Part 5
Specific Topics

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Chapter 38

Diagnosing and Improving Pharmacokinetic Performance

Overview

- *When pharmacokinetic (PK) parameters are poor, in vitro property data can be used to diagnose the limitations.*
- *High clearance results from metabolism (phases I and II), biliary excretion, transporters, renal extraction, and plasma hydrolysis.*
- *Low bioavailability results from first-pass metabolism, low solubility, low permeability, high efflux, and intestinal decomposition.*
- *The identified property limitation guides structure modifications, and the new analog can be checked in vitro for improvement.*

In vivo animal studies are performed during discovery for compounds having a wide range of pharmacokinetic (PK) properties, including those having PK parameters that differ greatly from the majority of commercial drugs (i.e., not drug-like). To make up for these limitations, doses can be increased or administered more frequently, less preferable dosing routes can be used (e.g., IV), or an unusual dosage form can be administered. This may be necessary for early pharmacology proof-of-concept studies. However, there are inevitable tradeoffs if such compounds move into development. Non–drug-like compounds may require IV dosing instead of the preferred PO route, complicated or expensive formulations for insoluble compounds, sustained release of rapidly cleared compounds, or prodrugs for insoluble or impermeable compounds. Often a discovery compound is found to not achieve sufficient PK performance in vivo to produce efficacy or meet PK advancement criteria.

In this common scenario, it is helpful to use PK parameters as a guide to diagnosing the underlying physicochemical, biochemical, and structural property limitations. This provides insights for informed decisions on specific structural modifications that can be made to improve the limiting property. In vitro assays are very helpful in determining if the modified structure has been improved for the limiting property. The improved compounds then can be tested in vivo to determine if the PK parameters have been improved.[1,2] A scheme for this strategy is shown in Figure 38.1.

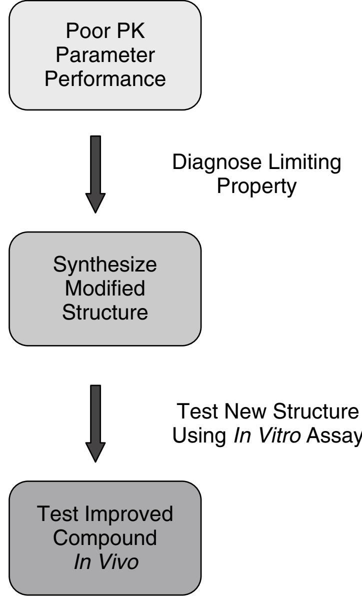

Figure 38.1 ► Scheme for diagnosis and improvement of a property that limits PK performance.

38.1 Diagnosing Underlying Property Limitations from PK Performance

The following sections list common PK issues that discovery project teams try to improve. The major possible causes of each of these limitations are provided as bullet points, and the in vitro assay/study that can be used to check for this limitation as a cause is indicated in brackets. The limiting causes are listed in terms of suggested priority of investigation in tracking down the cause. Strategies for modifying the structure to improve the property are provided in chapters on the individual properties.

38.1.1 High Clearance After IV Injection

- Liver metabolism (hepatic extraction)
  - Phase I metabolic stability [microsomal + NADPH stability; use PK species]
  - Phase II metabolic stability [microsomal + uridine diphosphate glucuronic acid stability; hepatocyte stability; use PK species]
- Liver biliary excretion (hepatic extraction)
  - Permeation into bile [drug and metabolite concentration in bile from bile duct cannulated PK animal]
  - Active transport into bile [P-glycoprotein ((Pgp) and other transporters in PK species]
- Renal extraction [drug and metabolite concentration in urine of PK animal; transporters involved in active secretion]
- Enzymatic hydrolysis in blood [plasma stability]

38.1.2 Low Oral Bioavailability

- High first-pass metabolism by liver and intestine; biliary extraction [phase I and II metabolic stability]
- Low intestinal solubility [solubility in simulated gastric fluid (SGF), simulated intestinal fluid (SIF), simulated intestinal bile salts–lecithin mixture (SIBLM), and pH 1–8 buffers;]
- Low intestinal permeability
  - Low passive diffusion [parallel artificial membrane permeability assay (PAMPA); Caco-2 A>B and B>A; MDCK;]
  - High Pgp efflux [MDR1-MDCKII Pgp assay; Caco-2 efflux ratio]
- Enzymatic or pH hydrolysis in intestine [stability in SGF, SIF, pH 1–8]

38.2 Case Studies on Interpreting Unusual PK Performance

Both transporter-mediated absorption and capacity-limited metabolism can lead to nonlinear PK profiles as illustrated by the following two case studies.

38.2.1 PK of CCR5 Antagonist UK-427,857

The compound shown in Figure 38.2 was found to have much higher C_{max} and area under the curve (AUC) (Table 38.1) when dosed in humans at 4.3 mg/kg than when dosed at 0.43 mg/kg after dose normalization.[3] Such behavior is indicative of saturation of an efflux transporter. With this diagnosis, Pgp efflux was studied using in vitro Caco-2 assay. P_{app} (A>B) was measured as $<1 \times 10^{-6}$ cm/s and P_{app} (B>A) was 12×10^{-6} cm/s, which was an efflux ratio ($P_{app,\ B>A}/P_{app,\ A>B}$) >10, indicating efflux. In follow-up studies, the Pgp inhibitor verapamil was found to reduce the efflux ratio, suggesting the compound was a Pgp substrate. The Pgp binding affinity was $K_m = 37\,\mu$M, and $V_{max} = 55$ nmol/mg/min. These findings were confirmed by in vivo studies using Pgp double knockout mice and wild type. Both C_{max} and AUC increased significantly in knockout mice compared to wild type (Table 38.2). This suggested the higher C_{max} and AUC at higher doses was caused by Pgp efflux in the intestine, which limited absorption at the lower dose, but was saturated at the higher dose, thus allowing higher absorption at the higher dose.

Log $D_{7.4}$ = 2.1
pKa = 7.3
MW = 514
Good solubility

H-bond Donor = 1
H-Bond Acceptor = 6
cLogP = 3.11
Low permeability

Figure 38.2 ► Structure and physicochemical properties of CCR5 antagonist UK-427,857.

TABLE 38.1 ▶ PK Parameters of CCR5 Antagonist UK-427, 857

Parameter	Human		Comments
Oral Dose (mg/kg)	0.43 (30 mg)	4.3 (300 mg)	
Elimination half-life (h)	8.9	10.6	
C_{max} (ng/mL), dose normalized	36	144	increased
AUC (ng.h/mL), dose normalized	272	537	increased
T_{max} (h)	2.9	1.6	decreased

TABLE 38.2 ▶ PK Parameters of CCR5 Antagonist UK-427, 857 in Pgp Knockout Mice

PO 16 mg/kg	C_{max} ng/mL	AUC ng . h/mL	Elimin. $T_{1/2}$ (h)
wild-type fvb mice	536	440	0.7
mdr1a/1b knockout	1119	1247	1
% Increase	108%	183%	

38.2.2 PK of Triazole Antifungal Voriconazole

Voriconazole (Figure 38.3) has good solubility and excellent oral absorption, with <7% eliminated unchanged through feces. It is mostly eliminated by hepatic clearance. Oral bioavailability of the compound was greater than 70% in human. Voriconazole produced an unusual nonlinear PK profile (Figure 38.4) following PO or IV administration in rat, termed the *hockey-stick profile*.[4] The PK characteristics are gender dependent. The analog compound shown in Figure 38.5 does not have the nonlinear PK characteristics because of low Log D (0.5), which results in elimination mostly by the kidney. Table 38.3 lists the gender-dependent PK parameters of voriconazole. PO AUC (normalized) at 30 mg/kg was higher than IV AUC at 10 mpk, resulting in >100% oral bioavailability (%F = 159%) in male rat. This suggested capacity-limited elimination due to saturation of metabolizing enzymes, which is facilitated by the good absorption resulting in high exposure in systemic circulation. For both IV and PO administration, AUC for multiple dosing was lower than for single dosing. This is because voriconazole induces metabolizing cytochrome P450 (CYP450)

Aq. Solubility = 0.7 mg/mL, Log $D_{7.4}$ = 1.8
Excellent absorption, <7% in feces unchanged
Oral Bioavailability >70%

Figure 38.3 ▶ Structure and physicochemical properties of triazole antifungal voriconazole.

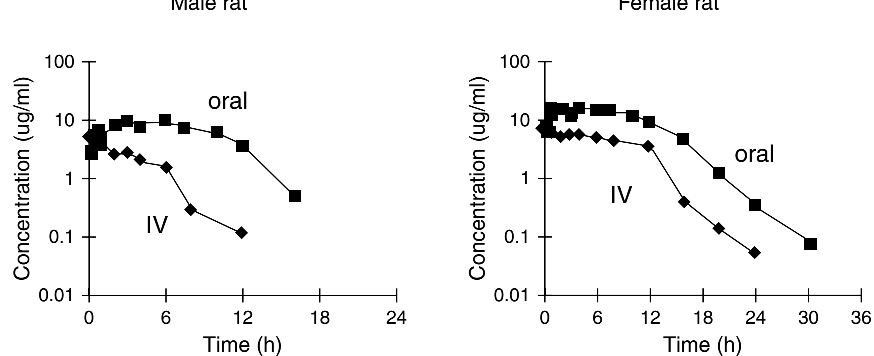

Figure 38.4 ► Nonlinear PK of voriconazole in rat. (Reprinted with permission from [14].)

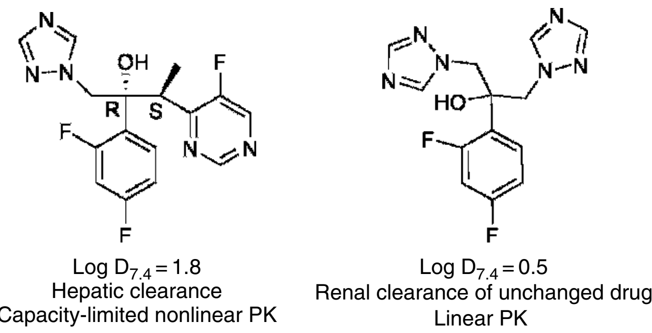

Figure 38.5 ► Effects of Log D on clearance and PK.

TABLE 38.3 ► Voriconazole: PK Data Interpretation in Rat

Sex	Male	Female	Comments
Plasma protein binding (%)	66	66	
IV			
Dose (mg/kg)	10	10	
Single dose AUC_t (ug .h/mL)	18.6	81.6	Gender dependent
Multiple dose AUC_t (ug .h/mL)	6.7	13.9	< S.D. CYP450 auto-induction
Oral			
Dose (mg/kg)	30	30	
Single dose C_{max} (ug/mL)	9.5	16.7	Gender dependent
Single dose T_{max} (h)	6	1	Gender dependent
Single dose AUC_t (ug .h/mL)	90	215.6	> IV, capacity-limited elimination
Multiple dose AUC_t (ug .h/mL)	32.3	57.4	< S.D. CYP450 auto-induction
Apparent bioavailability F (%)	159	88	Capacity-limited elimin. Good absorption

S.D., Single dose.

enzymes as indicated by the increase in liver weight and CYP450 enzymes with escalated doses (Table 38.4). As animals were exposed to voriconazole, more CYP450 enzymes were produced to metabolize the compound, resulting in quicker elimination. Hence, multiple dosing generated lower AUC than did single dosing.

TABLE 38.4 ► Voriconazole: Auto-Induction of CYP450s.

	Hepatic Microsomal Cytochrome P450 (nmol P450/mg protein)		Relative Liver weight		Voriconazole C_{max} (ug/mL)	
Dose (mg/kg)	Male	Female	Male	Female	Male	Female
Control	0.88	0.51	3.71	3.7	None	None
3	0.85	0.65	3.86	4.04	0.61	1.32
10	1.21	0.68	4.17	4.26	3.64	6.14
30	1.77	0.79	4.38	5.04	9.69	14.6
80	2.08	0.92	5.57	6.26	28.4	30.4

Problems

(Answers can be found in Appendix I at the end of the book.)

1. What dosing approaches can be tried to administer compounds that have poor absorption, short PK half-life, or low bioavailability after oral dosing?
2. What approach is preferable for enhancing absorption, PK half-life or bioavailability?
3. What physicochemical or biochemical properties, if they are low, will lead to poor bioavailability?
4. Which of the following properties can be a significant contributor to an observed high clearance in a PK study using an IV dose?: (a) low metabolic stability (liver), (b) low CYP inhibition, (c) high biliary excretion, (d) high plasma protein binding, (e) low renal extraction, (f) low plasma stability, (g) high RBC binding, (h) neutral pK_a, (i) hERG binding, (j) low stability at pH 4, (k) high phase I metabolism in intestinal epithelium, (l) high blood–brain barrier (BBB) brain to plasma ratio (B/P), (m) high renal extraction, (n) high metabolic stability (liver), (o) Pgp efflux.
5. Which of the following properties can be a significant contributor to an observed low oral bioavailability in a PK study using a PO dose?: (a) low metabolic stability (liver), (b) low CYP inhibition, (c) high biliary excretion, (d) high plasma protein binding, (e) low renal extraction, (f) low plasma stability, (g) high RBC binding, (h) neutral pK_a, (i) hERG binding, (j) low stability at pH 4, (k) high phase I metabolism in intestinal epithelium, (l) high BBB B/P, (m) high renal extraction, (n) high metabolic stability (liver), (o) low permeability, (p) low solubility, (q) Pgp efflux.
6. What effect may be observed if a compound is highly effluxed by Pgp in the intestine?: (a) high clearance, (b) oral dose-dependent C_{max}, (c) low V_d, (d) higher AUC at higher oral dose, (e) high AUC.

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Chapter 39

Prodrugs

Overview

- *Prodrugs have a structure that improves solubility, permeability, stability, or targeting to a tissue in order to improve pharmacokinetics.*
- *The pro-moiety is cleaved in vivo to release the active structure.*
- *Prodrugs can improve properties when no other structural modification is sufficient.*
- *The prodrug strategy is successful only a portion of the times it is used.*

Of all drugs worldwide, 5% are prodrugs. About half of the prodrugs are activated by hydrolysis, suggesting most of them are esters. Twenty-three percent of prodrugs are activated by biotransformation, meaning there is no pro-moiety. Prodrugs typically are developed to overcome pharmaceutical, pharmacokinetic (PK), and pharmacodynamic (PD) barriers. Many successful prodrugs are discovered by accident rather than by design.

There are many benefits to using the prodrug approach. It can be applied to improve solubility and passive permeability for absorption improvement. Prodrugs can be prepared to enhance transporter-mediated absorption and to improve metabolic stability. Certain prodrugs have been developed to reduce side effects. For example, most nonsteroidal antiinflammatory drugs (NSAIDs) are carboxylic acids and can cause GI irritation. The ester prodrugs of NSAIDs can overcome this side effect. Table 39.1 shows drug development barriers that have been overcome by various prodrug strategies.[1] Figure 39.1 shows examples of blockbuster prodrugs and their indications.[2] Prodrugs can be quite successful.

The prodrug approach has many challenges. Development programs for prodrugs are complex. Prodrugs tend to show interspecies and intraspecies variability due to differences in enzyme activity for prodrug activation. Enzymes that activate prodrugs might have genetic polymorphisms, which can cause variability from subject to subject. If two drugs are competing for the same enzyme, drug–drug interaction can result. Certain pro-moieties can have toxicity. Prodrug strategies are generally considered as the last resort to achieve pharmaceutical/PK properties that are incompatible with a given pharmacophore.

TABLE 39.1 ► Drug Development Barriers and Issues that Can be Overcome by Prodrug Strategies[1]

Barriers	Issues
Permeability	Not absorbed from GI tract because of polarity
	Low brain permeation
	Poor skin penetration
Solubility	Poor absorption and low oral bioavailability
	IV formulation cannot be developed
Metabolism	Vulnerable drug metabolized at absorption site
	Half-life is too short
	Sustained release is desired
Stability	Chemically unstable
	Better shelf life is needed
Transporter	Lack of specificity
	Selective delivery is desired
Safety	Intolerance/irritation
Pharmaceutics	Poor patient/doctor/nurse acceptance
	Bad taste or odor problems
	Painful injection
	Incompatibility (tablet desired but liquid is active)

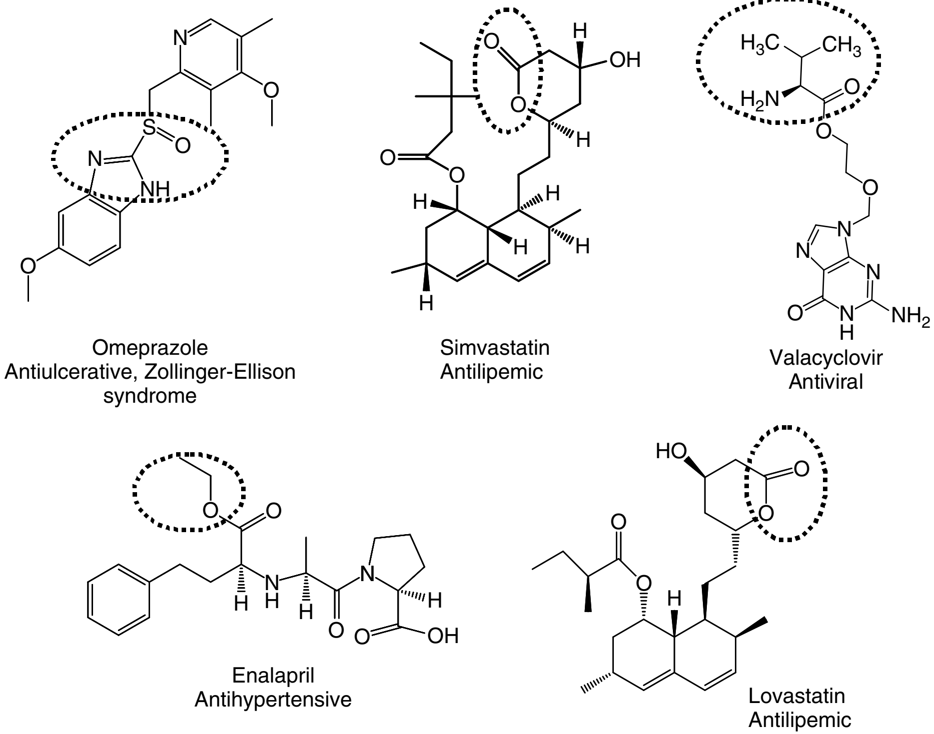

Figure 39.1 ► Blockbuster prodrugs and their indications.[2]

39.1 Using Prodrugs to Improve Solubility

Prodrug strategies can be applied to improve solubility. Table 39.2 shows commercially available prodrugs with improved solubility.[3] The structures of some prodrugs are illustrated in Figure 39.2. Prodrugs with a non-ionizable pro-moiety (e.g., glycol, polyethylene glycol [PEG], sugars) typically can improve solubility by two- to three-fold. Prodrugs with ionizable pro-moieties (e.g., phosphate) can increase solubility by orders of magnitude. There are three types of ionizable pro-moieties. Succinate-like derivatives were used early as prodrugs but

TABLE 39.2 ▶ Commercially Available Prodrugs with Improved Solubility[3]

Name	Solubility in water (mg/mL)
Clindamycin	0.2
Clindamycin-2-PO_4	150
Chloramphenicol	2.5
Succinate sodium	500
Metronidazole	10
N,N-dimethylglycinate	200
Phenytoin	0.02
Phosphate	142
Paclitaxel I	0.025
PEG-paclitaxel I	666
Celexicoxib	0.05
Parecoxib sodium	15

Figure 39.2 ▶ Structures of prodrugs with improved solubility.

are chemically unstable. Amino acid type, attached to hydroxyls (e.g., glucocorticoids) and phosphate type, attached to hydroxyls or amines (e.g., fosphenytoin) are common approaches used in the industry to increase solubility (Figure 39.3).

Amino-acid Type

Phosphate Type

Figure 39.3 ► Amino acid–type and phosphate-type prodrugs used to increase solubility.

The antitumor agent shown in Figure 39.4 is a weak base with p$K_a \leq 3.0$. The low solubility and weak basicity of the compound limited options for parenteral formulations. The novel sulfamate salt prodrug was prepared. Although the prodrug was more soluble, it was unstable and converted back to the parent under acidic conditions. Subsequently, amino acid–type prodrugs were synthesized with good solubility and stability. The IV formulation of the dihydrochloride salt was used in phase I clinical trials.

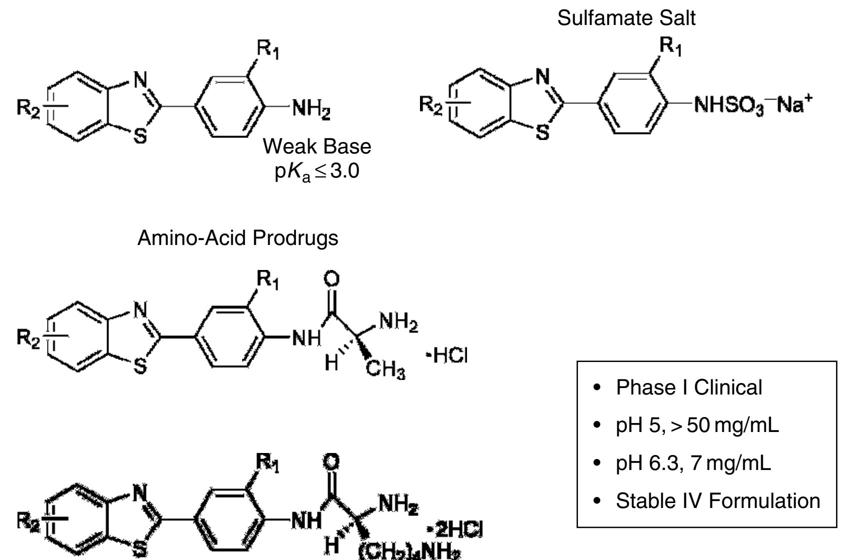

Figure 39.4 ► Amino acid–type and phosphate-type prodrugs used to increase solubility.[15]

The phosphate-type prodrug gained a lot of popularity when fosphenytoin (see Figure 7.25) was marketed.[4] The mechanism of this phosphate prodrug is illustrated in Figure 39.5. Its activation enhances absorption.[5] Phosphate prodrugs of amines are made

to increase solubility, due to the presence of the highly ionized species in the GI tract. The prodrug is hydrolyzed in the GI lumen by alkaline phosphatase, yielding hydroxymethyl amine intermediate and inorganic phosphate. The intermediate is highly unstable in physiological fluids and breaks down spontaneously to give the parent amine and one equivalent of formaldehyde. The parent amine can cross the GI membrane and be absorbed into systematic circulation. Figure 39.6 shows two examples of using phosphate prodrugs as potential approaches to enhance aqueous solubility of loxapine and cinnarizine. Both drugs (non-prodrugs) have low solubility with problematic formulation and erratic oral bioavailability.[5] One limitation of this approach is the formation of one equivalent of formaldehyde, which can have a toxic effect at a high dose or in chronic applications.

Figure 39.5 ► Mechanisms of phosphate prodrug used to increase aqueous solubility.[5]

Figure 39.6 ► Examples of using phosphate prodrug approach to increase solubility. Properties of the active (non-prodrug) are listed.[5]

39.2 Prodrugs to Increase Passive Permeability

Prodrug strategies are most commonly used to increase permeability of compounds by masking the polar functional groups and hydrogen bonds with ester or amide linkers and

increasing lipophilicity. Both permeability by passive diffusion and the transporter-mediated process (see Section 39.3) have been addressed with prodrug approaches.

Oral delivery of ester/amide prodrugs to the therapeutic target is confronted with many physiological, chemical, and biochemical barriers. In general, the highest oral bioavailability values that ester prodrugs can achieve clinically are 40% to 60%. This is due to incomplete membrane permeation, P-glycoprotein efflux, hydrolysis in the GI lumen and intestinal cells, nonesterase metabolism in the liver, biliary excretion, and metabolism of the parent.[6] Thus, a successful prodrug approach must consider the balance of all these issues. An ideal ester/amide prodrug should exhibit the following properties[6]:

- Weak or no activity against any pharmacological target
- Good chemical stability at physiological pHs
- Sufficient aqueous solubility
- High passive permeability
- Resistance to hydrolysis during absorption
- Hydrolyzed to parent rapidly and quantitatively after absorption
- The released pro-moiety has no toxicity or unwanted pharmacological effects

39.2.1 Ester Prodrugs for Carboxylic Acids

Simple alkyl esters are preferred for carboxylic acid prodrugs to increase passive diffusion permeability. Ethyl ester is the most common prodrug of this type. Other pro-moieties include aryl, double esters with diols, cyclic carbonates, and lactones. Examples of different types of prodrugs are shown in Figure 39.7.[2,6] Although simple esters are preferred, bioconversion of some simple alkyl or aryl esters is not mediated by esterases. This is nonideal for a prodrug approach because metabolism is nonproductive and leads to low systemic exposure.

Oseltamivir, ethyl ester   Benazepril, ethyl ester   Fosinopril, double ester

Figure 39.7 ► Examples of ester prodrugs of acids used to enhance passive permeability. Pro-moieties are circled.

Carbinicillin indanyl ester, aryl ester

Pivampicillin, double ester

Lenampicillin, cyclic carbonate

Lovastatin, lactone

Figure 39.7 ► ***Continued.***

Double esters are prepared in order to increase the recognition by esterases through the second ester. However, chemical stability of double esters is low, and the liberated aldehyde fragment can have toxicity. Cyclic carbonate prodrugs (e.g., lenampicillin) are designed to be labile in plasma to avoid nonproductive metabolism by cellular esterases. Prodrugs that hydrolyze in blood or plasma by blood-borne enzymes are beneficial to increase oral bioavailability and systemic circulation of the active principle. Double esters and cyclic carbonate prodrugs are designed for this purpose. Lactone prodrugs are developed for specific targeting. Bioconversion of lovastatin lactone (a hydroxymethylglutaryl coenzyme A [HMG-CoA] reductase inhibitor) to the active acid occurs in the liver, which is the site of action. Although the oral bioavailability of the compound is only 30% due to first-pass liver extraction, the high local concentration at the target organ (liver) results in good efficacy.[6]

39.2.2 Ester Prodrugs for Alcohols and Phenols

An increase in the lipophilicity of alcohols and phenols can often be achieved by preparing ester prodrugs using carboxylic acids. Examples are shown in Table 39.3.[1] The prodrugs showed increased corneal permeability, brain penetration, and oral absorption. Enhanced oral bioavailability of timolol prodrug was due to increased permeability and solubility as a result of decreased crystal lattice energy compared to the parent.

TABLE 39.3 ► Examples of Ester Prodrugs for Alcohols and Phenols

Prodrugs	Limitations of parent	Benefits of prodrug
Dipivaloyl-epinephrine	Log P = –0.04 Low corneal penetration	Log P = 2.08 Four- to six-fold increase in corneal penetration
Dibenzoyl-Amino-Dihydroxy-tetrahydronaphthalene (ADTN)	No CNS penetration	Reaches CNS
Butyryl-Timolol	Low oral exposure	High oral exposure Enable IV formulation

39.2.3 Prodrugs Derived from Nitrogen-Containing Functional Group

Because of the slow hydrolysis rate of amides in vivo, prodrugs using amide approaches are generally not recommended, except for activated amides, such as *N*-benzoyl- or *N*-pivaloyl derivatives. Imines and enamines, stabilized through hydrogen bonds and small peptide derivatives, can be effective prodrugs for amines. Carbamates can be used as prodrugs for amidines. For compounds containing acidic NH functional groups, sulfonamides, carboxamides, and carbamates are effective prodrugs. Figure 39.8 shows examples of this type of prodrug.

Figure 39.8 ► Examples of prodrugs for nitrogen-containing compounds.

39.3 Transporter-Mediated Prodrugs to Enhance Intestinal Absorption

Prodrugs can be designed to take advantage of the transporter-mediated process and enhance intestinal absorption. The transporters for which prodrugs have been made include peptide transporters, amino acid transporters, nucleoside transporters, bile acid transporters, and monocarboxylic acid transporters.[2,7,8] Table 39.4 lists examples of prodrugs that utilize transporter-mediated processes to enhance oral absorption.

Valacyclovir and valganciclovir are prodrugs of the natural amino acid valine.[9,10] They are substrates for the peptide transporters PEPT1 and PEPT2. The transporters increase oral absorption of the compounds.

Zidovudine is a synthetic nucleoside. It is converted by cellular kinases to the active metabolite zidovudine 5′-triphosphate. Zidovudine utilizes nucleoside transporters to increase oral absorption and cellular uptake.[11]

Enalapril is an angiotensin-converting enzyme inhibitor and a monoacid ester prodrug.[6] The oral bioavailability of the active principle diacid is only 3%, but the oral bioavailability of the monoacid is about 40%. This is because (1) an ethyl ester increases lipophilicity and results in increased transcellular absorption, and (2) PEPT1 transporter assists uptake of the compound.

TABLE 39.4 ► Transported-Mediated Prodrugs for Oral Absorption

Prodrugs	Transporters	Benefits of prodrug
Valacyclovir (Valtrex)	PEPT1 and PEPT2[10]	Oral bioavailability Three- to five-fold higher than acyclovir
Valganciclovir	PEPT1 and PEPT2[9]	Oral bioavailability Ten-fold higher than ganciclovir
Zidovudine (AZT, Retrovir)	Nucleoside transporter[11]	Oral bioavailability 64%[14]
Enalapril	PEPT1[6]	Oral bioavailability is 36%–44% due to increase in lipophilicity and transporter-mediated absorption. Oral bioavailability of diacid parent is 3%.

Most transporter-mediated prodrugs were discovered by accident. The specificity and capacity of the transporters determine the success of this approach. One should be aware that transporters can be saturated at high concentrations, and there is a potential for drug–drug interaction if two drugs compete for the same transporter.

39.4 Prodrugs to Reduce Metabolism

A prodrug approach can be used to prolong the half-life of the parent drug by masking the labile functional groups, such as phenolic alcohols, which are susceptible to phase II metabolism. They are essentially slow-release drugs. Figure 39.9 shows examples of prodrugs

with increased metabolic stability. Bambuterol is a dicarbamate prodrug of terbutaline. The phenolic alcohols are protected from phase II metabolism, and the carbamates are slowly hydrolyzed by nonspecific cholinesterases to release the parent terbutaline. The slow metabolism results in a longer half-life. Bambuterol is dosed once per day versus three times per day for terbutaline.

Figure 39.9 ► Examples of prodrugs used to reduce metabolism.[2]

Dopamine is not orally available because of rapid metabolism. It is extensively metabolized by *O*-sulfation, *O*-glucuronidation, and deamination in the intestine and liver. Docarpamine is an orally active dopamine prodrug. The bisethylcarbonates are hydrolyzed in the intestine, and the amide is converted in the liver.[12]

Levormeloxifene is an *O*-methylated prodrug of a selective estrogen receptor modulator. The compound is activated in vivo by oxidative demethylation. The prodrug enhanced oral bioavailability by protecting the metabolically labile site (OH).[2]

39.5 Prodrugs to Target Specific Tissues

Selective tissue delivery can increase therapeutic activity and reduce side effects. For example, PEG-conjugated anticancer prodrugs (e.g. PEG-paclitaxel) are found to selectively accumulate in tumor cells, prolong half-life, and improve efficacy. Prodrugs can also be used to target brain, bone, colon, and other specific tissues. Organ- or tissue-specific delivery is also known as the *magic bullet*.

Capecitabine is an orally active prodrug of 5-fluorouracil (5-FU).[13] Bioactivation of capecitabine is shown in Figure 39.10. It is first hydrolyzed in the liver by carboxylesterase and decarboxylated to 5′-deoxy-5-fluorocytidine, which is further converted to 5′-deoxy-5-fluorouridine by cytidine deaminase. Transformation of 5′-deoxy-5-fluorouridine to 5-FU occurs selectively in tumor cells by thymidine phosphorylase. Distribution of 5-FU to

tumor is impressive: six times higher than GI and 15 times higher than blood after oral administration of capecitabine.

Figure 39.10 ► Activation of the tumor-specific prodrug capecitabine to 5-fluorouracil.[13]

39.6 Soft Drugs

Soft drugs are discussed in Chapter 12.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. Which of these properties can be improved using prodrugs?: (a) toxicity, (b) permeability, (c) uptake transport, (d) hERG binding, (e) metabolic stability, (f) plasma protein binding, (g) solubility, (h) CYP inhibition?

2. How can the following structures be modified with pro-moieties to improve solubility?:

(A) (B) (C) (D)

3. How can the following structure be modified with a pro-moiety to improve permeability?:

4. What hydrolyzes phosphate prodrugs in the intestine?
5. How is the active carboxylic acid shown in Problem 3 released from the prodrug after absorption (permeation)?

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Effects of Properties on Biological Assays

Overview

- *Low-solubility compounds may precipitate in bioassays and produce erroneous data.*
- *Bioassay development should include optimization for low-solubility compounds.*
- *Serial dilutions should be performed in dimethylsulfoxide (DMSO), followed by dilution into assay buffer.*
- *Permeability can be used to interpret cell-based assay data.*
- *Solution stability checks compound stability under assay conditions.*
- *Some compounds are insoluble in DMSO stocks or can become less soluble with freeze–thaw cycles.*
- *Discussion of these issues among the project team leaders ensures optimum data.*

The measurement of drug-like properties accelerated during the 1990s for the purpose of reducing the attrition of clinical compounds during development, owing to poor biopharmaceutical properties.[1] In vitro property measurement provided a cost-effective and successful strategy for improving absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) properties and led to improved human pharmacokinetics (PK) and bioavailability.

In addition to PK, property measurement has benefited another major drug discovery area in an unexpected manner. The availability of property data led to the recognition that physicochemical and biochemical properties are also related to the performance of compounds in biological assays. For example, if compounds are insoluble in the bioassay matrix, then IC_{50} values will be wrong. If a compound has poor passive diffusion permeability, it will not penetrate the cell's membrane to interact with an intracellular target protein. If a compound is chemically unstable in the bioassay matrix, the data will be erroneous.

The logic of the intimate involvement of compound properties with biological testing is apparent by examination of the discovery biological testing process (Figure 40.1). Drug-like property activities began with living systems (animals to humans), by improving the delivery of compounds to the therapeutic target through improvement of PK and reduction of toxicity. The linkage of efficacy and PK is a central concept of drug discovery. However, efficacy in living systems is just one stage of the biological testing process. If earlier steps of biological testing are considered (cellular assays, enzyme assays, high-throughput screening [HTS]), it is apparent that drug-like properties also are linked to efficacy with in vitro biological

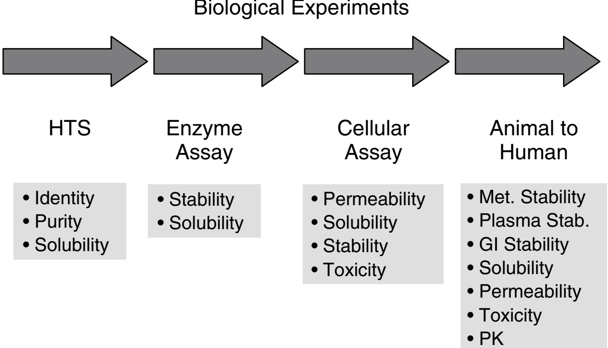

Figure 40.1 ► All stages of biological testing in drug discovery include property barriers.

tests. In each of these assays, compound properties affect exposure of the compound to the target.

The results of each assay are used to prioritize compounds in activity and selectivity. Fateful decisions on compound selection are made on the basis of biological data. Structure–activity relationships (SARs) are developed from biological data and are used to guide the activity optimization of leads. It is assumed that SAR is built on interaction with the therapeutic target alone. If the SAR is affected by solubility, permeability, or chemical stability, it will be misguided. It is crucial for the project team that SAR be founded only on activity. It would be unfortunate to overlook an important pharmacophore because the biological assay data were affected by properties. It is better to properly assess all compounds, even if they initially have poor properties, and then to improve properties of the active leads by structural modification. An active series is precious, and modern discovery scientists cannot afford to miss an opportunity because of inadequate experimental design.

Potential property barriers at each stage of biological testing are listed in Figure 40.1. HTS assay results can be affected by compound solubility, identity, and purity. Bench-top assays using enzymes and receptors also are affected by these properties. In addition, chemical instability of compounds in the bioassay medium can affect IC_{50}. Cell-based assays are additionally affected by permeability if the target is inside the cells. In vivo assays are subject to many of the property barriers discussed in Chapter 3.

This shift in perception of biological assays motivated considerable thinking about how to enhance discovery activities for the potentially major effect of properties. This has an impact on the following:

- Compound workflow: Store and handle compounds and solutions appropriately
- Experiment design: Optimize biological assays for properties
- Data interpretation: Recognize property effects; interpret results accordingly

Among the property issues, solubility in aqueous buffers and dimethylsulfoxide (DMSO) is the leading concern.[2,3] Biological assays universally rely on compound solubility in DMSO stock solutions and aqueous buffers. In recent years it has been recognized that many discovery compounds have low solubility. Low solubility in assay protocols results in falsely high IC_{50} values, low screening hit rates, poor SAR correlations, data inconsistency, differences in rank ordering between enzyme and cell-based assays, and poor in vitro ADME/Tox assay results. Recent studies indicate that discovery workflow could be improved by designing screening libraries with criteria for aqueous and DMSO solubility, improving

the storage and handling of DMSO stock solutions, assaying hits and leads for solubility early in discovery, improving assay protocols for compound dilution, and developing assays that are proven to work properly with insoluble compounds.

An example of solubility issues illustrates the concerns: low solubility of compounds in screening libraries can cause low HTS hit rates. It has been shown that screening libraries can contain a high percentage of low aqueous solubility compounds.[4] In one study, libraries of soluble compounds had a much higher hit rate (32%) in screens than for libraries containing a high percentage of insoluble compounds (4% hit rate). Low solubility causes lower concentrations in screening assays, so the activity is not adequately assessed. In such libraries, impurities can be more soluble than the main component. Thus, the impurity may be the cause of activity or a measured property, resulting in erroneous SAR or property conclusions.

The following sections discuss the effects of individual properties on biological assays. Successful actions that can be taken in discovery to deal with these problems are described and are summarized in Table 40.1.

TABLE 40.1 ► Approaches to Dealing with Solubility Limitations in Biological Assays

Biological assay aspect	Approach
Assay development	• Develop and validate assays to work with low-solubility compounds
Assay protocol	• Perform serial dilution in DMSO (not buffer) and transfer to assay media
	• Mix DMSO stock directly with assay media; avoid dilution in pure water
	• Screen at lower concentrations
	• In-well sonication to redissolve
	• Reduce or eliminate freeze–thaw cycles
	• Retest HTS hits using 0.1% Triton X-100 to break aggregates
	• Correct activity values with concentrations in assay media
Assay conditions	• Assess assay tolerance for media modifiers that enhance solubility; use maximum amounts
Sample handling	• Store DMSO plates at room temperature and use them for a minimum of time
	• Dissolve salts in 1:1 DMSO/water
	• Store stocks in 9:1 DMSO/water at 4°C
	• Store compounds in solid arrays

40.1 Effects of Insolubility in DMSO

It is often assumed that compounds are universally soluble in DMSO. However, this is not the case. DMSO solubility can be limited.[5,6] Compounds that have a strong molecular lattice for crystal packing can have low DMSO solubility. Compounds in this class have lower molecular weight (MW) and are rigid and hydrophilic, such as organic salts. A second compound class, which has DMSO solubility limitations, is not well solvated by DMSO because of high MW, high Log P, large number of rotatable bonds, or high solvent-accessible surface area. Low solubility in DMSO can result in compound precipitation. This will cause concentrations in the bioassay that are lower than expected and a measured IC_{50} that is higher than the actual value.

Another problem is decreasing concentration over time. Biologists often observe that a compound is more active when it is freshly prepared than after it has been stored for a

while. A primary cause of this phenomenon is precipitation of compound from the DMSO stock solution.

Common procedures can exacerbate this precipitation. Standard biological assay protocols dissolve compounds at a concentration of 10 to 30 mM in DMSO and then store the solutions at a cool temperature to reduce decomposition. Unfortunately, as many as 10% to 20% of compounds in libraries have DMSO solubility that is below this concentration,[7,8] and solubility drops further at reduced temperatures. The reduced concentrations of these DMSO stocks result in lower than expected concentrations in the biological assays using these stocks. Even if the compound has good intrinsic activity, it will appear to have low activity.

In addition, precipitate in the DMSO stock can have different effects when the stock is diluted for the assay (Figure 40.2). The IC_{50} dilution curve concentrations will be lower than planned when no precipitate is carried from the DMSO stock to the highest concentration aqueous solution, or when precipitate is carried over and does not dissolve. In these cases, the activity of the compound appears to be lower than it actually is. Conversely, the IC_{50} dilution curve concentrations will be higher than planned when precipitate is carried from the DMSO stock to the highest concentration aqueous solution and dissolves. In this case, the activity of the compound appears to be better than it actually is. Overall, precipitation of the DMSO stock will cause variable and unknown concentrations in the assay solutions that can make the compound appear more or less active than it actually is.

Another DMSO stock solution problem is "freeze–thaw cycles." When compound plates are reused, they are stored in the refrigerator. This is widely thought to reduce chemical decomposition. Unfortunately, the low temperature reduces the solubilities of compounds in solution.[9] The reduced solubility is favorable for crystal formation. These crystals usually have a lower solubility and dissolution rate than the amorphous material from which the

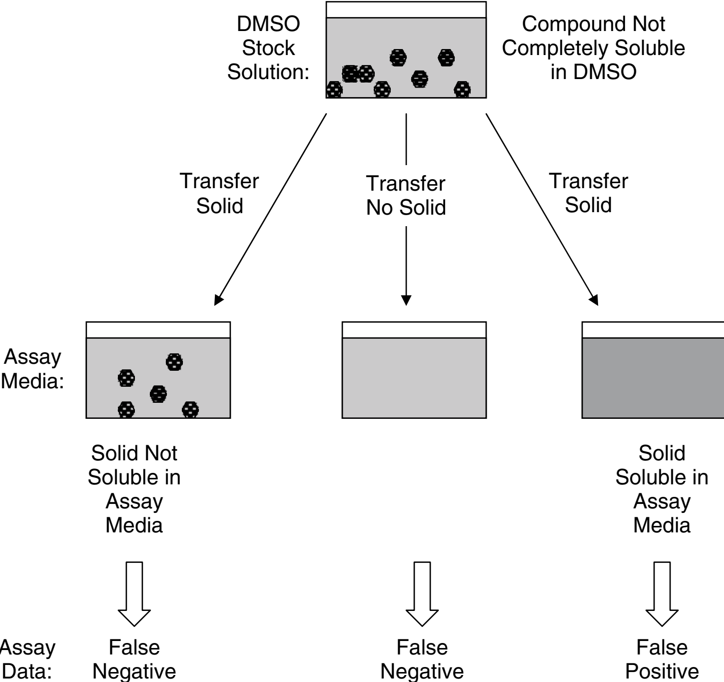

Figure 40.2 ▶ When a DMSO solution has undissolved particles, either false-negative or false-positive assay data can result, depending on whether solid is transferred and whether the solid is soluble in the assay media.

original DMSO solution was prepared.[5] Cooling also condenses water from the air into the DMSO (up to 10% w/w).[5,7,10–13] Unfortunately, many compounds have lower solubility in DMSO containing water than in DMSO alone.

40.2 Dealing with Insolubility in DMSO

One approach to the problem of low HTS hit rates caused by insoluble library compounds has been selecting only soluble compounds for screening libraries. Compounds are prescreened for solubility, and only those exceeding minimum DMSO or aqueous solubility criteria are placed in the screening library.[14]

Alternatively, insoluble salts can be dissolved in 1:1 DMSO/aqueous buffer, which increases the solubility of salts. Other water miscible organic solvents have been substituted for DMSO (e.g., methanol, ethanol, acetonitrile, tetrahydrofuran (THF), pyridine, dimethylformamide (DMF)).[15] With any solvent, it is important to determine the assay's tolerance to the organic solvent.

Sample storage approaches have been developed to reduce precipitation of DMSO stocks. One simple approach for reducing precipitation induced by freeze–thaw cycles is to use each DMSO solution only once. Individual-use tubes can be stored in automated systems, retrieved as needed, and then discarded after one use.[7] Another approach reduces freeze–thaw cycles by limiting the length of time that an individual DMSO solution is used (e.g., 2 weeks) and storing them at ambient temperature. This reduces the precipitation induced by cooling. Samples are not kept long enough for significant decomposition to occur at room temperature.[5]

Recognizing that water is difficult to keep out of DMSO solutions, some discovery organizations store compounds in 10% water/90% DMSO.[16] At a storage temperature of 4°C, the solutions remain liquid. Variability is reduced because there is no increase in volume from water absorption and no variable precipitation.

Alternatively, solutions can be prepared at lower concentrations (2 to 5 mM) to reduce DMSO precipitation.[4,16] A tradeoff of this approach is that the upper concentration of the assay is limited. For example, in a cell assay for which a maximum of 1% DMSO is tolerated,[17] only 1 μL of DMSO stock can be added to 99 μL of assay buffer, allowing an upper concentration of only 20 μM when a 2 mM DMSO stock is used. This can limit the determination of IC_{50}.

When compounds precipitate from DMSO or aqueous buffers, a low-energy sonicator has been successfully used to redissolve solids[7] or drive the solution to supersaturation. The energy is low enough to avoid compound decomposition.

New technologies for sample storage have begun to appear. NanoCarrier stores compounds in 1,536-well plates after evaporation of the DMSO to avoid limited DMSO solubility.[18] Compounds are stored as dry films in the DotFoil technology and rapidly dissolved for experiments.[19] Library compounds are spotted onto cards, dried, and sealed in lightproof pouches and inert atmosphere in the ChemCards product.[9]

It is useful for chemists to obtain an early estimate of the DMSO solubility of their project's compounds by using software.[6,8,20–22] Use of such tools can alert discovery scientists to compounds that may have low solubility in DMSO.

40.3 Effects of Insolubility in Aqueous Buffers

Biological assays typically test a compound's activity at various concentrations and determine IC_{50}. Typically, a high-concentration dilution (e.g., 100 μM) is made from DMSO stock into aqueous buffer, followed by serial dilution to the lower concentrations (Figure 40.3, *A*). Compounds with low aqueous solubility may not be fully soluble at the concentration of the highest aqueous solution. Serial dilution of this solution will produce a dilution curve that is

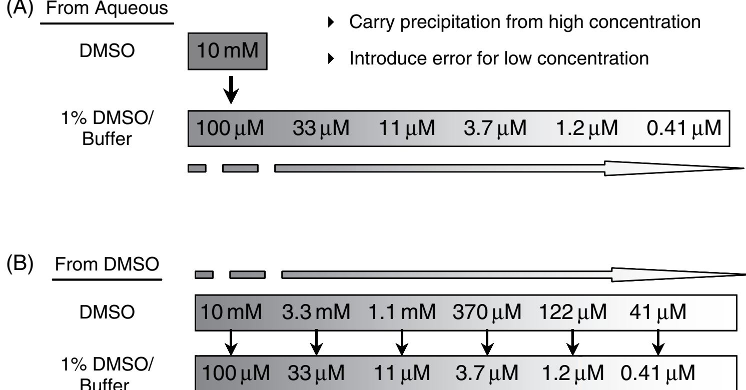

Figure 40.3 ► Schemes of serial dilution. **A,** Serial dilution in aqueous solutions can cause precipitation at the highest concentrations, which is carried to subsequent dilutions. **B,** Serial dilution in DMSO keeps compounds in solution, followed by transfer of a small aliquot of DMSO into the aqueous buffer. This also maintains the same DMSO concentration in each of the concentration solutions. (Reprinted with permission from [28].)

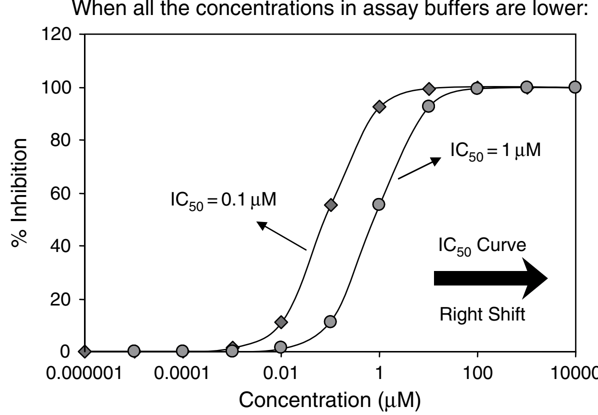

Figure 40.4 ► If the test compound is insoluble at the initial highest concentration of the dilution curve and serial dilutions are made by aqueous dilution (Figure 40.3, *A*), the IC_{50} curve is likely to be “right shifted,” causing compounds to appear less active.

shifted to lower concentration than planned (Figure 40.4). The apparent IC_{50} will be higher than the compound’s intrinsic IC_{50}, making the compound appear to be less active than it is.

Studies have indicated that about 30% of discovery compounds have an aqueous solubility <10 μM. This is the concentration that is commonly used for HTS.[23] Thus, it is likely that, for a portion of the library, HTS is providing IC_{50} values that are higher than the actual values.

An interesting result of limited aqueous solubility is unexplained discrepancies between different bioassays. Differences can be caused by the varying compositions of assay buffers. The composition differences occur because assays differ in their tolerance for DMSO and other components, but they also have an effect on the solubilities of compounds.[17] Some enzyme assays can tolerate up to 10% DMSO, but others can tolerate only up to 0.2% DMSO before the enzyme is inhibited. Significant differences in compound concentration can occur and result in differences in the assay results. An example of this is enzyme assays, which can tolerate additives, and cell-based assays, which have low tolerance. A low-solubility

Compounds	Solubility in Receptor Binding Assay Buffer (μM)	Solubility in Cell-based Assay Buffer (μM)
1	11	2.4
2	10	4.8
3	10	1.4
Buffer	5% BSA, 2.5% DMSO	0.1% DMSO

* Target assay concentration 10 μM

Figure 40.5 ► Cell-based assays customarily use fewer media modifiers, which can cause lower solubility and unexplained discrepancies between receptor/enzyme assays and cell-based assays.

compound's concentration in an enzyme assay containing 2.5% DMSO and 5% bovine serum albumin (BSA) can be 10-fold higher than in a cell-based assay with 0.1 % DMSO and 0% BSA (Figure 40.5).

In HTS, false ubiquitous hitters can be caused by compound aggregation at concentrations >10 μM.[24−26] The enzymes adsorb to the aggregates and appear to be competitively inhibited. False hits from aggregation will never be optimized by medicinal chemists.[27] Aggregates of 30- to 400-nm size pass through 0.2-μM filters (200 nm), so they are not removed by filtration. Aggregation may be triggered by supersaturation as concentrated DMSO solutions are added to aqueous buffers.[5] In one library of 1,030 compounds, the false hit rate was 19% at 30 μM but dropped to 1.4% at 5 μM, at which aggregation is less common.[27]

Not only activity and selectivity testing can be affected by low solubility. In vitro ADME/Tox assays are similarly affected. Erroneous data from metabolic stability, CYP inhibition, and hERG blocking assays can be generated because these assays are based on concentration. Low solubility can make these issues appear to be less of a problem.

40.4 Dealing with Insolubility in Aqueous Buffers

Various approaches are used to deal with insolubility of compounds in bioassays. Awareness of the potential problem is the first step. Once the discovery team realizes that biological assays may not perform properly with insoluble compounds, they can take steps to mitigate the situation. The most successful actions are as follows:

- Modify the dilution protocol to keep compounds in solution
- Assess compound solubility and concentrations
- Optimize assays for low-solubility compounds

In general, these are practical applications of physicochemical principles and best analytical chemistry practices.

40.4.1 Modify the Dilution Protocol to Keep Compounds in Solution

Serious problems can occur during the serial dilution protocol.[28] This is the first place to look to ensure bioassay reliability. It is important to perform the serial dilution in DMSO. Then a small volume of each DMSO dilution can be added to the aqueous assay matrix. This is illustrated in Figure 40.3, *B*. If the compound precipitates in the most concentrated

solutions, this will not affect the actual compound concentrations in the lower concentration solutions. If the serial dilution is performed from one aqueous solution to the next, then the errors in the highest concentration solution are propagated to the subsequent dilutions. Accurate preparation of the dilution curve is the most important action to take to ensure quality biological assay results.

Many biologists have observed that assay results are more reproducible if they mix the highest concentration solution up and down with the pipetter before transferring an aliquot to the next solution in the serial dilution. This is because the precipitated particles are broken up into smaller particulates and form more homogeneous suspensions. The problem with this approach is that particulates may not be transferred evenly. It is much better to use assay conditions that completely solubilize the compounds and entirely avoid particles. Also, if precipitation is occurring in the first aqueous solution, it may be useful to reduce the concentration of the highest concentration in the dose–response curve. This will reduce precipitation and make the ensuing dilutions more reliable.

Some biological protocols prepare a high-concentration aqueous stock solution from DMSO stock and then use the aqueous stock (instead of DMSO stock) to make the dilution curve. Unfortunately, this procedure increases the amount of time that low-solubility compounds remain at high concentration. It is ideal to directly add DMSO dilutions into assay media, that contains components, such as proteins, cellular material, lipid membranes, and microsomes, which help to solubilize low-solubility compounds. Addition of DMSO solution to the assay media may cause supersaturation, which is a solution with a higher concentration than would be reached at equilibrium. Supersaturation reduces the rate of precipitation, allowing time for the compound molecules to interact with the target. Usually the DMSO concentration must be kept low, so only a small volume of DMSO should be added to the media. Many workers mistakenly believe that if their manual pipetter has volume markings down to 0.5 μL, then they can accurately and precisely deliver this volume. Pipetting error ranges are much higher at the low-volume limit of the pipetter.

Another mistake is diluting DMSO solutions into pure water. It is much better to dilute into buffer. Pure water has no buffering capacity, so ionizable compounds tend to convert to the neutral state where they have a much lower solubility. Buffer will maintain the pH at the prepared value. Ionized molecules have much higher solubility than neutral molecules.

40.4.2 Assess Compound Solubility and Concentrations

It is useful to assess the solubilities of compounds early. Several commercial software programs predict aqueous solubility from the molecular structure.[21,29] Although these calculations may not be absolutely accurate (usually within 10-fold of the actual solubility) and provide equilibrium solubility predictions, they can provide chemists with an early warning of potential solubility issues. Furthermore, they typically provide a profile of solubility versus pH, so chemists can see the trends and preferable pHs.[30,31]

In addition to software, most discovery organizations are alerted to potential solubility limitations of compounds using high-throughput solubility assays. These assays are more reliable than software models. Most solubility assays use generic aqueous buffers and conditions (see Chapter 25).[32–34] Kinetic solubility methods, which use initial dissolution in DMSO and addition of this solution to aqueous buffers, mirror the biological assay protocols. This is more appropriate than thermodynamic (equilibrium) solubility methods, which use aqueous buffer added to solid compound. Some groups use high-performance liquid chromatography (HPLC) with a chemiluminescent nitrogen detector (CLND) for increased accuracy in quantitation of the actual compound concentration. This detector has a molar

response to compound concentration and does not require an analytical standard for quantitation. This distinguishes apparent concentration of added sample from actual concentration, which may be lower owing to impurities in the sample.[4,35–38]

For more thorough examination of solubility, it is good practice to use the conditions and protocol of the bioassay. This is because solubility is very dependent on the conditions of the assay media and the exact protocol. A customized solubility assay, using the bioassay conditions and protocol, is very useful.

Some organizations take an "adjustment" approach. The actual compound concentration in each of dilution curve solution is measured and the corrected IC_{50} calculated.[4] Although this approach is appealing, there are some drawbacks. If the measurement is done in a generic buffer, the concentrations will differ from those in assay media and may lead to an inaccurate IC_{50}. Also, the measurements are time and resource consuming, so they may not be efficient. This approach may be very appropriate if a key compound requires this level of accuracy, but it is inefficient if tens or hundreds of compounds are to be studied. Also, this approach could result in selection of several lead compounds with low solubility as the primary leads of a project. If the project team is never able to improve the solubility of these compounds, the team may be unnecessarily burdened with low-solubility leads. The most efficient approach is to optimize the assay protocol during assay development so that it works for low-solubility compounds and produces accurate activity without the need for burdensome analyses and IC_{50} correction to every tested compound.

40.4.3 Optimize Assays for Low Solubility Compounds

Solubility is strongly affected by assay media components (e.g., buffer, organic solvents, counter-ions, protein), dilution protocol, and incubation conditions (e.g., time, temperature). It is good practice to optimize the assay media components, percentages, dilution, and protocol during assay development to improve the solubilizing capabilities of the bioassay. Co-solvents (e.g., DMSO, methanol, ethanol, acetonitrile, DMF, dioxane) and excipients (e.g., cyclodextrin) have been used to improve solubility in bioassay media.[9,39,40] In many cases, the assay developer uses standard conditions or media components without testing for tolerance. The assay may tolerate a higher concentration of DMSO or other components, and this should be determined during assay development.

The ability of an assay to perform accurately for low-solubility compounds can be determined during assay development. A set of low-solubility compounds can be processed using the assay conditions and protocol. Their concentrations can be analyzed to determine if the assay is maintaining them in solution.

If compounds precipitate in aqueous buffer, in-well sonication can redissolve them.[7] A high-power sonicator with 96 individual probes can be used to simultaneously sonicate each well in a plate. This technique can salvage precipitated solutions, or it can ensure that all the wells are completely solubilized as part of the protocol. Precipitation is not always obvious by eye.

Running biological assays at a lower concentration can reduce precipitation. More compounds are soluble at a concentration of 3 μM than at 10 μM.

If the project team suspects that some of their hits may be due to aggregation, several approaches can help to identify the aggregators. Hits can be rescreened in the presence of 0.1% Triton X-100.[27,41] This detergent breaks up the aggregates. Another approach is to analyze the solutions using a dynamic light scattering plate reader, which detects aggregates. If the transition of the IC_{50} curve is sharper than a normal curve aggregation could be responsible because of the dependence of aggregation on concentration.[26] Screening can be performed at a lower concentration, at which aggregation occurs less frequently.

Finally, a computational model can help to identify structural features characteristic of aggregators.[27,42,43]

40.4.4 Effects of Permeability in Cell-Based Assays

Membrane permeability can greatly affect the observed biological activity of some compounds for intracellular targets. These compounds may have been very active in cell-free enzyme or receptor assays. However, their cellular membrane permeability may be low and reduce or eliminate their activity.

This effect usually is caused by low permeability by passive diffusion. Another well-known example is multidrug resistance cancer cells, which have high levels of P-glycoprotein and greatly reduce intracellular concentration by efflux. Most cell lines used in drug discovery have low levels of efflux transporters.

Compounds may have limited permeability in cell-based property assays. For example, measurement of metabolic stability using hepatocytes certainly is affected by the rate of enzymatic metabolism reactions, but it also can be affected by the hepatocyte membrane permeability of the compounds.

40.4.5 Dealing with Permeability in Cell-Based Assays

The simplest approach for checking passive diffusion permeability potential is structural rules. If polar surface area (PSA) is >140 Å^2, H-bond acceptor >10, H-bond donor >5, MW >500, or if the compound has a strong acid, it is possible that cell membrane permeability is limited.

High-throughput permeability assay data are useful. For example, if parallel artificial membrane permeability assay (PAMPA) permeability is low, then cell membrane permeability is likely to be low. Some chemists use Caco-2 data for estimating cell membrane permeability. It can be overkill to use the expensive Caco-2 assay for this purpose when less expensive assays could be used. Caco-2 is also more complex because it exhibits several permeability mechanisms that may be confusing to interpret for intracellular compound exposure.

Intracellular concentrations have been determined for this purpose (see Section 27.2.2). This is more common with radiolabeled compounds, but the availability of sensitive and rapid liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods make this more practical.

If reduced activity in cell-based assays is observed, the medicinal chemist can identify the structural cause of the low permeability. If the functional group is not crucial for the activity, then structural modification to reduce the polarity, hydrogen bonding, or size may greatly improve the permeability and cell-based activity. The permeability effect may be obvious by examining the activity differential between cell-free and cell-based assays to determine if there is a greater differential for the analogs that are permeability limited.

With limited resource investment, the permeability limitations of a series can be estimated, which can be useful for better understanding the behavior of the compounds.

40.4.6 Effects of Chemical Instability in Bioassays

Compounds may be chemically unstable in biological or property assays. They may react with an assay media component, they may be hydrolyzed, or a media condition (e.g., pH, temperature, light) can accelerate decomposition. Another complicating factor is that

decomposition products can be either more or less active than the tested compound, thus confusing the SAR. Moreover, compounds in the same series could have differences in chemical decomposition, which could be mistaken as SAR. Several decomposition reactions can occur, including hydrolysis, hydration, oxidation, and isomerization (see Chapter 13).

40.4.7 Dealing with Chemical Instability in Bioassays

When structural features indicate the potential for decomposition or when inconsistent data are generated that may be caused by chemical instability, it is prudent to test the stability in vitro (see Chapter 31). The test should be conducted under conditions as close as possible to the biological assay conditions (e.g., media, time, temperature). HPLC is useful for measuring the compound concentration over time. LC/MS is useful for rapidly identifying the reaction products, from which the decomposition mechanism can be inferred. The decomposition time course will provide a means for estimating the level of the effect of the decomposition on the bioassay activity.

Problems

(Answers can be found in Appendix I at the end of the book.)

1. What property barriers does a compound encounter in an in vitro cell-based assay for an intracellular therapeutic target?
2. Why might it be counterproductive to eliminate from further consideration all compounds that do not have good activity in an in vitro assay, regardless of whether the low activity is due to poor target binding, solubility, permeability, or stability?
3. What are the characteristics of two classes of compound with low DMSO solubility?
4. How might low solubility in DMSO cause either higher or lower aqueous assay concentrations than intended?
5. What are two negative effects of freeze–thaw cycles?
6. What approaches can help to better solubilize compounds in an organic stock solution?
7. Would low compound solubility in the aqueous assay media cause higher or lower IC_{50}?
8. What two general approaches can improve the characteristics of a biological assay to better assess compound activity?
9. A serial dilution should be performed by which of the following protocols?: (a) prepare a high-concentration DMSO stock solution and pipette as small of a volume as possible into the aqueous buffer, (b) make the initial dilution from DMSO as a high-concentration aqueous solution and dilute with aqueous buffer to subsequently lower concentrations, (c) make the initial dilution from DMSO as a high-concentration DMSO solution and dilute with DMSO to subsequently lower concentrations that are each diluted into aqueous buffer.
10. To measure the solubility of compounds in the biological assay, it is best to: (a) use a generic aqueous buffer, (b) use water, (c) use the solution used in the bioassay, (d) follow the protocol of the assay.

11. What components can be included in bioassay media to maximize the concentration of low-solubility compounds?

12. What tools can discovery scientists use to estimate if permeability is a potential problem for a lead series in a cell-based assay?

13. Define IC_{50} right shift. What is the cause?

14. What solubility-related problem can cause erroneous activity in cell-based assays that are not consistent with enzyme/receptor assays in which the compounds were previously tested? What can be done during assay development to improve this situation?

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Formulation

Overview

- *Formulation increases compound solubility to enhance absorption in vivo.*
- *Increased in vivo exposure provides more informative data for efficacy, pharmacokinetics, and toxicity during discovery.*

Formulation has numerous benefits in drug discovery and development. It enables efficacy, toxicity, and pharmacokinetic (PK) studies. Formulation can improve oral bioavailability, shorten onset of a therapeutic effect, enhance stability of drugs, and reduce dosing frequency. More consistent dosing can be achieved by reducing food effect through formulation. Formulation can reduce side effects (i.e., decreasing tissue irritation and improving taste). Tissue-(e.g., tumor) specific formulation can enhance efficacy and reduce toxicity. Novel formulations may be patentable.

Formulation is most commonly used to increase solubility and sometimes to improve stability (Figure 41.1). It is rarely used to enhance permeability because of toxicity.[1] Application of permeability enhancers is still an active research area. Enhancers work by opening the tight junctions between the cells to allow drugs to pass through by paracellular diffusion. However, this also allows toxic substances to go through and cause toxicity. Structure modification is required to increase permeability, and there are no formulation rescues.

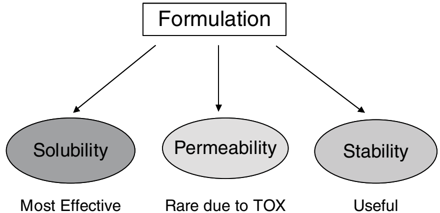
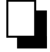


Figure 41.1 ► Applications of formulation.

Formulation in drug discovery faces many challenges.[2] Drug discovery typically has limited material. Only milligram quantities are prepared. This limits the options to screen for optimal formulations because insufficient material is available. Formulation in drug discovery requires short time lines. Usually dosing vehicles need to be developed in a few days for animal studies. There is not enough time to develop ideal formulation vehicles. At early stages of drug discovery, the compounds do not always have good potency. High doses typically are used in order to demonstrate efficacy and proof of concept. These factors make it more challenging for formulation to achieve high loading. At this stage, multiple animal

species and multiple routes of administration are explored to evaluate the drug effects. This requires development of various dosing vehicles and dosage forms.



41.1 Routes of Administration

The different routes of administration for commercial drugs are summarized in Table 41.1. Figure 41.2 shows the distribution of pharmaceutical sales for each delivery route.[3] The majority of the drugs (70%) are delivery by oral administration and 16% by injection.

TABLE 41.1 ▶ Comparison of Different Routes of Administration

Routes	Dosage form	Tonicity	Ideal pH	Bioavailability
Oral	Solid Suspension Solution	Not required	Not required	Incomplete absorption First pass in gut and liver
IP	Suspension Solution	Isotonic preferred	5–8	No first pass in gut Has first pass in liver
IV bolus	Solution Emulsion	Isotonic	5–8	Complete
SC	Solution Emulsion Suspension	Isotonic preferred	3–8	Incomplete absorption No first pass in gut and liver
IM	Solution Emulsion Suspension	Isotonic	3–8	Incomplete absorption No first pass in gut and liver

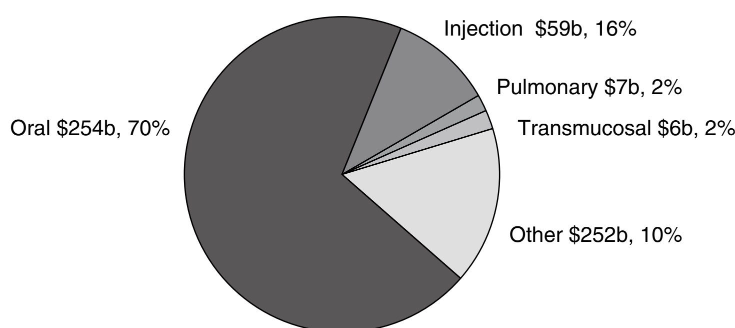


Figure 41.2 ▶ Distribution of pharmaceutical sales based on delivery route.[3]

Oral (PO)

Oral (PO) dosing is the most convenient, economic, safe, noninvasive route of administration. However, it requires patient compliance. PO tends to have limited and variable absorption for some drugs that have poor physiochemical properties, such as low solubility and/or low permeability. Drugs dosed PO are subject to first-pass metabolism in the gut and liver. They can have limited bioavailability due to absorption and metabolism barriers.

Gavage delivery is quite common for animal PO dosing. Drugs are delivered as a liquid into the stomach via a feeding needle.

Intravenous (IV)

Intravenous (IV) dosing has rapid onset and complete bioavailability. It precisely delivers the complete dose. IV requires that the dose be soluble and the solution be miscible with serum without precipitation. Oil/water emulsions and liposomal and nanoparticulate systems can be injected IV as long as the particle sizes are much smaller than erythrocytes. Certain formulations may cause hemolysis or precipitation after injection. An in vitro precipitation model can be used to determine whether compounds are likely to precipitate upon dilution and IV administration.[4,5]

IV delivery is commonly performed by needle injection into an easily accessible vein (e.g., tail, leg).

Intraperitoneal (IP)

Intraperitoneal (IP) injection is particularly useful in discovery laboratories for small animal studies, where it often is preferred over IV because of its ease of administration. The IP route also is used in clinical situations, particularly in intensive care units and during chemotherapy, where high concentration can be achieved locally while minimizing systemic side effects. IP bypasses first-pass metabolism from the gut but still is subject to first-pass metabolism by the liver[6] because absorption occurs via the portal system. Compounds that are very lipophilic will be quickly absorbed systemically by the IP route but not by the IM or SC route.

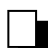
IP administration is performed by needle injection into the abdominal cavity. Both solution and suspension can be used for IP injection.

Subcutaneous (SC)

Drugs are injected in a subcutaneous (SC) site, which is beneath the skin, an area that is rich in fat and blood vessels. Solutions, suspensions, or implantation forms all can be used for SC delivery. Solutions are preferably isotonic. SC bypasses first-pass metabolism. Thus, SC is a useful route in proof-of-concept studies if a compound is highly metabolized. Injection volume is very small (0.5–2 mL) for SC delivery, so it typically is used for high-potency drug candidates.

Intramuscular (IM)

An intramuscular (IM) medication is given by needle into the muscle. It can be an isotonic solution, oil, or suspension. Drugs in aqueous solution are rapidly absorbed. However, very slow constant absorption can be obtained if the drug is administered in oil or suspended in other repository vehicles. IM administration of certain drugs can be painful. The drug might precipitate at the site of administration.



41.2 Potency Drives Delivery Opportunities

Routes of delivery are dependent on the potency of drug candidates. Figure 41.3 illustrates that the more potent the compound is, the more options are available for delivery. For very active compounds with a dose <10 mg (or 1 mg/kg), all the routes can be applied to deliver the drug. However, if the compound has an average potency with dose >100 mg (or 10 mg/kg), the route of administration is limited to only PO or IV. Therefore, potent compounds have more opportunities for delivery routes, whereas low-potency compounds have limited options.

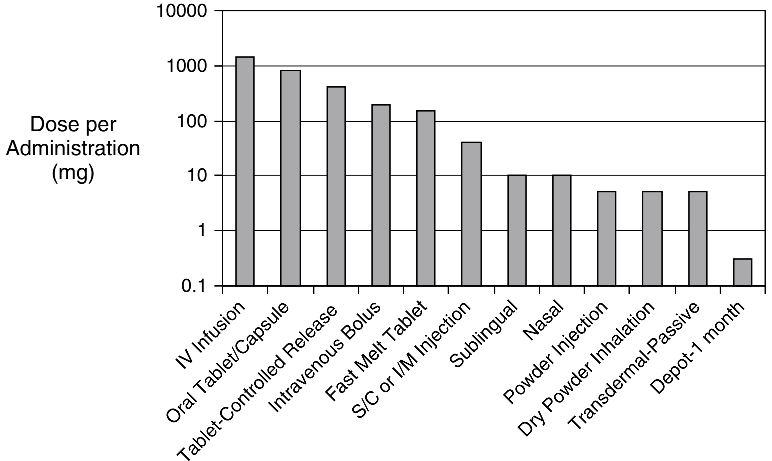
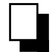


Figure 41.3 ► Drug potency drives delivery opportunities.



41.3 Formulation Strategies

Many strategies have been developed to formulate insoluble compounds, including pH adjustments, use of co-solvents, surfactants, and complexation agents, lipid-based formulation, and particle size reduction.

Adjust pH of Dosing Solution

For compounds containing ionizable groups, adjusting the pH of the dosing solution can favor the ionized form and increase solubility. The pH of the solution should be two units beyond the pK_a of the compounds to ensure complete ionization. For acids, basic buffers should be used; conversely, for bases, acidic buffers should be used. Table 41.2 summarizes common buffers used for pH adjustment to enhance solubility of ionizable compounds.[7] Dosing a basic amine in a citric acid solution is more likely to dissolve the compound and enhance in vivo exposure than dissolving the compound in water. Both in situ solution (salt formed in solution with counter-ion and pH adjustment) and salt form approaches can be used to increase solubility through ionization of acids and bases.

TABLE 41.2 ► Common Buffers for Formulation[7]

Buffering agents	pK_a(s)	Suitable for pH range	Commercial products
Maleic acid	1.9, 6.2	2–3	Teniposide
Tartaric acid	2.9, 4.2	2.5–4	Tolazoline HCI
Lactic acid	3.8	3–4.5	Ciprofloxacin
Citric acid	3.1, 4.8, 6.4	3–7	Labetalol HCI, Nicardipine HCI
Acetic acid	4.75	4–6	Mitoxantrone HCI, Ritodrine HCI
Sodium bicarbonate	6.3, 10.3	4–9	Cefotetan, Cyclophosphamide
Sodium phosphate	2.2, 7.2, 12.4	6–8	Warfarin, Vecuronium Br

Use Co-solvent

Co-solvents can help dissolve insoluble compounds by increasing solubility. Commonly used co-solvents and their toxicities are listed in Table 41.3. Examples of parenteral products

(administered IV in the clinic) containing co-solvents and surfactants are listed in Table 41.4. Co-solvent and pH adjustment can be used in combination to further enhance the solubility of insoluble compounds. Precautions should be taken when using co-solvent in animal models for efficacy studies, to minimize potential interference of pharmacological effects by the co-solvent.

TABLE 41.3 ► Commonly Used Co-solvents and Their Toxicities

	Mouse LD_{50} (g/kg)			Rat LD_{50} (g/kg)			
Co-solvents	PO	IV	IP	PO	IV	IP	Percent of commercial products containing
DMSO	8	2	1	7	1	4	
Glycerin	24	8	10	20	7	7	
Dimethylacetamide	4	6	9	126	6	9	< 3
Ethanol	29	9	10	–	7	10	< 10
Propylene glycol	5	3	3	5	3	3	~ 40
PEG 400	17	6	3	18	5	8	~ 50

TABLE 41.4 ► Examples of Parenteral Products Containing Co-solvents and Surfactants

Trade name	Generic name	Manufacturer	Cosolvents/surfactant	Routes
Sandimmune	Cyclosporin	Novartis	Cremophor EL 50% Ethanol 27.8%	IV infusion
Lanoxin	Digoxin	GSK	Propylene glycol 40% Ethanol 10%	IV, IM
Ativan	Lorazepam	Wyeth	PEG 400 18% Propylene glycol 80%	IM, IV
Taxol	Paclitaxel	BMS	Cremophor EL 50% Ethanol 50%	IV infusion

Utilize Surfactants

Surfactants can provide many benefits for formulations[8]: (1) increase solubility of drugs through micellization, (2) prevent precipitation due to surface properties, especially after dilution, (3) improve stability of drugs in solution by incorporating them into micelles, and (4) prevent aggregation in protein formulation due to interfacial properties. Commonly used surfactants are summarized in Table 41.5.[9] Table 41.6 lists examples of marketed drugs containing surfactants.

Suspension formulation (undissolved particles in liquid) using surfactant is most common in drug discovery (1) when solution formulation is not feasible due to the limit of solubility, and (2) for toxicity and chronic studies. Suspensions can be dosed through PO/gavage administration, IP, SC, or IM delivery. A typical suspension formulation includes a surfactant (e.g., Tween 80) to wet the surface of particles and a bulking agent (e.g., Methocel) to suspend the solid particles. For suspension formulation, it is critical to reduce the particle size of the solid material to enhance the surface area and dissolution rate in order to maximize exposure. The commonly used suspension formulations in drug discovery for PK and toxicokinetics (TK) studies is Tween 80 (0.1%–2%)/Methocel (0.5%–1%).

TABLE 41.5 ▶ Solubilizing Excipients Used in Commercially Available Solubilized Oral and Injectable Formulations[9]

Water-soluble co-solvent	Water-insoluble co-solvent	Surfactants
DMA	Beeswax	Cremophor EL
DMSO	Oleic acid	Cremophor RH 40
Ethanol	Soy fatty acids	Cremophor RH 60
Glycerin	Vitamin E	Tween 20
NMP	Castor oil	Tween 80
PEG 300	Corn oil	TPGS
PEG 400	Cottonseed oil	Solutol HS-15
Poloxamer 407	Olive oil	Span 20
Propylene glycol	Peanut oil	Softigen 767
HPβCD	Safflower oil	Labrasol
SBEβCD	Sesame oil	Labrafil M-1944CS
αCD	Peppermint oil	Labrafil M-2125CS
Phospholipids (HSPC, DSPG, DMPC, DMPG)	Soybean oil	PEG 400 monostearate
		PEG 1750 monostearate

TABLE 41.6 ▶ Marketed Parenteral Products Containing Surfactants[8]

Trade name	Generic name	Manufacturer	Surfactants	Routes
Cordarone X IV	Amiodarone HCl	Sanofi-Aventis	Tween 80 10% Dilute 1:50	IV infusion
Neupogen	Filgrastim	Amgen	Tween 80 0.004%	IV
Proleukin	Aldesleukin	Chiron	SDS 0.18 mg/mL Dilute 1:42	IV infusion
Calcijex	Calcitriol	Abbott	Tween 80 0.4%	IV

Lipid-based Formulation

There are four different types of lipid-based formulations depending on the percentage of oil, water-soluble and water-insoluble surfactants, and co-solvents.[10,11] Lipid-based formulation can increase the solubility of lipophilic compounds. The simplest lipid delivery system is to dissolve a drug into pure oil (e.g., vegetable oil). For example, oil solution is the standard method for administration of lipid-soluble vitamins, such as vitamins A and D. For more complex systems, compounds are dissolved in oil or lipids and then dispersed into aqueous buffers in the presence of surfactants, with or without co-solvents. They can form emulsions, micelles, or liposomes. Their structures are illustrated in Figure 41.4. Examples of marketed products using lipid-based formulations are listed in Table 41.7.

An emulsion is a dispersion of two immiscible liquids (e.g., oil and water) with a surfactant or emulsifier. Emulsifier coats the droplets to stabilize the emulsion by creating repulsion between the droplets. There are two common types of emulsions. Water-in-oil (W/O) emulsions typically are used in sustained release of steroids and vaccines by IM delivery. Oil-in-water (O/W) emulsions are one of the most commonly used lipid formulations for various routes of administration, such as SC, IM, and IV. Excipients for emulsion formulation are various oils of polar triglycerides (e.g., soybean oil, sesame oil, corn oil, safflower oil), an aqueous phase (saline, D5W and buffers), and an emulsifier (egg lecithin and Tween 80). Drugs are incorporated into the oil droplets, and most of the drop sizes are around 0.2 to 0.6 μM. Parenteral lipid emulsions normally are ready-to-use formulations and conveniently stored at room temperature. Drug emulsions normally are formulated to

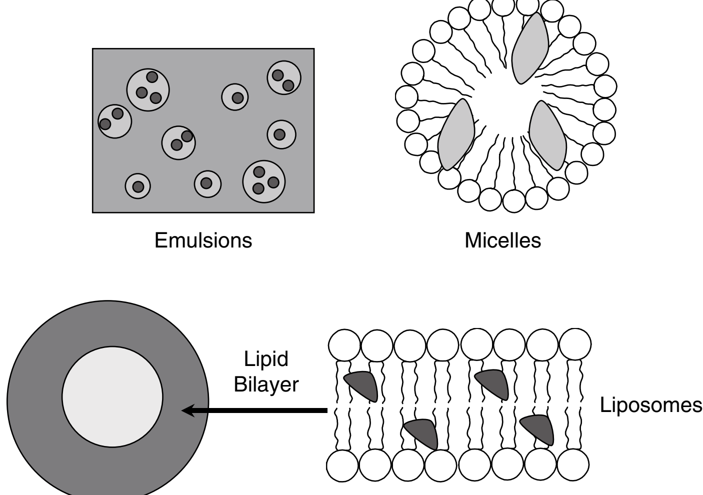


Figure 41.4 ► Structures of lipid-based formulations.

TABLE 41.7 ► Example of Commercially Available Injectable Lipid-based Formulation[8,18]

Solubilizer system	Trade name	Drug	Manufacturer
Lipid emulsion	Diazemuls	Diazepam	Dumex
Lipid emulsion	Diprivan	Propofol	AstraZeneca
Mixed micelles	Valium MM	Diazepam	Roche
Mixed micelles	Konakion/120	Vitamin K	Roche
Liposome	AmBisome	Amphotericin B	Gilead
Liposome	Doxil	Doxorubicin	Alza

isotonic concentration at pH 7 to 8, which reduces pain upon injection compared to solvent-based or solubilized formulations.[12] Loading of drugs for emulsion systems can be quite high and vary from 1 to 100 mg/mL, depending on formulation and process conditions.

Micelles are aggregates that self-associate to form a hydrophobic core and hydrophilic outer sphere. Lipophilic drugs can be incorporated into the core and, thus, dissolved in aqueous media. The sizes of micelles are approximately 5 to 50 nm.[13] Micelles typically consist of low–molecular-weight amphiphilic molecules, such as bile salts and phospholipids. The limitations of small molecular micelles are (1) low capacity for drug loading, (2) possible toxicity due to disruption of lipid bilayers, and (3) possible precipitation after injection due to breakdown of the micelles. Block copolymer micelles offer many advantages over traditional micellar formulations, with less toxicity, better stability, high loading, controlled-release properties, and targeted delivery.[13−17]

Liposomes are microscopic hollow spheres, typically made of bilayers of natural or semisynthetic phospholipids and/or cholesterol. Insoluble compounds can be solubilized in the hydrophobic space of liposomes. They vary in size (30 nm to 30 μM), bilayer rigidity, geometry, and charge. Beside the hydrophobic bilayer, the encapsulated aqueous compartment and the polar interface can be used to capture sparingly soluble compounds.[18] The versatility makes liposome delivery systems amenable to formulate a wide range of drug classes. Liposome technology currently is focused on applications in oncology, but it also can be used to formulate drugs to treat fungal, bacterial, and viral infections, alleviate pain, reduce inflammation, treat blood disorders, and for medical imaging and vaccines.[18]

Liposomes can be destabilized if overloaded with drug molecules. Drug loading of lipophilic drugs normally is substantially lower for liposomes than for emulsions. It typically is limited to high-potency compounds. Liposome formulations normally are lyophilized because of stability issues and require reconstitution before use.

Drug Complexation

Cyclodextrins are cyclic oligosaccharides consisting of (α-1,4)-linked α-D-glucopyranose units (Figure 41.5), with a hydrophilic outer surface and a lipophilic central cavity.[19] Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic poorly soluble compounds. An example of the guest–host complex of aspirin with β-cyclodextrin (βCD) is shown on Figure 41.6. Cyclodextrins are relatively large molecules with molecular weight between 1,000 and 2,000. The most abundant natural cyclodextrins are α-cyclodextrin (αCD), βCD, and γ-cyclodextrin (γCD), which contain six, seven, and eight glucopyranose units, respectively. Of these three cyclodextrins, βCD appears to be

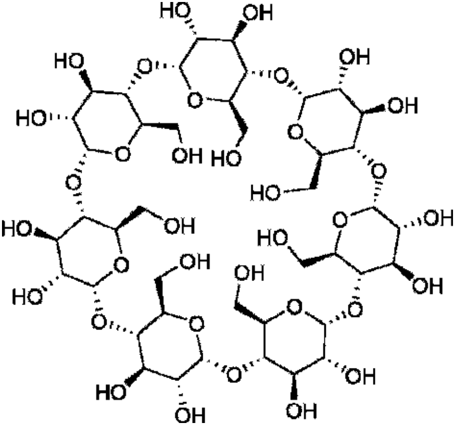


Figure 41.5 ► Structure β-cyclodextrin.

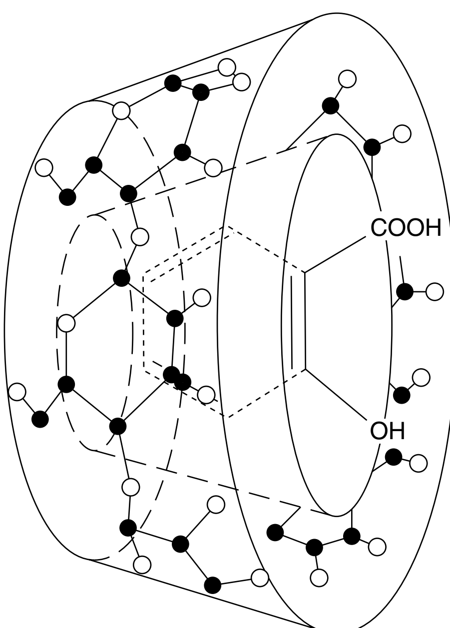


Figure 41.6 ► Structure of aspirin and β-cyclodextrin complex. (Reprinted with permission from [20].)

TABLE 41.8 ► Examples of Cyclodextrin-containing Pharmaceutical Products[19]

Drug/cyclodextrin	Trade name	Formulation
Alprostadil (PGE_1)/αCD	Prostavastin, Rigidur	IV solution
Itraconazole/HPβCD	Sporanox	Oral and IV solutions
Mitomycin/HPβCD	Mitozytrex	IV infusion
Voriconazole/SBEβCD	Vfend	IV solution
Ziprasidone mesylate/SBEβCD	Geodon, Zeldox	IM solution

αCD, α-Cyclodextrin; HPβCD, 2-hydroxypropyl-β-cyclodextrin; SBECD, sulfobutylether β-cyclodextrin.

the most useful pharmaceutical complexing agent because of its complexing abilities, low cost, and other properties.[20] Table 41.8 lists examples of marketed cyclodextrin-containing pharmaceutical products.[19] The major limitation of cyclodextrin complexes is toxicity, especially at high concentrations, which limits the dose level. Formation of cyclodextrin complexes requires specific molecular properties, and this approach may not work for certain compounds. Cyclodextrin derivatives with improved properties tend to be expensive.

Solid Dispersions

Solid dispersion systems can increase dissolution rate and bioavailability of water-insoluble drugs.[21–23] In solid dispersion systems, a drug may exist as an amorphous form in inert, hydrophilic polymeric carriers to form solid solutions (Figure 41.7). When they are exposed to aqueous media, the carriers dissolve, and the drug is released as very fine colloidal particles. This greatly reduces particle size and increases surface area, which results in improved dissolution rates and PO absorption. Furthermore, no energy is required to break up the crystal lattice of a drug (normally present in a crystalline solid dosage form) during the dissolution process. Drug solubility and wettability may be increased by surrounding hydrophilic carriers.

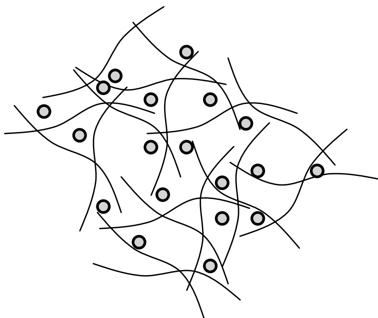


Figure 41.7 ► Amorphous solid solution.[22,24]

The methods used to prepare solid dispersions include the melting method, the solvent method, and the solvent wetting method.[23–25]Although solid dispersion is an area of active research, very few products relying on this technology have made it to the market (Table 41.9). The main reason is that solid dispersion is a high-energy metastable form. Phase separation, crystal growth, or conversion from the amorphous to the crystalline form during storage decrease solubility and dissolution rate and result in variable oral bioavailability.

Particle Size Reduction

If oral bioavailability is dissolution rate limited (not solubility limited), particle size reduction can increase the performance of the drug. The effect of particle size on the oral

TABLE 41.9 ▶ Examples of Marketed Products with Solid Dispersion Formulation[23]

Drug carrier	Trade name
Griseofulvin-poly(ethylene glycol)	Gris-PEG (Novartis)
Nabilone-povidone	Cesamet (Lilly)

bioavailability of a water-insoluble discovery compound is illustrated in Figure 41.8.[26] The smaller the particle size, the higher the in vivo exposure after PO dosing due to enhancement of dissolution rate. Microparticulate and nanoparticulate systems have particle sizes in the low micrometer to nanometer range. They can be delivered using all common routes of administration, that is, PO, injectable (IP, SC, and IM), and topical.

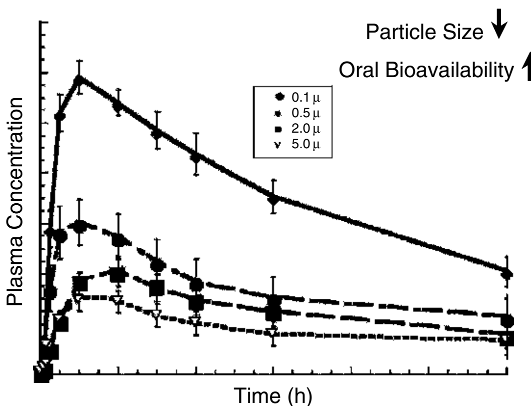


Figure 41.8 ▶ Effect of particle sizes on oral bioavailability of a discovery compound. (Reprinted with permission from [26].)

Many milling technologies have been developed for particle size reduction, such as the ball mill, fluid energy mill, cutter mill, hammer mill, pin mill, vibration mill, and media mill. Because only a small amount of material is available in drug discovery laboratories, industrial scale milling instruments may not be cost effective. A simple grinding apparatus, such as mortar and pestle or coffee mill, can be useful for reducing particle size to a narrow size distribution in order to increase oral bioavailability and reduce in vivo experimental variability due to wide particle size distribution. When particle size is small enough that dissolution is no longer a rate-limiting factor, variability due to food effect can be significantly reduced. A great advantage of nanoparticle technology is that drugs can be dosed at a significantly higher level than traditional approaches using co-solvents, for which dose is limited due to toxicity of the excipients. Figure 41.9 shows that a nanoparticle formulation of Taxol can be dosed at three times a higher level than the highest dose in the current commercial formulation of Cremophor EL/ethanol, which translates to greater efficacy.[26]

41.4 Practical Guide for Formulation in Drug Discovery

Although many advances have been made in formulating clinical dosages for humans, few reports have addressed formulation issues in preclinical studies.[2,7,27–29] The objective of preclinical in vivo studies affects formulation strategies.[29] For example, if the goal is to obtain an idea of oral efficacy, an optimal solution formulation is most effective in producing

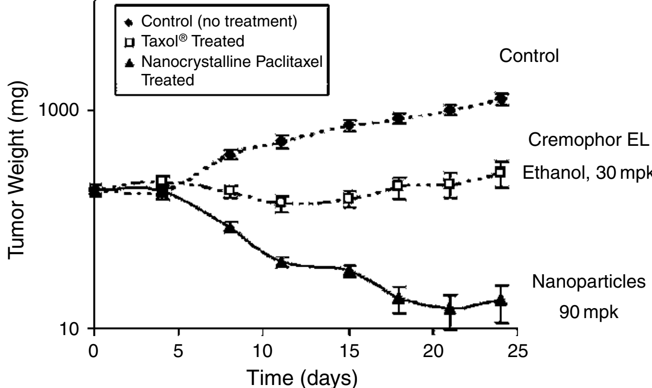


Figure 41.9 ▶ Effect of nanoparticles on efficacy of human lung tumor. (Reprinted with permission from [26].)

maximal exposure and reducing the variables in the studies compared to suspension or solid dosage form. On the other hand, if the purpose of an in vivo study is to explore the feasibility of developing the drug candidate into a commercial product, a more complex design is necessary using both solution and suspension formulation to determine both high and low solubility and dissolution effects on exposure. Different strategies are applied for PK, toxicokinetic, and pharmacodynamic studies.

41.4.1 Formulation for PK Studies

For early discovery PK screening, solution formulation is preferred to eliminate the effects of solubility and solid-state properties, such as crystal forms and particle size. Insufficient in vivo exposure will lead to a search for new and improved compounds through structural modification and the identification of reasons for low exposure (permeability, metabolism, etc.). Formulations should be tested for potential precipitation in simulated gastrointestinal fluids (simulated gastric fluid, simulated intestinal fluid, fasted state simulated intestinal fluid, fed state simulated intestinal fluid[30]). The same formulation should be used for the entire series of compounds within a discovery project for unbiased comparison and to minimize the potential impact of vehicle on the PK profiles. Although solution formulation is not always possible for all the compounds within a series, efforts should be made to formulate the first few compounds in the series to develop a robust vehicle for all the compounds in the projects. Table 41.10 lists possible vehicles for in vivo PK studies.[27]

TABLE 41.10 ▶ Possible Formulation Approaches for Discovery in Vivo PK Screening[27]

pH Adjustment and co-solvent	Surfactant solution	Lipid-based
pH buffers	Surfactants	Pure oil solution
Co-solvents:	Labrasol	Oil/buffer/surfactant
Polyethylene glycol	Tween 80	Emulsions
Propylene glycol	Cremophor RH 40	Micelles
Glycofurol 75	Lecithin	Liposomes
Glycerine		
Ethanol		
Transcutol		
pH buffers + co-solvents	Surfactant + co-solvents	

If compounds have excellent exposure using a solution formulation, PK studies with a suspension formulation should be initiated to (1) evaluate feasibility for solid dosage form, (2) predict exposure in toxicity studies, which is often performed in suspension formulation, and (3) measure exposure at maximum tolerated dose. If compounds have excellent exposure in a solution formulation but poor exposure with a suspension, physical properties can be modified to increase bioavailability by particle size reduction, such as micronization or nanoparticle technologies. Compounds with acceptable exposure in both solution and suspension formulations are considered for further development advancement.[27]

41.4.2 Formulation for Toxicity Studies

Suspension formulation with simple and safe excipients typically is used for acute and chronic toxicity studies. Vehicles often consist of a surfactant for wetting of particles (e.g., 2% Tween 80) and a bulking agent to increase viscosity and reduce sedimentation (e.g., 0.5% methylcellulose or hydroxyethyl cellulose). For hard-to-formulate or low systemic exposure compounds, due to low permeability or high metabolism, more sophisticated formulations (e.g., lipid-based or nanoparticle delivery systems) or a different route of administration (IV, IP, SC) can be used for toxicity studies.

41.4.3 Formulation for Pharmacological Activity Studies

Animal models are specific for the different diseases and therapeutic areas. Efficacy studies tend to be longer in duration and require more expensive animals (e.g., transgenic animals) compared to PK models. An optimal formulation is essential in order to demonstrate activity and proof of concept. Table 41.11 gives possible formulations for cardiovascular studies.[27]

TABLE 41.11 ► Possible Formulations for Efficacy Studies in Pharmacological Models[27]

Oral suspension	Oral solution	IV Solution
0.5% Hydroxyethylcellulose, wet milled	20%–50% PEG 400	20% PEG 400 in saline, 0.2 mL/kg (inject slowly)
0.5% Hydroxyethylcellulose/ 0.1% Tween 80, wet milled	3:1 (20%–50% glycofurol 75/Cremophor) to saline or buffer	50% PEG 400 in saline, 0.1 mL/kg (10-min infusion)
0.5% Hydroxyethylcellulose/ 50% Lipofundin, wet milled	Phosal 50 PG: mixture of 50% propylene glycol and 50% soybean lecithin	20%–50% glycofurol 75 in saline
N20 (10% soy bean oil in mixture)	Tween 80 (up to 10% in water)	20%–50% PEG/glycofurol/poloxamer 188 (39/10/1), in saline or buffer
Nanocrystals	1:1 Labrasol/Gelucire: diluted with 50%–90% water or buffer (emulsion)	3:1 (20%–50% glycofurol 75/Cremophor) to saline or buffer
	95:5 Miglyol/lecithin: to be homogenized with 50%–90% water (emulsion)	9:1 (20%–50% glycofurol 75/Solutol HS-15) to saline or buffer

Problems

(Answers can be found in Appendix I at the end of the book.)

1. What is the most common route of administration? What compound properties limit this route for some compounds?
2. What are advantages of IV administration?
3. Which of the following are advantages of formulation?: (a) reduce food effect, (b) reduce CYP inhibition, (c) reduce bioavailability, (d) increase stability, (e) achieve higher and earlier blood concentrations, (f) reduce phase II metabolism, (g) increase absorption.
4. What is an in situ salt, and how would you make one for a basic compound with $pK_a = 9.5$?
5. What three co-solvents are most commonly used in commercial drug products? What do they do?
6. Which of the following are effects of surfactants in formulations?: (a) incorporates insoluble compounds into micelles, (b) inhibits metabolism in the intestine, (c) surface effects reduce precipitation, (d) stabilizes particulate suspensions, (e) opens tight junctions for improved paracellular permeability.
7. What is a commonly used discovery formulation for in vivo dosing? What is the function of the components?
8. What different forms can lipid formulations take? Briefly describe each.
9. How do cyclodextrins work?
10. What aspects of a solid dispersion enhance intestinal solubility?
11. Why is it useful to reduce particle size?
12. Why is a solution formulation preferred for dosing in early discovery PK studies?
13. Why is it worthwhile to optimize a dosing formulation for efficacy studies?
14. What routes of administration can bypass first-pass metabolism?: (a) PO, (b) IP, (c) IM, (d) SC, (e) IV.

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