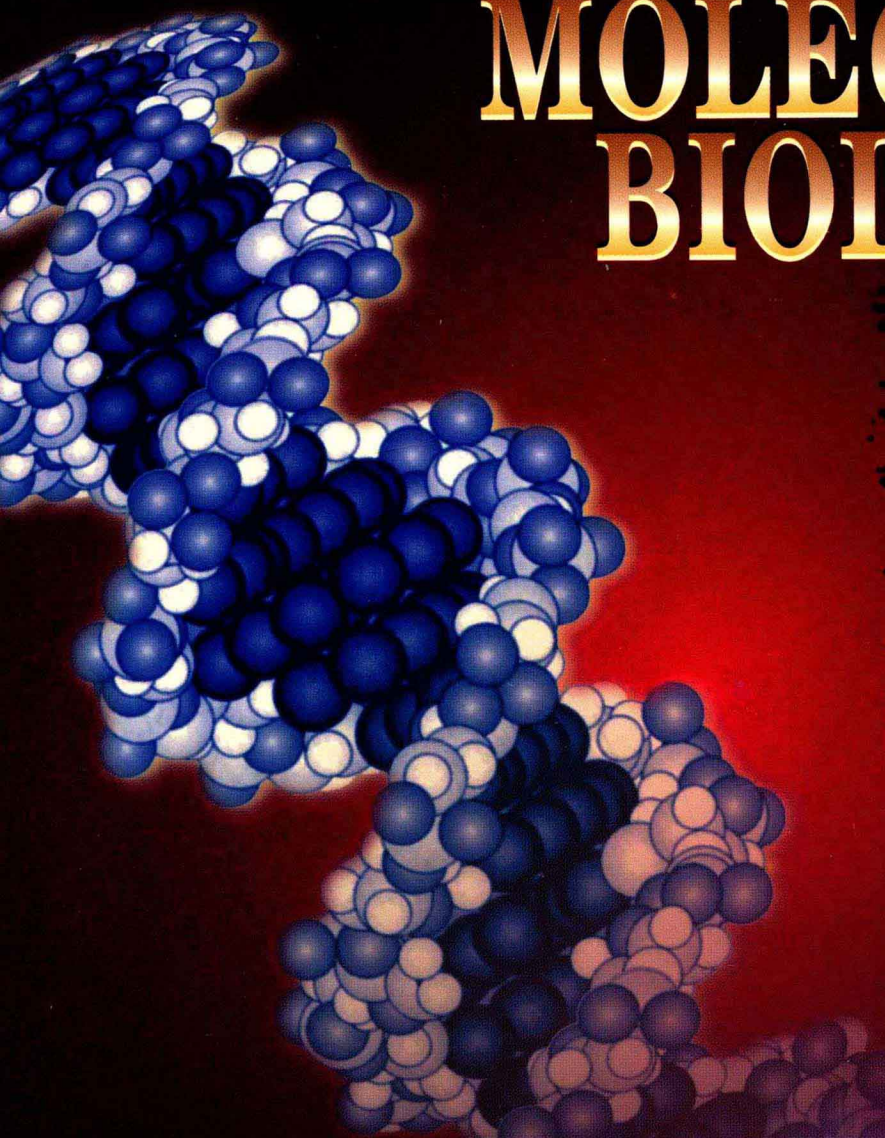
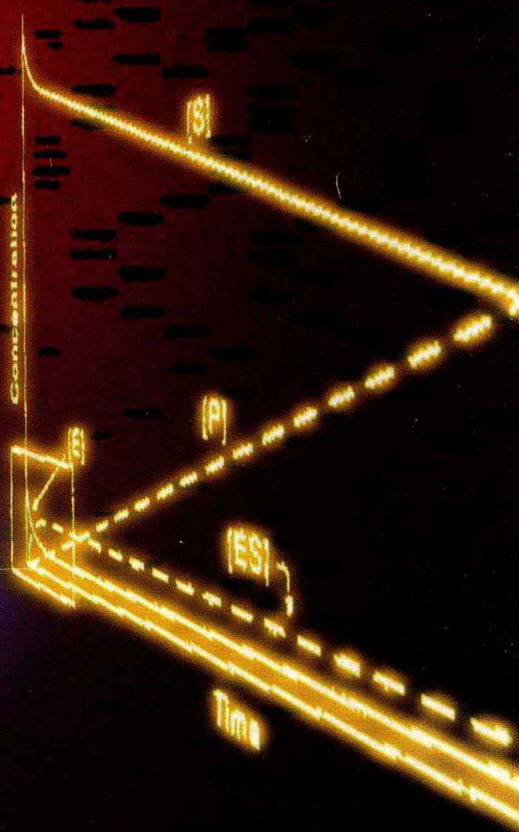
Experimental Approaches in BIOCHEMISTRY and MOLECULAR BIOLOGY





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Experimental Approaches in Biochemistry and Molecular Biology

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Certain laboratory exercises in this manual cannot be taught by an inexperienced instructor. To perform all the exercises, the instructor must be fully trained and familiar with NIH guidelines for recombinant DNA, as well as with the use and disposal of hazardous compounds and radionuclides. Most academic institutions possess an environmental health and safety office as well as a radiation safety office. These individuals are familiar with OSHA and NIH as well as Nuclear Regulatory Agency Guidelines for disposal of hazardous and radioactive wastes, respectively. In addition, the above institutions have guidelines for the use of electron microscope and animal care facilities. The latter involve an animal care committee. The institution where the exercises are performed must be equipped with chemical hoods that have been certified to pull at 100 FPM. In addition, the cloning protocols will require hepafiltered hoods, such as those manufactured by NuAire Flow.

Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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Dedication

Dr. Zeidan dedicates this manual to his wife, Nabila, and his children, Iman, 16, Angie 14, Ronnie 8, Amanda 7, and Nora 5, who patiently dealt with his need for years of scholarship. He renews his sincere thanks to the following for his intellectual development: Dr. Kerry T. Yasunobu, emeritus Professor of Biochemistry and Chair, University of Hawaii, School of Medicine, Department of Biochem and Biophysics; Dr. Lawrence H. Piette, Dean of the Graduate School at Utah State University; Dr. Lawerence J. Berliner and his colleagues; and in particular Dr. Simmon Kwok, Director of Reproductive Biology at the Medical Center of Albert Einstein, Philadelphia, Pennsylvania. Dr. Zeidan also thanks Dr. K. Watanabe and H. Ishizkai.

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Dr. Dashek dedicates this manual to his children, Kristin Ann Simpson and Karin Ann Waddil, who patiently dealt with his need for years of scholarship. He thanks the following for his intellectual development: Dr. Wm. F. Millington, Dr. Walter G. Rosen, Dr. D. T. A. Lamport, Dr. J. E. Varner, Dr. Lynn Margulis, and Dr. M. Schein. Dr. Dashek is indebted to the United States Department of Energy for financial support enabling certain of the polyphenol oxidase experiments described herein. Dr. Dashek extends his gratitude to Ms. Doris Donnell of the Morehouse School of Medicine for her patience and thoughtful clerical assistance. This manual would never have seen "light of day" without her dedication. He also thanks Ms. Kim Chancey of the University of Georgia for additional assistance.

Finally, Dr. Dashek is grateful to Dr. Terry L. Highley, Project Director, Biodeterioration of Wood, Forest Products Laboratory, USDA-Forest Service, for facilities and encouragement. He also expresses his appreciation to Dr. D. E. McMillin, Associate Professor of Biology at Clark Atlanta University in Georgia, for the preparation of Module 8.

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Preface

This laboratory manual is intended for undergraduate and beginning graduate students who are currently enrolled but have not taken an introductory course in biochemistry/biophysics. In addition, the manual is designed around modules that could be utilized as separates by students enrolled in traditional biochemistry/biophysics courses or alternatively those in basic health-oriented biochemistry courses. The latter is evident, as basic biomedical information is included in certain modules, thereby rendering this manual different from standard available biochemistry laboratory manuals. Furthermore, this manual presents biochemical techniques in application to "cutting-edge" research problems in biotechnology, life sciences, and biomedical sciences. In this connection, molecular biology methodologies are included, and thus this manual bridges the gap between traditional biochemistry/biophysics on the one hand and molecular biology on the other. This lab manual will be well-suited for biochemistry lab courses that are oriented toward biotechnology, since the use of antibodies, recombinant DNA, and batch culture techniques is presented here; the quantitative aspects of these disciplines have not been ignored.

The underlying theme centers about the purification, physiochemical properties, and overproduction of enzymes, certain of which are relevant to biomedical research. This manual will be particularly relevant to schools possessing preprofessional undergraduate and/or graduate professional curricula. With the proper selection of topics, depending upon the equipment available, this manual may also be used in two-year colleges or those four-year colleges lacking a research thrust. However, it should be emphasized that this manual is "geared" toward problem-solving oriented programs. An alternative outline that could be performed in a one-semester course that emphasizes spectrophotometry, electrophoresis, enzyme kinetics, and recombinant DNA techniques with less concern for antibodies and radioisotopes, and the more sophisticated biophysical approaches could involve selected modules as highlighted in the Table of Contents.

To test the student's comprehension and retention of the contents of the modules in relation to the theme, review/self-study questions are included. The manual is keyed to the up-to-date versions of certain traditional biochemistry textbooks such as Lehninger, Stryer, Zubay, and others. Relevant references are included. Certain two-year schools will not be able to perform the physiochemical experiments; however, the majority of the schools could perform the biochemistry/ molecular biology experiments.

Finally, the strengths of this manual are 1) an exposure and appreciation for the problem-solving, research-oriented approach; 2) the integration of traditional biochemistry/molecular biology and biophysics with basic medical approach, as well as an appreciation for some relevant environmental problems; 3) an introduction to classical and highly contemporary biochemical/biophysical techniques, as well as "state-of-the art" molecular biology methodologies centering about recombinant DNA techniques; and finally 4) an appreciation for the quantitative aspects of biochemistry. Relevant recent references, as well as enduring references from the research literature, are included.

In summary, students will find this manual a unique resource book throughout their careers, and instructors can avail themselves to a manual that fills the gap between traditional biochemistry/biophysics and biotechnology.

This edition was conceived while H. Zeidan and W. V. Dashek were Associate Professors of Biochemistry and Biology, respectively, in the Biomedical Sciences Program at Clark Atlanta University. The edition, which was written while H. Zeidan was on academic leave at the University of Texas, and W. Dashek was a visiting research botanist at the University of Georgia, will be followed by subsequent editions incorporating recent advances in the topics covered in the laboratory modules and other biomedical research topics.

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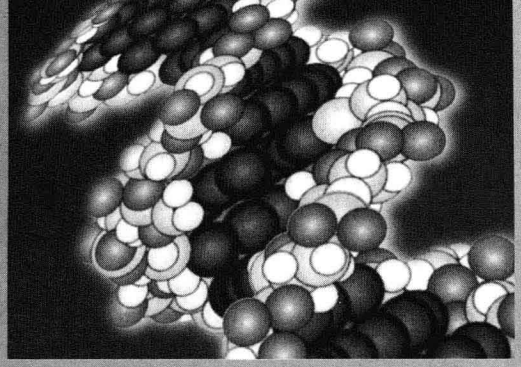

MODULE

1

Biochemical Calculations and Solution Preparation

Outline of Module

- Exponents
- Experimental measurements
- Biostatistical approaches
- Solution preparation
  - Concentrations based on volume
  - Concentrations based on weight
  - Acids and bases
  - Ionization of weak acids
- Laboratory buffers
  - Buffer capacity

Introduction

The objectives of this module are twofold. The first is to introduce the student to the mathematics and statistics used in biochemistry. A clear understanding of arithmetic and simple algebra is an essential component of biochemistry and molecular biology.[1–4] Therefore, sections I, II, and III discuss exponents, logarithms, experimental measurements, algebraic equations, and biostatistical approaches. Since biochemists are interested in studying reactions in solution, a survey of the various ways for expressing and interconverting concentrations of solutions forms the second objective. Section IV is devoted to these topics.

I. Exponents (Scientific Notation)

An exponent is a number that indicates how many times the base appears as a factor. For example, 8×8 can be indicated by 8^2; the exponent 2 appears as a superscript on the base 8. The base 8 is raised to the second power.

In scientific research, exponents are very useful, and the representation of either very large or very small numbers can be easily written. Any number can be represented in an exponential form. The ten (using the decimal system) is normally the base to use. The number 10,000 can be written as 1×10^4, 600,000 as 6×10^5, 0.00008 as 8×10^{-5}, and so on. Numbers that are integral (whole) as well as nonintegral powers of 10 can be represented. Generally, the exponential form is chosen so that it is the product of a number between 1 and 10 to some power. This exponential form is called scientific notation. The student can use the following two steps for writing a number in scientific notation.

1. Shift the decimal point so that the resulting number is between 1 and 10.
2. Determine the power of ten. To do this, count the number of positions the decimal point needs to be shifted to restore it to the original position. If this shift is to the right, the exponent will be positive, and if it is to the left, it will be negative.

Example 1: Convert 0.0000983 to scientific notation.

Solution:

1. Write as a number between 1 and 10:

$$9.83$$

2. The decimal point can be restored by shifting 5 places to the left. The exponent on 10 is –5, and thus the number in scientific notation is

$$9.83 \times 10^{-5}$$

Scientific notation is very useful in the mathematical operations of addition, subtraction, multiplication, and division provided that the student follows the following rules.

Addition and Subtraction

All the terms must have the same exponent. It is most common to convert all terms to the most positive exponent that appears. This gives the sum in scientific notation directly.

Example 2: Perform the following addition:

$$8.0 \times 10^{-4} + 6.5 \times 10^{-2} + 9.4 \times 10^{-6}$$

Solution:

$$0.08 \times 10^{-2} + 6.5 \times 10^{-2} + 0.00094 \times 10^{-2}$$

Remove the common factor, 10^{-2}, to yield

$$(0.08 + 6.5 + 0.00094) \times 10^{-2} = 6.580 \times 10^{-2}$$

Example 3: Perform the following subtraction:

$$5.94 \times 10^8 - 4.2 \times 10^6$$

Solution: Convert to the largest positive exponent, which is 8:

$$5.94 \times 10^8 - 0.042 \times 10^8$$

Remove the common factor, 10^8, to give

$$(5.94 - 0.042) \times 10^8 = 5.898 \times 10^8$$
$$= 5.90 \times 10^8$$

Multiplication

For multiplication of exponential numbers, the numerical portion is treated as usual, and the exponential part uses the law for multiplication of exponents. For the base 10 (decimal), it becomes

$$10^m \times 10^n = 10^{m+n}$$

The exponents are added algebraically to obtain the product in exponential form.

Example 4: Multiple 900×4000.

Solution: Convert to exponential numbers:

$$(9.0 \times 10^2) \times (4.0 \times 10^3)$$

Regroup the product:

$$9.0 \times 4.0 \times 10^2 \times 10^3$$

Multiply the parts:

$$36.0 \times 10^5$$

And convert to scientific notation:

$$3.6 \times 10^6$$

Example 5: Multiply 0.0085×3500.

Solution: Convert to scientific notation:

$$8.5 \times 10^{-3} \times 3.5 \times 10^{3}$$

Regroup the product:

$$8.5 \times 3.5 \times 10^{-3} \times 10^{3}$$

$$29.75 \times 10^{0} = 29.75$$

Division

For division of exponential numbers, the quotient for the numerical part is obtained by the rule for division of exponents:

$$\frac{10^{m}}{10^{n}} = 10^{m-n}$$

Example 6: Divide 6440 by 0.0025.

Solution: Convert all factors to exponential numbers:

$$\frac{6.44 \times 10^{3}}{2.5 \times 10^{-3}} = 2.576 \times 10^{0} = 2.58$$

II. Experimental Measurements

Significant Figures. The measurement of a physical quantity, no matter how precise, is still unreliable in a mathematical sense. A certain amount of error is associated with it. If, for example, the weight of a sample of a certain chemical is found to be 0.864 g and 0.868 g in two successive measurements, the answer is unreliable in the third decimal place. The measurement is said to contain three significant figures; that is, three of the digits are obtained with some reliability. The average weight obtained for the sample is 0.866 g, so the answer rounded off to the proper number of significant digits is 0.870 g. This problem illustrates the principle that the results of a calculation cannot be more reliable than the least reliable number used. Thus, we must drop digits that are not reliable. This process, which is called "rounding off," is governed by three rules.

1. If the digit to be dropped is less than 5, leave the last significant digit unchanged.
2. If it is more than 5, increase the last significant digit by one.
3. If the digit to be dropped is 5 followed by zero, the last remaining significant digit is left even.

Example 1: Indicate the number of significant digits in the following numbers:

1560.0, 16.8, 2.520, 0.0268, 0.02460

Solution:

Number	Significant Digits
1560.0	5
16.8	3
2.520	4
0.0268	3
0.02460	4

Example 2: Multiply 4.7×400. Give the answer to the proper number of significant digits.

Solution:

$$4.7 \times 400 = 1880$$

The minimum number of significant digits is 2, so that the product can be expressed to only 2 significant digits; thus,

$$4.7 \times 400 = 1900$$

The student may question how a number such as 1900 has only two significant figures. The answer to this question can easily be explained when the student writes this number in scientific notation, i.e., 19×10^{2}. This illustrates that only 1 and 9 are significant, and thus clarifies this point. In general, however, we assume the final zeros before the decimal point to be significant unless otherwise indicated. Final zeros after the decimal point are significant digits and should not be carelessly omitted. Thus, a reading of 10.0 g indicates that the weight is known to tenths of grams. Zeros appearing ahead of a number are not significant even though they may be to the right of the decimal point.

III. Biostatistical Approaches

Standard Deviation. Standard deviation, or root-mean-square error, is preferred for some types of measurements. The standard deviation, σ, is defined as:

$$\sigma = \pm\sqrt{\frac{(x_i - \text{a.m.})^2}{n-1}}$$

To understand the significance of standard deviation we need to examine the nature of the distribution function that determines the curve in Figure 1.1. Its

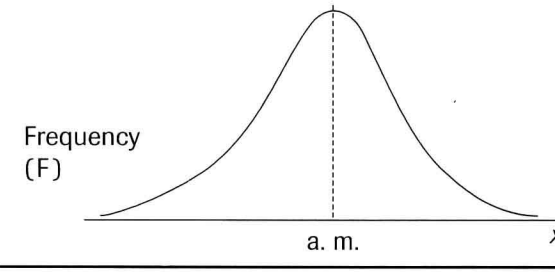

FIGURE 1.1 Gaussian/curve distribution.

mathematical representation for a large number of measurements is generally called the Gaussian distribution function,

$$F = \frac{1}{\sigma(2\pi)^{1/2}} e^{-\frac{(x_1 - \text{a.m.})^2}{2\sigma^2}}$$

where x_1, a.m. arithmetic mean and σ is as defined earlier, and F is the frequency of occurrence of a particular observation. Every experimental measurement that is made lies somewhere on this curve. The most probable value is the true value.

For a given range of X_1 about the true value, the probability that a measured value will fall in this range is proportional to the area under the curve. Since the standard deviation is a range of values spanning the true value, it is thus related to the probabilities for the Gaussian function, using a large number of measurements, as follows:

a.m. ± Q 68.3%

a.m. ± 2Q 95.4%

a.m. ± 3Q 99.7%

Thus, 95.4% of the measurements should be within two standard deviations, and 99.7% of the measurements should be within three standard deviations.

Example 1: The following data were gathered for the pH of a given buffer solution on six successive readings: 6.71, 6.75, 6.74, 6.77, 6.73, 6.74.

Solution: Evaluate the arithmetic mean and the sum of the square of the deviations from the arithmetic mean and the sum of the square of the deviations from the arithmetic mean.

Observations	$[X_1 - \text{a.m.}]$	$[X - \text{a.m.}]^2$
6.71	0.03	0.0009
6.75	0.01	0.0001
6.74	0.00	0.0000
6.77	0.03	0.0009
6.73	0.01	0.0001
6.74	0.00	0.0000
$\Sigma X_i = 40.44$	$[x_i - \text{a.m.}] = 0.08$	$\Sigma[x_i \text{ a.m.}]^2 = 0.0020$

The arithmetic mean is

$$\text{a.m.} = \frac{x_i}{n}$$

Substitute

$$\text{a.m.} = \frac{40.44}{6} = 6.74$$

The standard deviation is calculated by:

$$\sigma = \pm\sqrt{\frac{(x_i - \text{a.m.})^2}{n-1}}$$

Substitute

$$\sigma = \pm\frac{0.002}{5}$$

$$\sigma = \pm 0.0004$$

$$\sigma = \pm 2.0 \times 10^{-2}$$

The pH of the solution can then be expressed as

$$6.74 + 0.020$$

This means that if more measurements were made, then 68.3% would fall between a pH of 6.72 and 6.76.

Review Questions

1. Express the results of the following operations to the proper number of significant figures.
   a) $\frac{3.1 \times 3.57 \times 62.5}{14.6}$
   b) 0.0071 + 0.00364 + 0.00118
   c) 3120.1 + 610.3 + 56
2. Six separate determinations of the concentration of a solution of HCl gave these results: 0.1021, 0.1017, 0.1013, 0.1020, and 0.1016. Calculate the average deviation and standard deviation.
3. Calculate the average deviation and standard deviation for a radioactive sample that when counted gave the following data for 10 five-minute counts: 22,700, 21,650, 22,200, 23,100, 23,900, 22,000, 21,400, 22,300, 21,700, and 23,050.
4. The weight in grams of a sample of hexokinase on six successive weighings showed the following variations: 1.3146, 1.3131, 1.3137, 1.3135, 1.3141, and 1.3138. Express the average reading to the proper number of significant figures.
5. Five determinations of the molecular weight of the enzyme ribonuclease by ultracentrifugation gave the following results: 13,100, 13,640, 13,400, 13,250, and 13,790. Calculate the average deviation in molecular weight to the appropriate number of significant figures.

IV. Solution Preparation

Concentration Based on Volume

Concentrations based on the amount of dissolved solute per unit volume are the most widely used in biochemistry laboratories. The most common conventions are defined below.

Molarity (M) = the number of moles of solute per liter of solution.

To calculate M, we need to know the weight of dissolved solute and its molecular weight, MW.

$$\text{number of moles} = \frac{\text{wt}_g}{\text{MW}}$$

Dilute solutions are often expressed in terms of millimolarity, micromolarity, and so on, where

$$1 \text{ mmole} = 10^{-3} \text{ moles}$$

$$1 \text{ μmole} = 10^{-6} \text{ moles}$$

$$1 \text{ nmole} = 1 \text{ mμ mole} = 10^{-9} \text{ moles}$$

$$1 \text{ pmole} = 1 \text{ μμ mole} = 10^{-12} \text{ moles}$$

therefore,

$$1 \text{ mM} = 10^{-3} \text{ M} = 1 \text{ mmole/liter} = 1 \text{ μmole/ml}$$

$$1 \text{ μM} = 10^{-6} \text{ M} = 1 \text{ μmole/liter} = 1 \text{ nmole/ml}$$

$$1 \text{ nM} = 10^{-9} \text{ M} = 1 \text{ nmole/liter} = 1 \text{ pmole/ml}$$

Normality (N) = the number of equivalents of solute and its equivalent weight, EW.

$$\frac{\text{wt}_g}{\text{EW}} = \frac{\text{MW}}{n}$$

where n is the number of replaceable H^+ or OH^- per molecule (for acids and bases). The molarity and normality are related by:

$$N = nM$$

To calculate N, we need to know the weight of dissolved solute and its equivalent weight, EW.

$$\text{Equivalents} = \frac{\text{wt}_g}{\text{EW}}$$

One equivalent (i.e., the EW) of an acid or base is the weight that contains 1 gm (1 mole) of replaceable hydrogen, or 1 g– ion (1 mole) of replaceable hydroxyl. The EW of a compound involved in an oxidation-reduction reaction is the weight that provides or accepts 1 faraday (1 mole) of electrons. In general:

$$\text{EW} = \frac{\text{MW}}{\text{N}}$$

For example, 0.01 M solution of H_2SO_4 is 0.02 N.

Weight/Volume Percent (% w/v) = the weight in g of solute per 100 ml of solution.

Weight/volume percent is often used for routine laboratory solutions where exact concentrations are not too important.

Milligram Percent (mg%) = the weight in mg of a solute per 100 ml of solution.

Problem 1: a) How many grams of solid NaOH are required to prepare 500 ml of a 0.04 M solution? b) Express the concentration of this solution in terms of N, g/liter, % w/v, mg%.

Solution: a) The number of grams of NaOH equals the desired molarity (M) × the molecular weight for NaOH (40.0 g/mole). Number of grams to prepare 0.04 M solution = molecular weight of NaOH (40.0 g/mole) × 0.04 = 1.60 g.

Therefore, if you dissolve 1.60 g in deionized water and diluted to 1 liter, this will give you 0.04 M NaOH solution. If you dissolve 0.8 gram or 800 mg in deionized water and diluted to 500 ml, this also will give you 0.04 M NaOH solution.

b)

$$0.04 \text{ M NaOH} = 0.04 \text{ N NaOH}$$

$$1.60 \text{ g/liter} = 0.04 \text{ M NaOH}$$

$$1.60 \times 10^2 \text{ mg\%} = 0.04 \text{ M.}$$

Therefore, take 0.6 ml of the concentrated solution and dilute to 1.5 liters.

Concentration Based on Weight

Weight/Weight Percent (% w/w) = the weight in g of a solute per 100 g of solution.

Problem 2: Describe the preparation of 2 liters of 0.4 M HCl starting with a concentrated HCl solution (28% w/w HCl, SG = 1.15).

Solution:

$$\text{liters} \times \text{M} = \text{number of moles}$$

$$2 \times 0.4 = 0.80 \text{ mole HCl needed}$$

$$\text{wt}_g = 29.2 \text{ g pure HCl}$$

The stock solution is not pure HCl but only 28% HCl by weight.

$\therefore \frac{29.2}{0.28} = 104.3$ g of stock solution, we can calculate the volume required.

$$\text{vol}_{ml} = \frac{\text{wt}_g}{^-0} = \frac{104.3}{1.15} = 97.7 \text{ ml stock solution needed}$$

Therefore, measure out 90.7 ml of stock solution and dilute to 2 liters with water.

All of the above relationships (between weight, density, and percent w/w) can be combined into a single expression.

$$\text{wt}_g = \text{vol}_{ml} \times 0_{g/ml} \times \% \text{ (as decimal)}$$

where wt_g = weight of pure substance required in g
vol_{ml} = volume of stock solution needed in ml
% = fraction of total weight that is pure substance

$$\therefore \text{vol} = \frac{\text{wt}}{0 \times \%} = \frac{29.2}{1.15 \times 0.28} = 90.7 \text{ ml}$$

Molality (m) = the number of moles of solute per 1000 g of solvent.

Mole fraction (MF) = the fraction of the total number of moles per 1000 g of solvent.

Molality is used in certain physical calculations (e.g., calculations of boiling-point elevation and freezing-point depression). For dilute aqueous solutions, m and M will be quite close. In order to interconvert m and M, we need to know % w/w.

Mole fraction = the fraction of the total number of moles represented by the compound in question.

For example, in a solution containing n_1 moles of compound 1, n_2 moles of compound 2, and n_3 moles of compound 3, the mole fraction of compound 2, MF_2, is given by

$$MF_2 = \frac{n_2}{n_1 + n_2 + n_3}$$

Problem 3: a) Calculate the molarity of the concentrated stock HCl solution described in Problem 2. b) Calculate the mole fraction of HCl in solution.

Solution:

a) The solution contains 28% w/w HCl, or 28 g HCl per 100 g total, or 28 g HCl per (100 – 28) = 72 g water.

$$\frac{28 \text{ g HCl}}{72 \text{ g } H_2O} \times 1000 = 3.88.9 \text{ g HCl/1000 g } H_2O$$

$$\frac{wt_g}{MW} = \text{moles } \frac{388.9}{36.5} = 10.65 \text{ moles HCl/1000 g } H_2O$$

therefore, the solution is 10.65 m.

b) In 100 g of solution, for example, we have

$$\frac{28 \text{ g HCl}}{36.5 \text{ g/mole}} = 0.767 \text{ moles of HCl}$$

$$\frac{72 \text{ g } H_2O}{18 \text{ g/mole}} = 4.0 \text{ moles of } H_2O$$

$$MF_{HCl} = \frac{n_{HCl}}{n_{HCl} + n_{H_2O}} = \frac{0.767}{4.767}$$

$$MF_{HCl} = 0.161$$

Acids and Bases

An understanding of acid-base chemistry is essential if we are to appreciate the properties of biological molecules. A great many of the low molecular weight metabolites and macromolecular components of living cells are acids and bases, and thus, have the potential to ionize. The charges on these molecules are important factors in the rate of enzyme-catalyzed reactions, in the stability and conformation of proteins, in the interactions of macromolecules with each other and with small ions, and in the analytical and purification techniques used in the biochemical laboratory.

Bronsted Concept of Acids and Bases

An *acid* is defined as a substance that donates protons (hydrogen ions), and a *base* as a substance that accepts protons. When a Bronsted acid loses a proton, a Bronsted conjugated base is produced. The original acid and resulting base are referred to as a conjugate acid-conjugate base pair. The substance that accepts the proton is a different Bronsted base; by accepting the proton, another Bronsted acid is produced. Thus, in every ionization of an acid or base, two conjugate acid-conjugate base pairs are involved.

$$\underset{[\text{conjugate acid}]_1}{HA} + \underset{[\text{conjugate base}]_2}{B^-} \rightleftharpoons \underset{[\text{conjugate acid}]_2}{A^-} + HB$$

pH and pOH

pH is a shorthand way of designating the hydrogen ion activity of a solution. By definition, pH is the negative logarithm of the hydrogen ion activity. Similarly, pOH is the negative logarithm of the hydroxyl ion activity.

$$pH = -\log a_H^+ = \log \frac{1}{aH^+}$$

$$= -\log [H^+]$$

$$= \log \frac{1}{[H^+]}$$

$$pOH = -\log a_{OH}^- = \log \frac{1}{[OH^-]}$$

In dilute solutions of acids and bases in pure water, the activities of H^+ and OH^- may be considered the same as their concentrations.

$$pH = -\log [H^+] = \log \frac{1}{[H^+]}$$

$$pOH = -\log [OH^-] = \log \frac{1}{[OH^-]}$$

In all aqueous solutions the equilibrium for the ionization of water must be satisfied; that is, $[H^+][OH^-] = K_w = 10^{-14}$. Thus, if $[H^+]$ is known, we can easily calculate $[OH^-]$. Furthermore, we can derive the following relationship between pH and pOH:

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

Taking logarithms:

$$\log[H^+] + \log[OH^-] = \log K_w$$

$$-\log[H^+] = pH \qquad -\log[OH^-] = pOH \qquad -\log K_w = pK_w$$

$$\therefore pH + pOH = pK_w$$