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VOLUME 7
WATER QUALITY ANALYSIS

DESIGN MANUAL

Table of Contents

1 INTRODUCTION

1.1 GENERAL

Water in its chemically pure form occurs rarely in nature. In fact, water is commonly found to carry a variety of constituents. When in its precipitate form it reaches the surface of the earth, it has already collected a number of substances and properties that characterise natural water. Gases have been absorbed or dissolved, dust particles have been picked up, and it has obtained a certain temperature. In case of a high radioactive washout or high acidity pickup, atmospheric water may not even be clean in the general sense and may not be suitable for some uses.

Atmospheric water is subject to further changes of quality both upon reaching the earth's surface and during its travel underground. The ability to dissolve salts is gained in the topsoil where carbon dioxide is released by bacterial action on organic matter. The soil water becomes charged with carbon dioxide resulting in formation of carbonic acid. Under the acidic conditions that develop many soil and rock constituents are dissolved.

Man's influence on the quality of water is quite apparent and is now a major concern. Mixing with municipal and industrial waste waters may result in drastic changes in the water quality of natural waters. Agriculturally oriented activities such as irrigation, use of fertiliser, pesticides, herbicides, etc., may lead to diffuse pollution of both surface waters and groundwater. Irrigation return waters also tend to increase total salts in the receiving water. Construction schemes, such as those connected with river training, flood control, low flow augmentation, etc., considerably influence the quality regime. Mining activities often cause substantial water quality changes.

Manual on Hydrological Information Systems consists of several volumes: Volume 7 deals with "Water Quality Analysis" and consists of two parts:

1. **Design Manual** in which basic principal procedures are put in context.
2. **Operation Manual,** dealing with operational procedures.

This part of volume 7 is the Design Manual. This manual pertains to both surface water and groundwater quality analysis. Although groundwater quality analysis does not include certain parameters such as chlorophyll-a and other microbiological parameters, some groundwater laboratories also conduct analysis of surface water samples. Therefore this manual includes all information which is relevant for analysis of water quality. It is set up as follows:

- Chapter 1 gives an overview of the water quality parameters
- Basic concepts from chemical and microbiological analyses, with which an analyst must be thoroughly familiar to be able to conduct water quality analyses intelligently, are discussed in Chapter 2 and 3, respectively. Principles of instrumental analysis are given in Chapter 4. Understanding these concepts will also assist the analyst in interpreting the data.
- Chapter 5 gives a list of water quality parameters for which a water sample may be analysed. These are categorised according to their significance and techniques of analysis.
- Chapter 6 lists major laboratory equipment required to conduct various analysis. A discussion of pertinent specifications is also given.
- Guidelines for laboratory design are given in Chapter 7.
- Aspects of quality assurance in water quality analysis are given in Chapter 8.

1.2 OVERVIEW OF WATER QUALITY PARAMETERS

Pathogenic micro-organisms

Pathogenic or disease producing micro-organisms mostly originate from domestic wastewater. These include micro-organisms, which cause diseases of the intestinal tract. Some of these diseases classified according to their causative agent are given below:

Viruses : infectious hepatitis, poliomyelitis
Bacteria: cholera, bacterial dysentery, typhoid and paratyphoid.
Protozoa: amoebic dysentery, giardiasis
Helminth: hookworm, guinea worm, schistosomiasis.

The intestinal discharges (faeces) of infected individuals contains billions of the pathogen, which, if allowed to mix with a water supply source, may result in an epidemic. These diseases are called *water borne* diseases since their spread is mainly through a water pathway (sick individual-sewage-water supply source-healthy person). In 1960 in Delhi, there were more than 6000 cases of infectious hepatitis in a few months because of contamination of a raw water source with municipal sewage.

The presence or absence of the coliform group of bacteria determines the bacteriological quality of water. *Escherichia coli* is the most widely known member of the group. It is a normal inhabitant of intestines of both healthy and sick persons and therefore it is present in large numbers in domestic and municipal sewage. Its presence in water indicates contamination with sewage and of the possible presence of pathogenic micro-organisms of human origin.

Although tests are available for specific pathogenic micro-organisms, there is no way of knowing which pathogenic organism may be present in a sample of water. The cost of testing for all pathogenic organism is prohibitive. Furthermore, in an aquatic environment the die-off rate of *E. coli* parallels that of pathogenic organisms. For these reasons, *E. coli* has come to be used as an indicator for pathogenic organisms. *E. coli* is exclusively of faecal origin. Some coliform bacteria are normal inhabitants of soil and water. Results of bacteriological analysis therefore may be reported as total coliforms and faecal coliforms. The presence of coliforms in a water sample is determined by observing their growth in special culture media and making a statistical deduction regarding their number. The result is reported as the most probable number (MPN) /100 ml.

Organic matter

Most waters contain organic matter. In the environmental field organic matter is classified as that which is biochemically decomposable and that which cannot be decomposed. Common parameters of characterisation are: biochemical oxygen demand (BOD), chemical oxygen demand (COD) and volatile solids. While the BOD test measures biochemically decomposable or degradable organic matter, the other two measure total organics. It may be pointed out that the three parameters reflect the aggregate organic constituents.

The BOD test measures oxygen equivalence of organic matter. A schematic equation for the biochemical decomposition reaction may be written as:

$$\text{Organic matter} + O_2 \xrightarrow{\text{Micro-organisms}} CO_2 + H_2$$

Micro-organisms, mainly bacteria, utilise various types of waste organic matter as their food to obtain energy. In this process they decompose the organic matter to carbon dioxide and water in presence of oxygen. The amount of oxygen consumed is taken as a measure of the quantity of organic matter. The test is carried out in a laboratory representation of the aquatic environment; a water sample is added to oxygenated water and the loss of oxygen measured over a period of time.

The BOD test is the most important parameter to assess the pollution of water bodies by organic matter and its effect on the oxygen resources of streams and lakes. The BOD reaction, being biochemical in nature, proceeds slowly. The rate of reaction depends on the temperature and the population of bacteria that may be present in the sample or added as seed. The rate of reaction also depends on the amount of organic matter in the sample or its BOD value.

Dissolved oxygen

The DO level in a surface water body is an important indicator of its 'health'. Presence of DO in water is necessary for maintaining favourable conditions for growth and reproduction of a normal population of fish and other aquatic life. The absence of a low level of DO in surface waters indicates pollution by organic matter. Under such conditions organic matter is decomposed by anaerobic bacteria resulting in production of obnoxious end-products, such as mercaptans, hydrogen sulphide, ammonia, etc.

Nutrients

Nutrients are chemicals containing elements, such as nitrogen (N), phosphorus (P), carbon (C), sulphur (S), calcium (Ca), potassium (K), iron (Fe), manganese (Mn), boron (B) and cobalt (Co), that are essential for the growth of living things. Some of these are required only in very small quantities and are called micro-nutrients. Plants require relatively large amounts of C, N, and P. They obtain C from carbon dioxide and N and P from soil or water. N and P may be limiting in the aquatic or land environment; that is the concentration of one or other of these species may dictate the biomass of plant species which can survive in a particular water body.

Municipal and some industrial wastewaters contain N and P. Addition of such wastes to water bodies may result in algal blooms or eutrophication (unnaturally accelerated growth of algae). With excess growth of algae the available light becomes limiting and the algal cells begin to die. This increases the decomposable organic matter load on the water body resulting in consumption of oxygen and deterioration of quality of water.

Total dissolved solids

Water as it travels in the atmosphere, through ground or over the land, dissolves a large variety of substances or salts. These substances in solution exist in their ionic form. The major cations (positively charged ions) comprise calcium (Ca^{++}), magnesium (Mg^{++}), sodium (Na^{+}) and potassium (K^{+}) and the associated anions typically include sulphate ($SO_4^{=}$), bicarbonate (HCO_3^{-}) and chloride (Cl^{-}). The divalent cations (those having two positive charges) are responsible for the hardness of water. Other ions which may be present in smaller concentrations but can nevertheless be of environmental significance are B, fluoride (F^{-}), Fe^{++}, Mn^{++}, and nitrate (NO_3^{-}).

The aggregate salts are measured as total dissolved solids (TDS). As a rough approximation waters having less than 1500 mg/L TDS can be considered fresh waters.

Toxic metals and organic compounds.

A number of toxic metals and organic compounds may be added to water through anthropogenic activity. Some metals which are toxic even in small concentrations are arsenic (As - not a metal), cadmium (Cd), copper (Cu), chromium (Cr), mercury (Hg), lead (Pb), nickel (Ni) and zinc (Zn).

Examples of organic compounds which are environmentally significant and important from a water quality viewpoint are polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), pesticides, etc. Since these substances are toxic, even in very small concentrations, special care has to be taken in the collection of samples for their analysis. Further, advanced level instruments are needed for their determination.

2 BASIC CONCEPTS FROM CHEMICAL ANALYSIS

2.1 GENERAL

Laboratory analysts are required to communicate the results of analyses accurately and without any ambiguity. For this purpose, a specified system of units and symbols should be used consistently. Learning basic calculations and concepts helps in appreciating the various steps involved in the analytical procedures and understand the need to follow these steps precisely.

2.1.1 UNITS OF MEASUREMENTS

To develop a uniform method of reporting, the International System of Units (SI) is commonly used in most countries. Table 2.1 gives some of the common units used in chemical calculations and environment monitoring.

Quantity	SI unit	SI symbol
Length	meter	m
Mass	Kilogram	kg
Time	second	s
Temperature	Celsius	°C
Area	square meter	m^2
Volume	cubic meter	m^3
Velocity	meter per second	m/s
Flow rate	cubic meter per second	m^3/s
Concentration (w/v)	kilogram per cubic meter	kg/m^3

Table 2.1: Common SI units and symbols

In the environmental field it is quite common to encounter both extremely large quantities and extremely small ones. To describe such extreme values a system of prefixes is used. Commonly used prefixes and their meaning are given Table 2.2

Prefix	Symbol	Meaning
micro	μ	10^{-6}
milli	m	10^{-3}
centi	c	10^{-2}
deci	d	10^{-1}
deca	da	10
hecta	h	10^{+2}
kilo	k	10^{+3}
mega	M	10^{+6}

Table 2.2: common prefixes used with unit symbols

Example 2.1

4 kg of common salt is thrown in a tank containing 800m^3 of water. What is the resulting concentration of salt in mg/l and μg/L? (1m^3 = 1000 l)

4 kg/800m^3 x 10^6 mg/1 kg x 1m^3/1,000 L = 5 mg/L

5 mg/L x 1000 μg/mg = 5000 μg/L

Units of a quantity can be converted by multiplying the quantity by an appropriate “factor-label”. In Example 2.1, to convert kg it is multiplied by a factor 10^6/1 having a label mg/kg. Note that the value of factor-label fraction is one and that the label is chosen in such a way that it cancels the unit to be converted and replaces it by the desired unit.

Concentrations of substances in liquids are expressed as a ratio: mass of the substance in a given volume of water. They can also be expressed as a ratio of the mass of the substance to a specified mass of mixture or solution, usually as parts per million (ppm by weight).

If 1 L of a solution weighs 1 kg, for 1 mg/L we can write 1 mg/L x 1 L / 1,000 g x 1 g/ 1,000 mg = 1 mg/ 10^6 mg = 1 ppm. Therefore mg/L and ppm can be used interchangeably as long as the density of the solution can be assumed to be 1,000 g/L.

2.1.2 SIGNIFICANT FIGURES

If individuals in a group are asked to measure a line exactly 6 cm and 4 mm long using a scale marked in cm graduations only, they may report the result as 6.3, 6.2, 6.5, 6.4, 6.6 cm, etc. To avoid ambiguity in reporting results or in presenting directions for a procedure, it is the custom to use significant figures only. In a significant figure all digits are expected to be known definitely, except the last digit, which may be in doubt. Thus in the above example there are only two significant figures (the figure before the decimal point is certain, after the decimal point the figure is based on an estimation between to graduations of the scale). If more than a single doubtful digit is carried, the extra digit or digits are not significant.

Round off by dropping digits that are not significant. If digits greater than 5 are dropped increase the preceding digit by one unit; if the digit is less than 5, do not alter preceding digit. If the digit 5 is dropped, round off the preceding digit to the nearest even number: thus 2.25 becomes 2.2 and 2.35 becomes 2.4.

The digit 0 may at times introduce ambiguity. If an analyst calculates total residue of 1146 mg/L, but realises that 4 is somewhat doubtful and therefore 6 has no significance, he may round off the result and report it as 1150 mg/L. Obviously he can not drop the digit 0, although it has no significance. The recipient of the result will not know if the digit 0 is significant or not.

Zeros bounded by other digits only on the right side only are never significant. Thus, a mass of 21.5 mg has three significant figures. Reported in g, the value will be 0.0215, which will again have 3 significant digits.

In most other cases, there will be no doubt as to the sense in which the digit 0 is used. It is obvious that the zeros are significant in such numbers as 104,5.000 and 40.08.

A certain amount of care is needed in determining the number of significant figures to carry in the result of an arithmetic operation. When numbers are added or subtracted, the number that has fewest decimal places, not necessarily the fewest significant figures, puts the limit on the number of places that justifiably may be carried in the sum or difference. The sum 0.0072 + 12.02 + 488 = 500.0272, must be rounded off to 500, because one of the numbers, 488, has no decimal places.

For multiplication or division, round off the result of the calculation to as few significant figures as are present in the factor with the fewest significant figures. For example, for the calculation (56x0.003462x43.22)/1.684, the result 4.975740998, may be rounded off to 5.0, because one of the components, 56, has only two significant figures.

2.2 ELEMENTS, COMPOUNDS AND MOLECULAR WEIGHTS

Table 2.3 lists some basic information regarding elements that an environmental chemist may encounter. Certain groupings of atoms act together as a unit in a large number of compounds. These are referred to as radicals and are given special names. The most common radicals are listed in Table 2.4. The information regarding the valence and ionic charge given in the tables can be used to write formulas of compounds by balancing +ive and -ive charges. For example, sodium chloride will be written as NaCl, but sodium sulphate will be Na_2SO_4.

Most inorganic compounds when dissolved in water ionise into their constituent ionic species. Na_2SO_4 when dissolved in water will dissociate in two positively charged sodium ions and one negatively charged sulphate ion. Note that the number of +ive and -ive charges balance and the water remains electrically neutral.

Example 2.2

Write the molecular formula for aluminium sulphate (alum) given that the aluminium ion is Al^{3+}, the sulphate ion is SO_4^{2-} and that each molecule has 18 molecules of water of crystallisation. Calculate its molecular weight. What is the percentage of sulphur in the compound?

As the total number of +ive and -ive charges must be the same within a molecule, the lowest number of Al^{+++} and SO_4^{2-} ions which can combine together is 2 and 3 respectively so that:

Number of +ive charges on $2Al^{3+}$ = 6

Number of -ive charges on $3SO_4^{2-}$ = 6

Therefore the formula is $Al_2(SO_4)_3.18H_2O$

The molecular weight is

$2Al^{3+}$	= 2 x 27 = 54
$3SO_4^2$	= 3 x 96 = 288
$18H_2O$	= 18 x 18 = 324
Total	= 666

Percent sulphur = (3 x 32/666) x 100 = 14.4

The gram molecular weight of a compound is the summation of atomic weights in grams of all atoms in the chemical formula. This quantity of substance is also called a mole (mol). Some reagent grade compounds have a fixed number of water molecules as water of crystallisation associated with their molecules. This should also be accounted for in the calculation of the molecular weight.

Name	Symbol	Atomic Weight	Common Valence	Equivalent Weight
Aluminium	Al	27.0	3+	9.0
Arsenic	As	74.9	3+	25.0
Barium	Ba	137.3	2+	68.7
Boron	B	10.8	3+	3.6
Bromine	Br	79.9	1 -	79.9
Cadmium	Cd	112.4	2+	56.2
Calcium	Ca	40.1	2+	20.0
Carbon	C	12.0	4-	
Chlorine	Cl	*35.5*	1-	35.5
Chromium	Cr	52.0	3+	17.3
			6+	
Copper	Cu	63.5	2+	31.8
Fluorine	F	19.0	1-	19.0
Hydrogen	H	1.0	1+	1.0
Iodine	I	126.9	1-	126.9
Iron	Fe	55.8	2+	27.9
			3+	
Lead	Pb	207.2	2+	103.6
Magnesium	Mg	24.3	2+	12.2
Manganese	Mn	54.9	2+	27.5
			4+	
			7+	
Mercury	Hg	200.6	2+	100.3
Nickel	Ni	58.7	2+	29.4
Nitrogen	N	14.0	3-	
			5+	
Oxygen	O	16.0	2-	8.0
Phosphorus	P	31.0	*5*+	6.0
Potassium	K	39.1	1+	39.1
Selenium	Se	79.0	6+	13.1
Silicon	Si	28.1	4+	6.5
Silver	Ag	107.9	1+	107.9
Sodium	Na	23.0	1+	23.0
Sulphur	S	32.1	2-	16.0
Zinc	Zn	65.4	2+	32.7

Table 2.3: Basic information on common elements

Name	Formula	Atomic Weight	Electrical Charge	Equivalent Weight
Ammonium	NH_4^+	18.0	1+	18.0
Hydroxyl	OH^-	17.0	1-	17.0
Bicarbonate	HCO_3^-	61.0	1-	61.0
Carbonate	CO_3^{2-}	60.0	2-	30.0
Orthophosphate	PO_4^{3-}	95.0	3-	31.7
Orthophosphate,mono-hydrogen	HPO_4^{2-}	96.0	2-	48.0
Orthophosphate, di-hydrogen	$H_2PO_4^-$	97.0	1-	97.0
Bisulphate	HSO_4^-	97.0	1-	97.0
Sulphate	SO_4^{2-}	96.0	2-	48.0
Bisulphite	HSO_3^-	81.0	1-	81.0
Sulphite	SO_3^-	80.0	2-	40.0
Nitrite	NO_2^-	46.0	1-	46.0
Nitrate	NO_3^-	62.0	1-	62.0
Hypochlorite	OCl^-	51.5	1 -	51.5

Table 2.4: Common radicals in water

2.3 VOLUMETRIC ANALYSIS

2.3.1 EQUIVALENT WEIGHTS AND CHEMICAL REACTIONS

Table 2.3 and Table 2.4 also give the valence and equivalent weights of the listed substances. Valence is determined as (1) the absolute value of ion charge, (2) the number of H^+ or OH^- a specie can react with, or (3) the absolute value of change in charge on a specie when undergoing a chemical reaction. The equivalent weight is determined by dividing the atomic or molecular weight by the valence. A major use of the concept of equivalents is that one equivalent of an ion or molecule is chemically equivalent to one equivalent of a different ion or molecule.

Example 2.3

Express 120 mg/L Ca^{2+} concentration as $CaCO_3$.

120 mg Ca^{2+}/L = 120 mg Ca^{2+}/L x 1 meq/20 mg Ca^{2+} x 50 mg $CaCO_3$/1 meq

= 300 mg $CaCO_3$/L

A balanced chemical equation is a statement of combining ratios that exist between reacting substances. Consider the reaction between NaOH and H_2SO_4:

$$2NaOH + H_2SO_4 = Na_2SO_4 + 2\ H_2O \qquad (2.1)$$

It is seen that 2 moles (80g) of NaOH react with 1 mole (98g) of H_2SO_4. In terms of equivalents, the number of equivalents of NaOH (80 {molecular weight} divided by 40 {equivalent weight} = 2) is the same as that of H_2SO_4 (98 {molecular weight} divided by 49 {equivalent weight} = 2). Stated differently, in a balanced chemical reaction the number of equivalents of combining reactants is the same. This concept is utilised in the determination of unknown quantities in titrimetric analyses described in the following section.

2.3.2 TITRATIONS

Titrimetric or volumetric methods of analysis make use of standard solutions, which are reagents of exactly known strength. It involves determining the exact volume of the standard required to react completely with the unknown substance contained in a known weight or volume of the sample. The standard of highest known purity and which is stable under conditions of storage, is called a primary standard. If it is unstable, it is necessary to determine the purity of the standard periodically. Such a standard is called a secondary standard.

The strength of standard solutions is defined in terms of either normality (N) or molarity (M). A 1.0*N* solution contains one equivalent weight of the substance in 1L of the solution. For a given reaction, if one is fixed the other is also known. A 0.05M H_2SO_4 will be 0.1*N* (2 equivalents/ mole), since one mole of sulphuric acid combines with two moles of hydroxyl ion, Equation (2.1).

Example 2.4

Calculate the number of meq of H_2SO_4 present in 35 mL of 0.1N standard solution.

Solution:

The strength of 0.1*N* solution = 0.1eq/L = 0.1meq/mL

Therefore number of meq present in 35 mL = 0.1meq/ml x 35 mL = 3.5 meq.

One of the requirements of titrimetric analyses is that it should be possible to know the exact volume of the standard consumed by the unknown substance in the sample. This is achieved by using an indicator in the reaction mixture. The indicator causes a visual change in the appearance of the mixture as soon as the reaction is complete.

Example 2.5

Calculate the concentration of alkali present in a sample when 50 mL aliquot of the sample consumed 12.4 mL of 0.1N standard H_2SO_4. Express your result in meq/L, mg NaOH/L, mg $CaCO_3$/L.

Solution:

Standard acid consumed = 0.1 meq/mL x 12.4 mL = 1.24 meq

Therefore, the concentration of alkali in the sample

= 1.24 meq/50 ml x 1000 mL/1 L

= 24.8 meq/L

= 24.8 meq/Lx 40 mg NaOH/meq

= 992 mg/L as NaOH

= 24.8 meq/L x 50 mg $CaCO_3$/meq

= 1240 mg/L as $CaCO_3$

2.4 CHEMICAL EQUILIBRIA

It is possible to write an equation for a theoretical chemical equilibrium as follows:

$$A + B \leftrightarrow C + D \tag{2.2}$$

This means that the reaction is reversible (indicated by $\leftrightarrow$) and that the species C and D are in equilibrium with the species A and B. It is possible to disturb this equilibrium by a number of means including increasing the concentration of one of the species involved in the reaction. When this is done, Le Chatelier's principle states that:

> *'A reaction, at equilibrium, will adjust itself in such a way as to relieve any force, or stress, that disturbs the equilibrium'*

This means, for example, that if the concentration of, say, D is increased, the reaction will tend to move to the left thus producing more of the species A and B.

This leads to the concept of the equilibrium constant (K) for a reaction which is defined as:

$$K = \frac{[C]}{[A]}\frac{[D]}{[B]} \tag{2.3}$$

where the [C] is the molar concentration of C, [D] is the molar concentration of D etc.

Where different numbers of molecules are involved, the reaction becomes:

$$aA + bB \leftrightarrow cC + dD \tag{2.4}$$

The equilibrium constant (K) for the reaction is then defined as:

$$K = \frac{[C]^c}{[A]^a}\frac{[D]^d}{[B]^b} \tag{2.5}$$

where a, b, c, d are the number of molecules of species A, B, C and D involved in the reaction.

The above equations give good results for salts, acids and bases when concentrations are low (as they mostly are in aquatic environment) but become progressively less accurate as the concentration of the species increases. This is due to the fact that the 'activity' of the ions (a concept thought to be associated with ion interactions) needs to be taken into account at higher ion concentrations. This concept will be discussed later in section 2.6.

2.4.1 IONISATION EQUILIBRIA

For an ionic compound (AB), which dissolves in water (or any solvent), a general equation can be written as follows:

$$AB \leftrightarrow A^+ + B^- \tag{2.6}$$

The equilibrium for this equation is written as:

$$K = \frac{[A^+]\,[B^-]}{[AB]} \tag{2.7}$$

where $[A^+]$ and $[B^-]$ represent molar concentrations of the ionic species in solution and [AB] represents the concentration of the unionised compound

Because 'K' also describes the ionisation of 'AB', it can be called the 'ionisation constant' of the species. This ionisation constant concept can also be applied to the ionisation of any molecule which dissociates into its constituent ions.

2.4.2 ION PRODUCT OF WATER AND pH

Under normal circumstances, water dissociates into its component ions, namely hydrogen (H^+) and hydroxide (OH^-) ions as follows:

$$H_2O \leftrightarrow H^+ + OH^- \tag{2.8}$$

And the ionisation constant is given by:

$$K_a = \frac{[H^+]\,[OH^-]}{[H_2O]} \tag{2.9}$$

where $K_a = 1.8 \times 10^{-16}$ mole/L at 25 ^oC

The concentration of the species '$[H_2O]$' in the above equation is largely unchanged after ionisation and = 55.5 moles/L. The above equation, therefore, can be written as:

$$[H^+][OH^-] = K_w = 10^{-14} \quad (\text{at } 25^OC) \tag{2.10}$$

where K_w = the ion product of water

To eliminate the very small powers of ten in the above equation it is useful to introduce the following terminology:

$p(x) = -\log_{10}(x)$

which means:

$p(10^{-14}) = -\log_{10}(10^{-14}) = 14$

This can be applied to the ionisation equation of water at 25 ^{o}C:

$-\log_{10}([H^+][OH^-]) = -\log_{10}(K_w) = -\log_{10}(10^{-14})$

$pH + pOH = pK_w = 14$ (2.11)

Note that the term pH has now been introduced which is defined as:

$pH = -\log_{10}[H^+]$ (2.12)

For a neutral solution, that is one where the concentration of $[H^+]$ ions is equal to the concentration of $[OH^-]$ ions:

$[H^+] = [OH^-]$

therefore: $[H^+][OH^-] = [H^+][H^+] = [H^+]^2 = 10^{-14}$

and $[H^+] = 10^{-7}$

or: pH = 7 (i. e: the pH of a neutral solution)

From the above, it can be seen that:

- the pH scale runs from 0 (acid) to 14 (alkali)
- that when pH is measured it is actually the negative logarithm of the hydrogen ion concentration that is being determined
- that an acidic solution (pH: 0 – 7) has a greater concentration of hydrogen ions than hydroxide ions
- that an alkaline solution has a greater concentration of hydroxide ions than hydrogen ions

Example 2.6

Calculate the pH value for the following concentrations of hydrogen and hydroxyl ions:

$[H^+]$ = a. 0.001, b. 0.00005 and

$[OH^-]$ = a. 10^{-8}, b. 0.00005, c. 0.00001

Solution:

$[H^+]$ a. 0.001 $pH = -\log 10^{-3} = 3$

b. 0.00005 $pH = -\log 0.00005 = -\log(5 \times 10^{-5}) = -0.7 + 5 = 4.3$

$[OH^-]$ a. 10^{-8} $[H^+] \times 10^{-8} = 10^{-14}$, or $[H^+] = 10^{-6}$

Therefore pH = 6

b. 0.00005 $-\log[H^+] - \log[OH^-] = 14$

Therefore pH = 14 – 4.3 = 9.7

c. 0.00001 $pH = 14 + \log 10^{-5} = 14 - 5 = 9$.

pH indicators

A number of naturally occurring or synthetic organic compounds undergo definite colour changes in well-defined pH ranges. A number of indicators that are useful for various pH ranges are listed in Table 2.5. The indicators assume different hues within the specified pH range. These are used as liquid solutions or some as pH papers. The change in colour occurs over a wide range of pH change

and therefore pH value cannot be measured accurately. Further, the turbidity and colour of the sample may cause interference.

Indicator	Acid colour	Base colour	pH range
Methyl orange	Red	Yellow orange	3.1 – 4.6
Methyl red	Red	Yellow	4.4 – 6.2
Litmus	Red	Blue	4.5 – 8.3
Thymol blue	Yellow	Blue	8.0 – 9.6
Phenolphthalein	Colourless	Pink	8.2 – 9.8
Alizarin yellow	Yellow	Lilac	10.1 – 11.1

Table 2.5: Various pH ranges

pH meter

The use of colour indicators for pH measurements has, to some extent, been superseded by development of glass electrode. pH meters employing glass-indicating electrodes and saturated calomel reference electrodes are now commonly used. Such meters are capable of measuring pH within $\pm$ 0.1 pH unit. The electrodes, connected to the pH meters are immersed in the sample and the meter measures the potential developed at the glass electrode due to hydrogen ion concentration in the sample and displays it directly in pH units. The pH meters are also equipped with a temperature-compensation adjustment.

The electrodes should be carefully handled and should not be scratched by butting against the sides of the beaker containing the sample. Follow the manufacturer's instructions for their care during storage and use. For short-term storage, the glass electrode may be left immersed in pH 4 buffer solution., Saturated KCl is preferred for the reference electrode. The glass electrode needs to be soaked in water for at least 12 hours before it is used for pH measurement.

Buffer solutions & instrument calibration

pH meters have to be calibrated against solutions of known pH values. Standard buffers are used for this purpose. Buffers are solutions of chemicals of known pH which do not change their pH value upon dilution and resist change of pH when small amounts of acid or alkali are added to them. Buffer solutions usually contain mixtures of weak acids and their salts (conjugate bases) or weak bases and their salts (conjugate acids). Construction of buffers is discussed in Section 2.5.2.

2.4.3 IONISATION OF ACIDS AND BASES

The classical definition of acids and bases is given as:

- An *acid* is a compound that yields a hydrogen ion (H^+) when it is added to water.
- A *base* is a compound that yields a hydroxide ion (OH^-) when it is added to water.

Strong acids and bases completely dissociate in water:

- Hydrochloric acid (strong acid): $HCl \rightarrow H^+ + Cl^-$
- Sodium Hydroxide (strong base): $NaOH \rightarrow Na^+ + OH^-$

Weak acids and bases only partially dissociate in water. A weak acid often has a paired or 'conjugate' base, and vice versa. For example, boric acid is a weak acid, and borate is its conjugate base.

- Boric acid (weak acid): $H_3BO_3 \leftrightarrow H^+ + H_2BO_3^-$
- Borate (weak base): $H_2BO_3^- + H_2O \leftrightarrow H_3BO_3 + OH^-$

The general equation for an acid can be written:

$HA \leftrightarrow H^+ + A^-$

$$K_a = \frac{[H^+]\,[A^-]}{[HA]} \tag{2.13}$$

The value of K_a is defined for each different acid , e.g. for Boric Acid (H_3BO_3):

$H_3BO_3 \leftrightarrow H^+ + H_2BO_3^-$

$$K_a = \frac{[H^+][A^-]}{[HA]} = \frac{[H^+][H_2BO_3^-]}{[H_3BO_3]} = 10^{-9.24}$$

Values for some common weak acids are given in Table 2.6

Acid	Equilibrium Equation	K_a	K_a	pK_a	Significance
Acetic	$CH_3COOH \leftrightarrow H^+ + CH_3COO^-$	1.8×10^{-5}	$10^{-4.74}$	4.74	Organic Wastes
Ammonium	$NH_4^+ \leftrightarrow H^+ + NH_3$	5.56×10^{-10}	$10^{-9.26}$	9.26	Nutrient, fish toxicity
Boric	$H_3BO_3 \leftrightarrow H^+ + H_2BO_3^-$	5.8×10^{-10}	$10^{-9.24}$	9.24	Nitrogen Analysis
Carbonic	$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ $HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$	4.3×10^{-7} 4.7×10^{-11}	$10^{-6.37}$ $10^{-10.33}$	6.37 10.33	Buffering, precipitation
Hydrocyanic	$HCN \leftrightarrow H^+ + CN^-$	4.8×10^{-10}	$10^{-9.32}$	9.32	Toxicity
Hydrogen Sulphide	$H_2S \leftrightarrow H^+ + HS^-$ $HS^- \leftrightarrow H^+ + S^{2-}$	9.1×10^{-8} 1.3×10^{-13}	$10^{-7.04}$ $10^{-12.89}$	7.04 12.89	Odours, corrosion
Hypochlorous	$HOCl \leftrightarrow H^+ + OCl^-$	2.9×10^{-8}	$10^{-7.54}$	7.54	Disinfection
Phenol	$C_6H_5OH \leftrightarrow H^+ + C_6H_5O^-$	1.2×10^{-10}	$10^{-9.92}$	9.92	Tastes, industrial waste
Phosphoric	$H_3PO_4 \leftrightarrow H^+ + H_2PO_4^-$ $H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$ $HPO_4^{2-} \leftrightarrow H^+ + PO_4^{3-}$	7.5×10^{-3} 6.2×10^{-8} 1.3×10^{-13}	$10^{-2.12}$ $10^{-7.21}$ $10^{-12.32}$	2.12 7.21 12.32	Analytical buffer Plant nutrient

Table 2.6: Ionisation constants for common weak acids (25°C)

Many weak acids have corresponding (conjugate) weak bases. The base dissociates to form OH^- when added to water. For these weak bases, an ionization constant K_b can also be defined. For Boric Acid, the corresponding weak base is Borate, which has the following reaction:

$H_2BO_3^- + H_2O \leftrightarrow H_3BO_3 + OH^-$

The ionization constant for a weak base (K_b) is defined:

$$K_b = \frac{[HA]\,[OH^-]}{[A^-]} = \frac{[H_3BO_3]\,[OH^-]}{[H_2BO_3]} = 10^{-4.76} \tag{2.14}$$

Water (H_2O) is not included in the constant.

Values for some common weak bases are given in Table 2.7

Acid	Equilibrium Equation	K_b	K_b	pK_b	Significance
Acetate	$CH_3COO^- + H_2O \leftrightarrow CH_3COOH + OH^-$	5.56×10^{-10}	$10^{-9.264}$	9.26	Organic Wastes
Ammonia	$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$	1.8×10^{-5}	$10^{-4.74}$	4.74	Nutrient, fish toxicity
Borate	$H_3BO_3 + H_2O \leftrightarrow H_3BO_3^- + OH^-$	1.72×10^{-5}	$10^{-4.76}$	4.76	Nitrogen Analysis
Carbonate	$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$ $HCO_3^- + H_2O \leftrightarrow H_2CO_3 + OH^-$	2.13×10^{-4} 2.33×10^{-8}	$10^{-3.67}$ $10^{-7.63}$	3.67 7.63	Buffering, precipitation
Calcium Hydroxide	$CaOH^+ \leftrightarrow Ca^{2+} + OH^-$	3.5×10^{-2}	$10^{-1.46}$	1.46	Softening
Magnesium Hydroxie	$MgOH^+ \leftrightarrow Mg^{2+} + OH^-$	2.6×10^{-3}	$10^{-2.59}$	2.59	Softening

Table 2.7: Ionization constants for some common weak bases (25°C)

It is often useful to know that, for a weak acid and its associated base:

$$pK_a + pK_b = 14$$

which is the same as:

$$K_aK_b = K_w = 10^{-14}$$

So for boric acid, for example:

$pK_a = 9.24$ (from Table 2.6)

And for borate:

$pK_b = 4.76$ (from Table 2.7)

Thus, it can be seen that:

$9.24 + 4.76 = 14$

Acid-base reactions are very important in water quality chemistry. For example, the toxicity of ammonia to fish is affected by the reaction below, which is itself affected by pH of the water:

$$NH_3 + H^+ \leftrightarrow NH_4^+$$

At high pH (alkaline conditions), the reaction tends to produce more ammonia species (NH_3) which is toxic to fish, whereas at low pH (acid conditions) the ammonium species (the relatively non-toxic, NH^+_4) predominates.

Example 2.7

A eutrophic lake water sample contains 2.8 mg NH_3-N ($NH_3 + NH_4^+$)/L. High pH values are likely to occur due to photosynthetic activity. Determine the concentration of NH_3 specie at pH 7 and 9.5. Water quality standards for fish culture specify that NH_3 concentration should not exceed 1.5 mg/L.

Solution:

2.8 mg NH_3 - N/L = 2.8 mg NH_3 - N/L x 1g/10^3 mg x 1 mole/14 g NH_3 - N= 0.2x10^{-3} mole/L

From Table 2.6:

$[H^+][NH_3]/[NH_4^+] = 10^{-9.26}$

At pH 7:

$10^{-7} \times [NH_3]/[NH_4^+] = 10^{-9.26}$ or: $[NH_3]/[NH_4^+] = 10^{-2.26}$

and $[NH_3] + [NH_4^+] = 0.2 \times 10^{-3}$ or: $[NH_3] = 10^{-6}$ mole/L = 0.014 mg/L

At pH 9.5:

$10^{-9.5} \times [NH_3]/[NH_4^+] = 10^{-9.26}$ or: $[NH_3]/[NH_4^+] = 10^{0.24}$

and $[NH_3] + [NH_4^+] = 0.2 \times 10^{-3}$ or:$[NH_3] = 0.13 \times 10^{-3}$ mole/L = 1.8 mg/L

The maximum limit is likely to exceed at pH 9.5.

2.5 BUFFERING

A buffer solution is one which offers resistance to changes in pH when small amount of alkali or acid is added to it. Normally, buffer solutions are made up of weak acids and their salts or weak bases and their salts. In the laboratory they are used for calibrating pH meters.

2.5.1 ALKALINITY OF NATURAL WATER

Natural waters also normally possess a buffering capacity; that is they have the ability, due to the salts, acids and alkalis that they naturally contain, to resist pH changes. The capacity of a water to neutralise acid is called alkalinity.

Rain water, as it travels through the atmosphere, dissolves carbon dioxide to yield poorly ionised carbonic acid:

$H_2O + CO_2 \leftrightarrow H_2CO_3$

Carbon dioxide may also be dissolved as the water percolates through the soil, where carbon dioxide is available as the end product of microbial degradation of organic matter.

Carbonic acid is a diprotic acid. It contains two ionisable hydrogen ions. It reacts with soil and rock minerals to form soluble bicarbonates:

$H_2CO_3 + CaCO_3 = Ca^{++} + 2HCO_3^-$

Carbonic acid, bicarbonates and carbonates exist in equilibrium with each other according to the following ionisation and equilibrium relations:

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-, \frac{[H^+]\,[HCO_3^-]}{[H_2CO_3]} = K_1 = 10^{-6.37} \quad (2.15)$$

and

$$HCO_3^- \leftrightarrow H^+ + CO_3^-, \frac{[H^+]\,[HCO_3^{--}]}{[HCO_3]} = K_2 = 10^{-10.3} \quad (2.16)$$

The hydrogen ion concentration, which appears in both the equilibrium equations, controls the relative cocentrations of the three carbonate species. Figure 2.1 shows the distribution of the three species as a function of pH according to Equations 2.15 and 2.16.

Note that:

- at pH 11.5 and higher carbonate specie predominates
- at pH 10.3 concentrations of carbonate and bicarbonate specie are equal
- at pH 8.3, phenolpthalein end point, bicarbonate specie predominates, which reduces with decreasing pH
- at pH 4.5, bromcresol green or the alkalinity titration end point, carbonic acid or carbon dioxide species predominate

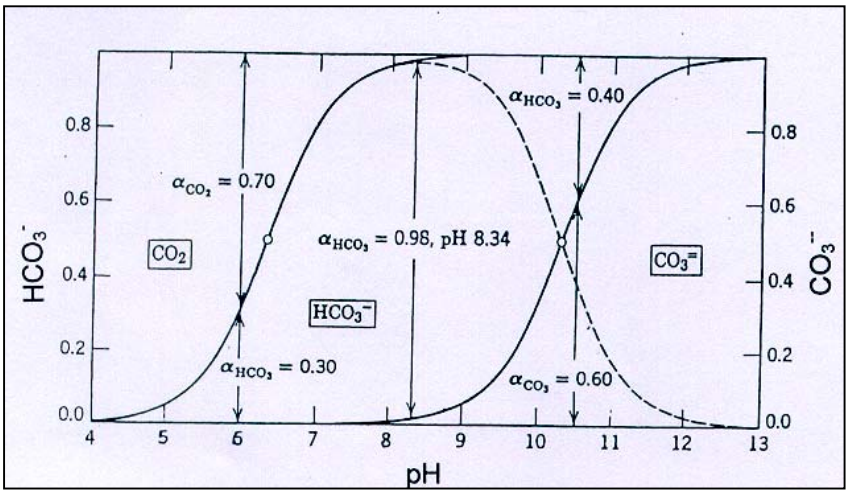


Figure 2.1: Carbonate species as a function of pH

If acid is added to a water at neutral or near neutral pH, the hydrogen ions (H^+) will be taken up by the bicarbonate ions (HCO^-_3) to produce more neutral carbonic acid (H_2CO_3). Assuming that the bicarbonate is not totally consumed, the addition of acid will cause only a slight increase in the hydrogen ion concentration of the water. Therefore the pH, which is a measure of hydrogen ion concentration, will also not change significantly. It is worth noting, however, that once nearly all the bicarbonate is consumed, and the buffering capacity is nullified, a relatively small addition of acid may cause a large pH change.

The above effect occurs when acid rain (rain made acidic by passing through atmospheric pollution such as sulphur dioxide) is continually deposited in a lake containing bicarbonate species. At first when this occurs, the lake water pH is unaltered due to the natural buffering capacity of the dissolved bicarbonate. However, once this buffering capacity is consumed, the lake rapidly becomes acidic and is unable to support certain forms of life.

Similarly, addition of an alkali will shift the equilibrium towards carbonate specie and the pH will increase only slightly. Only when nearly all the bicarbonate is consumed there will be a significant incease in the pH value.

Alkalinity Relationships

The alkalinity of a water is a measure of its capacity to neutralize acid. Bicarbonates represent the major form of alkalinity in natural waters. Other salts of weak acids, such as borates, silicates and phosphates, which may be present in small amounts, would also contribute to alkalinity. Salts of organic acids, for example, humic acid, which is quite resistant to biological oxidation, would also add to alkalinity.

For all practical purposes, the alkalinity in natural waters may be classified as, hydroxide, carbonate and bicarbonate alkalinity.

Hydroxide alkalinity: Water having pH well above 10 would have significant amount of hydroxide alkalinity. At a lower pH value, say the phenolpthalein end point, the hydroxide alkalinity may be taken to be insignifcant.

Carbonate alkalinity: Water having pH higher than 8.5 may have carbonate alkalinity. When the pH is lowered to 8.3 exactly one-half of the carbonate alkalinity is neutralised.

Bicarbonate alkalinity: Water having pH less than 8.3 would have only bicarbonate alkalinity.

The above concepts, in relation to titration of water samples with an acid to phenolphthalein and bromcresol green end points are illustrated in Figure 2.2. The distribution of various forms of alkalinity in samples may be calculated as described in Table 2.8.

Result of titration	Hydroxide alkalinity	Carbonate alkalinity	Bicarbonate alkalinity
P = 0	0	0	T
P < 1/2 T	0	2P	T - 2P
P = 1/2 T	0	2P	0
P > 1/2 T	2P - T	2(T - P)	0
P = T	T	0	0

*Key: P-phenolphthalein alkalinity, T-total alkalinity

*Table 2.8: Alkalinity Relationships**

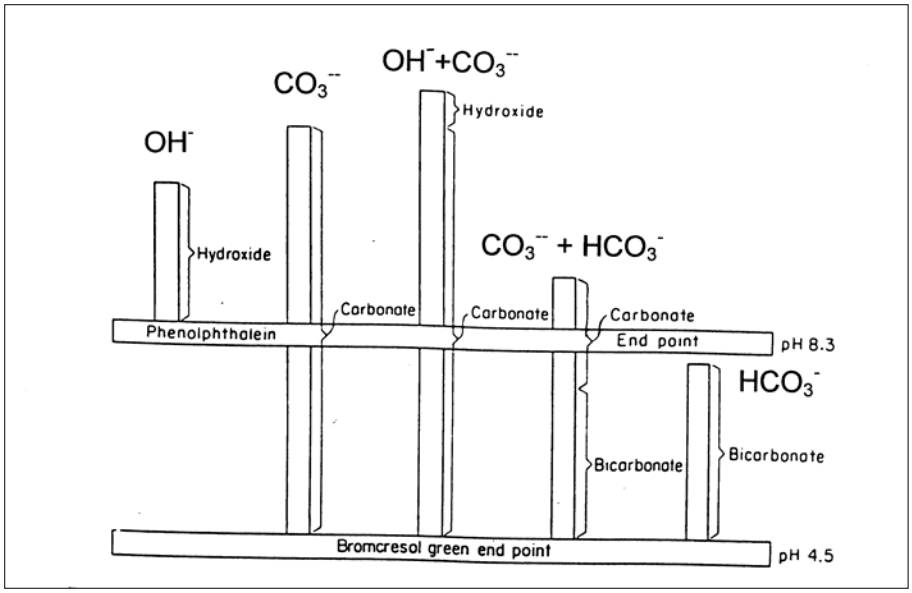


Figure 2.2: Titration of samples containing various forms of alkalinity

Example 2.8

Calculate the hydroxide alkalinity of water at pH 11 and 9. Express the result in terms of mg $CaCO_3$/L

Solution:

At pH 11: $[OH^-] = 10^{-3}$ mole/L x 1eq/1 mole x 50g $CaCO_3$/1eq x 10^3mg/1g = 50 mg $CaCO_3$/L

At pH 9: $[OH^-] = 10^{-5}$ mole/L x 1eq/1 mole x 50g $CaCO_3$/1eq x 10^3mg/1g = 0.5 mg $CaCO_3$/L

Example 2.9

The phenolphthalein and total alkalinity of a water sample is 40 and 120 mg as $CaCO_3$/L, respectively. Calculate the different forms of alkalinity in the sample.

Solution:

Since P < 1/2 T, from Table 3 the different forms of alkalinity are: hydroxide = 0, carbonate = 80 mg as $CaCO_3$/L and bicarbonate = 40 mg as $CaCO_3$/L

2.5.2 CONSTRUCTION OF BUFFERS

The following relation is obtained by taking logarithm of the equilibrium expression for ionisation of a weak acid (Equation 2.13) and rearranging:

$$pH = pK + \log\{[A^-]/[HA]\} \quad (2.17)$$

If the pK value is known (for example, Table 2.6), a buffer of predetermined pH value can be constructed by suitably proportioning the molar concentrations of the acid and its conjugate base.

Example 2.10

Calculate the quantities of acetic acid (AH) and sodium acetate (A^-) required to construct 500 mL of a 0.05 molar acetate buffer of pH 5. The pK value for acetic acid is 4.74.

Solution:

From Equation 2.17 $pH = pK + \log\{[A^-]/[HA]\}$

Substituting in the above equation one obtains:

$5 = 4.74 + \log\{[A^-]/[HA]\}$ or $\log\{[A^-]/[HA]\} = 0.26$ or $[A^-]/[HA] = 1.82$

Since molarity is 0.05: $[HA] + [A^-] = 0.05$

Therefore $[HA] + 1.82[HA] = 0.05$

or $[HA] = 0.0177$ and $[A^-] = 0.0323$ moles/L

For 500mL buffer: HA = 0.0088 and A^- = 0.0161 moles

Note that:

- In constructing a buffer of a given pH, select an acid or a base whose pK value is close to the pH value of the buffer, preferably within 1 pH unit range. The difference between pK and pH should never be more than 1.5 units. This will ensure that the pH variation is minimal when an acid or alkali is neutralised.
- It is also important to ensure that the selected compounds for constructing the buffer do not enter into any side reaction with the system, which is to be buffered.
- The molarity of buffer indicates the strength or the neutralising capacity of the buffer, i.e., the quantity of acid or alkali that it can neutralise without resulting in significant change in pH.

Table 2.9 gives the composition of some commonly used buffers. Buffers of different pH values are also available commercially.

S. No.	Buffer solution	pH at 25°C	Amount of salt to be dissolved in 1000 ml freshly boiled and cooled distilled water
1	0.05 M potassium hydrogen phthalate	4.00	10.12 g $KHC_8H_4O_4$
2	0.025 M potassium dihydrogen phosphate + 0.025 M disodium hydrogen phosphate	6.86	3.387 g KH_2PO_4 and 3.533 g Na_2HPO_4
3	0.01 M sodium borate decahydrate	9.18	3.80 g $Na_2B_4O_7.10\ H_2O$

Table 2.9: Buffer solutions of known pH

2.6 ACTIVITY COEFFICIENTS AND IONIC STRENGTH

As solutions of ionised materials become more concentrated, their quantitative effect in equilibrium relationships becomes progressively less. Thus, the effective concentration, or activity, of ions is decreased below that of the actual molar concentration. The activity of an ion or molecule can be found by multiplying its molar concentration by an activity coefficient, γ.

The activity coefficient is related to the ionic strength, μ, which is defined as

$$\mu = \tfrac{1}{2} \Sigma\, C_i Z_i^2 \qquad (2.18)$$

where C_i is the molar concentration of the ith ion and Z_i its charge and the summation extends over all the ions in solution.

The ionic strength can also be approximated by multiplying TDS in mg/L by 2.5×10^{-5}.

The activity coefficient is calculated by

$$\log \gamma = -0.5Z^2(\sqrt{\mu}/(1 + \sqrt{\mu})) \quad (2.19)$$

where Z is the charge on the ion for which the activity coefficient is being determined.

Example 2.11

Calculate the activity coefficients and activities of each ion in a solution containing 0.01 M $MgCl_2$ and 0.02 M Na_2SO_4.

Solution:

Set up a calculation table:

Ion	C	Z	CZ^2
Mg^{2+}	0.01	+2	0.04
Na^+	0.04	+1	0.04
Cl^-	0.02	-1	0.02
SO_4^{2-}	0.02	-2	0.08
		Σ	0.18

Calculate ionic strength from Equation 2.18:

$$\mu = \tfrac{1}{2} \Sigma C_i Z_i^2 = 1/2 \times 0.18 = 0.09$$

Calculate activity coefficients and activity for various ions from Equation 2.19:

$$\log \gamma = -0.5Z^2(\sqrt{\mu}/(1 + \sqrt{\mu})) = 0.115\ Z^2$$

Ion	γ	γC
Mg^{2+}	0.35	0.0035
Na^+	0.77	0.031
Cl^-	0.35	0.015
SO_4^{2-}	0.77	0.007

2.7 SOLUBILITY EQUILIBRIA

A knowledge of basic equilibrium chemistry is useful to the environmental chemist dealing with water pollution problems. This is because equilibrium chemistry:

- is an aid to the understanding of the fate of pollutants discharged into water
- enables quantitative determinations to be made of the relationships between various species present in polluted and natural waters
- helps to evaluate how best to treat contaminated waters

However, it is important to understand that the concepts detailed below are, in most cases, simplifications of what goes in the dynamic and multi-species systems, which make up natural waters. Further limitations are imposed by:

- the fact that unlike laboratory experiments, natural systems are subject to outside influences (e.g., sunlight)

- the fact that no account is taken of the kinetics (speed) of the reactions considered some of which may be very slow (such as some oxidation-reduction reactions) whilst some are very fast (certain acid-base reactions)
- the lack of accurate equilibrium constants for reactions in natural waters

Even given the above limitations, however, the concepts of equilibrium chemistry can indicate whether certain reactions are likely, the direction in which these reactions are likely to proceed and the relative position of the equilibrium with respect to the reactants and products.

2.7.1 SOLUBILITY PRODUCT

For an ionic solid AB, which dissolves in water (or any solvent) a general equation can be written as follows:

$$\underline{AB} \leftrightarrow A^+ + B^- \qquad (2.20)$$

The underscore represents solid or precipitated material.

The equilibrium for this equation can be treated as though the equilibrium were homogeneous in nature. It can be written as:

$$K = \frac{[A^+][B^-]}{[\underline{AB}]} \qquad (2.21)$$

where [ ] represents molar concentrations of the species.

However, the concentration of the solid can be regarded as constant in equilibrium calculations, since the rate of dissolution of the solid at equilibrium condition is equal to the rate of deposition of the ions.

$$K = \frac{[A^+]\,[B^-]}{[\underline{K_1}]}$$

Therefore:

where K_1 is a constant

or, replacing two constants with a new constant K_{sp}:

$$[A^+]\,[B^-] = K_{sp} \qquad (2.22)$$

The constant K_{sp} is called the solubility-product constant (sometimes simply the solubility constant) and is a measure of the extent of dissolution of a solid when it is dissolved. Tables of solubility product constants are available in the chemical literature and, for solutes which produce the same number of ions upon dissolution, can be compared to ascertain relative solubilities in moles/L.

Examples of solubility product constants are:

$AgCl \leftrightarrow Ag^+ + Cl^-$	K_{sp} (at 25°C) = 3 X 10^{-10}
$BaSO_4 \leftrightarrow Ba^{2+} + SO_4^{2-}$	K_{sp} (at 25°C) = 1 X 10^{-10}
$Ca\ CO_3 \leftrightarrow Ca^{2+} + CO_3^{2-}$	K_{sp} (at 25°C) = 5 X 10^{-9}
$MgCO_3 \leftrightarrow Mg^{2+} + CO_3^{2-}$	K_{sp} (at 25°C) = 4 X 10^{-5}
$Mg(OH)_2 \leftrightarrow Mg^{2+} + 2OH^-$	K_{sp} (at 25°C) = 9 X 10^{-12}

From the above solubility product constants it can be seen that magnesium carbonate ($MgCO_3$) is the most soluble followed by calcium carbonate ($CaCO_3$), silver chloride (AgCl) and finally barium sulphate ($BaSO_4$) being the least soluble.

Example 2.12

Calculate the solubility of $CaCO_3$ in mg/L in pure water.

Solution:

Assume that the solubility of $CaCO_3$ is p moles/L. Therefore the concentrations of both calcium and carbonate ions would be p moles/L each. Writing the equilibrium expression one obtains:

$p^2 = 5 \times 10^{-9}$ or $p = 7 \times 10^{-5}$ moles/L

or Solubility = 7×10^{-5} moles/L X 100g/mole X 1000 mg/g = 7 mg/L

Note that the solubility of a compound would be different in case the water contains any of the ions released by dissolution of the compound or if one of the released ions enters into a reaction with other constituents in the water. In the above example, if the water contains carbonate ion from any other source, say presence of sodium carbonate, the solubility of calcium carbonate would be lesser or if the carbonate ion obtained from dissolution of calcium carbonate is converted to bicarbonate, the solubility would be higher.

For environmental chemists the solubility product constant is a useful aid to understanding the fate of chemical species discharged to water bodies and, in particular, whether they are likely to be present as dissolved substances or solid particles.

2.7.2 LOGARITHMIC CONCENTRATION DIAGRAMS

Logarithmic concentration diagrams are useful in understanding solubility equilibrium of compounds when the water contains ions, which affect its solubility. Consider the case of solubility of $Mg(OH)_2$. The solubility is a function of hydroxyl ion concentration in water or its pH.

According to solubility equilibrium expression:

$$[Mg^{++}] [OH^-]^2 = 9 \times 10^{-12} \quad (2.23)$$

Substituting $10^{-14}/[H^+]$ for $[OH^-]$ and taking logarithms, the following expression is obtained:

$$\log[Mg^{++}] = 16.95 - 2pH \quad (2.24)$$

This is an equation to a straight line when $\log[Mg^{++}]$ is plotted against pH value. By substituting different values for pH in Equation 2.24 values of $\log[Mg^{++}]$ can be obtained:

pH	$\log[Mg^{++}]$
6	+4.95
8	+0.95
10	-3.05

Figure 2.3 shows a plot of the above relation. Note that as the pH increases from 6 to 10 the solubility decreases from $10^{4.95}$ moles/L to $10^{-3.05}$ moles/L. Further, it can be concluded that waters having pH and $\log[Mg^{++}]$ values plotting to the right of the line are supersaturated with Mg^{++} and precipitation will take place. Similarly, the waters plotting to the left of the line are undersaturated.

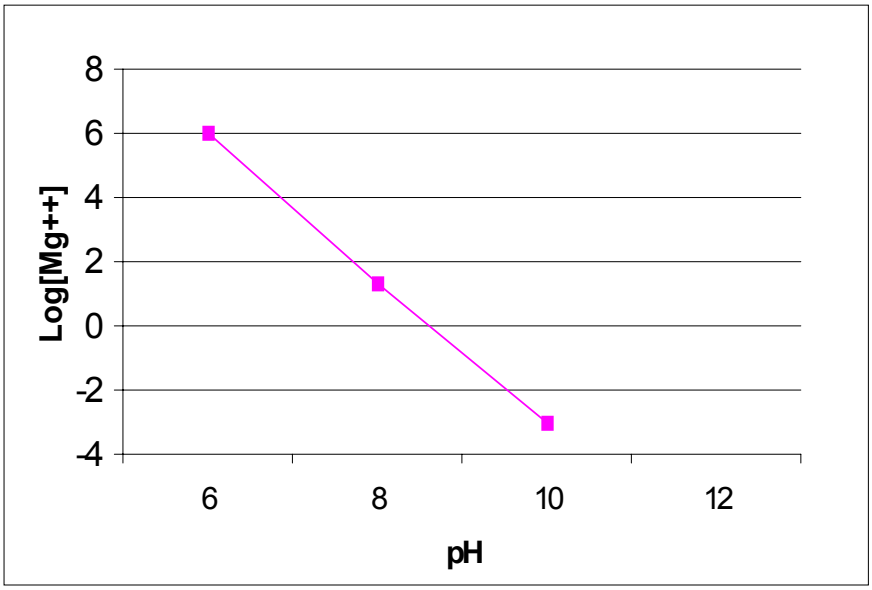


Figure 2.3: Logarithmic concentration vs. pH diagram

2.7.3 COMPLEX FORMATION

The total solubility of a compound increases if one of the ions start forming complexes with another specie present in the water or with the hydroxide or hydrogen ion contributed by the solvent water. The case of $Zn(OH)_2$ will be used as an illustration, which undergoes the following reactions:

$\underline{Zn(OH)_2} \leftrightarrow Zn^{++} + 2OH^-$	$K_{sp} = 8 \text{ X } 10^{-18}$
$Zn^{++} + OH^- \leftrightarrow ZnOH^+$	$K_1 = 1.4 \text{ X } 10^4$
$ZnOH^+ + OH^- \leftrightarrow Zn(OH)_2$	$K_2 = 1 \text{ X } 10^6$
$Zn(OH)_2 + OH^- \leftrightarrow Zn(OH)_3^-$	$K_3 = 1.3 \text{ X } 10^4$
$Zn(OH)_3^- + OH^- \leftrightarrow Zn(OH)_4^{--}$	$K_4 = 1.9 \text{ X } 10^1$

It is seen that as the solid zinc hydroxide goes into solution, the Zn ion is consumed to make 4 different hydroxide complexes in step-wise reactions. The dissolution continues till each of the 4 complexing reactions and the solubility product reaction attains equilibrium.

These equations can be written in the form of logarithmic concentration and pH relations as described in the earlier section. Then each species of Zn can be plotted as shown in Figure 2.4. The total solubility,C_T, at any pH can be obtained by summing the ordinates of each plot at that pH value.

Note that the solubility of total Zn first decreases with increase in pH and then it again increases. The minimum solubility occurs at pH 9.4 and is equal to $10^{-6.8}$ moles/L.

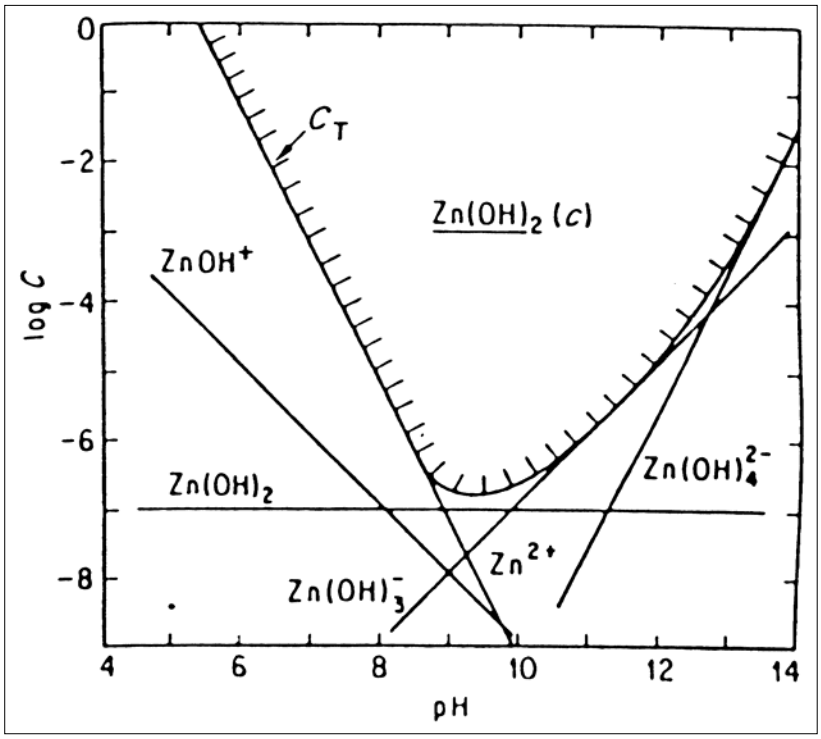


Figure2.4
Solubility of zinc

2.7.4 PARTITION EQUILIBRIUM

One of the aspects of equilibrium chemistry, which is particularly important when dealing with water pollution, is that of the partition of contaminants between solid and liquid phases of the water body. In this way, it is often possible to be more definite about the fate of pollutants when they are discharged to the aquatic environment.

For organic chemicals, a useful way of estimating the relative amounts of a particular compound which may be present in the liquid and solid phases of surface water is provided by the so-called octanol-water partition coefficient which is defined as follows:

$$K_{ow} = \frac{\text{Conc. in octanol } (\mu g/l)}{\text{Conc. in water } (\mu g/l)}$$

This coefficient, for which tables are available in the literature, can be used in concert with the solubility of the chemical in water to predict how the chemical is likely to be partitioned between the water and solid phases when discharged to surface waters. Thus, the higher the value of $K_o w$ is the greater the proportion of the chemical in the solid phase is likely to be.

Attempts have also been made to determine the partitioning of other chemicals, particularly metals, either theoretically or empirically. Such partition coefficients, whilst helpful at times, normally relate to quite strictly-defined conditions and are not, therefore, generally useful. Reference should be made to the surface water quality literature for further information in this regard.

2.8 REDOX EQUILIBRIA

Chemical reactions can be divided into two groups: (a) reactions in which no change of oxidation state occurs, such as precipitation of calcium carbonate when sodium carbonate is added to water containing calcium ions, neutralisation of carbonate with acid or ionisation of acetic acid in water and (b) reactions in which changes in oxidation state of the reactants occurs. The basic theory of redox reactions are discussed in this section.

2.8.1 GALVANIC CELLS

Redox reactions may be the result of direct transfer of electrons from donor to the acceptor. Recall that the loss of electrons from the donor results in its oxidation and the gain of electrons by the acceptor results in its reduction.

Thus, if metallic zinc is immersed in a copper sulphate solution the following reactions occur:

Oxidation : $Zn(s) \leftrightharpoons Zn^{2+} + 2e$ (2.25)

Reduction: $Cu^{2+} + 2e \leftrightharpoons Cu(s)$ (2.26)

Overall $Zn(s) + Cu^{2+} \leftrightharpoons Zn^{2+} + Cu(s)$ (2.27)

The individual oxidation and reduction reactions are called half-reactions, copper is deposited directly on solid zinc surface in the overall reaction.

The flow of electrons from Zn(s) to Cu^{2+} can be monitored if the two half-reactions are physically isolated from each other. Such an arrangement is shown in Figure 2.5 and is called galvanic or electrochemical cell. The anode of the cell is the electrode at which oxidation occurs (Zn), while cathode is the electrode at which reduction takes place (Cu). The cell can be considered to comprise two half-cells.

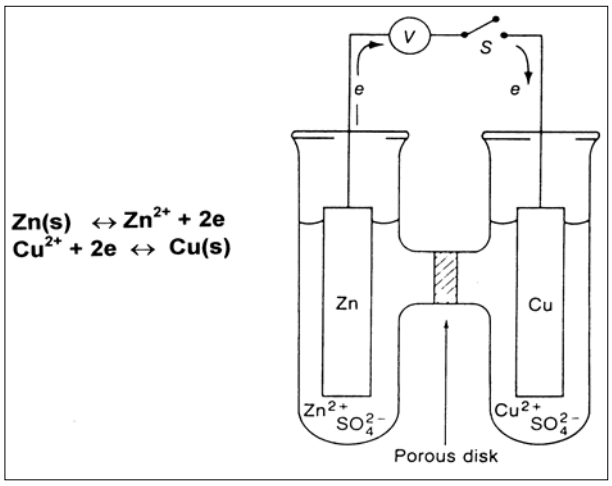


Figure 2.5: Galvanic Cell

Standard electrode potentials

It is obvious from Figure 2.5, that the potential of an electrode can be measured only against another half cell. An electrode potential is defined as the potential of a cell consisting of the electrode in question acting as cathode and a standard hydrogen electrode acting as anode.

Figure 2.6 illustrates a reference hydrogen electrode and a copper electrode for measurement of the electrode potential for the half-reaction given in Equation (2.26). Here instead of a porous disc, as in Figure 2.5, a salt bridge (a tube containing gelatin and saturated KCl) is provided to allow migration of negatively charged $SO^{=}_4$ from copper half cell and positively charged H^+ from hydrogen half cell to maintain electrical neutrality.

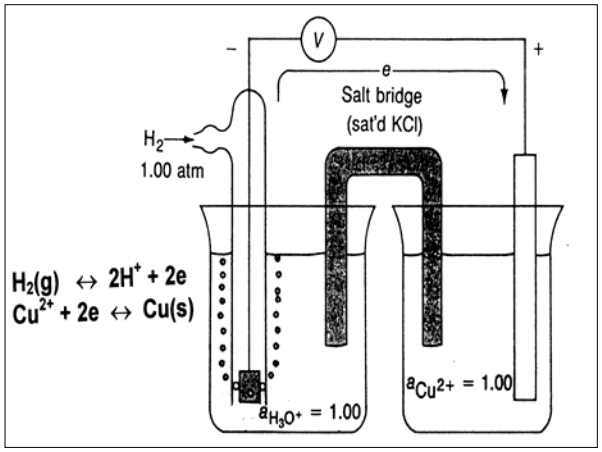


Figure 2.6:
Standard electrode potential definition

The half reaction at the reference electrode is :

$$H_2(g) \rightleftharpoons 2H^+ + 2e \qquad (2.28)$$

Note that:

- All species in the standard half-cells are at standard state (P = 1 atm, activities = 1).
- The reference hydrogen half-cell is assigned a potential of 0.0V.
- Copper electrode bears a positive charge and it functions as cathode while the hydrogen electrode functions as anode.
- The cell develops a potential of 0.334V and by definition is assigned a positive sign. The standard electrode potential is, therefore, +0.334V.

Replacement of Cu-Cu^{2+} half-cell with Zn electrode immersed in a solution with a Zn^{2+} activity of one results in a potential of 0.763V. However, in this case the Zn electrode acts as anode. The half-reactions become:

$$Zn(s) \rightleftharpoons Zn^{2+} + 2e \qquad (2.29)$$

$$2H^+ + 2e \rightleftharpoons H_2(g) \qquad (2.30)$$

Since the hydrogen electrode acts as cathode, the electrode potential is assigned a negative sign and is equal to -0.763V.

Table 2.10 lists a few standard electrode potentials for the purpose of illustration. A more complete list may be obtained from chemistry handbooks.

Half-reactions for standard electrode potential are always written as reductions. A negative sign of the numerical value of the potential signifies that the half-reaction when coupled with the hydrogen reference electrode would be an oxidation reaction.

Half-reactions	V
$O_2(g) + 4H^+ + 4e \leftrightharpoons 2H_2O$	+ 1.229
$Fe^{3+} + e \leftrightharpoons Fe^{2+}$	+ 0.771
$Cu^{2+} + 2e \leftrightharpoons Cu(s)$	+ 0.337
$SO_4^{2-} + 10H^+ + 8e \leftrightharpoons H_2S + 4H_2O$	+ 0.3
$2H^+ + 2e \leftrightharpoons H_2(g)$	0.000
$Cd^{2+} + 2e \leftrightharpoons Cd(s)$	- 0.403
$Zn^{2+} + 2e \leftrightharpoons Zn(s)$	- 0.763

Table 2.10 Standard electrode potentials, E^o_h at 25^0C

Entries in the table are according to the numerical values of the E^0_h. Among the listed reactions oxygen is the most effective electron acceptor.

The Nernst equation

For the generalised reversible half-reaction

$$aA + bB + \ldots + ne \leftrightharpoons cC + dD + .. \qquad (2.31)$$

where capital letters represents formulas for species, e is electron and a,b,c,d and n are number of moles, the potential E_h is given by

$$E_h = E^o_h - \frac{RT}{nF} \ln \frac{[C]^c\,[D]^d \ldots}{[A]^a\,[B]^b \ldots} \qquad (2.32)$$

where

E^o_h = standard electrode potential, V
R = gas constant, 8.316 $J.K^{-1}.mol^{-1}$
T = temperature, 298K (25^oC)
F = the faraday, 96487 coulombs
ln = the base for natural logarithms = 2.303 $\log_{10}$

Substitution of numerical values gives:

$$E_h = E^o_h - \frac{0.591}{n} \log \frac{[C]^c\,[D]^d \ldots}{[A]^a\,[B]^b \ldots} \qquad (2.33)$$

(The subscripts h indicate that the potential measurements are against hydrogen reference electrode).

Example 2.13

Generate Nernst expression for the half-reaction $Zn^{2+} + 2e \leftrightharpoons Zn(s)$

Solution:

The Nernst expression is:

$$E_h = E^o_h - \frac{0.591}{2} \log \frac{1}{[Zn^{2+}]}$$

The activity of elemental Zn(s) is unity. The electrode potential varies with the logarithm of reciprocal of molar Zn^{2+} concentration, assuming that the activity = molar concentration.

Cell potential

The potential of an elctrochemical cell is obtained by combining the electrode potentials of the two half cell processes.

$$E_{cell} = E_{cathode} - E_{anode} \quad (2.34)$$

Example 2.14

Calculate the cell potential for the cell shown in Figure 2.5. Assume that the reactants are at standard condition.

Solution:

The standard electrode potentials for the Cu and Zn half-cells from Table 2.10 are +0.337 and -0.763V, respectively. Since E^o_h value for Cu is higher it will act as cathode.

Therefore, E_{cell} = +0.337 - (-0.763) = 1.100V

In Example 2, E_{cell} may be written as E^o_{cell}, the standard cell potential, since E^o_h values were used in determining the cell potential. In case the reactants are not at standard condition, the E_h values can be calculated using the Nernst expression.

Example 2.15

Calcultate cell potential for the cell shown in Figure 2.5 when the activities of Zn^{2+} and Cu^{2+} are 0.1 and 0.01 mol/L

Solution:

The potential for Zn electrode is given by

$$E_{zn} = -0.763 - \frac{0.0591}{2}\log\frac{1}{10^{-1}} = -0.793\text{V}$$

The potential for Cu electrode will be

$$E_{cu} = -0.037 - \frac{0.0591}{2}\log\frac{1}{10^{-2}} = +0.278\text{V}$$

Therefore, the cell potential will be

E_{cell} = +0.278 - (-0.793) = 1.071V

2.8.2 EQUILIBRIUM STATE

Equilibrium constant

As a redox reaction proceeds, the concentration of the reactants decrease. Simultaneously the concentrations of products increase. In a galvanic cell, the potential decreases and ultimately at equilibrium it becomes zero. Thus,

$$E_{cell} = 0 = E_{cathode} = E_{anode} \quad (2.35)$$

Taking the example of Zn - Cu cell, therefore at equilibrium:

$$E_{Cu} - E_{Zn} = 0$$

or $$E^0_{cu} - \frac{0.0591}{2}\log\frac{1}{[Cu^{2+}]} - E^0_{zn} + \frac{0.0591}{2}\log\frac{1}{[Zn^{2+}]} = 0$$

or $$\frac{2\left(E^0_{cu} - E^0_{zn}\right)}{0.0591} = \log\frac{[Zn^{2+}]}{[Cu^{2+}]} = \log K_{eq} \quad (2.36)$$

where K_{eq} is the equilibrium constant for the overall reaction, Equation 2.27.

Note that the concentrations in Equation (2.27) would be the equilibrium concentrations. The value of K_{eq} can be calculated by substituting the values of standard potentials in the equation.

Redox potential

In an aqueous chemical system there may be a number of chemical species giving rise to corresponding half-reactions. Each half-reaction would develop a half-cell potential whose magnitude would depend on the standard electrode potential for the half-reaction and the activities of relevant species and temperature. If the chemical system is at equilibrium (analogue to zero potential of a galvanic cell), these potentials are equal.

As an example, a natural water may be at equilibrium with respect to the following half-reactions:

$Fe^{3+} + e \leftrightharpoons Fe^{++}$ and $SO_4^{2-} + 10H^+ + 8e \leftrightharpoons H_2S + 4H_2O$

Therefore, $E_{Fe} = E_S = E_{system}$

The potential of the system is called the redox potential. It is determined by immersing an inert electrode, usually platinum, in the chemical system in question and determining the potential difference with respect to hydrogen or other reference electrode of known potential.

Example 2.16

The redox potential of a sample of water containing Fe^{2+} and Fe^{3+} ions is 0.5volt. Which is the predominant specie of iron?

Write the Nernst expression for $Fe^{3+} + e \leftrightharpoons Fe^{2+}$

$$E_n = 0.5 = 0.77 - \frac{0.0591}{1} \log \frac{[Fe^{2+}]}{[Fe^{3+}]}$$

or $$\log \frac{[Fe^{2+}]}{[Fe^{3+}]} = \frac{0.027}{0.0591} = 4.58$$

or $$\frac{[Fe^{2+}]}{[Fe^{3+}]} = 10^{4.58}$$

Therefore, Fe^{2+} dominates

Stability field diagrams

Many of the redox reactions include H^+ or OH^- ions. Stability diagrams can be drawn to describe the relationship between E_h(cell potential with respect to hydrogen electrode), pH and stable chemical species for a particular chemical system.

For example, the ability of a natural environment to oxidise sulphur will vary with its redox potential. Sea water at the surface, where it is undergoing aeration, may have a potential of +0.3 volt contains sulphates, while near the bottom sediments in presence of organic sediments, the potential may be -0.5volt and sulphides may be present. The relevant half-reaction relating the oxidised specie of sulphur, SO_4^{2-}, to the reduced specie, H_2S, is:

$SO^{2-}_4 + 10H^+ + 8e \leftrightharpoons H_2S + 4H_2O$ (2.37)

Writing the Nernst expression for the above equation, relation between E_h and pH is obtained:

$$E_h = E_h^0 - \frac{0.0591}{8} \log \left\{ \frac{[H_2S]}{[SO_4^{2-}]} \cdot \frac{1}{[H^+]^{-10}} \right\}$$

or $$E_h = E_h^0 - 0.074pH - \frac{-.0591}{8} \log \frac{[H_2S]}{[SO_4^{2-}]} \quad (2.38)$$

Assuming that the ratio of $[H_2S]$ and $[SO_4^{2-}]$ is one and substituting the value of E^0_h from Table 2.10.

$$E_h = 0.3 - 0.074pH \quad (2.39)$$

A plot of Equation (2.39) is shown in Figure 2.6. If the E_h and pH value of a chemical system plot on this line both the species (H_2S and SO_4^{2-}) have the same concentration. However, if for example the E_h value is 0.1 and the pH is 7, which is a point on the right side of the plot, substitution of these values in Equation (14) gives the value of ration $[H_2S]$ / $[SO_4^{2-}]$ = 10^{-43}. Therefore, the plotting positions on the right side of the line show a predominance of SO_4^{2-} species in the water.

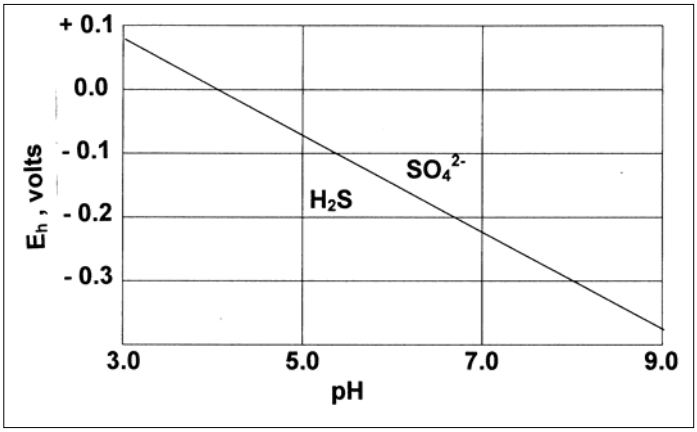


Figure 2.6:
E_h-pH diagram for sulphur species

The first step in constructing a stability field diagram is to identify the pertinent reactions. The reactions may not be limited to only redox reactions. The plotting equations are then developed. Unit activities may be assumed for all soluble species.

2.9 MAJOR IONS IN WATER

All waters in the environment contain dissolved salts. However, some species occur more frequently and at greater concentrations than others. This is illustrated in Table 2.11.

Major constituents (1.0 to 1000 mg/L)	**Secondary Constituents (0.01 to 10.0 mg/L)**
Sodium	Iron
Calcium	Strontium
Magnesium	Potassium
Bicarbonate	Carbonate
Sulphate	Nitrate
Chloride	Fluoride
Silica	Boron

Table 2.11: Major and Secondary Constituents of Groundwater

With regard to ions, Table 2.12 shows the cations and anions which normally constitute the major ions in water and it is these ions which are discussed in this module.

CATIONS	ANIONS
Calcium (Ca^{2+})	Bicarbonate (HCO_3^-) / Carbonate (CO_3^{2-})
Magnesium (Mg^{2+})	Sulphate (SO_4^{2-})
Sodium (Na^+)	Chloride (Cl^-)
Potassium (K^+)	

Table 2.12: Major Cations and Anions in Water

2.9.1 SOURCES OF THE MAJOR IONS

Rain water, as it passes through the air and through and over the land, dissolves many chemical species. Passing through the atmosphere, for example, it dissolves the gases which constitute air including nitrogen, oxygen and carbon dioxide. The fact that it dissolves carbon dioxide from the air is important because when carbon dioxide in present in water it forms carbonic acid and this acid enhances water's ability to dissolve chemical species (salts) contained in rocks and soil. In passing through polluted atmosphere it is also possible for the water to dissolve gases associated with pollution such as sulphur and nitrogen oxides. Some of these gases can also make the water acidic, further adding to the water's ability to dissolve salts.

By the time that rain water has passed over and through land to become groundwater or surface water it has normally acquired many dissolved chemical species. Clearly, the precise chemical composition of the water will depend upon the types of rock and soils with which the water has been in contact and this can be used to characterise a particular water by determining its chemical make-up. As a guide to this characterisation process Table 2.13 gives some of the primary sources of the major ions.

Major Ions	Some Primary Sources
Calcium	Amphiboles, feldspars, gypsum, pyroxenes, aragonite, calcite, dolomite, clay minerals
Magnesium	Amphiboles, olivine, pyroxenes, dolomite, magnesite, clay minerals
Sodium	Feldspars, clay minerals, halite, mirabilite, industrial wastes
Potassium	Feldspars, feldspathoids, some micas, clay minerals
Bicarbonate/Carbonate	Limestone, dolomite
Sulphate	Oxidation of sulphide ores, gypsum, anhydrite
Chloride	Sedimentary rock, igneous rock

Table 2.13: Primary Sources of the Major Ions

2.9.2 WATER CHARACTERISATION

It is possible to characterise waters by performing a chemical analysis of their major ions. Once this is done the results can be plotted in a variety of formats to allow comparison between different waters. Figure 2.7 shows how this can be done by means of a bar chart for 4 different samples. The cations are plotted as the left half of the bar and the anions as the right half. The height of the chart represents the total concentration of major ions in the water in milliequivalents per litre.

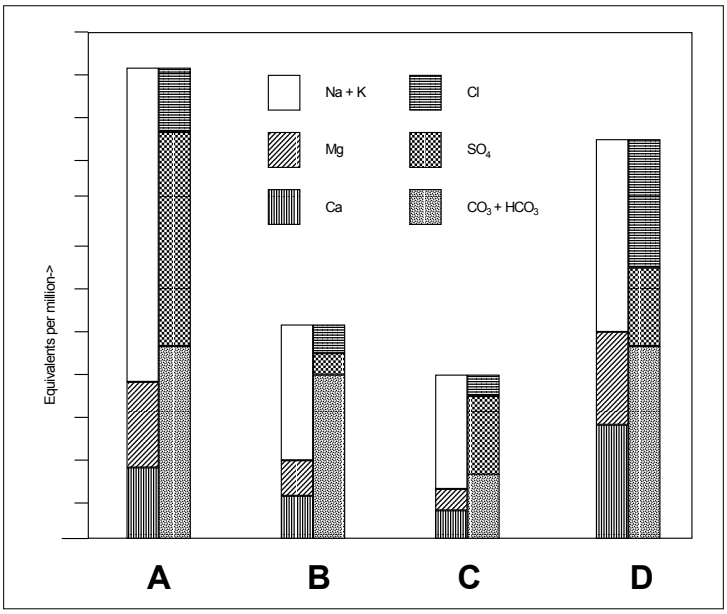


Figure 2.7:
Vertical bar graphs for characterising waters

A number of different types of plot can be constructed to show the same information but the type shown in Figure 2.7 is probably the easiest to use and understand and most common.

Figure 2.8 shows a trilinear diagram known as Piper or Hill diagram. Here cations, expressed as percentages of total cations in meq/L, plot as a single point on the left triangle; while anions similarly plot on the right triangle. These two points are then projected in the central diamond-shaped area. This single point is thus uniquely related to the total ionic distribution. In order to use this method, Na^+ and K^+ and CO_2 and HCO_3^- are combined. The points shown on the diagram correspond to the following analysis:

Ca^{++} = 30 %, Mg^{++} = 15% $Na^+ + K^+$ = 55%

SO_4^{--} = 10% Cl^- = 28% HCO_3^- = 62%

Such plots conveniently reveal similarities and differences among different samples because those with similar qualities will tend to plot together as groups.

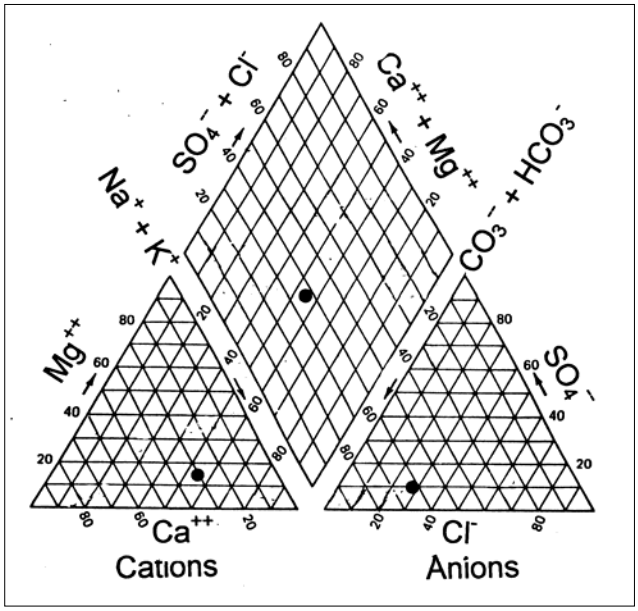


Figure 2.8:
Piper or Hill trilinear diagram for water quality

Another method of plotting chemical characteristics, devised by Stiff, is shown in Figure 2.9. The scale shown in the figure is used to plot the ion concentrations for a specific water sample. When the points are connected, the resulting pattern provides a pictorial representation of the water sample. Such plots are used to trace similar formation groundwaters over large areas.

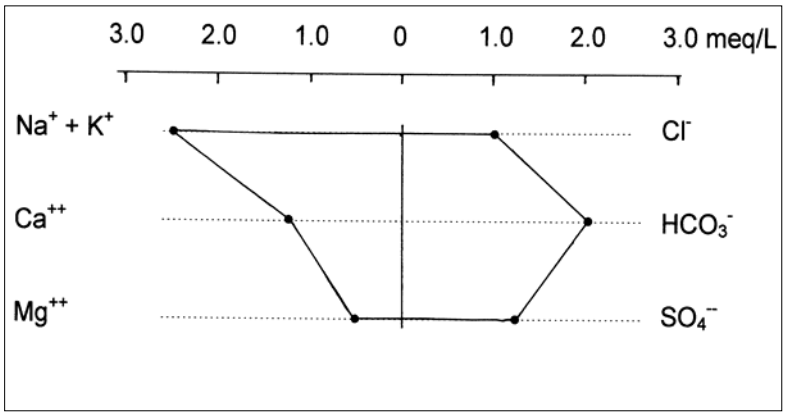


Figure 2.9:
Stiff pattern diagram

2.9.3 WATER QUALITY CONSEQUENCES OF THE MAJOR IONS

Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) ions are both common in natural waters and both are essential elements for all organisms. Calcium and magnesium, when combined with bicarbonate, carbonate, sulphate and other species, contribute to the hardness of natural waters. The effect of this hardness can be seen as deposited scale when such waters are heated. Normally hardness due to calcium predominates although in certain regions magnesium hardness can be high. In some river, hardness can vary seasonally reaching peak values during low flow conditions. It is possible to analyse waters to determine hardness and then classify them as shown in Table 2.14 below.

Hardness (mg/L as Ca CO_3)	**Classification**
0 – 75	Soft
75 – 150	Moderately hard
150 – 300	Hard
Over 300	Very hard

Table2.14: Hardness classification of water

In natural waters, calcium concentrations are normally below 15 mg/L although this can rise to 100 mg/L where waters are associated with carbonate-rich rocks. Magnesium concentrations also vary widely and can be from 1 to over 50 mg/L depending upon the rock types within the catchment.

All natural waters contain sodium ions (Na^+) as the element is one of the most abundant on the planet. High concentrations in inland waters, however, are normally associated with pollution from industrial discharges or sewage effluent or, in coastal areas, sea water intrusion. Normally, however, sodium concentrations are below 200 mg/L (this is also the World Health Organisation guideline limit for sodium in drinking water).

When water is to be used for irrigation purposes it is important to know the sodium concentration as sodium can have a negative effect on soil structure by defloculating it, which can affect plant growth. To evaluate the suitability of water for irrigation the Sodium Adsorption Ration (SAR) is used as follows:

$$SAR = \frac{Na^{+}}{\sqrt{\left(Ca^{2+} + Mg^{2+}\right)/2}}$$

where the concentrations of the ions in milliequivalents per litre are used.

From an international perspective, if the value of the SAR is less than 3 the water is suitable for irrigation use. Values from 3 to 9 represent some use restriction whilst SAR values in excess of 9 normally mean that water cannot be used for irrigation. In India, however, the SAR standard for irrigation water is set to 26 which reflects the fact that sodium does not build up in the soil and cause damage because every monsoon season the soil is thoroughly flushed and renewed.

A related parameter to asses the suitability of water for irrigation is sodium percentage:

$$Na\% = \frac{Na \times 100}{(Na + K + Ca + Mg)}$$

where all values are expressed in meq/L. It is recommended that sodium percentage should not exceed 60.

In waters where the bicarbonate content is high, there is a tendency for calcium and magnesium if present, to precipitate out as carbonates, thus increasing SAR. The residual sodium carbonate (RSC) is defined as:

$$RSC = (CO_3^{--} + HCO_3^{-}) - (Ca^{++} + Mg^{++}) \qquad (2.40)$$

where the concentrations of the ions in milliequivalents per litre are used.

If its value exceeds 2.5 meq/L the water is not suitable, 2.5 to 1.25 is marginal and less than 1.25 is safe.

Figure 2.10 shows a diagram for classification of irrigation waters proposed by US Department of Agriculture, which is widely used in India.

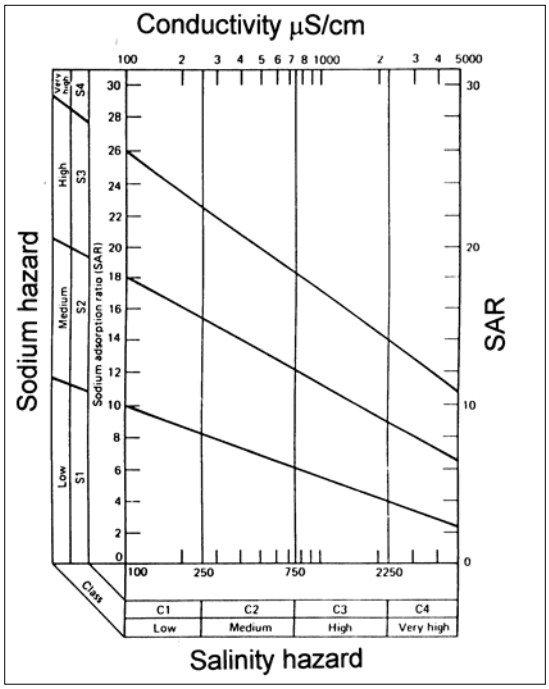


Figure 2.10
USDA classification for agricultural water

The concentration of potassium ions (K^+) in natural fresh waters is generally low (normally less than 10 mg/L). Sea water and brines contain much higher concentrations, however. Potassium ions are highly soluble and are essential for most forms of life. Potassium in the water environment is readily taken up by aquatic life, therefore.

A discussion of carbonates and bicarbonates in waters is given in Section 2.5.1. Bicarbonate concentrations in natural waters range from less than 25 mg/L in areas of non-carbonate rocks to over 400 mg/L where carbonate rocks are present. Carbonate concentrations in surface and ground waters by contrast are usually low and nearly always less than 10 mg/L.

Sulphate is present in all surface waters as it arises from rocks and from sea water which contains a high sulphate concentration. In addition to its role as a plant nutrient, high concentrations of sulphate can be problematic as they make the water corrosive to building materials (e.g., concrete) and are capable of being reduced to hydrogen sulphide (a toxic, foul-smelling gas) when zero dissolved oxygen conditions prevail in the water body. Normally, sulphate concentrations in surface waters are between 2 and 80 mg/L although they may exceed 1000 mg/L if industrial discharges or sulphate-rich minerals are present. The WHO guideline value for sulphate in drinking water is 400 mg/L.

Chlorides in fresh waters generally come from rocks, the sea, sewage and agricultural and industrial effluents. Fresh water concentrations of chloride are normally less than 40 mg/L and can be as low as 2 mg/L in waters, which have not been subject to pollution. Chloride concentrations over 100 mg/L give the water a salty taste.

Though nitrate is not listed as a major ion, high concentrations of nitrate upto 200 mg NO_3-N/L have been reported from many sites in the country where municipal wastewater or leachate from garbage dumps has contaminated the groundwater. Excessive amount of nitrate in drinking water causes methaemoglobinaemia in bottle fed infants. WHO has recommended a guideline value of 10 mg NO_3-N/L.

2.9.4 ION BALANCING

When a water quality sample has been analysed for the major ionic species, one of the most important validation tests can be conducted: the cation-anion balance.

The principle of electroneutrality requires that the sum of the positive ions (cations) must equal the sum of the negative ions (anions). Thus the error in a cation-anion balance can be written as:

$$\%\,\text{balance error} = \frac{\sum \text{cations} - \sum \text{anions}}{\sum \text{cations} + \sum \text{anions}} \text{ X } 100 \qquad (2.41)$$

where the ions are expressed in meq/L.

For groundwater and surface water, the % error should be less than 10. If it is greater, the analysis does not pass the validation check.

Example 2.17

A laboratory measures the following concentrations of ions in a sample of water. Perform the validation check.

Cation	Conc (mg/l)	Anion	Conc (mg/l)
Ca^{2+}	93.8	HCO_3^-	164.7
Mg^{2+}	28.0	SO_4^{-2}	134.0
Na^+	13.7	Cl^-	92.5
K^+	30.2		

Solution:

1 First the concentrations of cations and anions must be converted from mg/l to meq/l.

(a) This conversion is made using the mg/meq value for each major ion species. This value is equal to the atomic weight if species divided by the ion charge

For Calcium (Ca^{2+}):

- atomic weight = 40
- ion charge = 2
- mg/meq = 40/2 = 20

(b) Dividing the concentration (mg/l) by the mg/meq value for each species results in meq/l.

For Calcium (Ca^{+2}):

- Concentration (mg/l) = 93.8
- mg/meq = 20
- 93.8/20 = 4.69 meq/l

(c) A table should be completed with all the values per species, and the sum of cations and anions.

Cation		**Concentration**	
	(mg/l)	**(mg/meq)**	**(meq/l)**
Ca^{+2}	93.8	20.0	4.69
Mg^{+2}	28.0	12.2	2.3
Na^+	13.7	13.7	0.60
K^+	30.2	39.1	0.77
Total Cations			**8.36 meq/l**

Anion		**Concentration**	
	(mg/l)	**(mg/meq)**	**(meq/l)**
HCO_3^-	164.7	61.0	2.74
SO_4^{-2}	134.0	48.0	2.79
Cl^-	92.5	35.5	2.61
Total Anions			**8.14 meq/l**

2. Check accuracy (% balance error)

$$\%\,\text{balance error} = \frac{\sum \text{cations} - \sum \text{anions}}{\sum \text{cations} + \sum \text{anions}} \text{ X } 100$$

$$= \frac{8.36 \;-\; 8.14}{8.36 \;+\; 8.14} \text{X} 100 = 1.3\%$$

This is less than the allowed error, so the sample results can be accepted. If error > 10% then check results, and possibly re-analyse samples.

Note: An accurate ion balance does not necessarily mean that the analysis is correct. There may be more than one error and these may cancel each other out.

2.10 DISSOLVED OXYGEN

2.10.1 SOLUBILITY

Surface water in contact with air, dissolves oxygen through molecular diffusion. Dissolved oxygen (DO) levels in natural and wastewater depends on the physical, chemical and biological activities in water body. If the dissolved oxygen (DO) is not consumed by reactions in the water, it ultimately reaches saturation value. Table 2.15 gives saturation levels as a function of temperature and chloride concentration. The solubility of oxygen decreases as the concentration of dissolved solids increases. Chloride concentration is used as a measure of the seawater-freshwater mix in a sample. The chloride content of seawater is about 19,000 mg/L.

The solubility also depends on the partial pressure of oxygen. The solubility of atmospheric oxygen in fresh waters ranges from 14.6 mg/L at 0°C to about 7 mg/L at 35°C, under 1 atm. pressure.

For saturation at barometric pressures other than 760 mm,

$$C'_s = C_s \text{ x } \frac{P - p}{760 - p} \qquad (2.42)$$

C'_s = Solubility at barometric pressure P mm and given temperature, mg/L

C_s = Saturation at given temperature from Table 1, mg/L

p = Pressure of saturated water vapour at temperature of the water selected from table, mm.

In polluted waters the presence or absence of oxygen determines whether the decomposition of organic matter is brought about by aerobic or anaerobic organisms. Aerobic decomposition produces innocuous end products. In the absence of oxygen anaerobic organisms take over the decomposition process producing reduced compounds such as ammonia, hydrogen sulphide, mercaptans, etc., which cause environmental nuisance. Though the solubility of oxygen in water is very limited under ambient conditions, its presence is essential for the well-being of fish and normal aquatic life. Indian standards specify a minimum of 4 mg/L for such a purpose.

Measurement of DO is also important in monitoring and operation of aerobic wastewater treatment plants and corrosion prevention in water treatment and distribution systems.

Temp.°C	Dissolved Oxygen, mg/L				Vapour pressure mm Hg
	Chloride concentration in water mg/L			Difference per 100 mg chloride	
	0	5000	10000		
0	14.6	13.8	13.0	0.017	5
1	14.2	13.4	12.6	0.016	5
2	13.8	13.1	12.3	0.015	5
3	13.5	12.7	12.0	0.015	6
4	13.1	12.4	11.7	0.014	6
5	12.8	12.1	11.4	0.014	7
6	12.5	11.8	11.1	0.014	7
7	12.2	11.5	10.9	0.013	8
8	11.9	11.2	10.6	0.013	8
9	11.6	11.0	10.4	0.012	9
10	11.3	10.7	10.1	0.012	9
11	11.1	10.5	9.9	0.011	10
12	10.8	10.3	9.7	0.011	11

Temp.°C	Dissolved Oxygen, mg/L				Vapour pressure mm Hg
	Chloride concentration in water mg/L			Difference per 100 mg chloride	
	0	5000	10000		
13	10.6	10.1	9.5	0.011	11
14	10.4	9.9	9.3	0.010	12
15	10.2	9.7	9.1	0.010	13
16	10.0	9.5	9.0	0.010	14
17	9.7	9.3	8.8	0.010	15
18	9.5	9.1	8.6	0.009	16
19	9.4	8.9	8.5	0.009	17
20	9.2	8.7	8.3	0.009	18
21	9.0	8.6	8.1	0.009	19
22	8.8	8.4	8.0	0.008	20
23	8.7	8.3	7.9	0.008	21
24	8.5	8.1	7.7	0.008	22
25	8.4	8.0	7.6	0.008	24
26	8.2	7.8	7.4	0.008	25
27	8.1	7.7	7.3	0.008	27
28	7.9	7.5	7.1	0.008	28
29	7.8	7.4	7.0	0.008	30
30	7.6	7.3	6.9	0.008	32

Table 2.15: Saturation values of dissolved oxygen in water exposed to water saturated air containing 20.90 % oxygen under a pressure of 760 mm of mercury.

Example 2.18

Calculate the saturation DO level in water containing 600 mg/L chloride at 28°C and 710 mmHg barometric pressure. What is the percent saturation level if the DO content is 5.8 mg/L.

Solution:

From Table 2.15, at 28°C for 0 mg/L Cl^- concentration

Cs = 7.9 mg/L, the concentration decreases at the rate of 0.008 mg/L per 100 mg/L Cl^-

Therefore for 600 mg/L Cl^-, C_s = 7.9 – (0.008 x 6) = 7.85 mg/L

The table also gives saturated water vapour pressure as 28 mmHg

Therefore saturation value at 710 mmHg pressure from Equation (2.42)

$$C_s' = 7.85 \times \frac{710 - 28}{760 - 28} = 7.31\,\text{mg/L}$$

Therefore saturation level $= \frac{5.8}{7.31} \times 100 = 79\%$

2.10.2 WINKLER METHOD WITH AZIDE MODIFICATION

The method is based upon reactions that release iodine equivalent to the amount of oxygen originally present in the sample and titration of the liberated iodine. In the first step manganous sulphate and alkali iodide reagents are added. If no oxygen is present the manganous ion reacts only with the hydroxide ion to form a white precipitate of manganous hydroxide:

$$Mn^{++} + 2OH^- = Mn(OH)_2 \qquad (2.43)$$

If oxygen is present the manganous ion is oxidised and brown precipitate of manganese dioxide is formed:

$$Mn^{++} + 2OH^- + 1/2O_2 = MnO_2 + H_2O \quad (2.44)$$

Upon addition of sulphuric acid iodine is formed by oxidation of iodide:

$$MnO_2 + 4H^+ + 2I^- = Mn^{++} + I_2 + 2H_2O \quad (2.45)$$

Sodium thiosulphate standard solution is used to titrate iodine

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI \quad (2.46)$$

The end point of titration is obtained by first titrating iodine to a pale straw colour and then adding starch indicator which combines with iodine to give a blue colour. The titration is continued till the iodine complexed with starch is also reacted and the blue colour disappears.

Note that all the reagents except the standard solution are added in excess to ensure complete reactions.

Nitrites if present in the sample cause interference by oxidising iodide:

$$2NO_2^- + 2I^- + 4H^+ = I_2 + N_2O_2 + 2H_2O \quad (2.47)$$

N_2O_2 in turn is oxidised by oxygen entering the sample during titration:

$$N_2O_2 + 1/2O_2 + H_2O = 2NO_2^- + 2H^+ \quad (2.48)$$

Thus it becomes impossible to reach a definite end point and high results are obtained. In the azide modification, sodium azide added with the alkali reagent destroys the nitrite, if present:

$$NaN_3 + H^+ = HN_3 + Na^+ \quad (2.49)$$

$$HN_3 + NO_2^- + H^+ = N_2 + N_2O + H_2O \quad (2.50)$$

Sodium thiosulphate is a secondary standard. Because of its water of hydration it cannot be dried to a compound of definite composition. It also undergoes oxidation under storage. Therefore it is standardised against a primary standard, either dichromate or bi-iodate. Both react with iodide ion to release an equivalent amount of iodine:

$$Cr_2O_7^{--} + 6I^- + 14H^+ = 2Cr^{+++} + 3I_2 + 7H_2O \quad (2.51)$$

$$IO_3^- + 5I^- + 6H^+ = 3I_2 + 3H_2O \quad (2.52)$$

The released iodine is titrated with sodium thiosulphate.

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI \quad (2.53)$$

The reactions described above involve oxidation-reduction reactions. The valence number for the reactants in these reactions is equal to the number of electrons released or accepted by them. Since the equations are balanced, we can find the valence number from the number of electrons accepted

by iodine or released by iodide in each reaction. One iodine atom is reduced to iodide upon acceptance of one electron. Thus the valence number for thiosulphate is 1, for dichromate and for bi-iodate it is 6, Equations (2.46), (2.51) and (2.52), respectively.

2.11 BIOCHEMICAL OXYGEN DEMAND

The biochemical oxygen demand (BOD) test is an experimentally derived analytical method designed to give an indication of the polluting nature of organic matter in a sample of water. It measures aggregate organic matter. How this is achieved is described below.

When organic polluting matter is discharged to the aquatic environment it will normally degrade through the action of micro-organisms in the watercourse. In degrading the organic matter, micro-organisms take up atmospheric oxygen which is dissolved in the water. Such an uptake of dissolved oxygen (called an 'oxygen demand') can, if sufficient oxygen is lost, lead to the degradation of water quality as aquatic plants and animals (including fish) need to breathe this dissolved gas and will die or migrate if it is unavailable. If the dissolved oxygen is totally depleted, foul odours and unsightly conditions are created.

The BOD test was designed to provide a measure of this uptake of oxygen by attempting to recreate in the laboratory environment some of the conditions that prevail in nature. Not all such conditions can be recreated, however, and so the BOD test merely gives an indication of likely pollution. It does not attempt to describe it exactly.

2.11.1 THE BOD TEST

Once material, which has a biochemical oxygen demand, is discharged to a watercourse it begins to take up dissolved oxygen at a rate which depends on the temperature and the type of material discharged. Generally, simple, non-toxic organic chemicals are degraded more quickly than complex molecules and thus can exert their oxygen demand more quickly. This can be seen in Figure 2.11, which shows two samples that have been tested for oxygen demand over a number of days.

Sample 1 represents an effluent, which exerts its oxygen demand quickly, whereas Sample 2 consumes oxygen more slowly as seen by the flatter shape of the curve. Sample 1 could represent a discharge containing sugar, milk, blood, untreated sewage effluent or some other easily oxidised material. Sample 2, on the other hand, might represent a typical oxygen demand curve for an effluent containing more complex organic materials which are more difficult to oxidise such as industrial chemicals, solvents, fats or a treated sewage effluent.

Typically, oxygen demand curves are of the shape shown in Figure 2.11 because some material within the sample will be oxidised quickly, giving rise to the initially steep slope of the curve, and some material will be oxidised more slowly leading to the subsequent flatter appearance. The point at the end of each curve, where it approaches the horizontal, is sometimes known as the 'ultimate BOD (UBOD or BOD-U) of a sample; depending upon the organic material being tested it can take many days or even weeks to reach this point. For convenience, a twenty-day BOD (BOD_{20}) is often considered to be equal to the ultimate BOD. In Figure 2.11 you may note that both the samples probably have the same BOD-U value.

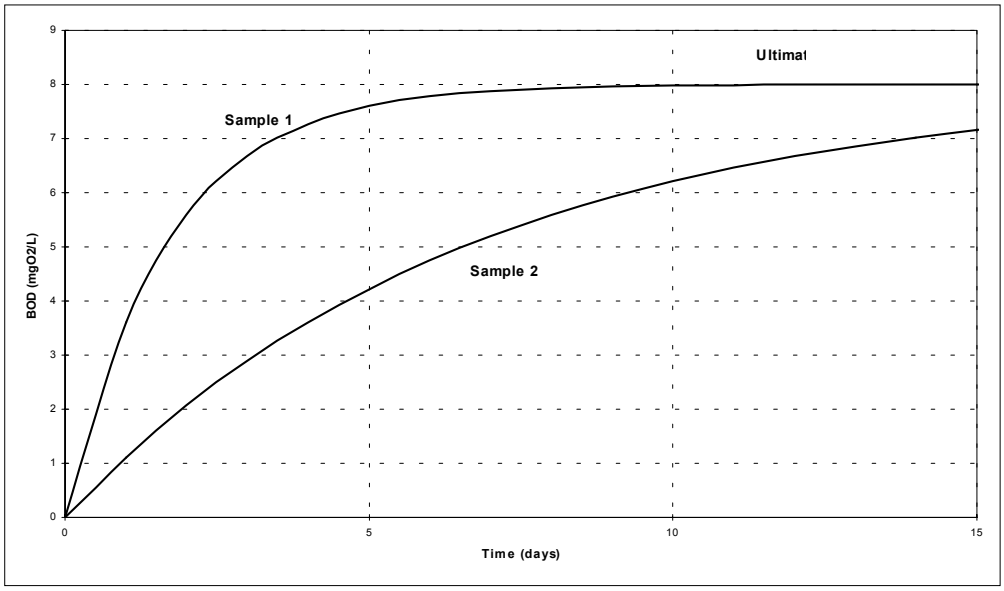


Figure 2.11: Oxygen Uptake Curves: Sample 1 - easily degradable organics, Sample 2 - difficult to degrade Organics

For the sake of convenience and reproducibility, the standard BOD test carried out in the laboratory is run at a constant temperature and is time limited. Often the test is carried out over five days at 20°C and is designated as 'BOD_5'. However, three day (BOD_3), seven day (BOD_7) and other period tests are also used. In India a three day BOD test at 27°C has been standardised (Bureau of Indian Standards). As can be seen from Figure 1, the three day test will normally result in lower BOD values than a five day test. When the temperature is increased from 20°C to 27°C the reaction proceeds at a faster rate and hence the difference between the 3 day and 5 day values is minimal.

When performed in the laboratory, the Indian Standard BOD test involves assessing the loss of dissolved oxygen in a sample incubated for three days at 27°C. This is done by comparing the dissolved oxygen concentration of a water sample, which has been incubated for three days with the dissolved oxygen concentration of the sample before incubation. The loss of dissolved oxygen over the three day period is then reported as the BOD (or more correctly BOD_3) of the sample.

Example 2.19

The following table gives DO values in BOD bottles, containing identical samples when incubated at 20 and 27 °C over a period of 5 days. It also gives the cumulative oxygen uptake values. Note that the BOD_3 27 °C and BOD_5 20 °C values are nearly the same.

Solution:

Day	DO, mg/ L 20 °C	Total DO consumed mg/L, 20 °C	DO, mg/L, 27 °C	Total DO consumed, mg/L, 27 °C
0	8.0	0	8.0	0
1	6.4	1.6	5.6	2.4
2	5.1	2.9	3.9	4.1
3	4.1	3.9	2.7	5.3
4	3.3	4.7	1.9	6.1
5	2.6	5.4	1.3	6.3

Factors Affecting the Oxygen Demand Rate

The rate at which organic matter is oxidised in the aquatic environment depends upon a number of factors including the following:

- the composition of the material
- the temperature
- the concentration of micro-organisms present

With regard to temperature, generally the rate of the BOD reaction increases with increasing temperature. In the laboratory, therefore, the BOD test is carried out at a standard temperature of 27°C in order that results are comparable with each other. It is important to remember, however, that in the environment the oxygen demand reaction may proceed at a greater or lesser rate depending upon the ambient temperature.

The concentration of micro-organisms present in the watercourse or water sample bottle also has an effect on the rate of the BOD reaction. Under normal circumstances there are usually sufficient numbers of suitable micro-organisms present for the BOD reaction to proceed. Occasionally, however, particularly if the water contains chemicals which are toxic to bacteria, no, or few micro-organisms are present to carry out the oxygen demand reaction. If the BOD of such a sample were to be determined, it would produce a result much lower than the concentration of organic matter in the sample would suggest. To prevent this false low result in the laboratory, the sample must be 'seeded' with suitable bacteria. Usually a small amount of settled raw sewage is used as a source of seed. In case the waste is toxic in nature an acclimated seed must be developed.

In order that a BOD test is successful, some residual DO must remain in the bottle after the incubation. Since DO saturation values are in the range of 8 to 9 mg/L, a sample whose BOD is higher has to be diluted.

Relationship of BOD to Other Water Quality Parameters

There are a number of other ways of measuring the amount of organic pollution in water. Two methods in particular are worthy of discussion in this respect as they are related in different ways to the BOD.

Chemical Oxygen Demand (COD) is also a measure of the organic pollution present in the water. Like BOD, it is a measure of the oxygen demand exerted by organic matter when it is discharged to the water environment. It differs from BOD, however, in the method of determination of the oxygen demand as this is ascertained by means of a chemical test. A strong chemical oxidising agent is used under extreme laboratory conditions to ensure that virtually all organic matter within the sample is oxidised during the analysis some of which may not be susceptible to bacterial decomposition. The amount of oxidant used during the test is then calculated. Some naturally occurring organic compounds such as celluloses, fulvic acids, lignins or many synthetic petrochemicals, are either nondecomposable or are degraded at a very slow rate by bacteria. For a given water sample, therefore, COD is always greater than BOD.

It is also possible to measure the Total Organic Carbon (TOC) content of a water sample. The analysis can be carried out in a number of ways and, as its name implies, measures all the carbon, which is bound up in the organic matter within the water sample. TOC is therefore related to BOD and COD as most of the oxygen demand measured during these analyses is due to organic carbon.

Due to the fact that the BOD of a sample can be related to both the COD and the TOC, it is sometimes possible to estimate the BOD from either the COD or the TOC. However, before this can be attempted, it is necessary to establish a relationship between these two parameters for a particular sampling point. This is done by carrying out a number of BOD and COD or TOC analyses on the sampling point under various conditions so that a reliable ratio can be established.

Sample Handling

Provided conditions are right, organic matter discharged to the aquatic environment will be continually oxidised and thus exert an oxygen demand. Once a sample of water containing organic material is taken from a water body there is nothing to stop the oxidation reactions within the sample bottle, thereby altering the BOD of the sample. To limit this change in the BOD, it is preferable if samples are analysed as soon as possible after collection. If they cannot be analysed immediately, samples should be stored at 4 to 5^{O}C. This greatly reduces the rate of oxidation reactions so that the BOD does not change significantly.

Interferences

The BOD test relies on micro-organisms degrading the organic matter present in the sample during the analysis. For this reason, the conditions of the analysis must allow the micro-organisms to grow, as far as possible, without undue environmental stress. If the pH of the sample is too low (below 6.5) or too high (above 7.5), the BOD analysis may be affected by the bacteria's ability to grow in such conditions. Therefore, samples with a pH below 6.5 and above 7.5 on reaching the laboratory, should be adjusted by the addition of acid or alkali, to a pH within the range 6.5 to 7.5.

As discussed above, the presence of toxic material in the BOD sample will also inhibit the analysis and will mean that the sample must be 'seeded' with bacteria in order for the test to proceed normally. If the toxicity of the sample is due to the presence of chlorine this should be neutralised by adding sodium thiosulphate or sodium bisulphite prior to 'seeding'.

Sometimes waters, particularly those containing high concentrations of algae, may be 'supersaturated' with dissolved oxygen (that is, due to pure oxygen production by algae during photosynthesis, the water has a higher concentration of oxygen than normal saturation value). If this is the case, the sample should be shaken in a partially filled bottle so that all excess oxygen is lost before the BOD analysis takes place. If such a procedure is not carried out , the excess oxygen may be lost during the BOD test leading to an incorrect result.

Organic compounds are not the only materials which, when discharged to the aquatic environment, have an oxygen demand. Most notably ammonia, either free or when released from nitrogen containing organic compounds, is also oxidised in watercourses resulting in depletion of dissolved oxygen. The oxidation of nitrogen compounds, carried out by nitrifying bacteria, (a process known as nitrification) in the BOD sample can be suppressed, by the addition of an inhibitory chemical, so that only the BOD resulting from the oxidation of carbon compounds is determined. If such a chemical is not added the resulting BOD may be a combination of the oxygen demand caused by both carbonaceous matter and ammonia based material in the sample. This is particularly the case for biologically treated secondary effluents. Oxygen consumption due to ammonia oxidation may be important in case of study of dissolved oxygen resources of receiving waters.

In many wastes where the concentration of nitrifying bacteria is relatively low (e.g., raw sewage, industrial effluents) the bulk of the oxygen demand will be due to carbonaceous material. The nitrification reaction starts only after most of the organic matter is oxidised which may take from 10 to 15 days.

The presence of algae in the BOD sample may also lead to false results if the bottles are not stored in the dark. This is because algae have the ability to produce oxygen during photosynthesis making it impossible to decide how much oxygen the organic matter consumed.

2.11.2 BOD PROGRESSION EQUATION

The BOD progression can be approximated by a first order reaction. In simple terms the oxygen demand exerted in a day is a constant fraction of the demand remaining.

Example 2.20

Calculate the oxygen demand exerted by a sample of industrial waste whose ultimate BOD is 256 mg/L, in 1, 2, and 3 days. Assume that 25% of remaining demand is exerted each day.

Solution:

Time day	BOD exerted each day mg/L	Cumulative BOD exerted mg/L	BOD remaining mg/L
(1)	(2)	(3)	(4)
0	0	0	256
1	64	64	192
2	48	112	144
3	36	148	108

(2) = 25% of (4) at (t-1),

(3) = (2) at t + (3) at (t-1),

(4) = 256 – (3) at t

The above relation can also be expressed in the form of an exponential equation:

$$Y_t = L_o (1 - e^{-kt}) \quad (2.54)$$

where,

Y_t = BOD exerted in time t, d

L_o = BOD-U, mg/L

k = BOD rate constant, d^{-1}

The rate constants for a waste, k_1 and k_2, at two temperatures T1 and T2, respectively can be related to each other by the equation:

$$k_1 = k_2\ 1.047^{(T1 - T2)}. \quad (2.55)$$

Example 2.21

Calculate the BOD progressions for 5 days at one day intervals for a water sample at 20 and 27 °C. Th BOD-U is 12 mg/L and the rate constant for 20 °C is 0.22 d^{-1}. Plot the results on a graph and compare BOD_3 at 27°C with BOD_5 at 20°C.

Solution:

(1) Calculate the BOD rate constant at 27 °C:

$k_{27} = k_{20}\ 1.047^{(T1-T2)} = 0.22 \times 1.047^{(27-20)} = 0.3\ d^{-1}$

(2) Set up calculation table:

t, d	T = 20 °C, k_{20} = 0.22 d^{-1}		T = 27 °C, k_{27} = 0.3 d^{-1}	
	$1-e^{-0.22t}$	$Y_t=12(1-e^{-0.22t})$	$1-e^{-0.3t}$	$Y_t=12(1-e^{-0.3t})$
1	0.1975	2.4	0.259	3.1
2	0.356	4.3	0.451	5.4
3	0.483	5.8	0.593	7.1
4	0.588	7.0	0.699	8.4
5	0.667	8.0	0.777	9.3

(3) Plot the Y, Oxygen consumed values against *t*, note that BOD 27°C is nearly same $BOD_5$20°C.

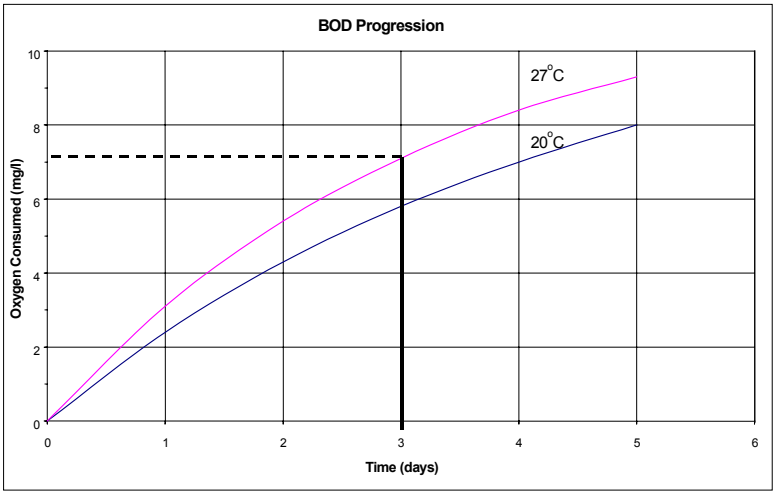


Figure 2.12: Calculated BOD progression at 20 and 27°C

2.11.3 SAMPLE DILUTION

In the determination of BOD in the laboratory it is necessary that excess dissolved oxygen is available during the period of incubation of the sample. In case the BOD value is more than the available oxygen, the BOD reaction will come to a stop and it will not be possible to estimate the total oxygen demand during the period of incubation. Tests in which at least 1 mg/L DO remains after 3 days of incubation period and at least 2 mg/L DO is consumed give the most reliable results. This ensures that the rate of BOD exertion is not limited by the available DO concentration and the difference in the initial and final DO concentration is sufficiently large to be statistically reliable.

The solubility of atmospheric oxygen at 27 °C, the temperature of incubation of the sample, is only about 8 mg/L. Therefore, samples that consume more than 7 mg/L DO during the incubation period of 3 days will not fulfil the condition that excess DO is always present. Such samples should be diluted. This is the case with sewage and many other waste liquids. Grossly polluted surface waters may have a BOD of more than 7 mg/L. Ordinarily, though, surface water samples do not require dilution if they are saturated with DO.

Care has to be taken that the water used for dilution does not contribute any BOD. Dilution water is prepared from distilled water that has been supplemented with phosphate buffer pH 7.2, and salts containing nitrogen, magnesium, calcium and iron as nutrients to provide an environment conducive to growth of micro-organisms. It is also aerated for a significant period of time to raise the concentration of DO to near saturation value.

The degree of dilution depends upon the expected BOD value of the sample. There are two procedures for dilution:

Using percent mixtures: A 1 to 2 L graduated cylinder is half filled with the dilution water, a predetermined volume of the sample is added and the volume is made up to the desired level with more dilution water. The dilution is expressed as percent mixture given by:

Percent mixture = {(Volume of sample)/(Volume of diluted sample)} x 100 (2.56)

Direct pipetting: A predetermined sample volume is pipetted directly into an empty BOD bottle of known capacity, usually 300 mL. The bottle is then filled with dilution water.

Based on knowledge of the approximate BOD of the sample, the required dilution can be determined from Table 2.16. In case nothing is known about the sample, more than one dilution may have to be tried. An idea of the strength of the waste may be obtained from the COD value of the waste.

If D_o and D_T are DO values initially and after time T, respectively, the BOD of the sample is given by:

$$BOD = \{(D_o - D_T)/(\text{Percent mixture})\} \times 100 \quad (2.57)$$

Using separate container		***Direct pipetting into 300mL bottles***	
Range of BOD	**% mixture**	**Range of BOD**	**mL Sample**
1,000 - 3,500	0.2	1,200 - 4,200	0.5
400 - 1,400	0.5	600 - 2,100	1.0
200 - 700	1.0	300 - 1,050	2.0
100 - 350	2.0	120 - 420	5.0
40 - 140	5.0	60 - 210	10.0
20 - 70	10.0	30 - 105	20.0
10 - 35	20.0	12 - 42	50.0
4 - 14	50.0	6 - 21	100.0
0 - 7	100.0	0 – 7	300.0

Table 2.16: Dilutions for samples having different BOD values.

Example 2.22

In a test for determination of BOD of a sample from a polluted river stretch, three dilutions were used. The observations and results of the calculations are given below. Calculate BOD.

Solution:

S No	% Mixture	Initial DO	Final DO	O_2 Con.	BOD
1	50	7.5	2.5	5.0	10.0
2	20	7.5	5.2	2.3	11.5
3	10	7.7	6.9	0.8	8.0

Accept the results at 1 and 2. Report the average value of 10.7 mg/L as BOD of the sample. Result at 3 is not acceptable since it does not meet the condition of minimum consumption of 2 mg/L.

Example 2.23

The BOD of a sample is estimated to be 30 mg/L. What dilution would you recommend? Calculate the quantities of the sample and the dilution water that should be mixed for the suggested dilution to conduct the test.

Solution:

From Table 2.16, two dilutions giving 10 or 20 % mixture seem to be suitable. However, if 20% dilution is used and the BOD turns out to be around 35 mg/L, the condition of the minimum DO of 1 mg/L at the end of the incubation period may not be met. Therefore adopt a 10% dilution.

It is advisable to incubate the diluted sample in duplicate. Therefore, for 3 BOD bottles (one to be titrated initially and the remaining two at the end of the incubation period) each of 300 mL capacity, a total of 900 mL diluted sample will be needed. Since some amount may be wasted, prepare 1000mL of dilution water. Therefore mix 100 mL of sample with 900 mL dilution water.

2.11.4 SEEDING

The purpose of seeding is to introduce a biological population capable of oxidising the organic matter in the sample. Seeding would not be necessary for domestic and municipal sewage, unchlorinated treated effluents and surface waters. When there is a reason to believe that the sample contains very few micro-organisms, for example as a result of chlorination, high temperature, extreme pH or because of the specific composition of some industrial wastes, the dilution water should be seeded.

Most often, the supernatant of fresh, settled sewage may be used as a seed. In cases where the sample may contain organic matter, which is hard to degrade, the seed may be developed by adding a small amount of soil to a portion of the sample and aerating it for 24 to 48 hours. Soil is a medium that supports a wide variety of micro-organisms capable of metabolising many different types of organic matter. Alternatively, water from a body of water receiving the waste may be used as a source of seed. A small volume of seed added to the dilution water, 4 – 6 mL per litre, would contain a sufficient number of micro-organisms adapted to the waste to carry out the oxidation of the organic matter.

Correction must be carried out to account for the oxygen consumed in oxidation of organic matter carried with the seed. The volume of seed added to the dilution water should be recorded and parallel seed control test should be run to determine the BOD of the seed.

If the seeding and dilution methods are combined, the following general formula is used to calculate the BOD:

$$BOD_{3.27}, mg.l^{-1} = \frac{(D_0 - D_T) - (B_0 - B_T)}{P} \quad (2.58)$$

where:

D_0	=	DO of diluted sample initially, mg/L
D_T	=	DO of diluted sample after 3 day incubation at 27°C, mg/L
P	=	decimal volumetric fraction of sample used (% mixture/100)
B_0	=	DO of seed control initially, mg/L
B_T	=	DO of seed control after incubation, mg/L
f	=	ratio of %seed in diluted sample to %seed in seed control

Example 2.24

A seeded BOD test was conducted on an industrial waste, estimated BOD 800 mg/L, using fresh settled sewage as the source for seed micro-organisms. The BOD of the seed was estimated as 150 mg/L.

Solution:

Seed control

Dilution used from Table 1 = 2%

Initial DO = 7.4 mg/L

Final DO = 4.4 mg/L

Sample

Dilution used from Table 1 = 0.5%

Amount of seed used for seeding the dilution water for the sample = 4 mL/L or 0.4%

Initial DO = 7.3 mg/L

Final DO = 2.3

Calculation

P = 0.5/100 = 0.005

f = 0.4/2.0 = 0.2

$$BOD = \frac{(7.3 - 2.3) - 0.2(7.4 - 4.4)}{0.05} = 880 mg/L$$

2.12 CHEMICAL OXYGEN DEMAND

The chemical oxygen demand (COD) analysis is designed to measure the maximum amount of oxygen that can be consumed by the organic matter in a sample of water. This is important because when organic polluting matter is discharged to the aquatic environment it will normally take up dissolved oxygen during its subsequent degradation thus reducing the amount of oxygen available for the respiration of fish and other aquatic life. The test is based upon the fact that all organic compounds, with a few exceptions, can be oxidised by the action of strong oxidising agents under acidic conditions.

One of the limitations of the COD test is its inability to differentiate between biologically oxidisable and biologically inert organic matter. In addition it does not provide any evidence of the rate at which the biologically active material would be stabilised under conditions that exist in nature. As a result COD values are greater than BOD values and may be much greater when significant amounts of biologically resistant organic matter, such as lignin, is present. Nevertheless, the COD test, in comparison to the BOD test gives a rapid indication of the amount of oxygen a sample will demand when released to the aquatic environment.

2.12.1 CHEMISTRY OF COD TEST

Potassium dichromate has been found to be the most suitable oxidant[1]. It is capable of oxidising a wide variety of organic substances almost completely to carbon dioxide and water. Certain organic compounds, particularly low molecular weight fatty acids are not oxidised unless silver sulphate catalyst is present. Aromatic hydrocarbons and pyridine are not oxidised under any circumstances.

In the chemical reaction electrons are released by the organic molecule (glucose) being oxidised, such as

$$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 24H^+ + 24e^- \quad (2.59)$$

The electrons are accepted by hexavalent chromium (Cr^{6+}) which is reduced to trivalent chromium (Cr^{3+}):

$$Cr_2O_7^{2-} + 14H^+ + 6e^- = 2Cr^{3+} + 7H_2O \quad (2.60)$$

The two half reactions can be added after multiplying Equation (2.60) by 4 for balancing of electrons to yield:

$$4Cr_2O_7^{2-} + 32H^+ + C_6H_{12}O_6 + = 8Cr^{3+} + 6CO_2 + 22H_2O \quad (2.61)$$

The following steps may be followed to balance redox (reduction-oxidation) reactions.

[1] Oxidation is defined as a loss of electrons, reduction as a gain of electrons. The term oxidation originates from a 'reaction with oxygen' (where the substance that was oxidised loses electrons to oxygen).

Write the two half reactions separately showing the chemical species undergoing the change in oxidation state, for each case. Recognise the change in the oxidation numbers of species undergoing redox reactions. In Equation 2.59, the oxidation number of carbon atoms in glucose molecule is 0. It changes to +4 in carbon dioxide. In Equation 2.60, the oxidation number of chromium changes from +6 to +3.

The oxidation numbers for chemical species may be determined in most cases by first assigning oxidation numbers1+ to H, 2- to O and 0 to whole molecules (other oxidation numbers which do not change and may be of use are: Ca & Mg 2+, K & Na 1+). Then summing the charges after taking into account the number of atoms in the specie and assigning an oxidation number to the element of interest to obtain the net charge on the specie.

Thus for the carbon atom in glucose: H~(12x1+) + O~(6x2-)=0, therefore oxidation number for carbon is 0, since the molecule has no net charge. Similarly for the chromium atom in dichromate ion: O~(7x2-)=14-, therefore the oxidation number for chromium atom is 6+ since there are two atoms and the net charge on the specie is 2-.

Add appropriate number of electrons to balance the change in the oxidation numbers in the two half reactions. Thus electrons were added to the right hand side of Equation 2.59, since carbon is oxidised releasing electrons and to the left hand side of Equation 2.60, since chromium is reduced while accepting electrons.

Add appropriate number of either H^+ or OH^- ions to the left or right hand side of the two half reactions to balance the charges on the ionic species and the electrons.

Add water molecules to balance H and O atoms in the two half equations.

Multiply the half equations by suitable numbers to obtain the same number of electrons in each case.

Add the two half equations.

In the laboratory the COD test is carried out by digesting the sample with excess potassium dichromate and silver sulphate catalyst in strong sulphuric acid solution at an elevated temperature. In order to ensure that volatile compounds present in the sample and those formed during the digestion reaction do not escape, the digestion flask is fitted with a reflux condenser. Organic matter in the sample reduces part of the potassium dichromate and the remaining excess dichromate is titrated with ferrous ammonium sulphate (FAS).

$$6Fe^{2+} + Cr_2O_7^{2-} = 6Fe^{3+} + 2Cr^{3+} + 7H_2O \qquad (2.62)$$

FAS is a secondary standard and must be standardised against a primary standard such as potassium dichromate.

Note that Equations 2.59 and 2.60 are half equations while Equations 2.61 and 2.62, are complete oxidation - reduction equations. All four equations are balanced in terms of charges and elements participating in the reaction.

Example 2.25

Calculate the normality of 0.1M potassium dichromate solution used for oxidation of organic matter in COD test.

Solution:

From Equation 1 it is seen that hexavalent chromium in one mole of dichromate ion is reduced to trivalent chromium by accepting 6 electrons. Therefore each mole of dichromate has 6 equivalents and 0.1 M solution will be 0.6 *N*

The oxidiseable organic matter in the sample is reported in terms of oxygen equivalent.

Example 2.26

Calculate COD of a sample of water if 20 mL of the sample consumed 1.6 mL of 0.25 N potassium dichromate solution.

Solution:

Potassium dichromate consumed = 1.6 mL X 0.25 meq/mL = 0.4 meq

Therefore COD of the sample = 0.4 meq/ 20 mL X 1000 mL/ L = 20 meq/L

In terms of oxygen, COD = 20 meq/L X 8 mg O_2/meq = 160 mg/L.

It is important that organic matter from outside sources is not included in the COD value. Therefore, a distilled water blank is run along with the sample and an adjustment is made in calculations accordingly.

A very marked change in oxidation - reduction potential occurs at the end point of the titration with ferrous ammonium sulphate. Ferroin, 1,10 phenanthroline sulphate, is used as an indicator. It gives a very sharp change to brown colour on complete reduction of the dichromate by ferrous ammonium sulphate.

Example 2.27

Derive a formula for calculating COD value from the following information:

N_1 = *normality of ferrous ammonium sulphate (FAS)*

N_2 = *normality of potassium dichromate,*

V = *mL volume of potassium dichromate taken for reaction for sample and blank,*

A = *mL volume of FAS used in titration for sample after digestion,*

B = *mL volume of FAS used in titration for blank after digestion,*

S = *mL volume of sample and blank*

Solution:

meq of oxidant taken

= $N_2.V$,

meq of oxidant remaining in the sample and blank flasks after digestion

= $N_1.A$ and $N_1.B$, respectively.

Therefore meq of oxidant consumed in the sample and blank flasks

= $(N_2.V - N_1.A)$ and $(N_2.V - N_1.B)$, respectively,

and meq of oxidant consumed by the organic matter in the sample

= $(N_2.V - N_1.A) - (N_2.V - N_1.B) = (B - A).N_1$.

Hence COD of sample

= $(B - A).N_1$ X 1000/S, meq/L = $(B - A).N_1$ X 8000/S, mg O_2/L

Interferences

Certain reduced inorganic compounds can be oxidised under the conditions of the test and thus can cause erroneously high results. Chlorides cause the most serious problem because of their normally high concentration in water and wastewater samples. This interference can be eliminated by the addition of mercuric sulphate to the sample, prior to addition of other chemicals. The mercuric ion combines with the chloride ion to form a poorly ionised mercuric chloride complex.

The amino nitrogen in organic compounds is converted to ammonia. Nitrite, however, is oxidised to nitrates. But nitrite seldom occurs in significant amounts in natural water. This also holds true for other possible interference such as ferrous iron and sulphides.

2.12.2 RELATIONSHIP OF COD TO OTHER WATER QUALITY PARAMETERS

As mentioned earlier the COD value of a sample is always higher than its BOD value. This is due to the fact that the COD test measures both the biologically degradable and biologically non-degradable organic matter. Further, COD test measures the total oxygen equivalents of the organic matter while the BOD test measures oxygen equivalents of organic matter which is degraded in 3 days. This 3 day value is about 70 % of the ultimate BOD. However COD data can be interpreted in terms of BOD values after sufficient experience has been accumulated to establish reliable correlation factors. For domestic wastewaters and many decomposable industrial wastes, COD is about 2.5 times the BOD. For unpolluted surface and ground waters this ratio is higher.

COD value can be related to total organic carbon (TOC) value also which is a measure of organic carbon atoms in a sample. However it should be noted that two compounds might have the same TOC value but different COD values. This is because the COD value depends on the oxidation state of the carbon atoms in the compound.

Where formula of the organic compounds present in the waste is known, the COD value can be related to the theoretical chemical oxygen demand (ThCOD) which is the oxygen required to completely oxidise the organic matter to carbon dioxide and water.

Example 2.28

Calculate the theoretical chemical oxygen demand of a sample of wastewater from an industry containing 890 mg/L of glycine, $CH_3.NH_2.CH.COOH$.

Solution:

Write a balanced equation for oxidation, taking amino nitrogen to be converted to ammonia and carbon atoms to carbon dioxide.

Balance hydrogen atoms by adding required number of water molecules to the right side of the equation. Count number of oxygen atoms needed for balancing and add them to the left side as oxygen demand.

$$C_3H_7O_2N + 3O_2 = 3CO_2 + NH_3 + 2H_2O$$

Therefore one mole of glycine (89 g) has a theoretical chemical oxygen demand of 3 moles of oxygen (96g) or the waste will have a demand of (96/ 89) X 890 = 960 mg/L.

Standard solutions of pure organic compounds are often used to evaluate the accuracy of laboratory measurements.

3 BASIC CONCEPTS FROM MICROBIOLOGY

3.1 INTRODUCTION

Microbiology is the study of organisms, called *micro-organisms,* that are too small to be perceived clearly by the unaided eye. If an object has a diameter of less than 0.1 mm, the eye cannot perceive it at all, and very little detail can be perceived in an object with a diameter of 1 mm. In general, organisms with a diameter of 1 mm or less are micro-organisms, and fall in to the broad category of microbiology.

Micro-organisms bring about a number of important chemical transformations in nature. Production of alcohol, making of cheese and yoghurt, and retting of flax are some of the processes mankind has harnessed since prehistoric times. Micro-organisms are also responsible for breaking down organic matter in nature.

Antony van Leeuwenhoek (1632 - 1723) first discovered the microbial world. Some early scientists proposed a theory of spontaneous generation of microbial life. Pasteur in 1862 using sterilized equipment showed that growth of micro-organisms was possible only if an opportunity for outside contamination was provided. Rise of microbiology has refined the traditional microbiological processes and has added entirely new processes such as production of organic acids, solvents, vitamins, antibiotics, etc. An entirely different dimension to traditional microbiology has been added through genetic engineering.

Micro-organisms exist everywhere in nature: soil, air, water, the table top, your stomach. Each different environment has a specific set of micro-organisms which is ideally suited to living in that environment.

The (micro) biological characteristics of water, specifically the resident aquatic population of micro-organisms, impact directly on water quality. The most important impact is the transmission of disease by pathogenic organisms in water. Other important water quality impacts include the development of tastes and odors in surface waters and groundwaters and the corrosion and biofouling of heat transfer surfaces in cooling systems and water supply and wastewater management facilities.

To help understand the impact of the micro-organisms present in water on humans and on water quality, the following topics will briefly be covered.

- the classification used to group micro-organisms
- the scientific nomenclature used to describe micro-organisms
- nutritional requirements
- micro-organisms in water and wastewater

3.1.1 CLASSIFICATION OF MICRO-ORGANISMS

Before the advent of microscopy and discovery of micro-organisms, living beings were classified as either plants or animals. As knowledge of microbes grew it became apparent that many did not fit into either traditional class. Table 3.1 shows the taxonomic groupings used to accommodate the micro-organisms.

The principal groups of organisms found in water may be classified as protists (higher and lower), plants and animals. Commonly, the organisms listed in the table are characterised as *procaryotic* or

eucaryotic depending on whether the nucleus within the cell is enclosed in a well-defined nuclear membrane.

The microscopic forms of life are collectively classified as protists. Many protists are unicellular, but even the multi-cellular ones lack the internal differentiation into separate cell types and tissue regions characteristics of plants and animals. The micro-organisms generally recognized as protists include algae, bacteria, fungi and protozoa. There is an additional group of pseudo- organisms that are not visible through a light microscope: the viruses.

Kingdom	Representative Member	Cell Classification
Animal	Crustaceans Worms Rotifers	
Plant	Rooted aquatic plants Seed plants Ferns Mosses	Eucaryotic cells: Cells contain a nucleus enclosed within a well-defined nuclear membrane
Protista (Higher)	Protozoa Algae Fungi (molds and yeasts)	
Protista (Lower)	Blue-green algae Bacteria	Procaryotic cells: Nucleus not enclosed in a true nuclear membrane.

Table 3.1: Taxonomic grouping of micro-organisms

Electron microscopy has shown that the protists can be classified in two categories, higher protists, which have an organized cell, or eucaryotic cell, and lower protists which have a simple cellular structure or procaryotic cell. The eucaryotic cell is the unit of structure in protozoa, fungi and most groups of algae whereas the procaryotic cell is the unit of structure in bacteria and blue-green algae. The viral particles have a still simpler structure, which can not be classified, as a cell.

Eucaryotic cell

An eucaryotic cell is about 20 μm in diameter or larger. The main units of cell organization are:

- Nucleus consisting of sub-units called chromosomes which are composed of deoxyribonucleic acid (DNA), contains the genetic information. The nucleus is contained in a membrane.
- Mitochondria and chloroplasts are sites of energy generation.
- Vacuoles and lysosomes are involved in ingestion and digestion of food.
- Cytoplasm contains a colloidal suspension of proteins, carbohydrates and important organelles such as endoplasmic reticulum, Golgi apparatus and ribosomes, which are involved in protein synthesis. Cytoplasm is also a means of locomotion in cells without cell walls by amoeboid motion.
- Flagella provide a means of locomotion for cells, which have a rigid cell wall.

Procaryotic cell

These are usually smaller than 5 μm in diameter and have much simpler structure:

- Nucleus contains a single long molecule of DNA and is not separated from cytoplasm by a membrane.
- Cytoplasm occupies most of the space and is relatively uniform in structure.

- Enzymes for respiration and photosynthesis are housed in the cell membrane, which also regulates the flow of materials into and out of the cell.
- Most cells are surrounded by a rigid cell wall.
- Procaryotes move by the action of flagella.

Viruses

Viruses have a simpler chemical structure. They consist only of a protein coat surrounding a single kind of nucleic acid, either DNA or ribonucleic acid (RNA). With the exception of enzymes, which aid in penetration of the host cell they are devoid of enzyme activity and consequently can not be considered true cells. When a virus injects its nucleic acid into the host cell, it takes over the regulation of the cell and directs it towards the production of more viruses. The cell fills up with the newly formed viruses and then bursts spilling the viruses into the medium where each particle can infect other host cells.

Nomenclature for Micro-organisms

The nomenclature for micro-organisms, as for all organisms, is based on a classification system of 7 steps:

Kingdom
Phylum
Class
Order
Family
Genus
Species

In this classification system, the scientific name for any organisms includes both the genus and the species name. The genus name is always given first, and is capitalized. The species name is not captalized. Both names are always written in *italics.*

As an example, the scientific name for human beings is *Homo sapiens*. That is, humans belong to the genus *Homo*, and the species *sapiens*. The full classification of human beings is:

Kingdom:	Animal
Phylum:	Chordata
Class:	Mammalia
Order:	Primate
Family:	Hominidae
Genus:	*Homo*
Species:	*sapiens*

An example of the scientific name for a common bacteria is *Escherichia coli* (also known as *E. coli*). This bacterium belongs to the genus *Escherichia* and the species *coli*.

3.1.2 NUTRITIONAL REQUIREMENTS

To continue to reproduce and function properly, organisms must have sources of energy and carbon for synthesis of new cellular material. Inorganic elements, N and P and other elements such as S, K, Ca, and Mg are also vital. Organisms may be classified according to their sources of energy and carbon as given below (Table 3.2):

Micro-organisms may be further classified as aerobic, anaerobic or facultative depending upon their need for oxygen.

Classification	Energy source	Carbon*	Representative organisms
Photoautotroph	Light	Carbon dioxide	Higher plants, Algae, Photosynthetic bacteria
Photoheterotroph	Light	Organic	Photosynthetic bacteria
Chemoautotroph	Inorganic matter	Carbon dioxide	Bacteria
Chemoheterotroph	Organic matter	Organic matter	Bacteria, fungi, protozoa, animals
* organisms that use carbon dioxide as their only source of carbon are called self-feeding (autotroph). Autotrophy does not refer to energy source.			

Table 3.2: Organism classification based on energy and carbon source

3.1.3 IMPORTANT TYPES OF MICRO-ORGANISMS

Bacteria

Bacteria are single cell protists. They are of the Kingdom of the lower protists, meaning they are procaryotic cells, with no clear nucleus, only a nuclear area. They use soluble food and reproduce by binary fission.

There are thousands of bacteria, but their general form falls in one of the four categories (Table 3.3):

- Spherical (cocci),
- cylinderical rods (bacilli),
- curved or helical rods (vibrio and spirilla), and
- filaments.

Most bacteria are about 0.5 to 1.0 micron in diameter. The spiral forms may be as long as 50 microns. The multicellular filamentous bacteria may be 100 microns or longer.

Shape	general name	size range
sphere	cocci (coccus, singular)	1-3 μm
rod	bacilli (bacillus, singuar)	0.3-1.5 μm (diameter)
curved rod or spiral	vibrio (curved)	0.6-1 μm (diam), 2-6 μm (length) up to 50
filament, (chains of	variety of names	100 μm and longer

Table 3.3: Classification of bacteria according to shape

Metabolically, most bacteria are heterotrophic. The autotrophic forms obtain energy by oxidation of inorganic substrates such as ammonia, iron and sulphur. There are a few autotrophic photosynthetic

bacteria also. Depending on their metabolic reaction, the bacteria may be classified as aerobic, anaerobic or facultative. Various pathogenic bacteria cause many diseases of man.

The group known as 'enteric bacteria' are a major cause of infection through food or water in all countries. The most common enteric bacteria are *Salmonella typhimurium, Escherichia coli,* and *Shigella* species. In all these infections, live bacteria must be ingested to produce disease. Following ingestion, symptoms begin after variable periods of time, usually 7 – 48 hours for *Salmonella typhimurium,* and 1-4 days for *Escherichia coli.* Abdominal pain and diarrhoea are the most prominent symptoms, but vomiting and fever are also common, persisting from several days to several weeks. Most adults recover, but loss of fluids can lead to death, especially for children and elderly people. Enteric bacteria act by colonizing the intestines and secreting toxins, namely enterotoxins. The resulting sickness is often known as gastro-enteritis.

Typhoid fever, which is caused by *Salmonella typhi*, is endemic to many areas. The disease is acquired by ingesting food or water contaminated with human faeces. Within 6 to 14 days after exposure, headache and fever develop. The fever can continue for several days and rise above 40 ^{o}C. In most cases *S. typhi* is shed in the feces for several weeks. Approximately 3 percent of the patients who recover become carriers of the disease and continue to shed the organism for extended periods.

Cholera is another major disease caused bacteria. Throughout history, it has been a major disease in India and other parts of Southeast Asia, but is also known in many other areas, where it occurs in epidemics. The disease is caused by the bacteria *Vibrio cholerae*, a motile, Gram-negative rod bacteria. The disease is acquired by ingesting food or water contaminated by faecal material. After an incubation period of 2-5 days, diarrhoea and abdominal pain begin suddenly, and vomiting can occur. The bacteria acts by colonizing the intestines and secreting cholera toxin.

Viruses

Viruses are sub-microscopic particles that are unable to replicate or adapt to environmental conditions outside a living host. Thus all viruses are parasites, and require a host organism to survive and reproduce. A virus consists of a strand of genetic material, DNA or RNA, within a protein coat. The particles doe not have the ability to synthesize new compounds. Instead, they invade living cells, where the viral genetic material redirects cell activities towards production of new viral particles, at the expense of the host cell growth. When the infected host cell dies, large numbers of viruses are released to infect other cells.

All viruses are extremely host specific. Thus a particular virus can attack only one species of organism. A number of viral diseases are commonly transferred via water. The viruses of most significance in water quality are the enteric viruses, i.e. those that inhabit the intestinal tract. Viruses ingested from water can result in a variety of diseases including hepatitis, and diseases of the central nervous system caused by the poliovirus, coxsackieviruses, and echoviruses.

Fungi

Fungi are of the Kingdom of the higher protists. They are aerobic, multicellular, non-photosynthetic organisms having eucaryotic cells (i.e. clear cell nucleus with nuclear membrane). Most fungi are *saprophytes,* obtaining their food from dead organic matter. Fungi are mostly multinucleate organisms which have a vegetative structure known as mycelium. The mycelium consists of a rigid, branching system of tubes, through which flows a multinucleate mass of cytoplasm. A mycelium arises by the germination and outgrowth of a single reproductive cell, or spore. Yeasts are fungi that can not form a mycelium and are therefore unicellular.

Together with bacteria, fungi are the principal organisms responsible for the decomposition of carbon in the biosphere. In contrast to bacteria, fungi can grow in low-moisture areas, and they can grow in

low-pH environments. Because of these characteristics, fungi play an important role in the breakdown of organic matter in both aquatic and terrestrial environments. As organic matter is decomposed, fungi release carbon dioxide to the atmosphere and nitrogen to the terrestrial environment.

Fungi vary in size from microscopic organisms to mushrooms, and are often divided into the following five classes:

1. Myxomycetes, or slime fungi
2. Phycomycetes, or aquatic fungi
3. Ascomycetes, or sac fungi
4. Basidiomycetes, including mushrooms
5. Fungi imperfecti, or miscellaneous fungi

With respect to water quality, the first two classes are the most important.

Algae

The name algae, is applied to a diverse group of eucaryotic micro-organisms that share some similar characteristics. Algae are unicellular or multi-cellular, autotrophic, photosynthetic protists. They are classified according to their photosynthetic pigment and taxonomic and biochemical cellular properties. They range in size from tiny single cells to branched forms of visible length.

Typically, algae are organisms which contain chlorophyll and are capable of photosynthesis. The presence of chlorophyll in the algae. is the principal feature used to distinguish them from fungi. In addition to chlorophyll, other pigments encountered in algae include carotenes (orange) and other colored pigments (red, brown, blue, yellow). Combinations of these pigments result in the various colors of algae observed in nature.

Algae, like all higher plants, utilize CO_2 and light for the synthesis of cell carbon, in a process known as photosynthesis. The light is absorbed by the cell pigments (e.g. chlorophyll or carotenoids), giving the energy for the photosynthetic reaction to occur. Oxygen is produced during the process. At night, algae utilize oxygen in the process of respiration. Respiration also occurs during the daytime, but the amount of oxygen consumed is less than the amount of oxygen produced by photosynthesis. Simplified reactions for photosynthesis and respiration are given below:

Photosynthesis: $6CO_2 + 6H_2O + \text{nutrients} \longrightarrow 6CH_2O + 6O_2$

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

The major groupings used to classify algae are given in Table 3.4.

Group	Descriptive name	aquatic habitat	description
Chlorophyta	Green algae	fresh and salt water	can be unicellular or multi-cellular
Chrysophyta	Diatoms, golden-brown algae	fresh and salt water	Mostly unicellular. Diatoms have shells composed mainly of silica.
Chryptophyta	Cryptomonads	salt water	
Euglenophyta	Euglena	fresh water	motile, colonial, unicellular and flagellated.
Cyanophyta	Blue-green	fresh and salt water	unicellular, usually enclosed in a sheath, no flagella. Can use N_2 from atmosphere as nutrient in cell synthesis.
Phaeophyta	Brown algae	salt water	
Pyrrhophyta	Dinoflagellates	fresh and salt water	
Rhodophyta	Red algae	fresh and salt water	
Xanthophyta	Yellow-green algae	fresh and salt water	

Table 3.4: Classification of major algae groups

Protozoa

Protozoa are single-cell micro-organisms of the Kingdom Protista. Protozoa are motile, unicellular, aerobic heterotrophs. They are an order of magnitude larger than bacteria and often consume bacteria as a food source. They are eucaryotic cells, but have no cell walls.

Most protozoa are free-living in nature, although several species are parasitic, and must live on or in a host organism. The host organism can vary from primitive organisms such as algae to highly complex organisms, including human beings. The 4 major groups of protozoa are given in Table 3.5.

Class	Mode of motility	Parasitic	Typical members	
			Name	Remarks
Ciliata	cilia (usually multiple)	No	*Paramecium*	free-swimming
Mastigophora	flagella (one or more)	usually parasitic	*Giardia lamblia* *Trypanosoma gambiense*	causes giardiasis causes African sleeping sickness
Sarcodinia	pseudopodia (amoeboid motion); some have flagella	some parasitic	*Entamoeba histolytica*	causes amoebic dysentery
Sporozoa	creeping, spore forming; often non-motile flagella at some stages	parasitic.	*Plasmodium vivax*	causes malaria

Table 3.5: Major groups of protozoa

Giardiasis is the most widespread of the protozoan diseases occurring throughout the world. This intestinal disease is caused by ingestion of food or water contaminated with faeces from humans or other mammals containing the flagellate (Mastigophora), *Giardia lambia*. *Giardia lambia* colonizes the small intestine and causes diarrhoea and abdominal pain.

Worms

A number of worms are of importance with respect to water quality, primarily from the standpoint of human disease. Two important worm categories (Phylum) are the Platyhelminthes and the Aschelminthes. The common name for the phylum Platyhelminthes is flatworm. Free-living flatworms of the class Turbellaria are present in ponds and quiet streams all over the world. The most common form is planarians. Two classes of flatworms are composed entirely of parasitic forms. They are the class Trematoda, commonly known as flukes, and the class Cestoda, commonly known as tapeworms.

The most important members of the phylum Aschelminthes are the nematodes. About 10,000 species of nematodes have been identified, and the list is growing. Most nematodes are free-living, but it is the parasitic forms which cause several human diseases:

- *Trichinella*, causes trichinosis
- *Necator*, causes hookworm
- *Ascaris*, causes common roundworm infestation
- *Filiria*, causes filariasis: blocking of the nymph nodes, causing permanent tissue damage (e.g. elephantitis).

Rotifers

Rotifers are the simplest of multi-cellular animals. The name is derived from the apparent rotating motion of the cilia located on the head of the organisms. Metabolically, rotifers can be classified as aerobic chemoheterotrophs. Bacteria are the principal food source for rotifers.

Crustaceans

Like rotifers, crustaceans are aerobic chemoheterotrophs that feed on bacteria and algae. These hard-shelled, multi-cellular animals are an important source of food for fish.

3.2 LABORATORY TECHNIQUES

Most bacteria in water are derived from contact with air, soil, living or decaying plants or animals, and faecal excrement. Many of these bacteria have no sanitary significance as they are not pathogenic and are not suspected of association with pathogenic micro-organisms. A variety of procedures have been used to measure the bacteriological quality of water:

1. Total plate count at 20^{o}C and at 37^{o}C.
2. Presence of coliform bacteria as indicators of sewage contamination
3. Identification of specific pathogenic bacteria
4. Employing miscellaneous indicators and serological methods

The specific identification of pathogenic bacteria requires large samples, a variety of laboratory procedures and is time consuming and costly. Such tests are therefore not applicable to routine testing and monitoring. Tests for coliform group of bacteria and in some cases the total plate count are considered adequate to indicate sanitary quality of water.

To aid identification the microbiologist normally uses techniques known as ‘isolation’ and ‘cultivation’. Isolation is the separation of a particular organism from a mixed population whilst cultivation is the growth of that organism in an artificial environment (often in an incubator at a constant temperature because many micro-organisms only grow well within a limited range of temperatures).

3.2.1 CULTURE MEDIA

Bacteria are grown in laboratory by providing them with an environment suitable for their growth. The growth medium should contain all the correct nutrients and energy source and should be maintained at proper pH, salinity, oxygen tension and be free of antibacterial substances.

Such media can be either liquid or solid. A solid medium is generally the corresponding liquid medium made up with a gelling agent. Agar extracted from certain sea weeds is a particularly useful gelling agent as it is hydrolysed by only a very few bacteria. It melts at 100 °C but does not solidify until below 40°C.

Growth in liquid media is demonstrated by turbidity. This may be accompanied by a change in colour of an indicator incorporated in the medium to detect acid or alkali production. Gas production resulting from break down of sugars, etc., in the medium may be detected by placing a small inverted test tube (Durham tube) in the main tube to trap some of the gas evolved.

In agar medium the bacteria are trapped on the surface or in the depth of the medium when it is in liquid state. Once the medium solidifies after it cools below 40^{o}C no movement is possible. The cells are only able to grow locally and a cluster of cells, or colony, visible to naked eye is eventually formed.

Liquid media, broths, are dispensed either into rimless test tubes closed by an aluminum cap or a plug of non-absorbent cotton, or into screw capped bottles. Solid, agar media are most commonly poured whilst liquid into shallow, covered dishes (petri dishes or plates) which may contain a small amount of sample.

Media may be selective or non-selective. A nonselective medium permits the growth of all species at the chosen pH, temperature, salinity and oxygen tension. Selective media may contain ingredients which are utilizable only by the selected group and may also contain additional ingredients toxic to others.

Frequently an enrichment medium is used. Such a medium contains a greatly increased amount of a particular ingredient required mainly by the organism to be enriched. The organism outgrows all other types and establishes itself as the dominant organism.

Sterilisation and aseptic techniques

The two most popular methods of laboratory sterilisation (that is, the process of destroying all life forms) both involve heat. Dry heat of 170°C for 90 minutes can be used to sterilise objects which are heat stable such as all glassware. Steam must be used for sterilising aqueous solutions and other items which cannot be placed in an oven. This type of sterilisation is normally carried out in an autoclave at a pressure of 1.06 kg/cm^2, which corresponds to a temperature of 121°C. The time required for steam sterilisation depends upon the material being sterilised, usually 20 - 30 minutes are sufficient.

Due to the fact that micro-organisms can be present virtually anywhere, it is important to take measures to avoid contamination of microbiological experiments with extraneous bacteria. The measures used to prevent this cross-contamination in microbiological laboratories are collectively known as aseptic technique.

Water samples or bacterial cultures are introduced into sterile media by aseptic procedures to ensure that stray bacteria are not introduced. Asepsis in achieved by:

- Washing one's hands and cleaning the bench area with swab soaked in methylated spirit.
- Working in a dust and draught free area.
- Not touching any part of the container, pipette, etc., which will come in contact with the sample or culture.
- Not removing the lid of a petridish or cap of a test tube longer than absolutely necessary.
- Lightly flaming the top of test tubes, ends of pipettes, necks and stoppers of bottles by a flame before and after adding or withdrawing samples or inocula.
- Transferring culture from one tube to a fresh tube (subculturing) using a wire loop sterilized by heating to redness and cooled.

3.2.2 IDENTIFICATION AND ENUMERATION

Microscopic examination

It is frequently necessary to look at bacteria under light microscope. Usually an oil immersion lens of X1000 magnification is used with a X10 eyepiece to give a magnification of about XI0,000. Before bacteria can be seen under the light microscope they are fixed to a microscope slide by spreading a loop full of cellular mass on a slide and lightly flaming and then stained with a suitable dye.

Gram stain is the most widely used staining method. Those bacteria which after staining with crystal violet resist decolorisation with acetone or alcohol and can not be counter stained are called Gram positive. Those which take up a counter stain such as safranin are called Gram negative. A slide prepared with a mixed culture will show Gram positive organisms blue and Gram negative pink coloured.

The identification of micro-organisms can be accomplished with microscopy The disadvantage of using a microscope for identification is that it can be time-consuming and require specialist knowledge. Further many different organisms are so similar in morphology that a positive identification cannot be made.

Metabolic reactions

Bacteria can be differentiated on the basis of their metabolic reactions and requirements for nutrition and growth environment. Microbiologists have devised series of tests to identify bacteria even up to the specie level. An organism may be identified on the basis of one or more of the following:

- ability to utilise a particular compound as a substrate for growth
- requirement of an essential compound for growth
- excretion of identified endproducts when using a given substrate
- ability to grow in the presence of a toxicant
- ability to grow in a given environment.

Counts in solid media

Plate count is the most common procedure. The basis of counting is to obtain colonies derived from each cell. These colonies are counted and the count per ml of sample is calculated. Obviously the colonies must be well separated otherwise it would be impossible to count them. A sample containing more than 300 cells per ml must be diluted when a standard 100 mm diameter petri dish is used. The diluent should be a standard salt solution. If the number of cells present is approximately known from previous work, it is not necessary to adhere to the procedure illustrated. For statistically reliable data the plate count should be between 30 and 300. Confidence (95% level) limits for the counts may be calculated from the formulae:

Upper limit = $N + 2\ (2 + \sqrt{N})$

Lower limit = $N - 2\ (1 + \sqrt{N})$

where N is the number of colonies counted.

The count per ml should be multiplied by the dilution factor.

Counts in liquid media

Counts in liquid media are not as accurate as counts in solid media but they are employed where the organism produces an easily identifiable end product, such as a gas or an acid. The principle employed is dilution to extinction. Broadly stated, if a series of culture tubes are inoculated with equal volumes of a sample then some tubes may show growth while some may not depending upon the concentration of bacteria in the sample and volume of the inoculum. It is then possible to statistically estimate the concentration of the bacteria. In order to increase the statistical reliability of the test a series of tubes are inoculated with varying volumes of the sample or diluted sample. The count thus obtained is called the Most Probable Number (MPN) of cells per stated volume of the sample. The procedure will be discussed in detail in the section on 'Coliforms bacteria'.

Sampling

Samples must be representative of the bulk from which they are taken. Sterilized bottles should be used and aseptic procedures should be followed. Samples from an open body of water should be collected 30 cm below the surface. Samples from water supply systems, which may contain chlorine, should be dechlorinated. Samples should be stored at 4 °C and analyzed within eight hours of collection.

When taking samples care should be taken to ensure that the inside surfaces of the bottle and cap are not touched by the sampler's hand or any other objects as this may lead to contamination of the sample. When the sample is collected, it is important that sufficient airspace (at least 2.5 cm) is left in the bottle to allow mixing by shaking prior to analysis.

Good Laboratory Practice

The use of good laboratory practice is an important factor in safeguarding the health and safety of laboratory personnel. It should be remembered that many of the bacteria which are cultured in aquatic microbiological laboratories are capable of producing disease in humans. This, coupled with the fact that, potentially more virulent, pure strains of such bacteria are often being produced, means that there is considerable risk to the health of microbiology laboratory workers if adequate precautions are not taken.

The basis of good practice in a microbiological laboratory can be summed up by the following:

- ensure all necessary equipment and media is sterilised prior to use
- ensure that all sterilised equipment and media is not re-contaminated after sterilisation by allowing it to touch, or rest on, any unsterilised surface
- frequently disinfect hands and working surfaces
- as far as possible, eliminate flies and other insects which can contaminate surfaces, equipment, media and also pass organisms to laboratory personnel
- never pipette by mouth samples which are suspected to have high bacterial concentrations
- wear appropriate protective clothing
- do not eat, drink or smoke in the laboratory
- sterilise contaminated waste materials prior to disposal
- take care to avoid operations which result in bacterial aerosols being formed

3.3 COLIFORM BACTERIA

The problem of waterborne diseases is relevant in many countries particularly the developing countries. The impact of high concentrations of disease-producing organisms on water users can be significant.

Disease producing organisms are also known as 'pathogens'. Pathogenic organisms can generally be classified as:

- pathogenic bacteria
- viruses
- parasites (e.g. protozoa and intestinal worms (helminths))

Some examples are listed in Table 3.6.

Type	***Examples***	***Diseases***
Pathogenic bacteria	*Vibrio cholerae*	cholera
	Salmonella species	typhoid
	Shigella species	dysentery
Viruses	Hepatitis A	hepatitis
	Polio viruses	polio
	Enteroviruses Echoviruses	central nervous system disorders
Intestinal parasites (protozoa and intestinal worms – helminths)	*Giardia lambia*	giardiasis (diarrhoeal disease)
	Entamoeba histolytica	amoebic dysentery
	Facultatively parasitic amoebae (*Naegloria* and *Hartmanella)* Helminths (e.g. whipworm, hookworm, dwarf tapeworm)	

Table 3.6: Classification of pathogenic micro-organisms

Many, but not all, of these pathogenic organisms are of faecal origin, The method of transmission of pathogens is through ingestion of contaminated water and food, and exposure to infected persons or animals. Infections of the skin, eys, ears, nose and throat may result from immersion in water while bathing. Specific modes of infection are:

- drinking water: municipal, domestic, industrial and individual supplies
- direct (primary) contact with polluted water: bathing
- secondary contact with polluted water: boating, fishing, clothes washing
- eating fish / shellfish, contaminated food.

Methods of measurement of bacteriological quality can be:

- direct analysis for pathogenic bacteria
- analysis for viruses
- analysis for intestinal parasites
- analysis for **indicator bacterial groups** which reflect the potential presence of pathogens, e.g. coliform bacteria

The determination of specific pathogenic bacteria, viruses, or parasites requires a high degree of expertise, especially when they are present in low numbers. The use of indicator bacterial groups has always been a favoured method.

3.3.1 INDICATOR BACTERIA

The group of coliform bacteria as an indicator of other pathogenic micro-organisms, specifically organisms of faecal origin, has had much emphasis in all countries. This is due primarily to the fact that the coliform bacteria group meet many of the criteria for a suitable indicator organism, and are thus a sensitive indicator of faecal pollution:

- they are abundant in faeces (normal inhabitants of intestinal tract)
- they are generally found only in polluted waters,
- they are easily detected by simple laboratory tests,
- can be detected in low concentrations in water
- the number of indicator bacteria seems to be correlated with the extent of contamination.

It is important to remember, however, that not all coliforms emanate from human faeces as they can originate from other mammalian species or from other environmental sources (e.g., bird droppings).

When coliforms are discharged to the aquatic environment they will tend to die at a rate which depends, amongst other things, on the temperature and turbidity of the water and the depth to which solar radiation penetrates. Therefore, it is not safe to conclude that the lack of coliforms in a water means that it has not been subject to faecal pollution.

Text books of microbiological analysis use differing terminology to refer to the coliform group of organisms. For this reason, it is necessary to be familiar with a number of terms which may be used in this context as follows:

Total coliforms

The Total coliform group comprises several distinct types (genera) of bacteria. These bacteria have been isolated from the faeces of humans and other warm-blooded animals. Some coliform bacteria are normal inhabitants of soil and water. In testing for coliforms, therefore, tests may be run in conjunction to verify their faecal origin. However, this group of bacteria is widely used as a measure of health hazard from faecal contamination. Pathogenic bacteria and viruses causing enteric diseases in humans also originate from faecal discharges of diseased persons. Consequently, water containing coliform bacteria is identified as potentially dangerous. The total coliform group comprises the aerobic and facultative, gram negative, nonspore-forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35 °C.

Faecal coliforms (Thermotolerant coliforms)

The Faecal coliform group of bacteria are indicative of faeces of humans and other warm blooded animals. The specific bacterium *Escherichia coli* is part of this group. The test for faecal coliform is at an elevated temperature, 44.5 °C, where growth of other non-faecal bacteria is suppressed. Only a small percentage of non-faecal bacteria may be also be identified in the faecal coliform test (<5%)).

Faecal streptococci

This group of bacteria includes several species or varieties of streptococci and the normal habitat of these bacteria is the intestines of humans and animals. Examples include *Streptococci faecalis* which represents bacteria of humans and *Streptococci bovis* and *Streptococci equinus* which represent bacteria that are indicators of cattle and horses.

Escherichia coli (E. coli)

This bacterium is a particular member of the faecal coliform group of bacteria; the presence of this organism in water indicates faecal contamination. The bacterium *E.coli* is exclusively of faecal origin.

E. coli reside in human intestinal tracts. They are excreted in large numbers in faeces, averaging about 50 million per gram. Untreated domestic wastewater generally contains 5 to 10 million coliforms per 100 ml.

The degree to which indicator organisms represent the presence of individual pathogens (such as Salmonella) has been the subject of continuing investigation. There does seem to be a general correlation between the concentration of Faecal coliform bacteria and the occurrence of *Salmonella*. When faecal coliform numbers are about 1000 per 100 ml, *Salmonella* occurrence is about 95 %

Relationships between total coliform and individual pathogens is not so quantitative. Thus the test of total coliform is not so effective as an indicator. The total coliform test is complicated by the presence of non-faecal bacteria. As a general rule, faecal coliform levels are about 20% of total coliform concentrations, although a wide spread exists.

Bacterial Standards

Many countries or international organisations have water quality standards for bacteria. A few are given in Table 3.7.

Use	Total Coliform MPN per 100 ml	Faecal Coliform MPN per 100 ml	Agency / Country
Public water supply	0	0	WHO, India
Drinking water source, no conventional. treatment, with disinfection	<50	no value	India
Drinking water source, with conventional treatment, and disinfection	5000	no value	India
Bathing, recreation water	5000 guide 10,000 mandatory	100 guide 2000 mandatory	Europe
Outdoor bathing (organized)	500	no value	India
Shellfishing	70	no value	US
	no value	14	Venezuela, Mexico

Table 3.7: Some Indian and international water quality standards for indicator bacteria groups.

3.3.2 COLIFORM ANALYSIS

The test for total coliform bacteria is usually conducted using a liquid culture. Enumeration employing solid culture media is not commonly done in India. The liquid culture 'multiple tube technique' consists of 2 stages:

1. presumptive test
2. confirmed test

The presumptive test is based on gas production during fermentation in enrichment medium which contains beef extract, peptone and lactose within 48 hour of incubation at 35^{o}C.

The confirmed test is used to substantiate or deny the presence of coliforms in a positive presumptive test. A small inoculum from a positive lactose broth is transferred to a tube containing brilliant green lactose bile broth. The green dye and bile salts in this broth inhibit non- coliform growth. The presence of coliform is confirmed by growth and gas production within 48 hour at 35^{o}C. The Most Probable Number (MPN) of total coliform is then calculated from the number of confirmed tubes.

Faecal coliform test

Sometimes a 'completed test' may be performed to determine the faecal origin of the coliforms giving positive confirmative test. These tests involve subculturing of the positive tubes on solid media and testing for further bio-chemical reactions.

Elevated temperature test for the separation of organisms of coliform group into those of fecal and nonfecal origin may also be performed. In this test, transfers from all positive presumptive tubes are made to culture tubes of EC medium which contains bile salts and sodium chloride as selective agents along with nutrients. The inoculated tubes are incubated at 44.5 ± 0.2 ^{O}C. Gas production within 24 hour is considered a positive reaction indicating coliforms of faecal origin.

Methods of Analysis

There are two basic analyses which can be performed to determine the presence of coliform bacteria. These are the 'multiple tube' technique and the 'membrane filter' method. A comparison of the two methods is given in Table 3.8 below.

Multiple Fermentation Tube Method	***Membrane Filter Method***
Slower: requires 48 hours for a positive or presumptive positive	More rapid: quantitative results in about 18 hours
More labour intensive	Less labour intensive
Requires more culture medium	Requires less culture medium
Requires more glassware	Requires less glassware
More sensitive	Less sensitive
Low precision	High precision
Difficult to use in the field	Can be adapted for field use
Applicable to all types of water	Not applicable to turbid waters
Consumables readily available	Cost of consumables is high
May give better recovery of stressed or damaged organisms	Poorer recovery because of direct inoculation in selective medium

Table 3.8: Comparison of coliform analysis methods

Multiple Tube Method

As referred to above, the multiple tube technique is applicable to many different water samples including those obtained from potable, fresh, brackish and salt waters. The test can also be used for the estimation of coliform bacteria in muds, sediments and sludges.

The method, which has been successfully used in many countries for the analysis of drinking and other waters, reports coliform results in terms of the 'most probable number' (MPN) of organisms. That is, the test gives the most likely number of coliform bacteria rather than the actual number.

The basis of the test is that multiple tubes of culture medium are inoculated with various dilutions of a water sample and incubated at a constant temperature for a given period of time. If coliforms are present in a tube this is detected by growth within the tube and the production of gas. Any gas produced is collected in an inverted gas collection tube placed within a larger test tube containing the culture medium. The result of the analysis, in terms of the most probable number of coliforms, depends upon the number of tubes which show a positive reaction.

Typically, the MPN value is determined from the number of positive tests in a series of 5 replicates made from 3 different dilutions or inoculation amounts (15 samples altogether). For example, sample inoculation amounts may be 10, 1 and 0.1 ml per test tube. The test method can be described as follows:

For drinking water, high numbers of coliform bacteria are not expected, so there is no need to make dilutions. Transfer a 10 ml sample into each of 10 test tubes containing a lactose culture medium and an inverted gas collection tube. MPN results can be read from Table 3.9.

For non-potable water, rivers, open wells and tanks transfer a 10 ml, 1 ml and 0.1 ml sample into 5 test tubes each (i.e. a total of 15 tubes). MPN results can be read from Table 3.10.

For non-potable and polluted waters, smaller volumes, i.e. 1 ml, 0.1 ml and 0.01 ml sample should be transferred into 5 test tubes each (i.e. a total of 15 tubes). Transferring small sample amounts is difficult, so first a series of dilutions is made . The next step is to transfer a 1 ml sample from each dilution to each of 5 test tubes containing a lactose culture medium and an inverted gas collection tube. The MPN value is read again from Table 3.10 and the result is multiplied by the dilution factor.

In each case, the inoculated tubes are incubated in an incubator or a water bath at 35 ± 0.5^{o}C for $24 \pm$ 2hours. The accumulation of gas in the inverted gas-collection tubes after 24 hours is considered to be a positive presumptive test for total coliform bacteria. The number of positive tubes is confirmed as described earlier.

For the combination of positive tubes not appearing in Table 3.10, or in case the table is not available, the following formula is used:

$$\text{MPN/100 mL} = \frac{\text{no. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

When using the above equation, remember that the count of positive tubes starts with the highest dilution in which at least one negative result has occurred.

When more than three test dilutions are incubated, the following rules are used in determining MPN value:

- Choose the highest dilution that gives positive results in all five portions tested or the largest number of positives and the two next higher dilutions.
- Where positive results occur in dilutions higher than the three chosen according to the above rule, they are incorporated in the results of the highest chosen dilution up to a total of five.
- If only one dilution gives a positive result, two dilutions immediately lower and higher giving zero positives should be chosen so as to keep the positive result in the middle of the series.

No of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index / 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	< 1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	> 23.0	13.5	Infinite

Table 3.9: MPN Index and 95% Confidence Limits for Various Combinations of Positive and Negative Results when Ten 10 mL Portions are used

Some examples for estimation of MPN for various cases are given in Table 3.11. The selected combinations are shaded. Calculations are explained below:

- Ex. 1 Regular reading of Table 3.10
- Ex. 2 Unusual combination, formula calculation: 1200/ (12x55.5)1/2 = 47
- Ex. 3 Adjust the selected positive tube set as 5-3-3, followed by regular reading of Table 3.10 x 100
- Ex. 4. Regular reading of Table 3.10 x 1000
- Ex. 5 Adjust the selected set as 5-0-5, which is unusual combination, formula calculation: $500/ (0.5x0.55)^{1/2}$=953
- Ex. 6 Adjust the selected positive tube set as 5-4-4, followed by regular reading of Table 3.10 x 100
- Ex. 7 Regular reading of Table 3.10 x 100
- Ex. 8 Regular reading of Table 3.10 x 10
- Ex. 9 Regular reading of Table 3.10
- Ex. 10 Adjust the selected positive tube set as 5-3-2, followed by regular reading of Table 6 x 10

Combination of Positives	MPN Index / 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0-0-0	< 2	-	-
0-0-1	2	1.0	10
0-1-0	2	1.0	10
0-2-0	4	1.0	13
1-0-0	2	1.0	11
1-0-1	4	1.0	15
1-1-0	4	1.0	15
1-1-1	6	2.0	18
1-2-0	6	2.0	18
2-0-0	4	1.0	17
2-0-1	7	2.0	20
2-1-0	7	2.0	21
2-1-1	9	3.0	24
2-2-0	9	3.0	25
2-3-0	12	5.0	29
3-0-0	8	3.0	24
3-0-1	11	4.0	29
3-1-0	11	4.0	29
3-1-1	14	6.0	35
3-2-0	14	6.0	35
3-2-1	17	7.0	40
4-0-0	13	5.0	38
4-0-1	17	7.0	45
4-1-0	17	7.0	46
4-1-1	21	9.0	55
4-1-2	26	12	63
4-2-0	22	9.0	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9.0	86
5-0-1	30	10	110

Combination of Positives	MPN Index / 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360
5-3-3	170	80	410
5-4-0	130	50	390
5-4-1	170	70	480
5-4-2	220	100	580
5-4-3	280	120	690
5-4-4	350	160	820
5-5-0	240	100	940
5-5-1	300	100	1300
5-5-2	500	200	2000
5-5-3	900	300	2900
5-5-4	1600	600	5300
5-5-5	≥ 1600	-	-

Table 3.10. MPN Index and 95% Confidence Limits for Various Combinations of Positive Results with Five Tubes per Dilution (10 mL, 1.0 mL, 0.1 mL)

Ex.No	10 mL	1 mL	0.1 mL	0.01 mL	0.001 mL	0.0001 mL	MPN index /100mL	MPN /100mL
1.	5/5	3/5	1/5	-	-	-	110	110
2.	4/5	3/5	5/5	-	-	-		46
3.	5/5	5/5	5/5	3/5	2/5	1/5	170	17000
4.	5/5	5/5	5/5	5/5	3/5	2/5	140	140000
5.	5/5	5/5	0/5	3/5	2/5	1/5		953
6.	5/5	5/5	5/5	4/5	3/5	1/5	350	35000
7.	-	5/5	5/5	2/5	0/5	-	50	5000
8.	-	5/5	4/5	2/5	0/5	-	220	2200
9.	0/5	1/5	0/5	0/5			2	2
10.	-	5/5	3/5	1/5	1/5	-	140	1400

Table 3.11: Examples for reading and calculating MPN values.

4 PRINCIPLES OF INSTRUMENTAL ANALYSIS

4.1 ELECTRICAL CONDUCTIVITY

As in the case of metallic conductors, electrical current can flow through a solution of an electrolyte also. For metallic conductors: current is carried by electrons, chemical properties of metal are not changed and an increase in temperature increases resistance. The characteristics of current flow in electrolytes in these respects are different: the current is carried by ions, chemical changes may occur in the solution and an increase in temperature decreases the resistance.

Electrical conductivity (EC) is a measure of the ability of water to conduct an electric current and depends on:

Concentration of the ions (higher concentration, higher EC)

Temperature of the solution (high temperature, higher EC)

Specific nature of the ions (higher specific ability and higher valence, higher EC)

Conductivity changes with storage time and temperature. The measurement should therefore be made in situ (dipping the electrode in the stream or well water) or in the field directly after sampling. The determination of the electrical conductivity is a rapid and convenient means of estimating the concentration of ions in solution. Since each ion has its own specific ability to conduct current, EC is only an estimate of the total ion concentration.

4.1.1 EQUATIONS AND DIMENSIONS

Ohm's law defines the relation between potential (V) and current (I). The resistance (R) is the ratio between V and I:

$$R = \frac{V}{I} \tag{4.1}$$

The resistance depends upon the dimensions of the conductor, length, L, in cm, cross-sectional area, A, in cm^2 and the specific resistance, ρ, in ohm.cm, of the conductor:

$$R = \rho \times \frac{L}{A} \tag{4.2}$$

In the present case our interest is in specific conductance or electrical conductivity (which is the preferred term), the reciprocal of specific resistance, κ, is 1/ohm.cm or Siemens per centimetre, S/cm, which can be thought of as the conductance offered by $1cm^3$ of electrolyte:

$$\kappa = \frac{1}{\rho} = \frac{L}{A} \times \frac{1}{R} \tag{4.3}$$

The resistance of the electrolyte is measured across two plates dipped in the liquid and held at a fixed distance apart in a conductivity cell. The ratio L/A for the cell is called cell constant, K_c, and has the dimensions 1/cm. The value of the constant is determined by measuring the resistance of a standard solution of known conductivity:

$$K_c = R\kappa \tag{4.4}$$

Unit of measurement and reporting

The two main units for expressing electrical conductivity are Siemens and mhos, where 1 Siemen = 1 mho.

A Siemen (or mho) is the reciprocal of resistance in ohm. Conductivity is preferably reported as micro-mho per cm at 25°C (μmho/cm). See Table 4.1 for conversions between commonly used units.

Multiply	by	to obtain
μS/m	0.01	μmho/cm
mS/m	10	μmho/cm
mS/cm	1000	μmho/cm
μS/cm	1	μmho/cm
mmho/cm	1000	μmho/cm

Table 4.1: Conversion table for units of electrical conductivity

4.1.2 APPARATUS

An apparatus called a conductivity meter that consists of a conductivity cell and a meter measures conductivity, Figure 4.1. The conductivity cell consists of two electrodes (platinum plates) rigidly held at a constant distance from each other and are connected by cables to the meter. The meter consists of a Wheatstone bridge circuit as shown in the figure. The source of electric current in the meter applies a potential to the plates and the meter measures the electrical resistance of the solution. In order to avoid change of apparent resistance with time due to chemical reactions (polarisation effect at the electrodes) alternating current is used. Some meters read resistance (ohm) while others read in units of conductivity (milli-Siemens per meter). Platinised electrodes must be in good condition (clean, black-coated) and require replating if readings of the standard solution become erratic. Replating should be done in the laboratory. The cell should always be kept in distilled water when not in use, and thoroughly rinsed in distilled water after measurement.

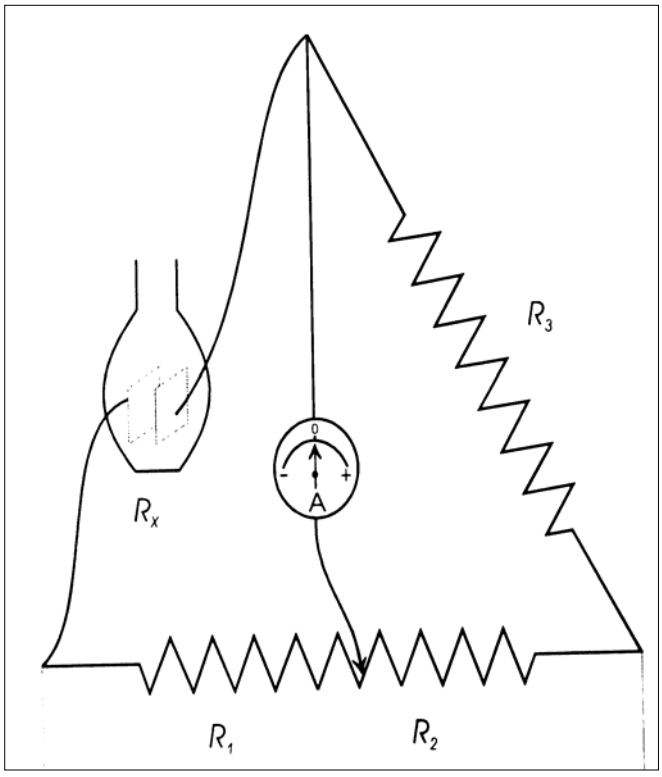


Figure 4.1:
Schematic diagram of a conductivity meter

The cell constant (calibration)

The design of the plates in the conductivity cell (size, shape, position and condition) determines the conductivity measured and is reflected in the so-called cell constant (K_c). Typical values for K_c are 0.1 to 2.0. The cell constant can be determined by using the conductivity meter to measure the resistance of a standard solution of 0.0100 mol/L potassium chloride (KCl). The conductivity of the solution (1412 μmho/cm at 25°C) multiplied by the measured resistance gives the value of K_c, Equation 4.4. The cell constant is subject to slow changes in time, even under ideal conditions. Thus, determination of the cell constant must be done regularly.

Temperature correction

Conductivity is highly temperature dependent. Electrolyte conductivity increases with temperature as follows:

$$\text{Electrical Conductivity (}\mu\text{mho/cm) at 25°C} = \frac{EC_m}{0.0191(t-25)+1}$$

EC_m = measured conductivity in μmho/cm at t°C

t = observed temperature of sample, °C

For natural waters, this temperature coefficient is only approximately the same as that of the standard KCl solution. Thus, the more the sample temperature deviates from 25°C the greater the uncertainty in applying the temperature correction. Always record the temperature of a sample (±0.1°C) and report the measured conductivity at 25°C (using a temperature coefficient of 0.0191/.°C)

Most of the modern conductivity meters have a facility to calculate the specific conductivity at 25°C using a built in temperature compensation from 0 to 60°C. The compensation can be manual (measure temperature separately and adjust meter to this) or automatic (there is a temperature electrode connected to the meter).

4.1.3 CONDUCTIVITY FACTORS

Current is carried by both cations and anions, but to a different degree. The conductivity due to divalent cations is more than that of mono-valent cations. However, it is not true for anions. The conductivity factors for major ions present in water are listed in Table 4.2

Ion	Conductivity Factor
Cations	
Ca^{2+}	2.60
Mg^{2+}	3.82
K^{+}	1.84
Na+	2.13
Anions	
HCO_3^-	0.715
Cl^-	2.14
SO_4^{2-}	1.54
NO_3^-	1.15

Table 4.2: Conductivity factors for ions commonly found in water

The conductivity of a water sample can be approximated using the following relationship

$$EC = \sum (C_i \times f_i) \qquad (4.5)$$

in which

EC = electrical conductivity, μmho/ cm

C_i = concentration of ionic specie i in solution, mg / L

f_i = conductivity factor for ionic specie i

Example 4.1

Given the following analysis of a water sample, estimate the EC value in μ mho/cm and mS/m.

Cations: Ca^{2+} = 85.0 mg/L, Mg^{2+} = 43.0 mg/L, K^+ = 2.9 mg/L, Na^+ = 92.0 mg/L

Anions: HCO_3^-=362.0 mg/L, Cl^-=131.0 mg/L, SO_4^{2-}=89.0 mg/L, NO_3^-=20.0 mg/L

Solution:

Using data of Table 4.2, set up the following calculation table

Ion	Conc., mg/L	Factor	Conductivity, μmho/cm
Ca^{2+}	85.0	2.60	221.0
Mg^{2+}	43.0	3.82	164.3
K^+	2.9	1.84	5.3
Na^+	92.0	2.13	196.0
HCO_3^-	362.0	0.716	258.8
Cl^-	131.0	2.14	280.3
SO_4^{2-}	89.0	1.54	137.1
NO_3^-	20.0	1.15	23.0
			Total 1285.8

Electrical Conductivity = 1285.8 μmho/cm = 1285.8 X 0.1 = 128.58 mS/m

4.1.4 USE OF EC MEASUREMENT

- **Check purity of distilled or de-ionised water**

Gradation of water	Use of water	EC (μmho/cm)	EC (mS/m)
Type I	use at detection limit of method	< 0.1	< 0.01
Type II	routine quantitative analysis	< 1	< 0.1
Type III	washing and qualitative analysis	< 10	< 1

Table 4.3: Gradation of water for laboratory use.

- **Relations with many individual constituents and TDS can be established.**

  The relationship between TDS (mg/L) and EC μmho/cm) is often described by a constant, that varies according to chemical composition: TDS = A x EC, where A is in the range of 0.55 to 0.9. Typically the constant is high for chloride-rich waters and low for sulphate-rich waters.

- **Check deterioration of samples in time (effect of storage)**

  If EC is checked at time of sampling and again prior to analysis in the laboratory, the change in EC is a measure for the 'freshness' of the sample.

Example 4.2

For the water sample given in the example in 4.1.3, calculate TDS and the corresponding constant 'A'.

Solution:

Ion	Conc. mg/L
Ca^{2+}	85.0
Mg^{2+}	43.0
K^{+}	2.9
Na^{+}	92.0
HCO_3^{-}	362.0
Cl^{-}	131.0
SO_4^{2-}	89.0
NO_3^{-}	20.0
$\sum$ = 824.9	

TDS in the sample = 824.9 mg/L. EC value = 1285.8 μmho/cm. (From example 4.1)

TDS = A x EC

824.9 = A x 1285.8

A = 0.64

4.2 ABSORPTION SPECTROSCOPY

Absorption spectroscopic methods of analysis rank among the most widespread and powerful tools for quantitative analysis. The use of a spectrophotometer to determine the extent of absorption of various wavelengths of *visible* light by a given solution is commonly known as *colorimetry*. This method is used to determine concentrations of various chemicals which can give colours either directly or after addition of some other chemicals.

As an example, in the analysis of phosphate, a reaction with orthophosphate is made, to form the highly coloured molybdenum blue compound. The light absorption of this compound can then be measured in a spectrophotometer.

Some compounds absorb light in other than the visible range of the spectrum. For example, nitrates absorb radiation of 220 nm wave length in the UV region.

4.2.1 THEORY

Absorption Spectroscopic methods of analysis are based upon the fact that compounds ABSORB light radiation of a specific wavelength. In the analysis, the amount of light radiation absorbed by a sample is measured. The light absorption is directly related to the concentration of the coloured compound in the sample.

The wavelength (λ) of Maximum Absorption is known for different compounds. For example, the coloured compound formed for analysis of Phosphate (molybdenum blue) has maximum light absorption at λ= 640 nm. Conversely, a minimum amount of light is *transmitted* through the compound at λ= 640 nm. This is shown schematically in Figure 4.2.

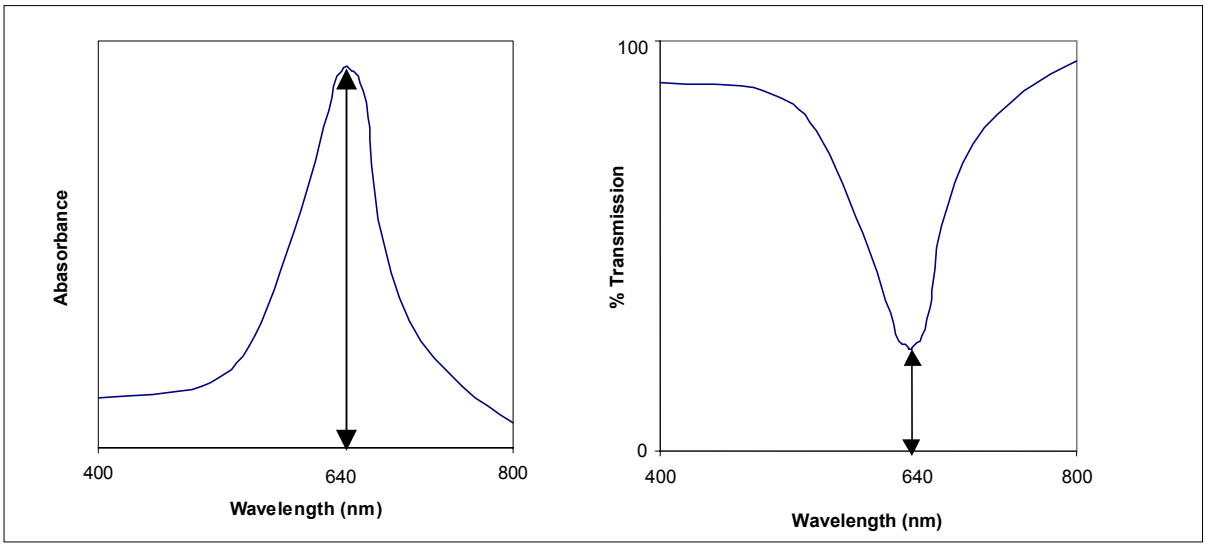


Figure 4.2: Light Absorption and Transmission by Phosphate-molybdenum blue compound. Schematic diagram showing maximum light absorption (and minimum light transmission) at λ= 640 nm.

The Beer-Lambert Law

The basis for Beer-Lambert Law is illustrated in Figure 4.3. The Absorbance (or optical density) and Transmission (or Transmittance) of light through a sample can be calculated by measuring light intensity entering and exiting the sample.

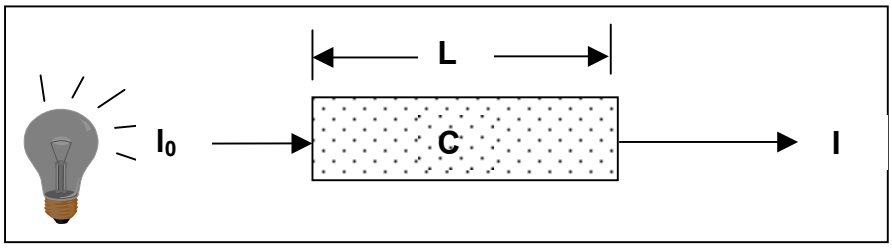


Figure 4.3: Incidence and transmission of light energy through a sample

The following terms are defined:

- Light Intensity entering a sample is "I_o"
- Light Intensity exiting a sample is "I"
- The Concentration of analyte in sample is "C"
- The length of the light path in glass sample cuvette is "L"
- "K" is a constant for a particular solution and wave length

The Beer-Lambert Law is given by the following equations:

$$\text{Light Absorbance (A)} = \log\left(\frac{I_o}{I}\right) = KCL$$

$$\text{Light Transmission (T)} = \log\left(\frac{I_o}{I}\right) = 10^{-KCL}$$

Plots of absorbance and transmission versus concentration of the analyte in sample according to Equations (4.6) and (4.7) is shown in Figure 4.4.

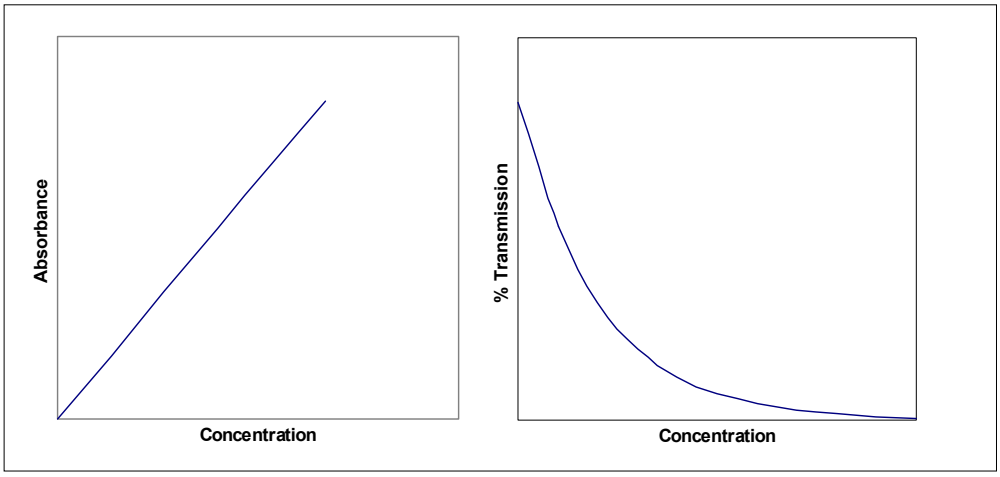


Figure 4.4: Beer-Lambert Law relates the amount of light Absorbance (A) by a solution to the Concentration (C) of a compound in solution and the length of light path:

- As Concentration (C) increases, light Absorption (A) increases, linearly
- As Concentration (C) increases, light Transmission (T) decreases, exponentially

4.2.2 SPECTROPHOTOMETER

All spectrophotometer instruments designed to measure the absorption of radiant energy have the basic components as follows (Figure 4.5):

1. a stable source of radiant energy (Light);
2. a wavelength selector to isolate a desired wavelength from the source (filter or monochromator);
3. transparent container (cuvette) for the sample and the blank;
4. a radiation detector (phototube) to convert the radiant energy received to a measurable signal; and a readout device that displays the signal from the detector.

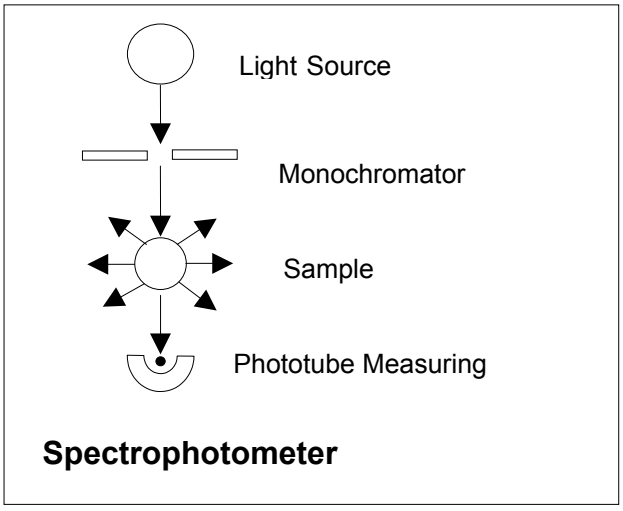


Figure 4.5: Components of a spectrophotometer

The energy source is to provide a stable source of light radiation, whereas the wavelength selector permits separation of radiation of the desired wavelength from other radiation. Light radiation passes through a glass container with sample. The detector measures the energy after it has passed through the sample. The readout device calculates the amount of light absorbed by the sample displays the signal from the detector as absorbance or transmission.

The spectrophotometers, which are used for such measurements may vary from simple and relatively inexpensive colorimeters to highly sophisticated and expensive instruments that automatically scan the ability of a solution to absorb radiation over a wide range of wavelengths and record the results of these measurements.

One instrument cannot be used to measure absorbance at all wavelengths because a given energy source and energy detector is suitable for use over only a limited range of wavelengths.

True linearity between absorbance and concentration according to Beer-Lambert Law requires the use of monochromatic light. In addition, a narrow band of light ensures a greater selectivity since substances with absorption peaks in other close by wavelengths are less likely to interfere. Further, it increases sensitivity as there is a greatest change in absorbance per increment of change in concentration of the analyte.

Both filters and monochromators are used to restrict the radiation wavelength. Photometers make use of filters, which function by absorbing large protions of the spectrum while transmitting relatively limited wavelength regions. Spectrophotometers are instruments equipped with monochromators that permit the continuous variation and selection of wavelength. The effective bandwidth of a monochromator that is satisfactory for most applications is about from 1 to 5 nm.

The sample containers, cells or cuvettes, must be fabricated from material that is transparent to radiation in the spectral region of interest. The commonly used materials for different wave length regions are:

Quartz or fused silica :	UV to 2 μm in 1R
Silicate glass:	Above 350 nm to 2 μm in 1R
Plastic:	visible region
Polished NaCl or AgCl:	Wave lengths longer than 2μm

Cuvettes are provided in pairs that have been carefully matched to make possible direct comparison of the transmission of light through the solvent and the sample. Accurate spectrophotometric analysis requires the use of good quality, matched cells. These should be regularly checked against one another to detect differences that can arise from scratches, etching and wear. The most common cell path for UV-visible region is 1cm. For reasons of economy, cylindrical cells are frequently used. Care must be taken to duplicate the position of such cells with respect to the light path; otherwise, variations in path length and in reflection losses will introduce errors.

4.2.3 MEASUREMENT PROCEDURES

As explained above, the Beer-Lambert Law forms the basis of the measurement procedure. The amount of light radiation absorbed by a compound is directly related to the concentration of the compound.

The general measurement procedure consists of 5 steps:

1. Prepare samples by reacting analyte with reagents to produce compounds or complexes which abort radiation.

2. Make series of standard solutions of known concentrations and treat them in the same manner as the sample.
3. Set spectrophotometer to λ of maximum light absorption
4. Measure light absorbance of standards
5. Plot standard curve: Absorbance vs. Concentration, as shown in Figure 4.6.

Once the standard plot is made, it is simple to find the concentration of an unknown sample: Measure the absorption of the unknown, and from the standard plot, read the related concentration (Figure 4.7).

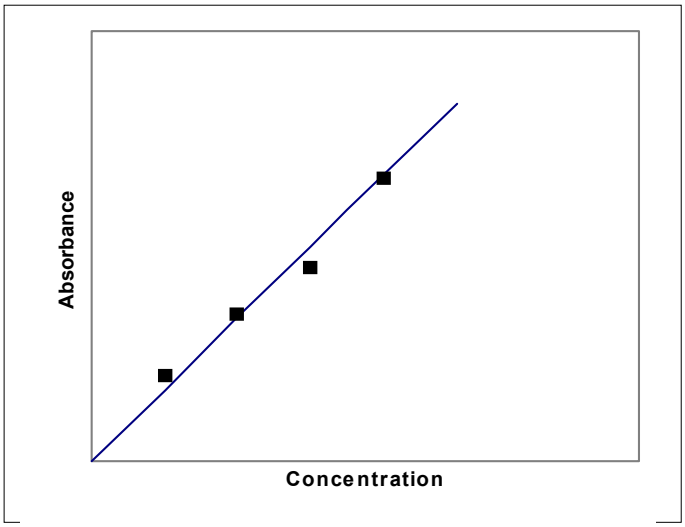


Figure 4.6:
Plot of the standard curve: showing the linear relation between light absorption and concentration of the standards

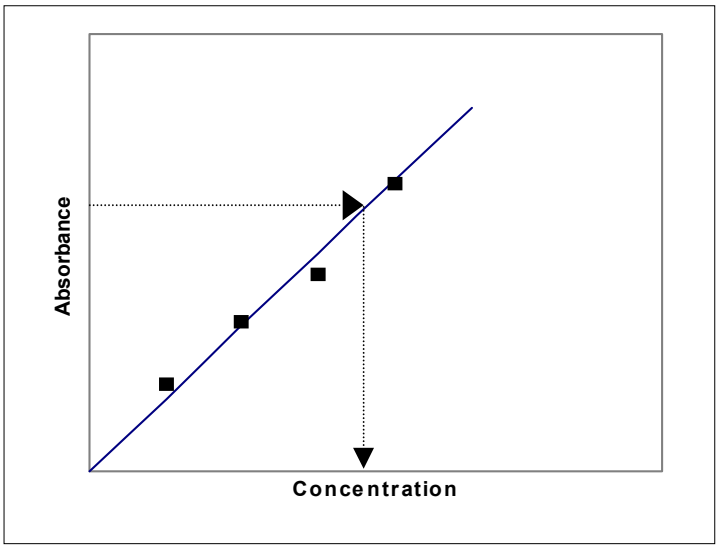


Figure 4.7:
Finding the concentration of an unknown sample from the standard curve.

Standard addition

Due to the fact that the overall composition of the sample is seldom the same as that of the calibration standard, in some cases, the absorption characteristics of the two may differ. Where such discrepancy is suspected, the standard addition approach may be used. Here, a known amount of analyte is added to a second aliquot of the sample. The difference in absorbance is used to calculate the analyte concentration of the sample as illustrated in Example 4.3.

Example 4.3

A 25 mL sample after treatment with reagents to generate colour for measurement of phosphate yielded absorbance of 0.428. Addition of 1.00 mL of a solution containing 5.0μg phosphorus to a second 25 mL aliquot and development of colour resulted in an absorbance of 0.517. Calculate μg phosphorus in each mL of sample.

Solution:

Correct absorbance for dilution:

Corrected absorbance = 0.517 (26.0/25.0) = 0.538

Absorbance caused by 5μg phosphorus = 0.538 - 0.428 = 0.110

Therefore, phosphorus in the sample = (5.0/0.11) 0.428

= 19.5μg, or 19.5/25 = 0.7μg/mL

4.2.4 OVERVIEW OF INDIVIDUAL METHODS

The general procedure can be followed for all spectrophotometer analyses. For analysis of specific compounds, the method of preparation of the colored compound, and the wavelength of maximum light absorption will vary. An overview is given in Table 4.4.

Parameter	λ	Method Name	SAP
Aluminum	535	Eriochrome Cyanine R Spectrophotometric	1.30
Boron	540	Curcumin Spectrophotometric	1.3
Chlorophyll a	750, 664, 65	Acetone Extraction Spectrophotometric	1.5
Flouride	570	SPADNS Spectrophotometric	1.11
Iron	510	Phenanthroline Spectrophotometric	1.13
Manganese	525	Persulphate Spectrophotometric	1.34
NH_3-N	640	Phenate Spectrophotometric	1.15
NO_3-N	220, 275	UV Spectrophotometric	1.16
NO_2-N	543	Sulphanilamide Spectrophotometric	1.17
o-PO_4	880	Ascorbic Acid Spectrophotometric	1.20
Total P	880	Digestion + Ascorbic Acid Spectrophotometric	1.39
Silica	815	Ammonium Molybdate Spectrophotometric	1.38
Sulphate	420	NOTE: preferred method for sulphate analysis is with nephelometer	1.26

Table 4.4: Overview of specific methods used for analysis of water quality parameters, and the wavelength of maximum light absorption

Spectrophotometric analysis has

- wide applicability
- high sensitivity: detection limit 10^{-5}M to 10^{-4}M range
- moderate to high selectivity
- good accuracy: relative error 1 to 3%
- ease and convenience, lends to automation

4.3 FLAME PHOTOMETRY

In Flame photometry, also known as atomic emission spectroscopy, the light radiation *emitted* by metal atoms when heated in a flame is measured. If a solution containing a metallic salt (or some other metallic compound) is aspirated into a flame (e.g. of acetylene burning in air), a vapour which

contains atoms of the metal may be formed. Some of these gaseous metal atoms may be raised to an energy level which is sufficiently high to permit the emission of radiation characteristic of the metal, e.g. the characteristic yellow colour imparted to flames by compounds of sodium. This is the basis of atomic flame emission spectroscopy, which is also referred to as flame emission spectroscopy (FES) or flame photometry.

Atomic emission spectroscopy is used in the determination of alkali metals as they are excited by the relatively low excitation energy of the flame. Sodium and Potassium are more frequently determined because of their importance in aquatic and biological systems. For these elements, the spectra are simple, consisting of only a few wavelengths.

4.3.1 FLAME PHOTOMETER

The basic diagram of a flame photometer is shown in Figure4.8. In this method of analysis, first an emission radiation is created:

- the solution being tested is first sucked into the instrument
- In the **atomiser**, air at a certain pressure is blown in. Two types of atomisers are available: Right angle type, and Concentric type. In both atomisers, a stream of air is blown across the opening of the sample inlet jet, creating a suction. The sample is drawn into the air stream, where it forms a fine mist. The sample is broken into fine particles and the metal salts get vaporised (atomised)
- Particles get carried with the compressed air towards to the **mixing chamber**. Here the compressed air carrying metal particles is mixed with the fuel gas. Fuel gas is typically acetylene. Ordinary cooking gas may also be used.
- The fuel gas mixture is fed into the **burner** for producing the flame.
- In the **flame** the metal particles emit radiation, producing a colour whose wavelength is characteristic of the element present in the sample. The intensity of the radiation varies with the concentration of the metallic ions in the solution.

After the radiation is emitted, it must then be measured:

- Radiation from the flame passes through a lens, a slit, a optical filter and falls on the photocell. The lens and filter isolate the particular wavelength characteristic of the metal ions being measured.
- The photocell measures the characteristic radiation.
- Amplifier and digital read-out give the radiation intensity
- Quantitative analysis is based on an empirical relation between the intensity (power) of the emitted radiation and the quantity of the corresponding elements in the sample.

Specific Analysis: Na^+ and K^+

Sodium and Potassium are routinely analysed with Emission Spectroscopy.

- Na is determined at λ = 589 nm; concentrations can be measured to $\pm$ 1 mg/l.
- K is determined at λ =766.5 nm; concentrations can be measured to $\pm$ 0.1 mg/l.
- Make a series of standard solutions, of known concentrations.
- Set Flame Photometer to correct λ
- Measure the emission intensity of the standards

- Plot a standard curve: Intensity vs. Concentration
- Measure intensity of unknowns, then read concentration from standard curve (Figure 4.9).

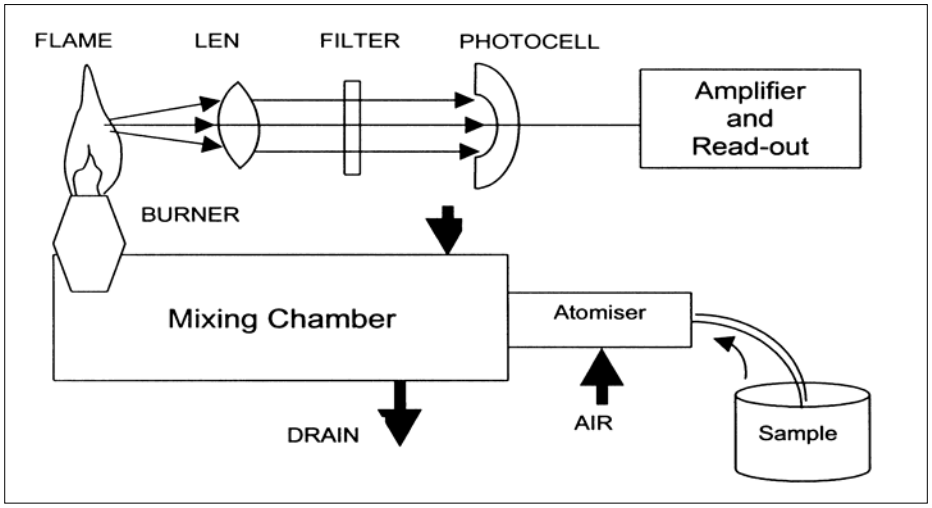


Figure 4.8: The main components of Flame Photometer

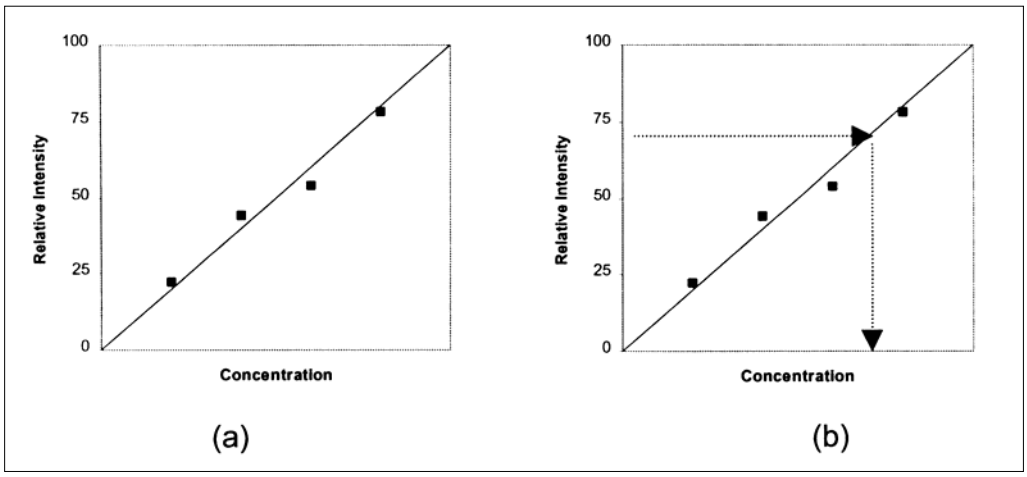


Figure 4.9 (a): a standard curve showing linear relation of emitted light intensity (I) with sample concentration (C) and (b): Reading the concentrations of an unknown using the standard curve after measuring the intensity

Interferences

At high concentrations of solutes, the analysis can be influenced by the composition of sample solutions. The concomitant elements may act upon the intensity of emission of test elements. It is seen that the emission intensity of any alkali metal increases in the presence of other alkali metals. Specifically, high concentrations of calcium and potassium can interfere with sodium analyses and high concentrations of calcium, magnesium and sodium can interfere with potassium analyses. Normally such interferences are not a problem. If such high concentrations of interfering ions are present, special analytical procedures can be followed, such as adding a “Radiation Buffer”.

4.4 NEPHELOMETRY AND TURBIDIMETERY

In Nephelometry, the light *scattered* by particles in water is measured. Suspension of particles in water interfering with passage of light is called turbidity. The turbidity of a sample may be measured either by its effect on the scattering of light, which is termed 'Nephelometery', or by its effect on transmission of light, which is termed 'Turbidimetery'. These methods use common electric photometers to measure light intensity. Nephelometric measurement is more sensitive for very dilute solutions, but for moderately heavy turbidity, either this or turbidimetric measurements can be made.

In turbidimetry, the amount of light passing through a solution is measured. The higher the turbidity, the smaller the quantity of light transmitted. The transmitted light is determined at 420 nm.

In nephelometry, on the other hand, the detecting cell is placed at right angles to the light source to measure light scattered by the turbidity particles.

4.4.1 NEPHELOMETER

The turbidimeter or nephelometer mainly consists of four parts – light source, optical components (e.g. slit), sample compartment and a photocell for the measurement of light either transmitted through the sample or scattered from the suspended particles in the sample. The photocell detects light and an electronic amplifier measures the light intensities. The principle measurement by photometer and nephelometer is illustrated in Figure 4.10.

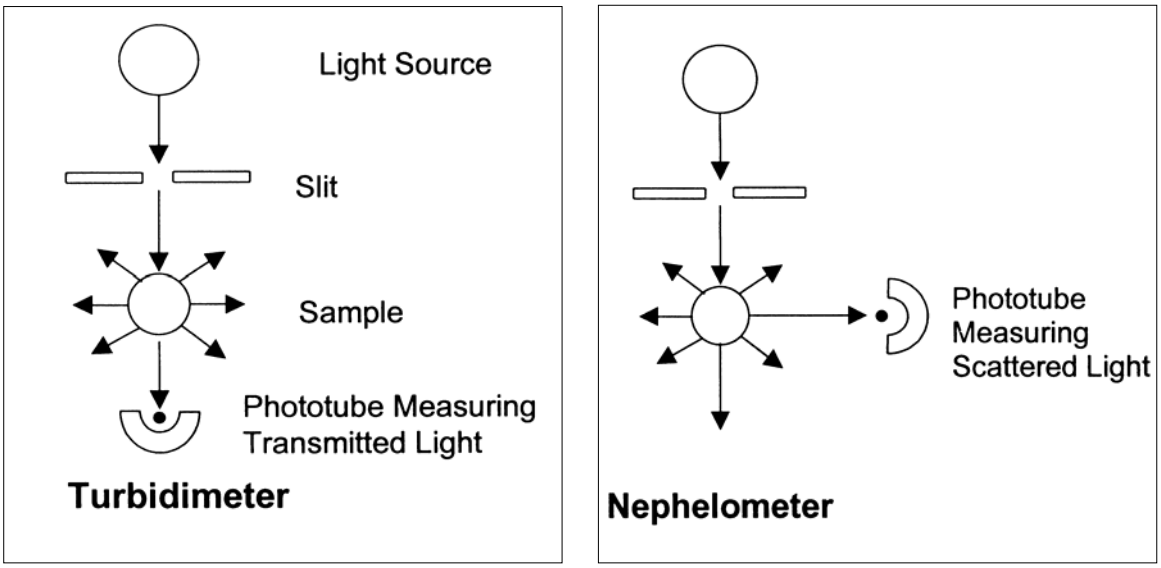


Figure 4.10: Diagram of Turbidity analysis by 'Turbidimetry' or 'Nephelometry'

Measurement of turbidity based on visual methods, such as Jackson's candle turbidimeter, although simple and cheaper, is not sensitive for low turbidities. The accuracy of visual instruments is also limited and depends upon the skill of the user's eye.

Turbidity Units

The standard Turbidity unit is called Nephelometric Turbidity Unit (NTU) because of the use of nephelometric method of measurement. The NTU has generally replaced the previous unit of 'Jackson candle turbidity units (JTU).

Calibration of Instrument and Measurement of Turbidity

Formazine is used as turbidity standard for calibration because it possesses the desired properties which other standards do not have. Formazin particles have uniform size and shape. Formazin can be synthesised and has reproducibility of one percent. Formazin stock solution has good stability (up to 1 year), thus making it an ideal turbidity standard. Calibration is made in NTU, the standard units of measurement.

Procedures for preparation of formazin stock standard, 4000 NTU, are in the Guidelines for Standard Analytical Procedures for Water Analysis. For each use, the dilute standards are prepared from stock solution, and the nephelometer must be calibrated according to manufacturer's operating instructions.

Interferences in Nephelometry:

Nephelometry is based on measuring light intensity. Several factors can interfere with the measurements:

- Dirty glassware
- Air bubbles in the sample
- Vibration that disturbs the surface visibility
- Colour of the sample

Maintenance

Cleanliness is of utmost importance in all turbidity measurements. All the glass and optical parts must be protected from dust and scratches. Even the finger marks on the sample tube bottoms, mirror, source lamps and reflectors may disturb the measurement, accuracy and calibration. To prevent the polish bottoms of the sample tubes and plungers from scratching, it is advisable to keep these items on a soft cloth or tissue paper when not in use.

The sample tubes and condensing lenses etc may be washed with mild detergent and rinsed with distilled water. The filters and mirrors may be cleaned using a soft tissue paper or air syringe and may be wiped occasionally with a soft tissue soaked with distilled water or mild detergent. Never immerse these items in water or cleaning solutions. Also wipe the outside of the sample tube before placing into the housing; otherwise it may keep the mirror wet for long time and damage it permanently.

4.5 POTENTIOMETRIC ANALYSIS

4.5.1 BASIC PRINCIPLES AND DEFINITIONS

Electrode: A simple electrode is a strip of metal immersed in a solution of its own ions. A chemical change will occur at an electrode, as certain ions in solution migrate towards the electrode. The reaction occurring at the electrode is a redox-reaction, where electron transfer is involved. Thus, a potential difference is established between the metal and the solution.

Half-cell: a single electrode dipping into a solution is also known as a half-cell. Each half-cell has a specific redox reaction occurring there.

Electrode potential: The electric potential of an electrode is the potential due to electron transfer in a redox reaction occurring at the electrode. The potential is measured against a reference half-cell or electrode.

Electrochemical cell (also known as 'galvanic cell'): The combination of 2 electrodes (half-cells), creates an electrochemical cell. If a voltmeter is connected across the electrodes, it can measure the electromotive force being generated by the electrochemical cell (E_{cell}).

Cell Potential: The electrochemical cell potential, E_{cell}, is equal to the difference of the 2 electrode potentials and can be measured in volts, see Figure 4.11.

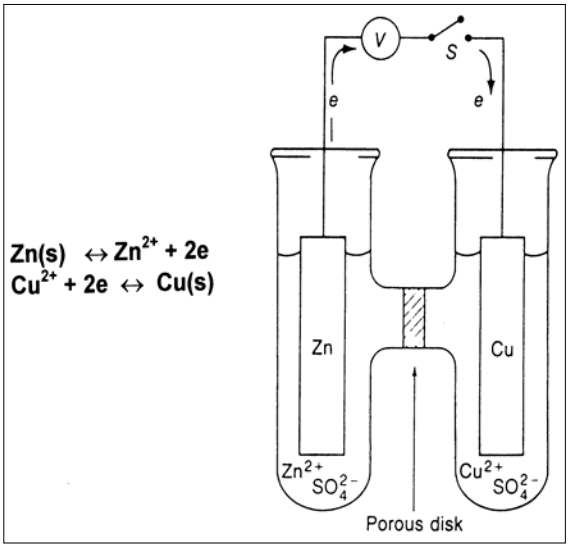


Figure 4.11:
A Galvanic Cell

$$E_{cell} = E_c - E_a \quad (4.7)$$

Where:

- E_{cell} = electrochemical cell potential (volts)
- E_c = the electrode potentials of the cathode
- E_a = the electrode potentials of the anode

Nernst Equation: For the generalised half-reaction

$$aA + bB\ldots + ne \leftrightharpoons cC + dD\ldots \quad (4.8)$$

the potential is related to the ionic activity of the solutes (approximated by their concentration) by the expression:

$$E = E^o - \frac{RT}{nF} \ln\left(\frac{[C]^c[D]^d}{[A]^a[B]^b}\right) \quad (4.9)$$

where:

- E = electrode Potential, volts
- E^o = a constant called standard electrode potential; it is equal to E when the activities of all reactants and products are one.
- R = the Gas Constant, 8.316 J oK-1 mol-1
- T = absolute temperature, ^{0}K

- n = number of moles of electrons that appear in the half reaction for the electrode process
- F = the faraday = 96487 coulombs
- [A], [B], [C], [D]= active ions in the solution
- a, b, c, d = mole concentrations of the respective ions

Substituting numerical values, simplifying and converting to base-10 logarithm provides:

$$E = E^{o} - \frac{0.059}{n} \log\left(\frac{[C]^{c}[D]^{d}}{[A]^{a}[B]^{b}}\right) \quad (4.10)$$

The Nernst equation indicates that the electrode potential varies with the log of ionic activities (concentration). In an electrochemical cell where the potential of one electrode is known (reference electrode), the measured cell potential can be related to the electrode potential at the indicator electrode. The indicator electrode potential, is in turn related to the ionic concentration of the species of interest.

If the cell potential is measured for standard solutions of the ion of interest, using its indicator electrode, and a calibration is made, the cell can be then used to measure the ion in unknown solutions.

In order to determine the potential in an electrochemical cell, a potential measuring device and two electrodes are necessary. These two electrodes are generally referred as *reference electrode* and *indicator electrode*. The potential of the reference electrode is independent of concentration of analyte or any other ion present in the sample.

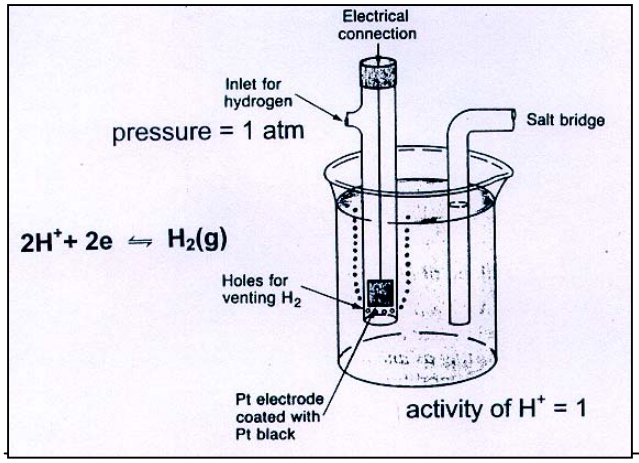
4.5.2 REFERENCE ELECTRODES

There are 3 common reference electrodes used for potentiometric analysis

- Hydrogen electrode
- Calomel electrode
- Silver – silver chloride electrode

Hydrogen Electrode

Figure 4.12 shows a standard hydrogen electrode. This reference electrode is used as the standard to which the potentials of all other electrodes are related. The hydrogen ion activity in a standard hydrogen-electrode is maintained at unity and the hydrogen gas partial pressure at one atmosphere. The electrode reaction is:



$$H_2(g) + 2e \leftrightharpoons 2H^{+}$$

Figure 4.12: Standard Hydrogen Electrode

By convention the potential of this half-cell is assigned a value of exactly zero at all temperatures. The flowing stream of gas needed for the operation of a hydrogen electrode is inconvenient as well as hazardous. Therefore, ordinarily such an electrode is not used.

Calomel Electrode

The calomel electrode is most widely used reference electrode due to its ease of preparation and constancy of potential. The calomel electrode is the reference electrode used in the electronic pH measurement. The calomel electrode is also used in oxidation-reduction measurements, and in most other electrochemical analyses for which a stable easy-to-use reference electrode is desired. A simplified diagram of the calomel electrode is shown in Figure 4.13.

It has a calomel half-cell in which mercury and calomel (mercury(I) chloride) are covered with potassium chloride solution of definite concentration (0.1 M,1 M or saturated). The inner tube containing $Hg.Hg_2Cl_2$ is connected to the outer tube containing KCl through a small opening. Contact with the analyte solution is made through a porous fibre wick or a fritted disc at the bottom of the outer tube. These electrodes are referred to as the decimolar, molar and the saturated calomel electrode depending on the concentration of KCl solution. The potential of each with respect to the hydrogen electrode is given in Table 4.5.

Concentration of KCl	**E_H in volts**
0.1 N	-0.3358
1.0 N	- 0.2824
Saturated	-0.2444

Table 4.5: Standard potentials of calomel reference electrodes at 25°

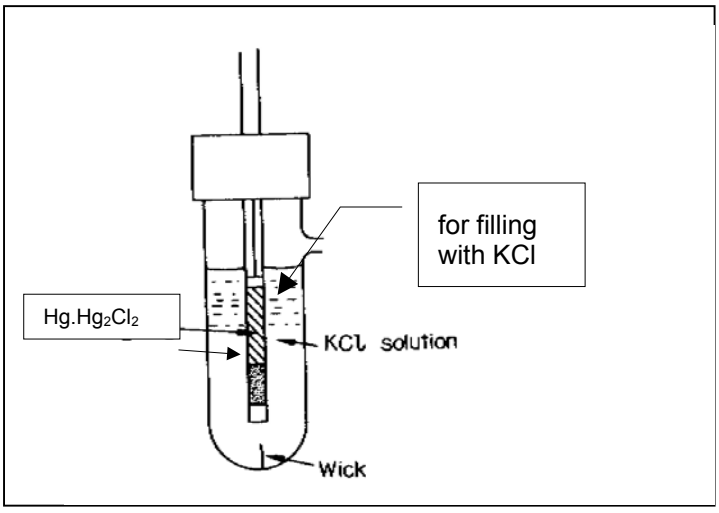


Figure 4.13:
Schematic diagram of the Calomel reference electrode

The electrode reaction is: $Hg_2Cl_2\ (s) + 2e^- \leftrightarrow 2Hg\ (liq) + 2Cl^-$

Silver-Silver Chloride Electrode

This electrode is another reference electrode consisting of a silver wire or a silver plated platinum electrode dipped in saturated solution of KCl in a tube. The tube is connected to analyte through a porous plug at the bottom. In practice, it is no longer commonly used and therefore is not discussed here.

4.5.3 INDICATOR ELECTRODES

An indicator electrode of a cell is one whose potential is dependent upon the activity (concentration) of a particular ionic specie whose concentration is to be determined. Various types of electrodes used for determination of different ions are discribed below.

Metal electrodes: A metal electrode consists of a metal in contact with its ions. For example, the half-reaction for the Cu electrode in Figure 4.11 is,

$$Cu^{2+} + 2e \leftrightharpoons Cu(s) \qquad (4.12)$$

and the Nernst expression is written as:

$$E = E^{\circ} - \frac{0.0529}{2} \log \frac{1}{[Cu^{2+}]} \qquad (4.13)$$

It is seen that the electrode potential is directly related to logarithm of inverse of concentration of Cu^{2+} ion in solution in contact with the electrode. A plot of E vs. log $[Cu^{2+}]$ would have a slope of +0.0295.

This type of electrode might be used for analysis of metals such as Ag, Cu, Hg, Pb and Cd. Metal electrodes for other elements cannot be used as they do not behave ideally and do not give reproducible results.

Another kind of metal electrode may be used for determination of an anion that forms a sparingly soluble precipitate on reaction with the electrode metal. For example, I^- can be determined using an Ag electrode. Here two equilibria are involved:

$$AgI(s) \leftrightharpoons Ag^+ + I^- \qquad (4.14)$$

and $$Ag^+ + e \leftrightharpoons Ag \qquad (4.15)$$

Combining the two equations one gets:

$$AgI(s) + e \leftrightharpoons Ag(s) + I^- \qquad (4.16)$$

The Nernst expression for this process is:

$$E = E^0_{AgI} - 0.0591 \log [I^-] \qquad (4.17)$$

The above equation shows that the potential of a silver electrode immersed in a tube containing a saturated solution of AgI is directly proportional to the logarithm of iodide concentration. A plot of E vs. log $[1^-]$ would have a slope of -0.0591.

The electrode is constructed by providing the tube containing the electrode and AgI solution with an agar plug saturated with AgI and supported on a porous disc at the bottom of the tube. This prevents

the loss of AgI solution from the electrode tube and at the same time provides contact with the analyte solution.

Redox electrode

A non-reactive metal electrode, such as platinum, may be used as indicator electrode for redox systems. Of itself, the electrode is inert; its potential depends solely on the potential of the chemical system with which it is in contact. As in the case of other indicator electrodes, it is used in conjunction with a reference electrode.

Glass membrane electrodes

Determination of pH involves measurement of the potential developed across a thin glass membrane that separates two solutions with different hydrogen ion concentrations. Indicator glass membrane electrodes for other univalent ions, such as Na^+, K^+, NH^+_4, etc., are also available.

The glass membrane electrode and also other types of membrane electrodes, which are discussed later, are fundamentally different from metal electrodes both in design and principle. Since pH is measured routinely potentiometrically in all laboratories, glass membrane electrode for pH measurement is described in detail.

Figure 4.14 shows an electrode system, comprising a reference saturated calomel electrode and a glass indicator electrode, for measurement of pH. The indicator electrode consists of a thin, pH-sensititve glass membrane sealed onto one end of a heavy-walled glass or plastic tube. A small amount of dilute HCl that is saturated with silver chloride is contained in the membrane bulb. A silver wire in this solution forms an internal silver-silver chloride reference electrode. An internal calomel reference electrode may also be used within the glass electrode in place of the silver-silver chloride electrode.

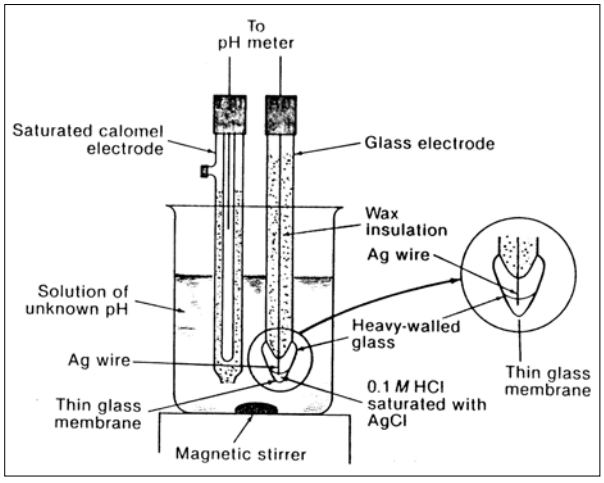


Figure 4.14:
Glass electrode system for measurement pH

The thin glass membrane responds to pH. The process involves ion exchange reactions on the two sides of the membrane in which H^+ ions in the solutions are exchanged with monovalent cations in the glass lattice. The electrode potential established on the pH electrode is a function of H^+ ion activities of the analyte, H_a^+, and the solution within the electrode, H_e^+, on the two sides of the glass membrane:

$$E_{ind} = K + 0.059 \log \frac{[H_a^+]}{[H_e^+]}$$

(4.18)

since $[H_e^+]$ is constant

$E_{ind} = K - 0.059\ pH$ (4.19)

The glass membrane of the pH electrode is of 0.03 to 0.1mm thickness. It is of silicate glass within which there are singly charged cations, such as sodium and lithium, that are mobile in the lattice and are responsible for electrical conduction. The surface of the membrane is hygroscopic and must be hydrated before it will function as a pH electrode. It loses pH sensitivity after dehydration by storage. The effect is reversible, however, and the response is restored by soaking in water.

Liquid membrane electrode

Liquid membrane electrodes have been developed for the direct potentiometric measurement of both polyvalent cations and polyvalent anions.

Figure 4.15 shows a schematic diagram of a liquid membrane electrode for calcium. It consists of a conducting porous membrane impregnated with a liquid ion exchanger that selectively bonds calcium ions, an internal solution containing a fixed concentration of $CaCl_2$ and a silver-silver chloride electrode. The similarity to the glass membrane electrode is obvious.

The exchange of Ca^{2+} ions at the two surfaces of the membrane differs as a consequence of the difference in Ca^{++} activity of the internal electrode solution and the external analyte. This results in the development of the electrode potential as in the case of the pH electrode.

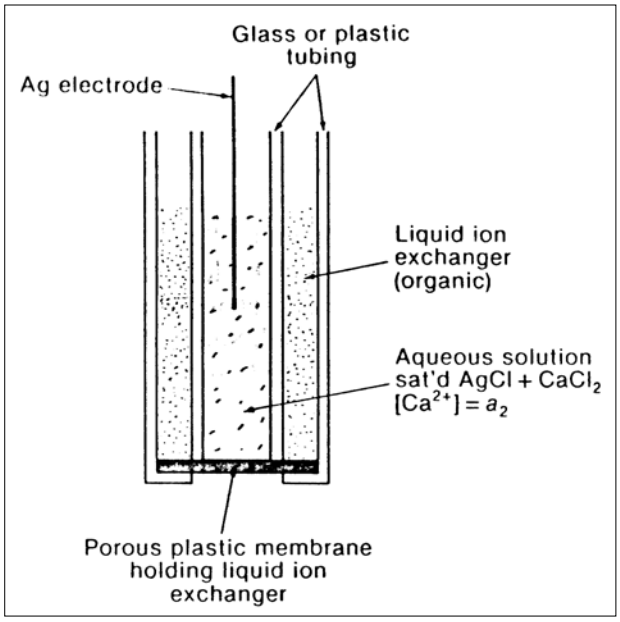


Figure 4.15:
Liquid membrane electrode for Ca^{2+}

Crystalline membrane electrodes

Many solid crystalline membranes, other than glass, have been developed for specific anions and cations, such as CN^-, F^-, Pb^{2+}, Cd^{2+}, etc. These also function on the same principle as the glass membrane and liquid membrane electrodes.

Gas-sensing probe

The electrodes belonging to this class are not strictly ion electrodes because they respond to dissolved molecular species.

Further, they represent a complete electrochemical cell rather than an electrode. They are, therefore, more properly called probes.

Figure 4.16 shows a schematic diagram of an oxygen probe based on principle of polarography. An inert metal such as gold or platinum serves as cathode and silver is used for the anode. These are electrically connected through an electrolyte solution. The cell is separated from the sample by means of a replaceable gas permeable membrane. Any oxygen that passes through the membrane is reduced at the cathode by externally applying a potential of about 0.5 to 0.8 volt across the anode and cathode. This causes a current to flow. The magnitude of the current is proportional to the amount of oxygen in the sample.

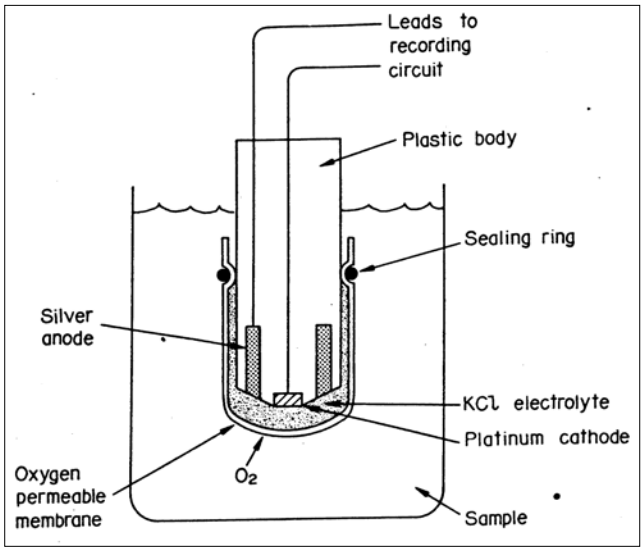


Figure 4.16:
Schematic diagram for oxygen probe

In a galvanic cell type probe, a basic electrolyte such as KOH and a lead cathod is used in place of the inert platinum cathode. This produces enough potential to reduce the oxygen and the magnitude of the current is proportional to the dissolved oxygen concentration in the analyte solution.

4.5.4 MEASUREMENT OF CELL POTENTIAL

An instrument for potentiometric measurements must not draw any current from the cell as it will change its potential. Historically, potential measurements were performed with a potentiometer, a nul-point instrument in which the unknown potential was just balanced by a standard reference potential. At null, no electricity is withdrawn from the cell whose potential is being measured.

The potentiometer has now been supplanted by electronic voltmeters, commonly called pH meters when used for measurement of pH or ion meters when used for measurement of other ions. These meters directly read the concentration or p-concentration of the chemical specie being measured after suitable calibration.

4.5.5 PERFORMANCE CHARACTERISTICS OF ELECTRODES

Temperature effect

It is necessary to correct for the temperature of the samples because the electrode potential is temperature dependent. The sample temperature should be measured independently and a temperature correction should be made with the in-built correction in the meter.

Response time

The response time of ion-selective electrode is the speed at which it responds (electrode potential reaches a steady state value), and depends upon the type of electrode and concentration of ion under measurement. The response time of solid – state electrode is generally of the order of 5-10 seconds. The response time of some liquid ion-exchanger electrode is longer, usually many seconds or even few minutes at low concentrations. When making a reading, enough time must be given for the electrode to reach a stable reading.

Potential drifts

The potential drifts of ion-selective electrodes is connected with changes in the surface structure of solid – membrane electrode due to contact with the electrolyte and due to dissolution of the ion exchanger of liquid-membrane electrodes. All electrodes show drifts with time and therefore need re-calibration periodically with standardising solutions. This should be done every few hours if making many analyses.

Sensitivity

The useful working range of the ion-selective electrodes is knows as sensitivity which is effectively governed by the properties of the membrane. The working range of electrode is determined from the concentration (mg/l) to which an electrode responds in a theoretical fashion (59.15 mV/ten fold change in concentration when one electron is involved in the reaction).

Selectivity & Interferences

Although intended to be specific to a single ionic species, most electrodes respond in varying degrees to the presence of other ions. Where severe interferences are likely to be encountered, there are two practical courses of action. Either the electrode must be calibrated in the presence of interfering ion or the interference must be eliminated by some other means such as buffering, precipitation or by adding a complexing agent.

Activities vs. Concentration

Electrodes measure activities and not concentrations. The activity varies with ionic strength. Thus, to use an electrode for concentration measurements, the electrode must be calibrated in a standard solution of similar ionic strength (see Section 4.5.6 Electrode calibration).

Effect of pH

The pH of the sample solution changes the chemistry of the analyte ion. For instance, at low pH values F^- ions are present as HF, which are not sensed by the fluoride electrode. Thus in such cases an appropriate pH adjuster should be added to samples to set up a reliable ion-selective electrode analysis.

Presence of complexing agent

A number of ions in water samples can be present in more than one form, such as complexes with other ions or molecules. Electrodes measure only the free ion in solution, and cannot sense bound or complexed ions.

Life - time

The life-time of an electrode is determined by the rate at which the liquid ion exchanger leaks through the membrane in liquid membrane electrodes, or the time required for crystal surface or the pressed pellet to become chemically fouled in solid membrane electrodes. Life-time markedly depends upon conditions of use. With good care, electrodes should provide many months or years of service. If electrode response drops off, rejuvenation of electrodes is possible by refilling the liquid internals, replacing sensing membrane of liquid membrane type electrode or mechanical polishing of solid – state membrane tip.

The use of ion-selective electrodes as an analytical techniques in water analysis is increasing. This is because of the significant advantages such as:

- They are not bothered by turbidity or colour of the sample
- Small volumes of samples can be analysed without loss of sample
- Measure species without upsetting chemical equilibrium
- Measurements are independent of volume of sample
- Concentrations can be read directly on specific ion meter
- Calibration curves can cover even six decades of concentration
- Electrodes are sensitive up to parts per billion

Further application of these electrodes especially for on-line analysis are very much in use because:

- They do not usually require extensive pre-treatment of the sample
- They have a relatively rapid response-time
- They provide an electrical analogue signal which does not need sophisticated signal conditioning circuits before a result can be displayed to the instrument user
- Considerable previous experience of on-line instrumentation for pH measurement is available, which is relevant to other electrodes also.

4.5.6 ELECTRODE CALIBRATION

Activity vs. concentration:

Electrode response is related to analyte activity rather than to analyte concentration. More often than not, activity coefficients are not available. The difference between activity and concentration is illustrated by Figure 4.17 in which the response of a calcium electrode is plotted against logarithmic functions of the calcium concentration and calcium activity. The electrode responds in an ideal fashion, i.e., constant slope, only when concentration values are changed to activity values.

An obvious way to convert potentiometric measurements from activity to concentration is to make use of an emperical calibration curve, such as the lower plot in Figure 4.17. For this approach to be successful, it is necessary to make the ionic composition of the standards essentially the same as that for the analyte solution. This is often done by swamping both sample and standards with a measured excess of an inert electrolyte, thus making the added effect of electrolyte from the sample matrix negligible.

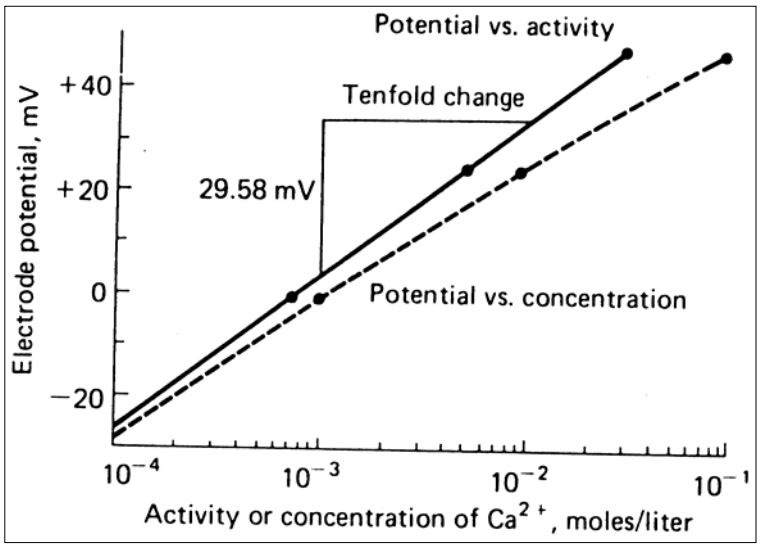


Figure 4.17:
Response of Ca electrode as functions of Ca^{++} ion concentration and activity

Standard addition method

Notwithstanding the advantages of direct potentiometric measurements, there are certain inherent limitations to the method. Principal among these is the existance of a 'liquid junction potential' that affects most potentiometric measurements. The junction potential, E_j, is created at the interface of the electrode and the analyte solution due to unequal mobility of various cations and anions, which modifies the observed potential, E_{obs}, according to the following expression

$$E_{obs} = \left(E_{ref} + E_j + E_{ind}\right) = E_{ref} + E_j\left(E^0 - \frac{0.0591}{n}\log\frac{1}{a}\right)$$

$$-\log a = \frac{E_{obs} - K}{0.0591/n} \qquad (4.20)$$

where a is the analyte activity, E_{ind} is the actual indicator electrode potential and K is a constant.

In case the effect of liquid junction potential is to be eliminated and where the effect of the matrix of the sample cannot be swamped by addition of an inert electrolyte, standard addition method may be used. It involves determining the potential of the electrode system before and after a measured volume of a standard has been added to a known volume of the analyte solution. As in the other method, if required, an excess of an electrolyte is added to the analyte solution to prevent a major shift in the ionic strength due to addition of the standard. The following example explains the calculations involved in the method.

Example 4.4

A lead ion electrode developed a potential of 0.4706 V (vs. Standard Calomel Electrode) when immersed in 50.00 mL of a sample. A 5.00 mL addition of standard 0.02000 M lead solution caused the potential to shift to 0.4490 V. Calculate the molar concentration of lead in the sample.

Solution:

Applying Equation 4.20 and assuming that the activity of Pb^{2+} is approximately equal to its molar concentration C_x, we may write

$$-\log C_x = \frac{E_1 - K}{\frac{0.0591}{2}}$$

Where E_1 is the measured potential (0.4706V)

After introduction of the standard addition, the potential is now E_2 (0.4490V) and

$$-\log\frac{50.00\,X\,C_x + 5.00\,X\,0.02000}{50.00+5.00} = \frac{E_2 - K}{\frac{0.0591}{2}}$$

Which can be written as:

$$-\log\left(0.9091C_x + 1.818\,X\,10^{-3}\right) = \frac{E_2 - K}{\frac{0.0591}{2}}$$

Subtraction of this equation from the first gives

$$-\log\frac{C_x}{0.9091C_x + 1.818\,X\,10^{-3}} = \frac{2(E_1 - E_2)}{0.0591}$$

$$= \frac{2(0.4706 - 0.4490)}{0.0591} = 0.7310$$

$$\frac{C_x}{0.9091C_x + 1.818\,X\,10^{-3}} = 0.7310$$

$$C_x = 4.06\,X\,10^{-4}M$$

4.5.7 RECOMMENDED PROBES UNDER HP

This section describes the use of probes recommended under HP. As stated earlier, while the probes have their advantages, their limitations must also be kept in mind when used in different situations.

For each case the manufacturer's instructions for calibration and operation of the probe and the ion-meter should be followed.

Dissolved oxygen probe

Application: DO probes are useful for on site measurements and when a large number of samples are to be analysed in the laboratory, for example, when determining BOD. For continuous DO measurement the potentiometric measurement is the only option.

Construction: The probe consists of two solid metal electrodes in contact with a supporting electrolyte separated form the test solution by a selective membrane permeable to molecular oxygen.

Interference: The probe exhibits a high temperature coefficient. The ion-meter may have in-built temperature correction or correction factor may be available from the manufacturer. Prolonged use of the probe in waters containing sulphides lowers the sensitivity of the probe.

Calibration: The probe may be calibrated by reading against air, a sample containing known DO concentration (determined by iodometric titration) or a sample containing zero DO (by adding sulphite to the sample). Periodic cross-check of calibration by different methods is recommended. Preferably calibrate using the sample water. In case the sample water contains interfering substances, calibrate with a sample of clean water containing approximately the same salt content.

Precautions: Change the probe membrane when the response becomes sluggish or erratic. Take care to avoid contamination of the sensing element and trapping of minute air bubbles while changing the membrane. Provide sufficient sample flow across the membrane surface while making measurements.

Fluoride electrode

Applications: Fluoride electrode has been used for determination of fluoride in drinking water supplies. The analyst should be aware of its limitations as described in the section on interference

below. Its suitability should be evaluated since even small errors can be critical in terms of suitability of a water for drinking purpose.

Construction: The key element in the fluoride electrode is the lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations, in the sample and the standard solution in the electrode. The electrode can be used with a standard calomel reference electrode.

Interference: Fluoride forms complexes with several cations notably aluminium and iron. At pH values lower that 5 poorly ionisable HF.HF complex is formed. Cyclohexylene-diamine-tetra acetic acid and a buffer can be added to the sample to complex the interfering ions. The buffer maintains a pH of 5. This pH also reduces the effect of the interfering ions. Samples having TDS in excess of 10,000 mg/L should be distilled.

Calibration: A calibration curve in the 0.2 to 2 mg/L F^- range may be prepared. The recommended complexing agent and buffer should be added to the sample as well as the standards. This would make the ionic strength of the sample similar to that of the standard.

Precautions: Treat the sample and the standard as described above. Avoid stirring before immersing electrode so as not to entrap air bubbles. Let electrode remain in solution until the reading is constant. Recalibrate frequently against atleast one standard.

Ammonia probe

Applications: The method can be used for a wide range of ammonia concentration from 0.03 to 1400 mg/L NH_3-N. It is suitable for coloured and turbid water samples.

Construction: The probe consists of a pH electrode and a reference electrode. It is provided with a membrane which allows permeation of NH_3. The permeated gas reacts with an internal solution and changes its pH, which is sensed by the pH electrode.

Interference: The probe has a high temperature coefficient. The temperature correction is generally available from the manufacturer. Amines cause a positive interference. Hg and Ag form complexes with ammonia. This can be overcome by adding NaOH/EDTA to the sample.

Calibaration: Calibrate the instrument using a series of standards in the range of 0.1mg/L - 1000 mg/L NH_3-N prepared from NH_4Cl. Sufficient quantity of NaOH is added to convert NH_4^+ to NH_3. In case presence of Hg or Ag is suspected in the sample ass NaOH/EDTA solution to the standard also. Same volume of the reagent should be added to the standards as well as the sample. A 10 fold increase in NH_3 concentration the potential change should be about 59mV.

Precautions: Calibration standards and samples should be at the same temperature. Do not add NaOH before immersing the probe to avoid loss of ammonia. Stir slowly and maintain the same speed while taking readings. For ammonia concentration below 1 mg/L, 2 to 3 minutes may be required to obtain a stable response from the electrode.

Nitrate electrode

Applications: Potentiometric analysis for nitrate is an alternative to UV absorption method where presence of organic matter in the sample interferes with the spectrophotometric method. It is also convenient and time saving when compared to cadmium reduction method. The use of the electrode may be limited in case of saline samples (see interferences below). The electrode responds to NO_3^--N concentration in 0.14-1400 mg/L range.

Construction: The electrode has a sensitive liquid ion-exchange membrane held in an inert membrane. It develops a potentia across the membrane whose one face is kept in contact with the analyte in the sample and the other in contact with an inner standard solution.

Interferences: Chloride and bicarbonate ions interfere when their weight raios to NO_3^--N are in excess of 10 and 5, respectively. Variation in pH gives erratic response. Other ions which may interfere are NO_2^-, CN^-, S^{2-}, Br^-, I^- and NO_2^-.

Calibration: A buffer is added to the standards and the sample to adjust the solutions to similar ionic strength. The buffer maintains a pH of 3, which eleminates HCO_3^-. The buffer also contains $Ag_2(SO_4)$ to remove interference from Cl^-, Br^-, S^{2-}, and CN^-, and sulfamic acid to remove NO_2^-.

Precautions: The standards and the samples should be at same temperature preferably 25^0C. The calibration curve, when mV potential reading is plotted as ordinate versus log NO_3^--N concentration as abscissa, should result in a straight line with a slope of $+57\pm3$ mV/decade. Check the electrode calibration several times a day using at least one standard.

Cyanide electrode

Applications: A preliminary step of distillation of sample and collection of CN^- in alkaline solution is required. The electrode responds to CN^- concentration in the range of 0.05 to 10 mg/L

Construction: The electrode uses a solid crystalline membrane sensitive to CN^-.

Interferences: Since the determination is made on the distillate, ordinarily interference from other ions is not expected. Lead carbonate may be added to absorbing solution if sulphides are expected in the distillate.

Calibration: Use a series of 4 or more standards in the range of 0.025-10 mg CN^-/L. Proceed from the lowest to the highest concentration while taking readings. Mix well using a magnetic stirrer. Allow at least 5 min for reaching equilibrium.

Precaution: HCN is an extremely toxic gas. Follow all precautions as detailed in the distillation step in standard procedures. Do not word with an acidified sample is open. The standards and the samples should be at the same temperature, preferably as close to 25°C as possible.

4.6 ATOMIC ABSORPTION SPECTROPHOTOMETRY

Atomic Absorption Spectrophotometry (AAS) is used for the determination of trace metals in water samples. It can also be used for analysing sediments and biological material after they have been digested with acid.

The advantages of atomic absorption spectrophotometry are that it is a simple, effective and relatively low-cost method of determining a wide range of metallic species. In addition, it can produce rapid results, particularly when many analyses of the same element need to be performed.

4.6.1 THE PRINCIPLE OF ATOMIC ABSORPTION SPECTROPHOTOMETRY

Heavy metal concentrations can be determined by either Flame Atomic Absorption Spectrophotometry (FAAS) or Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS).

AAS relies on the fact that atoms of all elements can absorb radiation of a particular frequency, and that this absorption is proportional to the concentration of the species that is present.

In order to analyse a sample, an AAS must first create atoms from the various chemical species in a sample (atomisation). This can be carried out in a number of ways but the most common method is to spray the sample into a flame, the heat from which is sufficient to break down the compounds in the sample into their constituent atoms. It is now possible for the atoms of one of the elements present in

the flame to be transformed to a more excited state by incident radiation of a particular wavelength – the wavelength being specific to the element being excited. In moving to a more excited state, the atoms in the sample absorb energy, the quantity of which is proportional to the concentration of atoms present. Absorption of energy is calculated by the difference in signal received, at a detector downstream of the flame, in the presence and absence of the test element.

4.6.2 THE ATOMIC ABSORPTION SPECTROPHOTOMETER

From the above discussion on the principles of AAS, it is clear that the essential features of an atomic absorption spectrophotometer.

- a source of energy of the appropriate wavelength for the element being detected
- a detector capable of receiving and quantifying radiation emitted by the source
- a means of atomising the sample

In addition, other devices may be present within the AAS including a monochromator, which passes light of only one frequency to the detector, and some means of enabling correction for background radiation to be carried out. This latter task is usually accomplished by pulsing the output of a single source, often by passing the beam through a spinning wheel divided into opaque and transparent segments (a 'chopper', as shown in Figure 4.18), or by having two energy sources (Figure 4.18).

The normal energy source of an AAS is a hollow cathode lamp. This device has a cathode made of the element to be determined. When power is applied, the lamp emits energy at wavelengths specific to the element of which its cathode is made.

The detector inside an AAS is often a photomultiplier tube. This device works by emitting electrons as radiant energy falls upon its detection surface. The design of the tube ensures that each electron released eventually generates many electrons within the device (multiplying) thus amplifying the original signal.

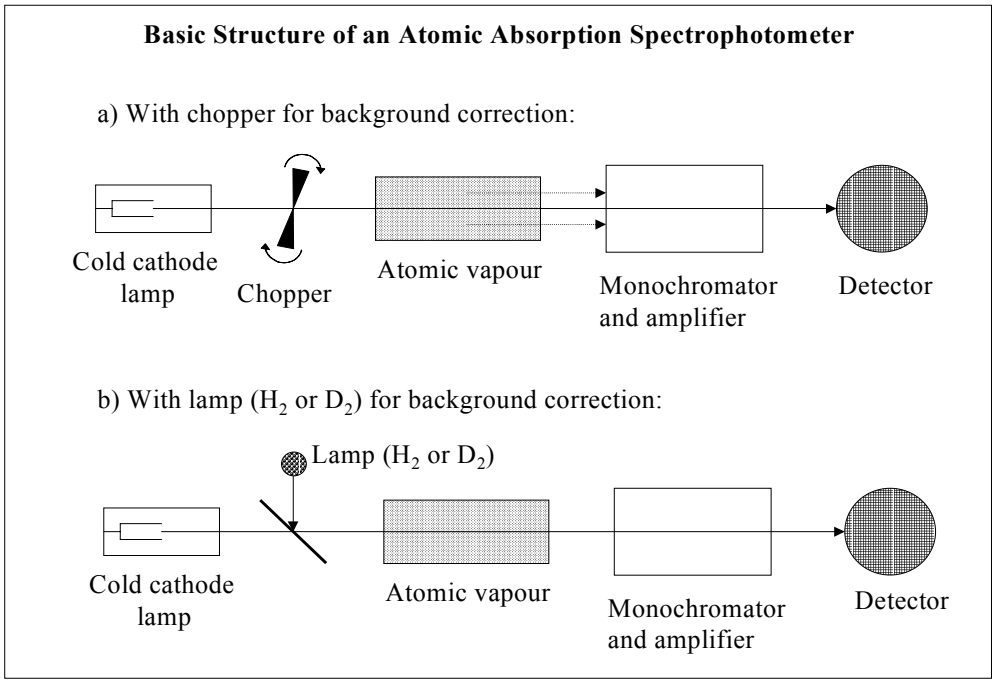


Figure 4.18: Basic Structure of an Atomic Absorption Spectrophotometer

A number of methods can be used for atomisation of the sample within an AAS as discussed below:

Flame Atomisation

As discussed above, a flame atomisation AAS uses a gas burner to atomise the element being analysed. It is possible to create this flame with a number of different gas mixtures each of which has a characteristic frequency and burning velocity. At present the most common flame gas mixtures for water analysis are air-acetylene and nitrous oxide-acetylene.

Whilst the flame represents a simple, convenient and reproducible source of heat, it does have its drawbacks including the following:

- the flicker and general instability of the flame can lead to a noisy signal
- sampling is relatively inefficient because two sequential processes (solvent evaporation followed by atomisation) must take place within a very short time
- the residence time of material in the flame is extremely short meaning that significant quantities of sample must be sprayed into the flame to achieve a continuous signal which can be measured
- only rarely can solid samples be atomised directly

Reactions taking place within the flame can give rise to interferences, the main ones being incomplete dissociation of materials or the formation of thermally stable compounds. Sometimes the latter problem can be overcome by changing the fuel to produce a hotter flame.

Flame interference can also arise from differences in the bulk property (viscosity, for example) of the liquids being analysed which result in changes to their mode of aspiration and transport through the flame. Due to this type of interference it is possible for two dissimilar liquids with identical concentrations of metals to give different AAS readings.

A further type of flame interference may arise during the analysis of calcium compounds. It is possible that, if the flame temperature is high enough, calcium atoms can become ionised thus diluting the concentration of analysable atoms in the flame. Normally, more easily ionised atoms (eg, sodium) are added to the sample to prevent this occurring.

The detection limits and operating conditions for FAAS determinations of a variety of elements are given in Table 4.6.

Element	Wave length (nm)	Flame gases	Detection limit (mg/l)	Optimum Concentration range (mg/l)
Ag	328.1	A-Ac	0.01	0.1-4
As	193.7	N-H	0.002	0.002-0.02
Cd	228.8	A-Ac	0.002	0.05-2
Cr	357.9	A-Ac	0.02	0.2-10
Cu	324.7	A-Ac	0.01	0.2-10
Hg	253.6	A-Ac	0.2	10-300
Mn	279.5	A-Ac	0.01	0.1-10
Ni	232.0	A-Ac	0.02	0.3-10
Pb	283.3	A-Ac	0.05	1-20
Se	196.0	N-H	0.002	0.002-0.02
Zn	213.9	A-Ac	0.005	0.05-2

Table 4.6: The detection limits and operating conditions for FAAS

Where:

A-Ac = Air – Acetlyene gas

N-H = Nitrogen-Hydrogen gas

Electrothermal Atomisation: Graphite Furnace

Electrical heating is now becoming a popular method of atomising samples for AAS analysis. The most common type of electrical heater is the graphite furnace which normally consists of a small tube of graphite which is heated by passing a large electrical current through it - the sample for analysis being previously introduced into the graphite tube.

The temperature of the graphite furnace is normally raised in three steps as below:

I. heated to approximately 300^{o}C for one minute to evaporate solvent

II. heated to approximately 1700^{o}C for one minute to burn off organic matter

III. finally heated to final temperature (often 3000^{o}C) for the analysis phase

In most models of AAS it is possible to vary both the temperatures and their residence times to suit the particular analysis being performed.

The main advantages of electrothermal atomisation are:

- higher sensitivity resulting in detection limits for water samples being from 20 to 1000 times better than flame techniques
- the ability to handle very small sample volumes
- low noise
- the ability to handle a wide range of samples including solids, organic solvents, viscous liquids and liquids with a high dissolved solids content

Disadvantages of electrothermal atomisation are that often precision is lower and interference more severe than with flame methods.

Similar interferences to those discussed under flame techniques arise with electrothermal methods (see above). A particular problem with graphite furnaces, however, is the formation of thermally stable carbon compounds due to reaction between elements in the sample and the graphite furnace. Such compounds tend to absorb or scatter radiation leading to analytical inaccuracies.

Cold-vapour Atomisation: for analysis of mercury compounds

This is a particular technique which is applied to the analysis of mercury compounds in water. It relies on the fact that mercury vapour can be released by chemical reaction at room temperature from a sample containing the metal. The sample is treated with an oxidising mixture of nitric and sulphuric acids to convert mercury to Hg^{2+}, followed by reduction to the metal with $SnCl_2$. The elemental mercury vapour is then swept with by bubbling a stream of inert gas through the mixture. This vapour is then measured in a 'flow-through' cell by a conventional AAS equipped with the appropriate accessories for this determination.

Hydride Atomisation

Hydride generation technique provides a method for introducing samples containing elements, such as arsenic, selenium and lead in to an atomiser as a gas. Such a procedure enhances the detection limit by a factor of 10 to 100. Volatile hydrides are generated by addition of sodium borohydrate to acidified sample. The volatile hydride is swept in to the atomisation chamber by an inert gas. The atomisation chamber is usually a silica tube heated to several hundred degrees.

4.7 GAS CHROMATOGRAPHY

Gas chromatography (GC), like all chromatography, is a separation technique; that is to say that it is a method of separating mixtures of chemicals rather than analysing them. However, virtually all commercially available gas chromatographs include a detector which can, in conjunction with the known chromatographic behaviour of the chemical being determined, both identify and quantify many different chemical species.

There are actually two gas chromatography techniques, gas-solid chromatography (GSC), in which the stationary phase is a solid, and gas-liquid chromatography (GLC) in which the stationary phase is a liquid held as a thin layer upon a solid. Of the two techniques, the latter tends to be the one that is used in water analysis and therefore the one which will be discussed as gas chromatography in the remainder of this section.

Gas chromatography is widely used in water analysis as it provides a quick and relatively inexpensive way of determining many organic compounds, particularly pesticides, polychlorinated biphenyls (PCBs) and volatile organic compounds (VOCs). It is, however, a sophisticated analytical technique in that it often requires an experienced operator to interpret and evaluate the results of analyses.

4.7.1 THE PRINCIPLE OF CHROMATOGRAPHY

There are many different chromatographic methods including gas chromatography, liquid chromatography, paper chromatography and thin-layer chromatography. All of these techniques consist of two immiscible phases, one of which is mobile and the other stationary. Stationary phases tend to be solids, or thin liquid films adsorbed onto solids, whilst mobile phases are normally gases or liquids. Separation of materials is achieved by utilising the fact that each chemical in a mixture will partition differently between the two phases and therefore make dissimilar progress through the chromatographic process.

Consider, for example, the case of a liquid flowing through a column packed with solid granular material. If a mixture of two chemicals is introduced into the liquid they will begin to flow through the column together. However, each chemical will have a different tendency (perhaps only very slightly different) to become adsorbed on the solid material in the column and this adsorption will impede progress of the material through the column. If the column is long enough and the potential sites for adsorption extensive enough (normally achieved by careful selection of the solid material), complete separation of the two materials can be achieved.

The tendency for a species to separate between two phases in chromatography can be expressed by the ‘partition ratio’ (k'), defined as:

$k' = n_s / n_m$

where n_s and n_m are the number of moles of solute present in the stationary (s) and mobile (m) phases

The larger the value of n_s (the number of moles in the solid phase), the longer the material will take to pass through the column.

The extent to which two substances (1 and 2) can be separated is expressed by the relative retention (α) expressed as:

$$\alpha = k'_2 / k'_1$$

The subscripts of 'k' in the above equation are assigned on the basis of faster speed through the column having the higher number so α is always be greater than 1.

4.7.2 GAS CHROMATOGRAPHY

In gas chromatography the mobile phase is a gas (most commonly helium but nitrogen, argon-methane and hydrogen are also used) and the stationary phase a liquid coated onto a solid. In a conventional packed column diatomaceous earth (also known as kieselguhr) is often used as the solid support material whilst in a so-called 'capillary column' the walls of the column itself form the solid surface. Many materials have been used as gas chromatography liquid phases including waxes, rubbers and glasses that are solid at room temperature but become liquids at the operating temperature of the GC.

A gas chromatograph is capable of analysing very small samples of liquids; in fact small samples must be used or the column will be overloaded and inadequate separation will result.

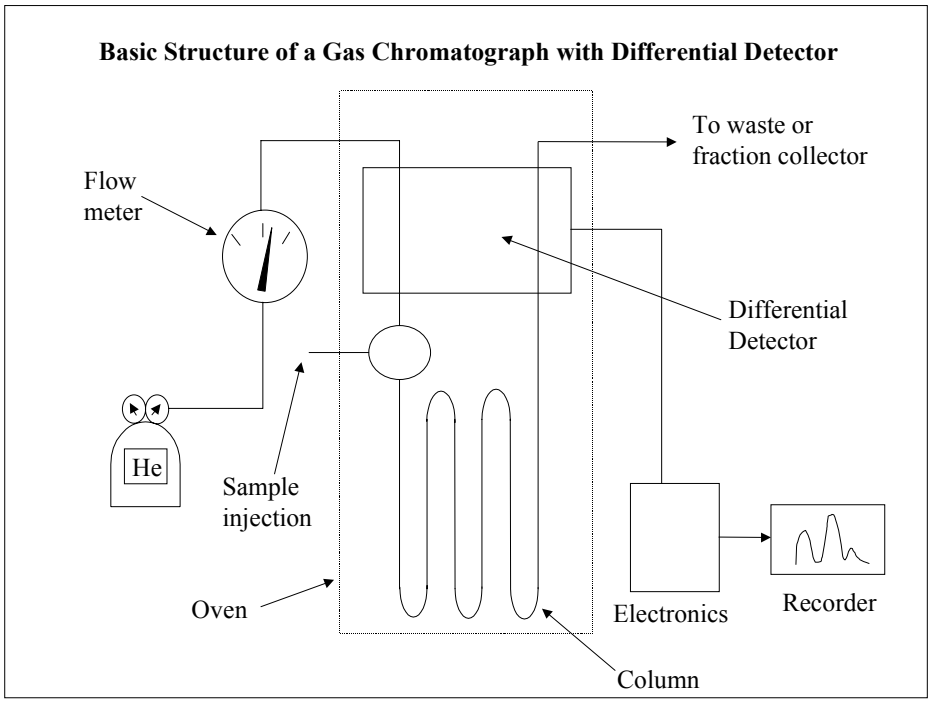


Figure 4.19: Basic Structure of a Gas Chromatograph with Differential Detector

A gas chromatograph (see Figure 4.19) normally consists of the following six components:

1. A supply of carrier gas
2. A sample injection system

3. The separation column
4. A detector
5. A chart recorder and its associated electronics
6. A temperature regulated oven to contain the column and detector

In order to better understand the workings of a GC, each of the above components will be considered in more detail below.

Carrier Gas Supply

As stated above, helium is the gas most frequently used due to the requirement that the carrier gas needs to be inert. The supply from a pressurised bottle, is delivered to the gas chromatograph through a flow meter to ensure that an optimum flow rate is maintained.

Sample Injection System

Due to the need to introduce a small volume of sample , material is often injected onto the column by means of a small diameter (0.01 to 50 μL) hypodermic syringe. However, special valves and flow splitting devices which can limit the quantity of material reaching the column are also used.

Separation Column

Chromatographic columns vary in length from less than 2 m to 50 m or more. They are constructed of stainless steel, glass, fused silica or Teflon tubes.

Packed columns: These columns typically have lengths of 2 to 3 m and inside diameters of 2 to 4 mm. The tubes are densely packed with a uniform finely divided packing material (solid support), that is coated with a thin layer (0.05 to 1 μm) of the stationary liquid phase.

The ideal solid support should be of small, uniform, spherical particles.150-250 μm dia, with good mechanical strength and a specific surface are of at least 1 m^2/g. In addition the material should be inert at elevated temperatures. The most widely used support is made from naturally occurring diatomaceous earth.

Desirable properties for the liquid phase include: the boiling point should be at least 100°C higher than the maximum operating temperature; thermal stability; chemical inertness: the values of k' and α for the solutes (analytes) should be within a suitable range to give acceptable resolution. Table 4.7 lists three commonly used stationary phases commonly used for analytes of environmental significance.

Capillary columns : The most widely used capillary columns have inside diameters of 260 and 320mm. Higher resolution columns with diameters of 150 and 200 μm are also available. Such columns require a sample splitter to inject a small size of sample. Recently, 530μm diameter megabore columns have also become available, which can take higher size samples, comparable to packed columns. The lengths of the columns range between 10 and 100m.

The stationary phase listed in Table 4.7 for packed columns are also used in capillary columns. The stationary phase may be directly coated on the wall of the capillary, or first the inner surface of the capillary is lined with a thin film of a support material, such as diatomasceous earth. The latter column holds a larger amount of stationary phase and can take larger size samples.

Stationary phase	Trade name	Maximum temperature, °C	Common applications
Polydimethyl siloxane	OV-1, SE-30	350	General purpose non-polar phase; hydrocarbons; PAHs; PCBs
Poly (phenylmethyl) Siloxane (50% phenyl)	OV-17	250	Durgs; pesticides
Poly (difluoropropyldi-methyl) siloxane	OV-210	200	Chlorinated aromatics; nitroaromatics; alkylsubstituted benzenes

Table 4.7: Common stationary phase for analysis of environmental samples

Detector

There are many detectors which can be used for gas chromatography. For water analysis, however, five principal types of detector are used as follows:

1. Electrolytic Conductivity Detector – this detector is much used to analyse environmentally significant compounds such as pesticides, herbicides and nitrosamines. It consists of a reference electrode, an analytical electrode, a gas-liquid contactor (where solvent is combined with the material coming off the column) and a gas liquid separator (where solvent and gas are separated). Detection is accomplished by measuring the difference in conductivity between the reference electrode, which is only exposed to solvent, and the analytical electrode, which is exposed to both solvent and material from the gas chromatography column.
2. Electron Capture Detector (ECD) – this detector is used to analyse compounds which have a high electron affinity such as chlorinated pesticides and other halogen-containing compounds. It works by passing the material emerging from the column over a radioactive beta particle emitter contained within a cell. The beta particles released create many more electrons in the cell and these are periodically collected by the application of short voltage pulses to the cell electrodes. In order to maintain a constant current within the cell, the frequency of the voltage pulses is varied and it is this variation in pulse frequency when the sample enters the cell that is proportional to the concentration of sample present. The sensitivity of this method of detection means that it can detect extremely small (sub-picogram) concentrations of material.
3. Flame Ionisation Detector (FID) – this detector responds well to carbon-containing compounds and is therefore widely used for the detection of organic material. It consists of small hydrogen/air flame into which the output from the gas chromatography column is routed. The method works because most organic compounds, when pyrolised in a flame, produce ionic species which conduct electricity. Therefore by monitoring the voltage across the flame it is possible to measure the quantity of organic material present. This type of detector is easy to use and rugged, has a high sensitivity, offers a large linear response and is low in noise. The principal disadvantage with a detector of this kind is that in detecting the sample it also destroys it.
4. Photoionisation Detector (PID) – Photoionisation is a the term for the dissociation of a molecule into its parent ion and an electron when a photon of light is absorbed. In a PID this phenomenon occurs when the material emerging from the GC column is irradiated by light from a UV lamp. The ions produced induce a voltage which is proportional to the concentration of material present. The photoionisation detector is non-destructive and has high sensitivity, low noise and excellent linearity.
5. Mass Spectrometer (MS) – it is possible to route the output from a gas chromatograph to a mass spectrometer. This device transforms molecules into ions which it then sorts through a series of magnetic filters and lenses by making use of their differential. mass-charge ratio. Identification is achieved by comparing the resulting spectrum against a library of known spectra. The mass spectrometer is an expensive device but is able to identify and quantify a wide variety of chemical species.

Chart Recorder

The chart recorder plots the results emerging from the detector as a series of peaks on a continuously moving sheet of paper. Normally, however, this type of chart records the concentration of material but does not record the absolute quantity of the material present. It is often necessary, therefore, to integrate the peak produced to obtain the total quantity of material which has entered the detector. Whilst it is possible to use manual methods to perform this integration, most modern GCs incorporate integration circuits into their electronic circuitry.

Temperature Regulated Oven

Gas chromatographs are equipped with a thermostatic oven to aid volatilisation of the sample and to ensure reproducibility of column retention times. Normally such ovens are held at a constant temperature but it is possible, with a programmable oven, to alter the temperature of the oven throughout the sample run to better separate the components of a mixture.

5 WATER QUALITY PARAMETERS

5.1 INTRODUCTION

In Chapter 1, Section 1.2 of this volume, an overview of water quality parameters was given. There is a large range of water quality parameters (sometimes also referred to as variables) that can be used to characterise waters. It is true, however, that some parameters are of special importance and deserve frequent attention. Further, depending upon the objective of characterisation of a water body, different suits of parameters may be selected. Tables 5.1 and 5.2 give a comprehensive list of standards adopted by various organisations for different uses of water.

This chapter discusses those parameters which can be determined in the laboratories contributing to the Hydrological Information System. The parameters are grouped and discussed according to their significance with the objective of providing information to help an appropriate selection of the parameters for a particular monitoring programme. Basic concepts from chemistry, microbiology and instrumental methods of analyses given in Chapters 2, 3 and 4 of this volume may also be reviewed for a better understanding of the significance of the parameters discussed here. Analytical methods which can be used for the determination of each parameter are also mentioned. Detailed analytical procedures are given in Volume 7, Water Quality Analysis, Operation Manual.

5.2 GENERAL

The parameters belonging to this group give a general picture of the quality of the water body. Except for the solids determination, analysis for the rest of the parametrs can be performed in the field also. These are, therefore also called ‘field parameters’.

5.2.1 TEMPERATURE

The temperature of a surface water body depends on its location, season and time of the day. The temperature of tropical and sub-tropical rivers may vary from 10 to 30 ^{o}C. Temperature of rivers receiving water from snow melt in their upper reaches may be even lower than 10 ^{o}C.

Warm temperatures result in:

- decrease of solubility of gases in water, such as, O_2, CO_2 and N_2
- increase in the metabolic and growth rates of the aquatic organisms
- increase in volatilisation and chemical reaction rates of substances
- increase the die-away rate of micro-organisms, which are not normal inhabitant of the aquatic environment

Considering the above factors, it is seen that the warm water environment, along with organic pollution, would lead to a greater stress on the oxygen resources of the stream. In the case of addition of nutrients, it would also lead to eutrophication of the water body.

5.2.2 COLOUR

Ordinarily, surface waters do not have any true colour. Naturally present minerals and humic acids in dissolved state may impart their characteristic hues. Presence of suspended matter may give an apparent colour. Green, yellow-brown or red colour may be the result of presence of different micro-organisms, particularly, algae. Presence of suspended, inorganic matter may also result in an apparent colour.

Water Quality Standards specified by Bureau of Indian Standards (Tolerance Limits) are defined below:

S. No.	Characteristics	Tolerance limits				
		A	B	C	D	E
1	pH	6.5 to 8.5	6.5 to 8.5	6.5 to 8.5	6.5 to 8.5	6.5 to 8.5
2	Dissolved Oxygen mg/L min	6	5	4	4	
3	Biochemical oxygen demand (5 days at 20°C) mg/L max	2	3	3		
4	Total Coliform organisms MPN/100 ml, max	50	500	5000		
5	Colour Hozen units, max	10	300	300		
6	Odour	Unobjectionable				
7	Taste	Tasteless				
8	Total dissolved solids, mg/L, max	500		1500		2100
9	Total hardness (as $CaCO_3$) mg/L, max	300				
10	Calcium hardness (as $CaCO_3$) mg/L max	200				
11	Magnesium (as $CaCO_3$) mg/L, max	100				
12	Copper (as Cu) mg/L, Max	1.5		1.5		
13	Iron (as Fe), mg/L, Max	0.3		50		
14	Maganese (as Mn) mg/L max	0.5				
15	Chlorides (as Cl) mg/L, max	250		600		600
16	Sulphates (as SO_4) mg/L, max	400		400		1000
17	Nitrates (as NO_3) mg/L, max	20		50		

S. No.	Characteristics	Tolerance limits				
		A	B	C	D	E
18	Fluorides (as F) mg/L, max	1.5	1.5	1.5		
19	Phenolic compounds (as C_6H_5oH) mg/L, Max	0.002	0.005	0.005		
20	Mercury (as Hg), mg/L, max	0.001				
21	Cadmium (as Cd), mg/L, max	0.01		0.01		
22	Selenium (as Se), mg/L, max	0.01		0.05		
23.	Arsenic (as As), mg/L, max	0.05	0.2	0.2		
24	Cyanides (as CN), mg/L, max	0.05	0.05	0.05		
25	Lead (as Pb), mg/L, max	0.1		0.1		
26	Zinc (as Zn), mg/L, max	15		15		
27	Chromium (Cr^{6+}) mg/L, max	0.05	0.05	0.05		
28	Anionic detergents (as MBAS), mg/L, max	0.2	1	1		
29	Polymuclear aromatic hydrocarbons (PAH) mg/L, max	0.2				
30	Mineral oil mg/L, max	0.01				
31	Barium (as Ba), mg/L, max	1				
32	Silver (as Ag), mg/L, max	0.05				
33	Pesticides	Absent				
34	Alpha emitters, uc/ ml, max	10^{-9}	10^{-9}	10^{-9}	10^{-9}	10^{-9}
35	Beta emitters, uc/ ml, max	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}
36	Insecticides			Absent		
37	Oils and grease, mg/L, max			0.1	0.1	
38	Free ammonia (as N), mg/L, max				1.2	
39	Free Carbondioxide (as CO_2) mg/ l				6	
40	Electrical conductance at 25°C, μmho/cm, max				1000	2250
41	Sodium adsorption ratio, max					26
42	Boron (as B), mg/L, max					2
43	Percent sodium, max					60

A Drinking water sources without conventional treatment but after disinfection;

B Outdoor bathing;

C Drinking water sources with conventional treatment followed by disinfection;

D Fish culture and wild life propagation; and

E Irrigation, industrial cooling and controlled waste disposal

Table: 5.1: Water quality standards specified by Indian Standards

Use	Drinking Water					Fisheries and aquatic life		
Variables	**WHO[1]**	**EU**	**Canada**	**USA**	**Russia[2]**	**EU**	**Canada[1]**	**Russia**
Colour (TCU)	15	20 mg l^{-1}Pt-	15	15	20			
Total dissolved solids (mg l^{-1})	1,000		500	500	1,000			
Total suspended solids (mg l^{-1})						25	Inc. of 10 or 10%[3]	
Turbidity (NTU)	5	4 NTU	5	0.5-10				
pH	< 8.0[4]	6.5[1] - 8.5[1]	6.5 - 8.5	6.5 - 8.5	6.0 - 9.0	6.5 - 9.0		
Dissolved Oxygen (mg l^{-1})					4.0	5.0 - 9.0	5.0 - 9.5	4.0[5] - 6.0
Ammoniacal nitrogen (mg l^{-1})					2.0	0.005 - 0.025	1.37 - 2.2[6.7]	0.05
Ammonium (mg l^{-1})		0.5			2.0	0.04 - 1.0		0.5
Nitrate as N (mg l^{-1})			10.0	10.0				
Nitrate (mg l^{-1})	50	50			45			40
Nitrite as N (mg l^{-1})			1.0	1.0				
Nitrite (mg l^{-1})	3(P)	0.1			3.0	0.01 - 0.03	0.06	0.08
Phosphorus (mg l^{-1})		5.0						
BOD (mg $l^{-1}O_2$)					3.0	3.0 - 6.0		3
Sodium (mg l^{-1})	200	150						120
Chloride (mg l^{-1})	250	25[1]	250	250	350			300
Chlorine (mg l^{-1})	5						0.002	
Sulphate (mg l^{-1})	250	250	500	250	500			100
Sulphide (mg l^{-1})			0.05					
Fluoride (mg l^{-1})	1.5	1.5	1.5	2.0	<1.5			0.75
Boron (mg l^{-1})	0.3	1.0[1]	5.0		0.3			
Cyanide (mg l^{-1})	0.07	0.05	0.2	0.2 (PP)	0.07		0.005	0.05
Trace Elements								
Aluminium (mg l^{-1})	0.2	0.2			0.5		0.005 - 0.1[7]	
Arsenic (mg l^{-1})	0.01 (P)	0.05	0.05	0.05	0.01		0.05	
Barium (mg l^{-1})	0.7	0.1[1]	1.0	2.0	0.7			
Cadmium (mg l^{-1})	0.003	0.005	0.005	0.005	0.003		0.0002 - 0.0018[8]	
Chromium (mg l^{-1})	0.05 (P)	0.05	0.05	0.1	0.05		0.02 - 0.002	0.02 - 0.005
Cobalt (mg l^{-1})					0.1			0.01
Copper (mg l^{-1})	2 (P)	0.1[1] - 3.0[1]	1.0	1	2.0	0.005 - 0.112[8.9]	0.002 - 0.004[8]	0.001
Iron (mg l^{-1})	0.3	0.2	0.3	0.3	0.3		0.3	0.1
Lead (mg l^{-1})	0.01	0.05	0.05	0.015	0.01		0.001 - 0.007[8]	0.1

Use	Drinking Water					Fisheries and aquatic life		
Variables	**WHO[1]**	**EU**	**Canada**	**USA**	**Russia[2]**	**EU**	**Canada[1]**	**Russia**
Manganese (mg l^{-1})	0.5 (P)	0.05	0.05	0.05	0.5			0.01
Mercury (mg l^{-1})	0.001	0.001	0.001	0.002	0.001		0.0001	0.00001
Nickel (mg l^{-1})	0.02	0.05			0.02		0.025 - 0.15[8]	0.01
Selenium (mg l^{-1})	0.01	0.01	0.01	0.05	0.01		0.001	0.0016
Zinc (mg l^{-1})	3	0.1[1] - 5.0[1]	5.0	5	5.0	0.03 - 2.0[8, 10]	0.03	0.01
Organic contaminants[11]								
Oil and Petroleum products (mg l^{-1})		0.01			0.1			0.05
Total pesticides (μg^{-1})		0.5	100					
Aldrin & dieldrin (μg^{-1})	0.03		0.7				4 ng l^{-1} dieldrin	
DDT (μg^{-1})	2		30.0		2.0		1 ng l^{-1}	
Lindane (μg^{-1})	2		4.0	0.2	2.0			
Methoxychlor (μg^{-1})	20		100	40				
Benzene (μg^{-1})	10			5			300	
Pentachlorophenol (μg^{-1})	9 (P)			10	10			
Phenols (μg^{-1})		0.5	2		1.0		1.0	1.0
Detergents (mg l^{-1})		0.2		0.5[12]	0.5			0.1

WHO: World Health Organisation
EU: European Union
BOD: Biochemical Oxygen Demand
TCU: True Colour Unit
NTU: Nephelometric turbidity Unit
(P): Provisional value
(PP): Proposed value

1. guideline value
2. some values not yet adopted but already applied
3. for effective disinfection with chlorine
4. i.e. above background concentration of ≤100.0 mg/L or > 100 mg/L respectively
5. lower level acceptable under ice cover
6. total ammonia
7. depending on pH
8. depending on hardness
9. dissolved only
10. total zinc
11. for some groups values are also set for individual compounds
12. foaming agents
13. for a single sample

Sources: Environment Canada, 1987

CEC, 1978, 1980

Committee for Fischeries, 1993

Gray, 1994

WHO, 1993

5.2.3 ODOUR

Fresh water is odour free. Presence of odour suggests higher than normal biological activity due to the presence of decomposable organic material contributed by human or industrial wastes or excessive growth of algae and other plants. Odour is caused by production of volatile organic compounds and inorganics, such as, NH_3 and H_2S. It is more pronounced when the dissolved oxygen in water is less than about 25% of its saturation value. Industrial wastes can also create odours directly.

5.2.4 SUSPENDED SOLIDS

Suspended solids in water may consists of silt, clay and other fine inorganic and organic particles. Planktons may also contribute to the suspended solid load. Normally, the particles range from 10 nm to 0.1 µm in diameter. Their concentration depends on the location of the water body and changes from season to season. During rainy season, it may change hourly during a rainfall event. It is greatly influenced by the catchment area characteristics.

Turbidity, which is a related parameter, is interference to the passage of light or scattering of light by suspended particles in a column of water. It is measured in nephelometric turbidty units (NTU). It may range from 1 to 1000 NTU.

5.2.5 TOTAL DISSOLVED SOLIDS

Total dissolved solids (TDS) refers to the residue left after evaporation of a known volume of water at 105 ^{o}C, which has been filtered through a standard filter. It is approximately equal to the total content of dissolved substances in a water sample since approximately half of the bicarbonate ion, which is one of the dominant ions in waters, is lost as CO_2 during evaporation process.

The TDS value for river waters depends largely on the ratio of the contribution of the overland flow to the subsoil flow. It may vary from less than 50 mg/L to a few thousand mg/L. Surface evaporation in arid climates and agricultural return waters increase the TDS considerably.

5.2.6 CONDUCTIVITY

Conductivity or electrical conductivity (EC) of natural water is due to the presence of salts, which dissociate into cations and anions. It is the ability of a solution to conduct current. The units of EC are µmhos/cm or µS/cm and is expressed at 25 ^{o}C. Even in cases where the chemical composition of water is represented almost exclusively by inorganic ions, the correlation between their content and EC may change considerably since different ions conduct electricity to different extents.

The value of EC may serve as an approximate index of the total content of dissolved substances in water samples. TDS, mg/L may be obtained by multiplying EC, µmhos/cm, by a factor ranging between 0.55 and 0.9. A commonly used value is 0.67. In order to increase the accuracy of the evaluation of the mineral content of waters from EC measurements, it is necessary to establish such correlations, for each body of water.

The conductivity of most fresh waters ranges from 10 to 1000 µmhos/cm. It is, at times, used as an indication of ingress of sea water in estuarine region of a river.

5.2.7 pH

The hydrogen ion concentration in water is expressed in terms of pH. It is defined as the logarithm of inverse of hydrogen ion concentration in moles/L. The pH value of natural waters mostly depends on free carbon dioxide, bicarbonates and carbonate ions. The equilibrium condition may be changed by the intensity of photosynthetic process (which consumes carbon dioxide) and the biochemical oxidation of organic substances (which produces carbon dioxide), as well as chemical conversions of some mineral substances, such as reduction-oxidation reactions of ammonia, sulphur containing minerals, iron, etc. The pH value is also affected by the presence of naturally present humic substances and various acids and alkalis, which may be discharged into the body of water through wastes.

Alkalinity and *acidity* are related parameters, which reflect the capacity of a water sample to neutralise acid or alkalinity, respectively. Measurement of these parameters along with pH may be required when solubility and ionic equilibria of various chemical species are under investigation.

5.2.8 DISSOLVED OXYGEN

The dissolved oxygen (DO) saturation cocentration of water varies with temperature, salinity and atmospheric pressure. In fresh waters, at sea level, it ranges from 15 mg/L at 0 ^{o}C to 7.5 mg/L at 30 ^{o}C. In water samples, it may be expressed in absolute terms as mg/L or as percent of saturation value.

Deviation in the concentration of DO from the saturation equilibrium value in a surface water body may exist due to aerobic biochemical oxidation of organic matter and photosynthetic activity of plants in water. These reactions, combined with atmospheric reaeration may result in establishing a different equilibrium concentration at a location, which may be below or above the saturation value.

Oxygen content of fresh, unpolluted water bodies, having normal biological activity, ranges from 80 to 100% of saturation DO level. Lower levels indicate presence of organic pollution. DO in grossly polluted waters may be less than 25% of the saturation value. At this level, a drastic shift from the biological community of fresh waters may be expected. The water also becomes turbid and foul smelling.

In the main current of a stream the DO is usually the same at all depths because of mixing. However, in still water areas there may be a stratification. This is particularly true for lakes. In eutrophic waters, the variation in DO with depth is very pronounced. Further, it is important to record the time of sampling since wide variation in DO at a location may occur over a 24 hour period.

5.3 NUTRIENTS

Nitrogen and phosphorus are essential for the growth of plants and animals. For this reason these elements are often identified as nutrients, or biostimulants when present in surface waters. It should be noted that both the organic and inorganic forms of these constituents are of importance.

5.3.1 NITROGEN COMPOUNDS

Concentrations of nitrogen compounds referred to in the following discussion are always expressed in terms of mg/L as N (sometimes also written as mg N/L).

Organic nitrogen

In nature, nitrogen is cycled between its organic and inorganic forms. Bacteria and plants are responsible for the production of organic compounds containing nitrogen (proteins) from inorganic forms of nitrogen. Animals, including humans, cannot utilise nitrogen from atmosphere or from inorganic compounds to produce proteins but must obtain nitrogen in organic form. Bacteria also break down organic nitrogen in animal and plant wastes and convert it into inorganic forms.

Organic nitrogen in unpolluted fresh waters is very low. Further, organic nitrogen is easily de-aminated to produce ammonia. It is, therefore, not a routine parameter for characterisation of waters. However, in grossly polluted waters and where nutrient budgets are studied, organic nitrogen may be measured.

Ammonia

Proteins are broken down by bacterial activity to ammonia and urea, the latter in turn is also hydrolysed to ammonia and carbon dioxide. Ammonia may also be discharged into water bodies through industrial wastes. In water, it exists both as molecular specie, NH_3 and in ionic form, NH_4^+. Total ammonia, or simply ammonia refers to the sum of the two. At pH 7 nearly all of the ammonia exists in the ammonium form. At higher pH values, the ionic form is converted to the molecular specie and may be lost from the aqueous phase through volatilisation.

Total ammonia concentration in surface waters typically range between 0.1 and 2 mgN/L. Higher values may indicate pollution. Concentrations of undissocated ammonia upto 1 mgN/L may be tolerated by most fishes. However, toxicity varies greatly from specie to specie.

Oxidised nitrogen

In aerobic environment, ammonia is biochemically converted to nitrite (NO_2^-) and then to nitrate (NO_3^-) by nitrifying bacteria. The total of nitrite and nitrate nitrogen is called oxidised nitrogen. The rate of conversion of nitrite to nitrate is much faster than the formation of nitrite from ammonia. Therefore, nitrite concentration is usually much lower compared to nitrate. Nitrates may also be contributed to fresh waters through igneous rocks, land drainage and agricultural run-off. Under anaerobic conditions, nitrates may be converted to nitrogen gas and would be lost from the aquatic system.

In fresh waters nitrate nitrogen concentration seldom exceeds 0.1 mgN/L. Higher concentrations indicate the effect of human activity. Concentrations greater than 5 mgN/L indicate a significant level of pollution. Though many factors influence eutrophication phenomenon, waters having oxidised nitrogen in excess of 0.2 to 0.5 mgN/L are prone to algal blooms provided other requirements for the growth of algae are met.

5.3.2 PHOSPHORUS COMPOUNDS

The phosphorus containing compounds of interest with respect to water quality include orthophosphates (PO_4^{3-}), polyphosphates ($P_3O_{10}^{5-}$, $(PO_3)_5^{3-}$, $P_2O_7^{4-}$) and organically bound phosphate. The concentration of phosphates is always expressed as P and not as PO_4^{3-}. The sources of phosphorus are mainly the drainage from phosphate bearing rocks, agricultural runoff and decomposing organic matter. Considerable amounts may be added through pollution from municipal and industrial wastes.

Orthophosphates and polyphosphates, which are the dissoved forms, are often the limiting factor in the process of photosynthesis. They are converted to organic forms during the growth of plants and are utilised to a point where insignificant, small concentrations are left. The main users of organic

phosphorus are zooplanktons and bacteria. They are also responsible for recycling of the organic form into the soluble form. As a result there can be a seasonal change in the concentration of soluble phosphorus in surface waters.

Concentrations of phosphorus compounds should be expressed in terms of mgP/L. In most natural surface waters the concentration of dissolved phosphorus may be 0.02 mgP/L or less, often as low as 0.001 mgP/L. Higher concentrations indicate presence of pollution and may result in the eutrophication of the water body.

5.4 ORGANIC MATTER

The content of organic matter in water is one of the most important parameters of the quality of water. The parameters discussed in the following section measure the aggregate organic substances. Usually the measurement includes both the suspended and dissolved forms. It serves as a useful pollution index of a body of water. Determination of individual organic compounds of interest, such as phenols, humic acids, pesticide, etc., is carried out through specific tests.

5.4.1 BIOCHEMICAL OXYGEN DEMAND

Micro-organisms utilise waste organic matter as food. In aerobic environment, the organic matter is biochemically converted to carbon dioxide and water. The biochemical oxygen demand (BOD) test measures the oxygen consumed in the reaction. The standard test is carried out under controlled laboratory conditions, at a constant temperature and over a specified time. Since not all organic matter is biochemically decomposable, the test measures the oxygen equivalence of the degradable matter only. Compounds such as cellulose, lignin and many synthetic petrochemicals are very resistant to biological breakdown.

Nitrification is the term applied to the biological oxidation of ammonia to nitrate. The oxygen consumed during the process is differentiated from that required for the oxidation of organic matter. It is called the nitrogenous BOD.

The BOD of unpolluted waters is usually less than 2 mg/L. Higher values indicate organic pollution from municipal or industrial wastes. The BOD of domestic and municipal wastes ranges between 150 and 400 mg/L. In slow moving streams, values greater than 8 mg/L indicate the possibility of on set of anaerobic conditions in the stream since the oxygen demand may exceed the supply of oxygen through atmospheric reaeration. Ammonia, if discharged with organic wastes, is oxidised only after the stabilisation of the major portion of the organic matter. The BOD test is used extensively in the modelling of oxygen concentration in rivers and streams subjected to pollution.

5.4.2 CHEMICAL OXYGEN DEMAND

The chemical oxygen demand (COD) test measures the oxygen equivalent of the organic matter using potassium dichromate, which is a strong oxidant. The oxidation is carried out at a high temperature in an acidic medium, in the presence of a catalyst, to ensure complete oxidation of all organic matter. Only aromatic hydrocarbons and pyridines are not oxidised.

One of the chief limitations of the COD test is its inability to differentiate between biologically oxidisable and biologically inert organic matter. In addition it does not provide any information regarding the rate at which the oxidation of biodegradable matter would proceed in nature.

The COD test is used extensively in surveys where industrial wastes are discharged in streams. In conjunction with the BOD test, the COD test is helpful in indicating toxic conditions and the presence of biologically resistant organic matter. Compared to the BOD test, it has better precision and can be completed in a shorter period of time.

The COD of unpolluted surface waters is typically lower than 20 mg/L, which is mainly due to the presence of humic substances and the normal biota of the water body. The COD value of domestic and municipal wastes ranges between 400 and 800 mg/L.

5.4.3 TOTAL ORGANIC CARBON

For smaller amounts of organic carbon, the instrumental, total otganic carbon (TOC) method has proved to be satisfactory. In one form of this test, first the acidified sample is purged to remove the inorganic forms of carbon. Then a small amount of sample is evaporated and oxidised catalytically at high temperature to carbon dioxide which is measure with an infrared analyser.

The TOC value of unpolluted surface waters is usually less than 10 mg/L. Note that while the COD and BOD tests measure the oxygen equivalence of carbon, the TOC test measures the carbon content. In most samples COD > BOD > TOC.

5.5 MAJOR IONS

All natural waters contain dissolved ionic constituents. Analyses of surface and groundwaters from various sources has shown that the following species represent the principal chemical constituents.

CATIONS	ANIONS
Calcium	Bicarbonate
Magnesium	Sulphate
Sodium	Chloride
Potassium	Nitrate

Typically the ionic species are derived from the contact of water from various mineral deposits. The occurrence of these ions and the consequences, if they exceed certain limits, with respect to water quality and use, are discussed in Section 2.9, of this volume.

5.6 OTHER INORGANICS

5.6.1 HYDROGEN SULPHIDE

In surface waters, hydrogen sulphide is formed under oxygen deficient conditions, which may develop in the benthic layers of slow moving streams, or when the oxygen demand exerted by decomposing organic matter is in excess of the reaeration rate of the stream. Under such conditions sulphates are reduced biochemically to hydrogen sulphide. Hydrogen sulphide is also produced from the decomposition of sulphur containing organic compounds.

Hydrogen sulphide is a weak acid, which ionises to yield hydrosulphide (HS^-), and sulphide (S^{2-}) ions. At pH 7, the concentrations of the molecular specie and the hydrosulphide ion are nearly equal. The concentration of sulphide ion becomes significant only at pH 10 or above. Under acidic conditions, the concentration of hydrogen sulphide predominates.

Hydrogen sulphide is extremely toxic to fish. Concentrations of total sulphides as low as 0.01 mg/L make the water unfit for uninhibited fish culture. Hydrogen sulphide also reduces the aesthetic value of the water body due to its foul odour.

5.6.2 SILICA

Silicon is widespread in earth's crust. Degradation of silica containing rocks results in the presence of silica in natural waters. It occurs as suspended particles, in a colloidal or polymeric state and as silicic acids or silicate ions.

The silica content of natural waters ranges between 1 and 30 mg/L. Silica is an essential element for aquatic plants, principally diatoms. Silica is also undesirable in industrial waters as it forms scales, which are difficult to remove.

5.6.3 FLUORIDES

Fluorides appear in unpolluted natural water as the result of the interaction of the water with fluorine containing minerals. Natural surface water contains fluorides in amounts which usually do not exceed 1 mg/L. Fluorides may also be contributed to surface waters through industrial wastes, such as, from glass industry and some ore enriching plants.

Fluoride, in concentration range between 1.5 and 2 mg/L in drinking water, results in mottling of teeth. Higher concentrations may cause bone diseases.

5.6.4 BORON

Boron may occur in natural waters through weathering of rocks, soil leaching, or find its way into a watercourse through industrial waste effluents. Many cleaning compounds contain boron. Concentrations in unpolluted waters do not exceed 0.1 mg/L and seldom reach 1 mg/L level.

Boron in excess of 2.0 mg/L in irrigation water is deleterious to plants. Some sensitive crops may be adversely affected by concentrations as low as 0.5 mg/L. Drinking waters should not contain more than 1.0 mg/L.

5.6.5 CYANIDE

'Cyanide' refers to all the cyanide groups in cyanide compounds. Cyanide occurs in water as weakly ionised hydrocyanic acid or as complex compounds with metals. They may enter fresh water through industrial wastes such as from the plating industry or leachates from garbage dumps.

Cyanides are extremely toxic. Drinking waters should not contain more that 0.5 mg/L cyanide. Concentration in surface waters supporting aqua culture should not exceed 0.005 mg/L.

5.7 TRACE ELEMENTS

In addition to the major and minor ionic species found in natural waters, a variety of inorganic species, principally heavy metals, of anthropogenic origin may also be found. The more important of these are given in Table 5.3. These constituents are of concern because of their toxicity to microoganisms, plants and animals. Typically the presence of these constituents is due to improperly processed industrial wastes.

Inorganic specie	Remarks
Arsenic	A
Cadmium	B, D
Chromium	B
Copper	B
Iron	C
Mercury	B, D
Manganese	C
Lead	B, D
Nickel	B
Selenium	A
Zinc	B, E

A – toxic, non-metal, B – toxic, heavy metal, C – not toxic in trace concentrations,
D – priority because of high toxicity, E – priority because of association with others.

Table 5.3: Toxic trace elements

Because of the long list of the parameters in this category, a few are included in monitoring programmes on priority basis. In case one or more of these may be found, samples for others may be analysed.

Metals in waters can exist in dissolved, colloidal and suspended forms, as precipitates or adsorbed on other particles. Speciation and partitioning of the metals in a natural aquatic environment is a rather complicated and poorly understood phenomenon. Specification studies should be carried out only under specially designed surveys. Further, as dissolved metals occur in very low concentrations, it is recommended that metals are measured as the total metal concentration in a water sample.

As a rule the ionic form of a metal is the most toxic form. However, there are exceptions, such as methylmercury formed by aquatic organisms.

The concentration of different metals in unpolluted waters varies over a wide range,0.001 – 0.1 µg/L. Tables 5.1 and 5.2 list concentrations which are allowable for different uses.

5.8 ORGANIC CONTAMINANTS

Thousands of organic compounds have been synthesised by man. This section briefly discusses those classes of organic compounds that have found their way into natural waters and whose presence is of concern from ecological standpoint. Of greatest concern are those organic compounds that may be carcinogenic and may cause mutations in humans and other living forms at extremely low concentrations.

5.8.1 PESTICIDES

Pesticides are toxic substances, primarily used for the control of diseases and pest organisms in agriculture. These chemicals can be grouped on the basis of their molecular structure: chlorinated hydrocarbons, organophosphates, carbamates and derivatives of urea. The major source of these chemicals in surface water is agricultural runoff. Representative members of each group are given Table 5.4.

Class	Function	Trade name
Chlorinated hydrocarbons	Pesticide	Aldrin, Chlordane, DDT, Dieldrin, Lindane, Eldrin, Heptachlor, Toxaphene
	Herbicide	2,4 – D; 2, 4, 5 – T, Silvex
Organophosphates	Pesticide	Diazinon, Malathion, Parathion
Carbamate	Herbicide	Carbyl
Urea derivatives	Fungicide	Ferbam, Zeram

Table 5.4: Representative pesticides

Pesticides and herbicides belonging to the chlorinated hydrocarbon group are most widely spread. They have been in use longer compared to others and also have greater resistance to breakdown from natural processes. Organophosphorus pesticides have relatively lower chemical and biological stability and decompose in the environment within a month.

Most of the pesticides are insoluble in water but highly soluble in hydrocarbos and fats. The have the ability to accumulate in fatty tissues of organisms, reaching a much higher concentration in certain aquatic biota compared to the surrounding water and sediments. Concentration of pesticides in water bodies may range from 10^{-5} to 10^{-2} mg/L. Due to their toxic nature, the maximum allowable concentrations given in Table 5.1 and 5.2 must be strictly adhered to.

5.8.2 SURFACTANTS

Surfactants comprise one of the most widely spread groups of substances which pollute the water. When soaps and synthetic detergents are added to water, the non-polar end of the molecule dissolves in the grease-like material, which is to be removed. The polar end resists this action, and assisted by mechanical action the grease film is broken and can be removed by washing. Earlier, synthetic detergents were mostly of the alkyl-benzene-sulfphonate (ABS) type, which were hard to decompose biochemically and, therefore, persisted in the environment for long periods. Later, linear alkyl-sulphonate (LAS) came into use, which are biodegredable.

Although surfactants are not highly toxic, they can adversely affect aquatic biota. Detergents can impart taste and odour to water at concentrations of 0.4 – 3 mg/L. The threshold concentration to foam formation is 0.1 – 0.5 mg/L. The term, methylene blue active substances (MBAS) is commonly used in reporting the presence or absence of detergents.

5.8.3 PHENOLS

The term phenols is usually restricted to the simplest hydroxy derivatives of benzene, whose hydrogen atoms have been substituted by one to three hydroxyl and a few simple alkyl and alkoxy group. Even with this simple definition, the composition of phenols in natural waters may be very complex. The properties of phenols and their influence on organisms also differ considerably.

Phenols are formed during metabolic processes of aquatic organisms, particularly during the biochemical breakdown of algae. Phenols may also enter the water from discharge of industrial wastes, such as, those from chemical, coal and oil producing industries. Phenols are biochemically degradable.

Phenolic compounds have a greater deleterious effect on water quality compared to pure phenols. Chlorophenols at 0.001 to 0.005 mg/L may affect taste of water and cause tainting of fish. Concentration of phenols in unpolluted waters is usually less than 0.02 mg/L. Toxic effect on fish may be observed at higher concentrations.

5.8.4 MINERAL OIL AND PETROLEUM PRODUCTS

Mineral oil and its derivatives are a complex and varied mixture of substances composed of various hydrocarbons and unsaturated heterocyclic compounds containing nitrogen, oxygen and sulphur. Mineral oil and petroleum products enter the natural water along with the wastes from oil producing and processing and chemical industries. Since hydrocarbons are the principal component of oils, the term 'petroleum products' is applied to this fraction to ensure comparability of analytical data.

The permissible concentration of mineral oil and petroleum products in drinking water supply source and fisheries production are generally between 0.01 and 0.1 mg/L. Concentrations of 0.3 mg/L or more can cause toxic effects in fresh water fish.

5.8.5 OTHER ORGANIC CONTAMINANTS

The classes of compound discussed above, by no means, comprehensively include all types of toxic organic contaminants.. There are many compounds which are known carcinogens. These are used in industrial processes and emitted in waste products. To name a few, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAH), benzene, toluene, dichloromethane carbon tetra chloride, trichloroeathane 1, 1, 1, whose presence in the aquatic environment has been recorded.

The above compounds are not included in monitoring programmes, routinely. It is difficult and expensive to monitor the presence of such micro pollutants, which may occur in concentrations of a few ng/L in water. Special survey programmes may be undertaken for their monitoring around the suspect sites.

5.9 BIOLOGICAL

5.9.1 COLIFORMS BACTERIA

Microorganisms are a valuable parameter of water quality in relation to drinking water quality. Although tests are available for specific pathogenic organism, there is no way of knowing which pathogenic organism is present in a sample. Also the cost of testing for all the pathogenic organisms is prohibitive. The sanitary quality of drinking water is therefore routinely measured on the basis of the presence or absence of indicator bacteria.

Since most of the common disease, such as typhoid, cholera, dysentery, infectious hepatitis, etc., affect the gastrointestinal tract, faeces of the affected persons contain large number of the causative agents of the diseases. Non-pathogenic bacteria are also excreted in even higher numbers in faeces of all persons. Some of these bacteria have been shown to be present exclusively in faecal matter. Presence of these indicator bacteria in water therefore can be taken to indicate the certain presence of faecal matter and the possible presence of pathogenic bacteria.

Escherichia coli and some related bacteria, together called 'faecal coliforms', which originate only from faeces are used as an indicator bacteria. The faecal coliforms are a part of a larger group known as 'total coliforms'.

Other members of the total coliform group originate from soil and decaying plant matter. As a general rule the faecal coliforms are about 20% of the total coliform concentration, although a wide spread exists depending on the general sanitary conditions in the area of monitoring.

In polluted waters, the die-away rate of faecal coliforms usually parallels that of most of the pathogenic organisms. However, it is possible, that some pathogens may survive for longer periods of time compared to faecal coliforms. Therefore, often the drinking water quality is judged on the basis of the presence or absence of total coliforms. This provides an additional factor of safety.

The count of coliform bacteria is determined statistically on the basis of analysis of different volumes of the same sample. The result is expressed in terms of most probable number (MPN) per 100 mL. The total count of coliforms in unpolluted stretches of rivers may vary between 10 to100 MPN/100 mL. In polluted reaches the count may increase to 10^3 MPN/100 mL or even 10^6 MPN/100 mL, depending on the extent of pollution.

5.9.2 CHLOROPHYLL 'A'

The concentration of photosynthetic pigment is used to estimate phytoplankton biomass. All green plants contain chlorophyll 'a', which constitutes roughly 1 – 2% of the dry weight of the planktonic algae. Rather than measuring population of different types of algae, which can be tedious, chloropyll measurement can be performed quickly in a water chemistry laboratory. Measurement of chlorophyll gives information regarding the trophic status of the water body.

5.10 ANALYTICAL METHODS

Table 5.5 gives a summary of the analytical methods for the determination of major parameters and requirement of special equipment, if any. Parameters, which can be determined with the support of a routine chemistry laboratory have not been included.

Parameter	Method	Equipment
Conductivity	I	Conductivity meter
pH	I	pH meter
DO	I, T	DO meter
Organic nitrogen	T	Kjeldahl digestion & distillation apparatus
Ammonia	P, T	Spectrophotometer
Oxidised nitrogen	P	UV, vis Spectrophotometer
Phosphorus	P	Spectrophotometer
BOD	T	BOD incubator
COD	T	COD digestion & distillation apparatus
TOC	I	TOC analyser
Calcium	T	
Magnesium	T	
Sodium	I	Flame photometer
Potassium	I	Flame photometer
Bicarbonate	T	
Chloride	T	
Sulphate	P	Nephelometer
Hydrogen sulphide	T	
Silica	P	Spectrophotometer
Fluorides	I	Ion meter
Boron	P	Spectrophotometer
Cyanide	I	Ion meter
Trace elements	I	Atomic absorption spectrophotometer
Pesticides	I	Gas chromatograph
Surfactants	P	Spectrophotometer
Phenols	P	Spectrophotometer
Mineral oil & petroleum products	G	
Coliforms	B	Steriliser, incubator
Chlorophyll	P	Spectrophotometer

I - instrumental, T - titrimetric, P - photometric, G - gravimetric, B - biochemical

Table 5.4: Major parameters, method and equipment requirement

6 EQUIPMENT SPECIFICATIONS

6.1 INTRODUCTION

Specifications for equipment for water quality laboratories were drafted by R D Directorate, CWC, in collaboration with the HP Consultants. These were approved by the 'Specifications Committee' constituted by the Commission for the HP. These are available in a separate volume titled 'Equipment Specifications, Surface Water'. The list of equipment concerning water quality laboratories included in the publication given at the end of this chapter. The specifications may be modified or specifications for new equipment may be added, as the need arises from time to time. This chapter discusses criteria for evaluating the performance characteristics of analytical instruments and observations on the specifications and use of some of the major equipment.

6.2 PERFORMANCE CHARACTERISTICS OF INSTRUMENTS

In order to select an analytical method and the corresponding instruments, the following factors must be considered:

- accuracy required
- size of sample
- concentration range of the analyte
- interference and the effect of sample matrix
- number of samples to be analysed and the speed and ease of analysis
- skill required of the analyst
- cost, both initial and operational, of the equipment, its availability, service facility and the maintenance requirements

Answers to the above points will depend on the location, the medium being sampled, the importance of the information being collected, availability of expertise and funds, etc.

The quantitative performance criteria of instruments that can be used for comparing various alternatives are discussed in the following sections. It is recommended that the reader may review the statistical concepts given in Chapter 8 of this volume for reference.

6.2.1 PRECISION

The precision of analytical data is the degree of mutual agreement among data that have been obtained exactly in the same way. Precision provides a measure of the random, or indeterminate error of an analysis. The measure of precision of a method can be determined from absolute standard deviation, relative standard deviation and coefficient of variation.

6.2.2 BIAS

Bias can be determined by analysing a standard reference material whose true value is known and comparing the result with the true value. Since the result would be subject to both the random error as well as the systematic error or bias, a sufficient number of replicate analyses are performed and the mean value is taken. Ordinarily, mean of 20 replicate analyses can be taken to eliminate the random error.

6.2.3 ACCURACY

The term accuracy is used to describe the total error of an observation, which is the sum of systematic error or bias and random errors. For an instrument, it may be expressed as a percent of full range output.

6.2.4 SENSITIVITY

The sensitivity of an instrument is a measure of its ability to discriminate between small differences in analyte concentration. The quantitative definition of sensitivity is called *calibration sensitivity*, which is equal to the slope of the calibration curve. Since most of the calibration curves in analytical chemistry are linear, the calibration sensitivity is independent of the concentration.

The calibration sensitivity does not take into account the precision of the individual measurements. Further, the slope of a calibration curve can be increased by increasing the gain of the instrument (though at the cost of precision of the individual measurements). To take this into account, *analytical sensitivity* is defined as the ratio of the slope to the standard deviation of the measurement. Analytical sensitivity is relatively insensitive to amplification factors, since at increase in gain there will also be an increase in the standard deviation of the measurement, thus leaving the analytical sensitivity more or less constant. Note that the analytical sensitivity of an instrument will change with the concentration of the analyte since standard deviation may vary with concentration.

6.2.5 RESOLUTION

Resolution is a term related to sensitivity. It is the smallest measurable variation in the response of a measuring system. It should not be confused with accuracy, which depends on how well the system was calibrated and the random errors involved in the measurement.

6.2.6 DETECTION LIMIT

The detection limit is the minimum concentration or mass of analyte that can be detected at a known confidence level. The signal for the limit should be greater than the blank signal. Usually, this limiting signal is taken as the sum of average signal for the blank and 3 times the standard deviation of the blank signal. The detection limit is read from the calibration curve for this signal. The confidence level for this limit is taken as 95% since the variation in the readings for the blank is not according to normal distribution.

6.2.7 CONCENTRATION RANGE

The lower limit for the concentration range is taken as the value corresponding to a signal equal to the average blank reading plus 10 times the standard deviation for the blank reading. Note that it is different from the detection limit. The higher limit is taken as the concentration at which the calibration departs from linearity.

To be useful, an analytical method should have a range of at least two orders of magnitude.

6.2.8 SELECTIVITY

Selectivity of a method refers to the degree to which the method is free from interference by other species that may be contained in the sample matrix. A numerical value for the selectivity can be obtained by defining *selectivity coefficient* as the ratio of calibration sensitivity of the interfering substance to that of the analyte. If there is no interference, it is zero. It can assume values of one or

more showing a high degree of interference. Selectivity coefficients have not been used widely except to characterise ion selective electrodes.

6.3 CONSIDERATIONS IN EQUIPMENT PROCUREMENT

6.3.1 GENERAL REMARKS

The instrument required to run the intended monitoring programme in principle follows from the list of variables to be monitored and their selected method of analysis and instruments involved. Beside these variable specific instruments a number of general instruments are required to furnish a laboratory, like a warm water geyser, generator, stabiliser for each electric instrument etc.

Glassware and chemicals are not part of the equipment listing, their need is described for each parameter in the Standard Analytical Procedures in Field Manual on Water Quality Analysis.

Groundwater laboratories sometimes do not need instruments related to analysis of silt related parameters and biological parameters. Examples of those instruments are: silto meter, turbidity meter (nephelometer).

6.3.2 REMARKS ON SELECTED EQUIPMENTS

Portable WQ Analysis Kit

The analysis kit is used to measure field parameters, pH, electrical conductivity, temperature and dissolved oxygen. The manufactures usually supply the kit as one assembly, with a common power souces and read out and different probes. The whole assembly is housed in a carrying case. It is advisable to purchase separate kits for individual measurements, except temperature, the temperature probe is required with each of the three separate kits. This may marginally increase the cost. However, otherwise if the circuitory for one of the parameters becomes defective and the kit has to be repaired, the other parameter also can not be measured.

Experience with DO probes available indigenously, shows that the instruments needs frequent calibration. Therefore arrangement for the field calibration, using Winkler, titrimetric method should always be made. However, this may not be necessary if the instrument can be calibrated against ambient air.

Sometimes suppliers of EC meters quote arrangement for the measurement of total dissolved solids also. This added features is of no value, since TDS is obtained by multiplying the EC value with an empirical constant, which differs based on the ionic composition of the dissolved salts.

Balance

Accurately measuring mass of substances is a fundamental requirement for almost all types of analyses. In the laboratory the mass is determined by comparing the weight of an object with the weight of a set of standard masses using a balance. Because acceleration due to gravity affects both the known and unknown to the same extent, equality of weight indicates equality of mass. The terminological distinction between mass and weight is, therefore, seldom made.

Analytical balances, with a resolution of 0.1 mg, are commonly used to determine the weight. For analyses for micro-level contaminants balances with a resolution on of 0.01 may be used. It is of paramount importance that balances are used and maintained with great care. It is advisable to buy a set of certified standard weights to check the bias of the instrument which may develop with use.

COD Digestor

In the COD test the water samples are reacted with the sample at an elevated temperature. In order that none of the reactants escape as vapours the reaction is carried out either in a closed*, closed reflux,* or in a flask fitted with a suitable condenser, *open reflux.*

The heating block and the reaction tubes for the closed reflux method, at present, are not available indigenously. The screw caps used to seal the tubes often start leaking after they are used three or four times. Their replacement becomes a problem. It is therefore recommended that the open reflux method may be adopted, for which the necessary glassware is available easily.

In the case of the open reflux method, the condenser may be either air cooled or water cooled. In case a dependable source of water supply is not available, air cooled condenser should be preferred.

Portable Dissolved Oxygen meter

See Portable Water Quality Analysis kit above.

Filtration Assembly for membrane filters

The coliform analysis can be performed either by multiple tube fermentation technique or by using membrane filters, which requires use of imported filters. The filters are not available easily in the market. Therefore it is recommended that the multiple tube fermentation method may be adopted for which glassware and chemicals are available indigenously.

Gas Chromatograph

A large number of gas chromatographic equipment of varying capability are available commercially. Since the instrument is a most often used research tool, new models are being added. The cost of different models may vary even an order of magnitude. For water quality laboratories, the specifications should strictly match the immediate use requirements. Otherwise, the suppliers have the tendency to load the instrument with additional accessories which may not be used at all.

The specifications should include spares such as O-rings, septum, syringes and columns. An order for standards should also be placed at the time of ordering the equipment.

6.4 IN SITU AND/OR CONTINUOUSLY MONITORING EQUIPMENT

Nowadays automatic multi-parameter instruments that digitally record variables like EC, pH, Temperature, Dissolved oxygen and Turbidity are available. Continuous measurement of parameters is a tempting alternative to laborious analytical work in the laboratory, but the following discussion will give a more factual look at the matter.

If the monitoring programme envisaged, such as is the case under Hydrology Project, aims at long term standardised measurements to establish baseline and trends, a vast number of parameters are of interest. Probes cannot measure all the parameters of interest since probes are available for a limited number of basic parameters only. Further, the probes cannot measure pollution related parameters like organic matter, trace elements, trace organics in the concentration range of interest. Probes also cannot measure microbiological parameters.

Since the required frequency of measuring in the environmental monitoring programme is mostly quite low, 1 to 4 times per year, the use of continuously measuring probes is not justified. Moreover,

maintenance and calibration of the probes is required once every few months or more frequently. As a consequence a field visit to the site remains necessary anyhow.

In short in situ probes measure parameters that are of limited interest since they are not pollution related and the frequency of registration is unnecessarily high.

6.5 EQUIPMENT SPECIFICATIONS LIST

Specifications for equipment for water quality laboratories were drafted by R D Directorate, CWC, in collaboration with HP Consultants. These were approved by the 'Specifications Committee' constituted by the Commission for the HP. These are available in a separate volume titled 'Equipment Specifications, Surface Water'. The list of equipment is given here.

Instruments are referred to by their name (given in the column 'title') and their unique identification number (given in the column 'ID'), which is the same as that in the 'Equipment Specifications, Surface Water' document.

The list also suggests the number of instruments required for level I field laboratories, and level II and II+ laboratories for both surface and groundwater agencies. Of course this is only a suggestion from which may be deviated if circumstances so dictate. The list furthermore suggests where the instruments may be procured from: ICB (international competitive bidding), NCB (National Competitive Bidding) and LS (Local Shopping). Note that the procurement suggestion is dated 1998 - 1999 and the situation may change.

Title	ID	SW-I	SW-II	SW-II+	Procurement
AIR CONDITIONER	40.001		1	1	LS
ANALYSIS KIT, WQ FIELD MEASUREMENTS	40.002	1	1	1	NCB
BALANCE, ANALYTICAL (ELECTRONIC)	40.004		1	1	NCB
BALANCE, ANALYTICAL (MECHANICAL)	40.005	1	1	1	LS
BALANCE, ANALYTICAL HIGH ACCURACY	40.006			1	ICB
BALANCE, GENERAL PURPOSE (MECHANICAL)	40.007	1	1	1	LS
CENTRIFUGE	40.008		1	1	NCB
COD DIGESTOR, OPEN REFLUX	40.009		1	1	NCB
CONDUCTIVITY METER (DIGITAL)	40.010		1	1	NCB
DEEP FREEZER	40.011		1	1	LS
DIGESTION & DISTILLATION UNIT, KJELDAHL	40.025		1	1	LS
DISSOLVED OXYGEN METER (PORTABLE)	40.012		1	1	NCB
DISTILLATION APPARATUS, CYANIDE	40.014			1	LS
DISTILLATION APPARATUS, FLUORIDE	40.015		1	1	LS
FILTRATION ASSEMBLY FOR MEMBRANE FILTERS	40.016		1	1	NCB
FIRE EXTINGUISHER, CO2	40.050		1	1	LS
FLAME PHOTOMETER (MICROPROCESSOR BASED)	40.017		1	1	LS
FUME CUPBOARD	40.018		1	2	LS
GAS CHROMATOGRAPH (PC-CONTROLLED)	40.019			1	ICB
HOT PLATE	40.020		2	2	LS
ICE BOX	40.021	1	1	1	LS
INCUBATOR, BACTERIOLOGICAL	40.022		1	1	LS
INCUBATOR, BOD	40.023		1	1	NCB
ION METER	40.024		1	1	ICB
LPG CONNECTION	40.026		1	1	LS
OVEN, GENERAL PURPOSE	40.027	1	1	1	LS

Title	ID	SW-I	SW-II	SW-II+	Procurement
OVEN, MUFFLE FURNACE	40.051		1	1	NCB
pH METER (DIGITAL)	40.028		1	1	NCB
PIPETTES & DISPENSERS, AUTOMATIC	40.029		1	1	NCB
REFRIGERATOR	40.030		1	1	LS
SAMPLER, DISSOLVED OXYGEN	40.013	1	1	1	LS
SHAKER, OSCILATORY	40.031		1	1	LS
SILTO METER	40.032		1	1	NCB
SOXHLET EXTRACTION UNIT	40.033		2	2	LS
SPECTROPHOTOMETER, ATOMIC ABSORPTION	40.034			1	ICB
SPECTROPHOTOMETER, UV-VISIBLE (PC-	40.035			1	ICB
SPECTROPHOTOMETER, UV-VISIBLE (MANUAL)	40.036		1	1	NCB
STABILISER (CVT)	40.037		1	1	LS
STERILISER, AUTOCLAVE	40.003		1	1	LS
STIRRER , MAGNETIC (WITH HOT PLATE)	40.038		2	2	LS
THERMOMETERS, SET OF	40.039	1	1	1	LS
TISSUE GRINDER	40.040		1	1	LS
TOOL KIT	40.041		1	1	LS
TURBIDITY METER (NEPHELOMETER)	40.042		1	1	NCB
VACUUM PUMP	40.043		1	1	LS
WATER BATH, BACTERIOLOGICAL	40.044		1	1	LS
WATER BATH, GENERAL PURPOSE	40.045		1	1	LS
WATER GEYSER (HEATER)	40.046		1	1	LS
WATER PURIFIER, DISTILLATION UNIT (AUTOMATIC)	40.047	1	1	1	LS
WATER PURIFIER, DOUBLE DISTILLATION UNIT	40.048		1	1	LS
WATER PURIFIER, ION EXCHANGE RESIN COLUMN	40.049			1	NCB

Table 6.1: Laboratory equipment specification list

7 LABORATORY DESIGN

7.1 INTRODUCTION

This chapter provides guidance to building a laboratory. In Figures 7.1 tot 7.3 example layouts for level I (field laboratory), level II and level II+ laboratory design are given.

Ten different functional units are distinguished in designing a laboratory. Each laboratory or room has work benches. The positioning and description of the provisions (like water, electricity, air conditioners etc.) is not designed in detail. Important points are however listed in the furniture and secondary equipment chapters. Special attention is paid to the safety and the working conditions.

7.2 DESIGN CONSIDERATIONS

The following functional units are planned in a fully equipped level II+ laboratory.

description	specific requirements
1. wet chemical laboratory	acid proof
2. instrumentation laboratory	clean air conditioned
3. bacteriological laboratory	clean
4. weighing and extractions	solid floor, clean, no open windows
5. rinsing room	water proof
6. sample storage room	cool, dry, dark
7. chemical storage room	cool, dry, dark
8. lab office	-
9. secretary	-
10. library/archive	-

Table 7.1: Laboratory equipment specification list

The laboratory should be designed in such a way that a minimum of sample movement is achieved. This means, that the pre-treatment is next to the instrumentation laboratory. Also the daily used chemicals are stored in the weighing room, etc.

Following approximate sizes of the laboratories are proposed:

Level	Area	Dimension
Level I field laboratory	30m^2	4 x 7m
Level II laboratory	200m^2	13 x 16m
Level II laboratory	300m^2	15 x 20m

Table 7.2: Functional units in a Level II+ laboratory

Proposed typical designs take into account the various activities and provide the minimum required area. However, these should also include toilets and emergency shower even if the laboratories are a part of a larger building. Need for separate rooms for storage of bottled gas and watch and ward is recognised. In view of budgetary restrictions, however, this may not be included.

A chemist should be associated with planning electrical fittings, water taps and drainage of the building.

Special attention must be given to make the laboratory building dust proof by providing double windows and should be thermally insulated where necessary.

7.3 SECONDARY EQUIPMENT

Secondary equipment is defined as all the provisions in a laboratory that make it possible to run the primary equipment (see Table 7.3, equipment list) for chemical analysis.

floor	The floor in the laboratory must be finished off with acid and caustic proof material (preferably tiles). Situate a floor sink in the wet chemical analysis laboratory and the rinsing room.
electricity	Voltage stabilisers for individual equipment or an overall voltage stabiliser are required. After a power-failure all electric equipment must be switched off be for the generator is started. The total amount of electricity depends on the total equipment of the laboratory.
water	Hot and cold water at sufficient points in the laboratory.
gas	Gas (LPG) supply may be provided from gas containers.
air conditioners	Air conditioners not only provide a suitable working temperature in summer. Since the air within a laboratory should be as clean as possible air-purification can be considered purchasing.
compressor	equipped for AAS and cleaning drying of equipment
fume exhaust	
tool kit	For small maintenance an reparations screwdrivers and e.g. a multi-meter are convenient
office equipment	Insufficient administration materials like computers, calculators, log-books and even pencils etc. can occur a bad functioning of the laboratory.

Table 7.3: Secondary equipment list for laboratory

Furniture

The wall benches and island benches should be provided with wall sockets or pillar sockets for electricity according instruments, which will be in use on the bench. Keep in mind that a pH-meter needs only one socket, but a full-equipped AAS needs at least 6 sockets for electricity. Also a water tap with a built-in sink complete with drainage pipe can be provided in a bench.

The mixed cold and warm water taps besides the island-benches and in the rinsing room shall be fitted with stainless steel sinks and table cover. The standard bench top material is 13 or 20 mm solid plastic, however depending upon the operation tiled tops also can be used (for example, the island benches for the wet chemical analyses tiled and the other benches all with solid plastic). The island benches and wall benches should be provided with under bench cupboards units.

The fume cupboards should be provided with an optimal and safe airflow pattern, a water tap with built-in sink and a double socket for electricity.

Sufficient stools should be present to guarantee convenient working conditions.

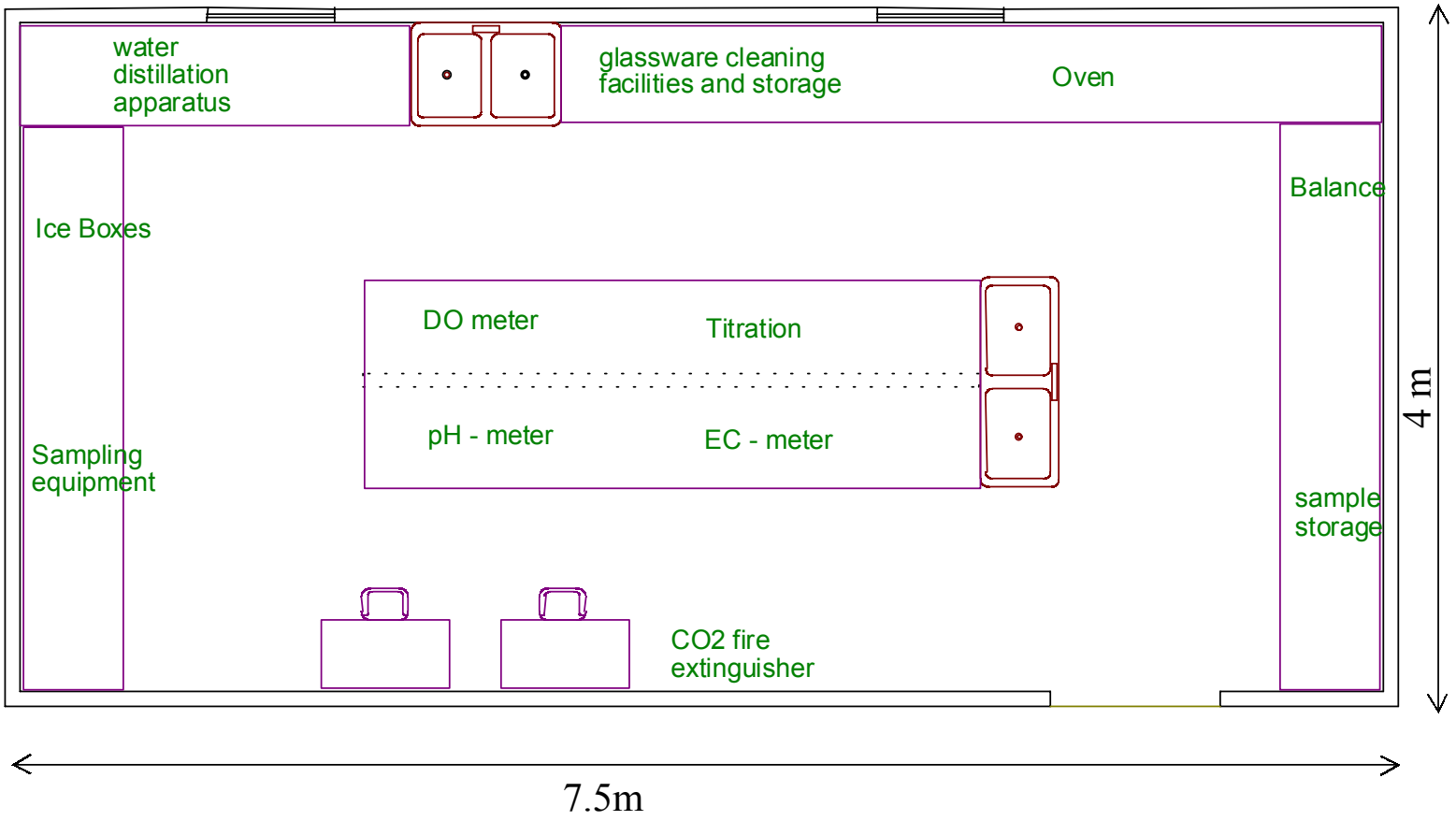
Safety facilities

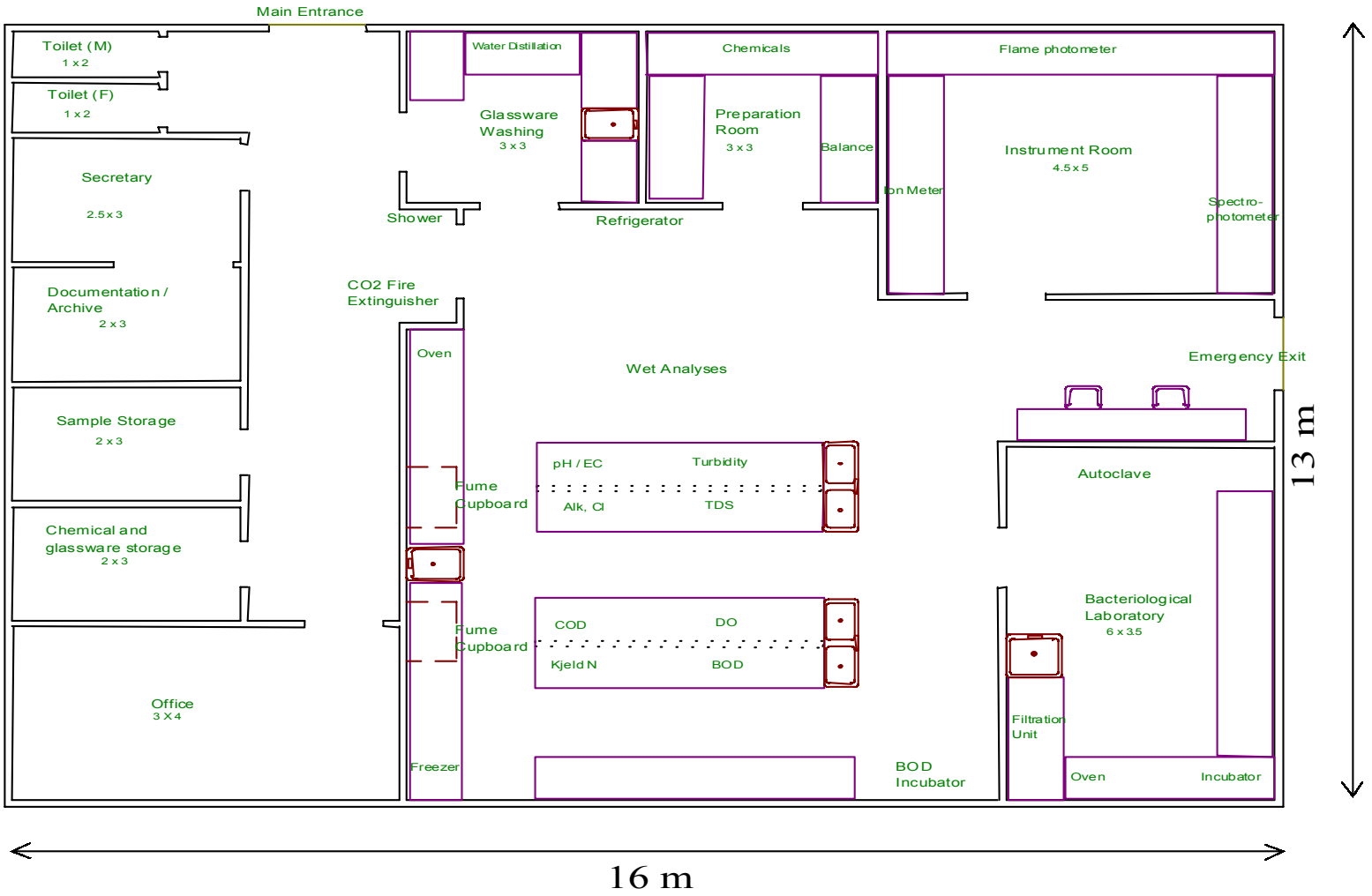
Fist of all safety is a matter of prevention. A clean well designed lab with skilled people following the right procedures is the best way to avoid accidents. Think of the following:

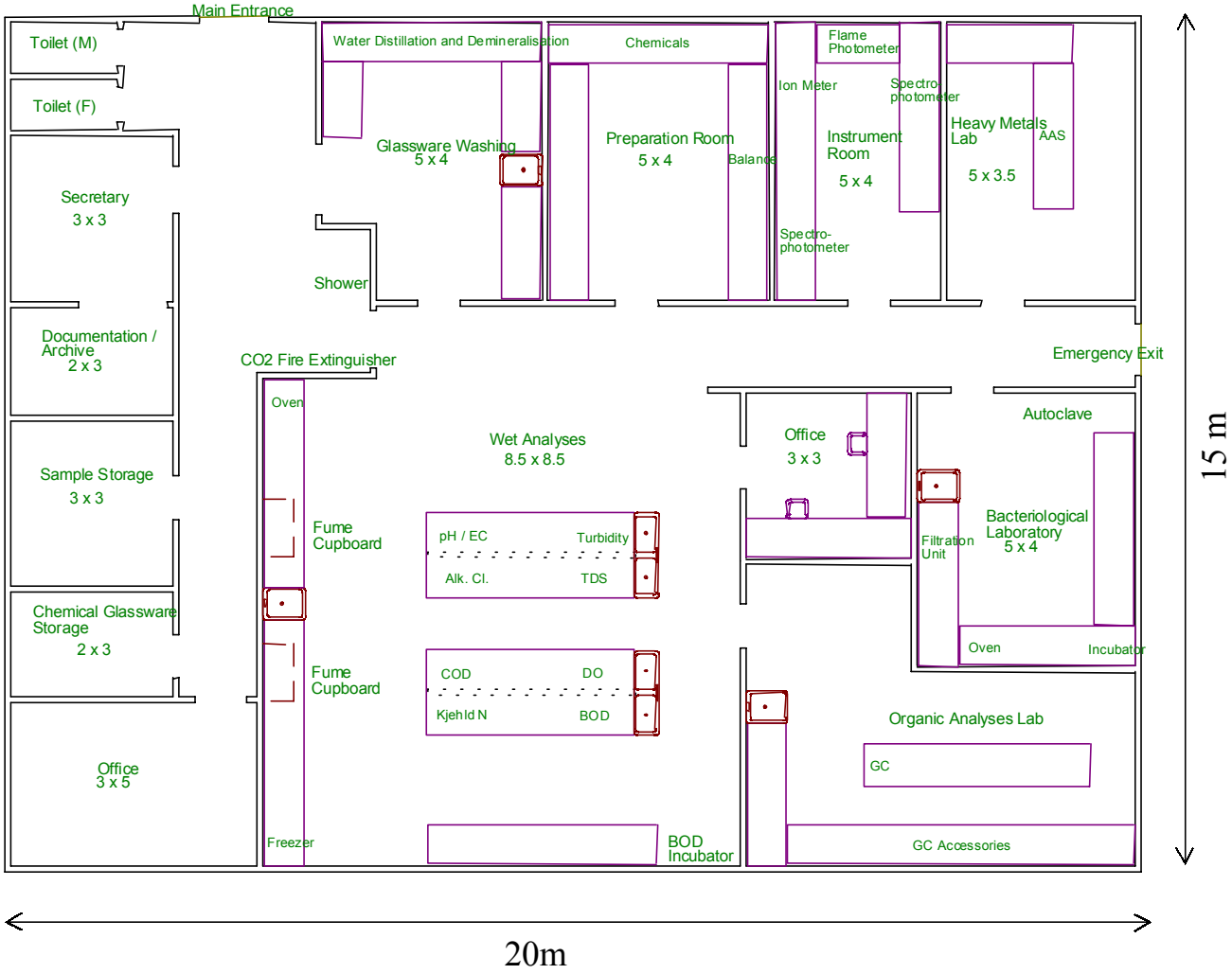
- sufficient light
- adequate working height of tables
- safety cards for chemicals
- fume cupboard
- gloves
- Eye protection (safety glasses)
- laboratory coats
- separated room for a break or lunch
- closed toilets

Should occur accidents then the following provisions will help to minimise the damage:
- eye-washer

- laboratory shower
- CO2 extinguisher
- an emergency exit
- alarm number and telephone
- first aid kit


water
distillation
apparatus
glassware cleaning
facilities and storage
Oven
Balance
Ice Boxes
DO meter
Titration
pH - meter
EC - meter
Sampling
equipment
sample
storage
CO2 fire
extinguisher
4 m
7.5m


Main Entrance
Toilet (M)
1 x 2
Toilet (F)
1 x 2
Secretary
2.5 x 3
Documentation / Archive
2 x 3
Sample Storage
2 x 3
Chemical and glassware storage
2 x 3
Office
3 X 4
Water Distillation
Glassware Washing
3 x 3
Shower
Refrigerator
Chemicals
Preparation Room
3 x 3
Balance
Flame photometer
Ion Meter
Instrument Room
4.5 x 5
Spectro-photometer
CO2 Fire Extinguisher
Oven
Wet Analyses
Emergency Exit
Fume Cupboard
pH / EC
Turbidity
Alk, Cl
TDS
Autoclave
Fume Cupboard
COD
DO
Kjeld N
BOD
Bacteriological Laboratory
6 x 3.5
Filtration Unit
Freezer
BOD Incubator
Oven
Incubator
13 m
16 m


Main Entrance
Toilet (M)
Toilet (F)
Secretary
3 x 3
Documentation /
Archive
2 x 3
Sample Storage
3 x 3
Chemical Glassware
Storage
2 x 3
Office
3 x 5
Water Distillation and Demineralisation
Chemicals
Flame
Photometer
Ion Meter
Spectro-
photometer
Glassware Washing
5 x 4
Preparation Room
5 x 4
Balance
Instrument
Room
5 x 4
Heavy Metals
Lab
5 x 3.5
AAS
Spectro-
photometer
Shower
CO2 Fire Extinguisher
Emergency Exit
Oven
Wet Analyses
8.5 x 8.5
Office
3 x 3
Autoclave
Fume
Cupboard
pH / EC
Turbidity
Alk. Cl.
TDS
Filtration
Unit
Bacteriological
Laboratory
5 x 4
Fume
Cupboard
COD
DO
Kjehld N
BOD
Oven
Incubator
Organic Analyses Lab
GC
Freezer
BOD
Incubator
GC Accessories
15 m
20m

8 QUALITY ASSURANCE/ QUALITY CONTROL

8.1 INTRODUCTION

Many studies have shown that analytical results are often subject to serious errors, particularly at the low concentrations encountered in water analysis. In fact, the errors may be so large that the validity of actions taken regarding management of water quality may become questionable.

Nutrients, N and P, in very small concentrations can cause eutrophication of waterbodies. An analytical quality control exercise (AQC) exercise conducted by United States Environmental Protection Agency (US-EPA) showed a wide variation in results when identical samples were analysed in 22 laboratories. The data obtained in Table 8.1.

Nutrient	Concentration, mg/L	Range of results, mg/L
Ammonia	0.26	0.09 - 0.39
Nitrate	0.19	0.08 - 0.41
Total phosphorus	0.882	0.642 - 1.407

Table 8.1: Results of analytical quality control exercise, 22 laboratories

It is seen that the range of values reported are significantly large, ±50% for ammonia and ±100% for nitrates, compared to the actual concentrations. Therefore, the need for nutrient control programme and its results become difficult to assess.

Many laboratories under Hydrology Project (HP) report total dissolved salts (TDS) calculated from the electrical conductivity (EC) value:

$$\text{TDS, mg/L} = A \times \text{EC, } \mu\text{mho/cm} \qquad (8.1)$$

where A is a constant ranging between 0.55 and 0.9 depending on the ionic composition of salts dissolved in the water.

An inter-laboratory AQC exercise conducted by Central Pollution Control Board (CPCB) showed that for measurement of EC of a standard solution, out of 44 participating laboratories only 34% reported values in the acceptable range. Figure 8.1.

Thus, the reliability of iso-concentrations of TDS in groundwaters, drawn based on data of several laboratories may become questionable on two counts; use of an arbitrary value for the constant A and variation in inter-laboratory measurements.

These examples amply demonstrate the need for quality assurance (QA) programmes.

This chapter discusses various aspects of a QA programme and AQC exercises that should be carried out by laboratory chemists engaged in water quality analysis. Since a knowledge of statistics is necessary for appreciating the exercises, an introductory section on some basic concepts from statistics have been included.

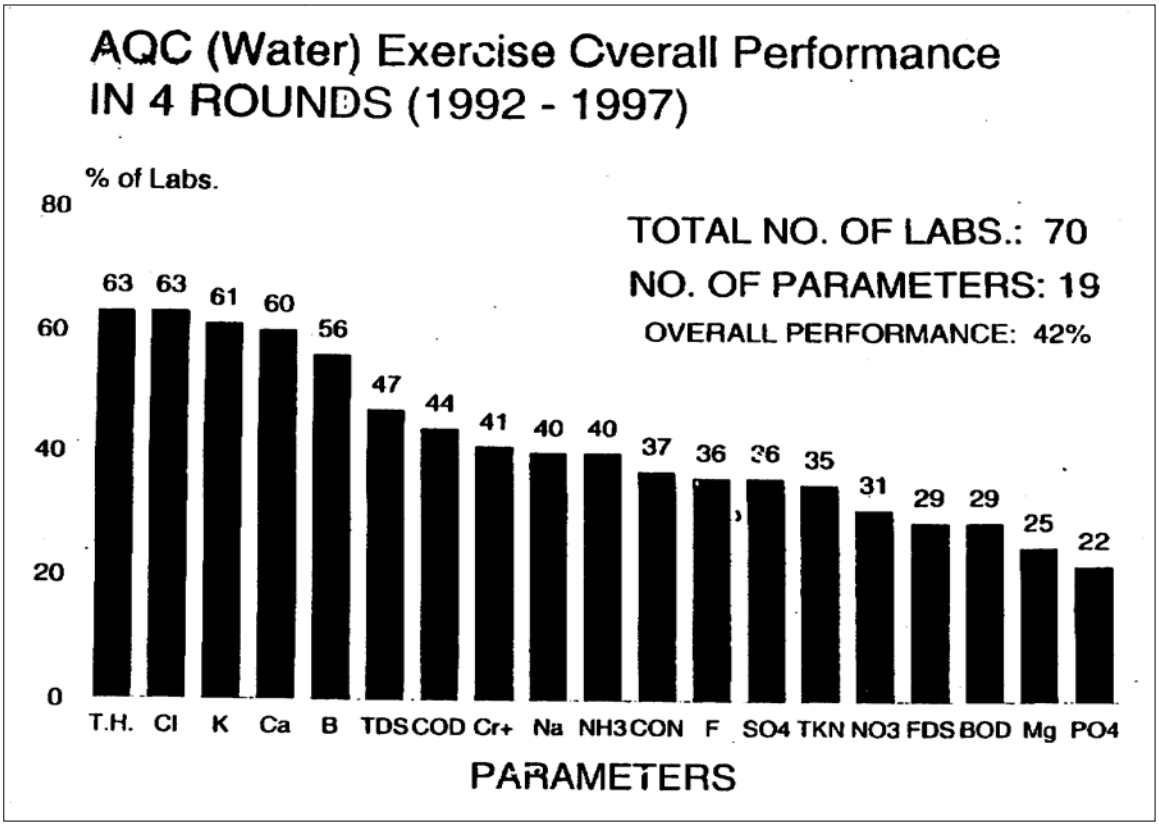


Figure 8.1: the overall performance of all 4 rounds of exercises carried out by CPCB in 8 slots during 1992 - 1997 covering 19 parameters. Laboratories found within the acceptable limits for all the 19 parameters

8.2 BASIC CONCEPTS FROM STATISTICS

Every physical measurement is subject to a degree of uncertainty. A result that is not particularly accurate may be of great use if the limits of the errors affecting it can be set with a high degree of certainty. Statistical methods are used to evaluate the probable errors in analytical measurements and interpretation of environmental data. This section discusses some basic concepts of statistics, which a chemist should be conversant with in order to evaluate the accuracy of the laboratory data and the estimation of the environmental characteristics based on the results of limited samples.

8.2.1 FREQUENCY DISTRIBUTION

Results of 44 replicate analyses for hardness of a sample of water are given in Table 8.2.

Class	Value	No. of Values in the Class	Frequency
(1)	(2)	(3)	(4)
51-52	51.8	1	0.0227
52-53	52.0, 52.8, 52.8	3	0.0682
53-54	53.1, 53.1, 53.3, 53.4, 53.5, 53.6, 53.6, 53.9	8	0.1818
54-55	54.0, 54.1, 54.3, 54.3, 54.4, 54.5, 54.5, 54.6, 54.7, 54.7, 54.9	11	0.2500
55-56	55.1, 55.1, 55.3, 55.3, 55.4, 55.4, 55.6, 55.7, 55.7, 55.8, 55.9, 55.9	12	0.2727

Class	Value	No. of Values in the Class	Frequency
(1)	(2)	(3)	(4)
56-57	56.2, 56.3, 56.7, 56.8, 56.9, 56.9	6	0.1364
57-58	57.3, 57.5	2	0.0454
58-59	-	0	
59-60	59.1	1	0.0227

Table 8.2: Results of 44 replicate analyses for hardness, mg/l as $CaCO_3$

The data are classified in 9 classes, column (1) and arranged in each class according to their magnitude, column (2). By convention an observation equal to the upper value of the class is counted in the next higher class. For example the value 52.0 is not placed in class 51-52 but in 52-53. The number of values in each class are given in column (3). The ratio of number of values in a class to the total number of observations is called frequency and is given in column (4).

A plot of data of column (1) vs. column (4) is called frequency distribution diagram and is shown in Figure 8.2. For clarity of presentation, the frequency value is multiplied by 100 and shown as %.

Note that if the number of observations is increased and the class interval is reduced, the histogram can be replaced by a continuous curve.

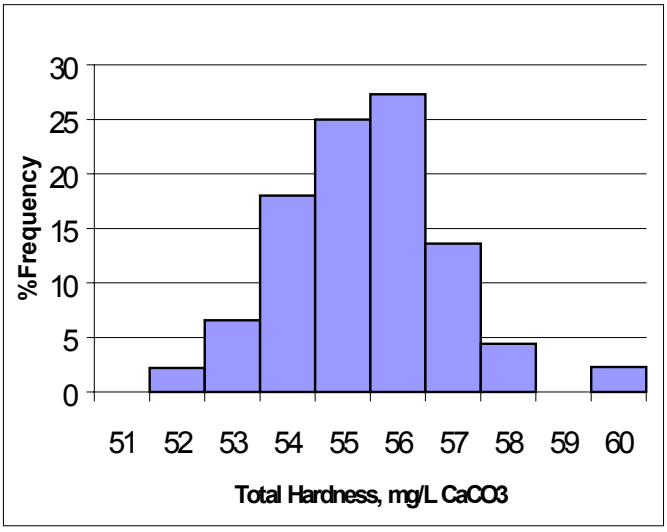


Figure 8.2:
Example of a frequency distribution diagram

Central Tendency

Arithmetic mean: The arithmetic mean, $\bar{x}$, of a set of data is calculated by adding all the observed values of variable (results of analyses) and dividing it by the total number of observations:

$$\bar{x} = (x_1 + x_2 + ...x_n)/n \qquad (8.2)$$

where x_1, x_2,.....x_n are the observed values and n is the total number of observations.

The arithmetic mean is the most common measure of the central tendency. The mean value of the data of Table 8.2 was calculated as 54.9

Geometric mean: When there are a few very high values or very low, such as in the cases of bacteriological analysis, the arithmetic mean is not necessarily representative of the central tendency. In such cases the geometric mean, g, is used:

$$g = \left(x_1 \times x_2 \times x_3 \ldots\ldots\ldots\ldots x_n\right)^{1/n} \quad (8.3)$$

Median: The median is the middle value of the set of data. If the sample size n is an odd number, one-half of the values exceed the median and one-half are less. When n is even, it is average of the two middle terms. For the data of Table 8.2, it is 54.8, which is the average of 54.7 and 54.9, the 22nd and the 23rd terms, when the data are arranged in ascending order.

Mode: The mode is the most commonly occurring value. It is not widely employed as it forms a poor basis for any further arithmetic calculations.

Standard deviation

The data of Table 8.2 show that 52% of observations lie in the range of 54 - 56 and 84% in the range of 53-57. This tendency of observations to cluster (or not to cluster) around the mean value, 54.9, is measured by *standard deviation*, s. Standard deviation is calculated as:

$$s = \sqrt{\{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + \ldots\ldots(x_n - \bar{x})^2\}/(n-1)}$$

Or
$$s = \sqrt{\frac{\sum x_i^2 - (\sum x_i)^2/n}{(n-1)}} \quad (8.4)$$

A small value of s signifies that most of the observations are close to the mean value. A large value indicates that the observed values are spread over a larger range. For example, if the data of Table 1 has more observations in 52-53 and 57-58 classes and correspondingly lesser number in the central classes 54-55 and 55-56, the frequency distribution diagram will be flatter and spread wider at the base and the value of s will be larger.

The standard deviation has the same units as the quantity measured. The standard deviation for the data of Table 1 was calculated as 1.545 mg/L.

The square of the standard deviation is called the *variance*. If the random distribution of data is due to r different reasons, the total variance is the sum of individual variance.

Normal Distribution

Most frequency distributions for *random* observations, when the total number of observations is very large tending to be the same as the population, N, conform to normal distribution. The distribution is given by the theoretical equation:

$$y = \frac{e^{-(x_i-\mu)^2/2\sigma^2}}{\sigma\sqrt{2\pi}} \quad (8.5)$$

where y = frequency of observations μ = the mean, and σ = standard deviation. Note the distinction made in the notations for number of observations, mean and standard deviation for a sample of a limited set of data used earlier.

The standard deviation is given by:

$$\sigma = \sqrt{\frac{\sum x_i^2 - \left(\sum x_i\right)^2 / N}{N}} \qquad (8.6)$$

For a normal distribution, 68.3 % of the total observations lie in the range $\mu \pm \sigma$, 95.5% in the range $\mu \pm 2\sigma$ and 99.7% in the range $\mu \pm 3\sigma$. This is illustrated in Figure 8.3.

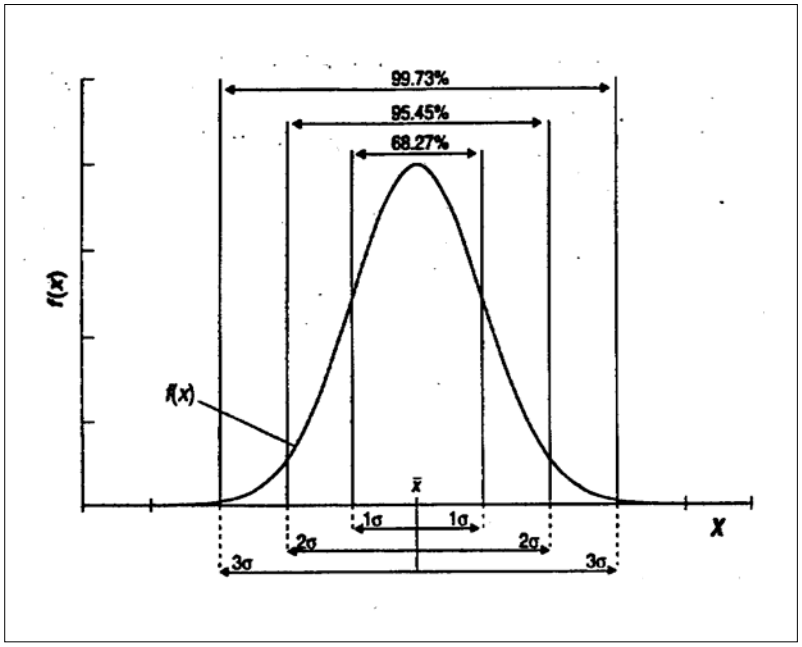


Figure 8.2:
Normal distribution curve and areas under curve for different distances

The number of observations between any two limits of the variable can be equated to the area bounded by the frequency distribution curve, the ordinates at these limits and the x axis.

When the data set is small, more often than not, sample mean, $\bar{x}$, will differ somewhat from the population mean, μ. Any error in $\bar{x}$ causes a corresponding error in the standard deviation calculated with Equation 8.5. There is a tendency for the standard deviation value calculated to be small as the number of measurements becomes smaller. It can be shown that this bias can be largely eliminated by substituting the *degree of freedom* as (n-1) in the calculation, Equation (8.4), which defines the standard deviation for a limited set of measurements.

8.2.2 PRECISION AND ACCURACY OF EXPERIMENTAL DATA

Classes of errors

The phenomena that are responsible for uncertainties in an analytical measurement can be divided in two broad categories: *determinate or system errors* and *indeterminate* or *random errors*. The total error is the sum of the two types of errors.

Determinate errors have assignable causes and are unidirectional. It may be possible to eliminate some of these, for example, errors because of improper calibration of the equipment, or presence of interfering substances in the sample. Errors inherent in the method, such as addition of a reagent in excess of theoretical requirement to cause an indicator to undergo colour change in a titration, may be difficult to eliminate.

Indeterminate errors are encountered whenever a measuring system is extended to its maximum sensitivity. The results fluctuate in a random manner about a mean value. The sources of these fluctuations can never be identified because they are made up of a myriad of instrumental, personal and method uncertainties that are individually so small that they can never be detected. For example, in he case of addition of an exact volume of reagent in an analysis through a pipette the uncertainties could be the time allowed for draining the pipette, the angle at which the pipette is held during delivery, temperature of the reagent, visual judgement of water level with respect to the graduation mark, etc. What is observed in the final result is then a summation of a very large number of minute unobservable uncertainties. The cumulative effect is likewise variable. Ordinarily they tend to cancel one other and thus exert a minimal effect. Occasionally, however, they act in concert to produce a relatively large positive or negative error.

Precision

The term precision is employed to describe the reproducibility of results. It can be defined as the agreement between the numerical values of two or more measurements that have been made in an identical fashion.

Absolute methods for expressing precision: The *absolute average deviation* from the mean is a common method for describing precision. The *spread* or *range* of a set of data is also a measure of precision and is simply the numerical difference of the highest and the lowest result. *Standard deviation*, described earlier, is a more significant measure of precision.

Example 8.1

From the data of replicate chloride analysis of a water sample given below, calculate the precision in terms of the average deviation from the mean:

Solution:

Analysis	Chloride, mg/L	Abs. dev. from the Mean, mg/L
1	24.39	0.077
2	24.19	0.123
3	24.36	0.047
Total	72.94	0.247
	Mean = 24.313	ave. dev. = 0.247/3 = 0.082

Relative methods for expressing precision: It is frequently more informative to indicate the relative precision. The relative parameters are dimensionless and therefore can be used to compare two ore more sets of data. Thus, for example, the *relative deviation* of analysis 1, in Example 8.1, is (0.077 X 100)/24.313 = 0.32%.

Relative standard deviation or *coefficient of variation* is defined as:

$$CV = \frac{100s}{x} \qquad (8.7)$$

Example 8.2

Monitoring results at three sampling locations are listed below. Compare the sampling records in terms of variability.

Solution:

	A	B	C
	40.0	19.9	37.0
	29.2	24.1	33.4
	18.6	22.1	36.1
	29.3	19.8	40.2
x	29.28	21.48	36.68
s	8.74	2.05	2.80
CV	0.30	0.10	0.07

In terms of the coefficient of variation the concentration at A varies the most, followed by B and then C.

Accuracy

The term accuracy is used to describe the total error of the observation, which is the sum of the systematic and random errors. It denotes the nearness of the measurement to its accepted value.

In Example 8.1, if the accepted value for the chloride concentration is 24.35 mg/L, *the absolute error* of the mean is 24.31 – 24.35 = - 0.04 mg/L.

8.2.3 PROPAGATION OF ERRORS

The indeterminate error or uncertainty is most commonly expresses as standard deviation. When the final result is computed from two or more data, each of which has an indeterminate error associated with it, the error of the result will depend on the arithmetic computations. The following examples illustrate the procedure to be followed:

Example 8.3:

For the sum

+ 0.50 (± 0.02)

+ 4.10 (± 0.03)

- 1.97 (± 0.05)

+ 2.63 (± ? ?)

Where the numbers in parantheses are the absolute standard deviations.

Solution:

Statistically the most probable standard deviation would be given by the square root of the sum of individual variances:

$$s = \sqrt{(\pm 0.02)^2 + (\pm 0.03)^2 + (\pm 0.05)^2}$$

$$= \pm 0.06$$

Therefore the result could be reported as:

2.63 (± 0.06)

Example 8.4

The case when products and quotients are involved is illustrated in the following calculations:

$$\frac{4.10\,(\pm 0.02)\times 0.0050\,(\pm 0.0001)}{1.97\,(\pm 0.04)} = 0.0104\,(\pm\,?)$$

Solution:

In this case it is necessary to calculate the relative standard deviations:

$$(S_a)_r = \frac{\pm\,0.02}{4.10} = \pm\,0.0049$$

$$(S_b)_r = \frac{\pm 0.0001}{0.005} = \pm 0.020$$

$$(S_c)_r = \frac{\pm 0.04}{1.97} = \pm 0.020$$

$$(S_y)_r = \sqrt{(\pm 0.0049)^2 + (\pm\,0.02)^2 + (\pm 0.02)^2} \quad = \quad \pm 0.029$$

The absolute standard deviation of the result will be:

S_y = 0.0104 × (± 0.029) = ± (0.0003)

Therefore the uncertainty of the result can be written as:

0.0104 (± 0.0003)

For calculations that involve both sums and differences as well as products and quotients, the uncertainties associated with the former are evaluated first and then the latter following procedures illustrated in Examples 8.3 and 8.4.

8.2.4 CONFIDENCE INTERVALS

The true mean of population, μ, is always unknown. However, limits can be set about the experimentally determined mean, $\bar{x}$, within which the true mean may be expected to occur with a given degree of probability. These bounds are called *confidence limits* and the interval between these limits, the *confidence interval*.

For a given set of data, if the confidence interval about the mean is large, the probability of an observation falling within the limits also becomes large. On the other hand, in the case when the limits are set close to the mean, the probability that the observed value falls within the limits also becomes smaller or, in other words, a larger fraction of observations are expected to fall out side the limits. Usually confidence intervals are calculated such that 95% of the observations are likely to fall within the limits.

For a given probability of occurrence, the confidence limits depend on the value of the standard deviation, s, of the observed data and the certainty with which this quantity can be taken to represent the true standard deviation, σ, of the population.

Confidence limits where σ is known

When the number of repetitive observations is 20 or more, the standard deviation of the observed set of data, s, can be taken to be a good approximation of the standard deviation of the population, σ.

However, it may not always be possible to perform such a large number of repetitive analyses, particularly when costly and time consuming extraction and analytical procedures are involved. In such cases, data from previous records or different laboratories may be pooled, provided that identical precautions and analytical steps are followed in each case. Further, care should be taken that the medium sampled is also similar, for example, analysis results of groundwater samples having high TDS should not be pooled with the results of surface waters, which usually have low TDS.

As discussed earlier, most of the randomly varying data can be approximated to a normal distribution curve. For normal distribution, 68% of the observations lie between the limits $\mu \pm \sigma$, 96% between the limits $\mu \pm 2\sigma$, 99.7% between the limits $\mu \pm 3\sigma$, etc., Figure 8.3. For a single observation, x_i, the confidence limits for the population mean are given by:

confidence limit for $\mu = x_i \pm z\sigma$ (8.8)

where z assumes different values depending upon the desired confidence level as given in Table 8.3.

Confidence level, %	Z
50	0.67
68	1.00
80	1.29
90	1.64
95	1.96
96	2.00
99	2.58
99.7	3.00
99.9	3.29

Table 8.3: Values of z for various confidence levels

Example 8.5:

Mercury concentration in the sample of a fish was determined to be 1.80 μg/kg. Calculate the 50% and 95% confidence limits for this observation. Based on previous analysis records, it is known that the standard deviation of such observations, following similar analysis procedures, is 0.1 μg/kg and it closely represents the population standard deviation, σ.

Solution:

From Table 8.3, it is seen that z = 0.67 and 1.96 for 50% and 50% confidence limits,respectively. Upon substitution in Equation 8.8, we find that

50% confidence limit = $1.80 \pm 0.67 \times 0.1 = 1.8 \pm 0.07$ μg/kg

95% confidence limit = $1.80 \pm 1.96 \times 0.1 = 1.8 \pm 0.2$ μg/kg

Therefore, if 100 replicate analyses are made, the results of 50 analysis will lie between the limits 1.73 and 1.87 and 95 results are expected to be within an enlarged limit of 1.6 and 2.0

Equation 8.8 applies to the result of a single measurement. In case a number of observations, n, is made and an average of the replicate samples is taken, the confidence interval decreases. In such a case the limits are given by:

confidence limit for $\mu = \bar{x} \pm z\sigma/\sqrt{n}$ (8.9)

Example 8.6

Calculate the confidence limits for the problem of Example 8.5, if three samples of the fish were analysed yielding an average of 1.67 μg Hg/kg

Solution:

Substitution in Equation 8.9 gives:

50% confidence limit = $1.67 \pm 0.67 \times 0.1/\sqrt{3} = 1.67 \pm 0.04$ μg/kg

95% confidence limit = $1.67 \pm 1.96 \times 0.1/\sqrt{3} = 1.67 \pm 0.11$ μg/kg

For the same odds the confidence intervals are now substantially smaller and the result can be said to be more accurate and probably more useful.

Note that Equation 8.9 indicates that the confidence interval can be halved by increasing the number of analyses to 4 ($\sqrt{4} = 2$) compared to a single observation. Increasing the number of measurements beyond 4 does not decrease the confidence interval proportionately. To narrow the interval by one fourth, 16 measurements would be required ($\sqrt{16} = 4$), thus giving diminishing return. Consequently, 2 to 4 replicate measurements are made in most cases.

Equation 8.9 can also be used to find the number of replicate measurements required such that with a given probability the true mean would be found within a predetermined interval. This is illustrated in Example 8.7.

Example 8.7

How many replicate measurements of the specimen in Example 8.5 would be needed to decrease the 95% confidence interval to ± 0.07.

Solution:

Substituting for confidence interval in Equation 8.9:

$0.07 = 1.96 \times 0.1/ \sqrt{n}$

$\sqrt{n} = 1.96 \times 0.1/0.07, \qquad n = 7.8$

Thus, 8 measurements will provide slightly better than 95% chance of he true mean lying within ± 0.07 of the experimental mean.

Confidence limits where σ is unknown

When the number of individual measurements in a set of data are small the reproducibility of the calculated value of the standard deviation, s, is decreased. Therefore, for a given probability, the confidence interval must be larger under these circumstances.

To account for the potential variability in s, the confidence limits are calculated using the statistical parameter t:

$$\text{confidence limit for } \mu = x \pm ts/\sqrt{n} \qquad (8.10)$$

In contrast to z in Equation 8.9, t depends not only on the desired confidence level, but also upon the number of degrees of freedom available in the calculation of s. Table 8.4 provides values for t for various degrees of freedom and confidence levels. Note that the values of t become equal to those for z (Table 8.3) as the number of degrees of freedom becomes infinite.

Degrees of Freedom	Confidence Level		
	90	**95**	**99**
1	6.31	12.7	63.7
2	2.92	4.30	9.92
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
8	1.86	2.31	3.36
10	1.81	2.23	3.17
12	1.78	2.18	3.11
12	1.78	2.16	3.06
14	1.76	2.14	2.98
∞	1.64	1.96	2.58

Table 8.4: Values of t for various confidence levels and degrees of freedom

Example 8.8:

A chemist obtained the following data for the concentration of total organic carbon (TOC) in groundwater samples: 13.55, 6.39, 13.81, 11.20, 13.88 mg/L. Calculate the 95% confidence limits for the mean of the data.

Solution:

Thus, from the given data, n = 5, $\bar{x}$ = 11.77 and s = 3.2. For degrees of freedom = 5 – 1 = 4 and 95% confidence limit, t from Table 8.4 = 2.78. Therefore, from Equation 8.10.

confidence limit for $\mu = x \pm ts/\sqrt{n}$ $= 11.77 \pm 2.78 \times 3.2/\sqrt{5}$

$= 11.77 \pm 3.97$

8.2.5 DETECTION OF DATA OUTLIERS

Data outliers are extreme (high or low) values that diverge widely from the main body of the data set. The presence of one or more outliers may greatly influence any calculated statistics and yield biased results. However, there is also the possibility that the outlier is a legitimate member of the data set. Outlier detection tests are to determine whether there is sufficient statistical evidence to conclude that an observation appears extreme and does not belong to the data set and should be rejected.

Data outliers may result from faulty instruments, error in transcription, misreading of instruments, inconsistent methodology of sampling and analysis and so on. These aspects should be investigated and if any of such reasons can be pegged to an outlier, the value may be safely deleted from consideration. However, this is to be kept in mind that the suspect data may rightfully belong to the set and may be the consequence of an unrecorded event, such as, a short rainfall, intrusion of sea water or a spill.

Number of observations	Q_{crit} (reject if $Q_{exp} > Q_{crit}$)	
	90% confidence	96% confidence
3	0.94	0.98
4	0.76	0.85
5	0.64	0.73
6	0.56	0.64
7	0.51	0.59
8	0.47	0.54
9	0.44	0.51
10	0.41	0.48

Table 8.5: Critical values for rejection quotient

Of the numerous statistical criteria available for detection of outliers, the Q test, which is commonly used, will be discussed here. To apply the Q test, the difference between the questionable result and its closest neighbour is divided by the spread of the entire set. The resulting ratio Q_{exp} is compared with the rejection values, Q_{crit} given in Table 8.5, that are critical for a particular degree of confidence. If Q_{exp} is larger, a statistical basis for rejection exists. The table shows only some selected values for number of observations and confidence level. Any standard statistical analysis book may be consulted for a complete set.

Example 8.9

Concentration measurements for fluoride in a well were measured as 2.77, 2.80, 2.90, 2.92, 3.45, 3.95, 4.44, 4.61, 5.21, 7.46. Use the Q test to examine whether the highest value is an outlier.

Solution:

$Q_{exp} = (7.46 – 5.21) / (7.46 – 2.77) = 0.51$

Since 0.51 is larger than 0.48, the Q_{crit} value for 96% confidence, there is a basis for excluding the value.

Other criteria that can be used to evaluate an apparent outlier are:

- Plotting of a scatter diagram indicating inter-parameter relationship between two constituents. If there is a correlation between the two constituents, an outlier would lie a significant distance from the general trend.
- Applying test for normal distribution by calculating the mean and standard deviation of all the data and determining if the extreme value is outside the mean ± 3 times the standard deviation limit. If so, it is indeed an unusual value. (A cumulative normal probability plot may also be used for this test.)
- When the data set is not large, calculating and comparing the standard deviation, with and without the suspect observations. A suspect value which has a considerable influence on the calculated standard deviation suggests an outlier.

8.2.6 REGRESSION ANALYSIS

In assessing environmental quality , it is often of interest to quantify relationship between two or more variables. This may allow filling of missing data for one constituent and also may help in predicting future levels of a constituent.

Regression analysis is focused on determining the degree to which one or more variable(s) is dependant on an other variable, the independent variable. Thus regression is a means of calibrating coefficients of a predictive equation. In *correlation* analysis neither of the variables is identified as more important than the other. Correlation is not causation. It provides a measure of the goodness of fit.

In the calibration step, in most analytical procedures, a 'best' straight line is fitted to the observed response of the detector system when known amounts of analyte (standards) are analysed. This section discusses the regression analysis procedure employed for this purpose.

Least-squares method is the most straightforward regression procedure. Application of the method requires two assumptions; a linear relationship exists between the amount of analyte (x) and the magnitude of the measured response (y) and that any deviation of individual points from the straight line is entirely the consequence of indeterminate error, that is, no significant error exists in the composition of the standards.

The line generated is of the form:

$$y = a + bx \qquad (8.11)$$

where a is the value of y when x is zero (the intercept) and b is the slope. The method minimises the squares of the vertical displacements of data points from the best fit line.

For convenience, the following three quantities are defined:

$$S_{xx} = \sum (x_i - \bar{x})^2 = \sum x_i^2 - (\sum x_i)^2/n$$

$$S_{yy} = \sum (y_i - \bar{y})^2 = \sum y_i^2 - (\sum y_i)^2/n$$

$$S_{xy} = \sum (x_i - \bar{x})\,(y_i - \bar{y}) = \sum x_i y_i - \sum x_i \sum y_i /n$$

Calculating these quantities permits the determination of the following:

1. The slope of the line b:

$$b = S_{xy}/S_{xx} \quad (8.12)$$

2. The intercept a:

$$a = \bar{y} - b\,\bar{x} \quad (8.13)$$

3. The standard deviation about the regression line, s_r:

$$s_r = \sqrt{\{(S_{yy} - b^2 S_{xx})/(n-2)\}} \quad (8.14)$$

4. The standard deviation of the slope s_b:

$$s_b = \sqrt{(s_r^2/S_{xx})} \quad (8.15)$$

5. The standard deviation of the results based on the calibration curve s_c:

$$s_c = (s_r/b) \times \sqrt{\{(1/m) + (1/n) + (y_c - y)^2/^2 S_{xx}\}} \quad (8.16)$$

where y_c is the mean of m replicate measurements made using the calibration curve.

Example 8.10:

The following table gives calibration data for chromatographic analysis of a pesticide and computations for fitting a straight line according to the least-square method.

	Pesticide conc., μg/L (x_i)	Peak area, cm^2 (y_i)	x_i^2	y_i^2	$x_i y_i$
	0.352	1.09	0.12390	1.1881	0.3868
	0.803	1.78	0.64481	3.1684	1.42934
	1.08	2.60	1.16640	6.7600	2.80800
	1.38	3.03	1.90140	9.1809	4.18140
	1.75	4.01	3.06250	16.0801	7.01750
Σ	5.365	12.51	6.90201	36.3775	15.81992

Therefore

$S_{xx} = \sum x_i^2 - (\sum x_i)^2/n$ =1.145365

$S_{yy} = \sum y_i^2 - (\sum y_i)^2/n$ =5.07748

$S_{xy} = \sum x_i y_i - \sum x_i \sum y_i /n$ =2.39669

and from Equations 8.12 & 8.13

b = 2.0925 = 2.09 and a = 0.2567 = 0.26

Thus the equation for the least-square line is

$$y = 0.26 + 2.09x$$

Note that rounding should not be performed until the end in order to avoid rounding errors.

The data points and the equation is plotted in Figure 8.4.

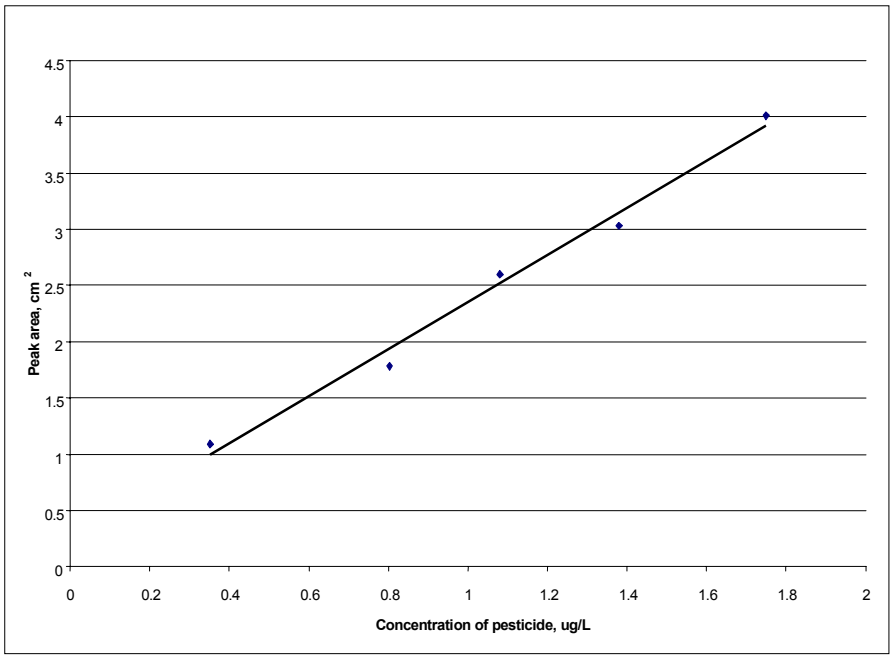


Figure 8.4: Calibration Curve

Example 8.11:

Using the relationship derived in Example 8.10 calculate

1. concentration of pesticide in sample if the peak area of 2.65 was obtained.

2. the standard deviation of the result based on the single measurement of 2.65.

3. *the standard deviation of the measurement if 2.65 represents the average of 4 replicates.*

Solution:

1. Substituting in the equation derived in the previous example

   2.65 = 0.26 + 2.09x

   x = 1.14 μg/L

2. Substituting in Equation 8.16 for m = 1

   $s_c = (0.144/2.09) \times \sqrt{\{(1/1) + (1/5) + (2.65 - 12.51/5)^2/2.09^2 \times 1.145\}} = \pm 0.08$

3. Substituting in Equation 8.16 for m = 4

   $s_c = (0.144/2.09) \times \sqrt{\{(1/4) + (1/5) + (2.65 - 12.51/5)^2/2.09^2 \times 1.145\}} = \pm 0.05$

8.3 QUALITY ASSURANCE/ QUALITY CONTROL PROGRAMME

The QA programme for a laboratory or a group of laboratories should contain a set of operating principles, written down and agreed upon by the organisation, delineating specific functions and responsibilities of each person involved and the chain of command. The following sections describe various aspects of the programmes

Sample control and documentation: Procedures regarding sample collection, labelling, preservation, transport, preparation of its derivatives, where required, and the chain-of-custody.

Standard analytical procedures: Procedures giving detailed analytical method for the analysis of each parameter giving results of acceptable accuracy.

Analyst qualifications: Qualifications and training requirements of the analysts must be specified. The number of repetitive analyses required to obtain result of acceptable accuracy also depends on the experience of the analyst.

Equipment maintenance: For each instrument, a strict preventive maintenance programme should be followed. It will reduce instrument malfunctions, maintain calibration and reduce downtime. Corrective actions to be taken in case of malfunctions should be specified.

Calibration procedures: In analyses where an instrument has to be calibrated, the procedure for preparing a standard curve must be specified, e.g., the minimum number of different dilutions of a standard to be used, method detection limit (MDL), range of calibration, verification of the standard curve during routine analyses, etc.

Data reduction, validation and reporting: Data obtained from analytical procedures, where required, must be corrected for sample size, extraction efficiency, instrument efficiency, and background value. The correction factors as well as validation procedures should be specified. Results should be reported in standard units. A prescribed method should be used for reporting results below MDL.

An important aspect of reporting the results is use of correct number of significant figures. In order to decide the number of significant digits the uncertainty associated with the reading(s) in the procedure should be known. Knowledge of standard deviation will help in rounding off the figures that are not significant. Procedures regarding rounding off must be followed.

Analytical quality control: This includes both *within-laboratory* AQC and *inter-laboratory AQC*.

Under the within-laboratory programme studies may include: recovery of known additions to evaluate matrix effect and suitability of analytical method; analysis of reagent blanks to monitor purity of chemicals and reagent water; analysis of sample blanks to evaluate sample preservation, storage and transportation; analysis of duplicates to asses method precision; and analysis of individual samples or sets of samples (to obtain mean values) from same control standard to check random error.

Inter-laboratory programmes are designed to evaluate laboratory bias.

It may be added that for various determinands all of the AQC actions listed may not be necessary. Further, these are not one time exercises but rather internal mechanisms for checking performance and protecting laboratory work from errors that may creep in. Laboratories who accept these control checks will find that it results in only about 5 percent extra work.

8.3.1 WITHIN - LABORATORY AQC

Shewhart control charts

If a set of analytical results is obtained for a control sample under conditions of routine analysis, some variation of the observed values will be evident. The information is said to be statistically uniform and the analytical procedure is said to be under statistical control if this variation arises solely from random variability. The function of a control chart is to identify any deviation from the state of statistical control.

Shewhart control chart is most widely used form of control charts. In its simplest form, results of individual measurements made on a control sample are plotted on a chart in a time series. The control sample is analysed in the same way as the routine samples at fixed time intervals, once or twice every week, or after 20 to 50 routine samples.

Assuming the results for the control sample follow the Normal frequency distribution, it would be expected that only 0.3% of results would fall outside lines drawn at 3 standard deviations above and below the mean value called upper and lower control limits, UCL and LCL, respectively. Individual results would be expected to fall outside these limit so seldom (3 out of 1000 results), that such an event would justify the assumption that the analytical procedure was no longer in statistical control, i.e., a real change in accuracy has occurred.

The chart is constructed from 20 or more replicate analysis results of a control or standard samples. Two lines are inserted on the chart at 2 standard deviations above and below the mean value called upper and lower warning limits, UWL and LWL, respectively. If the method is under control, approximately 4.5% of results may be expected to fall outside these lines.

This type of chart provides a check on both random and systematic error gauged from the spread of results and their displacement, respectively. Standard Methods lists the following actions that may be taken based on analysis results in comparison to the standard deviation.

Control limit: If one measurement exceeds the limits, repeat the analysis immediately. If the repeated analysis result is within the UCL and LCL, continue analyses; if it exceeds the action limits again, discontinue analyses and correct the problem.

Warning limit: If two out of three successive points exceeds the limits, analyse another sample. If the next point is within the UWL and LWL, continue analyses; if the next point exceeds the warning limits, discontinue analyses and correct the problem.

Standard deviation: If four out of five successive points exceed one standard deviation, or are in increasing or decreasing order, analyse another sample. If the next point is less than one standard deviation away from the mean, or changes the order, continue analyses; otherwise discontinue analyses and correct the problem.

Central line: If six successive points are on one side of the mean line, analyse another sample. If the next point changes the side continue the analyses; otherwise discontinue analyses and correct the problem.

Figure 8.5 to Figure 8.6 illustrate the cases of loss of statistical control for analysis of individual samples based on the above criteria.

Precision: The most important parameter to evaluate in the results is the precision. The statistical term to evaluate precision is standard deviation. The numerical value of the standard deviation depends on the average concentration (standard deviation also has the unit of concentration). Numerical values of standard deviations of low concentration solutions are usually smaller than those of solutions with higher concentrations. Therefore the coefficient of variation, defined earlier, should be used to evaluate precision. This is particularly useful when comparing results of analysis for samples having different concentrations. Before evaluating the results one should answer the question ‘what is the desired precision for an analyses?’. In fact this question should be answered by the so called ‘data users’. The use of the data determines the required precision, e.g. detection of trends may require more precise results (in order to actually detect small changes with time) than checking water for use, say for irrigation. Laboratory staff should always ask for the purpose for which they are performing the requested test.

As a minimum goal for precision, however, the precision that can be obtained by correctly and adequately following the method prescribed by the APHA Standard Methods for the examination of water and wastewater may be adopted

Calculating revised limits when continuing the exercise: Warning and control limits should be recalculated periodically. Especially when new techniques are introduced, the precision improves

when experience is gained with the technique. A good time for recalculating the control and warning limits is at the time when the control chart is full and a new graph has to be created anyway. At this point, use the 20 most recent data on the old chart for construction of LCL, LWL, average, UWL and UCL.

Errors that cannot be detected by within-laborartory AQC: The within-laboratory AQC exercise focusses mainly on precision. A laboratory on its own cannot detect many sources of bias. A good example to illustrate this is the total hardness method. If the analytical balance in a lab always reads 10% too much all solution prepared will have a 10% higher concentration: the Standard $CaCO_3$ solution, the EDTA titrant and also the control sample containing $CaCO_3$. This error can only be detected by analysing a sample prepared by a laboratory with a correctly functioning balance. The current laboratory will underestimate the concentration of such a inter-laboratory sample by 10% because their EDTA titrant is '10% too strong'.

In some cases freshly introduced bias may be detected. For example, if the measurements consistently fall on one side of the previously calculated mean, it indicates a freshly introduced bias.

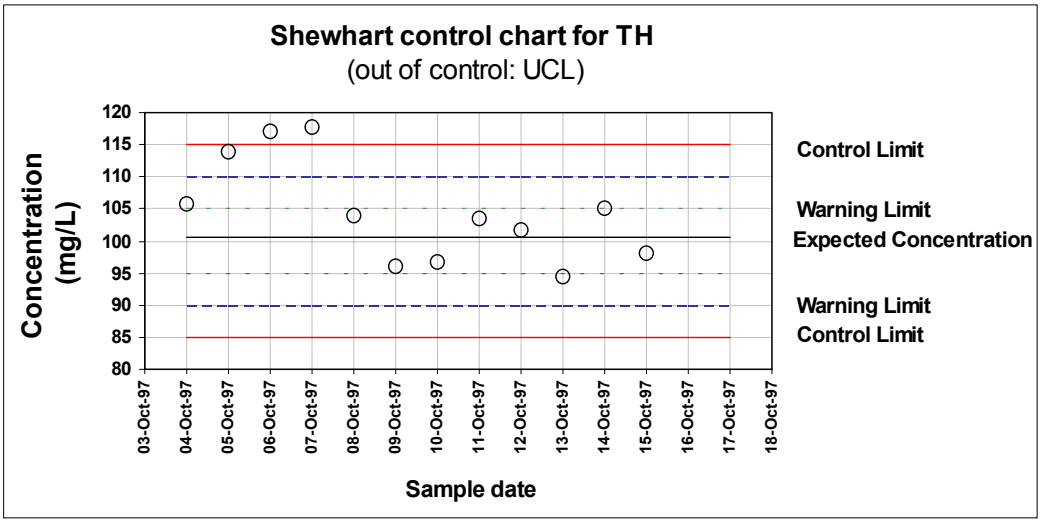


Figure 8.5: Example of loss of statistical control by the control limit criterion

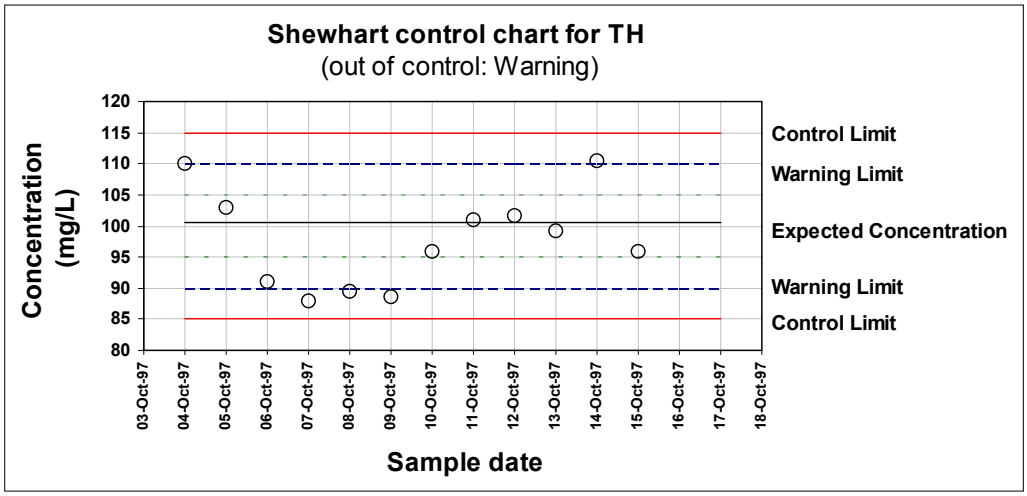


Figure 8.6: Example of loss of statistical control by the Warning limit criterion

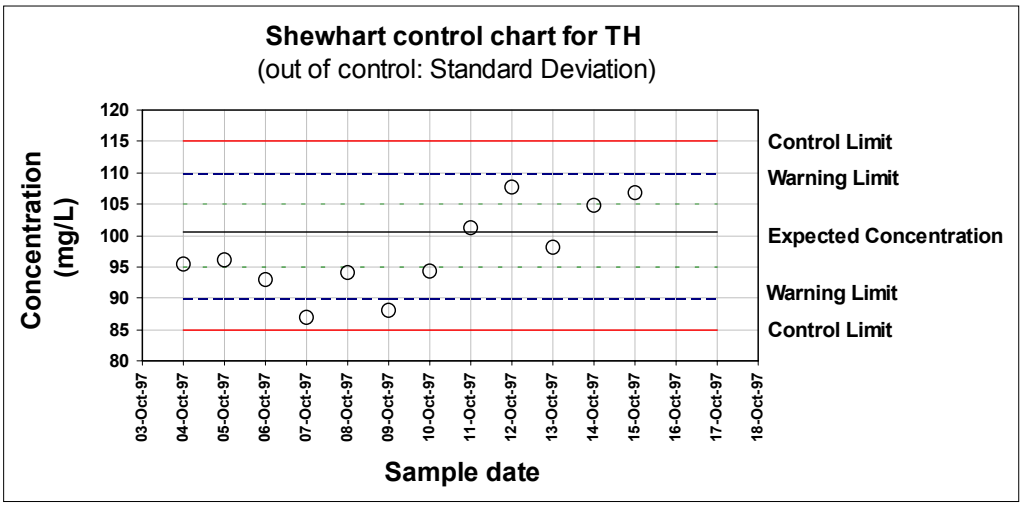


Figure 8.7: Example of loss of statistical control by the Standard Deviation criterion

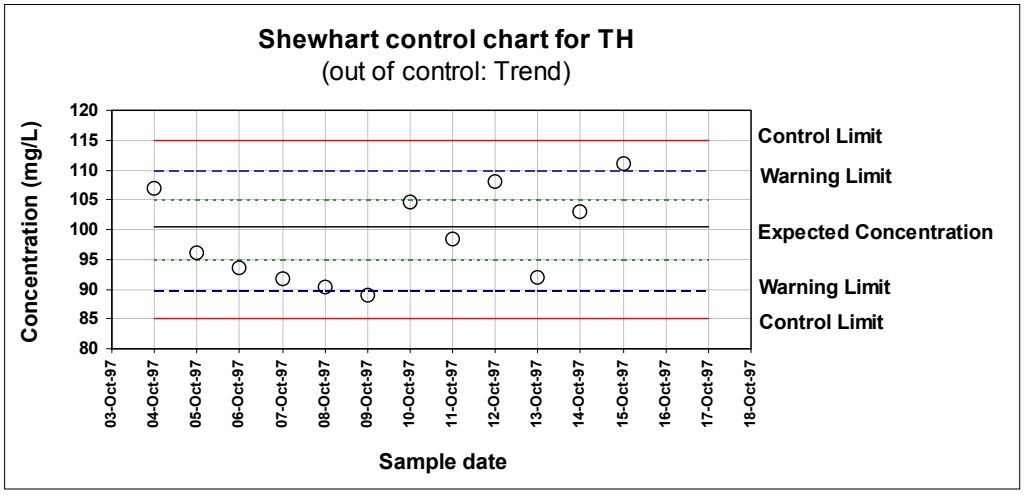


Figure 8.8: Example of loss statistical control by the Trend criterion

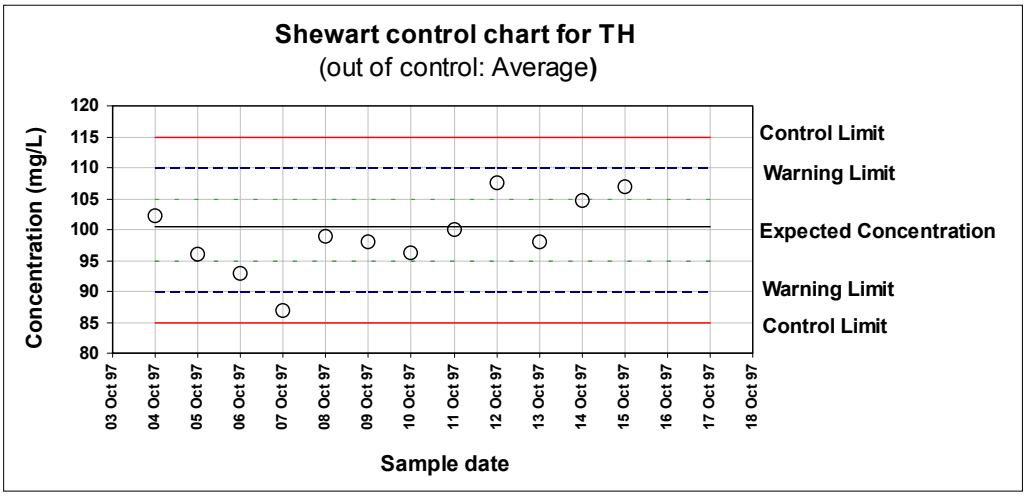


Figure 8.9: Example of loss of statistical control by the Central line) criterion

8.3.2 INTER-LABORATORY AQC EXERCISE

Objectives

The objectives of an *inter*-laboratory AQC programme are:

1 To test for possible bias in measurements in a laboratory.

2 To provide direct evidence of comparability of results among laboratories in a common water quality monitoring programme. Some related objectives and benefits are listed below:

- to assess the status of analytical facilities and capabilities of participating laboratories.
- to identify the serious constraints (random & systematic) in the working environment of laboratories.
- to provide necessary assistance to the concerned laboratories to overcome the short comings in the analytical capabilities.
- to promote the scientific and analytical competence of the concerned laboratories to the level of excellence for better output.
- to enhance the internal and external quality control of the concerned laboratories

Planning of exercise

An *inter*-laboratory AQC exercise should be planned carefully with complete instructions for the participating laboratories to avoid confusion and unnecessary correspondence. A co-ordinating laboratory is chosen to assume the overall charge of the exercise. Some aspects of planning are summarised below.

Co-ordinating Laboratory: The co-ordinating laboratory distributes identical portions of the same standard solution(s) or sample to each participating laboratory, which analyses the portion it receives. Results from the different laboratories are analysed by the co-ordinating laboratory to estimate the bias of results of each laboratory.

Thus, it is essential that a laboratory able to act in this co-ordinating role is available and has sufficient time and resource for the very careful work involved. Such a co-ordinating laboratory should be a member of the working group of the analysts. On satisfactory completion of the exercise, any of the participating laboratories, which qualifies may then also act as a co-ordinating laboratory.

Test samples: The objective of distributing a solution or sample is that each participating laboratory should receive and analyse a portion containing the same concentration of the determinand. For standard solutions, the co-ordinating laboratory should know this concentration to an accuracy appreciably better than that required of normal analytical results otherwise the results of the exercise will be worthless. The need for great care in the preparation and distribution of solutions cannot, therefore, be over-emphasised. Generally, it will often be desirable for the co-ordinating laboratory alone to make preliminary tests to ensure that its procedures do achieve the above requirement.

Purity of material used in preparation of standard solution: The chemicals used to prepare the standard test solutions should be of standard quality whose purity is guaranteed by a written specification; 99.5% or better purity is usually adequate. High purity water (de-ionised or distilled) is generally satisfactory, but absence of the determinand in such water should not be assumed.

Annexure I gives an example of the preparation of standard solutions for the analysis of the following 9 parameters. Note that two samples were prepared, one was 1.5 to 2 times more concentrated than the other in terms of the various determinands.

1. Conductivity (COND)
2. Total dissolved solids (TDS)
3. Total Hardness (TH)
4. Fluoride (F)
5. Sulphate (SO4)
6. Nitrate –N.(NO3-N)
7. Phosphate –P (PO4-P)
8. Sodium (Na)
9. Boron (B)

Errors in preparing the test solution or samples: In preparing a standard solution, it is useful that two analysts independently calculate the weight of standard material required in making up the desired volume of solution. A second analyst should check the balance readings when the standard material is weighed by the first analysis, and also independently calculate, the weight of material taken.

All apparatus used must be scrupulously clean and in particular, free from traces of the determinand of interest. Great care must be taken to avoid contamination of materials and apparatus before and during the preparation. Manipulations such as quantitative transfers and diluting solutions to a graduation mark must be conducted with the utmost care.

When the standard solution has been prepared a question arises whether the concentration of the solution should be checked by analysis. The approach recommended is to prepare the solution as a primary standard using all the classical precautions associated with such a preparation. The freshly prepared solution should be analysed for the determinand of interest, a sufficient number of replicates being made for the purposes of stability testing. The estimate of initial concentration also serves as a check for gross errors in the preparation. The true concentration for the collaborative test should, however, be taken as the nominal concentration of the solution as a primary standard and not the analytical result obtained in the concentration check.

Determinand stability and contamination: When the distribution is carried out, several portions of the solution should be retained at the co-ordinating laboratory for stability checks, and for replacements if required. The solution should be stored in containers of the type used in the distribution and under the storage conditions specified to participating laboratories. The concentration of the determinand of interest should be checked at the end of the collaborative exercise and should not have changed significantly from the initial value. For most determinands, this usually means 1% of the nominal concentration, and sufficient replicate analyses should be made to achieve that precision.

It is vitally important that the concentration of the determinand of interest in the samples should be stable throughout the period of the tests, and a preservative may some times be added to ensure this stability. However, some preservatives may cause interference in certain analytical methods, and so the possible effect of any proposed preservative on all methods of analyses must always be investigated carefully before the preservative is used.

The material of which sample bottles are made should neither absorb nor release the determinand, and bottles must be scrupulously cleaned to be free of the determinand of interest. Particular care is necessary for many trace impurities to ensure that bottle stoppers and caps are not a source of contamination.

Participating laboratories: The participating laboratories should be thoroughly familiar with the recommended analytical procedures for the parameters included in the exercise. They should have satisfactorily completed a within-laboratory exercise for the determinand producing results of acceptable precision.

The participating laboratories can easily assess sources of bias resulting from the use of impure chemicals, poor quality distilled water and sub-standard. If such errors are detected, they should be removed before starting the inter-laboratory exercise.

Proposal: The exercise is started by sending two samples by courier to the participating laboratories. The laboratories are requested to analyse both samples for various previously decided parameters. Sample of an instruction sheet and the reporting format, which may be sent with the samples is given in Annexure II.

Discussion of results

Reference value and acceptable range for reported values: The 'true' strength of the samples or the 'Reference value' can be determined in three different ways:

i. by the recommended analytical procedure in the reference laboratory

ii. from theoretical considerations assuming that the solutions were made correctly and that the purity of the chemicals used was as displayed on the bottle labels

iii. from the combined results of analysis of the participating laboratories.

It is recommended that the reference value against which the performance of the laboratories are to be judged is determined by the second method. However, to give a better acceptability of the standard reference value among the participating laboratories, the third method may also be adopted. The values obtained from the other two methods may then be used to check that the adopted value is not heavily biased due to the inclusion of some extreme values in the reported results.

Example 8.12 illustrates the procedure for calculating the reference value from the combined analysis results of the participating laboratories and the acceptable range.

Example 8.12

Annexe III gives conductivity values of two standard samples A and B, reported by the participating laboratories in an AQC exercise and the procedure for calculating the reference mean and the acceptable range. The theoretical conductivity and that determined by a reference laboratory for the two solutions were:

Solution	Conductivity, µmho/cm	
	Theoretical	Reference laboratory
A	335	340
B	190	198

The data were first subjectively scrutinised for removal of outliers. This was followed by calculating the mean, $\bar{X}$ and S of the remaining data and the 95 % confidence limits for the mean ($\bar{x} \pm 1.96S$). The data values within the 95 % confidence limits were filtered out for calculating the reference mean, X_R. The conductivity values for the solutions in this manner were determined to be:

Solution	Conductivity, µmho/cm
A	333
B	192

It is seen that these values are comparable to the theoretical values and those reported by the reference laboratory. These are therefore are acceptable.

In order to determine the acceptable range for the reported values, the standard deviation, SR, of the filtered data set after rejecting the outliers is multiplied by √2 to adjust the, 'Within Run Precision' to

'Between Day Precision', to obtain Sadj. This is done to account for the fact that the analyses were performed at different times at different locations. The acceptable range is then calculated as XR ± 3Sadj. The ranges were determined as:

Solution	Conductivity acceptable range, µmho/cm
A	306 – 360
B	178 - 207

Values which do not fall in the calculated ranges are not acceptable. A laboratory which reports values for *both* the samples within the acceptable ranges is considered to qualify in the exercise.

Youden 2-Sample Plots: An over all view of the performance of laboratories for each parameter can be obtained from Youden 2-sample plots. Figures 8.10 to 8.12 give the plots for 3 parameters covered under an exercise. For each parameter, the plot shows the value for sample A against that for sample B reported by a laboratory. Thus there is one data point for each laboratory for the two samples.

The acceptable limits for the two samples are also drawn on the plot as two parallel horizontal lines for the sample values plotted on the Y-axis and two parallel vertical lines for the sample values plotted on the X- axis.

The centres of the rectangular block created by the two sets of parallel lines is the reference value for each parameter. Results close to this point are considered to represent a high degree of accuracy.

The figures can be divided in 4 quadrants by drawing a vertical and a horizontal line through the reference value. If only random errors influence the determinations, the points would be expected to be randomly distributed in all the four quadrants. This is rarely seen. The points tend to concentrate in the first (++) or the third (--) quadrant, indicating that the laboratories tend to get, for both the samples, either high values or low values. This points to the dominant role of systematic error. If a point lies on a line of unit slope passing through the reference value, then the determination has only systematic error.

An estimate of the random error and systematic error components of the total error (reported value minus reference value) for the result of a laboratory can be obtained by drawing a perpendicular from its data point on the line of unit slope passing through the reference value point. The ratio of random error to the systematic error is equal to the ratio of the length of the perpendicular to the distance of the foot of the perpendicular to the reference value measured along the unit slope line.

It is advisable not to reveal the identity of the laboratories on the plot. Each laboratory, however, can recognise its own result in the plot.

Coefficient of Variation: The coefficient of variation of reported results, after excluding the outliers, gives an overview of the accuracy of the participating laboratories. It is expected that as the laboratories acquire better facilities and improve their technique, the coefficient of variation would decrease.

For the data of Example 8.12, the coefficient of variation for the conductivity values was calculated as 5.98 and 8.54, for samples A and B, respectively. It may be noted that it was higher for the more dilute solution.

Conclusion of the Exercise

It is the responsibility of the co-ordinating laboratory to collate and analyse the data as early as possible, preferably within one week of the last date decided for the receipt results. The last date should be realistic and sufficient allowance should be given for procedural delays. Often some participating laboratories would not respond in time. There is indeed no need to wait for their results. Probably they would not have analysed the samples.

A copy of the combined report should be sent to each laboratory, giving the reference values, number of laboratories qualifying, parameter-wise, in the exercise and the coefficient of variation for each determination. Along with the report, a separate letter should be addressed to each of the laboratories, individually, identifying probable sources of their errors and remedial steps.

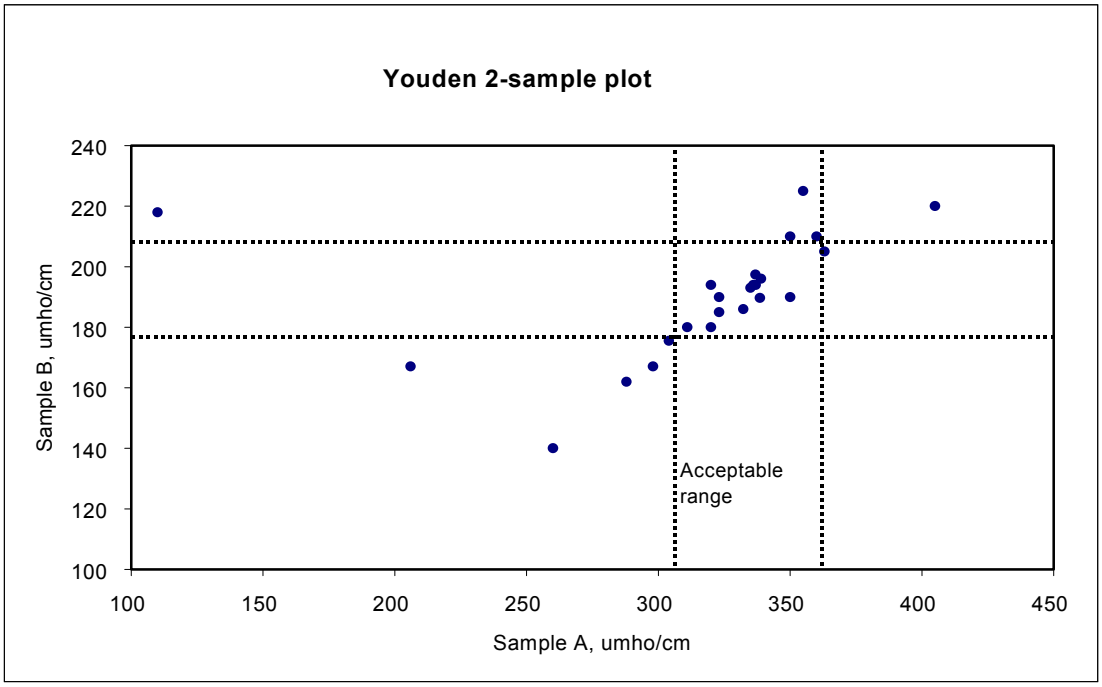


Figure 8.10: Performance of laboratories for conductivity

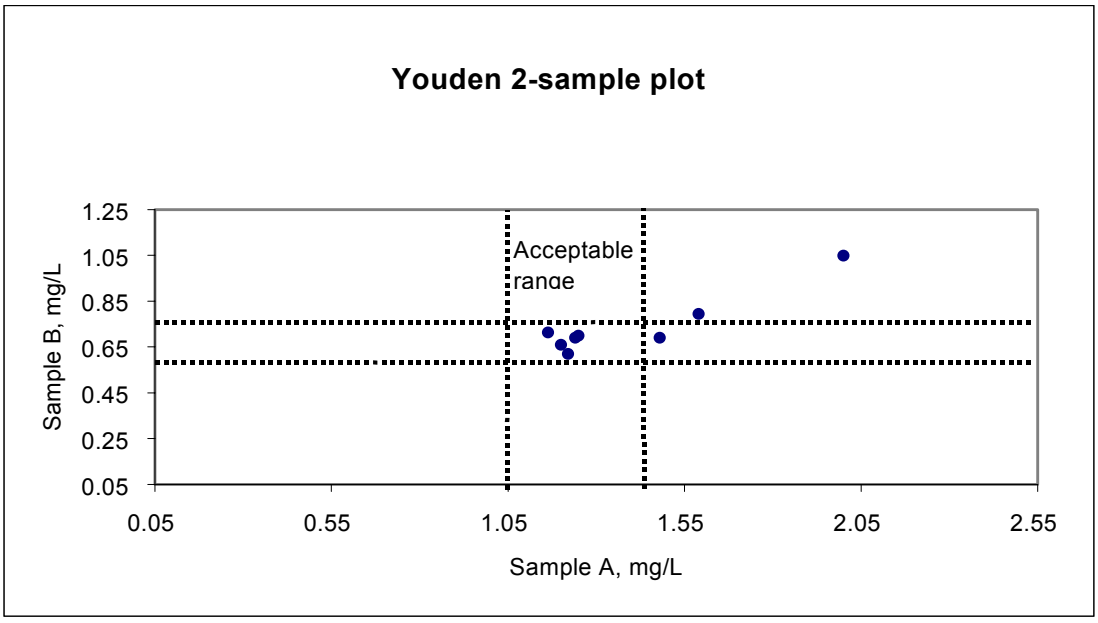


Figure 8.11: Performance of laboratories for Sodium

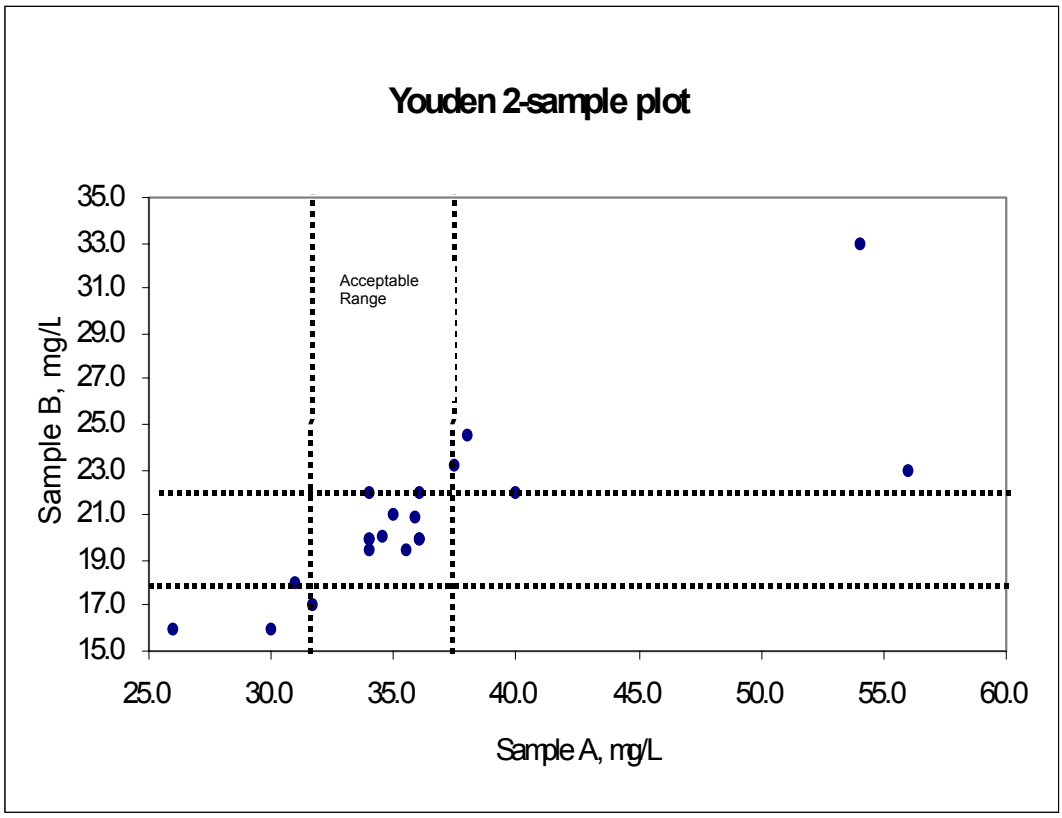


Figure 8.12: Performance of laboratories of Boron

Annex: I Composition of standard samples

INGREDIENT OF SOLUTIONS, Hydrology Project, December 1998

S.No.	Name of Chemical	Weight in gm.	Final Volume	Concentration
1.	MAGNESIUM SULPHATE ($MgSO_4$, $7H_2O$)	16.4	2 litres	800 ppm Mg and 3200 ppm SO_4
2.	CALCIUM CHLORIDE ($CaCl_2$, $2H_2O$)	14.7	2 litres	2000 ppm Ca 3500 ppm Cl
3.	SODIUM FLUORIDE (NaF)	2.21	1 litre	1000 ppm F
4.	POTASSIUM NITRATE (KNO_3)	7.214	1 litre	1000 ppm NO_3-N
5.	BORIC ACID (H_3BO_3)	5.716	1 litre	1000 ppm B
6	SODIUM CHLORIDE (NaCl)	12.717	1 litre	5000 ppm Na
7	POTASSIUM DIHYDROGEN PHOSPHATE (KH_2PO_4)	0.439	1 litre	100 ppm PO_4-P

PREPARATION OF SAMPLE – A

300 ml $MgSO_4$.$7H_2O$ + 200 ml $CaCl_2$.$2H_2O$ + 80 ml NaF + 160 ml KNO_3 + 60 ml H_3BO_3 + 200 ml KH_2PO_4 + 240 ml NaCl C ⟶ Final Volume 40 litres

PREPARATION OF SAMPLE – B

165 ml $MgSO_4$.$7H_2O$ + 100 ml $CaCl_2$.$2H_2O$ + 56 ml NaF + 100 ml KNO_3 + 30 ml H_3BO_3 + 120 ml KH_2PO_4 + 140 ml NaCl ⟶ Final Volume 40 litres

Annex II: Communication with the despatch of samples

CENTRAL REFERNCE LABORATORY

ANALYTICAL QUALITY CONTOL, (AQC/WATER) EXERCISE – DEC.,2000 FOR THE LABORATORIES OF SURFACE AND GROUND WATER DEPARTMENTS

GENERAL INSTRUCTIONS:

Note: Please read the following instructions carefully before starting analysis of samples
Two nos. of synthetic water samples (A & B) of one lit. each labelled with lab code are provided for analysing Conductivity, Total solids, Total Hardness, Sodium, Fluoride, Sulphate, Nitrate-N, Phosphate-P and Boron.

Both samples (A & B) are to be analysed separately for all 9 parameters as shown below.

S.No.	Parameter	Unit
01	Conductivity at 25°C	μ mhos/cm
02	Total Solids	mg/l
03	Total hardness as ca CO_3	mg/l
04	Sodium	mg/l
05	Fluoride as F	mg/l
06	Sulphate as SO_4	mg/l
07	Nitrate – N	mg/l
08	Phosphate – P	mg/l
09	Boron	mg/l

Note: Choose appropriate sample volume for each parameter for single run so that analysis can be done within the provided sample volume.

ANALYTICAL METHODS:

- You may choose any relevant method being followed in your laboratory for various parameters. However, the method is to be mentioned into the data report format.
- In case of spectrophotometric analysis method the standard graph and the factor used for calculation is to be submitted along with the data sheet.
- Brief outline of the procedures for each analytical parameter is to be provided as annexure along with the data sheets.

Please note the following points:

All the samples are to be analysed most preferably during 07th to 11th December' 00 for better comparison purpose of data obtained from various laboratories.

- **Report the analysis result in the enclosed Data Format Sheet only. Kindly avoid using separate typed data sheet.**
- Be sure that Lab code & sample code numbers are mentioned in the Data format sheet while sending the report.
- Be sure that all the units of various parameters are properly taken care while reporting data.
- Analysis report should be sent directly to the following address positively latest **by 25th December 2000, without fail.**

Dr. S. D. Bewtra, Additional Director Labs
3, Central Reference Laboratory
Olof Palme Marg, Delhi-110032, Fax: (011) 2320844, 2317079

DATA FORMAT SHEET

AQC/WATER	DEC. 2000

LAB CODE	

ANALYTICAL QUALITY CONTROL (AQC/WATER) EXERCISE – DEC.'2000 FOR THE LABORATORY OF SURFACE AND GROUND WATER DEPARTMENTS OF CENTRAL AND STATE AGENCIES

01	**Name of the organisation**	
02	**Address of the Laboratory with PIN code,** **Phone & Fax**	**PIN:**
		Phone: **Fax:**
03	**Samples analysed by:** **(Name & designation)**	**1.** **2.**
04	**Date of receipt of samples**	

RESULT

S. No.	Parameter	Sample Code		Method Adopted	Instrument Used	Calibration graph attached Yes/No	Date of Analysis
		A	B				
01	Conductivity at 25°(μ mhos/cm)					--	
02	Total Solids (mg/l)					--	
03	Total Hardness as $CaCO_3$ (mg/l)					--	
04	Sodium, (mg/l)						
05	Fluoride, (mg/l)						
06	Sulphate, (mg/l)						
07	Nitrate – N, (mg/l)						
08	Phosphate – P, (mg/l)						
09	Boron, (mg/l)						

Note: A copy of the standard Calibration graph wherever applicable as to be attached in annexure

Dated: Signature of Lab incharge

Annex III: Estimation of reference value and acceptable range for conductivity measurement for samples A and B

	COND-A0	COND-A1	COND-A2	COND-B0	COND-B1	COND-B2
	110.000			140.000		
	206.000			162.000	162.000	
	260.000			167.000	167.000	
	288.000	288.000		167.000	167.000	
	298.000	298.000		175.500	175.500	
	304.000	304.000		180.000	180.000	
	311.000	311.000		180.000	180.000	
	320.000	320.000		185.000	185.000	
	320.000	320.000		186.000	186.000	186.000
	323.000	323.000	323.000	189.700	189.700	189.700
	323.000	323.000	323.000	190.000	190.000	190.000
	332.300	332.300	332.300	190.000	190.000	190.000
	335.000	335.000	335.000	193.000	193.000	193.000
	336.000	336.000	336.000	194.000	194.000	194.000
	336.900	336.900	336.900	194.000	194.000	194.000
	337.000	337.000	337.000	194.000	194.000	194.000
	338.500	338.500	338.500	196.000	196.000	196.000
	339.000	339.000	339.000	197.400	197.400	197.400
	350.000	350.000		205.000	205.000	
	350.000	350.000		210.000	210.000	
	355.000	355.000		210.000	210.000	
	360.000	360.000		218.000	218.000	
	363.000	363.000		220.000	220.000	
	405.000			225.000	225.000	
Mean, X	316.696	330.985		190.358	192.548	
Std Dev, SD	56.974	19.791		19.227	16.453	
SD_R			6.333			3.449
$SD_{adj} = \sqrt{2}SD_R$			8.955			4.878
Lower limit = $X_R - 3SD_{adj}$			**306.545**			**177.777**
			360.277			**207.043**