The Tools of Biochemistry

Terrance G. Cooper

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University of Pittsburgh

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Preface

An essential condition for fruitful research is to have satisfactory techniques at one's disposal. Progress in biological sciences has largely depended on parallel advances in the technology of measuring and observing devices. Unfortunately, a lack of understanding of the tools used is commonly a weakness of many investigations. Careful evaluation is needed, not only of experimental data but also of the methods by which they were obtained.

The purpose of this work is to increase the number and quality of biochemical techniques available to the reader. Often students, during their undergraduate and graduate studies, become acquainted with only the techniques used in their immediate environments. They may find it difficult to apply entirely new approaches and techniques to a problem because they lack a general source of information that presents the potential and limitations of a particular method as well as an opportunity to use it under well characterized conditions. It is to this difficulty that I address this book. Although the text forms the basis of a senior level undergraduate course, it is intended also to serve as a ready source of useful information for graduate students and experienced investigators. Its purpose is to enlarge the sphere of the reader's experience, and its success will be measured by the degree to which this occurs.

The format used is to first present the theoretical basis and limitations of each technique. This section provides an understanding of the reasons for procedures that are subsequently performed but is not designed to be an absolutely comprehensive treatise; an experimental section follows in which a number of experiments using the method are described in detail. The particular experiments were selected because they are straightforward to execute and provide clear examples of data that can be obtained when the method is used properly. To permit application of this material to the greatest range of teaching and research requirements, each chapter is a self-contained unit. It is therefore possible to select those methods that are appropriate to the reader's needs and resources.

I wish to express my appreciation to Professor David Krogmann for providing the early stimulus and enthusiasm needed to undertake this work. Without his help it would still be just an idea. Many of my students and colleagues have read these chapters and offered advice for their

improvement. This and the many hours spent by my wife Carol reading and rereading the text have avoided countless errors, confusing statements, and insults to the English language. I did not, however, follow their good advice on all occasions and am solely responsible for any problems that still remain. For technical preparation of the manuscript I am indebted to Mrs. Sandra Wight. The many photographs generously provided by independent investigators and by manufacturers of scientific equipment and supplies enhance this work and are greatly appreciated. To the staff at John Wiley and Sons I am grateful for unlimited patience and assistance during development and publication of this work.

T. G. COOPER

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Chapter 1

Potentiometric Techniques

Most of the chemical reactions that comprise a living organism are profoundly influenced by hydrogen ion concentration. So important is this characteristic that multicelled organisms have evolved a variety of sophisticated methods to maintain the solutions in which their cells are bathed within rigid limits of hydrogen ion concentration. The same care exercised by living organisms to maintain acceptable hydrogen ion concentrations must be duplicated in the laboratory if meaningful insights are to be gained into the functioning of organisms and their components. The subsequent discussion employs the Brønsted–Lowry definition of acids and bases: an acid is a compound that donates protons and a base is one that accepts protons. This definition may also be formulated as an acid dissociating into a base and a proton:

$$\text{acid} \rightleftharpoons \text{base} + H^+ \qquad (1)$$

Therefore, HCl would be considered an acid and Cl^- would be its conjugate base.

Acid	Conjugate Base
HCl	Cl^-
CH_3COOH	CH_3COO^-
H_2CO_3	HCO_3^-
HCO_3^-	CO_3^{2-}
NH_4^+	NH_3

Acids and bases can be classified as strong or weak depending on the extent to which they ionize. A strong acid is one for which reaction 1

proceeds far to the right; that is, the acid is essentially totally ionized. For example, the hydrogen ion concentration of a $0.01M$ solution of the strong acid HCl is $0.01M$ because all of the acid is dissociated. On the other hand, a weak acid (such as acetic, boric, or carbonic) is one for which reaction 1 does not proceed significantly to the right.

pH CALCULATIONS

A Danish chemist, S. P. L. Sorensen, proposed a convenient notation for the hydrogen ion concentration of a solution. He defined the negative log of the hydrogen ion concentration as pH.

$$\text{pH} = -\log [\text{H}^+] \tag{2}$$

Therefore, for a $0.01M$ solution of HCl,

$$\text{pH} = -\log [10^{-2}]$$
$$= 2.0$$

The pH values most often encountered in biochemistry range from 4 to 11. Figure 1-1 depicts the relationship of pH to acidity and basicity or alkalinity.

It is clear that the pH of a $10^{-2}M$ solution of a strong acid is 2.0, but the

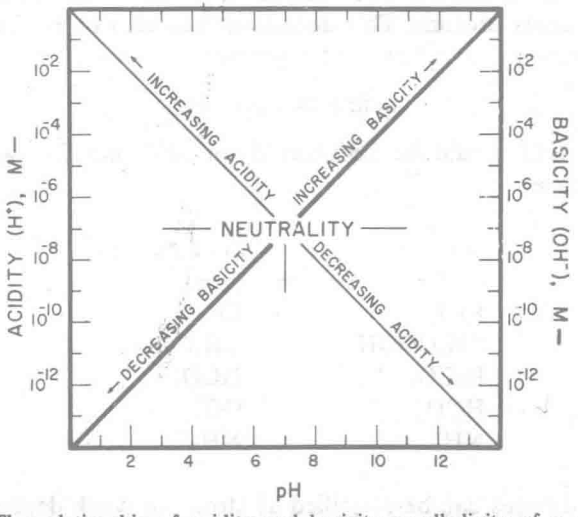

Figure 1-1. The relationship of acidity and basicity or alkalinity of a solution to its hydrogen ion and hydroxyl ion concentrations.

pH of a 0.01M solution of a strong base (reaction 3) is less obvious.

$$NaOH \rightleftharpoons Na^+ + OH^- \tag{3}$$

The hydroxyl ion concentration can be related to the hydrogen ion (H^+), or more accurately hydronium ion (H_3O^+), concentration by considering the dissociation of water:

$$H_2O \rightleftharpoons H^+ + OH^- \tag{4}$$

The equilibrium constant equation for this reaction is

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} \tag{5}$$

and thus

$$K_{eq}[H_2O] = [H^+][OH^-] \tag{6}$$

Since the concentration of water remains about constant throughout the ionization process this term can be combined with the equilibrium constant, generating a new constant, K_W:

$$K_W = [H^+][OH^-] \tag{7}$$

For pure water at 25°C both the hydronium and hydroxyl ion concentrations are equal to $1 \times 10^{-7}M$. Therefore,

$$K_W = (1 \times 10^{-7})(1 \times 10^{-7})$$
$$= 1 \times 10^{-14}$$

Since in aqueous solution, the product of the hydronium and hydroxyl ion concentrations must remain constant at $1 \times 10^{-14}M$, an increase in one term of equation 7 requires a corresponding decrease in the other term. Therefore, a 0.01M NaOH aqueous solution has a hydrogen ion concentration of

$$[H^+] = \frac{K_W}{[OH^-]} \tag{8}$$
$$= \frac{10^{-14}}{10^{-2}}$$
$$= 10^{-12}$$

and

$$pH = 12$$

Weak acids, by definition, are only partially ionized in aqueous solution.

$$HA \rightleftharpoons H^+ + A^- \tag{9}$$

The concentration of each species at equilibrium may be calculated from the dissociation constant of the acid as described by the equation

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

Rearrangement of equation 10 gives

$$[H^+] = \frac{[HA]K_a}{[A^-]} \tag{11}$$

If the negative logarithms of both sides of equation 11 are taken,

$$-\log [H^+] = (-\log K_a) + \left(-\log \frac{[HA]}{[A^-]}\right) \tag{12}$$

or

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{13}$$

In more general terms,

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{undissociated acid}]} \tag{14}$$

This equation is known as the Henderson–Hasselbach equation. If

$$[A^-] = [HA]$$

then

$$pH = pK_a \tag{15}$$

Many handbooks list dissociation constants (the equilibrium constant for the dissociation of acid into a proton and its conjugate base) as pK_a's.

Thus far it has been assumed that the molar concentration of any given ion is also its effective or active concentration. This, however, is true only at very low ion concentrations. As the number of ions in a given volume increases, the probability of ionic interactions also increases. These interactions tend to impede the movement of ions and hence decrease their effective concentration or activity. Activity is related to molar concentration by a normalization factor or activity coefficient

$$a_i = f_i[i] \tag{16}$$

where a_i is the activity of ionic species i, and f_i is the activity coefficient. When ions are separated from one another (at low concentration) f_i approaches unity; f_i decreases as the concentration of i increases. The distinction between the activity and molar concentration of an ion is significant because all potentiometric measurements of hydrogen ion concentration yield hydrogen ion activity, not concentration.