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Introduction

The stability of a drug product, which includes chemical and physical stability, is a key attribute for its efficacy and safety. This book focuses on the chemical aspect of drug stability. To have a drug product with desirable stability during production, shipment, storage, and throughout its expiration requires a clear understanding of drug degradation chemistry, so that a reasonably stable molecule can be selected as the pharmaceutical development candidate in the first place. This candidate will then be formulated with excipients to impart adequate pharmacokinetic properties onto the drug product. The excipients may stabilize, do nothing with, or destabilize the drug molecule. It would be desirable that the excipients would stabilize the drug molecule, or at least would not destabilize it. To achieve that goal, it is necessary to understand the chemical and physical interactions between the drug molecule and excipients, which also fall into the scope of drug degradation chemistry, or at least the part of the chemical interactions. In my previous book, *Organic Chemistry of Drug Degradation*, drug degradation chemistry was systematically summarized based on the types of degradation pathways such as hydrolytic, oxidative, and photochemical degradation. The current book, *Compendium of Drug Degradation Pathways*, is intended to be complementary to *Organic Chemistry of Drug Degradation* from the perspective of summarizing the known or reported degradation pathways of representative drugs in their entirety. There are more than 300 drugs that are discussed in the ensuing monographs. Before proceeding to the monographs, it would be helpful to have a brief overview of the regulatory perspective on drug stability, impurities, as well as drug degradation chemistry. Readers may need to refer to *Organic Chemistry of Drug Degradation* from time to time for a more comprehensive discussion on a particular degradation mechanism or pathway.

Drug Stability and Regulatory Requirements

The chemical stability of a drug is characterized by the persistence of its active ingredient(s), i.e., not degrading into degradation products, over its intended or registered shelf-life. The study of drug stability probably dates back to when

Compendium of Drug Degradation Pathways, First Edition. Min Li.

modern drug discovery and development started. The first publications on drug stability were released in the 1950s, according to Jamrógiewicz and Merchel [1]. In 1978, the pioneering book, *Chemical Stability of Pharmaceuticals*, was first published, and the second edition was released in 1986; the book provided "a collection of selected drug-stability data from the pharmaceutical literature" [2]. Nevertheless, it was not until 1987 when the US FDA issued the first guidance that required stability data in the submission of investigational new drug applications (INDs) as well as new drug applications (NDAs) [3]. Subsequently in April 1990, representatives of the regulatory agencies and industry associations from Europe, United States, and Japan convened in Brussels to inaugurate the international organization for pharmaceutical regulatory guidance – ICH, which stands for International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (formerly known as International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) [4]. In the early 1990s, ICH started to issue a series of guidance documents for performing the stability study and submission of stability data during the registration of new drug substances and products, starting from Q1, *Stability Testing of New Drug Substances and Products*. And the code Q1 was later revised to Q1A (and the most updated version is Q1A(R2)), as additional guidance documents on stability, Q1B through Q1E, were issued. The other ICH guidance documents that directly relate to the drug stability and drug impurities (including both process impurities and degradation products) are Q3A(R2), *Impurities in New Drug Substances*, and Q3B(R2), *Impurities in New Drug Products*. The above ICH guidance documents are summarized in Table 1.1 [5].

The main environmental factors influencing the drug stability over time are temperature, humidity, and light. Accordingly, Q1A(R2) stipulates the temperature and humidity requirements for typical long-term and accelerated stability studies to be carried out at 25°C, 60% relative humidity (RH) and 40°C, 75% RH, respectively, in order to assess the thermal stability of drug substances and products. For the drug

Table 1.1 ICH Guidance documents on drug stability and impurities.

Code	Document title
Q1A(R2)	Stability Testing of New Drug Substances and Products
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products
Q1C	Stability Testing for New Dosage Form
Q1D	Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products
Q1E	Evaluation of Stability Data
Q3A(R2)	Impurities in New Drug Substances
Q3B(R2)	Impurities in New Drug Products

Table 1.2 Storage conditions and time periods in Q1A(R2) and Q1B(R2) for stability studies of drug substances and products at the time of NDA submission.

Study[a]	Storage condition[b]	Minimum time period required at NDA submission
Long term	25°C, 60%RH	12 months
Intermediate	30°C, 65%RH	6 months
Accelerated	40°C, 75%RH	6 months

[a] Applicants of NDA may choose the intermediate condition as the long-term condition as appropriate, due to climate zone differences.
[b] The temperature variation allowed is ±2°C and the humidity variation allowed is ±5%.

substances or products that undergo significant degradation under the accelerated conditions of 40°C, 75% RH, an intermediate condition of 30°C, 65% RH is recommended for the stability study. Likewise, Q1B outlines the photolysis requirements, i.e., sources of light and duration of exposure, for photostability studies of drug substances and products, in order to assess the photostability of drug substances and products with and without packaging materials. The requirements of Q1A(2R) and Q1B are outlined in Table 1.2 [5].

The World Health Organization (WHO) also issued guidance on drug stability, particularly for Climate Zone III (hot and dry) and Climate Zone IV (hot and humid) regions, which provides the options of selecting 25°C/60%RH, 30°C/65%RH, or 30°C/75%RH as the long-term stability condition dependent upon the climate zones, where the drug products are intended to be marketed [6].

In order to help elucidate the intrinsic stability of drug molecules, stress testing, also referred to as forced degradation, is required in Q1A(R2), among which photostability testing is an integral part of the stress testing. The subject of stress testing will be discussed in detail later in this introduction.

On the other hand, Q3A(R2) and Q3B(R2) set the thresholds for impurities that need to be reported, identified, or qualified, once they exceed the respective thresholds, and these thresholds are summarized in Tables 1.3 and 1.4 [6].

Table 1.3 Thresholds for impurities in drug substances.

Maximum daily dose	Threshold (% of drug substance)		
	Reporting	Identification	Qualification
≤2 g	0.05%	0.10% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day intake (whichever is lower)
>2 g	0.03%	0.05%	0.05%

Table 1.4 Thresholds for degradation products in new drug products[a].

	Threshold (% of drug substance or total daily intake)		
Maximum daily dose	**Reporting**	**Identification**	**Qualification**
≤1g	0.1%	–	–
>1 g	0.05%	–	–
<1 mg	–	*1.0% or 5 μg TDI*[b]	–
1–10 mg	–	*0.5% or 20 μg TDI*[b]	–
>10 mg–2 g	–	*0.2% or 2 mg TDI*[b]	–
>2 g	–	*0.10%*	–
<10 mg	–	–	**1.0% or 50 μg TDI**[b]
10–100 mg	–	–	**0.5% or 200 μg TDI**[b]
>100 mg–2 g	–	–	**0.2% or 3 mg TDI**[b]
>2 g	–	–	**0.15%**

[a] The reporting thresholds are set based on two ranges of maximum daily doses (i.e., ≤ 1 g/day and >1 g/day), while both the identification threshold and qualification threshold are set based on four ranges of maximum daily doses.
[b] Whichever amount is lower; TDI, total daily intake.

Brief Overview of Drug Degradation Chemistry

The most frequently occurring drug degradation types are hydrolytic and oxidative in nature, followed by various other types, such as isomerization/rearrangement, elimination, decarboxylation, cyclization, dimerization, and photochemical degradation. The categorization of the degradation types is not absolute, as they may be interrelated, e.g., oxidation may trigger decarboxylation, cyclization, and dimerization, while oxidation, isomerization/rearrangement, elimination, decarboxylation, cyclization, and dimerization can often occur under photochemical degradation conditions.

Types of Functional Groups or Structural Moieties Susceptible to Hydrolytic Degradation

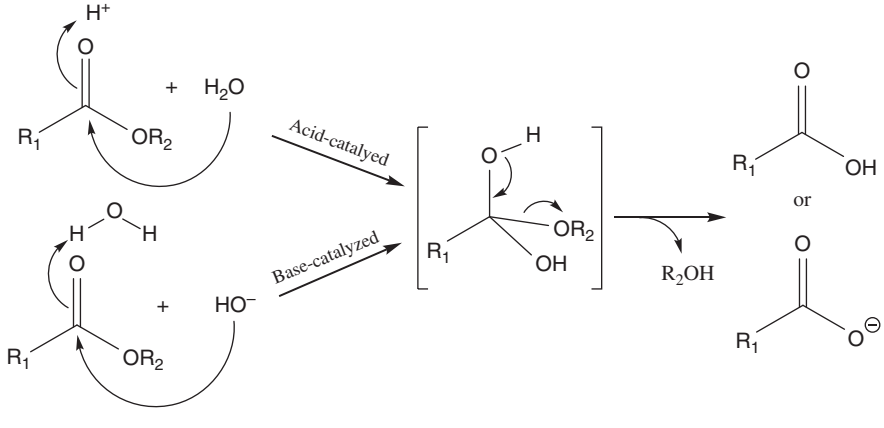
The susceptibility to hydrolytic degradation may be estimated based on the hydrolysis activation energy (*Ea*) or enthalpy of activation ($\Delta H^{\neq}$) of a compound bearing a hydrolyzable functional group [7]. Although *Ea* and $\Delta H^{\neq}$ are obtained from the different models describing the same reaction process, their numerical values are usually quite close to each other [8]. For this reason, these two types of values may be used interchangeably to evaluate the hydrolytic susceptibility of different hydrolyzable drug molecules. The lower the activation energy or enthalpy of activation, the easier it would be for the drug molecule to undergo hydrolytic degradation, and vice versa. The common functional groups or structural moieties in this category include, but are

not limited to, derivatives of carboxylic acid (esters, lactones, amides, imides, lactams, etc.), derivatives of phosphoric acid (esters and amides), carbamates, epoxides, ethers, polysaccharides, and nucleosides. The key factors that have impact on the intrinsic susceptibility of an organic compound to hydrolytic degradation are the steric and electronic factors surrounding the hydrolyzable moieties. While steric hindrance makes hydrolysis substrates of all types more stable, the presence of electron-withdrawing groups adjacent to the carbonyl groups of the hydrolysis substrates renders these carboxylic derivatives more susceptible to hydrolytic degradation [7]. From a different perspective, any factor destabilizing the transition states of hydrolytic degradation renders the hydrolysis substrates more resistant toward hydrolytic degradation [9, 10], while those (derivatives of both carboxylic and phosphoric acid) containing an easy-to-leave group are more susceptible to hydrolytic degradation [11, 12].

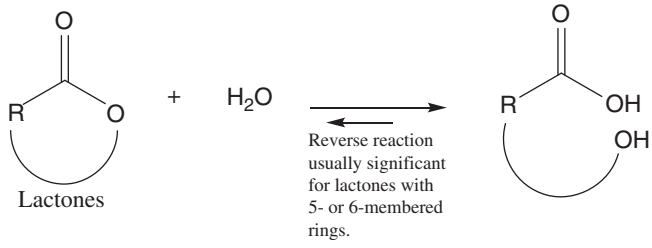
Carboxylic Esters and Lactones

Carboxylic esters, especially those without steric hindrance surrounding the ester linkage, are usually quite susceptible to hydrolytic degradation to produce carboxylic acids and alcohols, particularly under alkaline conditions (Scheme 1).

With increasing steric hindrance on either R_1 or R_2 group adjacent to the carbonyl group, carboxylic esters become more resistant toward hydrolysis. For example, while ethyl acetate only has a reported *Ea* value of 10 kcal/mol, hexyl acetate and *t*-butyl acetate have reported *Ea* values of 11.4 and 27 kcal/mol, respectively [13]. Aromatic carboxylic esters appear to be somewhat more stable than their alkyl counterparts, for example, the $\Delta H^{\neq}$ for hydrolysis of ethyl benzoate in a 0.1 M NaOH solution was reported to be approximately 82–84 kJ/mol (~20 kcal/mol) in the temperature range of 20–40°C, while the *Ea* of ethyl acetate was reported to be only 11.56 kcal/mol in a solution of 0.02 M NaOH [14, 15]. Lactones are cyclized esters, and particularly for lactones with a five- and six-membered lactone ring, their hydrolytic degradation under acidic conditions would usually reach an equilibrium point because the reverse reaction



Scheme 1 Hydrolysis of carboxylic esters.



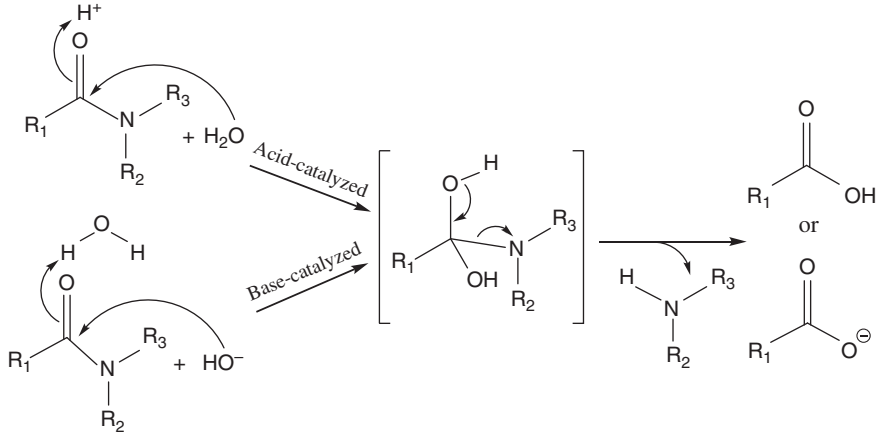
Scheme 2 Hydrolysis of lactones, and the reverse reaction would be significant for lactones with five- or six-membered rings, especially under acidic conditions.

(i.e., re-formation of the lactone ring) becomes increasingly significant as the hydrolysis goes on (Scheme 2) [16].

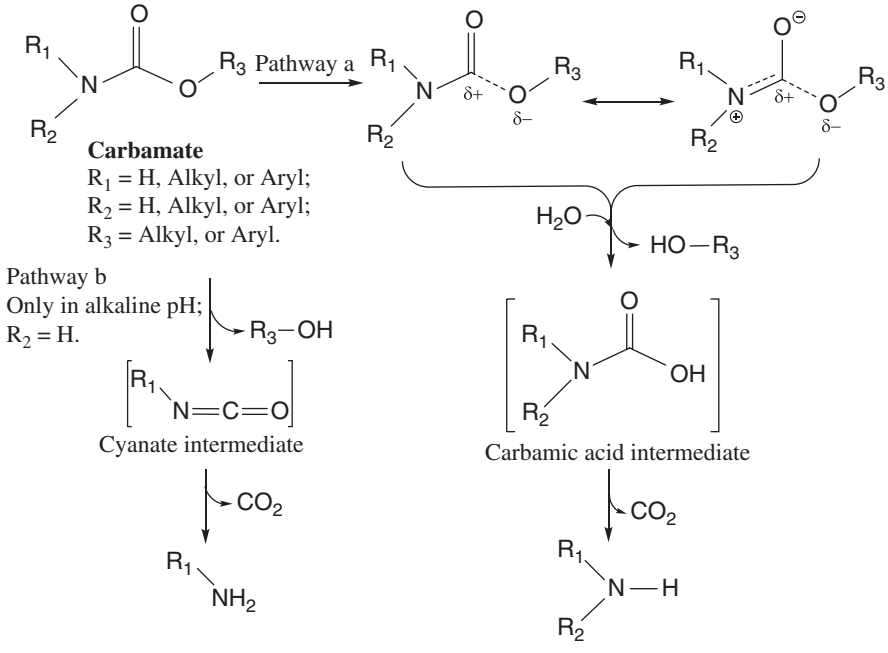
Carboxylic Amides and Lactams

Carboxylic amides and lactams are usually significantly more stable than the corresponding esters and lactones with regard to hydrolytic degradation, despite the fact that their hydrolysis mechanisms are similar to those of carboxylic esters and lactones, respectively (Scheme 3).

Consistently, typical hydrolysis activation energies of carboxylic amides and lactams are meaningfully higher, usually ~20 kcal/mol or higher. Nevertheless, if an electron-withdrawing group is present at the position alpha to the carbonyl, the amide becomes more susceptible to hydrolysis, because the carbonyl is more electron-deficient and thus more susceptible to attack by water or hydroxide ion. For example, the hydrolysis rate of trifluoroacetamide was reported to be several orders of magnitude higher than that of acetamide [17]. For this reason, the trifluoroacetyl group can be used as a protection group for amines in organic syntheses [18].



Scheme 3 Hydrolysis of carboxylic amides. R_1 = alkyl or aryl; R_2 = H, alkyl, or aryl; R_3 = H, alkyl, or aryl.



Scheme 4 Hydrolysis of carbamates.

Carbamates

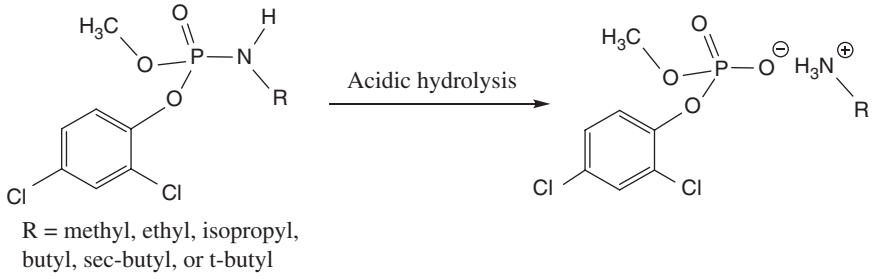
Carbamate is another carbonyl-based functionality often utilized in drug design [19]; structurally, it can be viewed as a hybrid of carbonyl ester and amide. A study by Dittert and Higuchi seemed to be indicated that the hydrolysis of a carbamate in alkaline solution could proceed via two mechanisms: the first one would start with the removal of the ester moiety, resulting in the formation of the unstable intermediate, carbamic acid, which quickly decomposes to an amine degradant and carbon dioxide (Pathway a, Scheme 4). The second one would involve the removal of the amide hydrogen, resulting in the formation of the isocyanate intermediate (Pathway b, Scheme 4) [20]. According to a hydrolytic stability study by Chapman in which *N*-phenyl-carbamates and *N*-cyclohexyl-carbamates were compared to their respective amide analogs, the carbamates were significantly more hydrolytically stable than the amides under acidic conditions, while the carbamates were slightly less stable than the amides under neutral conditions [21]. In a hydrolytic stability study of estramustine, an anticancer drug based on estradiol with a carbamate linkage to a nitrogen mustard, the hydrolytic enthalpy of activation was found to be 89.3 kJ/mol (21.3 kcal/mol) in the pH range of 1–9 [22]. This result is consistent with those obtained by Chapman, suggesting that the hydrolytic stability of carbamates is at least comparable to that of amides in general.

Phosphoryl Esters and Phosphoramidates

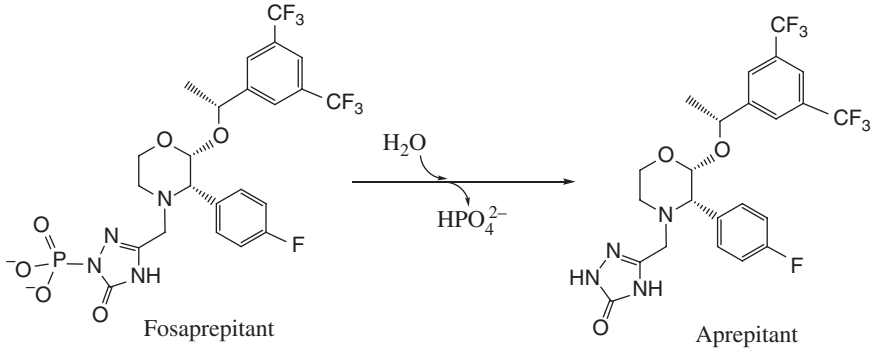
The phosphoryl functional group is often utilized to impart aqueous solubility to a drug molecule that contains either a hydroxyl or an amino group. In the former case, the resulting drug molecule is a phosphoryl monoester, while in the latter case, it is an

N-substituted derivative of phosphoramidic acid (amidophosphoric acid). Usually, the phosphoryl monoesters prepared from alkyl alcohols are quite stable hydrolytically. The hydrolysis activation energies of such phosphoryl monoesters are close to ~30 kcal/mol [23, 24]. On the other hand, *N*-substituted derivatives of phosphoramidic acid, or more commonly *N*-substituted phosphoramidates, are generally less hydrolytically stable than the structurally similar phosphates. For example, in a hydrolytic stability study of six 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates, the activation energies for the hydrolysis of the *N*-alkyl moiety (Scheme 5) in 0.19 M HCl solutions were found to be in the range of 10.1–13.2 kcal/mol [25]. Another example is fosaprepitant dimeglumine, which is the phosphoramidic pro-drug of aprepitant (Scheme 6). The activation energy for the hydrolysis of the phosphorylamidic linkage was determined to be 91 kJ/mol (22 kcal/mol) [26]. The above activation energies for the hydrolysis of the phosphorylamidic linkage are significantly lower than the activation energies of typical alkyl phosphoryl esters.

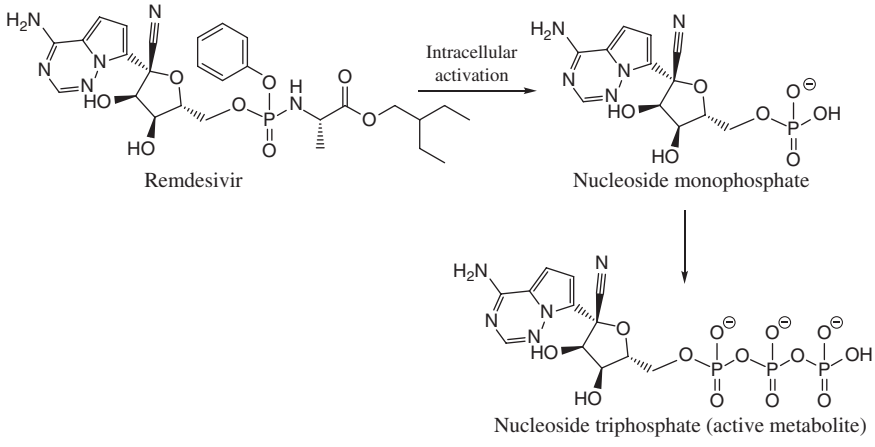
Nevertheless, the phosphoryl esters prepared from phenol or its analogs are not as stable hydrolytically as their alkyl counterparts, because phenol and its analogs are good leaving groups. For this reason, the phenyl phosphoryl functional group is usually used in the design of prodrugs where the free phosphate functionality needs to be released in vivo, such as in the case of anti-viral drug remdesivir (Scheme 7) [27].



Scheme 5 Hydrolysis of 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates.



Scheme 6 Hydrolysis of fosaprepitant. The counter ion, dimegluminium, is not shown for clarity.



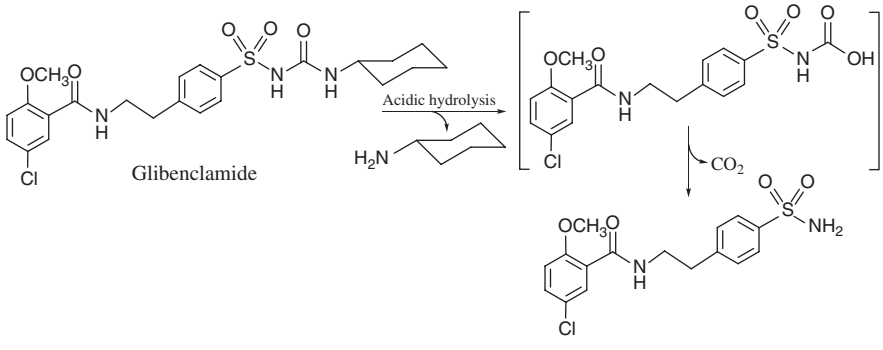
Scheme 7 Bio-activation of remdesivir, a prodrug containing phenyl phosphoramidate moiety.

Sulfonamides and Urea Derivatives

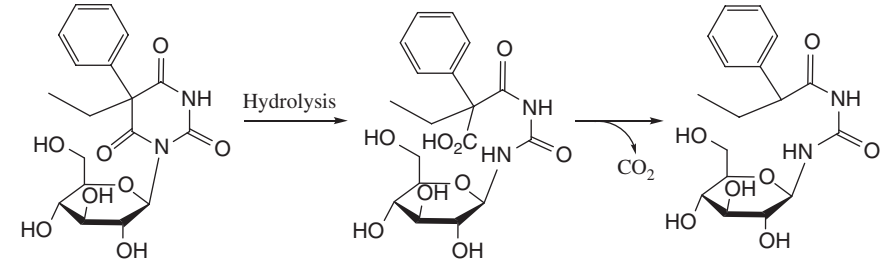
Theoretically, sulfonamide linkage is susceptible to hydrolysis; nevertheless, it is usually extremely stable under both acidic and alkaline hydrolytic degradation conditions, even under some very harsh conditions. The activation energy for a typical sulfonamide was estimated at greater than 30 kcal/mol [28]. Urea and its derivatives are generally resistant toward hydrolytic degradation, as evidenced by the fact that urea, the parent compound, was reported to have hydrolysis activation energies at approximately 30 kcal/mol in water and weakly to modestly acidic solutions [29]. Urea displayed pH-independent hydrolytic behavior under these conditions.

Imides and Sulfonylureas

Imides, particularly cyclic imides, are among the common building blocks utilized in drug design. The activation energies for hydrolysis of cyclic imides, such as phenobarbital and its derivatives (Scheme 8), were reported to be ~20 kcal/mol in



Scheme 8 Hydrolysis of glibenclamide under acidic conditions.

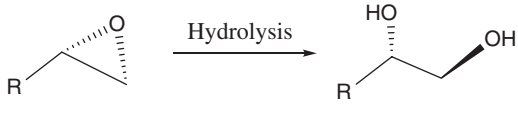


Scheme 9 Hydrolysis of phenobarbital *N*-glucoside.

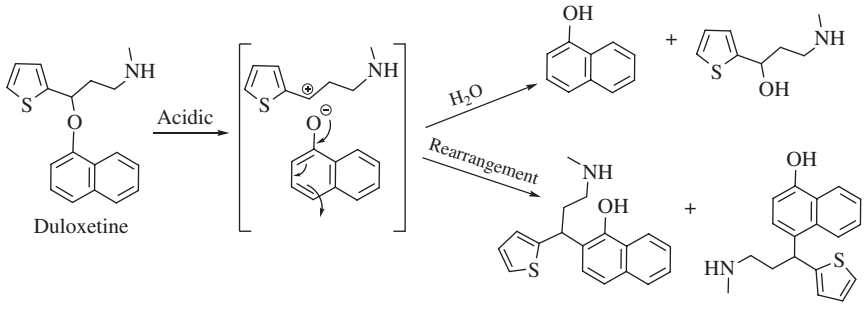
solutions of neutral pH [30], suggesting that they would be reasonably stable with regard to hydrolytic degradation under neutral conditions. On the other hand, sulfonylureas, such as glibenclamide, may be viewed as a class of hybrid imides. Due to the presence of the sulfonyl group, a strong electron-withdrawing group, the urea functionality becomes less resistant toward hydrolysis, particularly under acidic conditions (Scheme 9) [31]. Structurally similar sulfamylureas are also quite susceptible to acidic hydrolysis [32].

Epoxides and Ethers

Epoxides are electrophiles owing to the constrained tricyclic ether ring, which can be hydrolyzed or hydrated, particularly under acidic or alkaline catalysis, giving rise to *trans*-1,2-diol products (Scheme 10). The hydrolytic stability of epoxides depends upon the substituents surrounding the epoxide group [33]. For propylene oxide, a simple epoxide model compound, the hydrolysis activation energy was reported to be ~19.0–19.5 kcal/mol [34], suggesting it would be reasonably stable under uncatalyzed conditions. Ethers of other types are usually hydrolytically stable, except in cases where a relatively good leaving group coincides with a stabilizable transition state of the hydrolysis in the same molecular entity. The hydrolysis of duloxetine under acidic conditions is such a case in which 1-naphthalenol is a relatively good leaving group, while the resulting carbocation transition state can be stabilized by the neighboring thiophene group through conjugation; in addition to the straight hydrolytic products, the other two main degradants were isomers of duloxetine formed via rearrangement (Scheme 11) [35].



Scheme 10 Hydrolysis of epoxides.



Scheme 11 Degradation of duloxetine under acidic conditions.

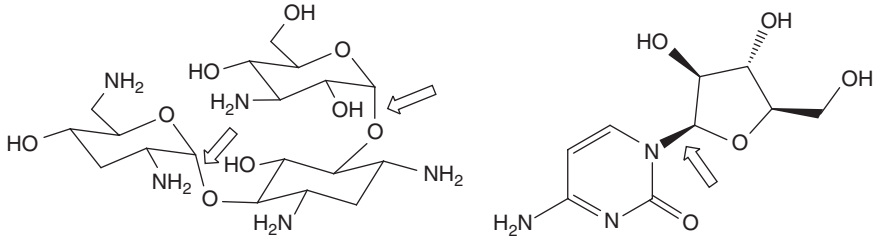


Figure 1.1 Structures of tobramycin and cytarabine. While the two glycosidic bonds in tobramycin may be susceptible to hydrolysis, the glycosyl bond in cytarabine is more resistant than the amino group toward hydrolysis.

Oligosaccharides/Polysaccharides and Nucleoside Analogs

The key linkage in oligosaccharides/polysaccharides and nucleosides is the glycosidic and glycosyl bond, respectively, with the glycosidic bond connecting the monosaccharide units of polysaccharides and glycosyl bond connecting the nucleobase (or its analog) and sugar moiety (Figure 1.1). The glycosidic and glycosyl bonds are usually quite stable hydrolytically; these bonds become less stable under alkaline conditions. For example, the hydrolysis activation energies for the glycosyl bond of tobramycin were reported to be 32 and 15 kcal/mol in 1 N HCl and 1 N NaOH solutions, respectively [36]. It needs to be pointed out that the glycosyl bonds of nucleosides are sometimes incorrectly referred to as glycosidic bonds.

Types of Functional Groups or Structural Moieties That Are Susceptible to Different Types of Oxidative Degradation

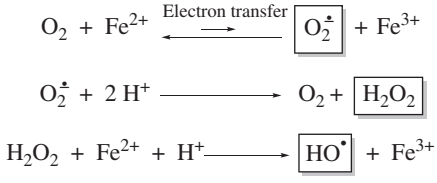
Oxidative degradation is another very common degradation pathway. The ultimate "oxidizing reagent" for drug oxidation originates from molecular oxygen, which constitutes ~21% of the atmosphere. The molecular oxygen at ground state is a diradical, which is a triplet species. Usually, the reaction between the triplet molecular oxygen and the vast majority of drug molecules, which are singlet species, is a kinetically forbidden process due to violation of the spin conservation rule [37]. The

activation of molecular oxygen is usually mediated by redox reactive transition metal ions, most notably ferrous ions (Scheme 12), during which process three reactive oxidative species (ROS), i.e., superoxide anion radical, hydrogen peroxide, and hydroxyl radical, are generated.

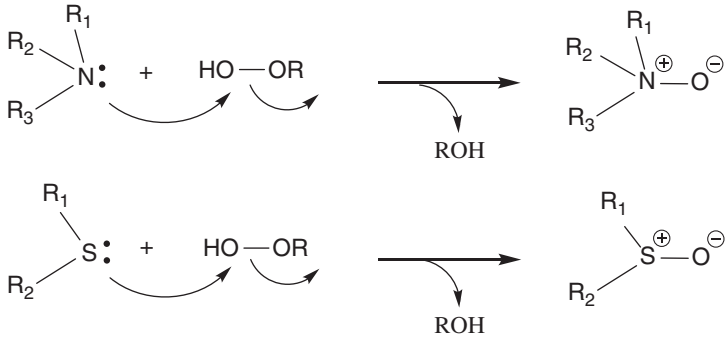
Nucleophilic Oxidation

Tertiary amines and thioethers, particularly the alkyl ones, are capable of reacting with hydroperoxide species (including the prototype hydrogen peroxide; Scheme 13), which are often present in polymeric excipients such as polyethylene glycol (PEG) and polysorbate (e.g., Twin 80), as a result of auto-oxidation [38].

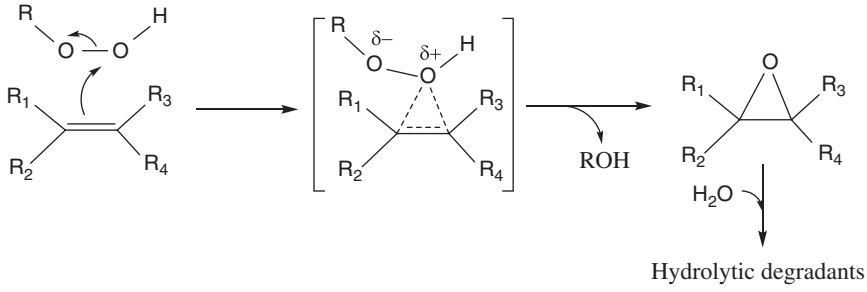
Certain compounds containing electron-rich carbon–carbon double bonds can also undergo analogous nucleophilic oxidation (Scheme 14); this type of compound



Scheme 12 Activation of molecular oxygen by ferrous ion Fe^{2+}, a redox reactive transition metal ion.



Scheme 13 Nucleophilic oxidation of tertiary amines and thioethers.



Scheme 14 Nucleophilic oxidation of compounds containing electron-rich carbon–carbon double bonds.

includes those that possess an indole ring, which can be viewed as an enamine (*N*-substituted carbon–carbon double bond).

Free-Radical-Mediated Oxidation

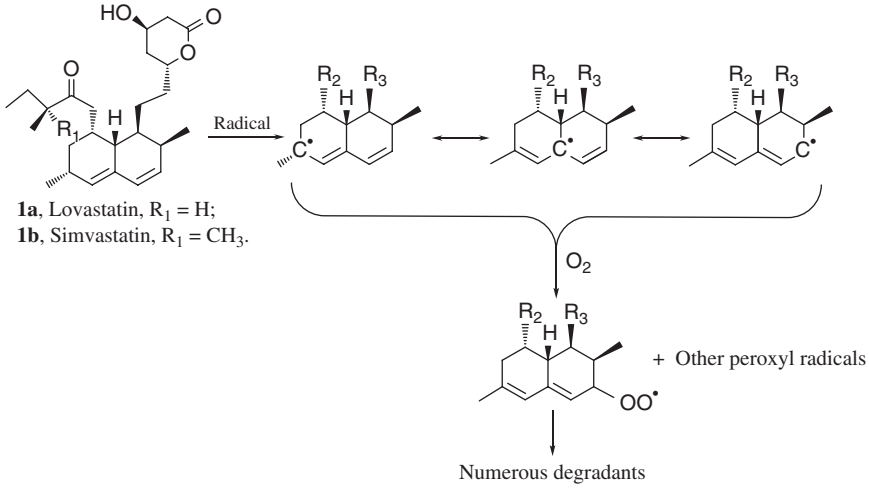
Free-radical-mediated oxidation is probably the most common and yet most complicated type of oxidation. The structural moieties that are susceptible to this type of oxidation include the CH or CH_2 positions that are alpha to carbon–carbon double bonds (particularly to conjugated carbon–carbon double bonds), CH or CH_2 positions that are alpha to a carbonyl group, and benzylic positions. An example is given for the facile free-radical-mediated oxidation of the butadiene moiety that is embedded in the core structures of lovastatin and simvastatin (Scheme 15) [39].

Base-Catalyzed Oxidation

This type of oxidation, also known as carbanion/enolate-mediated auto-oxidation, typically occurs in a group of compounds that contain somewhat "acidic" CH or CH_2 moieties. A classic example is the oxidation of rofecoxib (Scheme 16). The mechanism of the oxidation was originally proposed as free-radical mediated, but was later found to be mediated by carbanion/enolate [40, 41]. The rate of the base-catalyzed oxidation would be two orders of magnitude higher than that mediated by free radicals.

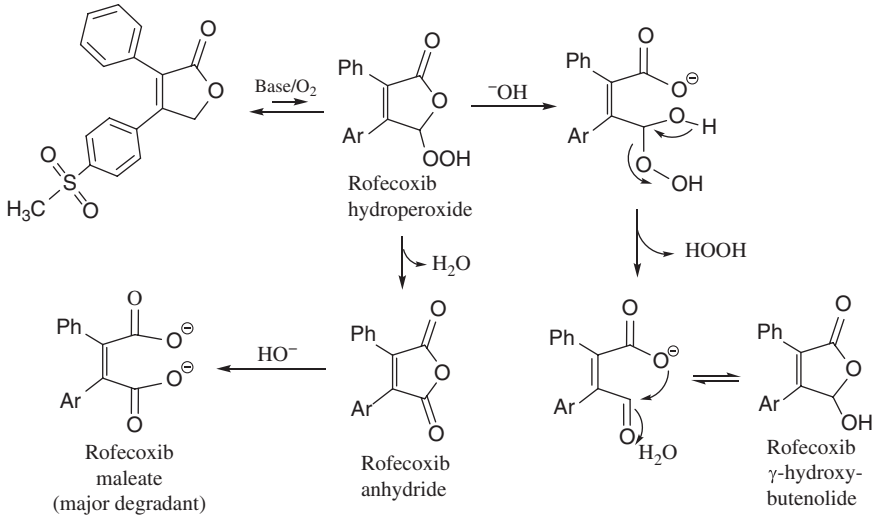
Oxidation Via the Udenfriend Reaction

The Udenfriend reaction is an oxidative degradation pathway that can occur in formulated drug products containing both a metal ion chelator and a reducing agent [38]. The role of the chelator, which is typically EDTA or citrate, is to form a chelating complex with the reduced form of a redox-reactive transition metal ion, most commonly ferrous ion (Fe^{2+}). The resulting complex, Fe^{2+}{EDTA},



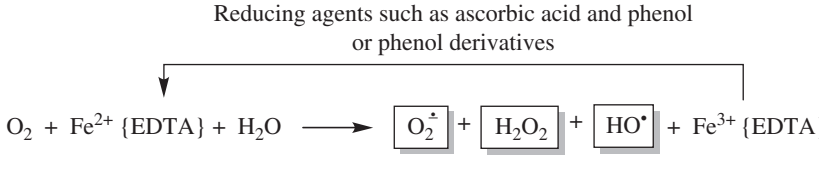
1a, Lovastatin, R_1 = H;
1b, Simvastatin, R_1 = CH_3.

Scheme 15 Free-radical-mediated oxidation of the butadiene moiety embedded in the core structures of lovastatin and simvastatin.



Scheme 16 Base-catalyzed oxidation of rofecoxib.

would be easier to transfer an electron to molecular oxygen than the unchelated Fe^{2+}, resulting in the formation of superoxide anion radical. The latter species can form hydrogen peroxide via disproportionation, and hydrogen peroxide can then, in turn, be transformed to hydroxyl radical via the Fenton reaction. During this process, which is the activation of molecular oxygen, two equivalents of Fe^{2+}{EDTA} are converted into the oxidized form of Fe^{3+}{EDTA}. In the presence of a reducing agent, such as ascorbic acid or a phenolic compound, Fe^{3+}{EDTA} can be reduced back to Fe^{2+}{EDTA}, causing the activation of molecular oxygen and subsequent oxidative degradation to continue until all the reducing agent is consumed. This whole process of the Udenfriend reaction can be illustrated in Scheme 17, which indicates that a reducing agent could actually promote oxidative degradation of a drug product if it also contains a chelating agent in its formulation [42–46].

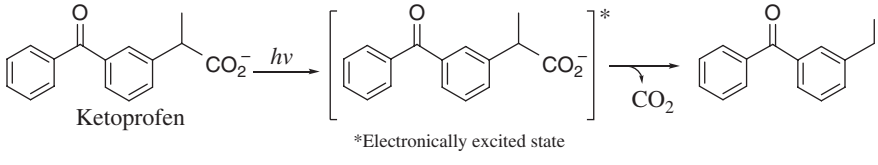


Scheme 17 Schematic presentation of the Udenfriend reaction in which molecular oxygen is activated continuously in the presence of a metal ion chelator and a reducing agent, resulting in the formation of three reactive oxygen species: superoxide anion radical, hydrogen peroxide, and hydroxyl radical.

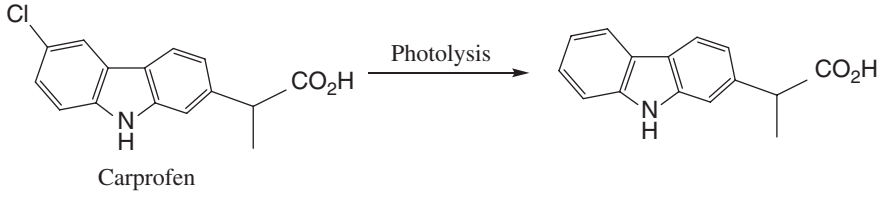
Types of Functional Groups or Structural Moieties That Are Susceptible to Photochemical Degradation

Photochemical reactions are those that take place at electronically excited states. Hence, the prerequisite for a drug molecule to undergo photochemical degradation is that it needs to have a chromophore that would have adequate absorption under the photolytic conditions, for the molecule to be elevated into electronically excited states. The excited states will return or relax to the ground state via several passages, one of which is a chemical transformation, i.e., a photochemical reaction, while others are physical processes such as light emission and collision-induced relaxation [47]. Some compounds lack appropriate chromophores but nevertheless can form chromogenic complexes with metal ions, thus making themselves photochemically vulnerable [44]. Some of the most common moieties that are susceptible to photochemical degradation include benzophenone and its analogs (any two aryl groups connected by a carbonyl), halogenated aryl compounds, and substituted alkenes (e.g., stilbene and its analogs). Several nonsteroidal anti-inflammatory drug (NSAID) molecules contain benzophenone or its analogous moieties in conjunction with a nearby carboxylic group; the resulting 2-arylpropionic acid moieties can usually undergo photochemical decarboxylation (Scheme 18) [48, 49].

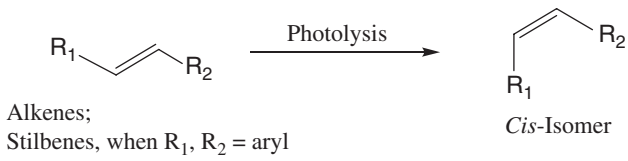
Halogenated aryl drugs, such as carprofen, are susceptible to photochemical dehalogenation (Scheme 19); nevertheless, such dehalogenation tends to occur in solution photolysis and usually would not take place in the solid state, except for iodoaryl compounds. In solution photolysis, if protic solvents such as water and methanol are used, the corresponding hydroxyl- and methoxyl-substituted photodegradants may also be formed. In such cases, the methoxyl-substituted photodegradants are artificial degradants as methanol is usually not a pharmaceutical excipient.



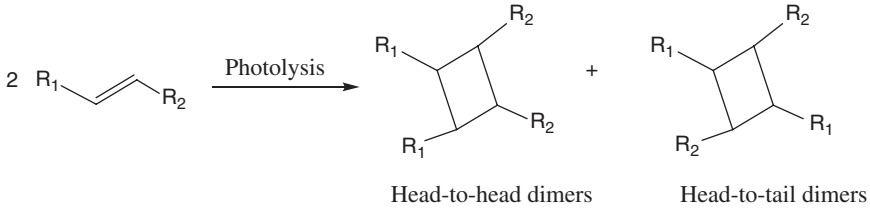
Scheme 18 Photodecarboxylation of ketoprofen, a 2-arylpropionic acid derivative of NSAID.



Scheme 19 Photo dehalogenation of carprofen.



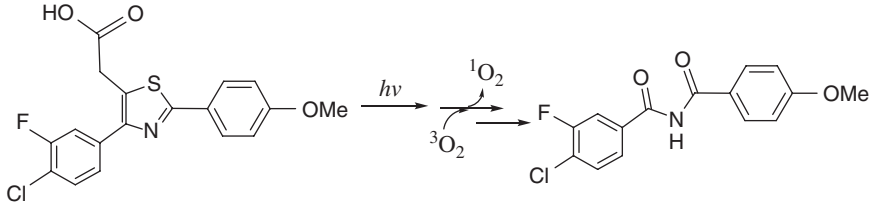
Scheme 20 Photoisomerization of alkenes, including stilbenes and analogs where R_1 and R_2 are aryl groups.



Scheme 21 2 + 2 Photodimerization of alkenes.

The carbon–carbon double bonds in alkenes can undergo photoisomerization (Scheme 20) and such isomerization becomes significant when the substituents of the double bonds render the resulting alkenes to have adequate absorption in UV and even visible regions. Analogous carbon–heteroatom double bonds can also be susceptible to similar photoisomeric degradation. Another important photodegradation pathway for alkenes is the 2 + 2 photodimerization (Scheme 21).

Drug molecules containing multiple aryl groups, either connected or fused, tend to undergo photosensitization during which process molecular oxygen, a triplet molecule, is excited to become very reactive singlet oxygen through collisional energy exchange with the excited state of the aryl rings. The singlet oxygen generated can easily inflict oxidative degradation on the drug molecule itself, such as in the example of an experimental drug containing a substituted thiazole ring (Scheme 22); this photodegradation pathway mediated via singlet oxygen is referred to as type II photosensitized oxidation [50].



Scheme 22 Photosensitized oxidation of an experimental drug containing an aryl-substituted thiazole ring.

Degradation Due to API-Excipient Interactions

The Maillard Reaction

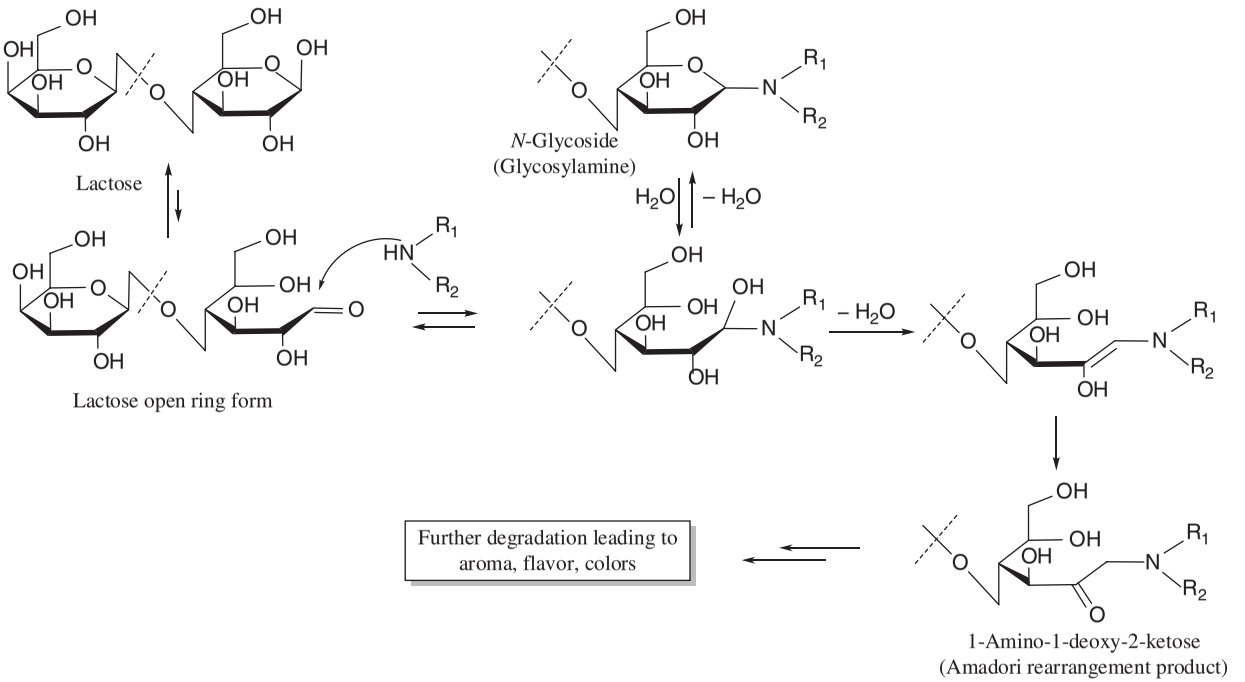
The most common interaction that occurs between active pharmaceutical ingredients (APIs) and excipients is probably the one between primary and secondary amine API molecules with reducing sugars such as lactose, which is known as the Maillard reaction. The Maillard reaction is a very complicated process consisting of hundreds of reactions and its pharmaceutical relevance is primarily limited to the first few steps leading to the formation of the so-called Amadori rearrangement products (Scheme 23) [51].

Degradation Due to Impurities in Excipients – Formaldehyde, Peroxides, Formic Acid, etc.

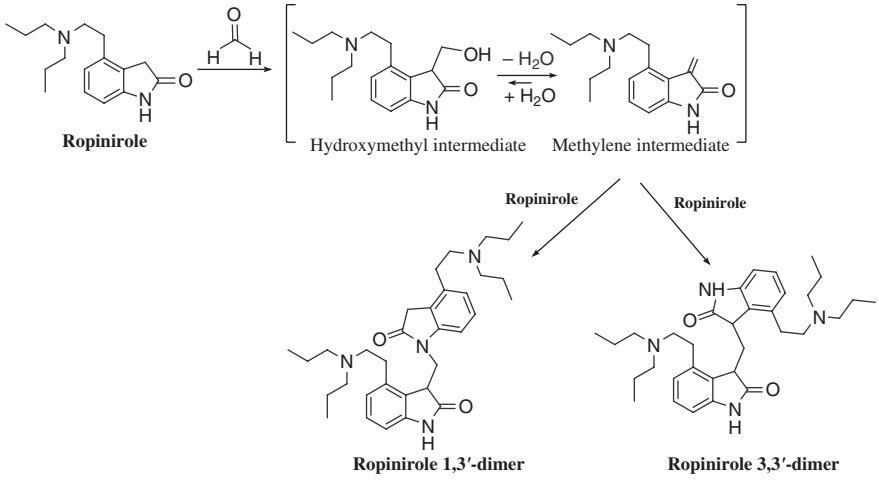
Pharmaceutical excipients contain impurities, some of which are of chemical reactivity and thus capable of reacting with API. The frequently seen impurities in excipients are formaldehyde, peroxides (including hydrogen peroxide), and formic acid, which result from auto-oxidative degradation of certain polymeric excipients such as PEG, polysorbate, and PVP (povidone) [51]. Formaldehyde is an electrophile that can react with API capable of nucleophilicity to form hydroxymethyl intermediates and then dimeric degradants, as in the case of two dimeric degradants of ropinirole (Scheme 24) [52]. Peroxides mainly inflict nucleophilic oxidative degradation of tertiary amines and thioethers (refer to Scheme 13). Formic acid can react with primary and secondary amine API to form *N*-formyl degradants, which is a partial source for this type of degradant, with the other source being from further degradation products of the Amadori rearrangement [53]. Formic acid appears to be an agent that may cause rare reductive degradation as in the case of *N*-methylvarenicline, a degradant of varenicline (Scheme 25) [54].

Formation of *N*-nitrosamines Due to the Reaction of Secondary Amine APIs or API-Related Fragments with Residual Nitrites in Excipients

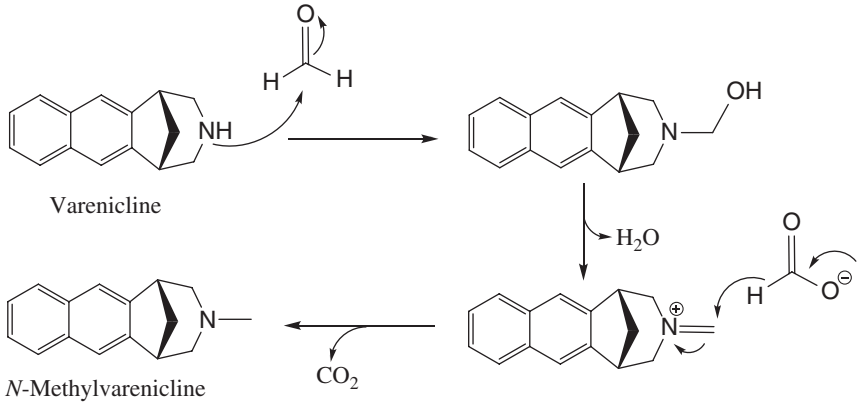
In mid-2018, it was reported to the US FDA and other regulatory agencies that *N*-nitrosodimethylamine (NDMA), an animal carcinogen, was present in valsartan in trace amounts by a manufacturer of the API [55]. Subsequently, NDMA and other low molecular weight *N*-nitrosamines (also referred to as small molecular weight *N*-nitrosamines), e.g., *N*-nitrosodiethylamine (NDEA) and *N*-nitroso-4-(methylamino) butyric acid (NMBA), were found in valsartan and other ARB drugs (angiotensin receptor blockers) [56]. These events prompted the regulatory agencies and the industry to look deeper into the root causes for the occurrence of *N*-nitrosamine impurities in drug products. Starting from 2019, NDMA was found to be present in drug classes other than ARBs, e.g., ranitidine, nizatidine, and metformin [57]. While NDMA is typically a process impurity for ARB drugs, it appears to be a degradant for the latter three drug products. In July 2021, it was announced that varenicline, a secondary amine smoking-cessation drug, contained *N*-nitroso varenicline by the original innovator of the drug [58]. Since that moment, it has become evident that the drugs with secondary amine APIs would be most



Scheme 23 The Maillard reaction between primary and secondary amine API with lactose, a very common reducing sugar excipient.



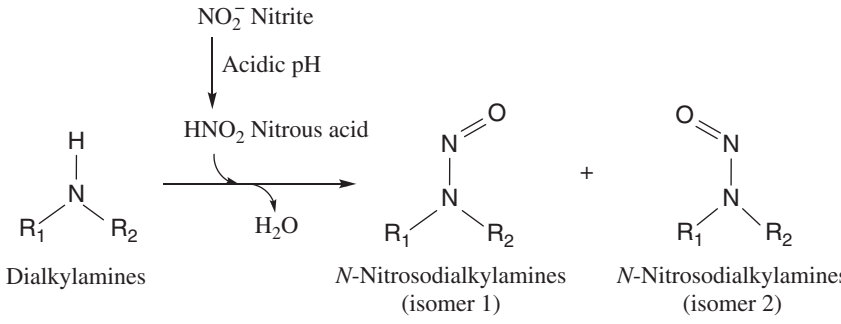
Scheme 24 Proposed mechanism for the formation of two ropinirole dimeric degradants [52].



Scheme 25 Formation of *N*-methylvarenicline.

vulnerable towards the formation of *N*-nitrosamine impurities, particularly for the drugs formulated with excipients that contain uncontrolled amounts of residual nitrites. This is because secondary amine APIs can readily react with residual nitrites, particularly in a somewhat acidic microenvironment of the formulations to form API-related *N*-nitrosamines (Scheme 26).

The *N*-nitrosamines of the secondary amine APIs and their secondary amine intermediates are now categorized as part of the "nitrosamine drug substance-related impurities" (NDSRIs), with the remaining part being *N*-nitrosamines of secondary amine fragments of the APIs, which are typically process impurities but could be degradants as well in certain cases. It is obvious that the highest risk for the



Scheme 26 Reaction of dialkylamines with nitrous acid to form *N*-nitrosodialkylamines. When $R_1 \neq R_2$, *E/Z* isomerism occurs. If R_1 or R_2 is replaced by an aryl substituent, the reactivity toward nitrous acid would be significantly reduced.

formation of NDSRIs comes from the secondary amine APIs, and therefore, as long as the secondary amine API-derived NDSRIs are controlled, the risk from their intermediates and fragments would be negligible since the amounts of the intermediates and fragments are usually three orders of magnitude lower than their APIs and all of them should have similar reactivity toward nitrous acid due to structural similarity. On the other hand, certain reducing agents, such as ascorbic acid and caffeic acid, can inhibit the formation of NDSRIs in drug products with secondary amine APIs [59]. For drug products with tertiary amine APIs, the main risk usually originates from their secondary amine impurities, despite the fact that the tertiary amine APIs could react with nitrous acid to eventually produce *N*-nitrosamines via a few intermediary steps. It seems that such multiplicity in the reaction mechanism would dramatically reduce the efficiency for the tertiary amine APIs to react with nitrous acid, which was reported to be only approximately 1/1,000 of the structurally similar secondary amines [60]. In the laboratories of the present author, we compared the efficiencies of triethylamine and diethylamine to react with nitrous acid, respectively, under the same conditions and found that triethylamine was only able to produce approximately 1/50 to 1/200 quantities of NDEA as compared to those by diethylamine [61]. An exception to the sluggishness of the tertiary amine reactivity toward *N*-nitrosamine formation was observed with those tertiary amines containing an electron-rich benzylic-type substituent [62].

Forced Degradation Study and Prediction of Real-Life Degradation Pathways

Forced degradation is also referred to as stress testing or simply stress. According to the ICH guidance document Q1(R2), *Stability Testing of New Drug Substances and Products*, "Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures

used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved" [5]. This statement clearly defines the purpose and significance of stress testing. Due to the complexity of the vast variety of drug molecules, ICH guidance only provides general principles for the stress testing of drug substances, except for the photolytic stress in solid state, where the light sources and duration for the exposure are clearly defined in Q1B [5]. At the high level, Q1A(R2) requires that the effect of temperature (typically in 10°C increments above that for the accelerated stability study), humidity (typically at 75%RH or higher), oxidation, and photolysis be evaluated on a drug substance. Furthermore, the susceptibility of the drug substance to hydrolysis under a wide range of pH also needs to be assessed.

In industry practice, there appears to be a wide variety of ways for performing stress testing or forced degradation [63, 64]. For the selection of hydrolytic conditions, although hydrochloric acid (HCl) and sodium hydroxide (NaOH) are usually the choices of selection for acidic and alkaline stress, respectively, the strengths of the acid and base, temperature, and duration of the hydrolysis can vary significantly without clear justification. With regard to oxidative stress, most studies seem to use hydrogen peroxide as the oxidizing reagent; nevertheless, hydrogen peroxide can usually only help predict nucleophilic oxidation of a drug molecule that contains tertiary amines, thioethers, or other structural moieties that are susceptible to nucleophilic oxidation, as explained above.

There have been efforts in trying to come up with best practices for performing forced degradation studies to maximize the predicting capability of stress testing, while minimizing the production of artificial degradation [65–68]. Such best practices focus on purposefully designed or mechanism-based stress conditions [68], which rely on the perceived degradation mechanism of the target drug molecule, as well as on the consideration of its degradation kinetics that would be correlated to the long-term and accelerated stability studies. Specifically, for the oxidative stress, not only hydrogen peroxide (typically not exceeding 3% concentration) needs to be used, but a free radical initiator such as AIBN or other initiators should also be employed for those drug molecules containing structural moieties or functional groups that are susceptible to free radical-mediated oxidation (e.g., benzylic positions, methylene positions alpha to double bonds and conjugated double bonds) [69]. Reynolds et al. reported the use of *N*-methylpyrrolidone (NMP)-water-air-heat system as a stress tool that can generate degradants from free radical-mediated and nucleophilic oxidation as well as hydrolytic degradation [70]. In the laboratories of the present author, benzoyl peroxide was also found to be capable of predicting oxidative degradants resulting from both free radical-mediated and nucleophilic oxidation [71]. For certain drug molecules, redox-active transition metal ion-mediated oxidation may be a significant pathway, although it was considered not relevant on a routine basis by certain researchers [68]. In those relevant cases, metal ions such as Fe^{2+}/Fe^{3+} may be used. As a matter of fact, metal-ion-mediated stress is a requirement by the Brazilian regulatory authority ANVISA [72]. For certain drug molecules containing "acidic CH or CH_2," it may be necessary to examine their susceptibility to base-catalyzed oxidation, which would be more favored in alkaline solutions prepared with a majority of aprotic solvents. The recommended conditions for oxidative stress studies are

Table 1.5 Recommended conditions for oxidative stress studies.

Type of oxidation	Recommended conditions	Notes
Nucleophilic	0.1%–3% H_2O_2, room temperature, 24 hours, protected from light	Higher temperature or under light may induce irrelevant degradation
Free radical-mediated	AIBN or benzoyl peroxide	For a mostly aqueous system, 4,4′-azobis(4-cyanovaleric acid) (ACVA), a water-soluble analog of AIBN, can be used [63]
Base-catalyzed	Aprotic solvent–water system with 1% of 1 M NaOH solution in methanol	Most common aprotic solvent: acetonitrile
Metal-ion-catalyzed	5 mM $FeSO_4$ and/or $FeCl_3$, 30°C, 2 weeks, protected from light	A combination of Fe^{2+}/Fe^{3+} may be used

Table 1.6 Recommended conditions for hydrolytic stress studies.

Type of stress	Recommended conditions	Notes
Acidic	0.1 N HCl, 60°C, 2 days or 1.0 N HCl, 60°C, 2 hours	H_2SO_4 may be used in place of HCl
Alkaline	0.1 N NaOH, 60°C, 2 days or 1.0 N NaOH, 60°C, 2 hours	KOH may be used in place of NaOH

summarized in Table 1.5, based on the recommendations reported in the literature as well as on the experience in the laboratories of the present author.

For the reagents of hydrolysis, 0.1 N HCl and 0.1 N NaOH solutions are generally used as the starting point [63]. While the temperature and duration of the hydrolytic stress can vary dramatically, the recommended conditions based on the literature as well as the experience at the laboratories of the present author are provided in Table 1.6; these conditions are usually strong enough to hydrolyze a typical amide bond (*Ea* ~20–25 kcal/mol) within the desirable range of ~5–20%.

For the stress of solid drug substances under dry heat or heat/moisture conditions, 60°C for 3 weeks would be approximately equivalent to 40°C for 6 months, assuming the type of degradation follows the Arrhenius equation with an activation energy (*Ea*) of ~25 kcal/mol. For the photo stress of solid drug substances, the photolysis conditions recommended by ICH Q1B are generally followed. In cases where photo stress study in solution state may be needed, the photolysis may be conducted with laboratory UV lighting or sunlight (Table 1.7).

There is no specific guidance for the stress testing of the finished drug products other than the photo stress testing, which is outlined in ICH Q1B. The heat and heat/moisture stress conditions typically used for API (e.g., those listed in

Table 1.7 Recommended conditions for stress studies in solid state.

Type of stress	Recommended conditions	Notes
Dry heat	60°C, ambient moisture, 3 weeks	[a]
Heat/high moisture	60°C, minimum 75% RH, 3 weeks	[a]
Heat/low moisture	60°C, 30% RH, 3 weeks	[a]This stress may be adequate for certain molecules susceptible to dehydration
Photolysis	Per conditions outlined in ICH Q1B	With and without packaging

[a] Assuming *Ea* of ~25 kcal/mol.

Table 1.7) can generally be directly applicable to the finished drug products. On the other hand, stress in solutions is generally not quite relevant to the finished drug products unless the dosage form is in liquid formulation. Preferably, the forced degradation study for solid dosage forms can be combined with the compatibility study that is typically conducted during the formulation development stage. The compatibility study is usually performed on a one-to-one basis between the API and each excipient. Sometimes, compatibility study on a one-to-two or more basis may be warranted.

Overall, the practices in stress or forced degradation studies vary significantly among the practicing scientists, despite the efforts to streamline a best practice that would be applicable in the vast majority cases. Nevertheless, it is now generally agreed that the level of the degradation should be controlled in ~5–20% range in order to avoid excessive degradation, which typically would result in irrelevant degradation profiles. While artificial degradants usually cannot be avoided completely, preferably the degradation profiles obtained under stress conditions will include the real degradation profiles, the ones obtained under the long-term stability conditions. At the same time, the practicing scientists should be able to distinguish the artificial degradants, such as the ones derived from the involvement of the organic cosolvents used to solubilize the drug substances in solution stress studies, from the real degradants.

Concluding Remarks

This introduction is intended to provide a quick overview of the most common drug degradation chemistry and pathways. For an in-depth knowledge in this regard, readers are encouraged to refer to the relevant chapters of *Organic Chemistry of Drug Degradation*. The 300+ drug molecules covered in the monographs of the current book, *Compendium of Drug Degradation Pathways*, would still represent a tiny fraction of all the drug products on the market. The present author hopes that readers can extrapolate from the examples in the monographs to other drug molecules based upon structural similarity and analogy of degradation chemistry.

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