

REVIEW ARTICLE

## Hydrolysis in Pharmaceutical Formulations

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### ABSTRACT

*This literature review presents hydrolysis of active pharmaceutical ingredients as well as the effects on dosage form stability due to hydrolysis of excipients. Mechanisms and measurement methods are discussed and recommendations for formulation stabilization are listed.*

### INTRODUCTION

#### Scope

Hydrolysis is the cleavage of a chemical species by water. Since water, either as a solvent or in the form of moisture in the air, contacts most pharmaceutical dosage forms to some degree, the potential for this degradation pathway exists for most drugs and excipients. In this review article, many aspects of hydrolysis are discussed, mainly with a focus on pharmaceutical formulations. In addition, the use of hydrolysis to effect desired changes is reviewed briefly with respect to prodrugs and

controlled release systems. Methods of predicting and measuring hydrolysis are covered such that a formulator is aware of hydrolysis problems early in the formulation process. The mechanisms of drug hydrolysis are outlined as well as the role of excipient hydrolysis in affecting drug stability or dosage form performance. Finally, most of the methods available for alleviating hydrolysis problems are discussed, including formulation modifications and packaging options.

The drug types included in this review span the range from small molecules to large molecules such as DNA and proteins. Dosage forms are divided into two broad categories: liquids and solids. Solid dosage forms can be

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further divided into traditional solids, such as tablets and capsules, and more complex solid formulations such as lyophiles. Liquids can be divided into aqueous based and non-aqueous dosage forms. The latter includes dispersions, oils, and polar solvents such as PEG400 and alcohols. Although there is no explicit discussion on the various uses of these dosage forms (e.g., transdermal, suppositories, depots, etc.), the general discussions of this review should provide the formulator with sufficient information to address hydrolysis issues for most systems.

### **Preliminary Screening for Hydrolytic Instability**

#### **Purposeful Degradation (1)**

Independent of the ultimate dosage form, forced drug degradation by exposure of drug solutions to acidic or basic conditions is useful to predict the primary hydrolytic drug degradation products. Such studies ensure that degradation products can be identified chromatographically, so that their presence in drug product stability samples can be determined, and will give some indication of a drug's propensity for undergoing hydrolysis. In contrast, stability studies (see the "Liquid and Lyophile Dosage Form Stability Screening" and "Solid Dosage Form Stability Screening" sections) focus on rates of degradant formation, where the degradants themselves are often identified by purposeful degradation studies. Drug concentrations of at least 1 mg/mL in 1 *N* acidic and 1 *N* basic conditions are recommended for acid–base stress testing. In some cases, a co-solvent may be necessary to achieve these concentrations. In exceptional cases, lower concentrations may be necessary, though this can make the process more difficult since it requires correspondingly more sensitive assays. Care should be taken to choose non-reactive co-solvents. For example, methanol and other alcohols should generally be avoided for acidic conditions if the compound contains a carboxylic acid, ester, amide, aryl amine, or hydroxyl group due to possible reaction of the drug with the alcohol.

The acid and base stressed conditions should result in approximately 10–20% degradation of the drug substance or represent a reasonable maximum condition achievable. If this level of degradation is not achieved, additional hydrolysis experimentation should be performed at no more than 70°C for one week total reaction time. It is not recommended to go above this level of stress for typical drug substance materials since

excessive acid–base stress can produce non-predictive samples, and may require unnecessary effort in the HPLC method development.

#### **Liquid and Lyophile Dosage Form Stability Screening**

Since hydrolytic instability is frequently pH dependent (as discussed in the "pH Rate Profiles" section), a pH vs. stability curve is usually required as part of the process of developing a liquid formulation, especially in aqueous vehicles. Storing the product at elevated temperatures is the standard method for accelerating hydrolysis. Generally, three months at 50°C and six weeks at 70°C are considered indicative of long-term stability. For feasibility work, sampling at shorter time points can indicate whether hydrolytic stability will be a concern. Similarly, the product can be autoclaved at 121°C for an extended period of time, usually 20–30 min; however, this large temperature extrapolation back to room temperature could be misleading due to any shift in mechanism, change in rate-limiting step, or shift in pH (due to temperature effects on the buffer). Non-linearity in a plot of the log of the rate constant,  $k$ , vs.  $1/T$  (where  $T$  is the absolute temperature) can indicate such a situation. In particular, a bowl-shaped curve indicates a change in mechanism and a dome-shaped curve implies a change in the rate-determining step. Single temperature measurements, which essentially assume a common activation energy with no change in rate-limiting steps, are particularly dangerous in terms of predicting lower temperature stability. Although a full Arrhenius plot is often impractical, using at least three temperatures, with a minimal extrapolation to the desired temperature, is preferred.

For lyophilized amorphous formulations, activation energies of hydrolysis are generally similar enough to those in solution (2) that they can be used for preliminary formulation development; though exceptions with lyophile (3,4) or solution (5) having a higher activation energy have been reported. The temperature range for a stability study should be below the glass transition temperature,  $T_g$  [measurable by differential scanning calorimetry (DSC), modulated DSC (6), critical molecular mobility by NMR (7), thermally stimulated current (8), or dielectric analysis (5)] to avoid any Arrhenius slope changes through the phase transition. In addition, the temperature should be below the temperature where the bulking agent decomposes, since the decomposition products could be reactive with the active pharmaceutical ingredient (API).

## Solid Dosage Form Stability Screening

### *Bulk Crystalline Active Pharmaceutical Ingredient*

Initial solid-state screening for hydrolytic instability first involves the use of pure bulk drug substance. The bulk drug form or forms are placed in appropriate packaging configurations and exposed to “accelerated” conditions of increased temperature and humidity. Typical accelerated conditions for pharmaceuticals are 30°C/60% RH, 40°C/75% RH, and 50°C/20% RH. While these are common conditions for stability challenges of pharmaceuticals, any specific combination of temperature and humidity can usually be achieved by employing programmable temperature ovens and sealed systems under humidity environments dictated by saturated salt solutions (9). Often, multiple sets of samples are screened using conditions that are “open,” that is, under conditions where humidity can equilibrate between the sample and the environment in the stability chamber. Such open conditions are associated with containers that have some amount of water vapor transport, e.g., polyethylene bottles, bags, and open-topped glass vials with gauze coverings. Under “closed” conditions, equilibration with the environment in the stability chamber is limited to the slow moisture transport through the packaging such that the humidity is determined by the moisture sealed in the container. Typical closed conditions employ crimp-sealed teflon-stoppered glass vials or other tightly sealed glass containers, foil lined high density polyethylene (HDPE) packaging, or foil–foil blister packaging (see Sec. “Packaging” for a discussion of packaging).

Since different physical (e.g., polymorph) and salt forms of an API can have significantly different hydrolysis rates and profiles, all available forms of the API should be screened in the accelerated temperature–humidity protocols. It is often useful to characterize the API being screened using such physical measurements as powder x-ray diffraction (PXRD), DSC, differential thermal analysis (DTA), thermal gravimetric analysis (TGA), and photomicroscopy. This analysis ensures that the lot of API being analyzed is of a single, stable polymorphic form since morphology plays an important role in hydrolytic reactivity (10). Different salt and hydrate forms of the drug can result in different hydrolytic reactivity based on several factors. For salt forms, differences in aqueous solubility, microenvironmental pH, and hygroscopicity can lead to differences in reactivity (see the “Hydrolysis of Crystalline Solids” section). For hydrated forms, the degree to which the waters of hydration are held in the crystal lattice affects

reactivity (see the “Water Activity” section) (11,12). For these hydrated forms, TGA/DTA and DSC can be used to determine the transition temperature where water molecules are lost, so that screening temperatures can be selected to be below these temperatures and therefore be predictive of room temperature behavior. Screening hydrated forms at high temperatures may serve to dehydrate crystals, even under relatively high humidity conditions, thereby affecting reactivity in a nonlinear and nonpredictive fashion.

An important aspect in estimating the reactivity of crystalline materials is the amount of amorphous material in the form studied (see the “Hydrolysis of Crystalline Solids” section), so that both processed (e.g., milled or ground) and unprocessed material should be included in the screening studies. Since amorphous regions within a crystal matrix can be considerably more hygroscopic than the crystalline regions (13), water within a system can be distributed unevenly resulting in significant plasticization of amorphous regions. This in turn can lead to higher hydrolysis rates than anticipated for purely crystalline materials. Often, information on the hydrolytic reactivity of amorphous regions can be investigated simply by screening samples of amorphous bulk, derived from lyophilization or spray drying, in the accelerated high temperature and humidity conditions discussed above. Preparation and screening of samples with known amounts of amorphous and crystalline character may be helpful in elucidating the effects of amorphous content on reactivity in mostly crystalline matrices.

Comparisons among the stabilities of an API form at corresponding open and closed conditions at different temperatures make it possible to decouple the effects of humidity and temperature. Generally, an increased hydrolytic decomposition is noted in open packaging configurations and increases with increasing humidity. For ionizable compounds, the stability of different salt forms may relate to the effective local pH around the API due to buffering effects of the counterion. For some API systems, the hydrolysis follows standard Arrhenius kinetics (14) at constant relative humidity over certain temperature ranges; however, this is case specific and should be investigated for an API on an individual basis. Deviations from Arrhenius behavior in crystalline solids can occur because of changes in rate-determining steps, changes in the physical forms of the materials, changes in the mechanisms of hydrolysis, and changes in the water activity with temperature (see the “Water Activity” section).

Quantitative models for the kinetics of drug decomposition in the crystalline state (10,15–17) include effects of temperature, crystal morphology, concen-

tration of nucleation sites, water vapor pressure, and moisture content. In some cases, the equations and parameters have been optimized to model only a specific region of the decomposition profile (usually the initial decomposition) or for specific accelerated conditions (at a particular temperature–humidity condition). For example, in studying the decomposition of the water soluble systems of propantheline bromide and meclizine hydrochloride, the equation parameters change depending on whether that stability screening is performed at humidity conditions above or below the critical relative humidity (CRH) of the system. Here, the CRH is defined as the humidity at which the solid starts to take up bulk moisture, a factor related to the relative humidity over a saturated solution of the compound at that temperature. Thus, the relative factors that are important in the decomposition of a particular system may need to be identified before effective modeling of the hydrolytic decomposition of a specific system can be achieved.

#### *Excipient Blends*

In addition to screening the bulk API, binary or ternary mixtures of the API with common pharmaceutical excipients should be screened using the open and closed high temperature–humidity conditions described above. Excipient mixtures can be screened, as with bulk API, in appropriate packaging configurations. While it is possible to screen for stability of the API with excipients using aqueous suspensions of the components, it has become common to evaluate the stability of these drug–excipient mixtures in both uncompressed powder blends and compacted tablets. To stress the hydrolytic instability in a blend, interfacial contact of the drug with excipients can be increased using a low-drug loading of 10–30%.

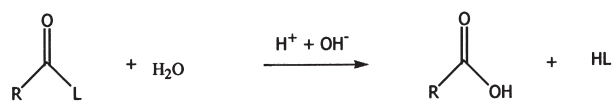
With excipients present in closed systems, moisture can be brought into an otherwise dry system thereby influencing hydrolysis. This phenomenon is related to the amount of moisture the excipients contain initially and the relative moisture affinity of the excipients and the API. The moisture affinity is related to particle size, amorphous content, processing conditions, and aqueous solubility. Since currently there is no good database on excipient moisture affinities, hydrolysis rates due to this term are difficult to predict. Measurement of the water content of excipients may be useful to assess prior to initiation of excipient blend stability studies. Typical water content values for common excipients are shown in Table 1. Measurement of the relative humidity in a

closed system can be indicative of water being brought into an otherwise dry system.

Several articles describe the effects of excipients on the solid-state hydrolytic decomposition of aspirin. In studies involving compacts of aspirin and sodium bicarbonate (19), two distinct topochemical reaction patterns of decomposition are noted at low and high temperatures. This emphasizes that crystal morphology, particle-size distribution, and morphological distributions play important roles in hydrolysis rates. Additional studies using blends of aspirin with metal stearates (20) illustrate that these excipient blends predict solid-state decomposition products of commercial aspirin tablet formulations more accurately than do the stability studies of the pure API due to specific interactions of the API with the stearates.

### MECHANISMS OF HYDROLYSIS

The chemistry of hydrolysis is largely the chemistry of an electrophilic carbon atom (often a carbonyl group carbon) with a good leaving group (21–23). There are cases (e.g., acetals, ketals, hemi-acetals, hemi-ketals, imines, alkyl halides, phosphate esters, and sulfate esters) where a carbonyl group is not present, though these are less common in drug substances. Table 2 offers examples of potentially hydrolyzable functional groups and examples of drugs containing these functional groups. The nature of the leaving group, the presence of acidic or basic catalysts, and the electrophilicity of the carbonyl group (or other center) play a fundamental role in modulating the reactivity. A general scheme of the reaction is depicted below, where  $\text{OH}^-$  acts as a nucleophile and L is a leaving group (charged or neutral). This reaction can also be classified as an acyl transfer to water (or hydroxide).



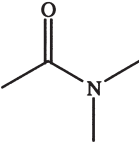
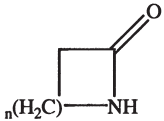
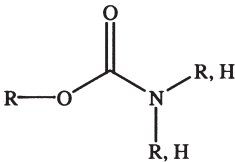
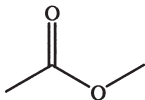
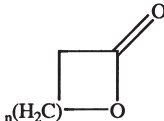
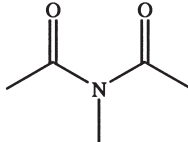
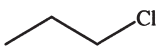
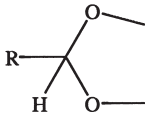
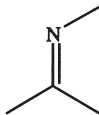
Since water cleavage involves loss of a functional group, the stability of the eliminated molecule will affect the reactivity. For this reason, amides are generally less hydrolytically reactive than esters since  $\text{RO}^-$  is generally more stable than  $\text{R}_2\text{N}^-$  [note that the relative  $\text{pK}_a$  values of the conjugate acids are 18–19 for alcohols and  $\geq 25$  for amines (21)]. Some functional groups are particularly sensitive to hydrolysis, and their presence may lead to

**Table 1**  
Typical Moisture Content of Common Tableting Excipients (18)

Excipient	Moisture Content (wt%)	Comments
Calcium phosphate, dibasic anhydrous	0.1–0.187	Moisture surface adsorbed; does not form hydrate
Calcium phosphate, dibasic dihydrate	20	H <sub>2</sub> O lost < 100°C
Microcrystalline cellulose (MCC)	5	Moisture content varies with grade; hygroscopic
Colloidal silicon dioxide	0–15 (<80% RH); 80 (>80% RH)	Absorbs H <sub>2</sub> O without liquification; hydrophobic surface treatments decrease hygroscopicity
Crospovidone	≤60%	Hygroscopic
Hydroxypropyl methyl cellulose	0–10 (<80% RH); 35 (>80% RH)	Moisture content depends greatly on previous storage conditions
Lactose, anhydrous	≤1	Varies with manufacturer and grade
Lactose, monohydrate	4.5–5.5	Varies with manufacturer and grade
Magnesium stearate	5–15 (<75% RH); 35 (>75% RH)	Varies with manufacturer and grade
Mannitol	0–1 (<75% RH); 10 (>75%RH)	Should be stored tightly closed under low RH
Methylcellulose	≤5	Slightly hygroscopic; store in cool, dry area; tightly closed
Sodium lauryl sulfate (SLS)	≤5	Non-hygroscopic
Sodium starch glycolate (Explotab)	5–20 (<75% RH); 60 (>75% RH)	Cakes if exposed to high humidity
Stearic acid	<0.1	Non-hygroscopic

**Table 2**

*Potentially Hydrolyzable Functional Groups in Pharmaceuticals and Example Drugs in Rough Rank Order from Most to Least Stable*

Functional Group	General Structure	Example
Amide		Acetaminophen, chloramphenicol, oxazepam, chlordiazepoxide
Lactam		Penicillin G ( $\beta$ -lactam antibiotics)
Carbamic Ester		Loratadine, pipazetate
Ester		Atropine
Lactone		Warfarin
Imide		Barbiturates
Alkyl Chloride		Chlorambucil
Acetal		Erythromycin
Imine		Diazepam, oxazepam, chlordiazepoxide

hydrolytic instability even in the absence of catalysts. These groups include acetals, ketals, hemi-acetals, hemi-ketals, imines, alkyl halides, phosphate esters, and sulfate esters. Since there are many factors related to the chemical structure of a drug and its environment in the dosage form, the presence of potentially hydrolytically reactive sites should be viewed in terms of interpreting the experimental findings rather than as a prediction of hydrolytic instability. Some reactions are extremely slow and unlikely to be an issue. However, since pharmaceutical standards for degradation are very exacting, even relatively slow rates can be problematic. For example, the half-life for hydrolysis of an unactivated amide (a tripeptide) at neutral pH and room temperature is approximately 7 years with a pseudo first-order rate

constant of  $3 \times 10^{-9} \text{ sec}^{-1}$  (24), yet even this slow rate corresponds to more than 10% degradation in 2 years and would generally be unacceptable for pharmaceutical applications. Rate constants for hydrolysis of several drugs are listed in Table 3.

Often, the rate of hydrolysis in the absence of catalysts is slow such that most reactivity is associated with catalytic reactions. For example, ester hydrolysis is generally slow due to the poor leaving-group ability of alkoxide and requires chemical or enzymatic catalysis. The same is true for non-activated amides, while for imines, hydrolysis occurs readily without catalysts. A general rank order of hydrolytic reactivity is as follows:  $\text{RC}=\text{NR}' > \text{RCO}_2\text{COR}' > \text{RCOSR}' > \text{RCO}_2\text{R}' > \text{RCONH}_2$ .

Table 3

*Examples of Pseudo First-Order Rate Constants for Hydrolysis of Drugs*

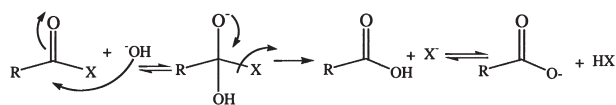
Drug	Type	T (°C)	pH	k (sec <sup>-1</sup> )	Notes
Acetaminophen (23)	Amide	25	6	$1 \times 10^{-9}$	
Chloramphenicol (23)	Amide	25	6	$7.5 \times 10^{-9}$	
Oxazepam (23)	Amide	70	3.2	$1.3 \times 10^{-4}$	Primary hydrolysis
				$9 \times 10^{-7}$	Secondary hydrolysis
			10.9	$4.1 \times 10^{-4}$	Primary hydrolysis
				$3.6 \times 10^{-5}$	Secondary hydrolysis
		85	2	$\sim 1 \times 10^{-2}$	Primary hydrolysis
				$\sim 6.3 \times 10^{-5}$	Secondary hydrolysis
			5.5	$\sim 1.6 \times 10^{-3}$	Primary hydrolysis
				$4 \times 10^{-6}$	Secondary hydrolysis
			11	$\sim 2.5 \times 10^{-2}$	Primary hydrolysis
				$\sim 1 \times 10^{-6}$	Secondary hydrolysis
Atropine (23)	Ester	40	2	$\sim 10^{-9}$	Concomitant dehydration at pH 2–4
			4	$\sim 10^{-10}$	Concomitant dehydration at pH 2–4
			9	$\sim 10^{-5}$	
Chlordiazepoxide (23)	Imine	80	1	$\sim 8 \times 10^{-5}$	
			3	$\sim 3 \times 10^{-5}$	
			5	$\sim 5 \times 10^{-5}$	
			7–11	$\sim 6 \times 10^{-5}$	
Erythromycin (23)	Acetal	60	5	$\sim 1.3$	
			7	$\sim 1.6 \times 10^{-2}$	
			8	$\sim 4 \times 10^{-2}$	
			10	$\sim 0.5$	
Chlorambucil (23)	Alkyl chloride	25	2	$\sim 2 \times 10^{-5}$	
			3	$\sim 6 \times 10^{-5}$	
			5–10	$\sim 2 \times 10^{-4}$	
Diazepam (23)	Imine	80	2	$\sim 1 \times 10^{-3}$	Primary hydrolysis
				$\sim 1 \times 10^{-6}$	Secondary hydrolysis
			4–8	$< 1.7 \times 10^{-7}$	
			10	$\sim 1 \times 10^{-6}$	
Phenobarbital (23)	Imide	80	7	$2.6 \times 10^{-6}$	



### Specific Base Catalysis

Specific base catalysis involves reaction dependent on the specific base hydroxide. This reaction depends only on the pH of a solution, not on the total amount of base present. General base catalysis, in contrast, depends on the total concentration of bases present, including hydroxide (see the “General Base” section). The two can therefore be distinguished by measuring reaction rates at the same pH but with different buffer concentrations (25).

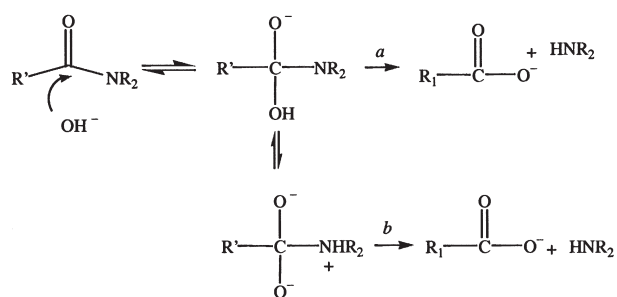
The most common situation for base-catalyzed API hydrolysis involves rate-limiting hydroxide attack on a carbonyl to form a tetrahedral intermediate. This is followed by elimination to give an anionic leaving group and the carboxylic acid in a mechanism referred to as  $B_{AC}2$  for base catalyzed, acyl cleavage, bimolecular (21):



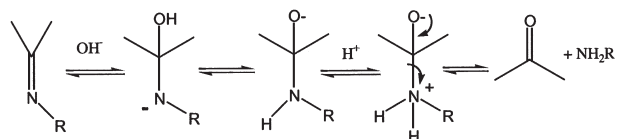
Electron withdrawing substituents near the carbonyl group (i.e., at the R substituent) facilitate hydrolysis by making the carbonyl more electrophilic (22,26). The substituent effects become attenuated with increasing distance from the carbonyl group. Leaving group ability becomes significant in the overall reaction when loss of that group is sufficiently slow to become rate-limiting. Leaving group ability will generally correlate with the  $pK_a$  of the conjugate acid. That is, the stronger the basicity of the leaving group, the poorer it is as a leaving group. For example, the leaving group ability corresponding to increasing reactivity and acidities of the conjugate acids follow in the order  $R = \text{alkyl (ketones)} < R = \text{amine amide)} < R = \text{alkoxy ester)}$ . In alkaline environments, hydrolysis is essentially irreversible. For carbonyl groups that are part of ring structures (e.g., lactones and lactams), opening the ring can relieve strain, which provides an additional driving force for the reaction (27,28). This strain will be minimal for five- and six-membered rings.

As with esters, most amides hydrolyze via the  $B_{AC}2$  mechanism; however, the reaction requires greater catalysis and/or higher temperatures since  $\text{NH}_2^-$  is a very poor leaving group (21). In some cases, amide hydrolysis may progress through a dianion intermediate leading to a reaction rate that is higher than first order with respect to hydroxide ion (29). The nature of the amine leaving group determines whether the dianionic

intermediate will follow path a or b (in the scheme below). For example, electron-withdrawing R-substituents will stabilize the negative charge on the nitrogen, making  $\text{NR}_2^-$  a good enough leaving group to allow for path a, while in many cases, protonation of the nitrogen allows for elimination of an amine along path b (30).



Compounds containing carbon–nitrogen double bonds are also susceptible to hydrolysis. Aliphatic imines are easily hydrolyzed by water alone whereas compounds such as aromatic imines, oximes, hydrazones, and semicarbazones are more difficult to hydrolyze and usually require acid or base catalysis (21). A general scheme for the hydrolysis of a carbon–nitrogen double bond in the neutral to alkaline pH region is shown below (31):



$R = \text{alkyl, aryl} = \text{imine}; R = \text{OH} = \text{oxime}; R$

$= \text{NH}_2 = \text{hydrazone}; R = \text{NHCONH}_2$

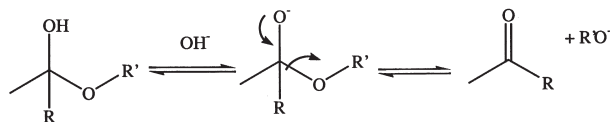
$= \text{semicarbazone}$

The rate of hydrolysis of a specific compound will vary with the reaction conditions, and in the case of imines, whether the carbon–nitrogen double bond is originally from a weakly or strongly basic amine (32). The rate-determining step in hydrolysis of these compounds in the neutral to alkaline pH region is the initial attack of the water or hydroxide ion. For some imines, the hydrolysis rate in the alkaline pH region may seem pH independent since the concentrations of hydroxide ion and of protonated imine vary in equal and opposite directions (31).

A number of drugs (e.g., macrolide antibiotics) contain hemi-acetal and hemi-ketal functionalities, both of which



are subject to base-catalyzed hydrolysis. The mechanism for base-catalyzed hydrolysis of hemi-acetals ( $R = H$ ) and hemi-ketals ( $R = \text{alkyl}$ ) is shown below (21,22):

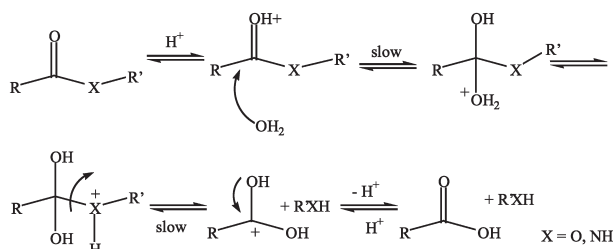


For acetals ( $R = H$ ) and ketals ( $R = \text{alkyl}$ ), only acid-catalyzed hydrolysis occurs (see the "Specific Acid Catalysis" section).

### Specific Acid Catalysis

Specific acid catalysis, that is catalysis by hydronium ion, can involve cleavage of the leaving group, or for the case of an ester, of the alkyl-oxygen bond, assuming the alkyl group can form a stable carbocation. In general, acid-catalyzed mechanisms are reversible such that products can slow the reaction as they build up.

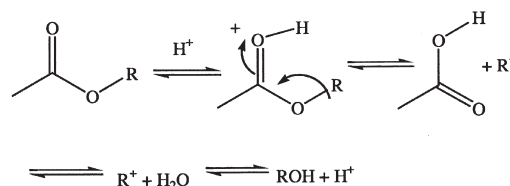
The most common mechanism for acid-catalyzed hydrolysis of a carboxylic acid derivative is known as  $A_{AC}2$  for acid catalyzed, acyl cleavage, bimolecular illustrated below:



As indicated, the rate-determining step in this scheme depends on both the nucleophile (water) and the substrate. The first event in this mechanism is the protonation of the carbonyl oxygen, followed by slow addition of water to yield a tetrahedral carbon. A proton shift then takes place (not necessarily intramolecularly) followed by the slow elimination of the protonated leaving group (the alcohol for an ester, or an amine for an amide). The hydrolysis of amides requires significantly stronger conditions than for esters. This is due to the fact that the electron-donating nitrogen substituent stabilizes the ground state of the carbonyl group via resonance of the  $\pi$ -system, and this stabilization is lost in going to the tetrahedral intermediate.

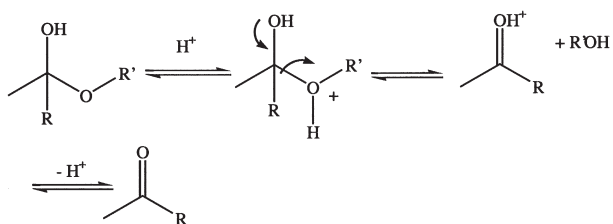
The result is a higher activation barrier to form the intermediate and an overall slower hydrolysis rate.

For esters, a rarely seen mechanism is possible when a stable carbocation can be formed by cleavage of the alkyl to oxygen bond. Examples include allylic, benzylic, and tertiary alkyl group esters. In the mechanism shown below, a cation is formed from the protonated substrate in the rate-determining process, then intercepted by water to yield the alcohol:

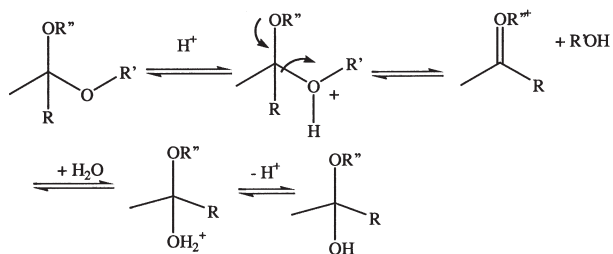


This mechanism is known as the  $A_{AL}1$  mechanism for acid catalyzed, alkyl cleavage, unimolecular. The rate-limiting process is analogous to an  $S_N1$  substitution reaction. Although amides can theoretically be similarly hydrolyzed, this mechanism is only observed under extreme conditions (33).

A number of drugs (e.g., macrolide antibiotics) contain acetal, hemi-acetal, ketal, and hemi-ketal functionalities, all of which are subject to acid-catalyzed hydrolysis. The mechanism for acid-catalyzed hydrolysis of hemi-acetals ( $R = H$ ) and hemi-ketals ( $R = \text{alkyl}$ ) is shown below (22):

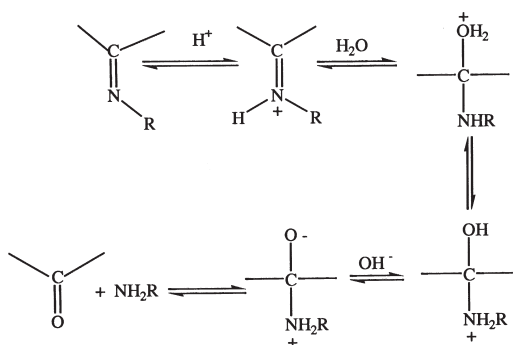


For acetals ( $R = H$ ) and ketals ( $R = \text{alkyl}$ ), hydrolysis yields the corresponding hemi-acetal or hemi-ketal as shown below:



These species will undergo further hydrolysis to yield the corresponding aldehydes or ketones.

Another class of hydrolyzable functional groups is represented by the  $C=NR$  group, which is common to imines ( $R$  is alkyl or aryl), oximes ( $R$  is  $OH$  or  $OR'$ ), hydrazones ( $R$  is  $NH_2$ ), and semicarbazones ( $R$  is  $NHCONH_2$ ). The scheme below depicts the general mechanism for acid-catalyzed hydrolysis of these groups (21):



These hydrolyses are generally reversible. The mechanism consists of addition of a water molecule to the protonated substrate, followed by elimination of the amine from a tetrahedral intermediate. In acidic solutions, the rate-determining step is the breakdown of the tetrahedral, zwitterionic intermediate (31,34).

### Buffers and Other Catalysts

General acid–base catalysis is the most encountered catalytic behavior of buffer species (25). Such catalysis can be detected on pH rate profiles (see the “pH Rate Profiles” section) when the slope of the curve at acid catalysis and base catalysis regions deviates from  $-1$  and  $+1$ , respectively. A commonly observed kinetic phenomenon when general acid–base catalysis is involved is a change in rate-determining steps, often revealed by a dome shaped curve when plotting  $\log k$  vs. the  $pK_a$ s of different buffer species (Brønsted plot). The three mechanisms associated with this catalysis are described in the sections that follow.

#### General Base

Buffers can act as bases to remove a proton from water to facilitate hydroxyl attack on a carbonyl carbon. When the rate-limiting step is removal of the proton rather than water attack on the drug, the hydrolysis

reaction rate will depend on the total base concentration rather than just that of the hydroxide ion. The result is a general base catalysis of the hydrolysis (35).

#### General Acid

Protonation of a carbonyl oxygen facilitates nucleophilic attack (as discussed in the “Specific Acid Catalysis” section). When the protonation, rather than the addition of water, is rate limiting, there will be a rate dependence on the total concentration of acids rather than just on the specific acid, hydronium ion.

#### Nucleophilic Catalysis

Nucleophilic catalysis occurs when a nucleophile adds to a carbonyl to form an intermediate that is more susceptible to hydrolysis than the original species. This circumstance is rarely encountered with pharmaceuticals. A well-studied example of nucleophilic catalysis is the imidazole-catalyzed hydrolysis of esters and amides with activated acyl groups (36,37). Nucleophilic catalysis is more common in phosphate hydrolysis, especially in DNA hydrolysis where the specific orientation of phosphate groups enables reaction (see the “Hydrolysis of Macromolecules” section).

#### Metal Catalysts

The rate of solution hydrolysis of esters (38), phosphate esters (39), amides (40), anhydrides (41), and mixed anhydrides can often be enhanced by the addition of metal ions to the medium. The most effective metal catalysts are generally divalent metal ions such as  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Zn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ , and  $Cd^{2+}$ . Catalysis is often most effective when the molecule is capable of chelating the metal such that the metal ion is held near the reacting site of the molecule (42). Chelation can occur whenever a molecule has negative charges or dipoles (such as with carbonyls) arranged such that two can ionically bind the metal ion. In addition to small molecules, proteins and nucleic acids are especially prone to metal ion binding (see the “Hydrolysis of Macromolecules” section). Examples of divalent metal-catalyzed hydrolysis include the deactivation of  $\beta$ -lactam antibiotics, such as penicillin and cephalosporin (43); carboxypeptidase A (38,41), a Zn-containing metallo-enzyme that catalyzes the hydrolysis of amide peptide bonds; and ribonuclease, which utilizes a divalent cationic metal ion to catalyze hydrolysis of RNA (44).

Divalent metals catalyze hydrolysis under both acidic and basic conditions. The divalent metal ion ligates a

carbonyl oxygen making the carbon more electrophilic. This serves to raise the concentration of the tetrahedral intermediate (see the “Specific Base Catalysis” and “Specific Acid Catalysis” sections). Metal ion complexation to the leaving group can also accelerate decomposition of the tetrahedral intermediate. Both processes lead to overall increases in the rate of hydrolysis, sometimes by orders of magnitude.

For liquid formulations, addition of ethylene diamine tetraacetate (EDTA) will often eliminate metal catalysis by competitive binding to the metal ions provided the pH is above about 5. With solid dosage forms, competitive chelators such as EDTA will be most effective if in the same phase as the drug. Although this sometimes occurs by simple mixing, often the chelator must be applied from solution.

### pH Rate Profiles

To develop an oral or parenteral solution formulation of a drug susceptible to hydrolysis, an understanding of the effects of pH and buffers is essential. In the most general sense, the rate of hydrolysis will depend on five

rate constants (23,45,46): an acid-catalyzed rate  $k_H +$ , a base-catalyzed rate  $k_{OH} -$ , an uncatalyzed rate  $k_0$ , a rate with acidic buffers  $k_{GA}$ , and a rate with basic buffers  $k_{GB}$ . Those rates that are dependent on pH (acid and base catalysis) are independent of the remaining rates; that is, for different buffer concentrations and buffer types, a minimum reaction rate will occur at the same pH. This allows for an optimization process as follows:

1. keeping buffer concentration constant, optimize solution pH for maximal stability; and
2. keeping the pH constant, optimize the buffer type and concentration for maximal stability.

In analyzing the hydrolysis reaction rate dependence on pH, it is often useful to plot the logarithm of the observed reaction rate (initial reaction rate) against the pH. The resulting graph will typically show a minimum at the most stable pH with a slope of +1 and -1 in the basic and acidic regions indicating acid and base catalysis. This is shown in Fig. 1 (curve 1) with pH 7 as the most stable condition. In most cases, however, the position of greatest stability is on the acidic side since base catalysis rates tend to be higher than acid catalysis

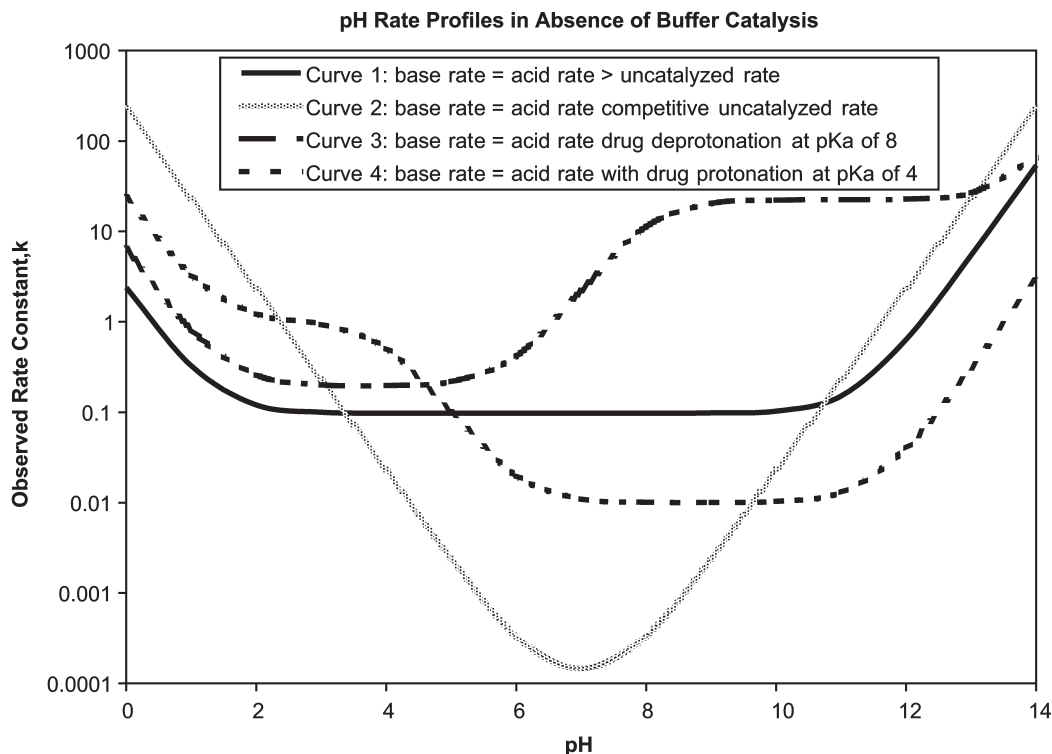


Figure 1. pH rate profiles in absence of buffer catalysis.

rates. If the uncatalyzed rate constant is sufficiently high, the pH rate profile will indicate a flat region of maximum stability (Fig. 1, curve 2). For a drug having an acidic or basic functional group, the ionization of that group can affect the rate of hydrolysis by effectively changing the electrophilicity of the hydrolyzing moiety (often a carbonyl). The degree to which these groups affect the reaction rate depends on the electronic overlap of the group with the carbonyl. In general, the greater the distance of the acidic or basic group from the carbonyl, the lower the effect on the hydrolysis rate. For ionizable drugs with  $pK_a$  values in the range of the study, a characteristic inflection in the pH rate profile may be observed at a pH value equal to the  $pK_a$ . The pH rate profile (Fig. 1, curve 3) for a drug that is a monoprotic weak acid is described in Eq. (1):

$$k_{\text{obs}} = k_{\text{H}^+}[f_{\text{HA}}] + k_1 f_{\text{HA}} + k_2 f_{\text{A}^-} + k_3 [\text{OH}^-] f_{\text{HA}} + k_{\text{OH}^-} [\text{OH}^-] f_{\text{A}^-} \quad (1)$$

where,  $f_{\text{HA}}$  and  $f_{\text{A}^-}$  are fractions of drug present in un-ionized and ionized states, respectively. These will depend on the pH of the solution and the  $pK_a$  of the drug. Similarly, the pH rate profile (Fig. 1, curve 4) for a drug that is weakly basic with one  $pK_a$  is described in Eq. (2):

$$k_{\text{obs}} = k_{\text{H}^+}[f_{\text{BH}^+}] + k_1 [\text{H}^+] f_{\text{B}} + k_2 f_{\text{BH}^+} + k_3 f_{\text{B}} + k_{\text{OH}^-} [\text{OH}^-] f_{\text{B}} \quad (2)$$

where,  $f_{\text{B}}$  and  $f_{\text{BH}^+}$  are fractions of drug present in un-ionized and ionized states.

When buffer species act as catalysts (see the “Buffers and Other Catalysts” section), the pH-rate profile will show deviations from the slopes of  $\pm 1$  observed with only specific (hydroxide and hydronium ion) catalysis (47,48). Buffer concentrations can also affect the ionic strength of the solution thereby affecting the drug hydrolysis rate (see the “Ionic Strength” section). Since the buffer effect on the hydrolysis rates can be significant, it is critical when formulating solutions of hydrolytically susceptible drugs that buffer type and concentration be examined.

### Ionic Strength

The ionic strength of a medium can affect the rates of reactions by stabilizing or destabilizing the transition state with respect to the starting materials (49,50). This effect is strongest when the drug molecule is ionically

charged such that bringing hydroxide or hydronium in contact involves a change in charge separation. In addition, higher ionic strength can affect the acidity and basicity of ions by stabilizing charge separation, that is, by favoring free ions vs. ion pairs. In the end, however, ionic strength effects on hydrolyses of drug molecules in realistic formulations tend to be small [see for example Refs. (50–52)]. When ionic strength does affect the hydrolysis rates, it generally is found to increase the rate [see for example Refs. (53–55)]. These effects can be evaluated by keeping a constant pH and buffer concentration while increasing the concentration of a non-buffering salt such as sodium or potassium chloride. In cases where rates are significantly affected, this factor must be accounted for in the formulation development generally by keeping the buffer concentration low.

### Computational Modeling

Computational modeling of hydrolysis reactions can be helpful in rationalizing degradation products, especially secondary degradation. Commercial computer programs designed to predict the synthetic outcome of a reaction include EROS (Elaboration of Reactions for Organic Syntheses) (56), WODCA (Workbench for the Organization of Data for Chemical Applications) (57), LHASA (Logic and Heuristics Applied to Synthetic Analysis) (58), and CAMEO (Computer Assisted Mechanistic Evaluation of Organic Reactions) (59). Predictions can be more accurate with a given calculational hydrolysis reaction condition, independent of the true reaction behavior. For example, hydrolysis of amides are predicted by CAMEO more accurately using basic than acidic conditions (60). For this reason, the use of these programs requires a history of connections between experiments and calculations. The use of such programs for predicting rates of hydrolyses has also proved limited to date. Computational modeling is therefore most suited to helping explain observed products rather than a priori estimating products and rates in real systems. These explanations can at times be useful when developing formulation solutions.

### Water Activity

In solid materials, water is either tightly bound as a crystal hydrate or more loosely entrapped in the amorphous portion of the material (13,61–64). Depending on the degree to which the water is bound, its availability for effecting hydrolysis will change. In solid-state systems with both amorphous and crystalline

regions, most of the unbound water, i.e., water available for chemical reactions, will partition to the amorphous regions, making these regions more prone to hydrolysis (65).

Water activity ( $A_w$ ) is a measurement that indicates how tightly water is bound. The water activity of a substance is defined as the equilibrium ratio of the moisture vapor pressure above a substance vs. that over a pure water control at the same temperature. In practice, water activity is obtained by placing a sample in an airtight enclosure and measuring the dew point, that is, the temperature where the moisture in the air is saturated (66). Although traditional methods for measuring water content in pharmaceutical formulations involve weight loss on drying and Karl–Fischer techniques, these techniques fail to provide an indication of how much water is available for reaction. For predicting drug stability, water activity will in general show a better correlation with hydrolytic activity. Though not widely used in the pharmaceutical industry, water activity is commonly used for predicting stability in the food industry (67–71). Based on this, an increased use of water activity measurements in understanding hydrolytic reactivity is anticipated in the pharmaceutical industry (13,62,72–75).

For hydrolysis reactions where water is involved in the rate-limiting step ( $B_{AC2}$  from “Specific Base Catalysis” and  $A_{AC2}$  in “Specific Acid Catalysis”), the hydrolysis rate will depend on the water activity around the drug. For solutions, this will translate to the water concentration, while in solids, this will be the moisture content in the same phase as the drug. The moisture content in a solid will equilibrate with the moisture in the environment as a function of the water activity. For temperature extrapolations, it is therefore preferable to keep the water activity of the air constant, generally by keeping the relative humidity constant. Since the solubility of water in the excipients and drug may vary with temperature, the water distribution within the dosage form may vary with temperature such that Arrhenius behavior is not observed. Table 1 shows typical water contents for a number of excipients, but does not indicate the water activity (availability) associated with the excipients since, unfortunately, such data are not available at this time. As an example of the influence of water availability on hydrolysis rates, compacts of aspirin containing dibasic calcium phosphate dihydrate degrade approximately 10 times faster than formulations containing lactose and two-fold faster than formulations containing microcrystalline cellulose (76). Interestingly, the rate of degradation does not correlate with total water content in the compacts in that

the lactose compact contains the most water, yet shows little degradation; however, a lower water activity associated with lactose provides less water to partition into amorphous regions and effect hydrolysis than with dibasic calcium phosphate dihydrate, where the water is more loosely bound.

### Hydrolysis of Crystalline Solids

The majority of mechanistic studies on hydrolysis of crystalline pharmaceutical compounds in solid dosage formulations indicate that in most, if not all, cases hydrolysis occurs only in amorphous regions, at crystal defect sites or at the surface of crystals (10,13,14,66,77). Hydrolysis in crystalline domains, though possible, is hindered by three factors.

1. Crystal packing stabilization of the starting material adds reorganization energy to the activation energy for hydrolysis since most molecules do not remain stabilized in a crystal lattice throughout the course of the reaction. As the reaction proceeds, a heterogeneous mixture of crystalline reactants and products plus amorphous mixtures thereof is often formed (78). This high-energy mixture can slow reaction due to the additional activation needed to reach this state (in contrast to solution).
2. Limited mobility raises the activation energy for such bimolecular processes as hydrolysis by making collisions more difficult. In addition, to the extent that attack on an electrophilic center requires a particular site on the molecule be available, crystal orientations may not be optimal for a given hydrolysis.
3. Water within a crystal lattice tends to be bound as hydrates rather than available for reaction (see the “Water Activity” section). For amorphous drug substances (or regions on a crystal) or for crystal defect sites, preferable sorption of water by these less ordered systems can plasticize the solid leading to more water uptake and increased molecular mobility. This in turn facilitates hydrolysis.

Because of the above factors, it is often important to determine the source of non-crystalline domains in drug products made from crystalline drug substances. It should be remembered that only a small percentage of drug need be in an amorphous form to constitute an issue for shelf life. We can broadly divide these non-crystalline domains into two categories, equilibrium and non-equilibrium (high energy) forms. Equilibrium amorphous



forms are created when drug has solubility in the excipients. Under the stresses induced by various processes (e.g., granulation and tableting), molecular mixing between drug and excipients can occur to give a new amorphous phase. The tendency for this to happen will depend on the mutual solubility of the drug and the excipient, and on the physical interactions between the two during the processes. In some cases, moisture can act to increase both the phase compatibility of the drug and excipients, and to allow more molecular interactions between the two. Such formulation variables as drug and excipient particle size can increase surface contact points between drug and excipient, which increases the rate of molecular mixing. For example, the rate of aspirin hydrolysis is 10-fold greater in tablets containing powdered vs. granular magnesium stearate (79), and five-fold greater in tablets containing dibasic calcium phosphate dihydrate with a particle size of 10 vs. 40  $\mu\text{m}$  (80). In general, molecular interactions can be studied by DSC, where phase compatibility can be assessed.

Non-equilibrium drug forms include amorphous forms and crystal defects. Although these forms can involve excipients, they do not require them as with equilibrium amorphous forms. Because these non-equilibrium forms are at a higher energy than the crystalline form of the drug, they are more reactive (lower change in energy going to a common transition state). These amorphous and crystal defect regions on crystalline drugs can be formed by mechanical energy that can break apart a crystal lattice. Such forces occur in granulations, and tableting is sufficient to induce defects and amorphous regions in many crystalline drugs.

Both equilibrium and non-equilibrium amorphous drug forms will generally have greater mobility for both drug and water molecules. This mobility will increase greatly when the amorphous material is at a temperature above the glass transition temperature,  $T_g$ , of the material. Since plasticization by a soluble material, such as water, will lower the  $T_g$ , the mobility (and hence hydrolysis rates) can rise dramatically as a function of relative humidity.

In many cases, it is difficult to distinguish between the equilibrium and non-equilibrium situations. Both depend on processing to either allow mixing or force break-up of a crystal lattice, and both will generally increase with greater stresses during processing. Generally, if a pure drug is sensitive (i.e., hydrolyzes more rapidly) to processing conditions without excipients, there is a likelihood that the drug is prone to formation of non-equilibrium amorphous material. If the sensitivity is greatly increased in the presence of excipients, drug solubilization should be

suspected. In this case, thermal analysis to determine phase compatibility may be helpful. In practical terms, excipients with a hydrophilic–lipophilic balance less matched to drugs could potentially alleviate the problem. Excipients that have lower molecular motion during processing (e.g., crystalline excipients) should also lower the interactions; however, flow during processing is often necessary for good mechanical performance in such operations as tableting.

### Hydrolysis of Lyophiles

Lyophilization (freeze-drying) is a common method of stabilizing drugs that are unstable in solution. Lyophilization consists of freezing, primary drying (ice sublimation), and secondary drying (removal of unfrozen water if any), which are combined in one operational cycle. Lyophilized materials can be produced in either crystalline or amorphous form. Crystalline drug is generally more chemically stable than its amorphous form (81); however, solubility or ease of reconstitution may necessitate the use of amorphous material. Even partially crystalline formulations may reduce hydrolysis rates (82). In many cases, however (especially with proteins), it is practically impossible to crystallize a drug during lyophilization. The majority of commercial lyophilized pharmaceuticals are probably amorphous or partially amorphous. Lyophilized formulations contain a relatively low amount of residual water, usually 1–5% by weight; however, this amount is usually adequate to cause a pharmaceutically significant amount of hydrolysis (greater than about 0.2% by weight). Examples of drugs that undergo hydrolysis in freeze-dried materials include peptides and proteins (3,83–88), esters (such as aspirin, methylprednisolone succinate, moexipril, heroin, aspartame) (4,5,89–92),  $\beta$ -lactams (89,93,94), and carbohydrates (95–99). Though hydrolysis does occur in these systems, in general the rates of hydrolysis reactions in the freeze-dried state are significantly lower than in solution. For example, a ten- to one hundred-fold decrease in the hydrolysis rate of moexipril was observed on going from solution to lyophile (91).

There are three variables that have a significant impact on hydrolysis rates and should be considered in development of freeze-dried formulations. It should be noted, however, that it is not obvious what the effects will be on any given molecule such that these must be determined experimentally for each specific drug.

1. *Water content.* The rate of hydrolysis decreases with decrease in water content. However,



quantitative relationships between water content and hydrolysis rates may be complex. When water is solely a reactant, a linear relationship between water concentration and reaction rate is observed. Change in water content, however, often leads to changes in physical properties of the material such as molecular mobility, microenvironmental pH, and microenvironmental polarity (100), complicating the water dependence. For example, an exponential increase in the hydrolysis rate was observed for aspartame degradation in a lyophile as a function of water activity (water content) (4). For development of a new product, it is recommended that a water target level of less than 1 wt% be used to minimize hydrolysis issues. In certain cases, the water can be distributed such that a higher percentage is associated with the drug. When a crystalline bulking agent (such as mannitol or glycine) is used, the effective water concentration in a drug is usually much higher than the average water content measured in the formulation, since the crystalline domains will contain relatively little water (see also the “Water Activity” section).

2. *The pH of the solution before lyophilization.* The reactivity of lyophilized formulations often depends on the pH of the solution before lyophilization. As a general rule, the pH sensitivity of solid-state hydrolyses resembles that in solution; however, when going to a non-aqueous environment such as in the lyophile, there are often shifts in the pH of maximum stability to hydrolysis (91,101). Therefore, it is recommended that tests be conducted for lyophilized materials with the solution pH adjusted to plus or minus 2 pH units from the

pH for minimal solution hydrolysis rates. Usually, the pH of solution before lyophilization and solution obtained by reconstitution of lyophilized cake are very similar. However, if a volatile acid or base (such as HCl) is used to adjust the pH, lyophilization may result in significant pH changes (91).

3. *Excipients (buffers, bulking agents).* The bulking agents and buffers can exert various stabilizing or destabilizing effects on the API. Agents that do not form a single phase with the API will have little influence on the API stability except to the extent that there is partitioning of water in the system. When the bulking agent is in a single phase with the API, it will be stabilizing if it lowers the water activity near the drug (see the “Water Activity” section), or decreases the local mobility by increasing the  $T_g$  of the drug and thereby decreases its free volume. Conversely, the additives will be detrimental to hydrolytic stability if they increase the water activity or increase mobility by lowering the  $T_g$ . The most common amorphous bulking agent in lyophiles is sucrose, which often stabilizes drugs to hydrolysis (93), presumably by decreasing the free volume. Though buffers may induce catalysis of hydrolysis (see the “Buffers and Other Catalysts” section) in lyophiles, the buffer choice is often dictated by the need for a low crystallization potential and high collapse temperature (see Table 4). Crystallization of a buffer component during freezing is usually undesirable because it can result in significant pH changes (105). Materials with higher collapse temperatures can be freeze-dried at higher temperatures, hence providing a faster and more robust lyophilization cycle. Phosphate

Table 4

*Crystallization Potential and Collapse Temperatures of Common Buffers Measured by DSC*

Buffer	Crystallization Observed?	Collapse Temperature (°C)	Reference
Citric acid/sodium	No (pH 2.5–7)	– 50 (pH 2.5); – 35 (pH 4); – 42 (pH 7)	(102)
Citric acid/potassium	No (pH 2.5–7)	– 48 (pH 2.5); – 32 (pH 4); – 60 (pH 7)	(102)
Tartaric acid/sodium	Yes (pH 2–4); no (pH 5)	– 35 to – 40 (pH 3–5)	(102)
Succinic acid/sodium	Yes (pH 4–6)		(102)
Glycine/sodium	Yes (pH 10)		(103)
Glycine/HCl	Yes (pH 3)		(103)
L-histidine/sodium	No (pH 4–8)	– 47 (pH 4.0) – 32 (pH 7.7)	(104)
Malic acid/sodium	No (pH 2–6)	– 49 (pH 2); – 43 (pH 4); – 50 (pH 6)	(102)

buffer, which is one of the most common buffers in liquid pharmaceutical formulations, has a high crystallization potential, often making it undesirable for lyophilized formulations subject to hydrolysis (106).

## Hydrolysis of Macromolecules

Macromolecules, most commonly proteins and nucleic acids (DNA), are becoming increasingly common as APIs. These are typically administered parenterally, often as complexes to assist entry into target cells. For both proteins and DNA, hydrolysis represents a significant mode of degradation. Problems resulting from hydrolysis of DNA include loss of transcriptional activity due to excessive strand breaks, especially if the breaks occur in either the promoter region or the gene sequence (107). Depending on the protein and hydrolysis site, hydrolysis can result in loss of protein activity.

Macromolecules can be considered different from small molecules based on three factors.

1. Though the primary functional groups within DNA and proteins undergo hydrolytic degradation similar to conventional small molecule pharmaceuticals, this reactivity is affected by the secondary and tertiary structures of the macromolecules. Therefore, equivalent functional groups within a macromolecule may hydrolyze at dramatically different rates depending on the overall structure. For example, metal cations can bind to macromolecules, bringing the metals into the proximity of hydrolytically active sites (see Sec. "Metal Catalysts"). This effect can make the macromolecules especially prone to metal catalyzed hydrolysis.
2. A single macromolecule will have numerous potential hydrolysis sites. For some macromolecules, a single hydrolysis can result in inactivation of the entire macromolecule. This can make the overall hydrolytic sensitivity of a macromolecule orders of magnitude greater than for the analogous small molecule (at least on an equal weight basis).
3. Hydrolytic cleavage of some macromolecules can lead to strain relief. For example, hydrolysis of supercoiled (plasmid) DNA can lead to unwinding of the DNA. This provides an added driving force for the hydrolysis and makes single hydrolytic events have large influences on the overall drug activity.

Structurally, DNA has very stable phosphodiester bonds and hydrolytically labile *N*-glycosyl bonds (see Fig. 2). The hydrolytic resistance of the phosphodiester bond is due to the lack of the 2'-OH group of the ribose sugar. The presence of the 2'-OH on ribonucleic acids (RNA) leads to stronger base-sugar *N*-glycosyl bonds, but weaker *N*-glycosyl bonds. This major difference affects the stability of deoxyribonucleic and ribonucleic acids differently, and accounts for the greater stability of DNA (108).

Conventional double-stranded B-DNA has the hydrophobic bases positioned in the internal core of the double helix, essentially free from solvent contact. In aqueous formulations, the exterior surface of the DNA double helix is completely solvated by water due to the favorable ion-dipole interactions between water and the highly anionic phosphate surface charge of the phosphate backbone. This accessibility of water to the macromolecule's surface manifests itself in an apparent four-fold increase in the rate of hydrolysis of single-stranded DNA compared to double-stranded DNA (108).

Hydrolytic degradation of double-stranded DNA is a two-step process that ultimately results in a single strand break. The first step of the pathway is a rapid depurination of either the guanine or adenine purine residues resulting in apurinic sites along the DNA strand, with the guanine residue leaving most rapidly (109). Pyrimidine bases, thymine, and cytosine can also be hydrolytically cleaved, but their release occurs at only 5% of the purine rate (108). Following depurination, the DNA undergoes a  $\beta$ -elimination reaction that results in a single stranded nick (see Fig. 3). Kinetically, the rate of depurination is more rapid than the  $\beta$ -elimination reaction, which occurs in the order of several days (110). This apparent rate difference is due to the equilibrium established between the low reactivity cyclic form (approximately 99%) of the base-free sugar and the reactive acyclic aldehyde form (approximately 1%) of the sugar (110).

The initial depurination step is acid catalyzed and the  $\beta$ -elimination is base catalyzed. Data show that physiological pH to slightly basic is optimal for stability. The overall rate of degradation is governed by the following equation (111):

$$SB = k_1(bp)t - \left[ \frac{k_1(bp)t}{(1 + k_2t)} \right]$$

where SB is the number of strand breaks,  $k_1$  is the rate constant for the depurination step,  $k_2$  is the rate constant for the  $\beta$ -elimination, and bp is the number of base pairs.

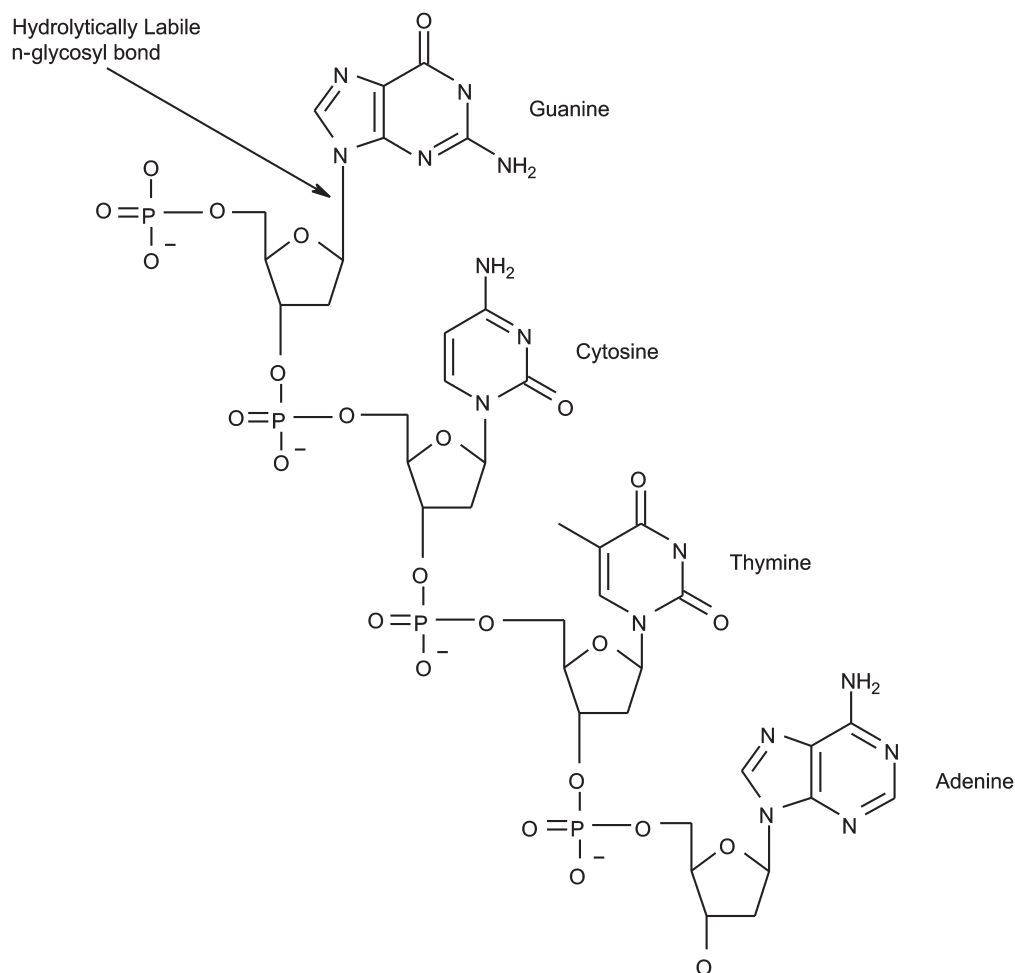


Figure 2. Structure of DNA.

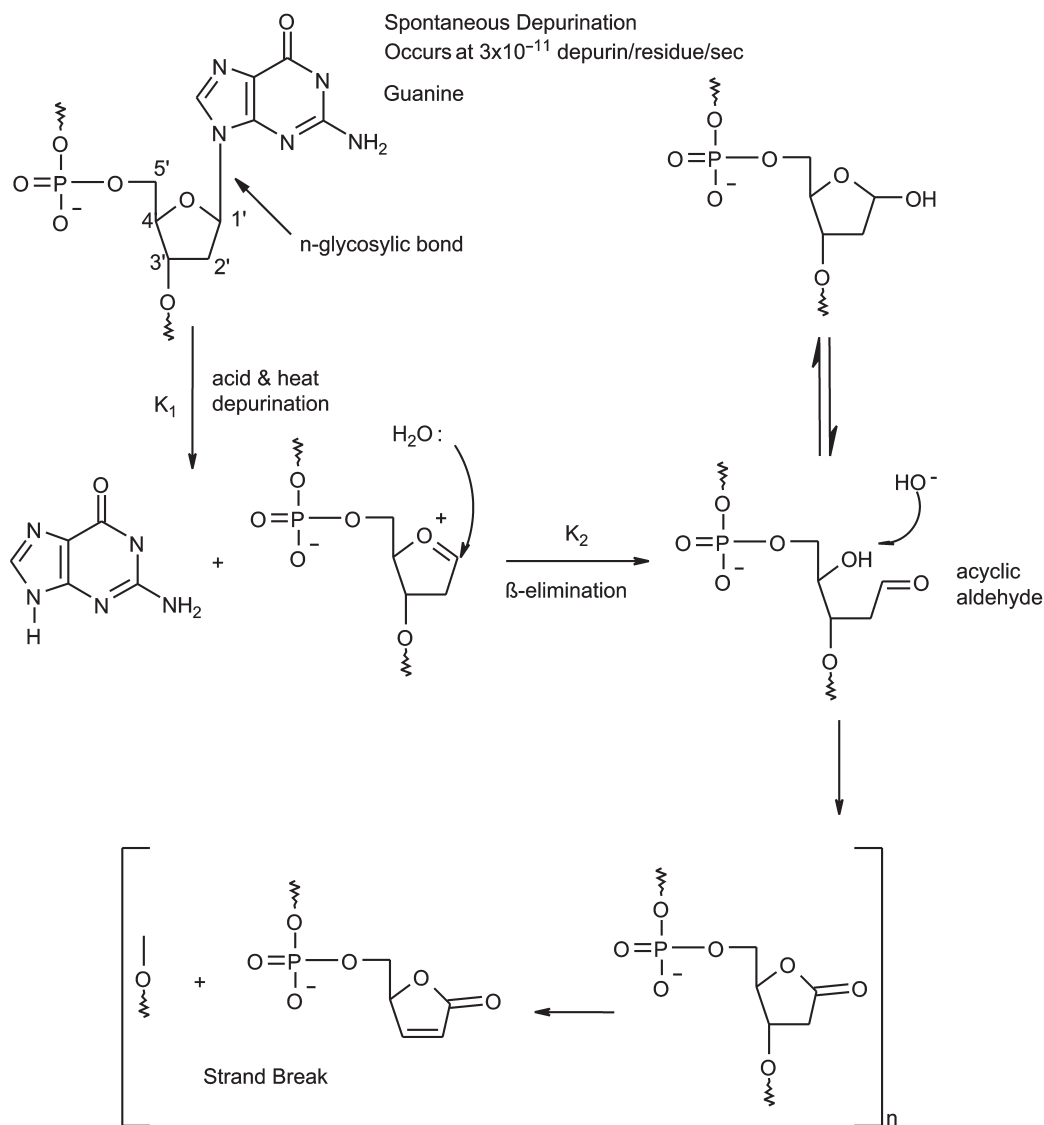
A more elaborate equation accounting for the presence of oxidative degradation is also available (112).

Proteins undergo nonenzymatic hydrolytic deamidation primarily at asparagine and glutamine residues, resulting in formation of aspartate and glutamate, respectively (113). Generally, hydrophobic amino acids decrease hydrolytic reactivity, while hydrophilic amino acids, in particular threonine or serine residues, increase the reactivity. This effect can involve secondary and tertiary structural features that bring water into the vicinity of susceptible amino acid residues. Proton donors neighboring either the glutamine or asparagine can act as catalysts for the hydrolysis (see the "General Acid" section). Additionally, folding of the protein may bring these groups near hydrolytically sensitive amino acids (113). As with most amide hydrolyses, the deamidation of proteins is catalyzed by acids or bases

(see the "Specific Acid Catalysis" section) resulting in greatest stability at approximately neutral pH (113).

### Prodrugs

Prodrug versions of APIs are often developed when the parent compound has poor or limited intestinal absorption, is metabolized in the gastro-intestinal tract, or to increase API solubility for parenteral drug products (114–119). For absorption purposes, prodrugs serve to mask very polar groups, thereby increasing the  $\log P$  (lipophilicity) of the compounds and increasing their permeation through the lipophilic intestinal mucosa. Prodrugs can also stop or slow gastric and intestinal metabolic degradation, thereby allowing absorption to occur before the drug degrades. Most prodrugs available in the market today mask carboxylic acids or alcohols,



**Figure 3.** Mechanism of DNA hydrolysis.

forming esters, which are cleaved hydrolytically to regenerate the API (see Table 5).

Hydrolysis of a prodrug to generate the API is typically mediated by esterases. The hydrolytic lability of these ester groups can be mediated by steric and electronic factors associated with typical ester cleavage (see “Specific Base Catalysis” and “Specific Acid Catalysis”), but can also be enzyme receptor site specific. Thus, hydrolytic lability may need to be optimized around enzyme binding to achieve sufficient parent API release. Parenteral prodrugs are typically screened in human plasma (pH 7.4) to determine

stability (expressed as in vivo half-lives), while oral prodrugs are additionally screened through simulated gastric fluids with enzymes and intestinal homogenates to assess stability.

Design of robust, marketable prodrugs requires that they be hydrolytically unstable in vivo, but hydrolytically stable in the required dosage form (as indicated by stability studies such as described in the “Preliminary Screening for Hydrolytic Instability” section), an often precarious balance. Because of the conflicting needs for dosage form stability and biological activity, use of biological enzymes to effect the in vivo hydrolysis

**Table 5**  
*Examples of Esters Used as Prodrugs*

Drug or API	Ester	References
Drugs with carboxyl groups		
L-Dopa	Methyl ester	(120)
Non-steroidal anti-inflammatory drugs (NSAIDs)	Methyl esters	(121)
Amino acids	Glycolic and lactic acid esters	(122)
Ibuprofen	Guaiacol esters	(123)
Indomethacin	Triglycerides, phenyl esters, glycolic acid esters	(124–127)
Drugs with hydroxyl groups		
Acyclovir	Amino acid and hemi-succinate esters	(128)
Epinephrine	Dipivolate	(129)
Digitoxigenin	Amino acids esters	(130)
Isoproterenol	Diethyl and dipivaloyl esters	(131)
Oxazepam and lorazepam	Aliphatic and amino acid esters	(130–135)

becomes especially important since it provides a necessary differentiation in rates.

### HYDROLYSIS OF EXCIPIENTS

In addition to drug hydrolysis, excipient hydrolysis can sometimes be a concern. Frequently, the excipient is present at high concentrations so that even a small percentage of excipient degradation can impact the purity of the drug product, affect its properties, or affect the performance of the dosage form.

#### Non-polymeric Excipients

Table 6 summarizes information on non-polymeric excipient hydrolysis. Such hydrolysis causes four general issues in drug products.

1. Excipient hydrolysis can generate reactive byproducts that can affect the drug stability. The primary excipient functional groups that are susceptible to hydrolysis are amides and esters, which yield carboxylic acids and either alcohols or amines. These groups have the potential to further react with either the drug or other excipients. Depending on the relative reaction rates of the excipient hydrolysis and subsequent byproduct reaction with the drug, the reactive byproduct can be consumed as quickly as it is formed (if excipient hydrolysis is rate-limiting). Drug reaction will then be pseudo first-order with respect to the concentration of the excipient. Otherwise, the consumption of the

reactive product is rate-limiting and this intermediate will build up until the formation and consumption steps are equal. This will appear as a bi-modal reaction rate for drug degradation with rapid initial hydrolysis of the excipient followed by a slower terminal rate. In aqueous solution, alcohol and carboxylic acid products can react with reactive drugs such as imines. In solids and non-aqueous solutions, even less reactive drugs can react with the products. Even in aqueous solution, amines can react with drugs having electrophilic moieties. In cases where the drug has a carboxylic acid or ester group, the result of this process is *trans*-amidation from an amide excipient to the drug.

2. Excipient hydrolysis can shift the pH of a solution or potentially affect the microenvironmental pH in a solid. When hydrolysis of an ester excipient occurs, the carboxylic acid product lowers the pH of its surroundings. When an ester is a co-solvent (e.g., ethyl oleate or benzyl benzoate), its concentration can be high enough that even trace amounts of hydrolysis can cause substantial pH shifts. Even with buffers present to stabilize a solution pH, the pH may still shift substantially; i.e., the amount of acid formed can exceed the buffer capacity of the system.
3. Excipient hydrolysis can change the physical and pharmaceutical characteristics of the product. Although this effect is more common with polymeric excipient hydrolysis (see the “Polymeric Excipients” section), hydrolysis of some additives can affect flow, disintegration, and dissolution

**Table 6**  
*Non-polymeric Excipients with Hydrolyzable Functionalities*

Compound	Use	Bond	Hydrolysis Information	Ref.
Aspartame	Sweetener	Ester, Amide	Hydrolyzes with moisture; half life <20 days at pH 1, 7, 8; 250 days at pH 5	(18,136,137)
Benzyl Benzoate	Solvent	Ester	May hydrolyze when cosolvent with water	(138)
Chlorhexidine	Antimicrobial preservative; antiseptic	Imine	Hydrolyzes in aqueous solution; 1.6% hydrolysis at pH 9, 30 min at 120°C.	(18,139,140)
Dibutyl Sebacate	Plasticizer	Ester	Stable, not reactive with water	(18)
Docusate Sodium	Anionic surfactant; wetting agent	Ester	Stable in the solid state when stored at room temperature; solutions hydrolyze at high and low pHs	(18)
Gallates	Antioxidant	Ester	Hydrolyze rapidly enzymatically	(141,142)
Glyceryl Monostearate	Emollient, emulsifying agent; solubilizing agent; stabilizing agent; sustained-release agent; tablet and capsule lubricant	Ester	Slow hydrolysis under alkaline conditions	(18)
Isopropyl Myristate	Emollient; skin penetrant; penetration enhancer; solvent	Ester	Resistant to hydrolysis	(18)
Lactitol	Sweetening agent; tablet and capsule diluent	Ether (hemi-acetal) linkage	In acidic solution, slowly hydrolyzes to sorbitol and galactose; stable as a solid under humid conditions	(18)
Lecithin	Emollient; emulsifying agent; solubilizing agent	Ester	Hydrolyzes enzymatically	(143)
Medium Chain Triglycerides	Emulsifying agent; solvent; suspending agent; therapeutic agent	Ester	Hydrolyzes enzymatically to give acids	(18)
Palmitates	Emollient; oleaginous vehicle; solvent, antioxidant	Ester	Resistant to hydrolysis	(18)
Parabens	Antimicrobial preservative	Ester	Aqueous solutions at pH 3–6 autoclaved for 20 min at 120°C without decomposition and stable (<10% decomposition) for 4 yrs at room temperature; aqueous solutions at pH 8 or above undergo rapid hydrolysis (10% or more after 60 days at room temperature)	(143–146)
Phthalates	Solvent, plasticizer	Ester	Stable to hydrolysis except under extreme conditions.	(147)
Propylene Carbonate	Gelling agent; solvent	Ester	Hydrolyzes rapidly in the presence of strong acids and bases	(148)
Sodium Lauryl Sulfate	Anionic surfactant; detergent; emulsifying agent; skin penetrant; tablet and capsule lubricant; wetting agent	Sulfate Ester	Stable under normal storage conditions, undergoes hydrolysis in solution under extreme conditions (e.g., pH 2.5 or below)	(18,149)
Triacetin	Humectant; plasticizer; solvent	Ester	Stable but subject to imidazole and lipase catalyzed hydrolysis	(18)
Triethyl Citrate	Plasticizer	Ester	Stable	(18)



rates, often by interaction with the API or other excipients.

4. Flavors frequently contain esters. Hydrolysis often will not only decrease the pleasant odor and taste, but also impart an unpleasant odor and taste to the formulation.

### Polymeric Excipients

A wide variety of polymeric excipients are prone to hydrolysis (see Table 7). Many of the excipients that have some sensitivity to hydrolysis are cellulose derivatives. Pharmaceutical cellulosic derivatives involve esters or hemi-acetals of cellulose, both of which are susceptible to hydrolysis. Examples of such derivatized cellulose derivatives include cellulose acetate, cellulose acetate phthalate (CAP), and hydroxypropyl methyl cellulose (HPMC). Hydrolysis of this class of compounds leads to elevated amounts of free acid and changes in solubility and viscosity. These changes can in turn affect the in vivo drug release performance.

The cellulose backbone itself is formed by condensation of sugars with hemi-acetal linkages. As such, strand scission occurs upon hydrolysis, generally under acidic conditions. Such chain scissions result in changes in drug release properties. Non-cellulose derivatives also hydrolyze to reduce molecular weight. For example, alginic acid, a linear glycuronan polymer, is prone to hydrolytic strand scissions (156,157). For certain controlled release, injectable dosage forms, hydrolysis can affect the release rate. One example is the copolymer polylactide-co-glycolide (PLGA). Since the drug release profile in this case is directly dependent on the polymer molecular weight, hydrolysis eventually changes the in vivo performance of the dosage form (158) (see also the “Hydrolytic Degradation for Controlled Release” section).

For viscosity-based oral liquids or IM formulations, hydrolysis of the viscosity-modifying agents can affect the delivery or release rates. These viscosity-modifying agents are usually long chain polymers or natural gums. Hydrolysis can cause the chain lengths to shorten and decrease the viscosity.

The presence of functional groups typically associated with hydrolytic degradation in polymers does not necessarily mean that hydrolysis will be a problem. For example, the ester groups in polyacrylates and polymethacrylates are very resistant to hydrolysis (159).

### Hydrolytic Degradation for Controlled Release

Often, the same functional groups that are responsible for problematic hydrolytic degradation of certain excipients are responsible for their proper function. For example, CAP and hydroxypropyl methyl cellulose phthalate (HPMCP) are both enteric coating materials. Enteric coating agents allow drug to be released at intestinal pHs, but not at lower pHs of the stomach. Both ionization and enzymatic hydrolysis contribute to the dissolution of these enteric polymers in the intestine (160). Hydrolysis during shelf life can lead to the enteric polymer not being a sufficient barrier to gastric juices. For this reason, formulation and packaging (see the “Packaging” section) with such polymers may require more rigorous water exclusion than with formulations not including these polymers.

Biodegradable systems used for controlled release devices employ hydrolysis as the release mechanism (161). Hydrolytically unstable bonds can be part of the polymer backbone with drug interspersed among polymer chains, part of a side chain linked to a hydrophobic agent, part of a crosslinked network, or a part of the polymer that is linked to the active agent as a prodrug (158). The most common hydrolyzable biodegradable polymers are PLGA copolymers. Degradation of these polyesters is autocatalytic and dependent not only on the chemistry and concentration of the ester in the polymer, but also the extent of crystallinity. Other types of biodegradable polymers using hydrolysis include poly-ortho esters, polyacetals, and poly(glutamic acid) (162,163). Erodable hydrogels typically consist of cross-linked polymer matrices with hydrolytic instability at either crosslinks or the polymer backbone. The hydrolysis kinetics for such polymers depends on the degree of water exposure, which often results in different rates for material near the surface vs. that in the bulk. In vivo drug release depends on polymer degradation to enable drug release, either from a matrix or in the form of polymer-encapsulated drug.

### HANDLING HYDROLYSIS PROBLEMS

In the section below, general solutions are suggested for hydrolysis problems. Since each drug is a special case, the specific solutions (if any) that apply must be determined experimentally for the specific drug and formulation.

**Table 7**  
*Polymeric Excipients That Potentially Hydrolyze*

Excipient	Uses	Bond	Reactivity	Hydrolysis Effects	Ref.
Cellulose acetate	Coatings, matrix for CR; diluent; taste-mask	Ester linkages to acetyl groups	Slow hydrolysis at high temp./humidity	Acetic acid formation	(18,150)
Cellulose acetate phthalate (CAP)	Enteric coatings; matrix for CR	Ester linkages to acetyl and phthalyl groups	Slow hydrolysis at high temp./humidity; Slower release observed from coated tablets after 126 days	Phthalic, acetic acid formation; changed enteric performance	(18,151,152)
Hydroxypropyl methyl cellulose phthalate (HPMCP)	Enteric coatings; matrix for CR; binder; microcapsule base	Ester linkages to phthalyl groups	Stable 3–4 yrs/RT, for 2–3 mos/40°C/75% RH, and as coating for 126 d/37°C. 8–9% hydrolyzed in 10 d/60°C/100%RH.	Phthalic acid formation; changed enteric performance	(18,151–154)
Methylcellulose	Binding agent; disintegrant; matrix for CR; taste mask; sealant	Glucose–glucose acetal linkages	Hydrolyzes pH < 3	Decreased MW; changed matrix performance	(18,155)
Hydroxyethyl cellulose	Thickening agent; binder; film coating agent	Glucose–glucose acetal linkages	Hydrolyzes pH < 5	Decreased MW (loss of viscosity)	(18)
Hydroxypropyl cellulose	Binder, coating, matrix for CR; thickening agent	Glucose–glucose acetal linkages	Hydrolyzes at low pH	Decreased MW (loss of viscosity)	(18)
Alginate acid	Binder/disintegrant; matrix for CR	Acetal linkages	Hydrolyzes slowly at warm temperatures	Decreased MW (loss of viscosity)	(156,157)
PLGA	Controlled release matrix	Ester	Hydrolyzes slowly	Loss of CR functionality	(158)

## Liquid Dosage Forms

### pH

The pH rate profile (see the “pH Rate Profiles” section) can be used to determine the shelf life at the pH value of maximal stability. Even if a shelf life of more than two years can be achieved at the pH of maximum stability, a significant sensitivity to changes in shelf life with pH can lead to robustness issues, despite a good buffer system. In contrast, if a shelf life of more than two years can be achieved over a large pH range, then formulation pH can be selected based upon other factors such as solubility and activity of the drug, injection site toleration, and stability and solubility of other excipients.

In addition to stability for solution formulations, a primary consideration for oral and parenteral formulations should be the solubility of the drug and excipients at the pH of maximum stability. If the drug does not have adequate solubility at that pH, various solubilization techniques such as cosolvents, complexation, and surfactants may be needed. With these solubilization methods, it may be necessary to re-evaluate the effect of pH on degradation and the resultant shelf life. Solubilization techniques have been known to increase or decrease hydrolysis rates (45,164), or to change the pH of maximum stability.

For oral solution and suspension products, not only does the pH affect the hydrolysis rate of the drug, but also the excipient stability (see the “Non-polymeric Excipients” section), product taste, and the potential for precipitation of the drug in the stomach, resulting in bioavailability issues. For parenteral products, pH not only affects drug stability, but also injection site precipitation, irritation, and pain. These problems will often occur when the pH is significantly different from the physiological pH (165). In addition, injection site precipitation can lead to an alteration in the pharmacokinetic profile. These factors become important considerations since rarely is the pH value of maximum stability and solubility close to pH 7.

If the desired stability along with the above-mentioned considerations are not achieved in a solution formulation for an oral or parenteral product, other formulation approaches may be necessary. For parenteral products, lyophilized formulations are often useful since this decouples the stability from injection site issues. Similarly, for oral products, powders for constitution allow stability with a dry powder, but provide for acceptable performance at the time of dispensing. This strategy has been successful for numerous commercial

oral antibiotic products. With lyophilized formulations, selecting the pH of the solution from which the lyophile is prepared can affect the product stability (see the “Hydrolysis of Lyophiles” section). The selection of the pH of the reconstitution vehicle will often be dominated by non-stability considerations since the solution need not be stable over an extended period.

### Buffers

An appropriate buffer must be selected to maintain the solution pH of a product at the pH of maximum stability since shifts in pH can dramatically decrease stability. This reduction in shelf life due to shifts in pH can be predicted using the pH rate profile for the drug (see the “pH Rate Profiles” section). The primary criteria for selection of a buffer are the pH range and buffer capacity [which is maximal at a pH value equal to the  $pK_a$  of the buffer (164)] for the buffer. Table 8 lists commonly used buffers along with their  $pK_a$  values and optimal pH ranges. For lyophilized formulations, buffer crystallization potential and collapse temperature should also be considered (see “Hydrolysis of Lyophiles” and Table 5).

The buffering capacity can be increased by using a higher concentration of a buffer; however, drug hydrolysis catalyzed by buffers may become an issue (see the “Buffers and Other Catalysts” section). For

**Table 8**

*Common Buffers Used in Parenteral Formulations [Derived from Refs. (165,166)]*

Buffer	$pK_a$	Buffering Range (pH units)
Glycine	2.34, 9.8	1.5–3.5, 8.8–10.8
Glutamic acid	2.1, 4.3, 9.7	2–5.3
Phosphoric acid	2.1, 7.2, 12.7	2–3.1, 6.2–8.2
Tartaric acid	3.0, 4.3	2.0–5.3
Citric acid	3.14, 4.8, 6.4	2.1–7.4
Lactic acid	3.1	2.1–4.1
Malic acid	3.4, 5.1	2.4–6.1
Benzoic acid	4.2	3.2–5.2
Ascorbic acid	4.2, 11.6	3.2–5.2
Succinic acid	4.2, 5.6	3.2–6.6
Adipic acid	4.41, 5.28	3.4–6.3
Acetic acid	4.8	3.8–5.8
Triethanolamine	8.0	7–9
Tromethamine (TRIS)	8.1	7.1–9.1
Diethanolamine	9.0	8.0–10.0

parenteral products, high buffer capacity can lead to an inability for adjustment to physiological pH in vivo, an effect associated with substantial pain and irritation at an injection site.

Use of co-solvents, surfactants, and complexing agents to solubilize a drug may also influence the buffering capacity and final pH of a formulation by altering the effective  $pK_a$  of the buffer and/or directly interacting with the buffer components. Hence, in such a formulation, the final pH and buffer capacity should be measured and its effect on stability and shelf life of the drug evaluated. In case of an adverse interaction, a suitable alternative buffer may be necessary.

The selection of a buffer and its concentration can also be influenced by the ionic strength contribution of the buffer and its resulting effect on drug stability (see the "Ionic Strength" section). The effect of ionic strength on the drug degradation rate can be studied by monitoring stability in a series of solutions of identical pH and buffer concentrations but with increasing ionic strength by adding salts such as potassium chloride or sodium chloride. When ionic strength effects are significant, the formulation may require a minimal buffer concentration to maintain pH without itself affecting the stability. For formulations that need to be isotonic, there are limited options available for balancing the conflicting needs.

#### Formation of Suspensions

For hydrolytically labile drugs in liquid formulations, formation of suspensions can be an option for increasing stability (46). Reducing drug solubility in the formulation minimizes the concentration of drug in solution and thereby reduces the overall degradation rate, since the reaction rates in the less mobile solid state are often orders of magnitude slower. Neglecting the hydrolysis rate in the solid, the rate of degradation in a suspension is determined by the solubility of the drug at saturation. This leads to a zero-order reaction rate, often significantly lower than the rate for drug at higher concentrations in solution. This method has been demonstrated by converting highly water-soluble but extremely labile penicillin G into poorly water-soluble procaine penicillin G, which maintains the same hydrolytically active center (41). The shelf life of a procaine penicillin G suspension is significantly longer than that of a penicillin G solution. In this case, the rate of hydrolysis is proportional to the extremely low concentration of procaine penicillin G in solution. This method has been used successfully for many penicillin and cephalosporin derivatives, a number of which have

been commercialized. However, parenterally, suspensions can be administered by intramuscular and subcutaneous routes only, and may be limited by the need for sterile bulk. Formation of drug suspensions requires either drug substance modification such as insoluble or sparingly soluble salt formation or use of non-solvents, which may not be preferred as the first choice for stabilization against hydrolysis.

#### Non-aqueous Solutions

One option for stabilizing APIs in solution is to replace some or all of the water with protic, dipolar aprotic or pure organic solvent. These serve to stabilize the drug by several mechanisms, as discussed below.

Organic solvents serve to decrease the concentration of water (lower the water activity, as discussed in Sec. "Water Activity"), and thereby to depress the reaction rate. Although with some solvents, in particular alcohols and amines, solvolysis can replace hydrolysis as a degradation mechanism, for most formulations, residual water-induced hydrolysis remains the major concern.

Since polar functionalities involved in the transition state of hydrolyses are stabilized by protic solvents (such as water), aprotic solvents can decrease the reaction rate. This effect is countered by the greater nucleophilicity of hydroxide anion when more poorly solvated (167). The net result is therefore difficult to predict and must be evaluated on a case-by-case basis. Retardation of hydrolysis rates in alcohol–water mixtures have also been attributed to variation in solvent structure (168).

#### Surfactants

For hydrophobic drugs, solubilization in surfactant based micelles or liposomes can provide protection from catalytic acid and base, thereby stabilizing the drug. This effect has been most pronounced with non-ionic surfactants; however, effects have been observed with charged micelles and liposomes (169–172). Decreases in hydrolysis rates from zero to about four-fold are generally observed. With more polar drugs, the molecules are presumably less deeply enveloped in the hydrophobic core of the micelles and the resulting stability improvement is diminished.

#### Lyophilization

By removing most of the water and reducing the mobility of the drug, lyophilization often results in increases in drug stability at the cost of using a more complex manufacturing and end-use procedure. For

many drugs, lyophilization remains the preferred method for providing a formulation stable to hydrolysis as discussed in the “Hydrolysis of Lyophiles” section.

### Solid Dosage Forms

Several options for fixing hydrolysis problems with solid dosage forms are listed below.

1. *Excipient Choice.* Excipients vary in the amount of water they bring to the system (see Table 1), how strongly they hold onto the water (see the “Water Activity” section), and in their tendency to dissolve some of the drug (see the “Hydrolysis of Crystalline Solids” section). In general, more brittle excipients are desirable since their reduced flow during processing can reduce molecular interactions with a crystalline drug; however, other formulation considerations (e.g., processability) may make such excipients undesirable.
2. *Acidity of Formulation.* Since hydrolysis can be catalyzed under both acidic and basic conditions (see “Specific Base Hydrolysis,” “Specific Acid Hydrolysis,” and “pH Rate Profiles”), excipient–drug mixtures that have a microenvironmental pH different than optimal will reduce the dosage form stability. Using the pH of maximum stability in solution, appropriate buffers can be used, preferably in a form able to chemically interact at the drug–excipient interface. One way of potentially achieving such an intimate interaction is to provide the buffer in solution as part of a wet granulation.
3. *Drug Form.* Since crystalline drugs are generally more resistant to hydrolysis (see the “Hydrolysis of Crystalline Solids” section), drug forms (salts, polymorphs, etc.), which have stronger lattice energies (higher melt temperature), will tend to be more stable. In a similar fashion, drug forms that are more hydrophobic may provide greater hydrolytic stability.
4. *Processing Condition.* Processing generally increases the hydrolysis of drugs by increasing the amount of amorphous drug present. This can sometimes be decreased by reducing the stress on the API by, for example, reducing tableting forces. When the amorphous material is created by direct interactions of an excipient with the API (i.e., when the two form a single phase), reducing interactions of the API with the excipient can

reduce hydrolysis. This can be accomplished by increasing the particle sizes of the API and excipient, or by physically separating the two in separate granulations (173).

### Packaging

#### Barriers

For products susceptible to hydrolysis, packaging may represent a reasonable option for stabilization. In using packaging, one must contend both with the water vapor available in the head-space and the dosage form itself as well as the water vapor permeation through the container walls and cap. For both liquid and solid dosage forms, it is possible to package under low humidity conditions such that the contribution from the head-space can be minimal. The permeation of water vapor into bottles or other packaging depends on the material. Table 9 lists the rates of water vapor transmission through a number of commonly used packaging materials. As can be seen, only glass and foil provide true barriers to water vapor transmission. To get an idea of how much water enters a plastic bottle, the following calculation is shown for a 60-cc HDPE bottle assuming no permeation through the cap (preferably sealed with a heat induction seal, HIS), an interior relative humidity of 0%, and an external relative humidity of 90%, both at 38°C:

$$\text{Surface Area} = 100 \text{ cm}^2$$

$$\text{Wall Thickness} = 0.9 \text{ mm}$$

$$\begin{aligned} \text{Amount of water} &= (\text{transmission})(\text{surface area}) \\ &\quad \times (\text{time})/(\text{thickness}) \\ &= (0.12 \text{ g mm}/(\text{m}^2 \text{ day})) \\ &\quad \times (0.01 \text{ m}^2)/(0.9 \text{ mm}) \\ &= 1.3 \text{ mg water/day} \end{aligned}$$

Even at the less severe external condition of 40°C/75% RH, the permeation rate will be about 1 mg/day. If the initial moisture content in the bottle is zero, then the relative humidity inside the bottle will rise to over 50% RH within about one day. This suggests that the use of standard bottle plastic as a sole means of protection of a dosage form from ambient moisture is unlikely to be successful.



**Table 9**  
*Water Vapor Transmission Rate for Common Packaging Materials*

Material	Water Vapor Transmission [g mm/(m <sup>2</sup> day)]	
	at 38°C and 90% RH	Reference
PVC	1.8	(174)
Polypropylene	0.54	(174)
HDPE	0.12	(175)
Aclar UltRx	0.006	(175)
Aclar 22A	0.011	(175)
Nylon 6	7.5–7.9	(175)
Oriented PET	0.39–0.51	(175)
Cold Formed Foil Blister	<0.005	(176)

An option for solid dosage forms that provides better moisture barrier characteristics as well as allowing single dose use without exposing the bulk to moisture is blister packaging (177–182). Although this option can be significantly more expensive than bottle packaging, for some drugs this cost can be absorbed. One can either start with a preformed shape or mold the container to the tablet or capsule in the process of packaging. With the former option, there will be a greater amount of water vapor trapped with the dosage form; however, the barrier properties are generally superior. The water barrier properties for a number of blister packaging options (typically multilayer coextrusions or laminates) have been studied under various temperature and humidity conditions (180). Typically, the higher the barrier properties, the greater the cost. In addition, many blister packaging materials that are good for moisture, are poor for oxygen. Because of the high surface area of a blister package per dose, moisture permeability will be a problem under challenging conditions with all the blisters except foil. In particular, some blisters become markedly more permeable at higher temperatures (180). In terms of maintaining a low relative humidity inside a blister under conditions of high moisture in the external environment, only foil–foil blisters are likely to be adequate.

#### Desiccants

Desiccants represent a valuable packaging option for solving hydrolysis problems with solid dosages. The type and capacity of desiccant can be adjusted to provide adequate protection from degradation of a dosage form over its shelf life. Desiccants vary in their water

adsorption capacity and rates. Desiccants also vary in their ability to remove moisture as a function of relative humidity in the air. When using moisture-permeable packaging (plastic bottles), the system will reach a steady-state condition where the amount of moisture permeation through the bottle matches the moisture adsorption rate. This steady-state moisture level will differ with different adsorbants. For example, with such desiccants as silica gel, the adsorption is efficient at high humidity but very poor at low humidity, while for molecular sieves, the efficiency at high humidity is less than that for silica gel, but better than silica gel at low humidity. The result is that for molecular sieves, the steady-state humidity is lower; however, for reduction of high humidity, one needs to use more adsorbant. In addition, because of its greater adsorption ability at low humidity, molecular sieves need more special handling to avoid losing capacity during packaging operations. Various desiccant options are summarized in Table 10. Desiccation rates also depend on the form the desiccant comes in. Sachets of desiccants have high permeation rates and consequently can desiccate more rapidly than can cartridges or canisters; however, insertion of the desiccant into bottles on packaging lines is typically slower for sachets than for cartridges or canisters.

A very approximate amount of desiccant needed for a system can be estimated based on the data in Table 10 and the water permeation rates from Table 9. For example, the amount of water entering a 60-mL HDPE bottle at 40°C/75%RH outside, 40°C/20%RH inside the bottle will be approximately half the rate calculated in the “Barriers” section (i.e., 0.65 mg/day) or 475 mg in two years. This estimate overstates the water transmission rate at room temperature; however, using this



**Table 10**  
*Desiccant Options for Use with Pharmaceutical Products [Derived from Refs. (183)]*

Desiccant Type	$\text{g}_{\text{H}_2\text{O}} \text{ Adsorbed/g}_{\text{Adsorbent}}$ (25°C/75% RH)	$\text{g}_{\text{H}_2\text{O}} \text{ Adsorbed/g}_{\text{Adsorbent}}$ (25°C/20% RH)	$\text{g}_{\text{H}_2\text{O}} \text{ Adsorbed/g}_{\text{Adsorbent}}$ (25°C/10% RH)	Hr. to 1/2 Capacity (25°C/75% RH)	Approx. RH Reached (%)
Silica gel	0.33	0.12	0.05	1.2	10–20
Clay	0.26	0.11	0.08	1.5	10–20
Molecular sieves	0.22	0.18	0.15	0.5	2–10
CaSO <sub>4</sub>	0.10	0.05	0.03	1.2	15–30
CaO	0.28	0.28	0.27	27	1–25

estimate it would take at least 260 mg of silica gel desiccant to maintain a 20% RH in the bottle. Since commercial desiccant canisters used for pharmaceutical products usually have 1–2 g of desiccant, in most cases there is adequate drying capacity to handle the shelf life of the API in the bottle.

### CONCLUDING REMARKS

Though, in general, the mechanisms of hydrolysis are well established, the particular issues intrinsic in dosage form development are difficult to predict. This is partly due to the heterogeneity of many dosage forms. Currently, stabilization methods exist for many hydrolysis problems for both liquids and solids; however, often fixing hydrolysis introduces new problems to the complex systems involved in dosage forms. Each drug or drug candidate presents its own set of constraints, making formulation development unique for each situation. For this reason, this review offers a broader perspective on hydrolysis and dosage form development.

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