Laboratory Manual
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Laboratory Report Writing


1. Introduction

As a student, you will be required to submit essays, laboratory and project reports to your lecturers for assessment. In future years, as a researcher, technician, teacher, academic, industrialist, civil servant, media correspondent, author, salesperson or politician, you may be required to write a range of scientific text targeted at a specific audience. This prospect may terrify you; many people regard writing as difficult, and something to be delayed or avoided. In fact, scientific writing is a skill, which, like tying your shoelaces or performing titration, can mastered with practice and perseverance. Like any other skill, scientific writing can be developed into something that will give you confidence, satisfaction and pleasure.

At undergraduate level, laboratory reports are very important components of assessed work, and consequently, it is worth trying to produce good quality reports. As chemist, laboratory reports, are written for several reasons. One reason is to communicate the laboratory work to management. In such situations, management often bases company decisions on the results of the report. Another reason to write laboratory reports is to archive the work so that the work will not have to be done in the future. Laboratory reports are intended to demonstrate some or all of the following:

- you have performed and understood an experiment;
- you have some knowledge of the theoretical basis of the experiment;
- you can process/interpret the data obtained from an experiment;
- you can relate fundamental or derived laws to the outcome of the experiment;
- you can present these ideas/results in an appropriate context and can evaluate their significance.

2. Effective Scientific Writing

1. Remember the purpose of your writing – communicate clearly, concisely and accurately.
2. Consider your audience (tutor/lecturer) and the assessment criteria.
3. Use appropriate format.
4. Plan and arrange your ideas in a logical order.
5. Treat what you write first as draft.
6. Make sure your grammar, spelling and punctuation are correct.
7. Ensure the first draft is clear enough.
8. Re-read and edit your first draft as necessary.
9. Proof-read the final draft, correcting any remaining mistake.
3. **Grammar and Style**

All the text in your report should be grammatically correct, properly punctuated and comprise complete sentences. The overwhelming majority of scientific reports are written using the impersonal Third Person / Past Simple Tense / Passive Voice form, avoiding, if possible, the use of the personal pronouns (I, we, or you). The following examples illustrate what is intended:

*Preferred*  
“The samples were stored at 0 °C”

*Not preferred*  
“I stored the samples at 0 °C”

4. **Presentation**

Laboratory reports should be good to look at; a well-presented report will please the reader, give him/her confidence in the report and will aid assessment. A cover page will aid the presentation of your work, as well as providing important information to your assessor. The cover page should have (Figure 1):

- Course title and code;
- Number of experiment;
- Your report title;
- Your name and matric number;
- Name of your group members
- Date of submission;
- Name of Lecturer / Tutor.

---

**SCES3311 Environmental Chemistry II**

**Experiment 1**

**WATER SAMPLING, TOTAL DISSOLVED SOLIDS AND TOTAL SUSPENDED SOLIDS**

Name: ________________________________

Matric No: ________________________________

Group member: (1) ________________________________

(2) ________________________________

Date of Submission: ________________________________

Lecturer: ________________________________

Tutor: ________________________________

---

Figure 1: Example of cover page.
Laboratory reports should always use SI units. Unit is very important for all measurement. Without units much of our work as scientists would be meaningless. We need to express our thoughts clearly and units give meaning to the numbers we calculate. Knowing the units of measurement that correspond with a number can give you so much more information than a digit sitting there by itself. Units can:

- Help to show another person the exact amount you have;
- Assist in solving a mathematical problem, especially in chemistry, where you can follow the units to get to the answer;
- Show which measurement system the person is using (i.e. metric or standard).

Proper pagination of your reports will assist you to structure your work, as well as being good practice. It will also assist the reader / assessor to ‘navigate’ your report, thus making it easier to find relevant sub-sections, table, figures, etc. Pages containing preliminary information (e.g. cover page) are paginated in small Roman numerals (I, ii, iii, etc.), whereas pages of the main body of the report are given in Arabic numerals (1, 2, 3, etc).

5. Structure of the Laboratory Report

Basic structure for laboratory reports:

- Cover page (refer to section 4)
- Aims / Objectives of the Experiment
- Introduction
- Materials and Methods (Experimental)
- Results
- Discussion
- Conclusions
- References
- Appendices (if related)

5.1 Aims / Objectives of the Experiment

The aims or objectives of the experiment should clearly and briefly state the purpose of undertaking experiment. They usually include specific overall aims of the experiment. For example, in Experiment 4 that measures the oxygen content of water, the principal objective may be

- To determine the dissolved oxygen content of samples of tap water and river water using Winkler method.

You should always refer back to your aims in the Conclusions section of your report and comment upon whether they have been achieved satisfactory.

5.2 Introduction

The introduction should establish the context of the experiment, and explain the rationale for undertaking it (i.e. why is it worth doing at all). Here, you should provide some background information on the problem under investigation, such as the source of the pollutant under investigation and any potential health/environmental effects. This section can also involve a
description of the theory relating to the experiment and the experimental technique(s) to be used. It should leave the reader with the feeling that the report has a general relevance and that to read on would be worthwhile.

5.3 Materials and methods

This section should contain a concise but adequate description of all of your experimental materials and procedures so that your results could be verified independently. Materials, too, should be as fully described as is necessary for replication. The details of the apparatus / instrument (e.g. UV-Vis Spectrophotometer; GC-FID, AAS, etc) used should be included at this section. There is also no need to repeat routine instructions for using apparatus or equipment where they are well-known or available in manufacturers’ instruction. Figure 2 shows the example of the description for chemicals and instruments.

Any form of sampling procedures must be very fully described – both the sampling techniques and the sampling strategy. Sampling usually undertaken to obtain some estimate relating to a population. Similarly, locations and study areas should be described well enough for a reader to duplicate, locate or visualize.

Figure 2: Example of writing the description for chemicals and instruments

5.4 Reporting Results

Clearly, the Results are an exceptionally important part of your report and great care should be taken in their presentation. Over the years, a number of conventions have developed in the reporting of results. It is important to open your Results section with appropriate text rather than by just presenting tables of data. A table must follow, and never precede, the first reference to it in the text. You should not leave it to the reader to interpret tables – that is your job. An acceptable format is of the type, “The data presented in Table 1 show that ……” . Indeed, the reader should be able to appreciate the significance of the result without reference to any table of data; the data are evidence to support your statements. While tables are used to present the data, figures can be helpful in interpreting them.

Tables

Tables are the main vehicles for conveying data to the reader. A table can be considered as a complete entity, in a sense, should be able to exist separately in the text. A well-constructed table does not need a lengthy explanation on how it is to be interpreted but should be self-explanatory and be characterized by its simplicity and unity. The caption (on top of the table)
is clearly important if the table is to stand as a separate entity. Table 3 is a well laid out and clear example.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average value</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Total alkalinity (mg CaCO₃ L⁻¹)</td>
<td>8857</td>
<td>1480</td>
</tr>
<tr>
<td>Carbonate alkalinity (mg CaCO₃ L⁻¹)</td>
<td>450</td>
<td>490</td>
</tr>
<tr>
<td>Bicarbonate alkalinity (mg CaCO₃ L⁻¹)</td>
<td>8374</td>
<td>1917</td>
</tr>
<tr>
<td>Total ammonia nitrogen (mg [N-NH₃] L⁻¹)</td>
<td>1998</td>
<td>387</td>
</tr>
<tr>
<td>Chloride (mg L⁻¹)</td>
<td>3196</td>
<td>862</td>
</tr>
<tr>
<td>Dissolved Reactive Phosphorus (mg L⁻¹)</td>
<td>7.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Total Solids (mg L⁻¹)</td>
<td>9390</td>
<td>2087</td>
</tr>
<tr>
<td>Total Suspended Solids (mg L⁻¹)</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>DOC – dissolved organic carbon (mg L⁻¹)</td>
<td>935</td>
<td>71</td>
</tr>
<tr>
<td>COD chemical oxygen demand (mg L⁻¹)</td>
<td>3332</td>
<td>523</td>
</tr>
<tr>
<td>BOD – biochemical oxygen demand (mg L⁻¹)</td>
<td>141</td>
<td>45</td>
</tr>
</tbody>
</table>

**Figures / Graphs**

Laboratories exercises will often involve the production of graphs from the data collected. A graph can provide much more information than a set of data. It gives a visual representation of trends and relationships, and permits the prediction of what happens between the known points. Graphs are commonly labeled as Figure in lab reports. As tables, appropriate captions (or titles) should be added at the bottom of the graph (refer to Fig. 3). Well-drawn graphs can greatly enhance the effectiveness of display and interpretation of the results presented in a report.

![Graph](image)

**Fig. 3.** Removal of COD for various mixing times and stirring speeds (experimental conditions: pH = 4.0, FeCl₃ dosage = 1400 mg L⁻¹ and temperature = 25°C).

### 5.5 Discussion

The Discussion should draw all the threads of the report together and is, arguably, the most important part of the report. The discussion offers the widest scope for individual freedom of expression, and may include items such as the following:

- A comparison of the results with those obtained or published elsewhere;
- A discussion of the significance of the data in an appropriate context;
- Comments on the value of the results in a wider scientific, environmental or even commercial context.
- A discussion of the possible limitations of the methods;
• Comments upon the precision, reproducibility or repeatability) of the results, as well as on the accuracy, if known.
• A discussion of effectiveness and limitations of the experiment and any statistical treatment of the data.

Attention should be drawn to any fault/problems with the chemicals or equipment used and to any deficiency in the assumptions upon which the experiment is based. Modifications and improvements should be included if appropriate.

5.6 Conclusions

The Conclusion section should summarize the main findings of the experiment. It is not a summary of your work programme or a description of the research carried out. It is often helpful to use ‘bullt points’, each no more than two or three lines, to summarize your results. This enables you, lecturer and tutor to see, at a glance, whether you have addressed all of the important areas and helps you to check that you have covered everything that you wanted to and listed in the objectives.

5.7 References

Citing references

References may be cited in the text in a number of ways, depending upon your style of writing or the context of your reference. However, there are convention that should be followed, as shown below – note the use of brackets.

• Natural levels of carbon monoxide are low, typically in the range 20 – 200 ppb (Grimes and Clement, 1993).
• Kinnear (1998) describes a system for sampling PM10 on an hourly basis, while Hegarty et al. (2001) describe a system for the continuous sampling of PM10. [Note: “Hegarty, Scanlon and Chan (2001) is written as Hegarty et al. (2011)]

If reference has two authors or less, the family name of all author(s) should be mentioned in the reports. If a reference has more than two authors, only the first is mentioned with “et al.” “et al. translates as “and others”.

You may want to cite an official or company report, or government paper, where there is no specified author or the authorship belongs to a committee. In such cases, you normally cite the body responsible for publishing the paper or report. Thus, in the text, the body responsible for publishing the paper is cited with the year of publication, e.g. (EvironTech Ltd, 2000).

The Reference Section

The Reference section must include details of all references that have been cited in text. It does not include peripheral reading. The details of each reference include the following: name(s) of the author(s) (surname first, with a comma), the year of publication, and the title of the publication. In the case of books and reports, the name of the publisher and place of publication is also given, There is more than one way of presenting this information; the following example illustrate the use of upper and lower case letters, italics, punctuation marks and general layout.
Books


Book Chapters

XYZ, F. M. (Year Published). Title of chapter In F. M. XYZ Editor (Ed.), Title of book/anthology (pp. Pages). Publisher City, State: Publisher.

Article in Journals

XYZ, F. M., & ABC, F. M. (Year Published). Article title. Journal Name, Volume (Issue), Pages.

Websites


6. **PLAGIARISM**

Plagiarism is the representation of another person’s published or unpublished work or ideas as your own by using an extensive unacknowledged quotation. In academia, plagiarism carries heavy penalties; your mark for any assessed work may be significantly reduced and you may be open to accusations of academic misconduct. However, this does not mean that all of your work must be completely original; expressing views that are influenced by other authors is a consequence of shared knowledge and reflection of wide reading. In order to avoid accusations of plagiarism, you should clearly reference sources by using the conventions outlined above.
Laboratory Report Marking Scheme

Section 1 Lab Performance (Total 20%)

1. Pre-entering lab (5%)

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No preparation of experimental procedure, no proper attire-shoes; goggle; lab coat.</td>
</tr>
<tr>
<td>1-2</td>
<td>Summary of procedures too brief, lack of details and confusing; incomplete safety attire.</td>
</tr>
<tr>
<td>3-5</td>
<td>Presents easy to follow steps in lab experimental, logical and adequately detailed; safety attire checked.</td>
</tr>
</tbody>
</table>

2. Skill & Techniques (15%)

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No skill is demonstrated.</td>
</tr>
<tr>
<td>1-5</td>
<td>Wrong glassware used, wrong technique, spillage and wasting of chemicals.</td>
</tr>
<tr>
<td>6-10</td>
<td>Right glassware used, incorrect or lack of lab technique.</td>
</tr>
<tr>
<td>10-15</td>
<td>Presents correct lab skill, clean and tidy.</td>
</tr>
</tbody>
</table>

Section 2: Lab report (Total 60%)

<table>
<thead>
<tr>
<th>Section</th>
<th>Total Mark</th>
<th>Rubric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
|             | 0-1        | • No title, or  
|             |            | • Too brief (e.g. “Lab report”; “Mercury in fish”; Ascorbic acid in fruits”, etc). |
|             | 2-3        | • Too long, or  
|             |            | • Does not identify the complete subject of study  
|             |            | (E.g “Determination of mercury”; “Determination of lead”, etc). |
| Objective   | 15         |        |
|             | 0          | • Section missing completely. |
|             | 1 - 7      | • Be too vague, ambitious or broad in scope.  
|             |            | • Just repeat each other in different terms.  
|             |            | • Just be a list of things related to the topic.  
|             |            | • Contradict with methods.  
|             |            | • Does not identify subject of study. |
|             | 8 - 15     | • Concise and brief.  
|             |            | • Be interrelated and describes how to achieve that objective.  
|             |            | • Clearly identify the subject of study.  
<p>|             |            | • Related to the experiment that has been done. |
| Introduction| 10         |        |
|             | 0          | • Section missing completely. |
|             | 1 - 5      | • Background info only from lab manual |</p>
<table>
<thead>
<tr>
<th>Section</th>
<th>Total Marks</th>
<th>Score</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>10</td>
<td>0</td>
<td>- Section missing completely.</td>
</tr>
<tr>
<td></td>
<td>1 - 5</td>
<td></td>
<td>- One or more subsections (e.g. chemicals or instrumentation) are missing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Confusing statement.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Parts have been included under the wrong subsection.</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td></td>
<td>- Contains all of the relevant information about the method used; clearly and systematically described in such a way that a reader could replicate the study from the description.</td>
</tr>
<tr>
<td>Results</td>
<td>20</td>
<td>0</td>
<td>No Discussion section.</td>
</tr>
<tr>
<td></td>
<td>1-6</td>
<td></td>
<td>- Very lack attempt to relate experiment findings and collected data.</td>
</tr>
<tr>
<td></td>
<td>6-12</td>
<td></td>
<td>- Showing attempt to discuss the findings and collected data, but using inaccurate theories and justifications.</td>
</tr>
<tr>
<td></td>
<td>13-20</td>
<td></td>
<td>- Able to demonstrate analysis skill in discussing the results, including the inaccuracies of data, using logic and appropriate statements to justify the experiment outcome.</td>
</tr>
<tr>
<td>Discussion</td>
<td>20</td>
<td>0</td>
<td>No Discussion section.</td>
</tr>
<tr>
<td></td>
<td>1-6</td>
<td></td>
<td>- Very lack attempt to relate experiment findings and collected data.</td>
</tr>
<tr>
<td></td>
<td>6-12</td>
<td></td>
<td>- Showing attempt to discuss the findings and collected data, but using inaccurate theories and justifications.</td>
</tr>
<tr>
<td></td>
<td>13-20</td>
<td></td>
<td>- Able to demonstrate analysis skill in discussing the results, including the inaccuracies of data, using logic and appropriate statements to justify the experiment outcome.</td>
</tr>
<tr>
<td>Safety caution</td>
<td>5</td>
<td>0</td>
<td>Section is not present.</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td></td>
<td>- Sentences are not in complete, focusing on minor or lack important steps.</td>
</tr>
<tr>
<td></td>
<td>4-5</td>
<td></td>
<td>- Tabulate at least 3 major and most important safety caution.</td>
</tr>
<tr>
<td>Conclusions</td>
<td>10</td>
<td>0</td>
<td>- Section missing completely.</td>
</tr>
<tr>
<td></td>
<td>1 – 5</td>
<td></td>
<td>- Conclusion is drawn but not supported by experimental evidence.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- No sensible conclusion is drawn.</td>
</tr>
</tbody>
</table>
No clear evidence of a thorough understanding of the experiment and/or theory behind the experiment.

6 – 10

- Conclusion is drawn and supported by experimental evidence.
- Sensible conclusion is drawn.
- Shows clear evidence of a thorough understanding of the experiment and/or theory behind the experiment.

References

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Unable to answer any questions.</td>
</tr>
<tr>
<td>1-5</td>
<td>Very little attempt to answer question correctly.</td>
</tr>
<tr>
<td>6-10</td>
<td>Most answers are incorrect, and some are irrelevant to the question type.</td>
</tr>
<tr>
<td>11-15</td>
<td>Some answers maybe very short or incomplete.</td>
</tr>
<tr>
<td>16-20</td>
<td>Questions are answered to the best of abilities and answers match the question types.</td>
</tr>
</tbody>
</table>

Late Report  -1 marks / day

*For Section 3 Assessment-it is up to the lecturer in-charge to decide whether want to carry out a simple test or not. If choose not to, the 20% marks will be allocated back to Section 2-Lab report.
WATER SAMPLING, TOTAL DISSOLVED SOLIDS AND TOTAL SUSPENDED SOLIDS

1. INTRODUCTION

A) Water Sampling

Samples collected for analysis should be obtained in such a way as to provide the most representative sample possible. In general, samples should be taken near the center of the body of water and entirely below the surface. It is difficult to obtain a truly representative sample when collecting surface water samples. More meaningful results are commonly obtained by carrying out a series of tests with samples taken from several locations and depths and at different times. The results can then be used to establish patterns applicable to that particular body of water (Boehnke and Delumyean, 2000).

Generally, as little time as possible should elapse between collecting the sample and carrying out the analysis. Depending on the nature of the test, special precautions in handling the sample may be necessary to prevent natural interferences, such as bacterial growth or the loss of dissolved gases. Table 1-1 gives detailed information for preserving samples. When studying a particular aquatic ecosystem, an environmental scientist learns as much about the system as possible. This knowledge helps to explain results and aids in locating areas of the system thus should be studied (Boehnke and Delumyean, 2000).

B) Total Suspended Solids

The total suspended solids (TSS) test is one of the most common determinations made in wastewater treatment plants. The test is not intended to measure the concentrations of specific chemical substances, but rather give an empirical estimate of water quality by measuring the amount of suspended foreign materials present. It is determined from the weight gain of a filter after drawing a known volume of water through the filter.

All streams carry some suspended solids under natural conditions. However, if concentrations are enhanced through anthropogenic perturbations, this can lead to alterations to the physical, chemical and biological properties of the waterbody. Physical alterations caused by suspended solids are such as reduced penetration of light, temperature changes, and infilling of channels and reservoirs when solids are deposited (Bilotta and Brazier, 2008). These physical alterations are associated with undesirable aesthetic effects, higher costs of water treatment, reduced navigability of channels and decreased longevity of dams and reservoirs (Bilotta and Brazier, 2008).

C) Total Dissolved Solids

Material that cannot be removed by a filter of a particular porosity is said to be "dissolved." Many, although not all of these species, are inorganic salts or weak organic acids, which ionize in water. The principal constituents are usually calcium, magnesium, sodium, and potassium cations and carbonate, hydrogen carbonate, chloride, sulfate, and nitrate anions. The presence of dissolved solids in water may affect its taste. High TDS concentrations can be measured gravimetrically, although volatile organic compounds are lost by this method (WHO, 2003). The palatability of drinking water has been rated by panels of tasters in relation to its TDS level as follows:
Table 1-2: Water quality according to TDS concentration (WHO, 2003).

<table>
<thead>
<tr>
<th>Classification of water</th>
<th>Concentration of TDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>&lt; 300 mg/L</td>
</tr>
<tr>
<td>Good</td>
<td>between 300 and 600 mg/L</td>
</tr>
<tr>
<td>Fair</td>
<td>between 600 and 900 mg/L</td>
</tr>
<tr>
<td>Poor</td>
<td>between 900 and 1200 mg/L</td>
</tr>
<tr>
<td>Unacceptable</td>
<td>&gt; 1200 mg/L</td>
</tr>
</tbody>
</table>

Table 1-1: EPA recommended preservation methods of water and wastewater samples (Boehnke and Delumyea, 2000).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preservation Method</th>
<th>Container*</th>
<th>Maximum Holding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity/Alkalinity</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>14 days</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>BOD</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>48 hours</td>
</tr>
<tr>
<td>COD</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>Chloride</td>
<td>None</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>Residual chlorine</td>
<td>None</td>
<td>P, G</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>None</td>
<td>G with Glass Top</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Fluoride</td>
<td>None</td>
<td>P</td>
<td>28 days</td>
</tr>
<tr>
<td>Mercury</td>
<td>Nitric acid to pH &lt; 2</td>
<td>P, G</td>
<td>6 months</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>P, G</td>
<td>48 hours</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>48 hours</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>G</td>
<td>28 days</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>pH</td>
<td>None</td>
<td>P, G</td>
<td>Analyze immediately</td>
</tr>
<tr>
<td>Ortho-Phosphate</td>
<td>Filter on site</td>
<td>P, G</td>
<td>48 hours</td>
</tr>
<tr>
<td>Phosphorus, total</td>
<td>Sulfuric acid to pH &lt; 2</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>Solids</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>7 days</td>
</tr>
<tr>
<td>Specific conductance</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>28 days</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>7 days</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Store at 4°C</td>
<td>P, G</td>
<td>48 hours</td>
</tr>
<tr>
<td>Purgeable aromatic</td>
<td>Store at 4°C</td>
<td>G, Teflon-Lined Septum</td>
<td>14 days</td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>HCl to pH 2</td>
<td>G, Teflon-Lined Cap</td>
<td>7 Days until extraction</td>
</tr>
<tr>
<td>Phenols</td>
<td>Store at 4°C</td>
<td>G, Teflon-Lined Cap</td>
<td>40 Days after extraction</td>
</tr>
<tr>
<td>PCBs</td>
<td>Store at 4°C</td>
<td>G, Teflon-Lined Cap</td>
<td>Same as above</td>
</tr>
<tr>
<td>Phthalate esters</td>
<td>Store at 4°C</td>
<td>G, Teflon-Lined Cap</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td>Store in dark</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P is plastic and G is glass.

2. LEARNING OBJECTIVES
   i. To introduce the methods used in water sample collection
   ii. To use a collected water samples to determine total suspended solids (TSS) and total dissolved solid (TDS).

3. METHODOLOGY

   A) Water Sampling

   1. Clean a 1 L plastic bottle by washing with 10% hydrochloric acid, followed by thoroughly rinsing with DI water.
   2. Collect a sample of water from about 1 m below the surface of a river or pond, from a boat dock, or from another convenient location.
   3. Device for collecting water samples is shown in Figure 1-1.
   4. In your laboratory notebook record all conditions under which the sample was obtained (air and water temperatures, weather conditions, tide, etc.).
   5. It is good practice to obtain a second sample on a collection trip to serve as a reference. This sample should be collected, handled, and stored the same way as the sample of interest.

   Figure 1-1: Van Dorn sampler

   B) Determination of Total Suspended Solids (TSS) according to EPA Method 160.2 (EPA, 2015)

   1. Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus.
   2. 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.
   3. Remove filter from membrane filter apparatus and dry in an oven at 103-105°C for one hour.
   4. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter with forceps or tongs only.
   5. For collected water sample, assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.
   6. Shake the sample vigorously and quantitatively transfer 500 mL of sample volume to
the filter using a graduated cylinder.
7. Remove all traces of water by continuing to apply vacuum after sample has passed through.
8. With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. **RECORD THE EXACT VOLUME OF DISTILLED WATER!!**
9. Remove all traces of water by continuing to apply vacuum after water has passed through.
10. Carefully remove the filter from the filter support. Alternatively, remove filter from membrane holder. Dry at least one hour at 103-105°C.
11. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained.

![Figure 1-2: Suction filtration system with membrane holder](image)

**Figure 1-2: Suction filtration system with membrane holder**

C) **Determination of Total Dissolved Solids (TDS) according to ASTM D5907-03 (Environmental Express, 2016)**

1. Mix your sample (from Section B) thoroughly and measure out a portion expected to contain between 2.5 and 200 mg residue.
2. Transfer the total filtrate, including the washings, to your evaporating dish and record the sample volume.
3. Evaporate the sample to dryness on a steam bath or in a drying oven (103-105°C).
4. Dry the dishes for at least one hour at 180 ± 2°C.
5. Remove dishes from the oven and place in a desiccator until at room temperature.
6. Weigh each dish on a balance to the nearest 0.0001 g and record the weight.

**REPORT**

Calculate the concentration of TSS and TDS in the unit of mg/L.

4. **REFERENCE**


The pH, Buffer Capacity and Alkalinity of Environmental Waters

1. INTRODUCTION

The Origin of Natural Acidity (by Boehnke and Delumyea, 2000)

In this section, we examine factors that affect the pH of natural waters, which are often somewhat basic. Most acidity in natural waters is due to carbon dioxide which dissolved in water and produces hydronium ion:

\[ \text{CO}_2 (g) + \text{H}_2\text{O} \leftrightarrow \text{H}^+ (aq) + \text{HCO}_3^- (aq) \]

This reaction can be considered to occur in two stages. **Stage I** is the establishment of equilibrium between atmospheric and aqueous carbon dioxide. The amount of carbon dioxide that dissolves in water is governed by Henry's law, which takes the form

\[ [\text{CO}_2(aq)] = K_H P(\text{CO}_2) \]

where \( K_H \) is the Henry's law constant and \( P(\text{CO}_2) \) is the partial pressure of carbon dioxide. For carbon dioxide at 25°C, \( K_H = 3.4 \times 10^{-2} \text{ mol/L • atm} \). The concentration of \( \text{CO}_2 \) in the atmosphere is about 350 ppm. In the gaseous state, 350 ppm of \( \text{CO}_2 \) means 350 molecules per \( 1 \times 10^6 \) molecules of air, and since moles and molecules are proportional,

\[ \frac{350}{1000000} \equiv \text{mol \ CO}_2/\text{mol} \equiv \text{air \ mol \ fraction \ CO}_2 = P(\text{CO}_2)/P(\text{air}) \]

Thus \( P(\text{CO}_2) = 3.5 \times 10^{-4} \text{ atm when } P(\text{air}) = 1.0 \text{ atm and } [\text{CO}_2(aq)] = 1.2 \times 10^{-5} \text{ mol/L at } 25^\circ \text{C from Stage I.} \)

**Stage II** is the dissociation of dissolved carbonic acid, according to

\[ \text{H}_2\text{CO}_3(aq) \leftrightarrow \text{H}^+(aq) + \text{HCO}_3^- (aq) \quad K_{a1} = 4.5 \times 10^{-7} \]

\[ \text{HCO}_3^- (aq) \leftrightarrow \text{H}^+ (aq) + \text{CO}_3^{2-} (aq) \quad K_{a2} = 4.7 \times 10^{-11} \]

Since \( K_{a1} >> K_{a2} \), the pH of the system is primarily due to the first equilibrium. Therefore, \( K_{a1} = [\text{H}^+][\text{HCO}_3^-]/[\text{H}_2\text{CO}_3] = [\text{H}^+]^2/1.2 \times 10^{-5} = 4.5 \times 10^{-7} \), and \([\text{H}^+] = 2.3 \times 10^{-6} \), giving a pH of 5.63. This is the expected pH for pure water in equilibrium with atmospheric carbon dioxide at 25°C.

Although the pH of a natural water is affected by the carbon dioxide acidity of rain, a third factor to be considered is the background carbonate level that is due, in part, to the dissociation of calcium carbonate in soil. When rain falls on land, it first percolates through topsoil, where its pH may drop by another unit due to the large quantity of carbon dioxide produced by bacteria. However, much of the earth's crust contains calcium carbonate (ultimately derived from marine organisms). The effect of calcium carbonate on the pH of environmental waters is due to three factors: (1) calcium carbonate is sparingly soluble in water; (2) the carbonate ion is a moderately strong base, where bicarbonate is only a weak base, and (3) dissolved carbonate is in equilibrium with carbon dioxide in some gases and in bodies of water.
The net result of these factors is that the pH of natural waters will be somewhat basic instead of the acidic pH from dissolved CO$_2$ alone. To illustrate, first consider the dissociation of calcium carbonate,

$$\text{CaCO}_3 (s) \leftrightarrow \text{Ca}^{2+} (aq) + \text{CO}_3^{2-} (aq)$$

where $K_{sp} = [\text{Ca}^{2+}][\text{CO}_3^{2-}] = 4.6 \times 10^{-9}$ at 25° C. If this were the only process to occur, the pH would remain unchanged.

However, since carbonate ion is a Bronsted-Lowry base, according to

$$\text{CO}_3^{2-} (aq) + \text{H}_2\text{O} (l) \leftrightarrow \text{HCO}_3^- (aq) + \text{OH}^- (aq)$$

the observed pH is due to not only atmospheric carbon dioxide, but also to the carbonate from the hydrolysis of CaCO$_3$(s), which increases pH.

The hydrolysis constant for the last reaction is found as follows:

$$K_h = \frac{K_w}{K_{a2}} = \frac{[\text{HCO}_3^-][\text{OH}^-]}{[\text{HCO}_3^{2-}]} = \frac{1.0 \times 10^{-14}}{4.7 \times 10^{-11}} = 2.1 \times 10^{-4}$$

When the solubility product equilibrium is combined with the hydrolysis equilibrium, the net result is

$$\text{CaCO}_3 (s) + \text{H}_2\text{O} (1) \leftrightarrow \text{Ca}^{2+} (aq) + \text{HCO}_3^- (aq) + \text{OH}^- (aq)$$

Because when equilibria are added the new equilibrium constant is the product of the individual equilibrium constants, the equilibrium constant for the last equilibrium is $K' = K_{sp} \times K_h = 4.6 \times 10^{-9} \times 2.1 \times 10^{-4} = 9.7 \times 10^{-13}$. Since $[\text{Ca}^{2+}][\text{HCO}_3^-][\text{OH}^-] [\text{OH}^-] = 9.7 \times 10^{-13}$, $[\text{OH}^-] = 9.9 \times 10^{-5}$ and pH = 10.00. Thus, without atmospheric carbon dioxide, the pH of natural waters in contact with calcium carbonate would be quite high. Now consider both equilibria simultaneously,

$$\text{H}_2\text{CO}_3 (aq) \leftrightarrow \text{H}^+ (aq) + \text{HCO}_3^- (aq)$$

$$\text{CaCO}_3 + \text{H}_2\text{O} \leftrightarrow \text{Ca}^{2+} (aq) \text{HCO}_3^- (aq) + \text{OH}^- (aq)$$

where $K_{st} = 4.5 \times 10^{-7}$, $K' = 9.7 \times 10^{-13}$, and $K = 1/K_w = 1.0 \times 10^{14}$, respectively for the three equilibria. The overall description of the three simultaneous processes is found by summing the equilibria to give,

$$\text{CaCO}_3(s) + \text{H}_2\text{CO}_3(aq) \leftrightarrow 2\text{HCO}_3^-(aq) + \text{Ca}^{2+}(aq)$$

for which $K'' = K_{st} \times K' \times K = 4.4 \times 10^{-5}$. 

Chemistry Department, University of Malaya
Now, since $[\text{HCO}_3^-] = 2 \times [\text{Ca}^{2+}]$, $[\text{Ca}^{2+}] (2 \times [\text{Ca}^{2+}])^2 / 1.2 \times 10^{-5} = 4.4 \times 10^{-6}$, then $[\text{Ca}^{2+}] = 5.1 \times 10^{-4} \text{ mol/L}$. In terms of ppm CaCO$_3$, the calcium level is $(5.1 \times 10^{-4} \text{ mol/L})(100 \text{ g/mol})(1000 \text{ mg/g}) = 51 \text{ ppm}$ — a reasonable value based on actual levels found. Finally, to calculate the expected pH, the last equilibrium is used and we examine the bicarbonate produced to see if it is a stronger acid or a stronger base. As an acid,

$$\text{HCO}_3^- (aq) \leftrightarrow \text{H}^+ (aq) + \text{CO}_3^{2-}(aq) \quad K_{a2} = 4.7 \times 10^{-11}$$

and as a base,

$$\text{HCO}_3^- (aq) + \text{H}_2\text{O}(l) \leftrightarrow \text{H}_2\text{CO}_3(aq) + \text{OH}^-(aq) \quad K_b = 2.2 \times 10^{-8}$$

since $K_b = K_{b1} = K_w / K_{a1}$. Since $K_b >> K_{a2}$, $K_{a2}$ can be ignored and the pH is calculated using the expression for $K_b$.

Then, $K_b = [\text{H}_2\text{CO}_3][\text{OH}^-]/[\text{HCO}_3^-] = 2.2 \times 10^{-8}$. From Henry's law we found that $[\text{H}_2\text{CO}_3] = 1.2 \times 10^{-6}$ and we also showed that $[\text{HCO}_3^-] = 2 \times [\text{Ca}^{2+}] = 2(5.1 \times 10^{-4}) = 1.0 \times 10^{-3}$. Since $K_b$ is so small, we can use $(1.2 \times 10^{-6})[\text{OH}^-] / 1.0 \times 10^{-3} = 2.2 \times 10^{-8}$. This gives $[\text{OH}^-] = 1.8 \times 10^{-6}$, and finally pH equals 8.26. This is remarkably close to the pH of many natural waters. Over a 10-year period in a major stream in the southeastern United States, Boehnke and Delumyea (2000) found an average pH close to 8.0, with little variation, except after periods of substantial rainfall.

**Alkalinity and Buffering Capacity of Natural Water (by Ibanez et al., 2008)**

This experiment will allow the determination of the alkalinity and buffering capacity of water samples from different natural sources. The buffering capacity is the ability to neutralize the pH and the resistance to change in it due to the small acidic or basic inputs or discharges. When a system is poorly buffered, the addition of even small amounts of an acid or a base will noticeably alter its pH, but when a system is well buffered, the same addition barely modifies its pH (i.e., it becomes relatively insensitive to the addition of small amounts of acids or bases). The buffering capacity of a system is defined as the moles/L of strong acid (or strong base) needed for a change in one pH unit of a solution. A typical buffer is formed by a combination of a weak acid (or base) with its corresponding salt. For example:

$$\text{HA} \overset{\text{H}^+}{\rightleftharpoons} \text{H}^+ + \text{A}^-$$

The equilibrium (acidity) constant is:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

from which we can derive the equation of $pK_a = -\log K_a$ with respect to the pH:

$$pH = pK_a + \log \left( \frac{[\text{A}^-]}{[\text{HA}]} \right)$$

This is known as Henderson-Hasselbalch equation and it is built under the assumption that $[\text{H}^+]$ or $[\text{OH}^-] \ll [\text{HA}]$ and $[\text{A}^-]$, where HA = weak acid, and A$^-$ = the corresponding anion generated from the salt. In a well-buffered system, the greatest resistance to changes in pH will occur when the ratio of concentrations of the acid and its salt are approximately equal and
therefore the $pK_a$ will be equal to its pH. From the above equation it is clear that this occurs at $[A^-]/[HA] = 1$.

By knowing the pKa of the buffering acid, one can estimate the pH at which its greatest buffering capacity will be centered. The pH of a buffer solution is affected by two factors: the concentration ratio, $[A^-]/[HA]$ (i.e., the inverse ratio of the acid to the conjugate base), and the strength of the parent acid or base. The stronger the parent acid or base in the buffer solution, the more extreme will the buffer's pH value be.

The buffering capacity depends on the concentration of the buffer, and on the type and concentration of the acid or base to be added to the buffered solution. In selecting the right working buffer for a specified pH, it is common to consider that its pKa must be at least one pH unit above or below the working pH.

The buffering capacity in natural waters is mainly due to the carbonate system and its equilibria. Therefore, it is important to know the alkalinity of the system, because this will provide the capacity for neutralizing an acid. The expression for alkalinity (i.e., dissolved species only) or acid neutralizing capacity (ANC) (i.e., the whole sample) is generally based on the carbonate system:

$$\text{Alkalinity} = \text{ANC} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+]$$

and this property is expressed as mg/L (or in eq/L, in the case of ANC) of the equivalent calcium carbonate. The ANC of natural water systems depends on the composition of the watershed. If there are minerals with poor solubility in the surrounding soil, the ANC will be low, whereas if calcareous minerals are present, there will be a high ANC. Some dissolved organic substances derived from decaying plant materials may also contribute to the ANC capacity of the water.

2. LEARNING OBJECTIVES
   i. Making pH measurements on environmental waters and rain waters.
   ii. To determine the buffer capacity of natural water
   iii. To determine the alkalinity of natural water

3. METHODOLOGY
   A) pH and Buffer Capacity of Natural Water
   1. Collection of water samples: In this experiment, you will use water samples (filtrate) collected in Experiment 1 and rainwater. Collect a sample of rainwater (if possible) using a standard rain collector. After collection, store all water samples in a refrigerator at 4°C. Allow the samples to reach the room temperature before use.
   2. Take a sample of deionized water that saturated with atmospheric carbon dioxide. You can prepare the sample by bubbling the deionized water with air for 1 hour.
   3. Calibrate the pH meter and measure the pH of the water samples.
   4. Use 100 mL of one environmental sample to determine buffer capacity. Select a sample expected to be high in buffer capacity. Set up a clean burette and a magnetic stir plate. Fill the burette with 0.01000 M HCl and take the initial volume reading. Add the sample to the titration beaker (250 mL) and measure the initial pH of the sample. Add 1 mL increments of 0.01000 M HCl. Measure the pH after each addition, stirring with a magnetic stir bar. Continue to a pH of about 4.0.
   5. If the volume needed to reduce the pH by 1 unit is too small, increase the sample size and repeat the titration (called a potentiometric titration). Add either 0.5 or 1.0 mL increments of titrant at a time. It is advantageous to plot pH (as ordinate) versus volume of titrant (as abscissa) as the titration progresses to determine where less (or greater) volume increments should be used.
B) **Alkalinity**

I. **Standardization**

1. Prepare a 0.1 M sodium hydroxide solution. Quickly weigh about 4 g of sodium hydroxide pellets using a small beaker and transfer to a 1 L plastic bottle. Fill the bottle with 1 L of DI water and mix thoroughly. Standardize this solution. (An alternative procedure is to dilute 8 g (about 5-6 mL) of 50% (w/w) sodium hydroxide to 1 L with DI water. This procedure eliminates sodium carbonate as an impurity since it is insoluble in the concentrated base.)

2. Prepare 0.1 M HCl (for alkalinity greater than 20 mg/L). In a fume hood, measure out 8.3 mL of concentrated HCl using a 10 mL graduated cylinder and dilute to 1 L in a glass or plastic bottle. Mix well.

3. Prepare 0.02 M HCl. Dilute 200 mL of the 0.1 M HCl to 1 L using volumetric flasks.

4. Standardize the 0.1 M NaOH against primary standard potassium acid phthalate (KHP). Weigh accurately (to 0.1 mg) three samples of KHP (previously dried) weighing about 0.5 g each (0.49-0.51 g). Quantitatively transfer the KHP to 250 mL Erlenmeyer flasks and dissolve in about 75 mL DI water. Add 3 drops of phenolphthalein indicator and titrate with the 0.1 M NaOH until the faintest pink persists for 30 seconds. You must rinse the buret with three 10 mL portions of the sodium hydroxide before use.

5. Use the standardized 0.1 M NaOH solution to titrate 25.00 mL (pipetted) aliquots of the 0.1 M HCl diluted to about 75 mL with DI water. Do three determinations of the molarity of the HCl and use the average value in subsequent calculations. (An exercise at this point to get a feel for the number of trials needed to obtain a reasonable standard deviation is to continue titrating 25.00 mL aliquots and calculating the standard deviation after each measurement [after three] until a reasonable standard deviation is obtained. That is, if the average molarity is 0.102 then the standard deviation might be 0.003 M, and the relative standard deviation is about 3%.)

II. **Indicator Titration for Alkalinity**

1. Do not filter, dilute or concentrate the selected water samples before testing. Since a large range of alkalinity is possible, made a rough measurement using 0.1 M HCl titrant. This allows for adjusting sample size so that the titration volume is greater than 10 mL, but less than 50 mL. For sample with titrant volume used is very small, a 0.02 M HCl solution is used with an appropriate sample size. Thus, the “test titration” with 0.1 M HCl is a guess to see what concentration of acid is needed.

2. Pipet 100 mL of sample (or the appropriate amount determined in a test titration) into a 250 mL Erlenmeyer flask. Add 3-5 drops of methyl orange.

3. Rinse a buret with three 10 mL portions of 0.1 M HCl. Fill with the acid and record the initial volume. Titrate the sample with the standardized 0.1 M HCl to the endpoint (which is orange to red), and record the final volume.

4. If the alkalinity is less than 20 mg/L, as determined by the test titration, use 0.02 M HCl and adjust the sample size if necessary. Titrate the sample as in Parts 1-2.

5. Do two additional titrations on the same sample according to Steps 1 and 2.

6. Repeat Steps 1-3 for a second type sample. You may use tap water, bottled drinking water (ground water).
III. Potentiometric Titration

1. Choose one of the environmental samples studied by indicator titration to examine in this part of the experiment. Use pipet to measure out 100 mL of sample into a 250 mL beaker. Lower the pH electrode into the sample, being certain that the bulb of the glass electrode is completely covered.

2. Using a magnetic stir bar and plate, obtain a potentiometric titration curve by adding standard 0.1 M HCl from a buret in either 0.5 mL or 1.0 mL increments, stirring and measuring the pH, until a pH of 4.0 is obtained. Record the pH after each addition of titrant.

4. REPORT

A) pH and Buffer Capacity of Natural Water

1. Tabulate your pH values, the type of sample, and expected pH values as presented in Introduction. Explain any differences between measured pH values and expected values.

2. Prepare a plot of pH versus volume of titrant for the environmental samples studied. Submit a copy of the titration curve.

3. Use the titration curve to determine the volume of titrant needed to decrease the initial pH by 1.00 unit. Use this volume to calculate the buffer capacity. The buffer capacity is the number of moles of the acid needed per liter of sample needed for this change in pH. Compare your result with the buffer capacity of a carbonate/bicarbonate system. You can obtain the buffer capacity of a carbonate/bicarbonate system through literature search.

B) Alkalinity

1. The molarity of sodium hydroxide can be obtained from the stoichiometric titration reaction, 
   \( \text{NaOH} + \text{KHP} \rightarrow \text{H}_2\text{O} + \text{KNaP} \). At the endpoint, the moles of base are equal to the moles of acid. Also, mol NaOH = \( \left( \frac{\text{mL}}{1000} \right) \times \text{(M)} \) and mol KHP = mass/molar mass. Therefore, the molarity of NaOH is given by,

   \[ M = \left( \frac{\text{mass \ KHP}}{204.23} \right) / \left( \frac{\text{mL \ NaOH}}{1000} \right) \]

   Report each individual molarity and the average of the values for NaOH.

2. Report the titration volumes and the calculated molarities of the 0.1 M HCl, and report the mean molarity.

3. If the 0.02 M HCl was used, report its molarity (from standardization or by calculation from the dilution of a standard solution).

4. The alkalinity for both the indicator and potentiometric methods is given by

   \[ [\text{Alk}] = \frac{\frac{1}{2}(\text{mL \ HCl})(\text{M}_{\text{HCl}})(100.0 \text{ mg / mmol})}{\text{L of sample}} \]

   when the alkalinity is expressed in mg CaCO\(_3\)/L. The volume of titrant is the volume of HCl needed to achieve a pH of 4.3 and 100.0 mg/mmol is the molar mass of CaCO\(_3\) (g/mol = mg/mmol). Report the alkalinity of the water samples. Compare the alkalinity values obtained from both indicator and potentiometric methods.

5. Discuss the alkalinitities for the two sample types and discuss their difference.
5. REFERENCE


DETERMINATION OF WATER HARDNES OF NATURAL WATERS – CONVENTIONAL EDTA COMPLEXOMETRIC TITRATION

1. INTRODUCTION

The hardness of water is originally defined in terms of its ability to precipitate soap. Calcium and magnesium ions are the principal causes of hardness in water, although iron, aluminum, manganese, strontium, zinc, and hydrogen ions are also capable of producing the same effect. The total hardness of water is now defined as the amount of calcium and magnesium present and is expressed as ppm calcium carbonate.

The procedure for determining both calcium and magnesium, when present together, is found in many schemes of applied analysis, including the analysis of minerals, blood serum, and food, and is the standard method for determining water hardness.

The hardness test is one of the most commonly performed analyses in the water industry. High levels of hardness are undesirable and must be removed before the water is used by the beverage, laundry, metal-finishing, dyeing and textiles, food, and paper pulp industries. Hardness levels greater than 500 ppm calcium carbonate are undesirable for domestic use and most drinking water supplies average about 250 ppm. Table 3-1 lists the various classes of hardness.

<table>
<thead>
<tr>
<th>Hardness Range (ppm CaCO₃)</th>
<th>Hardness Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 50</td>
<td>Soft</td>
</tr>
<tr>
<td>51 – 150</td>
<td>Moderately hard</td>
</tr>
<tr>
<td>151 – 300</td>
<td>Hard</td>
</tr>
<tr>
<td>&gt; 300</td>
<td>Very hard</td>
</tr>
</tbody>
</table>

Metal ions act as Lewis acids. Anions or molecules with unshared pairs of electrons can act as Lewis bases and covalently bind to metal ions. The electron pair donors are called ligands, and the species formed in the reaction are known as complex ions if ionic or complexes (or coordination compounds) if neutral. Ligands that bind to the metal at more than one coordination site are called polydentate. The ethylenediaminetetraacetate ion (EDTA) is an important polydentate ligand. This species has six donor atoms and is thus hexadentate. It reacts with many metal ions in a 1:1 ratio to form very stable complexes, as in the equation:

\[
\text{Co}^{3+} + [\text{EDTA}]^{4-} \rightarrow [\text{Co(EDTA)}]^3-
\]

EDTA is a tetraprotic acid and is frequently represented as H₄Y. The usual form of EDTA is the disodium salt, Na₂H₂Y. When this form is used as the titrant in a complexometric titration, the titration reaction is:

\[
\text{Na}_2\text{H}_2\text{Y (aq)} + \text{M}^{2+} (aq) \rightarrow \text{MY}^{2+} (aq) + 2 \text{H}^+ (aq) + 2 \text{Na}^+ (aq)
\]

Since hydronium ion is produced, a buffer is necessary since calcium and magnesium ions must be titrated at high pH for stable complexes to be formed and for the proper functioning of the indicator.
The indicators used for EDTA titrations are called metallochromic indicators, and for the most part they are weakly acidic organic dyes. They include Eriochrome Black T (EBT). EBT functions by forming a colored metal complex, MEBT\(^{-}\), at the start of the titration. As long as some metal remains unchelated by EDTA, the solution being titrated remains the color of MEBT\(^{-}\) complex. At the equivalence point, EDTA removes the metal ion from the indicator-metal complex by chelating it, and the solution changes color:

\[
Na_2H_2Y + MEBT^- \rightarrow HEBT^{2-} + MY^{2+} + H^+ + 2 Na^+
\]

The hardness due to calcium and magnesium ions separately can be determined by using the fact that at very high pH, magnesium forms the insoluble hydroxide, Mg(OH)\(_2\), whereas calcium remains in solution. The calcium can then be titrated with standard EDTA and its concentration determined. If another sample is titrated with EDTA at a lower pH, both calcium and magnesium ions react. The magnesium hardness is found by the difference in titrant volume used for the two samples. Some ions, notably iron (III), block the indicator by combining irreversibly with it. In this case the interfering ion must either be removed or chemically tied up before titrating with EDTA.

2. LEARNING OBJECTIVES
   i. To measure the hardness of environmental waters using conventional EDTA complexometric titration.

3. METHODOLOGY
   1. In this experiment, you will use samples collected in Experiment 1, tap water, and any other sample of interest.
   2. Prepare a 250 mL EDTA standard solution with the concentration of 0.01 M using Na\(_2\)EDTA. For more accuracy, standardize the EDTA.
   3. Pipet 10.00 mL of filtered water into a 250 Erlenmeyer flask, and dilute to about 50 mL with DI water. Add 15 mL of pH 10 buffer and mix thoroughly. Add 4 drops of EBT indicator and titrate with standard 0.01 M EDTA until a pure blue color, with not tinge of purple. Repeat this procedure for two additional samples, increasing the volume of sample if the titrant used is less than 10 mL.
   4. Repeat step 3 for other samples.

4. REPORT
   1. Report the concentration of the standard EDTA.
   2. Report the total hardness, in ppm CaCO\(_3\), for each determination. Since number of moles of EDTA = number of moles of metal from the titration reaction, the moles of calcium carbonate are equal to the moles of EDTA used in a titration. This is finally converted into mg CaCO\(_3\)/L of sample.
   3. Report the mean, standard deviation and %RSD for each type of sample analyzed. Discuss the precision of this method.
   4. Compare your hardness results with those given in Table 3-1 and classify the hardness of your samples accordingly.

5. REFERENCE

DETERMINATION OF DISSOLVED OXYGEN (DO) AND CHEMICAL OXYGEN DEMAND (COD) OF NATURAL WATERS

1. INTRODUCTION

A) Dissolved Oxygen

The level of DO in water is one of the most important parameters in determining its quality, because it indirectly indicates whether there is some kind of pollution. Common processes that pollute surface waters include the discharge of organic matter derived from municipal sewage or industrial wastes, and runoff from agricultural lots and livestock feedlots. In addition, the release of warm or hot discharges from industrial cooling towers induces what is known as thermal pollution. Such discharges directly affect the level DO in water bodies, which is crucial for the survival of aerobic organisms and aquatic fauna such as fish; in fact, excessive pollution has caused massive fish deaths. In the long run, the discharges of organics or of nutrients favor the accelerated eutrophication or productivity process with algal blooms. As a consequence, there will be a lowering of the DO content (or DO level) and the "death" of the aquatic system (Ibanez et al., 2008).

The measurement of the DO is also important to determine whether a water system is predominantly aerobic or anaerobic, predict the survival of aquatic organisms, and predict whether aerobic biological processes can take place for transforming the biodegradable organic contaminants discharged in water. When there is an organic discharge, the DO decreases rapidly due to the action of the aerobic microorganisms that consume oxygen during the metabolic degradation of organic matter. Consequently, the presence of dissolved oxygen is critical for the self-cleansing of the water system, and in combination with the presence of CO₂, it is also critical for the determination of the corrosive character of water on materials such as iron and other metals (Ibanez et al., 2008).

In this experiment, DO will be determined using Winkler method. The principle of analysis is based on the oxidation of iodide ion to iodine by DO. The amount of iodine generated is then determined by titration with standard thiosulfate solution. The endpoint is determined by using starch as a visual indicator.

B) Chemical Oxygen Demand (COD)

COD is a measurement of the oxygen required to oxidize soluble and particulate organic matter in water (Real Tech Inc, 2015). COD is a common parameter used to measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in mg/L, which indicates the mass oxygen consumed per liter of solution. The method used in this experiment involves using an excess amount of strong oxidizing reagent, potassium dichromate Cr₂O₇²⁻, to oxidize the organic matter in solution to carbon dioxide and water under acidic conditions. The test also involves a silver sulfate to encourage oxidation of certain organic compounds and mercury (II) sulfate to reduce the interference from oxidation of chloride ions (Real Tech Inc, 2015). The sample is reflux for 45 min. The remaining Cr₂O₇²⁻ is determined using titration method. The amount of oxygen required is calculated from the quantity of chemical oxidant consumed.
2. **LEARNING OBJECTIVES**
   I. To determine the dissolved oxygen of water samples by using Winkler Method.
   II. To determine the chemical oxygen demand (COD) of water.

3. **METHODOLOGY**

   **A) Determination of DO**

   1. **Collection of sample** — Collect the 3 different samples by a narrow necked 200-300 cm$^3$ glass bottle having an accurately fitting ground glass stopper. If the water from a tap, pass the water down a glass tube to the bottom of the bottle and allow water to overflow for 2-3 minutes before insertion of the stopper. When sampling stream water, displace the water in the bottle several times, before collecting the sample. The water temperature, weather conditions and nature of the water sample at the time of sampling should be recorded. Avoid inclusion of air bubbles in the sample bottle.

   2. **Standardisation of Sodium Thiosulphate** — Mix 5 cm$^3$ of potassium iodide solution (10% w/v) and 10 cm$^3$ of the dilute sulphuric acid (1:3 v/v ) and add 2 cm$^3$ of 0.025 mol dm$^{-3}$ potassium iodate solution in that order in a glassstoppered flask. Add about 100 cm$^3$ of distilled water. Titrate immediately with sodium thiosulphate solution until the colour is pale yellow. Add 2 or 3 drops of starch solution (freshly prepared) and continue the titration until the blue colour just disappears.

   3. **PROCEDURE for the Determination of Dissolved Oxygen in Water** — Carefully remove the stopper from the sample bottle and add in turn 1 cm$^3$ manganous sulphate solution followed by 1 cm$^3$ alkaline-iodide-azide solution. When introducing various reagents into the full bottle of sample, the tips of the pipettes should be well below the surface of the liquid. Replace the stopper carefully after each addition so as to avoid inclusion of air bubbles. Thoroughly mix the contents by inversion and rotation until a clear supernatant water is obtained.

   Add 1 cm$^3$ concentrated sulphuric acid with the tip of the pipette below the level of solution and again replace the stopper. Mix well by rotation until the precipitate has completely dissolved. Pipette into a 250 cm$^3$ conical flask 100 cm$^3$ of the solution and immediately titrate it against standard sodium thiosulphate (0.0125 mol dm$^{-3}$) using freshly prepared starch solution as the indicator (add when solution becomes pale yellow). Carry out the titration in duplicate.

   **B) Determination of COD**

   1. **Collection of Sample** — Use the collected water samples and any other sample.

   2. **Standardization of Ammonium Iron (II) Sulphate** — Add 10 cm$^3$ concentrated sulphuric acid carefully to 20 cm$^3$ water and cool. Add 2 cm$^3$ potassium dichromate and titrate with ammonium iron(II) sulphate using drops of ferroin as indicator. The colour changes from bluish-green to reddish-brown.

   3. **Procedure for Determination of COD** — Introduce 10.0 cm$^3$ of the water sample into 100 cm$^3$ round-bottomed flask, and add 2 cm$^3$ potassium dichromate, 2.5 cm$^3$ mercuric sulphate solution, 10-15 cm$^3$ concentrated sulphuric acid containing silver sulphate, and an anti-bumping rod. Heat to gentle, but steady boiling over an electric hot plate or
heating mantle and under a reflux condenser. After exactly 45 minutes boiling, allow to cool briefly, wash 20 cm³ distilled water through the condenser into the flask and the cool completely in cold water. Add 2 drops of ferroin solution and titrate the excess potassium dichromate with ammonium iron (II) sulphate until the colour changes from bluish-green to reddishbrown. Determine a blank with 10.0 cm³ distilled water under exactly the same conditions.

4. REPORT

1. Explain the reaction involved in the determination of dissolved oxygen in water using Winkler methods. Establish the relationship: 10 cm³ of 0.0125 mol dm⁻³ sodium thiosulphate = 1 mg O₂.

2. Report the result in mg dm⁻³ of COD and DO as well as percentage of O₂ saturation by referring to the following table:

   Table 4-1 Oxygen content in Air-Saturated Water.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ content (mg/kg)</td>
<td>11.2</td>
<td>10.2</td>
<td>9.1</td>
<td>8.3</td>
<td>7.6</td>
<td>7.1</td>
</tr>
</tbody>
</table>

5. REFERENCE


SPECTROPHOTOMETRY, COLORIMETRY, AND ABSORPTION SPECTRA: DETERMINING IRON IN NATURAL WATERS

1. INTRODUCTION

Background of Colorimetric and Spectrophotometric Analysis

Colorimetric and spectrophotometric methods are perhaps the most frequently used and important methods of quantitative analysis. These methods are based on the absorption of light by a sample. The amount of radiant energy absorbed is proportional to the concentration of the absorbing material, and by measuring the absorption of radiant energy it is possible to determine quantitatively the amount of substance present.

Colorimetric and spectrophotometric methods of analysis have been worked out for most of the elements and for many types of organic compounds. Methods based on the absorption of light are well suited to the determination of sample constituents from trace levels up to amounts of 1-2% but are not as frequently used for the analysis of larger (macro) quantities of substances.

The fundamental law on which colorimetric and spectrophotometric methods are based is the Bouguer-Beer or Lambert-Beer law, usually referred to simply as Beer's law. In mathematical form this Law is

\[ A = abc \]

where \( A \) is the absorbance, \( a \) is the absorptivity, \( b \) is the internal cell length, and \( c \) is the concentration of the solution. When the concentration is expressed in \( \text{mol/L} \), Beer's law is written

\[ A = \varepsilon bC \]

where \( \varepsilon \) is called the molar absorptivity, or the extinction coefficient, and \( C \) is the molarity. Typically, \( b \) is measured in \( \text{cm} \), and therefore \( \varepsilon \) has units of \( \text{M}^{-1}\text{cm}^{-1} \).

The colorimeter, or spectrophotometer, is an important analytical instrument that makes possible a quantitative measurement of the light that passes through a solution. The first step in an analysis is the determination of the optimum wavelength to use for the analysis. The analyte must appreciably absorb light at the wavelength chosen. In a colorimeter exact wavelengths are not used, but rather small bands of wavelengths and the wavelength chosen for analysis must be such that the absorbance does not change rapidly with the wavelength. If all the wavelengths in this narrow band are absorbed to nearly the same extent, the result is the same as if we isolated a single wavelength to use. Therefore, for an analysis have chosen a flat portion of the absorption spectrum (a plot of absorbance versus wavelength). Absorption spectra for metal analysis is shown in Figure 5-1.
Instruments that measure the absorption of radiant energy, spectrophotometers, have five essential components, as shown in Figure 5-2. For instruments that are used in the visible region of the spectrum, tungsten filament bulb is used as the source. The wavelength of light that enters the system is limited by means of a filter or a monochromator. The amount of light that enters the system is controlled with a variable slit or other means. The light then passes through the sample solution held in a glass cell called cuvette (quartz must be used in the ultraviolet region). Finally, the transmitted light strikes a phototube or other transducer (such as a photodiode), that converts it into an electric current. The current produced is a function of the radiant power of the light striking the transducer. The current is amplified and is then measured by a meter or a digital readout.

The advantage of a colorimeter is its relatively low cost and simplicity of operation. However, most colorimeters are not able to automatically change wavelength. The output from the source is not constant for all wavelengths, and this necessitates an adjustment in slit width or the sensitivity whenever the wavelength is changed. Also, colorimeters are single-beam instruments and therefore cannot automatically correct for the intensity changes in the light source and variations in detector sensitivity when the wavelength is changed.

The absorbance of a "reagent blank" must be determined at the start of an analysis to correct for any light absorption by the solvent or reagents.
Steps in an Analysis

If the analyte is colored, a colorimeter is used for the analysis and the cuvettes can be made of optical glass. If the analyte is not colored, but has an absorption in the ultraviolet, an ultraviolet spectrophotometer is used for the analysis and the cuvettes must be made of quartz or fused silica. In either case, the procedure for an analysis is the same, with the exception of the wavelength region scanned. In the visible region the wavelength range scanned is 760-400 nm, whereas in the ultraviolet region the wavelength range scanned is 400-200 nm.

1. **Formation of a Light-Absorbing Species** - When a species to be analyzed is not colored and must be analyzed using a colorimeter, it is transformed into a light-absorbing species. (Alternatively, if an ultraviolet spectrophotometer is available and if the species has a functional group that absorbs in the ultraviolet, the use of this instrument may be the easiest way to analyze the sample.) One straightforward way to obtain a colored species is to form a complex. Some metals form highly colored complexes with thiocyanate, \( r \) for example. A second way to produce a colored species is to transform a metal from a low oxidation state to a higher oxidation state by using an oxidizing agent. Chromium(III), which is only faintly colored, is transformed by oxidizing agents into chromate, \( \text{CrO}_4^{2-} \) or dichromate, \( \text{Cr}_2\text{O}_7^{2-} \), both of which are intensely colored. Some other types of reactions also produce colored species.

2. **Measuring the Absorption Spectrum** - The absorbance of the analyte solution is determined as a function of wavelength. The results are plotted (if a recording instrument is not used), preferably using computer software. Ideally, the most intense peak is chosen for the analysis, since it would be the most sensitive to the lowest concentrations. However, if the most intense peak is also sharp, it is better to choose a smaller, broader peak. The solutions should not contain suspended matter or colloids, which scatter light and distort absorbance measurements.

3. **Preparation of a Calibration (Beer's Law) Plot** - A series of standard solutions of the analyte is prepared spanning the concentrations expected. The instrument is adjusted to the wavelength chosen for the analysis, \( \lambda \) and the absorbance of each standard is measured. A plot of absorbance (ordinate) versus concentration is made, preferably using a computer. A least-squares analysis is carried out to obtain the equation of a straight line from which solution concentrations are calculated from measured absorbances. The correlation \( r \) coefficient from this analysis indicates the precision of the results. Curvature of the plot may indicate a change of equilibrium position of the analyte species with dilution and may have to be taken into account. A Beer's law plot is illustrated in **Figure 5-3**.

4. **Measuring the Sample** - The absorbance of the sample is measured at the wavelength used for the calibration. The concentration of the analyte is found from the Beer's law plot (either by estimating directly from the plot or by calculation using the straight-line Beer's law equation). The analyte concentration should be between the extreme limits of the plot; if not, its concentration or the concentrations of the standards should be adjusted accordingly.
Iron is found throughout the environment, often in large amounts. It enters the hydrosphere through the weathering of iron salts and minerals. Both iron(II) and iron(III) are found dissolved in water, often in colloidal form, or as inorganic and organic iron complexes. There are many industrial sources of iron, including canneries, tanneries, textile mills, shipping, and metal-cleaning operations.

Large concentrations of iron discharged into a stream or lake may have deleterious effects on aquatic life. A limit of 0.3 mg/L of iron is recommended for food and dairy product processing, soft drink manufacture, rind brewing, mainly because of taste.

Iron is a vital element in the respiratory processes of many animals, including humans. The human body has a great demand for iron, and 4 grams are found in the average human. Iron-containing proteins transport oxygen, catalyze the decomposition of peroxides, and play an essential role in the body's energy-generating processes. It is possible to ingest too much iron, which may cause liver damage. Thus, iron vitamin supplements contain cautionary statements.

A simple but sensitive procedure for the colorimetric determination of iron entails chelating ferrous iron with three molecules of 1,10-phenanthroline (phen) in a solution buffered at low pH,

$$\text{Fe}^{2+} + 3 \text{phen} \rightarrow [\text{Fe(phen)}_3]^{2+}$$

The orange-red complex has an absorption maximum at 510 nm.

A preliminary acid digestion of the sample is carried out in a fume hood to destroy organic matter and also to remove cyanide and nitrate which interfere with the analysis. Hydroxylamine hydrochloride is then added to reduce all iron(III) to iron(II), which is the effective complexing species. Then an excess of phen is added to the sample at a pH between 3.5 and 4.5. The low pH prevents other metals from precipitating and provides rapid reaction and color development. The concentration range of this method is 0.025-3.0 mg/L. Concentrations greater than 3.0 mg/L can be determined after diluting.
An advantage of the 1,10-phenanthroline method is its use of slightly acidic media. This prevents not only the precipitation of hydroxides, but also the phosphates and other anions of many metals. It is necessary that iron be present in a form that reacts completely with 1,10-phenanthroline in a reasonable period of time. This means that iron must not be bound to pyrophosphates or other ligands that form stable complexes; also, phosphate precipitates that contain iron must be prevented from forming. Therefore, the usual procedures in which sodium acetate is used to adjust the pH to 3.5-4.5 are not adequate for biological samples due to the possibility of precipitating ferric and aluminium phosphates. This is avoided by using sodium citrate.

2. LEARNING OBJECTIVES
   I. To illustrate the general principles of absorption spectrophotometry by demonstrating Beer's law.
   II. To measure the concentration of iron in water.

3. METHODOLOGY

   Preliminary Work

   1. Attempt to form a complex between thiocyanate ion and iron(II) by mixing together 5mL each of 0.01 M KSCN and 0.01M Fe(NO₃)₂.

   2. Repeat Step 1, this time using 0.01 M Fe(NO₃)₃ in acid in place of 0.01 M ferrous ion.

   3. Use a colorimeter or a recording spectrophotometer to measure the absorption spectrum of the solution prepared in Step 1 and 2, scanning from 760 to 400 nm. If necessary, dilute the solution to bring its absorbance within the range of the instrument. Use water as the reference.

   4. Repeat Steps 1 and 3 using Fe(II) nitrate and 5 mL of 0.3% 1,10-phenanthroline solution instead of thiocyanate.

   Procedure for Iron in Water

   1. Preparation of Standard 100 ppm Iron Solution - Weigh 351 mg of high quality ferrous ammonium sulfate hexahydrate, FeSO₄·(NH₄)₂SO₄·6H₂O, and quantitatively transfer to a 500 mL volumetric flask. Add 50 mL DI water followed by 1 mL of concentrated sulfuric acid. Dilute to the mark with DI water and mix thoroughly.

   2. Preparation of Standard Solutions - Prepare four standard solutions of iron(II) having the following No concentrations: 0.5, 1.0, 2.0, 3.0 and 5.0 ppm. Pipet 0.5, 1.0, 2.0, 3.0 and 5.0 mL of 100 ppm stock solution into 100 mL volumetric flasks and dilute to the mark with DI water.

   3. Obtaining a Beer's Law Plot - Transfer a 5 mL aliquot of the 0.5 ppm iron standard to a 125 mL Erlenmeyer flask and test the pH with test paper. If greater than 4.5, add enough 0.2 M sulfuric acid dropwise by using a buret to bring the pH to about 3.5, record the volume of sulfuric acid. Add sodium citrate (259 g/L) buffer dropwise to bring the pH to about 4.5 and again record the volume of sodium citrate buffer. Pipet 1 mL of 10% hydroxylamine
hydrochloride and 3 mL of 1,10-phenanthroline into the sample, mix, and allow 5 minutes for color development. Use the same volume of sulfuric acid and sodium citrate for the remaining four standard solutions, followed by 3 mL of 0.3% 1,10-phenanthroline and 1 mL of 10% hydroxylamine. Mix well. After adjusting the 0 and 100%T on the colorimeter at 512 nm, use water as the reference and measure the absorbance of each standard. (A reagent blank can also be used if desired. This consists of all substances added to the sample, is treated the same way as the sample, and accounts for any absorbance due to these materials.)

4. Analysis of Samples - Natural and tap water samples often have less than 0.5 ppm iron. A water faucet that has not been used for some time may furnish a good sample for iron analysis. Some well waters are high in iron content as well. However, even very dilute samples are within the range of this experiment. Determine the iron in several environmental water samples. Treat samples the same way as the standards, adding sulfuric acid initially, if necessary, followed by citrate buffer, reducing agent and the indicator. Use 5 mL samples and adjust the pH for each sample individually.

4. REPORT
1. Submit the two absorption spectra. From the iron-phenanthroline absorption spectrum decide what wavelength(s) can be used for analysis. Which particular peak, if there is more than one, would be best for an iron analysis?
2. Briefly discuss the effect of the ligand on the wavelength and the maximum absorbance of the peaks.
3. Calculate the extinction coefficient for the largest peak in the absorption spectrum of the iron-phenanthroline complex. (An excess of 1,10-phenanthroline was used.) The cell path length is exactly 1.0 cm. What quantitative information does this provide?
4. Prepare a Beer's law plot for the standard solutions (absorbance as ordinate versus concentration as T abscissa). Carry out a least-squares analysis and determine the slope, the y-intercept, and the correlation coefficient for the best straight line; comment on the linearity of the plot.
5. Use your Beer's law plot to determine the concentration (in mg/L) of iron in each sample studied. The concentration of a sample can be obtained directly from the Beer's law plot. Alternatively, the equation of a straight line can be obtained from the slope and y-intercept and the concentration calculated from the sample's absorbance. If duplicate determinations were done, report average values and the individual values of concentration and discuss the reproducibility.
6. Discuss the magnitude of the iron levels with respect to the sampling site.

5. REFERENCE


1. INTRODUCTION

Background of Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy (AAS) is an important analytical technique for the quantitative and qualitative analysis of metal ions. It is useful for measuring the presence and amounts of toxic metal cations in environmental samples, as well as for interrogating the metal content of ores in the mining industry to evaluate the economical worth of pursuing their extraction. With proper sample preparation method, the metal content of various samples such as minerals, biological samples, agricultural samples, petroleum, air particulate matters and water can be determined using AAS. This experiment is intended to illustrate the basic procedures used to analyse real samples for metals at the low part per million level.

Free atoms cannot undergo rotational or vibrational energy transitions, as molecule can. Only electronic transitions can occur when energy is absorbed or emitted. Because electronic transitions are discrete (quantized), line spectra are observed.

There are various ways of obtaining free atoms and measuring the radiation they absorb or emit. In flame spectrometry, a solution is aspirated into a flame and the compounds present are thermally dissociated into atomic vapour. The heat of the flame first causes the solvent to evaporate. The micro-crystals produced are partially (or wholly) dissociated into the elements in the gaseous state. Some of the atoms thus produced can absorb radiant energy of a particular wavelength and become excited to a higher electronic state. When these atoms fall back to lower energy levels and emit light, this provides the basis for a very sensitive analytical technique, atomic emission spectroscopy. In this method, high-temperature electric arcs or plasmas are used to maximize the production of excited atoms. The term "Atomic absorption" refers to the absorption of energy from a light source, with a consequent decrease in the radiant power transmitted through the flame. The measurement of this absorption corresponds to AAS.

The majority of atom in a flame are in the ground state; thus, most electronic transitions originate from this state. A partial energy-level diagram for sodium is shown in Figure 6-1. There are several possible transitions for sodium, but the primary line is at 589 nm.
More than 60 elements can be determined by AAS, many at the part per billion level. Only metals and metalloids can be determined directly by usual flame methods because the resonance lines for non-metals occur in the vacuum ultraviolet region of the spectrum. Table 6-1 lists the atomic absorption detection limits and wavelengths used for several environmentally important elements. For analytical measurements, the concentrations should be at least 10 times the detection limit since, by definition, the precision at the detection limit is no better than ±50%.

Table 6-1 Wavelengths and Detection Limits for Various Elements.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Detection limit (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>328.07</td>
<td>1</td>
</tr>
<tr>
<td>Ca</td>
<td>422.67</td>
<td>2</td>
</tr>
<tr>
<td>Cd</td>
<td>228.80</td>
<td>1</td>
</tr>
<tr>
<td>Co</td>
<td>240.72</td>
<td>2</td>
</tr>
<tr>
<td>Cr</td>
<td>357.87</td>
<td>2</td>
</tr>
<tr>
<td>Cu</td>
<td>324.75</td>
<td>4</td>
</tr>
<tr>
<td>Fe</td>
<td>248.33</td>
<td>4</td>
</tr>
<tr>
<td>Hg</td>
<td>253.65</td>
<td>500</td>
</tr>
<tr>
<td>K</td>
<td>766.49</td>
<td>3</td>
</tr>
<tr>
<td>Mg</td>
<td>285.21</td>
<td>3</td>
</tr>
<tr>
<td>Mn</td>
<td>279.48</td>
<td>0.8</td>
</tr>
<tr>
<td>Na</td>
<td>589.00</td>
<td>0.8</td>
</tr>
<tr>
<td>Ni</td>
<td>232.00</td>
<td>5</td>
</tr>
<tr>
<td>Pb</td>
<td>283.31</td>
<td>10</td>
</tr>
<tr>
<td>Sn</td>
<td>235.48</td>
<td>50</td>
</tr>
<tr>
<td>Zn</td>
<td>213.86</td>
<td>1</td>
</tr>
</tbody>
</table>
Atomic absorption uses essentially monochromatic radiation to excite vaporized atoms in their ground state. The instrument consists of a light source, a cell (consisting of the aspirated sample), a monochromator, and a detection system. The instrument is shown in Figure 6-2.

The source, usually a hollow cathode tube, emits essentially line radiation of the same wavelength as that being absorbed by the element under study. This is accomplished by making the source out of the sample element. Thus, if iron is to be determined, a lamp having an iron cathode is used. The sample is nebulized into a premixed gas-air burner designed for a long path length. The radiation then passes into a monochromator and is measured at the detector. The amount of radiation absorbed is proportional to the concentration of the element in the sample. A calibration curve is obtained by measuring the absorbance of a series of standard solutions.

2. LEARNING OBJECTIVES
I. To introduce one of the most important methods of environmental chemistry, atomic absorption spectroscopy. This instrumental method is used to detect metals and metalloids down to the ppb level. The method is fast and accurate and can be made to be essentially free of interferences.

3. METHODOLOGY
1. Collect two soil samples. Dry the samples at 110 °C for 3 hours.
2. Tare a labeled 150 mL beaker on an analytical balance and scoop in 10 g of soil sample. Weigh the sample to the nearest 0.1 mg. Repeat for a different environmental sample.
3. In a fume hood, add 10 mL of DI water and 10 mL of high-purity (for trace metal analysis) concentrated nitric acid to each sample. Add the acid slowly if there is frothing.
4. Prepare blank by adding 10 mL of DI water and 10 mL of high-purity concentrated nitric acid to another 150 mL beaker. Treat the blank identically to the samples.
5. Cover the beakers with watch glasses and gently swirl to mix. Heat on a hotplate to just below the boiling point and continue heating for 30 minutes. If necessary, add an additional 10 mL of acid if it appears that organic matter has not decomposed. Also add additional water if there is much evaporative loss. *NO₂ fumes (brown colour fumes) given off by decomposing nitric acid are extremely toxic. Avoid breathing in any of these fumes.
6. Remove the beakers from the hotplate, add 10 mL DI water to each sample, and allow to cool to room temperature. Filter each sample, including blank, using filter paper, catching the filtrate in a 100 mL volumetric flask. Rinse the beaker twice with small portion of DI water, adding the rinses to the funnel. Finally, rinse the funnel twice, using small amounts of DI water from a squeeze bottle. Bring the volume in the flask up to the mark, stopper, and mix thoroughly. The solution should be clear and devoid of any particles. Transfer the samples and blank to plastic bottles if they are not going to be immediately analyzed.

7. Select 3 metals. For each of the selected metal, prepare five standard solutions having concentration of 1, 2, 3, 5, 10 ppm. Prepare 100 mL of each standard and transfer to plastic containers for storage if samples and standards are not going to be immediately analyzed.

8. Analyze the samples and standard solutions with AAS.

9. Analysis of soil samples should be performed in triplicate.

4. REPORT

1. Prepare a calibration plot of absorbance versus concentration of each selected metal.

2. Use the calibration plots to calculate the concentration of metals (in ppm).

3. Calculate the (mg of metal)/(kg of sediment) for each metal and for each sample.

4. Do your values fall within expected values, or are they much higher than normal background levels? Discuss.

5. REFERENCE

QUANTITATIVE DETERMINATION OF OIL AND GREASE IN WATER

1. INTRODUCTION

Oil and grease (O&G) are made up primarily of variety of substances including fuels, motor oil, lubricating oil, hydraulic oil, cooking oil, and animal-derived fats (Boehnke and Delumyea, 2000; StormwaterRX, 2016). Sources of oil and grease are mainly anthropogenic. The O&G are a class distinguished on the bases of its solubility characteristics and not its chemical composition. O&G are nonpolar organic substances that are very soluble in nonpolar organic solvents such as n-hexane.

O&G is environmentally important because of the large number of sources and their toxicity to the environment. The concentration of O&G is often measured within a body of water, lakes, river, stormwater runoff, and wastewater. In some countries, regulatory bodies set limits in order to control the amount of O&G entering the aquatic environment through industrial and wastewater treatment plant discharges, and also to limit the amount of O&G present in drinking water (Pisal, 2009).

2. LEARNING OBJECTIVES

I. To introduce a method for the determination of O&G in water. This standard method involving extraction and gravimetric determination was developed by the United States Environmental Protection Agency and it has been classified as Method 1664.

II. To introduce quality control of analytical method.

3. METHODOLOGY

This method is suitable for the determination of n-hexane extractable material (HEM; oil and grease) in surface and saline waters and industrial and domestic aqueous wastes. It is not applicable to measurement of materials that volatilize at temperatures below approximately 85 °C. This method is capable of measuring HEM and SGT-HEM in the range of 5 to 1000 mg/L, and may be extended to higher levels by analysis of a smaller sample volume collected separately. Use the water sample collected in Experiment 1 for this experiment.

A. Determination of the background concentration of HEM and Percent Recovery

A.1 For the preparation of spiking solution, place 200 ± 2 mg steric acid and 200 ± 2 mg Hexadecane in a 100 mL volumetric flask and fill to the mark with acetone.

A.3 Analyze the sample aliquot (1000 mL) according to the procedure beginning in Section C to determine the background concentration of HEM (B)

A.4 Spike the additional sample aliquot (1000 mL) with spiking solution at 1 to 5 times the background concentration. Determine the concentration of HEM after spiking (A).

A.5 Calculate the percent recovery (P) of HEM in the sample by using the following equation:

\[
P = \left[ \frac{(A - B)}{T} \right] \times 100\%
\]

where T = True concentration of the spike
B. Determination of Precision and Recovery

B.1 Using a pipet, spike 10.0 ± 0.1 mL of the spiking solution into 1000 mL of reagent water (water free from O&G) to produce Precision and Recovery Standard (PAR) concentrations of approximately 20 mg/L each of hexadecane and steric acid.

B.2 Determine the concentration of HEM in FOUR PAR standard according to Section C. Using the results, compute the average percent recovery ($X$) and the standard deviation ($s$) of the percent recovery ($s$) for HEM.

B.3 Compare $s$ and $X$ with the corresponding limits for initial precision and recovery in Table 7-1. If $s$ and $X$ meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, $s$ exceeds the precision limit or $X$ falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test. Use the following equation for calculation of the standard deviation of the percent recovery:

$$s = \sqrt{\frac{1}{(n-1)} \sum (x_i - X)^2}$$

where: $n$ = Number of samples; $x_i$ = % Recovery in each sample

| Table 7-1 Acceptance Criteria for Performance Tests |
|---------------------------------|-------------------|
| **Initial precision and recovery** | **Limit (%)**      |
| HEM Precision (s)                | 11 or below        |
| HEM Recovery (X)                 | 83 - 101           |
| **Matrix spike**                 |                   |
| HEM Recovery                     | 78–114             |

C. Extraction of HEM

C.1 Bring the samples to room temperature.

C.2 Verify that the pH of the sample is less than 2 using pH paper. If the sample is at neutral pH, add 5-6 mL of 6 N HCl solution to the 1-L sample. Replace the cap and shake the bottle to mix thoroughly. Check the pH of the sample. If necessary, add more acid to the sample and retest.

C.3 Tare a clean boiling flask containing 3–5 boiling chips. Place the flask containing the chips in an oven at 105–115 °C for a minimum of 2 h to dry the flask and chips. Remove from the oven and immediately transfer to a desiccator to cool to room temperature. When cool, remove from the desiccator with tongs and weigh immediately on a calibrated balance.

C.4 Pour the sample into the separatory funnel. Add 30 mL of n-hexane to the sample bottle and seal the bottle with the original bottle cap. Shake the bottle to rinse all interior surfaces of the bottle, including the lid of the bottle cap. Pour the solvent into the separatory funnel.

C.5 Extract the sample by shaking the separatory funnel vigorously for 2 minutes with periodic venting into a hood to release excess pressure. Allow the organic phase to separate from the aqueous phase for a minimum of 10 minutes. Drain the aqueous layer (lower layer) into the original sample container. Drain a small amount of the organic layer into the sample container to minimize the amount of water remaining in the separatory funnel.

C.6 Place a filter paper (Section 6.5.2) in a filter funnel (Section 6.5.1), add approximately 10 g of anhydrous Na$_2$SO$_4$, and rinse with a small portion of n-
hexane. Discard the rinsate.

C.7 Drain the n-hexane layer (upper layer) from the separatory funnel through the Na₂SO₄ into the pre-weighed boiling flask containing the boiling chips.

C.8 Repeat the extraction twice more with fresh 30-mL portions of n-hexane, combining the extracts in the boiling flask.

C.9 Rinse the tip of the separatory funnel, the filter paper, and the funnel with 2–3 small (3–5 mL) portions of n-hexane. Collect the rinsings in the flask.

C.8 Connect the boiling flask to the distilling head apparatus and distill the solvent by immersing the lower half of the flask in a water bath with the temperature not more than 70 °C. When the flask appears almost dry, remove the distilling head. Sweep out the flask for 15 seconds with air to remove solvent vapor. Using tongs, immediately remove the flask from the heat source and wipe the outside surface dry to remove moisture and fingerprints. [Inspect the residue in the boiling flask for crystals. Crystal formation is an indication that sodium sulfate may have dissolved and passed into the boiling flask. This may happen if the drying capacity of the sodium sulfate is exceeded or if the sample is not adjusted to low pH. If crystals are observed, redissolve the extract in n-hexane, quantitatively transfer through a filter into another tared boiling flask, and repeat the distillation procedure]

C.9 Dry the boiling flask for 30 - 45 minutes in an oven maintained at 70 ± 2 EC. Cool to room temperature in a desiccator and maintain in the desiccator for at least 30. Remove with tongs and weigh immediately. Repeat the cycle of drying, cooling, desiccating, and weighing until the weight loss is less than 4 % of the previous weight or less than 0.5 mg, whichever is less.

C.10 Carried out the extraction in triplicate for sample.

4. REPORT

Calculate the concentration of HEM ("oil and grease") in the sample per the following equation:

\[
HEM \ (mg/L) = \frac{W_h(mg)}{V_s(L)}
\]

where:
\[W_h = \text{Weight of extractable material}\]
\[V_s = \text{Sample volume}\]

5. REFERENCE


EPA, 1999. Method 1664, Revision A: N-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry, Engineering and Analysis Division, U.S. Environmental Protection Agency.
ANALYSIS OF ENVIRONMENTAL HYDROCARBONS USING SIMPLE EXTRACTION AND ANALYSIS BY GAS CHROMATOGRAPHY COUPLED WITH FLAME IONIZATION DETECTOR

1. INTRODUCTION

A wide variety of analytical methods involving various instruments are currently used in the examination of environmental samples. This instruments are such as GC, GC–MS, high-performance liquid chromatography (HPLC), size exclusion HPLC, infrared spectroscopy (IR), supercritical fluid chromatography (SFC), thin layer chromatography (TLC), ultraviolet (UV) and fluorescence spectroscopy, isotope ratio mass spectrometry, and gravimetric methods. Among these instruments, GC technique is the most widely used and it is very hard to imagine an environmental laboratory without gas chromatograph (Beškoski et al., 2012). The GC-FID technique is a common routine technique for the quantitative analysis of all the non-polar hydrocarbons which are extracted using different sample preparation methods. It is also used for rapid semiquantitative assessments of the successfulness of bioremediation treatments of polluted environments (water, soil, sediments) or the decomposition of hydrocarbon materials originating from oil under natural conditions or historical pollution (Beškoski et al., 2012).

In this experiment, you are going to determine the hydrocarbons (HCs) in soil samples using GC-FID. The HCs most readily identifiable by gas chromatograph are the normal alkanes. These HCs are separated on the basis of their boiling points, the lower boiling point compounds elute from the column first and the least volatile ones elute at a later time. Recently, contaminated sediment samples often result in gas chromatograms with alkane peaks ranging from fewer than 10 to more than 30 carbon atoms. Figure 8-1 shows the gas chromatogram of an environmental sample that illustrates the spacing of the n-alkane peaks from a relatively clean environment. Figure 8-2 shows the chromatograms of the HCs from a contaminated. The hump, also called UCM (unresolved complex mixture), is characteristic of petrogenic HCs.

![Gas Chromatogram for Soil Sample](image)

**Figure 8-1** GC chromatogram for a soil sample dominated by plant wax n-alkanes (Zech et al., 2011)
Figure 8-2 GC chromatogram for a soil sample contaminated by petrogenic n-alkanes (Monza et al., 2013)

Due to the great range of alkanes usually present, temperature programming is necessary to decrease retention times for the larger alkanes. This is also necessary to keep peaks sharp. In temperature programming the temperature is increased at a certain rate (experimentally determined) until it reaches maximum value, which is often held for a period of time to allow the less volatile components to elute (Boehnke and Delumyeya, 2000).

Because HCs are flammable, flame-ionization detection is used to detect HCs emerging from the column. In a FID a very hot flame is produced by burning hydrogen in air. The high temperature causes the production of ions as an eluting compound is burned. The ions are collected at a charged electrode and the resulting current is measured with an electrometer amplifier. The burner jet is made the negative electrode, and a loop of inert metal surrounding the flame is made positive. The sensitivity of the FID to organic species is about proportional.

Quantitation of the gas chromatogram can be done manually using peak heights, but it is better to use a computerized data-handling system, preferably one that can be used to calculate results and change the attenuation after the chromatographic run.

Figure 8-3 The schematic diagram of FID (LibreText, 2016)
2. LEARNING OBJECTIVES

The objective of this experiment is to introduce one of the most important analysis methods used in environmental chemistry, flame-ionization detection gas chromatography (FID/GC). You will also learn how to extract trace amounts of hydrocarbons as well as the higher levels that would be found in soil samples.

3. METHODOLOGY

A. Sample Collection and Storage

Avoid using plastic devices for sample collection or storage. Plastics contain plasticizers that can interfere with the analysis. In fact, if sediment samples are collected from an urban stream, plasticizers will frequently be extracted with the HCs, and they often dominate the gas chromatogram. Since many bacteria metabolize HCs, samples should be extracted soon after collection. Otherwise, they must be stored in a refrigerator at 4°C. Collect 2 soils samples from two different locations where one of the sample must be roadside soils.

B. Sample Extraction and Preparation for Gas Chromatography Analysis

If the sample has a substantial HC level, a simple hexane/methanol extraction will suffice.
1. A dry soil sample with mass of about 10 g (weighed to 0.1 mg) is weighed into a tared 150 mL beaker.
2. Add 50 mL solution of pesticide grades hexane and methanol (4:1) to the beaker followed by 0.25 mL of the internal standard (will be provided by lab assistant) with a micropipette. A small stir bar is added and the beaker is put on a magnetic stir plate in a fume hood. A watch glass is placed on the beaker and the sample is stirred for about 0.5 hour.
3. After extraction, the sample is gravity filtered into a 150 mL beaker. The extraction beaker and funnel are rinsed with two 5 mL portions of hexane.
4. The combined filtrate and rinsings are dried with anhydrous sodium sulfate, using a magnetic stir bar to stir (if required). The sample is then filtered into a 150 mL beaker, rinsing the previous beaker and funnel with two 5 mL portions of hexane.
5. At this point, nitrogen gas could be used to evaporate excess solvent, or heating bath at 60 °C. However, it is convenient and much less expensive to evaporate the solvent in a fume hood for 6-12 hours.
6. The sides of the beaker are occasionally rinsed down with hexane, and the evaporation is continued until the final volume is 1 to 2 mL. Transfer the extract to a 1.5 mL vial.
7. Analyze the sample with GC-FID. You are required to do a literature search to obtain a suitable temperature program for the analysis. Discuss the obtained temperature program with your lecturer before analysis.

4. REPORT

1. Give full sampling details.
2. According to the peak of IS and the data for standard n-alkanes (will be provided), identify the n-alkanes in the samples.
3. Submit your chromatogram(s) and the report(s) generated by the data station. Describe the pattern of aliphatic peaks and whether or not there is a hump in the chromatogram.
4. Calculate the concentration of each n-alkane in the samples.
5. Use the report generated by the data station for the environmental sample to obtain the sum of the ppm of the alkanes of odd numbers of carbon atoms and do the same for the alkanes.
with an even number of carbon atoms. The carbon preference index (C.P.I.), is the ratio of odd to even hydrocarbons. Find this ratio for your sample. A large C.P.I indicates HCs of biogenic origin. Petrogenic HCs, on the other hand show little preference for either odd or even HCs and the C.P.I. is closer to unity.

6. Discuss the extent of HC contamination of the samples.

5. REFERENCE


