

A Textbook of  
**P**HARMACEUTICAL  
**A**NALYSIS

**3**rd Edition

**Kenneth A. Connors**

**Rx**

# *A Textbook of Pharmaceutical Analysis*

*Third Edition*

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*Professor of Pharmacy*

*The University of Wisconsin*



*A Wiley-Interscience Publication*

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*A Textbook of  
Pharmaceutical Analysis*

*To Some of My Students*

## *Preface*

The course in pharmaceutical analysis ("drug assay") retains a place in the undergraduate curriculum, despite its apparent inutility to the practicing pharmacist, for two reasons. The pharmacist is primarily an expert on drugs, and an important aspect of the development, production, distribution, and use of drugs is their analysis. This component of the pharmacist's professional field should be revealed to the student, even though few practicing pharmacists will ever need to analyze a drug sample. The second reason is that much of the subject matter of pharmaceutical analysis is fundamental to techniques and results of other fields in the curriculum, such as biochemistry, medicinal chemistry, and pharmaceutics. This is a preparatory or service function of the course.

Because pharmaceutical analysis deals with bulk materials, dosage forms, and, more recently, biological samples in support of biopharmaceutical and pharmacokinetic studies, I regard it as a branch of pharmaceutics rather than of pharmaceutical chemistry (its traditional assignment). This is important only in that students and teachers should keep in mind the close relationship between the analytical support and drug delivery, and pharmaceutics is the study of drug delivery systems.

In this book I try to give a sufficiently systematic and detailed account of modern pharmaceutical analysis to permit the pharmacy student and the pharmacist to understand most analyses in principle and many of them in detail. The material in this book will enable the student to approach with understanding the official compendia, reference works in analysis, specialized monographs, and advanced textbooks in analytical chemistry. On the other hand, this is not a catalog of assay methods for specific drugs, nor is it a commentary on the official volumes. It is a textbook.

Although the treatment is aimed at the undergraduate, in some sections I have gone beyond this level; and this further development may be helpful to graduate students and research analysts without lessening the value of the book in the undergraduate course. The treatment is in some places simp-

lified, but I hope that it is in no place wrong as a consequence of simplification. It should not be necessary, for the student wishing to go beyond this level, to "unlearn" anything in this book.

I have made frequent reference to the analytical literature, for several reasons. First, of course, is the traditional courtesy and requirement of giving credit, and the associated opportunity to acquaint students with this tradition; second, for both students and instructors immediate access is provided to more detailed accounts and other points of view; and third, many of these citations reveal the current importance and range of applications of a technique in pharmaceutical analysis.

This edition differs from the preceding one primarily in its presentation of new chapters on immunoassay, derivative formation, and statistics, and an expanded discussion of liquid chromatography. I have greatly benefited from discussions with colleagues and students, some of whom have provided new data and experimental work; and I especially wish to thank Jordan L. Cohen, Perri Corrick-West, Berry J. Kline, Paul A. Kramer, James W. Munson, Nita K. Pandit, David D. Pendergast, William R. Porter, Thomas W. Rosanske, and Peter G. Welling.

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*Madison, Wisconsin  
June 1982*

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# Part One: Titrimetric Analysis

The basic experimental operation in titrimetric analysis is called a *titration*. In a titration, a solution of one reactant of accurately known concentration (the *titrant*, or *standard solution*) is added to a second reactant, the solution of sample whose amount or concentration is to be determined. Titrant is added to the sample until the amount of titrant added is chemically equivalent to the amount of sample. The stage at which this equivalence occurs is called the equivalence point of the titration, and its experimental estimate is called the titration *end point*. From the amount of titrant used to reach the end point, from its concentration, and from the known stoichiometry of the titration reaction, the amount of sample substance can be calculated. Usually the volume of titrant is measured, and then titrimetric analysis is also called volumetric analysis.

This part of the book describes many of the chemical reactions that form the basis of successful titrations, and methods for end point detection. Titrimetry is a classical method of analysis, but even in modern analytical practice it is very important, as can be seen by browsing through the *United States Pharmacopeia/National Formulary* (the legal compendium of specifications and standards for drug purity and quality). Later chapters describe newer methods of end point detection and applications of titrimetry to more complicated analytical problems.

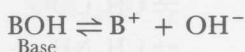
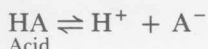
A brief description of the experimental technique and calculational basis of volumetric analysis is given in Appendix A. Chapter 27 treats the statistical analysis of experimental data; this material is useful in interpreting the results of titrimetric analysis, and it probably should be reviewed several times during one's progress through this book, since it applies to all kinds of quantitative experimentation.



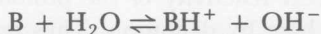
# 1 AQUEOUS ACID-BASE TITRATIONS

## 1.1 Acid-Base Equilibria

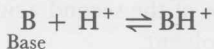
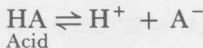
*Definitions of Acids and Bases.* The first successful acid-base theory is due to Arrhenius, who defined an acid as a substance that when dissolved in water gives rise to hydrogen ions, and a base as a substance that in water yields hydroxide ions.



This theory was broadened to include those substances that increase the hydroxide ion concentration of a solution but that do not themselves contain the hydroxyl group; amines, for example, are such substances. The concept of hydrolysis, or reaction with water, was adopted to account for this behavior, and these substances were referred to as pseudo bases.



The Arrhenius theory, which was introduced just before the turn of the century, leads to an adequate quantitative description of acid-base behavior in aqueous solution and is still widely taught and used. It lacks generality, however; in particular, it does not account for manifestations of acid-base reactions in nonaqueous solvents. In 1923 Bronsted (and, independently, Lowry) proposed new definitions of great generality, as follows: an acid is a species that can yield a proton, and a base is a species that can accept a proton.



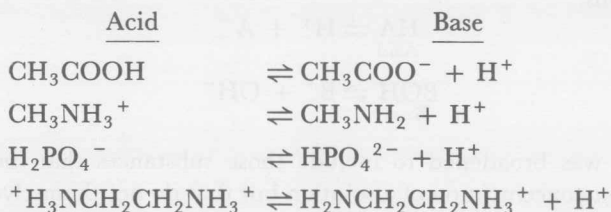
This definition of acid is slightly different from that in the Arrhenius theory.

In the older theory, the acid yields a hydrogen ion in aqueous solution (that is, the definition requires water as the solvent), whereas a Bronsted acid yields a proton, and this definition is independent of the solvent. A major difference between the theories is in their concepts of the base. Notice that in the system  $HA \rightleftharpoons H^+ + A^-$  the anion  $A^-$  is acting as a base, since it accepts a proton. Also, in the reaction  $B + H^+ \rightleftharpoons BH^+$ , the cation  $BH^+$  acts as a Bronsted acid. In general, we may write



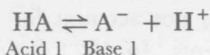
and an acid-base pair related by this equation is known as a *conjugate acid-base pair*.

One result of this definition is that an acid or a base can have any charge, the only limitation being that the charge of an acid is greater than that of its conjugate base by one positive unit. For example, each of the following equilibria fits the general Eq. 1.1; acids and bases that are neutral, positive, and negative are shown:

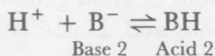


Notice that the solvent has not yet been invoked in these descriptions. In fact, Eq. 1.1 will not be observable in the absence of a substance to accept the proton because of the great reactivity of the proton. This second proton-accepting substance is a base by definition. Thus two conjugate acid-base systems are necessary in order to observe a reaction, and an acid-base reaction is a proton transfer from one system to the other.

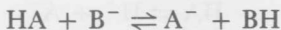
1st system:



2nd system:



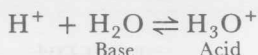
Overall reaction:



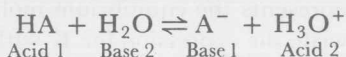
Often the solvent assumes the role of the second acid-base pair if it has acid-base properties. Water is such a solvent.



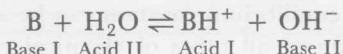
But water can act as both an acid and a base (it is *amphoteric*).



This dual behavior means that water can be the second conjugate acid-base system for solutes that are acids and for solutes that are bases.

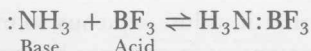


and



The Bronsted acid-base theory is very useful in describing reactions in many solvents besides water. Throughout this book acid-base phenomena will be considered in terms of this theory.

A third theory of acid-base behavior was suggested by G. N. Lewis in 1923. An acid is defined as an electron-pair acceptor and a base as an electron-pair donor. This involves no change in the concept of a base, since every proton acceptor is also an electron-pair donor. The concept of the acid is altered markedly, however, to include some substances that do not contain hydrogen. In the Lewis sense the reaction



is an acid-base reaction. It has become common to refer to such nonprotonic acids as "Lewis acids." The Lewis theory has helped to describe many phenomena (such as indicator color changes) that can take place in nonprotonic systems but that exhibit all the characteristics of acid-base reactions.

Table 1.1 gives a comparison of these three important theories, with equivalent definitions enclosed by dashed lines (actually, the Arrhenius and Bronsted definitions of acid are not precisely equivalent, as was pointed out previously).

*Acid-Base Dissociation Constants.* For a reversible chemical reaction

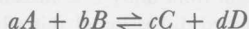


TABLE 1.1

*Comparison of Acid-Base Theories*

Theory	Acid	Base
Arrhenius	Proton donor	Hydroxide donor
Bronsted	Proton donor	Proton acceptor
Lewis	Electron-pair acceptor	Electron-pair donor

the quantity  $K$ , defined by Eq. 1.2, is a constant at constant temperature and pressure.\*

$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b} \quad (1.2)$$

In this equation  $[A]$  represents the equilibrium molar concentration of  $A$ , etc. It is customary to write the expression for  $K$  with the products (that is, the species on the right-hand side of the equation) in the numerator.

The concept of the equilibrium constant can be applied profitably to the quantitative description of all types of chemical equilibria, and much of this book is devoted to such applications. In the present case acid-base equilibria in aqueous solution are treated. Consider the dissociation of the weak acid  $HA$ ,



By analogy with Eq. 1.2 we define the *acid dissociation constant*†  $K_a$ .

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} \quad (1.3)$$

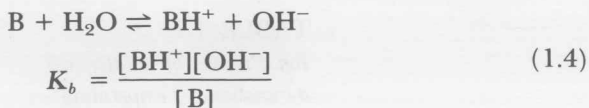
This definition implies that in dilute aqueous solution the concentration of water is essentially a constant, so it need not be explicitly incorporated. The species  $H_3O^+$  is called the *hydronium ion*; there is considerable experimental evidence that this is the form in which the proton exists in aqueous solution [1]. The acid dissociation constant is always written with the hydronium ion concentration in the numerator.

In a similar manner, an equilibrium constant  $K_b$ , called the *base dissociation constant*, can be defined for all weak bases.‡

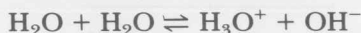
\* This statement is not strictly true. For  $K$  to be a constant, each concentration  $c$  must be replaced by the corresponding activity  $a$ , where  $a = fc$ . The proportionality constant  $f$  is called the activity coefficient; its deviation from unity is a measure of the deviation of the solution from ideal behavior. A more detailed discussion of this effect is given in Chapter 6. In this chapter we assume that all activity coefficients equal unity.

† *Ionization* is the formation of ions, whereas *dissociation* is the separation of species. These terms are usually used as synonyms in discussions of aqueous solutions, since in dilute aqueous solution every ionized species dissociates. (See p. 51 for a discussion of nonaqueous electrolyte behavior.) Sometimes ionization is restricted to those reactions in which charged species are formed; that is, separation of a neutral molecule into a cation and an anion is called ionization, whereas separation of a cation into another cation and a neutral molecule is called dissociation. We shall usually use the term *dissociation*.

‡ In the Arrhenius theory,  $K_b$  was labelled  $K_h$  and called the *hydrolysis constant*, since it represented reaction with water. In the Bronsted theory, the concept of hydrolysis (in this sense) is unnecessary.



Water, too, is capable of dissociation.



This equilibrium is characterized by the important dissociation constant  $K_w$ :

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-] \tag{1.5}$$

where, as before, the concentration of water is considered to be constant.  $K_w$  is the *ion product*, or autoprotolysis constant, of water. This is a very valuable quantity because it relates the  $K_a$  of a weak acid and the  $K_b$  of its conjugate base. Consider the acid HA and its conjugate base  $\text{A}^-$ :

$$\begin{aligned}
 K_a &= \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]} \\
 K_b &= \frac{[\text{HA}][\text{OH}^-]}{[\text{A}^-]}
 \end{aligned}$$

Multiplying these two constants gives

$$K_w = K_a K_b \tag{1.6}$$

which holds for any conjugate acid-base pair. Since  $K_w$  is accurately known over a wide range of temperature, this means that it is necessary only to know *either*  $K_a$  or  $K_b$  for a conjugate pair; the unknown constant may then be calculated with the aid of Eq. 1.6. It is, of course, essential that all quantities in this equation refer to the same temperature.

The numerical values of most dissociation constants are very small numbers; hence it is convenient to employ logarithms when manipulating them. The symbolism

$$\text{p}K = -\log K$$

is widely used. Thus Eq. 1.6 may be written

$$\text{p}K_w = \text{p}K_a + \text{p}K_b$$

for any conjugate acid-base pair. Table 1.2 lists values of  $\text{p}K_w$  at numerous temperatures; the effect of temperature upon  $\text{p}K_w$  is very marked.

For weak acids it is usual to express the acid dissociation constant as either  $K_a$  or  $\text{p}K_a$ . It is *not* common, however, to find the  $K_b$  or  $\text{p}K_b$  of weak bases tabulated. Rather, we usually encounter the  $K_a$  or  $\text{p}K_a$  of the conjugate acid of the weak base. (This is often referred to, erroneously, as the “ $\text{p}K_a$  of the base.”)

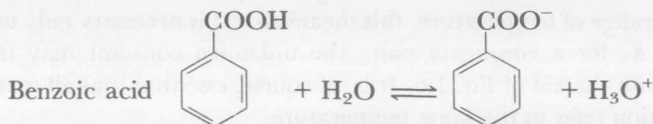


TABLE 1.2  
*Ion Product of Water as  
 a Function of Temperature<sup>a</sup>*

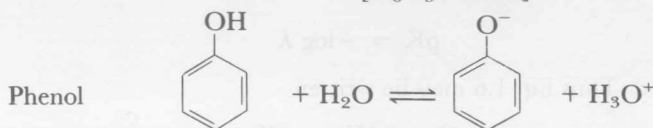
$t$ ( $^{\circ}\text{C}$ )	$\text{pK}_w$
0	14.94
10	14.54
20	14.17
25	14.00
30	13.83
40	13.54
50	13.26
60	13.02

<sup>a</sup> From Harned, H. S. and B. B. Owen, *The Physical Chemistry of Electrolytic Solutions*, 3rd ed., Reinhold Publishing, New York, 1958, p. 638.

The formulation of acid dissociation constants for some specific substances is shown below.\* All of these equations have the form of Eq. 1.3.



$$K_a = \frac{[\text{H}_3\text{O}^+][\text{C}_6\text{H}_5\text{COO}^-]}{[\text{C}_6\text{H}_5\text{COOH}]}$$



$$K_a = \frac{[\text{H}_3\text{O}^+][\text{C}_6\text{H}_5\text{O}^-]}{[\text{C}_6\text{H}_5\text{OH}]}$$

\* An acid with a single dissociable proton is called a *monoprotic acid* or a *monobasic acid*, the latter designation indicating that one molecule of the acid can transfer one proton to a base; similarly, a base that can accept a single proton is a *monoacidic base*. Polybasic acids (and polyacidic bases) can donate (or accept) more than one proton per molecule.