. Digestion reagent
   - In a 1 L volumetric flask, dissolve 134 g potassium sulfate and 7.3 g CuSO₄ in 800 mL ammonia free distilled water, add slowly 134 mL concentrated sulfuric acid into this solution, let cool, dilute to mark, invert to mix or,
   - As an alternative to prepare reagents by weight/weight, add directly 6.7 g K₂SO₄, 0.365 g CuSO₄ or 0.57 g CuSO₄.5H₂O and 6.7 mL concentrated sulfuric acid into a 300 mL Kjeldahl tube containing the sample.
3. Sodium hydroxide sodium thiosulfate reagent:
   Weigh 500 g of sodium hydroxide pellets into a 2 L beaker. Add ammonia free distilled water to cover pellets. Weigh 25 g of sodium thiosulfate (Na₂S₂O₃.5H₂O) and transfer into the beaker. Stir with a glass rod. Allow solution to cool and transfer to 1000 mL volumetric flask using a short-stemmed funnel. Dilute to volume with distilled water, rinse of the beaker and funnel. Put a stopper and mix thoroughly.
4. Mixed indicator solution:
   - Dissolve 200 mg of methyl red in 100 mL 95% isopropyl or ethyl alcohol.
   - Dissolve 100 mg of methylene blue in 50 mL 95% isopropyl or ethyl alcohol.
   - Combine the two solutions into one beaker. Stir to mix and store in a stoppered reagent bottle. Prepare fresh once a month.
5. Indicating boric acid solution:
   Weigh 20 g of boric acid into a 1-liter beaker. Add approximately 500 mL of distilled water and stir with a glass stirring rod to bring into the solution. Pipet 10 mL of the mixed indicator solution into the beaker. Transfer into a 1-liter volumetric flask and dilute to volume with distilled water washing of the beaker. Stopper and mix. Store in a glass-stoppered reagent bottle. Prepare once a month.
6. Standard sulfuric acid, 0.02 N (prepare and standardize as directed in alkalinity test)

D. PROCEDURE

1. Digestion step.
Add 25 mL sample into a 300 mL Kjeldahl flask. Adjust pH to 7 with sulfuric acid or sodium hydroxide.
Add carefully digestion reagent (6.7 mL concentrated H2SO4, 6.7 g K₂SO₄, 0.365 g CuSO₄ or 0.57 g CuSO₄.5H₂O). Add few glass beads and after mixing heat under a hood or with suitable ejection equipment to remove acid fumes. Digest for 30 min at 200 °C and continue to boil briskly at 400 °C for one hour while the volume is greatly reduced and copious white fumes are observed (fumes may be dark for samples high in organic matter). As digestion continues, colored or turbid samples will turn clear or straw-colored. After digestion, let flask and its contents cool, dilute to 25 mL with water and mix. Tilt flask and carefully add 50 mL hydroxide-thiosulfate reagent to form an alkaline layer at flask bottom and to remove residual chlorine at the time of collection. Connect flask to steamed-out distillation apparatus and shake flask to ensure complete mixing pH should exceed 11.

2. Distillation step:
Transfer the mixed sample into 500 mL Erlenmeyer flask, add distilled water. Adjust pH with 6 N NaOH using the distillation apparatus. Collect the distillate in 500 mL Erlenmeyer flask containing 50 mL indicating boric acid solution by running the distillation apparatus for 15 min. Lower the collected distillate free of contact with the delivery tube and continue distillation during the last minute or two cleanse the condenser. Titrate with 0.02 N sulfuric acid standard solution to a pale-lavender color end point. A reagent blank must be run with each series of samples using distilled water as sample.
E. CALCULATION

$$\text{mg TKN/L} = \frac{(D-E) \times 280}{\text{mL of sample}}$$

where;

D = mL of 0.02 N sulfuric acid used for filtration of sample
E = mL of 0.02 N sulfuric acid used for filtration of blank

F. REFERENCES

TOTAL KJELDAHL NITROGEN

FLOWCHART OF THE EXPERIMENT
TOTAL KJELDAHL NITROGEN
7.7. EXPERIMENT 7
DETERMINATION OF PHOSPHATE BY ASCORBIC ACID METHOD

A. PURPOSE
To determine phosphorous in water and wastewater by using ascorbic acid method.

B. THEORY
Phosphorous compounds of wide variety are very important in environmental engineering practice. Inorganic compounds of phosphorous are the phosphate or their molecularly dehydrated forms usually referred to as polyphosphate or condensed phosphate. Organically bound phosphorous is not very important. Polyphosphates are used in some public water supplies as a means of controlling corrosion.

Since all surface water supplies support growth of minute aquatic organisms such as algae, nitrogen and phosphorous are both essential. The critical level of inorganic phosphorous for algal bloom is 0.005 mg/L under summer growing conditions.

Domestic wastewater is relatively rich in phosphorous compounds that are contributed by human wastes as a result of the metabolic breakdown of proteins and elimination of the liberated phosphates in the urine.

Most heavy duty synthetic detergent formulations contain large amounts of polyphosphates as ‘builders’.

Orthophosphates (H$_2$PO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$) can be measured quantitatively by gravimetric, volumetric or colorimetric methods. Three colorimetric methods are used. They are essentially the same in principle but differ in the nature of the agent added for final color development. Phosphate ion combines with ammonium molybdate under acid conditions to form a complex compound.

\[
\text{PO}_4^{3-} + 12 \text{ (NH}_4\text{)}_2\text{MoO}_4 + 24 \text{ H}^+ \rightarrow (\text{NH}_4\text{)}_3\text{PO}_4.12\text{MoO}_3 + 21 \text{ NH}_4^+ + 12\text{H}_2\text{O}
\]

ammonium phosphomolybdate

The Mo contained in ammonium phosphomolybdate is reduced by ascorbic acid to produce a blue-colored solution. The colored compound is referred to as heteropoly blue. The amount produced is proportional to the amount of phosphate present and can be measured colorimetrically.
C. APPARATUS AND MATERIAL

1. Spectrophotometer, Hach Model with calibrated meter scale.

2. Acid-washed glassware

D. REAGENTS

1. Sulfuric acid solution, 5N:
Dilute 70 mL conc. H₂SO₄ with distilled water to 500 mL.

2. Potassium antimony tartarate solution:
Dissolve 1.375 g K(SbO)C₄H₂O₆.1/2H₂O in 400 mL distilled water in a 500 mL volumetric flask and dilute to volume.

3. Ammonium molybdate solution:
Dissolve 20 g (NH₄)₆Mo₇O₂₄.4H₂O in distilled water and store in plastic bottle at 4°C.

4. Ascorbic acid, 0.1 M:
Dissolve 1.76 g ascorbic acid in 100 mL distilled water.

5. Combined reagent:
Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N H₂SO₄, 5 mL potassium antimonyl tartarate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. Mix after addition of each reagent.

6. Stock phosphate solution:
Dissolve in distilled water 219.5 mg anhydrous KH₂PO₄ and dilute to 1000 mL; 1.00 mL = 50.0 g PO₄³⁻ -P.

7. Standard phosphate solution:
Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1 mL = 2.50 µg P.
E. PROCEDURE

- Pipet 50.0 mL sample into a clean dry test tube or 125 mL Erlenmeyer flask.
- Add 1 drop of phenolphthalein indicator.
- If a red color develops, add 5N H₂SO₄ solution just to discharge the color.
- Add 8.0 mL combined reagent and mix.
- After at least 10 min but no longer than 30 min, measure the absorbance of each sample at 880 nm.
- Prepare a calibration curve from a series of six standard solutions.

F. CALCULATION

\[
	ext{mg/L PO}_4^-\text{P} = \frac{\text{mg P}}{1000/\text{mL sample}}
\]

G. RESULTS AND DISCUSSION

Discuss the results obtained from the experiment.

Discuss the other phosphate determination methods; their advantages and advantages.

H. REFERENCES

2. Water Analysis Hand Book, HACH.
DETERMINATION OF PHOSPHATE

FLOWCHART OF THE EXPERIMENT
<table>
<thead>
<tr>
<th>mL of Standard Solutions in 100 mL</th>
<th>Absorbance ($\lambda=\text{nm}$)</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>2 mL Standard Phosphate solution</td>
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<td></td>
</tr>
<tr>
<td>4 mL Standard Phosphate solution</td>
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</tr>
<tr>
<td>6 mL Standard Phosphate solution</td>
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<td>8 mL Standard Phosphate solution</td>
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</tr>
<tr>
<td>10 mL Standard Phosphate solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE</td>
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</tbody>
</table>
DETERMINATION OF PHOSPHATE
7.8. EXPERIMENT 8

Part 1: COLOR

A. PURPOSE

To measure the true color of the water sample by Visual Comparison method.

B. THEORY

The term ‘color’ is used here to mean true color, which is the color of water from which turbidity has been removed. The term ‘apparent color’ includes not only color due to substances in solution, but also that due to suspended matter. Apparent color is determined on the original sample without filtration or centrifugation. In some highly colored industrial wastewaters color is contributed principally by colloidal or suspended material. In such cases both true color and apparent color should be determined.

C. APPARATUS AND MATERIAL

1. Nessler tubes, matched, 50-mL, tall form
2. pH meter, for determining sample pH

D. REAGENTS AND SOLUTIONS

1. Hydrochloric acid, HCl, 1+1
2. Distilled water
3. Potassium Chloroplatinate
4. Crystallized Cobaltous Chloride

Preparation of Standards

1. If a reliable supply of potassium chloroplatinate cannot be purchased, use chloroplatinitic acid prepared from metallic platinum. Do not use commercial chloroplatinic acid because it is very hygroscopic and may vary in platinum content. Potassium chloroplatinate is not hygroscopic.
2. Dissolve 1.246 g potassium chloroplatinate, K₂PtCl₆ (equivalent to 500 mg metallic Pt) and 1.00 g crystallized cobaltous chloride, CoCl₂.6H₂O (equivalent to about 250 mg metallic Co) in distilled water with 100 mL conc HCl and dilute to 1,000 mL with distilled water. This stock standard has a color of 500 units.

3. If K₂PtCl₆ is not available, dissolve 500 mg pure metallic Pt in aqua regia with the aid of heat; remove HNO₃ by repeated evaporation with fresh portions of conc. HCl. Dissolve this product, together with 1.00 g crystalized CoCl₂.6H₂O, as directed above.

4. Prepare standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 and 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0 and 7.0 mL stock color standard with distilled water to 50 mL in Nessler tubes. Protect these standards against evaporation and contamination when not in use.

**E. PROCEDURE**

1. Observe sample color by filling a matched Nessler tube to the 50-mL mark with sample and comparing it with standards.

2. Look vertically downward through tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid.

3. If turbidity is present and has not been removed, report as ‘apparent color’. If the color exceeds 70 units, dilute sample with distilled water in known proportions until the color is within the range of the standards.

4. Measure pH of each sample.

**F. CALCULATION**

1. Calculate color units by the following equation

\[
\text{Color units} = \frac{A \times 50}{B}
\]

where,

A: estimated color of a diluted sample and

B: mL sample taken for dilution

2. Report color results in whole numbers and record as follows:
3. Report sample pH.

**G. REFERENCES**

COLOR

FLOWCHART OF EXPERIMENT
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Color Units</th>
</tr>
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<tr>
<td>ST2</td>
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<td>ST4</td>
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<tr>
<td>Tap water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>
7.8. EXPERIMENT 8

Part 2: TURBIDITY

A. PURPOSE

To measure turbidity of drinking water sample as a quality-control parameter for aesthetic reasons by Nephelometric method.

B. THEORY

Turbidity in water is caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds, and plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. Correlation of turbidity with the weight concentration of suspended matter is difficult because the size, shape, and refractive index of the particulates also affect the light-scattering properties, such as those of activated carbon, may absorb light and effectively increase turbidity measurements.

C. APPARATUS AND MATERIAL

1. Turbidimeter

2. Sample tubes, clear colorless glass: Keep tubes scrupulously clean, both inside and out, and discard when they become scratched or etched. Never handle them where the light strikes them. Use tubes with sufficient extra length, or with a protective case, so that they may be handled properly. Fill tubes with samples and standards that have been agitated thoroughly and allow sufficient time for bubbles to escape.

D. REAGENTS AND SOLUTIONS

1. Turbidity-free water: Turbidity-free water is difficult to obtain. The following method is satisfactory for measuring turbidity as low as 0.02 NTU.
Pass distilled water through a membrane filter having precision-sized holes of 0.2 µm; the usual membrane filter used for bacteriological examinations is not satisfactory. Rinse collecting flask at least twice with filtered water and discard the next 200 mL. Some commercial bottled demineralized waters are nearly particle-free. These may be used when their turbidity is lower than can be achieved in the laboratory. Dilute samples to a turbidity not less than 1 with distilled water.

2. Stock turbidity suspension:
   - Solution I – Dissolve 1.000 g hydrazine sulfate (CAUTION: Carcinogen; avoid inhalation, ingestion, and skin contact.), \((\text{NH}_2\text{)}_2\text{H}_2\text{SO}_4\), in distilled water and dilute to 100 mL in a volumetric flask.
   - Solution II – Dissolve 10.00 g hexamethylenetetramine, \((\text{CH}_2\text{)}_6\text{N}_4\), in distilled water and dilute to 100 mL in a volumetric flask.
   - In a 100-mL volumetric flask, mix 5.0 mL Solution I and 5.0 mL Solution II. Let stand 24 h at 25 ± 3 °C, dilute to mark, and mix. The turbidity of this suspension is 400 NTU.

3. Standard turbidity suspension: Dilute 10.00 mL stock turbidity suspension to 100 mL with turbidity-free water. Prepare daily. The turbidity of this suspension is defined as 40 NTU.

4. Alternate standards: As an alternative to preparing and diluting formazin, use commercially available standards such as styrene divinylbenzene beads if they are demonstrated to be equivalent to freshly prepared formazin.

5. Dilute turbidity standards: Dilute portions of standard turbidity suspension with turbidity-free water as required. Prepare daily.

E. PROCEDURE

Turbidimeter calibration:

1. Follow the manufacturer’s operating instructions. In the absence of a precalibrated scale, prepare calibration curves for each range of the instrument.

2. Check accuracy of any supplied calibration scales on a precalibrated instrument by using appropriate standards.
3. Run at least one standard in each instrument range to be used.

4. Make certain that turbidimeter gives stable readings in all sensitivity ranges used.

**Measurement of turbidities less than 40 NTU:**

1. Thoroughly shake sample.

2. Wait until air bubbles disappear and pour sample into turbidimeter tube.

3. When possible, pour shaken sample into turbidimeter tube and immerse it in an ultrasonic bath for 1 to 2 s, causing complete bubble release.

4. Read turbidity directly from instrument scale or from appropriate calibration curve.

**Measurement of turbidities above 40 NTU:**

1. Dilute sample with one or more volumes of turbidity-free water until turbidity falls between 30 and 40 NTU.

2. Compute turbidity of original sample from turbidity of diluted sample and the dilution factor. For example, if five volumes of turbidity-free water were added to one volume of sample and the diluted sample showed a turbidity of 30 NTU, then the turbidity of the original sample was 180 NTU.

**F. CALCULATION**

1. Calculate nephelometric turbidity units (NTU) by the following equation

   \[
   \text{Nephelometric turbidity units (NTU)} = \frac{A \times (B + C)}{C}
   \]

   where,

   A: NTU found in diluted sample,

   B: volume of dilution water, mL, and

   C: sample volume taken for dilution, mL
2. Report turbidity readings as follows:

<table>
<thead>
<tr>
<th>Turbidity Range</th>
<th>Record to the Nearest NTU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1.0</td>
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<tr>
<td>1 - 10</td>
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<td>10 - 40</td>
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<td>100 – 400</td>
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<tr>
<td>400-1000</td>
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<tr>
<td>&gt;1000</td>
<td>100</td>
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</table>

G. REFERENCES

TURBIDITY

FLOWCHART OF EXPERIMENT
## TURBIDITY

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1</td>
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<td>ST3</td>
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<td>ST5</td>
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</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>
A. PURPOSE

To determine the dissolved or emulsified grease or oil content of a water sample by Partition-Gravimetric method.

B. THEORY

The term grease applies to a wide variety of organic substances that are extracted from aqueous solution or suspension by hexane or trichlorotrifluoroethane (Freon). Hydrocarbons, esters, oils, waxes and high molecular weight fatty acids are the major materials dissolved by these solvents. The method of determining grease by means of solvent extraction does not measure low molecular weight hydrocarbons such as gasoline.

The terms oil represents a wide variety of substances ranging from low to high molecular weight hydrocarbons of mineral origin. It includes all glycerides of animal and vegetable origin that are liquid at ordinary temperatures.

C. APPARATUS AND MATERIAL

1. Separatory funnel, with Teflon stopcock.
2. Distilling flask, 125 mL
1. Water bath

D. REAGENTS AND SOLUTIONS

1. Hydrochloric acid, HCl, 1+1
2. Hexane
3. Filter paper, Whatman No: 40, 11 cm.
4. Sodium sulfate, anhydrous crystal.
E. PROCEDURE

1. Collect about 1L of sample in a wide mouth glass bottle
2. Set acidity to pH 2 or lower, generally 5 mL HCl is sufficient
3. Transfer the sample to a separatory funnel.
4. Carefully rinse the sample bottle with 30 mL hexane and add solvent washings to the separatory funnel. Shake vigorously for 2 min.
5. Allow the layers to separate. Drain the hexane layer through a funnel containing solvent-moistened filter paper into a clean, tared distilling flask.
6. If a clear solvent cannot be obtained, add 1 g Na₂SO₄ to the filter paper cone and slowly drain the emulsified solvent onto the crystals. Add more Na₂SO₄ if necessary.
7. Extract twice more with 30 mL hexane each but first rinse the sample container with each solvent portion.
8. Combine the extracts in the tared distilling flask and wash the filter paper with an additional 10 to 20 mL hexane.
9. Distill the hexane from the extraction flask in a water bath at 80-85 °C.
10. Place the flask on a warm steam bath for 30 min and if necessary draw air through the flask by means of an applied vacuum for the final 1 min.
11. Cool in a desiccator for exactly 30 min and weigh

F. CALCULATION

\[
\text{mg/L grease or oil} = \frac{A - B}{\text{mL sample}} \times 1000
\]

where;

A: total gain in weight of the tarred flask

B: weight of tarred flask
G. REFERENCES

OIL AND GREASE

FLOWCHART OF EXPERIMENT
OIL AND GREASE

Sample Volume:

Tare mass of evaporating dish:

Mass of evaporating dish + Oil:
7.9. EXPERIMENT 9
Part 1: CHLORIDE

A. PURPOSE

To measure the chloride of the water sample by Argentometric method.

B. THEORY

Chloride, in the form of Cl- ion, is one of the major inorganic anions in water and wastewater. In potable water, the salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg/L chloride/L may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1,000 mg/L when the pre-dominant cations are calcium and magnesium.

The chloride concentration is higher in wastewater than in raw water because sodium chloride (NaCl) is a common article of diet and passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewerage system. It also may be increased by industrial processes.

A high chloride content may harm metallic pipes and structures, as well as growing plants.

C. APPARATUS AND MATERIAL

1. Erlenmeyer flask, 250 mL
2. Buret, 50 mL

D. REAGENTS AND SOLUTIONS

1. Potassium chromate indicator solution: Dissolve 50 g K₂CrO₄ in a little distilled water. Add AgNO₃ solution until a definite red precipitate is formed. Let stand 12 hr, filter, and dilute to 1 L with distilled water.
2. Standard silver nitrate titrant, 0.0141 N: Dissolve 2.395 g AgNO₃ in distilled water and dilute to 1000 mL. Standardize against 0.0141 N NaCl by the procedure described in below (Titration part); 1.00 mL = 500 µg Cl. Store in a brown bottle.

3. Standard sodium chloride, 0.0141 N: Dissolve 824.0 mg NaCl (dried at 140 °C) in distilled water and dilute to 1,000 mL; 1.00 mL = 500 µg Cl.

4. Special reagents for removal of interference:
   - Aluminum hydroxide suspension: Dissolve 125 g aluminum ammonium sulfate, AlK(SO₄)₂·12H₂O, in 1 L distilled water. Warm to 60 °C and add 55 mL conc ammonium hydroxide (NH₄OH) slowly with stirring. Let stand about 1 hr, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.
   - Phenolphthalein indicator solution.
   - Sodium hyroxide, NaOH, 1N.
   - Sulfuric acid, H₂SO₄, 1N.
   - Hydrogen peroxide, H₂O₂, 30%.

E. PROCEDURE

Sample Preparation
1. Use a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL Al(OH)₃ suspension, mix, let settle, and filter.
2. If sulfaide, sülfite, or thiosulfate is present, add 1 mL H₂O₂ and stir for 1 min.

Titration
1. Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H₂SO₄ or NaOH if it is not in this range. Add 1.0 mL K₂CrO₄ indicator solution. Titrate with standard AgNO₃ titrant to a pinkish yellow end point. Be consistent in end-point recognition.
2. Standardize AgNO₃ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.
F. CALCULATION

Calculate chloride by the following equation

\[ \text{mg Cl/L} = \frac{(A - B) \times N \times 35,450}{\text{mL sample}} \]

where,

A: mL titration for sample

B: mL titration for blank, and

N: normality of AgNO₃

\[ \text{mg NaCl/L} = (\text{mg Cl/L}) \times 1.65 \]

G. REFERENCES

CHLORIDE

FLOWCHART OF EXPERIMENT
CHLORIDE
7.9. EXPERIMENT 9
Part 2: SULFATE

A. PURPOSE

To measure the sulfate of the water sample by Gravimetric Method with Ignition of Residue.

B. THEORY

Sulfate is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage wastes may contribute large amounts of sulfate through pyrite oxidation. Sodium and magnesium sulfate exert a cathartic action.

C. APPARATUS AND MATERIAL

1. Steam bath.
2. Drying oven, equipped with thermostatic control.
3. Muffle furnace, with heat indicator.
4. Desiccator.
5. Analytical balance, capable of weighing to 1.0 mg.
6. Filter. Use one of the following:
   - Filter paper, acid-washed, ashless hard-finish, sufficiently retentive for fine precipitates.
   - Membrane filter, with a pore size of about 0.45 µm.
7. Filtering apparatus, appropriate to the type of filter selected. (Coat holder used for the membrane filter with silicone fluid to prevent precipitate from adhering.)
D. REAGENTS AND SOLUTIONS

1. Methyl red indicator solution: Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL.
2. Hydrochloric acid, HCl, 1+1
3. Barium chloride solution: Dissolve 100 g BaCl$_2$.2H$_2$O in 1L distilled water. Filter through a membrane filter or hard-finish filter paper before use; 1 mL is capable of precipitating approximately 40 mg SO$_4$.
4. Silver nitrate-nitric acid reagent: Dissolve 8.5 AgNO$_3$ and 0.5 mL conc HNO$_3$ in 500 mL distilled water.
5. Silicone fluid.

E. PROCEDURE

1. Removal of silica: If the silica concentration exceeds 25 mg/L, evaporate sample nearly to dryness in a platinum dish on a steam bath. Add 1 mL HCl, tilt dish on a steam bath. Add 1 mL HCl, tilt dish, and rotate it until the acid comes in complete contact with the residue. Continue evaporation to dryness. Complete drying in an oven at 180$^\circ$C and if organic matter is present, char over flame of a burner. Moisten residue with 2 mL distilled water and 1 mL HCl, and evaporate to dryness on a steam bath. Add 2 mL HCl, take up soluble residue in hot water, and filter. Wash insoluble silica with several small portions of hot distilled water. Combine filtrate and washings. Discard residue.

2. Precipitation of barium sulfate: Adjust volume of clarified sample to contain approximately 50 mg sulfate ion in a 250 mL volume. Lower concentrations of sulfate ion may be tolerated if it is impracticable to concentrate sample to the optimum level, but in such cases limit total volumeto 150 mL. Adjust pH with HCl to pH 4.5 to 5.0, using a pH meter or the orange color of methyl red indicator. Add 1 to 2 mL HCl. Heat to boiling and, while stirring gently, add warm BaCl$_2$ solution slowly until precipitation appears to be complete; then add about 2 mL in excess. If amount of precipitate is small, add a total of 5 mL BaCl$_2$ solution. Digest precipitate at 80 to 90 $^\circ$C, preferably overnight but for not less than 2 hr.

3. Filtration and weighing: Mix a small amount of ashless filter paper pulp with the BaSO$_4$, quantitatively transfer to a filter at room temperature. The pulp aids filtration and reduces the tendency of the precipitate to creep. Wash precipitate with small
portions of warm distilled water until washings are free of chloride, as indicated by testing with AgNO₃-HNO₃ reagent. Dry filter and precipitate and ignite at 800 °C for 1 hr. Do not let filter paper flame. Cool in dessicator and weigh.

F. CALCULATION

1. Calculate sulfate by the following equation

\[ \text{mg SO}_4/L = \frac{\text{mgBaSO}_4 \times 411.6}{\text{mLsample}} \]

G. REFERENCES

SULFATE

FLOWCHART OF EXPERIMENT
SULFATE
7.10. EXPERIMENT 10
PART 1: DETERMINATION OF IRON

A. PURPOSE

To determine the iron concentration in water samples using phenanthroline method.

B. THEORY

Iron in water may be in true solution, in a colloidal state that may be peptized by organic matter, in the inorganic or organic iron complexes, or in relatively coarse suspended particles. It may be either ferrous or ferric, suspended or filterable.

On exposing to air or addition of oxidants, ferrous iron is oxidized to ferric state. Under reducing conditions, iron exists in the ferrous state. In the absence of complex forming ions ferric ion is not soluble unless the pH of the water is very low. For natural and treated waters, the 1,10-phenanthroline is specific for measuring Fe$^{2+}$, all the iron in the form of Fe$^{3+}$ must be reduced to the ferrous state. This is most readily accomplished by using hydroxylamine as the reducing agent. The reaction involved may be represented as follows:

$$4\text{Fe}^{3+} + 2\text{NH}_2\text{OH} \rightarrow 4\text{Fe}^{2+} + \text{N}_2\text{O} + \text{H}_2\text{O} + 4\text{H}^+$$

Three molecules of 1,10 phenanthroline is required to chelate or from a complex ion with each Fe$^{2+}$ ion. The reaction may be represented as shown in the equation.

Strong oxidizing agents, cyanide, nitrite, phosphates, chromium, zinc, cobalt, nickel can be accepted as interfering substances; bismuth, cadmium, molybdate and silver precipitate phenanthroline. In the former case addition of excess hydroxylamine can eliminate errors and in the latter case excess of phenanthroline is required or the extraction method may be used. The method can be used to determine the iron concentrations between 0.02-4.0 mg/L directly and higher concentrations can be determined by dilution. The minimum detectable quantity is 50 µg at 510 nm with a 1 cm cell.
C. APPARATUS AND MATERIALS

1. Spectrophotometer
2. Acid washed glassware. All glassware should be washed with conc. HCl and rinsed with distilled water before use.

D. REAGENTS

1. Hydrochloric acid; HCl (conc)
2. Hydroxylamine solution: Dissolve 10 g NH₂OH.HCl in 100 mL distilled water.
3. Ammonium acetate buffer solution: Dissolve 62.5 g NH₄C₂H₃O₂ in 37.5 mL distilled water. Add 175 mL conc. (glacial) acetic acid.
4. Sodium acetate solution: Dissolve 20 g NaC₂H₃O₂.3H₂O in 80 mL distilled water
5. Phenanthroline solution: Dissolve 100 mg 1,10-phenanthroline monohydrate C₁₂H₈N₂.H₂O in 100 mL distilled water by stirring and heating to 80 °C (do not boil) or add 2 drops of conc. HCl.
6. Stock iron solution: Add slowly 20 mL conc. H₂SO₄ to 50 mL distilled water and dissolve 1.404 g Fe(NH₄)₂(SO₄)₂.6H₂O. Add 0.1 N KMnO₄ dropwise until a faint pink color persists. Dilute to 1000 mL with distilled water. 1.00 mL = 200 µg Fe
7. Standard iron solution: Dilute 50.00 mL stock solution to 1000 mL with distilled water.

E. PROCEDURE

1. Preparation of calibration curve, range 0-100 µg Fe/100 mL final solution:
   - Pipet 2.0; 4.0; 6.0; 8.0; 10.0 mL standard iron solution into 100 mL volumetric flasks.
   - To each flask add:
     - mL NH₂OH.HCl solution,
     - 10.0 mL sodium acetate solution,
     - 10.0 mL phenanthroline solution
     - Pipet 2.0; 4.0; 6.0; 8.0; 10.0 mL standard iron solution into 100 mL volumetric flasks.
• Dilute to volume (100 mL) with distilled water. Mix thoroughly and let stand for 10 min.
• Prepare a reference blank by treating distilled water as above except standard iron solution.
• Measure the absorbance of each solution at \( \lambda = 510 \) nm against reference blank in 1.0 cm cell.

2. Determination of iron concentration of unknown sample by absorbance measurement:
• Mix the sample thoroughly
• Measure 50.0 mL in 125 mL Erlenmeyer flask,
• Add 2 mL conc. HCl and 1 mL hydroxylamine solution.
• Add a few glass beads and heat to boiling until the volume is reduced to 15-20 mL. Cool to room temperature. Transfer to 100 mL volumetric flask.
• Add 10 mL ammonium acetate buffer solution and 4 mL phenanthroline solution.
• Dilute to volume (100 mL) with distilled water. Mix thoroughly and let stand for 10 min for max. color development
• Measure the absorbance of sample at 510 nm against reference blank in 1 cm cell.
• Determine mg Fe in sample used from the calibration curve.

F. CALCULATION

\[
\text{mg Fe/L} = \frac{\mu g \text{ Fe (in 100mL final volume)}}{\text{mL sample}}
\]
DETERMINATION OF IRON

FLOWCHART OF THE EXPERIMENT
### Experimental Data of Calibration Curve for Iron Determination

<table>
<thead>
<tr>
<th>mL of Standard Solutions in 100 mL</th>
<th>Absorbance ($\lambda = 510\text{nm}$)</th>
<th>Concentration</th>
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</thead>
<tbody>
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<td>SAMPLE</td>
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</table>
7.10. EXPERIMENT 10

PART 2: DETERMINATION OF MANGANESE BY PERSULFATE METHOD

A. PURPOSE

To determine manganese by using persulfate as the oxidizing agent acting upon soluble manganous compounds to form permanganate.

B. THEORY

Manganese exits in the soil principally as manganese dioxide which is very insoluble in water containing carbon dioxide. Under reducing conditions, the manganese in the dioxide form is reduced from an oxidation state of IV to II and solution occurs. Manganese concentration in water supplies seldom exceeds a few mg/L. When water is exposed to air, due to oxidation of manganese and iron to the Fe (III) and Mn (IV) states, it becomes turbid and highly unacceptable from the aesthetic viewpoint. The oxidation rates are not rapid, therefore reduced forms can persist for some time in aerated waters (pH<9). The rates may be increased by the presence of certain inorganic catalysts or through the action of microorganisms.

Two colorimetric methods are recommended (perodite method and persulfate method). Both depending upon the oxidation of manganese from its lower oxidation state to VII where it forms the highly colored permanganate ion. The color produced is directly proportional to the concentration of manganese present. Chlorides interfere because of their reducing action in acidic medium.

Persulfate method is preferred because the use of mercuric ion can control interference from a limited chloride ion concentration.

Persulfate oxidation of soluble manganese compounds to form permanganate is carried out in the presence of silver nitrate. The resulting color is stable for at least 24 hours if excess persulfate is present and organic matter is absent. The minimum detectable quantity is 30 µg Mn, corresponding to 300 µg/L, at 525 nm with a 1 cm cell.
For wastewaters containing organic matter, use preliminary digestion with nitric and sulfuric acids (HNO₃ and H₂SO₄). If large amounts of Cl⁻ are also present, boiling with HNO₃ helps to remove it. Interfering traces of Cl⁻ can be eliminated by HgSO₄ in the special reagent.

Samples that have been exposed to air may give low results due to precipitation of manganese dioxide (MnO₂). Addition of 1 drop of 30% H₂O₂ to the sample, after adding the special reagent, re-dissolves the precipitated manganese.

C. APPARATUS

Spectrophotometer, for use at λ = 525nm, providing a light path of 1 cm or longer.

D. REAGENTS

1. Special reagent:

   Dissolve 75 g mercuric sulfate (HgSO₄) in 400 mL conc HNO₃ and 200 mL distilled water. Add 200 mL 85% phosphoric acid (H₃PO₄) and 35 mg silver nitrate (AgNO₃). Dilute the cooled solution to 1L.

2. Ammonium persulfate, (NH₄)₂S₂O₈, or potassium persulfate (K₂S₂O₈) solid

3. Standard manganese solution:

   Prepare a 0.1 N potassium permanganate (KMnO₄) solution by dissolving 3.2 g KMnO₄ in distilled water and making up to 1L. Age for several weeks in sunlight or heat for several hours near the boiling point, then filter through a finefritted-glass filter crucible and standardize against sodium oxalate as follows:

   Weigh several 100 to 200 mg samples of NaC₂O₄ to 400 mL beakers. To each beaker, add 100 mL distilled water and stir to dissolve. Add 10 mL 1+1 H₂SO₄ and heat rapidly to 90 to 95 °C. Titrart rapidly with the KMnO₄ solution to be standardized, while stirring, to a slight pink end point color that persists for at least 1 min.
Do not let temperature fall below 85 °C. If necessary, warm beaker contents during titration; 100 mg NaC₂O₄ will consume about 15 mL permanganate solution. Run a blank on distilled water and H₂SO₄.

Normality of KMnO₄ = \frac{g \text{ Na}_2\text{C}_2\text{O}_4}{(A - B) \times 0.06701}

Where:

A= mL titrant for sample and

B= mL titrant for blank

Average results of several titrations. Calculate volume of this solution necessary to prepare 1 L of solution so that 1.00 mL = 50.0 µg Mn, as follows:

mL KMnO₄ = \frac{4.55}{\text{normality KMnO}_4}

To this volume add 2 to 3 mL conc. H₂SO₄ and NaHSO₃ solution dropwise, with stirring, until the permanganate color disappears. Boil to remove excess SO₂, cool and dilute to 1000 mL with distilled water. Dilute this solution further to measure small amounts of manganese.

1- Hydrogen peroxide, H₂O₂, 30 %.
2- Nitric acid, HNO₃, conc.
3- Sulfuric acid, H₂SO₄, conc.
4- Sodium oxalate, Na₂C₂O₄, primary standard.
5- Sodium bisulfite:

Dissolve 10 g NaHSO₃ in 100 mL distilled water.

**E. PROCEDURE**

Preparation of calibration curve, range 100-500 µg Mn/100 mL final volume:

1. Pipet 2.0, 4.0, 6.0, 8.0, 10.0 mL standard Mn solution into 100 mL volumetric flasks and dilute to the volume with distilled water.
2. Add 5 mL special reagent and 1 drop H₂O₂ to each flask.
3. Concentrate to 90 mL by boiling or dilute to 90 mL.
4. Add 1 g (NH₄)S₂O₈ or K₂S₂O₈, bring to boiling and boil for 1 min. Do not heat on a water bath. Remove from heat source, let stand 1 min, then cool under the tap. (Boiling too long results in decomposition of excess persulfate and subsequent loss of permanganate color; cooling too slowly has the same effect).

5. Dilute to 100 mL with distilled water free from reducing substances and mix.

Treatment of sample:

Prepare the sample as above and measure the manganese with one of the methods below.

Determination of manganese in standards and samples:

- Nessler tube comparison:

Use standards prepared as above and containing 5 to 100 microgram Mn/100 mL final volume. Compare samples and standards visually.

- Spectrophotometric determination:

Use a series of standards from 0 to 1500 µg Mn/100 mL final volume. Make spectrophotometric measurements at λ = 525nm against a distilled water blank. The following table shows light path length appropriate for various amounts of manganese in 100 mL final volume:

Prepare a calibration curve for absorbance against miligrams of manganese and determine mg Mn in sample used from the calibration curve.

<table>
<thead>
<tr>
<th>Mn range (µg)</th>
<th>Light path (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-200</td>
<td>15</td>
</tr>
<tr>
<td>20-400</td>
<td>5</td>
</tr>
<tr>
<td>50-1000</td>
<td>2</td>
</tr>
<tr>
<td>100-1500</td>
<td>1</td>
</tr>
</tbody>
</table>

F. CALCULATIONS

\[
\text{mg Mn/ L} = \frac{\mu g \text{ Mn (in 100mL final volume)}}{\mu g \text{ Mn (in 100mL final volume)}}
\]
DETERMINATION OF MANGANESE

FLOWCHART OF THE EXPERIMENT
Experimental Data of Calibration Curve for Manganese Determination

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QUANTITATIVE ANALYSIS OF METALS (Fe-Mn)
REFERENCES


