Protein binding
Chapter 10

PROTEIN BINDING OF DRUGS

Many drugs interact with plasma or tissue proteins or with other macromolecules, such as melanin and DNA, to form a ***drug-macromolecule complex***. The formation of a drug protein complex is often named ***drug-protein binding***. Drug-protein binding may be a reversible or an irreversible process. ***Irreversible* drug-protein binding is usually a result of chemical activation of the drug, which then attaches strongly to the protein or macromolecule by covalent chemical bonding**. Irreversible drug binding accounts for certain types of drug toxicity that may occur over a long time period, as in the case of chemical carcinogenesis, or within a relatively short time period, as in the case of drugs that form reactive chemical intermediates**. For example, the hepatotoxicity of high doses of acetaminophen is due to the formation of reactive metabolite intermediates that interact with liver proteins.**

Most drugs bind or complex with proteins by a reversible process. ***Reversible drug protein binding*** implies that the drug binds the protein with weaker chemical bonds, such as **hydrogen bonds or van der Waals forces.**

The amino acids that compose the protein chain have hydroxyl, carboxyl, or other sites available for reversible drug interactions.

Reversible drug -protein binding is of major interest in pharmacokinetics. The protein-bound drug is a 1-**large complex** that cannot easily transverse cell or possibly even capillary membranes and therefore has a restricted distribution. Moreover, the protein-bound drug is usually 2-**pharmacologically inactive**.

In contrast, the free or unbound drug crosses cell membranes and is therapeutically active. Studies that critically evaluate drug-protein binding are usually performed ***in vitro*** using a purified protein such as albumin. Methods for studying protein binding, including equilibrium dialysis and ultrafiltration, make use of a semipermeable membrane that separates the protein and protein-bound drug from the free or unbound drug.

By these *in vitro* methods, the concentrations of bound drug, free drug, and total protein may be determined. Each method for the investigation of drug-protein binding *in vitro* has advantages and disadvantages in terms of cost, ease of measurement, time, instrumentation, and other considerations.

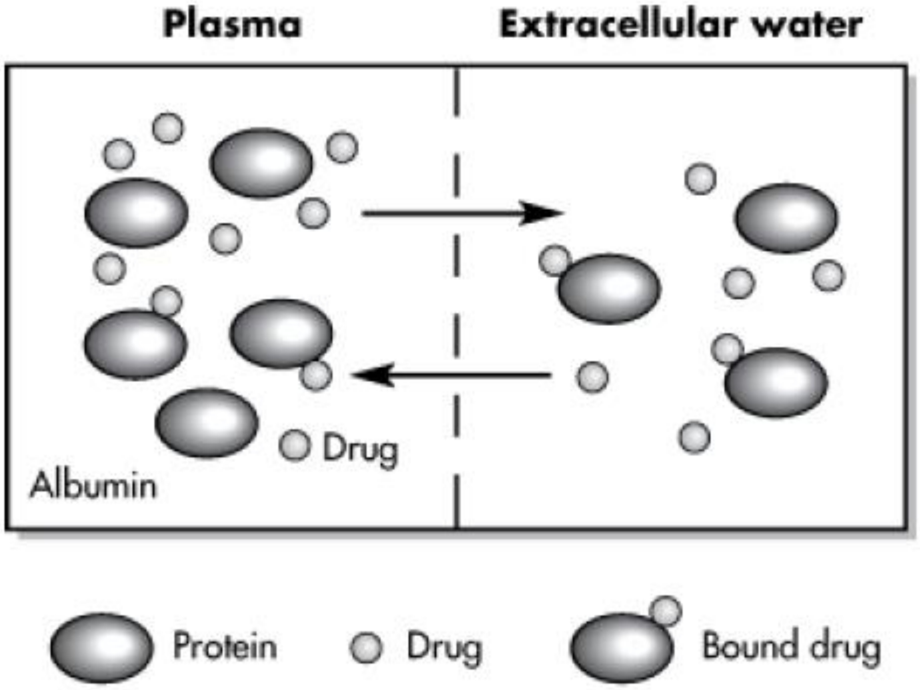


Diagram showing that bound drugs will not diffuse across membrane but free drug will diffuse freely between the plasma and extracellular water.

Drugs may bind to various macromolecular components in the blood, including **albumin, α1-acid glycoprotein, lipoproteins, immunoglobulins (IgG), and erythrocytes (RBC).**
Albumin is a protein with **a molecular weight of 65,000-69,000 Da** that is synthesized in the **liver** and is the **major component** of plasma proteins responsible for reversible drug binding. In the body, albumin is **distributed in the plasma and in the extracellular fluids of skin, muscle, and various other tissues.** Interstitial fluid albumin concentration is about 60% of that in the plasma. **The elimination half-life of albumin is 17-18 days**. Normally, albumin concentration is maintained at a relatively constant level of 3.5-5.5% (weight per volume) or 4.5 mg/dL. **Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances**. As a transport protein for endogenous substances, albumin complexes with free fatty acids (FFAs), bilirubin, various hormones (such as cortisone, aldosterone, and thyroxine), tryptophan, and other compounds. **Many weak acidic (anionic) drugs bind to albumin** by electrostatic and hydrophobic bonds. Weak acidic drugs such as salicylates, phenylbutazone, and penicillins are highly bound to albumin. **However, the strength of the drug binding is different for each drug.**

α1-*Acid glycoprotein* is a globulin with a molecular weight of about **44,000 Da**. The plasma concentration of α1-acid glycoprotein is low (0.4-1%) and **binds primarily basic (cationic) drugs** such as propranolol, imipramine, and lidocaine.

Lipoproteins are macromolecular **complexes of lipids and proteins and are classified according to their density and separation in the ultracentrifuge**. The terms VLDL, LDL, and HDL are abbreviations for very-low-density, low-density, and high-density lipoproteins, respectively. **Lipoproteins are responsible for the transport of plasma lipids to the liver and may be responsible for the binding of drugs if the albumin sites become saturated.**

Erythrocytes**, or red blood cells (RBCs),** may bind both endogenous and exogenous compounds. RBCs consist of about **45% of the volume of the blood**. **Phenytoin, pentobarbital, and amobarbital are known to have a RBC/plasma water ratio of 4 to 2, indicating preferential binding of drug to the erythrocytes over plasma water**. **Penetration into RBC is dependent on the free concentration of the drug.** In the case of phenytoin, RBC drug level increases linearly with an increase in the plasma-free drug concentration. Increased drug binding to plasma albumin reduces RBC drug concentration. With most drugs, however, binding of drug to RBC generally does not significantly affect the volume of distribution, because the drug is often bound to albumin in the plasma water.

For drugs with strong erythrocyte binding, the hematocrit will influence the total amount of drug in the blood. For these drugs, **the total whole-blood drug concentration should be measured.**

Effect of Protein Binding on the Apparent Volume of Distribution

The extent of drug protein binding in the plasma or tissue affects *V*D. Drugs that are highly bound to plasma proteins have a low fraction of free drug (*f*u = unbound or free drug fraction) in the plasma water. The plasma protein-bound drug does not diffuse easily and is therefore less extensively distributed to tissues. Drugs with low plasma protein binding have larger *f*u, generally diffuse more easily into tissues, and have a greater volume of distributions. Since the apparent volume of distribution is influenced by lipid solubility in addition to protein binding, there are some exceptions to this rule. However, when several drugs are selected from a single family with close physical and lipid partition characteristics, the apparent volume of distribution may be explained by the relative degree of drug binding to tissue and plasma proteins.

Effect of Changing Plasma Protein: An Example

The effect of increasing the plasma α1-acid glycoprotein (AAG) level on drug penetration into tissues may be verified with **cloned transgenic animals that have 8.6 times the normal α1-acid glycoprotein levels.** In an experiment investigating the activity of the tricyclic antidepressant drug imipramine, equal drug doses were administered to both normal and transgenic mouse. **Since imipramine is highly bound to AAG, the steady state imipramine serum level was greatly increased in the blood due to protein binding.**

Imipramine serum level (transgenic mouse): 859 ng/mL
Imipramine serum level (normal mouse): 319.9 ng/mL
Imipramine brain level (transgenic mouse): 3862.6 ng/mL
Imipramine brain level (normal mouse): 7307.7 ng/mL

However, the imipramine concentration was greatly reduced in the brain tissue because of higher degree of binding to AAG in the serum, resulting in reduced drug penetration into the brain tissue. The volume of distribution of the drug was reported to be reduced. The antidepressant effect was observed to be lower in the transgenic mouse due to lower brain imipramine level. This experiment illustrates that high drug protein binding in the serum can reduce drug penetration to tissue receptors for some drugs .

For a drug that **distributes into the plasma and a given tissue in the body**, the amount of drug bound may be found by Equation 10.7. Because drug may bind to both plasma and tissue proteins, the bound and unbound drug concentrations must be considered. **At steady state, unbound drug in plasma and tissue are in equilibration.**

$$D_B = V_p C_p + V_t C_t \qquad (10.7)$$

$$C_u = C_{ut}$$

Alternatively,

$$C_p f_u = C_t f_{ut} \qquad (10.8)$$

or

$$C_t = C_p \frac{f_u}{f_{ut}} \qquad (10.9)$$

where all terms refer to steady-state condition: *f*u is the unbound (free) drug fraction in the plasma, *f*ut is the unbound drug fraction in the tissue, *C*u is the unbound drug concentration in the plasma, and *C*ut is the unbound drug concentration in the tissues. Substituting for *C*t in Equation 10.7 using Equation 10.9 results in

$$D_B = V_p C_p + V_t \left[C_p \left(\frac{f_u}{f_{ut}} \right) \right] \qquad (10.10)$$

Rearranging,

$$\frac{D_B}{C_p} = V_p + V_t\left(\frac{f_u}{f_{ut}}\right) \qquad (10.11)$$

$$D_B = V_P C_P + V_t\left[\, C_P\left(\frac{f_u}{f_{ut}}\right)\right]$$

Because $D_B/C_p = V_{app}$, by substitution into Equation 10.11, V_{app} may be estimated by Equation 10.12:

$$V_{app} = V_p + V_t\left(\frac{f_u}{f_{ut}}\right) \qquad (10.12)$$

Equation 10.12 relates the amount of drug in the body to plasma volume, tissue volume, and fraction of free plasma and tissue drug in the body. Equation 10.12 may be expanded to include several tissue organs with *V*ti each with unbound tissue fraction *f*uti.

$$V_{app} = V_p + \sum V_{ti}\left(\frac{f_u}{f_{uti}}\right)$$

where *V* ti = tissue volume of the *i*th organ and *f*uti = unbound fraction of the *i*th organ.
The following are important considerations in the calculation of *V*app.

1. The volume of distribution is a constant only when the drug concentrations are in equilibrium between the plasma and tissue.

2. Values of *f*u and *f*ut are concentration dependent and must also be determined at equilibrium conditions.

3. Equation 10.12 shows that *V*app is an **indirect measure of drug binding in the tissues** rather than a measurement of a true anatomic volume.

$$V_{\mathrm{app}} = V_{\mathrm{p}} + V_{\mathrm{t}}\left(\frac{f_{\mathrm{u}}}{f_{\mathrm{ut}}}\right) \qquad (10.12)$$

4. When *f*u and *f*ut are unity, Equation 10.12 is simplified to

$$\frac{D_{\mathrm{B}}}{C_{\mathrm{p}}} = V_{\mathrm{p}} + V_{\mathrm{t}}$$

When no drug binding occurs in tissue and plasma, the volume of distribution will not exceed the real anatomic volume. **Only at steady state are the unbound plasma drug concentration, *C*u, and the tissue drug concentration, *C*ut**, **equal**. At any other time, *C*u may not equal to *C*ut. **The amount of drug in the body, *D*B, cannot be calculated easily from *V*app and *C*p under nonequilibrium conditions**. **For simplicity, some models assume that the drug distributed to a tissue is approximated by the drug present in the fluid of that tissue.**

RELATIONSHIP OF PLASMA DRUG-PROTEIN BINDING TO DISTRIBUTION AND ELIMINATION

In general, drugs **that are highly bound to plasma protein have reduced overall drug clearance.** For a **drug that is metabolized mainly by the liver, binding to plasma proteins prevents the drug from entering the hepatocytes, resulting in reduced drug metabolism by the liver.** In addition, molecularly bound drugs may not be available as substrates for liver enzymes, thereby further reducing the rate of metabolism.

Protein-bound drugs act as larger molecules that cannot diffuse easily through the capillary membranes in the glomeruli. **The elimination half-lives of some drugs, such as the cephalosporins, which are excreted mainly by renal excretion, are generally increased when the percent of drug bound to plasma proteins increases**. The effect of serum protein binding on the renal clearance and elimination half-life on several tetracycline analoges.

(**For example, doxycycline, which is 93% bound to serum proteins, has an elimination half-life of 15.1 hours, whereas oxytetracycline, which is 35.4% bound to serum proteins, has an elimination half-life of 9.2 hours**.)

On the other hand, drug that is both extensively bound and actively secreted by the kidneys, such as penicillin, has a short elimination half-life, because active secretion takes preference in removing or stripping the drug from the proteins as the blood flows through the kidney.

Relationship between *V*D and Drug Elimination Half-Life

Drug elimination is governed mainly by renal and other metabolic processes in the body. However, extensive drug distribution has the effect of diluting the drug in a large volume, making it harder for the kidney to filter the drug by glomerular filtration. Thus, the *t* 1/2 of the drug is prolonged if clearance (*Cl*) is constant and *V*D is increased according to Equation 10.14. *Cl* is related to apparent volume of distribution, *V*D, and the elimination constant *k*, as shown in Equation 10.13.

$$Cl = kV_D \qquad (10.13)$$

$$t_{1/2} = 0.693\frac{V_D}{Cl} \qquad (10.14)$$

Cl= K Vd

KINETICS OF PROTEIN BINDING

The kinetics of **reversible drug-protein binding for a protein with one simple binding site can** be described by the *law of mass action*, as follows:

Protein + drug ⇔ drug–protein–complex

$$[P] + [D] \Leftrightarrow [PD] \qquad (10.15)$$

From Equation 10.15 and the law of mass action, an **association constant, *K*a, can be expressed as the ratio of the molar concentration of the products and the molar concentration of the reactants**.

This equation assumes only one-binding site per protein molecule.

$$K_a = \frac{[PD]}{[P][D]} \qquad (10.16)$$

The extent of the drug-protein complex formed is dependent on the association binding constant *K*a. The magnitude of *K*a yields information on the degree of drug protein binding. Drugs strongly bound to protein have a very large *K*a and exist mostly as the drug-protein complex. With such drugs, a large dose may be needed to obtain a reasonable therapeutic concentration of free drug.

Most kinetic studies *in vitro* use purified albumin as a standard protein source, because this protein is responsible for the major portion of plasma drug-protein binding. Experimentally, both the free drug [*D*] and the protein-bound drug [*PD*], as well as the total protein concentration [*P*] + [*PD*], may be determined.

To study the binding behavior of drugs, a determinable **ratio** ***r*** is defined, as follows:

$$r = \frac{\text{moles of drug bound}}{\text{total moles of protein}}$$

Because moles of drug bound is [*PD*] and the total moles of protein is [*P*] + [*PD*], this equation becomes

$$r = \frac{[PD]}{[PD] + [P]} \qquad (10.17)$$

$$K_a = \frac{[PD]}{[P][D]} \qquad (10.16)$$

According to Equation 10.16, [*PD*] = *K* a[*P*][*D*]; by substitution into Equation 10.17, the following expression is obtained:

$$r = \frac{K_a[P][D]}{K_a[P][D] + [P]}$$

$$r = \frac{K_a[D]}{1 + K_a[D]} \qquad (10.18)$$

This equation describes the simplest situation, in which **1 mole of drug binds to 1 mole of protein in a 1:1 complex**. This case assumes only one independent binding site for each molecule of drug. **If there are *n* identical independent binding sites per protein molecule, then the following is used:**

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad (10.19)$$

In terms of K_d, which is $1/K_a$, Equation 10.19 reduces to

$$r = \frac{n[D]}{K_d + [D]} \qquad (10.20)$$

Protein molecules are quite large compared to drug molecules and may contain more than one type of binding site for the drug. If there is more than one type of binding site and the drug binds independently on each binding site with its own association constant, then Equation 10.20 expands to

$$r = \frac{n_1 K_1[P]}{1 + K_1[D]} + \frac{n_2 K_2[P]}{1 + K_2[D]} + \cdots \qquad (10.21)$$

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad (10.19)$$

where the numerical subscripts represent different types of binding sites, the *K*'s represent the binding constants, and the *n*'s represent the number of binding sites per molecule of albumin.

These equations assume that each drug molecule binds to the protein at an independent binding site, and the affinity of a drug for one binding site does not influence binding to other sites.

In reality, drug-protein binding sometimes exhibits a phenomenon of *cooperativity*.

For these drugs, the binding of the first drug molecule at one site on the protein molecule influences the successive binding of other drug molecules

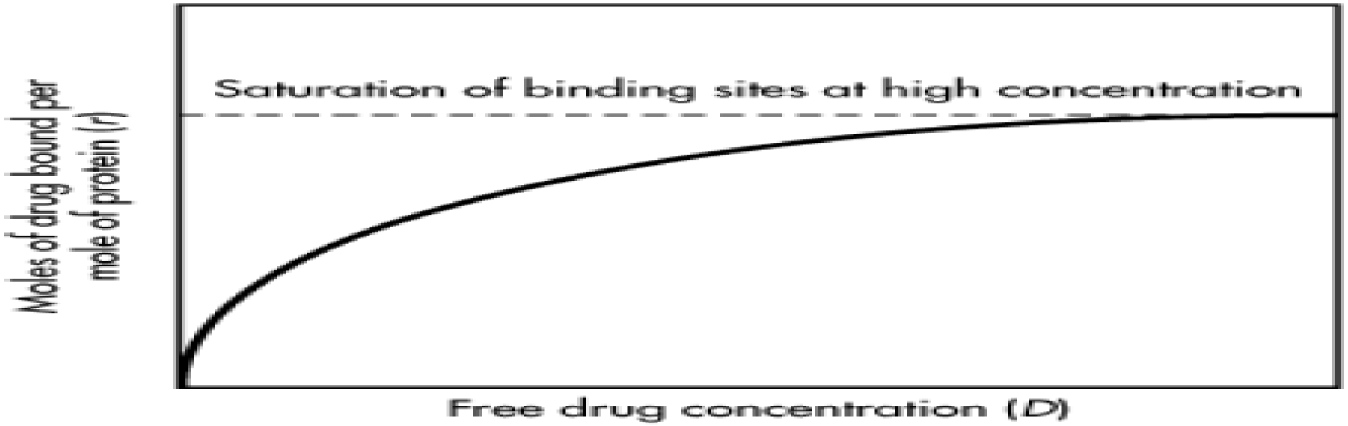
DETERMINATION OF BINDING CONSTANTS AND BINDING SITES BY GRAPHIC METHODS

In-Vitro Methods (Known Protein Concentration)

A plot of the ratio of *r* (moles of drug bound per mole of protein) versus free drug concentration [*D*] is shown below. Equation 10.20 shows that as free drug concentration increases, the number of moles of drug bound per mole of protein becomes saturated and plateaus.

Because of nonlinearity in drug-protein binding, Equation 10.20 is rearranged for the **estimation** of ***n* and *K*a**, using various graphic methods as discussed in the next section.

Figure 10-13.



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Pharmacokinetics, 5th Edition: http://www.accesspharmacy.con

In terms of K_d, which is $1/K_a$, Equation 10.19 reduces to

$$r = \frac{n[D]}{K_d + [D]} \qquad (10.20)$$

The values for the association constants and the number of binding sites are obtained by various graphic methods. The **reciprocal of Equation 10.20 gives the following equation**:

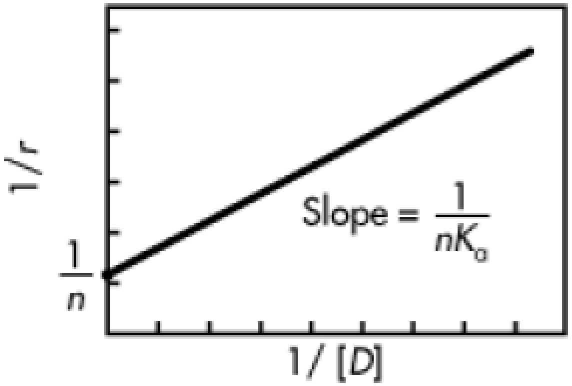
$$\frac{1}{r} = \frac{1 + K_a[D]}{nK_a[D]} \qquad (10.22)$$

$$\frac{1}{r} = \frac{1}{nK_a[D]} + \frac{1}{n}$$

A graph of 1/*r* versus 1/[*D*] is called a *double reciprocal plot*. The *y* intercept is 1/*n* and the slope is 1/*nK*a.

From this graph, the number of binding sites may be determined from the *y* intercept, and the association constant may be determined from the slope, if the value for *n* is known.

Figure 10-14.



If the graph of 1/*r* versus 1/[*D*] does not yield a straight line, then the drug-protein binding process is probably more complex. Equation 10.20 assumes **one type of binding site and no interaction among the binding sites.**

Another graphic technique called the *Scatchard plot*, is a rearrangement of Equation 10.20. **The Scatchard plot spreads the data to give a better line for the estimation of the binding constants and binding sites**. From Equation 10.20, we obtain

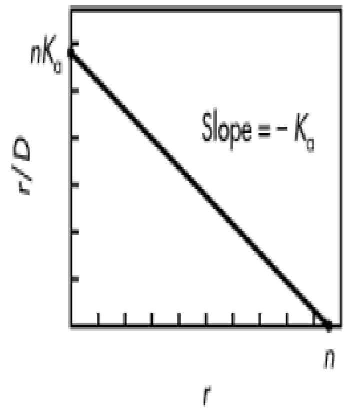
$$r = \frac{nK_a[D]}{1+K_a[D]}$$

$$r + rK_a[D] = nK_a[D] \qquad (10.23)$$

$$r = nK_a[D] - rK_a[D]$$

$$\frac{r}{D} = nK_a - rK_a$$

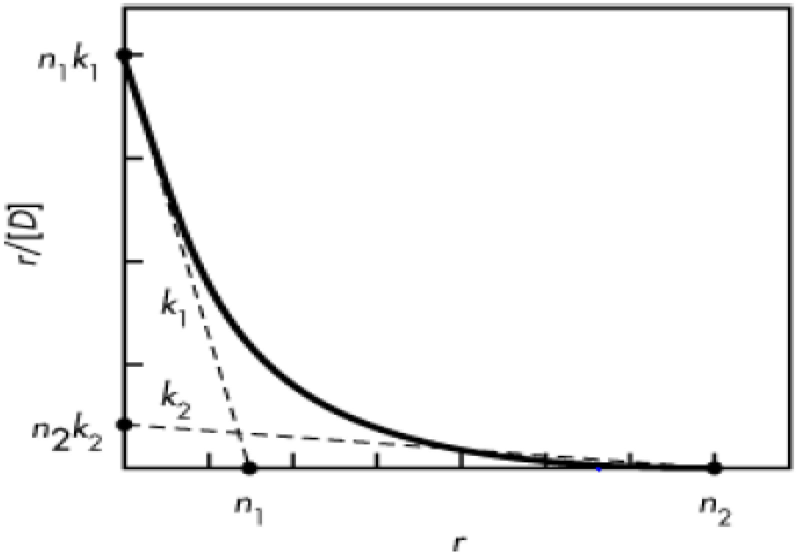
Figure 10-15.



A graph constructed by plotting *r*/[*D*] versus *r* yields a straight line with the intercepts and slope shown in and

Some drug-protein binding data produce Scatchard graphs of curvilinear lines. The **curvilinear line represents the summation of two straight lines that collectively form the curve. The binding of salicylic acid to albumin is an example of this type of drug-protein binding in which there are at least two different, independent binding sites (n_1 and n_2), each with its own independent association constant (k_1 and k_2).**
Equation 10.21 best describes this type of drug-protein interaction.

Figure 10-17.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

Hypothetical binding of drug to protein. The *k*'s represent independent binding constants and the *n*'s represent the number of binding sites per molecule of protein.

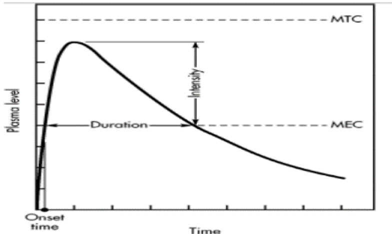
When a highly protein-bound drug is displaced from binding by a second drug or agent, a sharp increase in the free drug concentration in the plasma may occur, leading to toxicity. **For example, an increase in free warfarin level was responsible for an increase in bleeding when warfarin was co-administered with phenylbutazone, which competes for the same protein-binding site.** Recently, studies and reviews have shown that the clinical significance of warfarin protein binding and its impact on bleeding are less prominent, adding other factors and explanations.

Albumin has two known binding sites that share the binding of many drugs. **Binding site I** is shared by phenylbutazone, sulfonamides, phenytoin, and valproic acid. **Binding site II is** shared by the semisynthetic penicillins, probenecid, medium-chain fatty acids, and the benzodiazepines. **Some drugs bind to both sites.**
Displacement occurs when a second drug is taken that competes for the same binding site in the protein as the initial drug.

Drug Distribution and Pharmacodynamics

A decrease in protein binding that results in increased free drug concentration will allow more drug to cross cell membranes and distribute into all tissues, as discussed above. More drug will therefore be available to interact at a receptor site to produce a more intense pharmacologic effect. **Clinically, the pharmacodynamic response is influenced by both the distribution of the drug and the concentration of the unbound drug fraction**. The drug dose and the dosage form must be chosen to provide sufficiently high unbound drug concentrations so that an adequate amount of drug reaches the site of drug action (receptor). The onset of drug action depends on the rate of the free (unbound) drug that reaches the receptor and **produces a *minimum effective concentration* (MEC) to produce a pharmacodynamic response**. The onset time is often dependent on the rate of drug uptake and distribution to the receptor site.

The intensity of a drug action depends on the total drug concentration of the receptor site and the number of receptors occupied by drug. To achieve a pharmacodynamic response with the initial (priming) dose, the amount (mass) of drug when dissolved in the volume of distribution must give a drug concentration MEC at the receptor site. Subsequent drug doses maintain the pharmacodynamic effect by sustaining the drug concentration at the receptor site. Subsequent doses are given at a dose rate (eg, 250 mg every 6 hours) that replaces drug loss from the receptor site, usually by elimination. **However, redistributional factors may also contribute to the loss of drug from the receptor site.**



NONLINEAR PHARMACOKINETICS: INTRODUCTION

In previous chapters, linear pharmacokinetic models using simple first-order kinetics were introduced to describe the course of drug disposition and action. **These linear models assumed that the pharmacokinetic parameters for a drug would not change when different doses or multiple doses of a drug were given**.

With some drugs, increased doses or chronic medication can cause deviations from the linear pharmacokinetic profile previously observed with single low doses of the same drug.

(This *nonlinear* pharmacokinetic behavior is also termed *dose-dependent pharmacokinetics.)*

Many of the processes of drug absorption, distribution, biotransformation, and excretion involve enzymes or carrier-mediated systems. For some drugs given at therapeutic levels, one of these specialized processes may become saturated. As shown in , various causes of nonlinear pharmacokinetic behavior are theoretically possible. 1-**Besides saturation of plasma protein-binding or carrier-mediated systems, drugs may demonstrate nonlinear pharmacokinetics due to a 2- pathologic alteration in drug absorption, distribution, and elimination.**

For example, **aminoglycosides may cause renal nephrotoxicity, thereby altering renal drug excretion.** In addition, **gallstone obstruction of the bile duct will alter biliary drug excretion**. In most cases, the **main** pharmacokinetic outcome is a change in the apparent elimination rate constant.

A number of drugs demonstrate ***saturation*** **or** ***capacity-limited metabolism*** **in humans**. Drugs that demonstrate saturation kinetics usually show the following characteristics.

1. Elimination of drug does not follow simple first-order kinetics-that is, elimination kinetics are nonlinear.

2. The elimination half-life changes as dose is increased. Usually, the elimination half-life **increases** with increased dose due to saturation of an enzyme system. **However, the elimination half-life might decrease due to "self"-induction of liver biotransformation enzymes, as is observed for carbamazepine.**

3. The area under the curve (AUC) is not proportional to the amount of bioavailable drug.

4. The saturation of capacity-limited processes may be affected by other drugs that require the same enzyme or carrier-mediated system (ie, competition effects).

5. The composition and/or ratio of the metabolites of a drug may be affected by a change in the dose

Because these drugs have a changing apparent elimination constant with larger doses, prediction of drug concentration in the blood based on a single small dose is difficult. Drug concentrations in the blood can increase rapidly once an elimination process is saturated. In general, **metabolism (biotransformation) and active tubular secretion of drugs by the kidney are the processes most usually saturated**. shows plasma level-time curves for a drug that exhibits *saturable* kinetics.

When a large dose is given, a curve is obtained with an initial slow elimination phase followed by a much more rapid elimination at lower blood concentrations (curve *A*).

With a small dose of the drug, apparent first-order kinetics are observed, because no saturation kinetics occur (curve *B*).

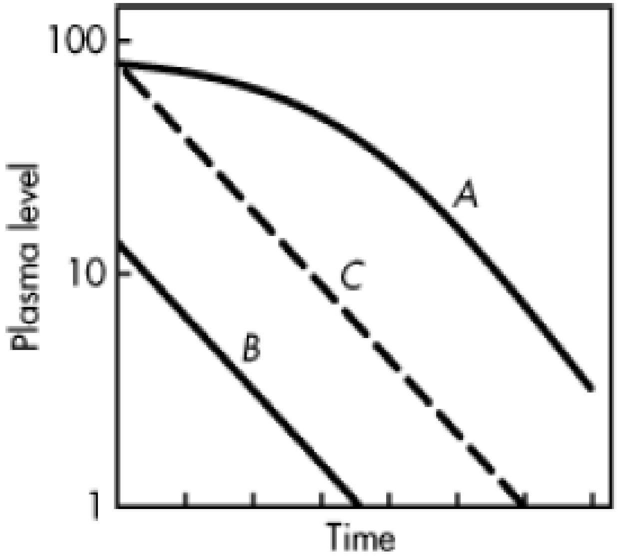
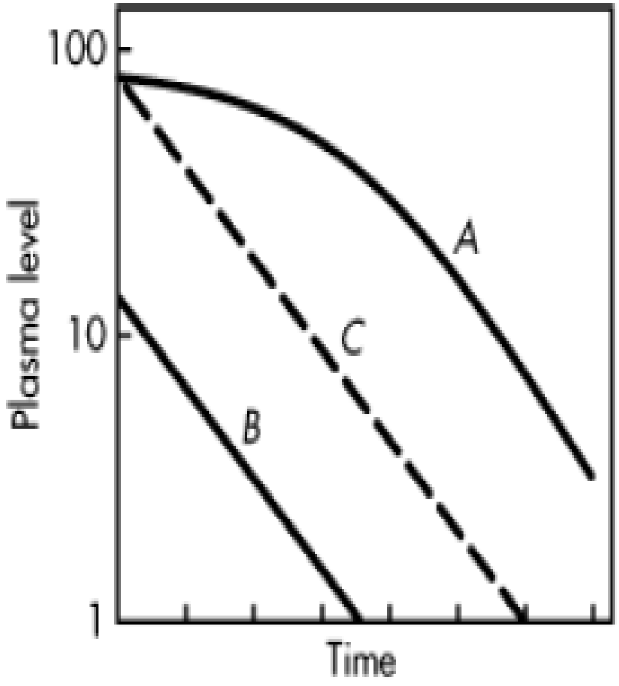


Figure 9-1.



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Plasma levelâ€"time curves for a drug that exhibits a saturable elimination process. Curves *A* and *B* represent high and low doses of durg, respectively, given in a single IV bolus. The terminal slopes of curves *A* and *B* are the same. Curve *C* represents the normal first-order elimination of a different drug.

In order to determine whether a drug is following dose-dependent kinetics, the drug is given at various dosage levels and a plasma level-time curve is obtained for each dose. The curves should exhibit parallel slopes if the drug follows dose-independent kinetics. Alternatively, a plot of the areas under the plasma level-time curves at various doses should be linear.

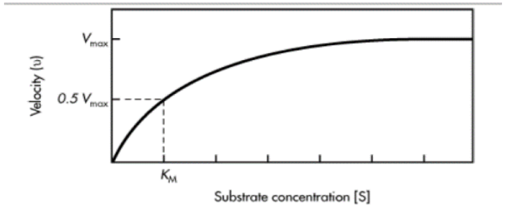
SATURABLE ENZYMATIC ELIMINATION PROCESSES

The elimination of drug by a saturable enzymatic process is described by ***Michaelis-Menten kinetics***. If *C*p is the concentration of drug in the plasma, then

$$\text{Elimination rate} = \frac{dC_p}{dt} = \frac{V_{max} C_p}{K_M + C_p} \tag{9.1}$$

where *V*max is the maximum elimination rate and *K*M is the Michaelis constant that reflects the *capacity* of the enzyme system. It is important to note that *K*M is not an elimination constant, but is actually a hybrid rate constant in enzyme kinetics, representing both the forward and backward reaction rates and equal to the drug concentration or amount of drug in the body at 0.5*V* max.

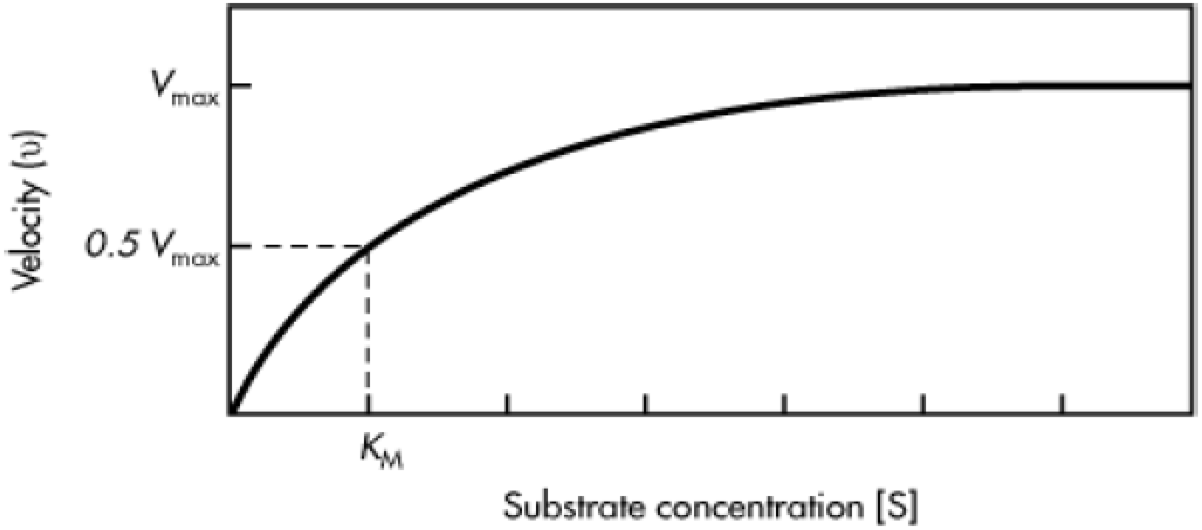
The values for *K* M and *V* max are dependent on the nature of the drug and the enzymatic process involved



The elimination rate of a hypothetical drug with a *K*M of 0.1 g/mL and a *V* max of 0.5 g/mL per hour is calculated in by means of Equation 9.1. Because the ratio of the elimination rate to drug concentration changes as the drug concentration changes (ie, *dC* p/*dt* is not constant, Eq. 9.1), the rate of drug elimination also changes and is not a first-order or linear process.

In contrast, a first-order elimination process would yield the same elimination rate constant at all plasma drug concentrations. At drug concentrations of **0.4-10 g/mL**, the enzyme **system is not saturated and the rate of elimination is a mixed or nonlinear process .**

At **higher drug concentrations, 11.2 g/mL and above**, **the elimination rate approaches the maximum velocity (*V* max) of approximately 0.5 g/mL per hour. At *V* max, the elimination rate is a constant and is considered a zero-order process.**



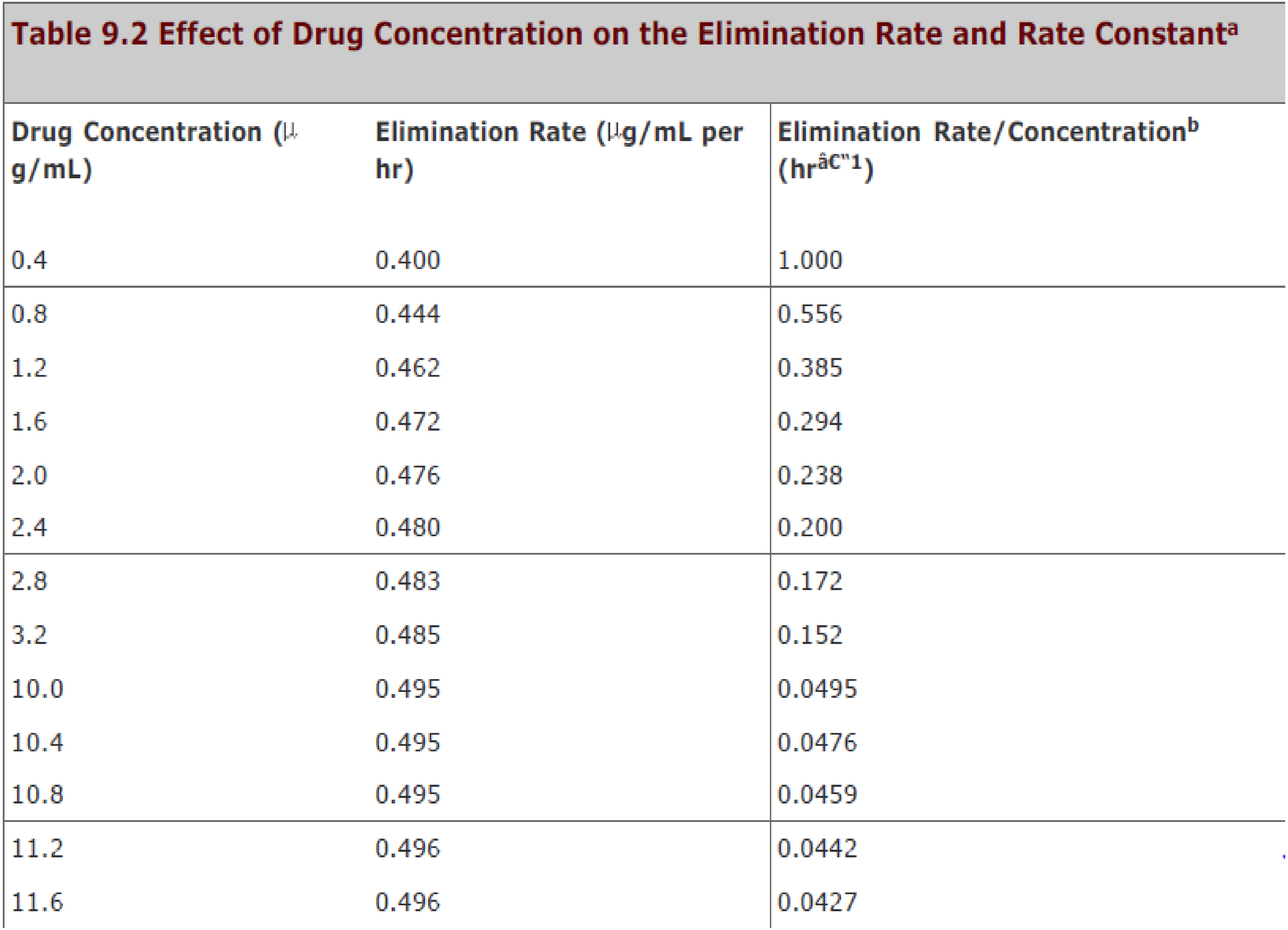


Table 9.2 Effect of Drug Concentration on the Elimination Rate and Rate Constant[a]

Drug Concentration (µg/mL)	Elimination Rate (µg/mL per hr)	Elimination Rate/Concentration[b] (hr$^{\text{â€"}1}$)
0.4	0.400	1.000
0.8	0.444	0.556
1.2	0.462	0.385
1.6	0.472	0.294
2.0	0.476	0.238
2.4	0.480	0.200
2.8	0.483	0.172
3.2	0.485	0.152
10.0	0.495	0.0495
10.4	0.495	0.0476
10.8	0.495	0.0459
11.2	0.496	0.0442
11.6	0.496	0.0427

Equation 9.1 describes a nonlinear enzyme process that encompasses a broad range of drug concentrations. When the drug concentration **Cp is large in relation to *K*M (*C* p >> *K* m), saturation of the enzymes occurs and the value for *K*M is negligible. The rate of elimination proceeds at a fixed or constant rate equal to *V* max.**

Thus, elimination of drug becomes **a zero-order** process and Equation 9.1 becomes:

$$\text{Elimination rate} = \frac{dC_p}{dt} = \frac{V_{max} C_p}{K_M + C_p} \qquad (9.1)$$

$$-\frac{dC_p}{dt} = \frac{V_{max} C_p}{C_p} = V_{max} \qquad (9.2)$$

[redacted]

of elimination rate to drug concentration has a constant value of 1.1 hr^{-1} . Mathematically, when *C* p is much smaller than *K* M, *C* p in the denominator is negligible and the elimination rate becomes first order.

$$-\frac{dC_p}{dt} = \frac{V_{\max} C_p}{C_p + K_M} = \frac{V_{\max}}{K_M} C_p \qquad (9.3)$$

$$-\frac{dC_p}{dt} = k' C_p$$

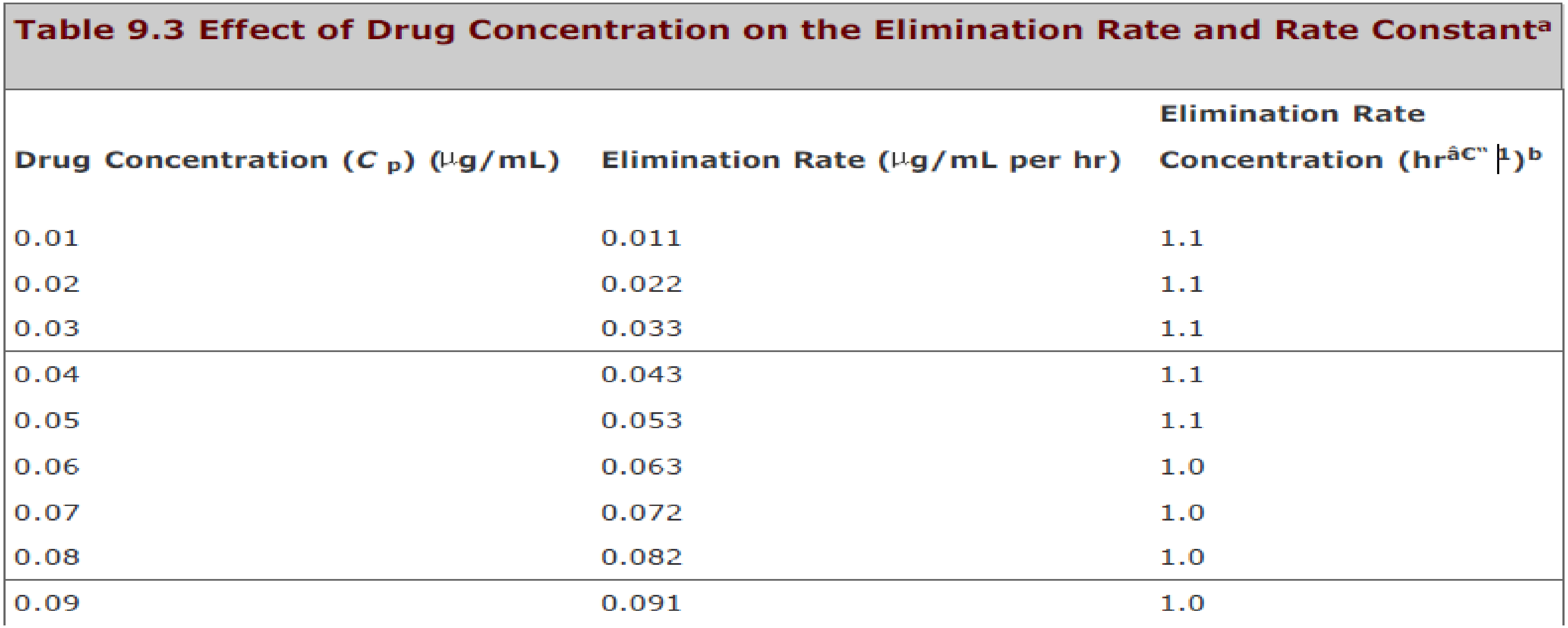


Table 9.3 Effect of Drug Concentration on the Elimination Rate and Rate Constant[a]

Drug Concentration (C_p) (µg/mL)	Elimination Rate (µg/mL per hr)	Elimination Rate Concentration (hr$^{\text{âC" }1}$)[b]
0.01	0.011	1.1
0.02	0.022	1.1
0.03	0.033	1.1
0.04	0.043	1.1
0.05	0.053	1.1
0.06	0.063	1.0
0.07	0.072	1.0
0.08	0.082	1.0
0.09	0.091	1.0

A *K*M = 0.8 g/mL, *V* max = 0.9 g/mL per hour.

$$k' = \frac{V_{\max}}{K_M} = \frac{0.9}{0.8} = {\sim}1.1\ \text{hr}^{-1}$$

This calculation confirms the data in , because enzymatic drug elimination at drug concentrations below 0.05 µg/mL is a first-order rate process with a rate constant of 1.1 hr^{-1}. Therefore, the *t* 1/2 due to enzymatic elimination can be calculated:

$$t_{1/2} = \frac{0.693}{1.1} = 0.63\ \text{hr}$$