

REVIEW ARTICLE

Stabilization of Pharmaceuticals to Oxidative Degradation

Kenneth C. Waterman,* Roger C. Adami,
Karen M. Alsante, Jinyang Hong, Margaret S. Landis,
Franco Lombardo, and Christopher J. Roberts

*Pfizer Global Research and Development, Eastern Point Road,
Groton, CT 06340*

ABSTRACT

A guide for stabilization of pharmaceuticals to oxidation is presented. Literature is presented with an attempt to be a ready source for data and recommendations for formulators. Liquid and solid dosage forms are discussed with options including formulation changes, additives, and packaging documented. In particular, selection of and methods for use of antioxidants are discussed including recommended levels.

INTRODUCTION

Scope

This review article sets the stage for a pharmaceutical formulator to deal with the problem of drug-product chemical instability. In particular, this article focuses on one of the more common modes of degradation in drug products, namely oxidation. Methods are suggested for establishing that oxidation is indeed the problem, and what the particular oxidative pathway is for degradation. Although each new drug presents unique challenges, guidance is provided for resolving this problem based on the best information currently available.

This review is organized to provide information on recognizing and predicting drug oxidation in dosage

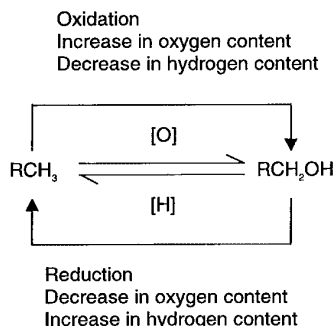
forms. Relevant oxidation mechanisms and various remedies are discussed for the different dosage forms including traditional liquid and solid dosage forms containing small molecules, as well as formulations containing protein and DNA-based pharmaceuticals. This review also provides a decision tree for addressing oxidative degradation, along with a detailed table of antioxidant additives and their commercial precedence. Other approaches discussed include packaging, counterions and pH, and mitigation of impurities.

Formal Oxidation (Recognition of Oxidation)

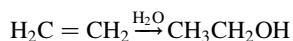
Oxidation is defined broadly as the loss of electrons from a molecule (increase in oxidation number). For organic molecules, this can be restated as an increase in

*Corresponding author. Fax: (860) 441-3972; E-mail: ken_waterman@groton.pfizer.com

oxygen or decrease in hydrogen content (1). Alternatively, oxidation can be defined as a reaction that increases the content of more electronegative atoms in a molecule (2). With organic systems, these electronegative heteroatoms are generally oxygen or halogens.



When a compound is oxidized, another compound must be reduced. Hydration and dehydration are not oxidation/reduction reactions, though they add oxygen, since the reaction is essentially an internal oxidation and reduction: one carbon atom is oxidized while another is reduced. The net change to the oxidation state of the molecule is therefore zero. In the example below, ethylene is hydrated to ethanol. While the carbon on the left gains a hydrogen atom and is therefore effectively reduced, the carbon on the right gains an oxygen atom and is effectively oxidized. The net change to the molecule is zero.

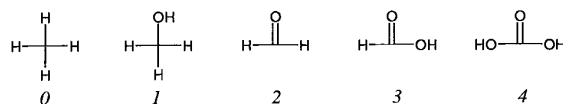


If there is any doubt as to the oxidation status of a molecule, the following procedure helps to identify the oxidation state of a given compound (3):

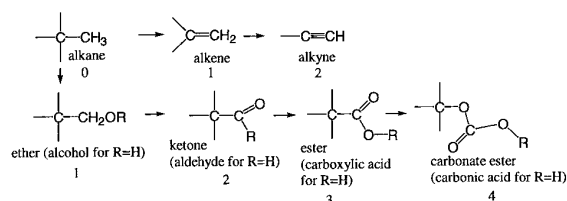
1. Imagine a water molecule being added onto all unsaturated bonds in that molecule. If there is a ring in the molecule, open the ring with a water molecule.
2. Count the number of heteroatoms in the water addition product. This is the oxidation number of that molecule.
3. By comparing the oxidation numbers for reactants and products, one can determine if the reaction represents an oxidation.

The italicized numbers in the following scheme represent the corresponding oxidation numbers for the

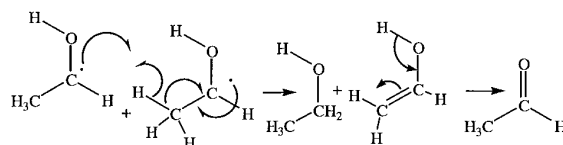
structures shown below:



Listed below are the general structures and names of some common pharmaceutically relevant species in the order of increasing oxidation states with the oxidation numbers listed below each species:



To study an oxidation reaction mechanism, it is important to understand the electron transfer between the species involved by tracking the fate of electrons (using Lewis structures and arrows). For single-electron transfers, single-headed arrows are used, while for two-electron processes, double-headed arrows are used. As an example of tracking an electron transfer, disproportionation of two radicals is shown with arrows indicating the "flow" of electrons:



Preliminary Screening for Oxidative Instability

Solid Dosage Forms

There is currently no best practice purposeful degradation protocol to study oxidative degradation in solid-state drug substances or drug products. As a result, solid-state reactivity of the drug substances towards oxidation in drug products is explored most readily using excipient compatibility screening under thermal/humidity challenge conditions. Although there has been some discussion on using ternary mixtures of drug and excipients (with statistical design) (4), currently, binary mixtures of drug compound and tableting excipients (binders, fillers, disintegrants, etc.) are used commonly in preliminary screening experiments. Samples are prepared using standard mortar and pestle geometric dilution techniques (i.e., over a wide range of drug-to-



excipient ratios). The mechanical forces involved in using the mortar and pestle are hoped to mimic some of the forces involved in milling and tableting, which often lead to amorphous or disordered drug regions (see "Oxidation in Solid Dosage Forms"). These blends are stored under different temperature and humidity conditions (indicated by the relative humidity, RH), typically 5°C, room temperature/ambient humidity, 40°C/closed bottle, 40°C/75% RH open bottle, 50°C/closed bottle and 50°C/20% RH open bottle. These studies provide an indication of the oxidative instability of a drug in a binary mixture where the pure drug substance may be quite stable. Such studies help to identify particular excipients that may need to be avoided. Unfortunately, these blend stability studies do not, in general, predict rates for the system after granulation, milling, and tableting, and can therefore only be used qualitatively.

If oxidative instability is suspected, studies can be performed to determine if molecular oxygen is involved in the oxidation process. Stability challenges that involve filling the headspace of vials with nitrogen (negative control) and pure O₂ (positive control) are often useful to determine if molecular oxygen is involved in the degradation. If the headspace environments have little or no effect on the oxidative reactivity of the drug substance in these stability screenings, molecular oxygen may be involved in the reaction but not in the rate-limiting oxidation step, or the level of oxygen may still be sufficiently high that the reaction readily occurs. More thorough removal of oxygen in the headspace can be accomplished by a freeze–pump–thaw cycle. Alternatively, oxidation may be related to highly reactive impurities (peroxides, superoxides, hypochlorites, formic acid) present in the excipients as manufacturing-related impurities (see "Detecting and Controlling Impurities"). Such drug oxidative instability will often show itself in the form of greater decomposition rates for more dilute drug mixtures. Pre-treatment of the excipients with heat and radical scavengers such as nitric oxide or benzoquinone may also be helpful in implicating these impurities as the source of oxidative instability. While these techniques may be helpful in determining the causes and mechanisms of a drug oxidation, they have not been adapted to use in the actual solid dosage form stabilization.

Liquid Dosage Forms

Liquid dosage form oxidative stability screening generally involves examining the drug stability under a

number of conditions. These conditions will vary depending on the type of dosage form (oral, parenteral, etc.) and limitations due to solubility and decomposition pathways competitive with oxidation. Among the factors to evaluate for a new drug candidate are the following:

1. *Acidity.* The pH can impact the oxidative stability of ionizable drugs (see "Acidity and pH Effects").
2. *Concentration.* Excipient impurities will have more relative impact on dilute solutions than on concentrated solutions.
3. *Temperature.* Although Arrhenius behavior is seldom observed, an indication of oxidative stability is found by examining the reactivity over the range 5–70°C.
4. *Oxygen in the headspace.* Both oxygen enriched and inert atmosphere samples can provide an indication of the tendency for a drug to oxidize in a particular formulation.
5. *Photo-oxidation.* The stability of the drug in the presence of light and oxygen can be important. In addition, the use of added sensitizers (such as rose bengal) in examining the photostability of the drug can help determine whether the mechanism involves singlet oxygen.
6. *Metals.* Addition of metal ions to solutions can indicate whether there is a tendency for the drug to be catalytically oxidized (see "Catalysis"). Typically, FeCl₃ or CuCl₂ are added at levels less than 100 ppm.
7. *Packaging.* For parenteral dosage forms, a range of stoppers should be examined. For oral dosage forms, both plastic and glass bottles should be evaluated (see "Packaging/Liquid Dosage Forms").

General Issues

It is difficult to use the Arrhenius equation to describe oxidative instability, or accelerated screening methods in general to predict room temperature shelf-life. This can be due to the following factors:

1. Instability may be related to the amount of a peroxide impurity present in the particular lot of the excipient (see "Detecting and Controlling Impurities"). This could be correlated with the manufacturing processes involved, age of the excipient, and the conditions (temperature, humidity, sunlight) under which the material was stored. Oxidation rates can be very rapid at early time



- points when peroxide impurities are plentiful and plateau or fall off after the impurity is consumed by the degradation reaction.
2. If the generation of radicals is rate limiting, the kinetics can show autocatalysis; i.e., the rate of drug degradation increases as the radical concentration increases.
 3. There can be more than one mechanism involved in the degradation with the differences in activation parameters similar enough that temperature changes can essentially lead to different mechanisms dominating.
 4. In the solid state, temperature-sensitive properties of the drug product such as percent amorphous content, degree of hydration of the components, and molecular mobility may affect the oxidative degradation.
 5. The permeability of oxygen through packaging is temperature dependent.
 6. The solubility of oxygen in excipients (solvents) is inversely temperature dependent.

Detection and identification of oxidative degradants is aided by the use of mass spectrometry. Table 1 gives some characteristic mass peaks that may be associated with a drug substance that has undergone some type of oxidative reaction. Often, tandem liquid chromatography-mass spectrometry/mass (LC-MS/MS) techniques can be used in identifying specific sites of oxidation within complex molecules.

Predicting Oxidation

Purposeful Degradation (5,6)

Oxidative studies executed to force drug substance degradation are useful to predict primary oxidative

degradants in drug products. Published stability guidelines (7) suggest the use of a concentrated oxygen atmosphere to generate oxidative degradation products for chromatographic identification. Since degradants can arise from reaction of the drug product with molecular oxygen (see "Autoxidation—Chain Processes" and "Electron Transfer") or with oxidizing agents present in the formulation (usually peroxides, see "Peroxides and Other Oxidizing Agents"), it is important to conduct purposeful degradation studies with oxygen as well as hydrogen peroxide. Hydrogen peroxide is often non-predictive of molecular oxygen reactions (8) because it does not involve the radical chain process common with the oxygen-based reactions (see "Autoxidation—Chain Processes"). Hydrogen peroxide stress testing is useful in drug-product studies where hydrogen peroxide itself is an expected impurity in an excipient (see "Formation and Presence of Oxidants in Excipients" and "Detecting and Controlling Impurities").

For an oxygen-atmosphere purposeful degradation study, a solvent must be chosen that solubilizes the drug sufficiently (1–10 mg/mL) and ideally mimics the proposed formulation. Although ideally one would use protic solvents to mimic common protic excipients, this is complicated by the tendency for alcohols to slow oxidation reactions by competing with the drug for initiator radicals (9). For this reason, the polar aprotic solvent acetonitrile is often used in place of alcohols in model studies. A co-solvent may be necessary to achieve a sufficiently high concentration.

Radical initiators can be effective in accelerating autoxidation (see "Initiation"), thereby allowing for easier characterization (10–12). Although electron-transfer (see "Peroxides and Other Oxidizing Agents") rather than free-

Table 1
Possible Products of Drug Oxidation Based on Mass Spectral Data

Mass Spectral Analyses	Possible Products
Parent – 2	Oxidation of –CH(OH)– to –CO–, primary or secondary amine to imine
Parent + 14	Oxidation of –CH to –C(O)–
Parent + 15	Oxidation of secondary amine to N-oxide
Parent + 16	Hydroxylation or conversion of –C–H to –C–OH; epoxidation of a double bond; sulfide to sulfoxide conversion; tertiary amine to N-oxide
Parent + 32	Hydro- or endoperoxide formation; sulfide to sulfone conversion



radical propagation may dominate the mechanism in the dosage form, in most cases the products (though not their relative distributions) are the same in either case. Because the addition of initiators allows for generation of 10–20% degradation product within 10 days (using 1–10 mol% radical initiator in the presence of pressurized oxygen), it is generally advantageous to use this approach along with appropriate controls.

To perform an autoxidative degradation study, the drug substance is dissolved in an appropriate solvent and transferred to a reaction vessel pressurized at 50–300 psi O₂ to increase the oxygen concentration in solution, and heated to form radicals from the initiator (see Table 2 for a list of typical initiators).

In carrying out a purposeful oxidative degradation study, it is critical to run the appropriate controls in order to get a better mechanistic understanding of whether the degradation results from a thermal, free-radical, or nonfree-radical process. Controls for these experiments include the following:

1. drug with oxygen without initiator;
2. drug with initiator purged of oxygen (with nitrogen or argon);
3. drug without initiator purged of oxygen (at the reaction temperature); and
4. initiator at appropriate level without drug substance to determine if any observed high pressure liquid chromatography (HPLC) peaks result from oxidation products that are not drug substance related.

Redox Potential

As discussed in “Formal Oxidation (Recognition of Oxidation)”, oxidation involves the (formal) loss of

electrons. A compound’s oxidation potential (or redox potential) gives its thermodynamic tendency to lose an electron. A classical way to determine the redox potential and provide some kinetics for the decomposition of the oxidized species, involves the use of cyclic voltammetry (CV). Sweep rates of several thousand volts per second are possible with adequate instrumentation, which offers the opportunity of clocking the lifetime of radicals generated by oxidation, even for very fast processes (13). Because of the preparation and analysis time involved, CV techniques are more appropriate for detailed mechanistic investigations rather than for fast preliminary screening of drug candidates.

A comparison based on the use of known standards (antioxidants, drugs, and general organic compounds) can be used to assess the relative ease with which a compound undergoes electron transfer. This allows a prediction of stability based on a compound’s redox potential and the relative stability of standards with similar potentials. A compound, which by comparison to known oxidatively labile compounds yields a low oxidative potential (more easily oxidized), is likely to be prone to oxidative degradation. In Table 3 are tabulated some redox potentials of reference compounds and rough potentials of some oxidatively labile groups (the lower the potential, the more readily the species will be oxidized). Molecules that are generally stable to electron-transfer oxidation could still be unstable to oxidation by hydrogen-atom abstraction (see “Propagation”). These data allow for general rules for predicting electron-transfer based oxidative stability:

1. oxidation potential ≥ 1300 mV: stable to electron-transfer oxidation;
2. oxidation potential between 850 and 1300 mV: depends on specific drug and formulation; and

Table 2

Free-Radical Initiators Useful in Purposeful Oxidative Degradation Studies (from Waco Pure Chemical Industries)

Initiator	Chemical Abstract Service (CAS) #	Temperature for 10 hr $T_{1/2}$ (°C)	Solubility (mg/mL)
2,2'-Azobis(<i>N,N'</i> -dimethyleneisobutyramidine)dihydrochloride	27776-21-2	44 (H ₂ O)	35.2 (H ₂ O)
4,4'-Azobis(4-cyanopentanoic acid)	61630-29-3	69 (H ₂ O)	1 (H ₂ O)
2,2'-Azobis(2-amidinopropane)dihydrochloride	2997-92-4	56 (H ₂ O)	23.2 (H ₂ O)
2,2'-Azobis(2-methyl- <i>N</i> -hydroxymethyl) propionamide))	61551-69-7	86 (H ₂ O)	2.4 (H ₂ O)
2,2'-Azobisisobutyronitrile (AIBN)	927-83-3	65 (toluene)	7.5 (MeOH)
2,2'-Azobis(2,4-dimethylvaleronitrile)	52406-55-0	51 (toluene)	22 (MeOH)



Table 3
Selected Potentials for Antioxidants and Drugs

Compound	Oxidizable Functional Group	Oxidative Peak Potential	Observed Stability Towards Oxidation
Dextromethorphan	Tertiary amine	1400 (14)	Stable (9)
Amitriptyline	Tertiary amine	1300 (15)	Stable (16)
Fluoxetine	Secondary amine, benzylic ether	1300 (15)	Stable (17)
Flurazepam	Tertiary amine, imine	900 (15)	Stable (18)
Captopril	Thiol	~900 (19)	Unstable (20)
Chlorpromazine	Thioether	840 (15)	Unstable (20)
Morphine	Allylic alcohol, phenol	820 (21)	Unstable (20)
L-Ascorbic acid	Allylic alcohol	700 (21)	Unstable (20)
Vitamin E	Phenol	700 (21)	Unstable (20)
Phenylbutazone	3,5-Dioxopirazolidine	660 (22)	Unstable (9)
Tetracycline(s)	Phenol, enols, tertiary amine	550 (23)	Unstable (20,24)

The potentials were obtained under different conditions, and are intended only as illustrative examples. All potentials are in mV vs. Ag/Ag⁺ reference electrode.

3. oxidation potential ≤ 850 mV: easily oxidized.

Identifying Oxidative Reactivity Based on Structure

Autoxidation

Assuming autoxidation of a drug molecule occurs by an initial abstraction of a labile hydrogen atom followed by reaction with molecular oxygen (see "Autoxidation—Chain Processes"), oxidative degradation should be linked to the lability of hydrogen atoms within the molecular framework. The lability of hydrogen atoms within a molecular structure is described by the corresponding bond-dissociation energies; i.e., the lower the bond energy, the more likely a hydrogen atom will be abstracted by a radical. Table 4 shows the representative bond-dissociation energies of some common functional moieties.

A qualitative way to estimate hydrogen atom lability is to evaluate the stability of the radical that is formed. In general, the more stable the resulting radical, the easier for the hydrogen atom to be abstracted from the corresponding position on the molecule. The rules of radical stability are similar to the rules that govern reactive center stability (i.e., cations and anions). In general, for sp³ hybridized centers, tertiary centered radicals are more stable than secondary centered radicals which are more stable than primary radicals so that the corresponding hydrogen atom abstraction will occur most readily from tertiary hydrogens, and

least readily from primary hydrogens. This stability order is supported by the bond-dissociation energies in Table 4, where the C–H bond energy for ethane is less than the C–H bond energy for methane. With sp² and

Table 4
Representative Hydrogen Bond-Dissociation Energies for Some Common Structural Moieties (25,26)

Species	Bond-Dissociation Energy (kcal/mol)
CH ₃ –H	104
CH ₃ CH ₂ –H	98
CH ₂ =CH–H	103
CH ₂ =CHCH ₂ –H	85
PhCH ₂ –H	85
H ₂ N–H	103
CH ₃ NH–H	92
CH ₃ O–H	102
PhNH–H	80
HO–H	119
HOCH ₂ –H	93
HOCH(CH ₃)–H	90
CNCH ₂ –H	86
HO–OH	51
CH ₃ O–OCH ₃	36
HOO–H	90



sp hybridized centers, stabilization of radicals is generally poor due to the orthogonal arrangement of the π -orbitals with the unpaired electron. Referring to Table 4, the hydrogen bond energy for ethylene is similar to that of methane.

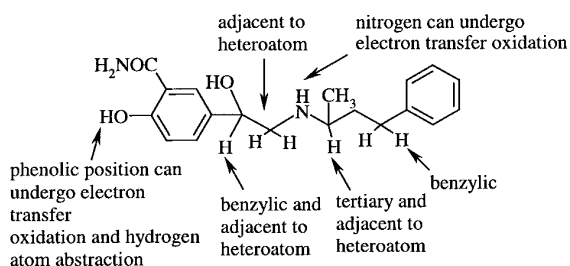
Particularly stable radicals are formed from hydrogen atom abstraction of allylic or benzylic positions. These methylene/methine positions adjacent to aromatic or vinylic systems are very susceptible to hydrogen-atom abstraction because the resulting radical centers are stabilized through delocalization (see Table 4). Similarly, hydrogen atom abstraction from carbons adjacent to heteroatoms (nitrogen or oxygen) is facile due to electronic stabilization of the product.

Other Oxidation Mechanisms

Oxidation can also occur via an electron-transfer reaction (see "Electron Transfer") to form reactive radical anions or cations. Nitrogen (amines), sulfur heteroatoms (sulfides, disulfides, and sulfoxides), and oxygen-based anions (phenol anions) are common sites for electron-transfer induced oxidation, producing final products such as N-oxides, sulfoxides, sulfones, and ketones. These sites can also act as nucleophiles to react with peroxide impurities in the formulation (see "Peroxide Reactions with Drugs") giving similar products.

Example

As an example of using these rules to predict reactive centers, Labetalol is shown below with the potentially labile hydrogen atoms and other oxidation sites indicated:



Computational Modeling

Computational prediction of degradation can be extremely helpful in the understanding of degradation

mechanisms and the characterization of degradation products found by purposeful degradation studies (see "Purposeful Degradation"). Several commercial computer programs designed to predict reaction products are available including elaboration of reactions for the organic syntheses (EROS) (27), workbench for the organization of data for chemical applications (WODCA) (28), and logic and heuristics applied to synthetic analysis (LHASA) (29). Since these programs are parameterized around the solution chemistry reactions, they may be less predictive for reactivity of drug molecules in the solid state. The EPIWIN (estimation program interface for Windows™) (30,31) is designed to predict the reactivity of organic molecules towards hydroxyl radicals ($\text{HO}^\bullet$). This approach can be useful in estimating the reactivity of a given molecule towards hydrogen abstraction; however, its use in predicting actual degradation products and reaction rates has not been reported. One program found to be particularly useful is computer assisted mechanistic evaluation of organic reactions (CAMEO) (32). The CAMEO program computationally predicts the products of organic reactions given the starting materials, reagents, and conditions including the oxidative conditions. Solvent variation (protic/aprotic) and temperature changes can be considered, as well as more than one equivalent of reagents. The CAMEO degradation prediction results can be used as an initial guide to possible decay products. The CAMEO predicted decay products may not in reality be observed during the stability studies, and some degradants can be formed, that are not predicted in the calculations. In general, however, the CAMEO algorithms have been biased to predict more products than are likely to be observed. It is also likely that certain products predicted can undergo further decomposition. For example, primary and secondary hydroperoxides predicted in many oxidation processes typically undergo further reactions in actual degradation studies to produce ketones and alcohols. Although not reported in the literature, CAMEO allows for a standard oxidative set of reagents and conditions to be created and used routinely to predict oxidation products. This approach would allow for consistency for all compounds being evaluated. The reagents and conditions used do not necessarily need to mimic the real stability conditions, but rather could be predictive of those conditions. In spite of CAMEO's limitations (only available for Macintoshes, limited to 64 total atoms), future adaptations promise to make this and other computational methods more routinely useful for pharmaceutical scientists.



MECHANISMS OF OXIDATION

Autoxidation—Chain Processes

The free-radical process of autoxidation consists of a chain sequence involving three distinct types of reactions: initiation, propagation, and termination.

Initiation

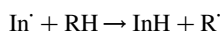
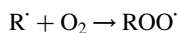
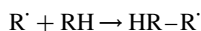
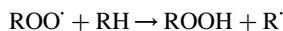
Initiation produces a free radical to begin a chain reaction (11,33,34). Initiation can occur due to homolytic cleavage of a weak bond such as with a polymeric excipient, by electron-transfer processes (see "Electron Transfer"), by light-induced reactions, or by metal catalysis (see "Catalysis").

In the solid state, special issues exist with regard to initiation. In particular, homolytic bond cleavage to give a pair of radicals (caged radicals) will generally not lead to free radicals since cage escape is strongly dependent on the diffusional mobility within a solid matrix. Because of this, initiation is slow to nonexistent in the solid state, though any radicals generated tend to survive for long periods since termination is slow. At this point, it remains unknown how much drug oxidation in the solid state is a product of chain processes vs. reaction of drug with oxidant impurities (see "Peroxides and Other Oxidizing Agents") and mobile active oxygen species (hydroxyl, peroxy, and superoxide) generated by metal catalysis.

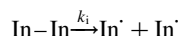
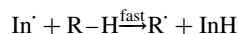
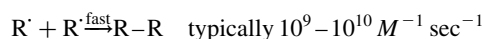
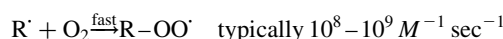
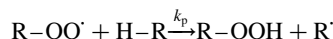
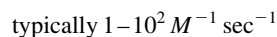
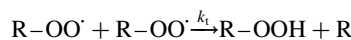
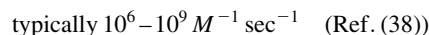
Propagation (11,34)

Once a free radical is produced, its fate can include propagation by hydrogen-atom abstraction from the drug or excipient (solvent), propagation by a radical addition reaction to the drug or excipient, propagation by reaction with molecular oxygen to form a peroxy radical, rearrangement, cyclization, or termination (see "Termination"). The peroxy radical itself can propagate through the same types of reactions.

Propagation:



Though hydrogen-atom abstraction from the drug is formally an oxidation process, it is not an autoxidation process since there is no oxygen in the reaction path. When oxygen is absent, the drug radical will reform the drug by abstraction of a hydrogen atom from other molecules in the system. When oxygen is involved, however, the hydroperoxide product rarely loses an oxygen molecule, such that irreversible drug decomposition occurs. In solution, reaction of a radical with molecular oxygen typically occurs with no activation barrier; i.e., the reaction occurs at the diffusion-controlled rate of approximately $10^9 \text{ M}^{-1} \text{ sec}^{-1}$ depending on the solvent viscosity (35,36). In solution, under normal atmospheric conditions, the rate-limiting step is hydrogen-atom abstraction, therefore the resulting rate expression is determined by the rate of radical formation (initiation), propagation (typically $10^4 - 10^{-7} \text{ M}^{-1} \text{ sec}^{-1}$) (37,38) and termination, but is not dependent on the concentration of oxygen in solution. This is shown in the following rate expression for chain oxidation (37,38):



$$-\frac{d[\text{RH}]}{dt} = k_p \left(\frac{k_i}{k_t} \right)^{1/2} [\text{RH}]$$

One of the by-products of this chain process is a hydroperoxide of either the drug or an excipient, which itself can act as an oxidant for the drug (see "Peroxides and Other Oxidizing Agents"). With oxygen present, the radical propagation (with oxygen consumption) can lead to large turnovers where every step creates a degradation product of the drug. Eventually, the build-

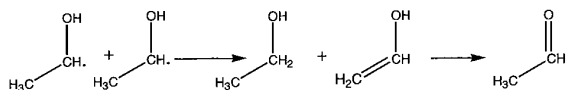


up of hydroperoxides can lead to changes in the reaction mechanism, where the hydroperoxide competes with the drug in the hydrogen-atom abstraction step.

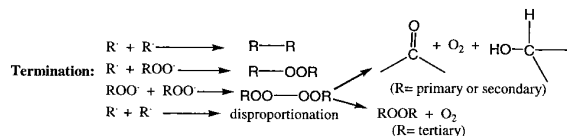
In the solid state, propagation is hindered by low mobility. Reactions therefore tend to occur over extremely short distances with fewer chain propagation steps. Alternatively, solid-state oxidation can be propagated through diffusible species such as peroxide and superoxide. Although the products from these processes are often the same as for the chain process described above for solutions, the mechanism and rates in the solid state can be significantly different (see "Oxidation in Solid Dosage Forms"). Another implication of having fewer propagation steps in the solid state compared to the solution state is that antioxidants designed to intercept the chain (see "Chain Terminators") may be considerably less effective in the solid state. The low mobility of the solid state requires that an antioxidant be dispersed into each small domain to quench the reaction effectively.

Termination (11,34)

Termination of propagating radicals occurs on combination of two radicals to form nonradical products. This can manifest itself in radical combination reactions where two radicals form a new bond joining them together or by disproportionation where one radical is reduced while the other is oxidized. This latter process typically involves hydrogen-atom donation from one radical to another. This process is illustrated in the case of ethanol where oxidative disproportionation leads to recovered ethanol plus acetaldehyde:



Termination in solid dosage forms is generally similar to that in solution, though with different rates. The overall termination steps are shown below (39):

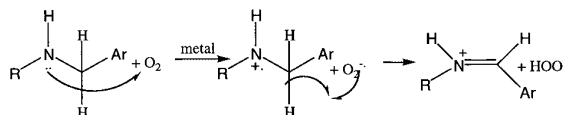


In most environments, the concentration of $\text{R} \cdot$ will be low due to its rapid reaction with oxygen. This

effectively means that most termination will occur via dimerization of peroxy radicals (third reaction above). Primary and secondary peroxy radicals undergo further decomposition to alcohol, aldehyde, and ketone products via termination. In the solid state, termination is again hindered by the inability of radicals to collide because of low mobility. This can lead to higher steady-state concentrations of radicals than seen in solution, and permits their ready detection by electron spin resonance spectroscopy.

Electron Transfer

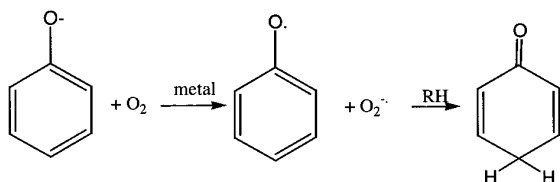
Direct and catalyzed electron-transfer processes can be important mechanisms of drug oxidation (2). Oxidation by electron transfer can occur under two conditions: 1. Electron-transfer based oxidation of a radical can occur to produce the radical cation of the drug, which can then undergo further decomposition. A drug radical may be formed in a chain process such as shown in "Autoxidation—Chain Processes" or may itself be the result of an electron-transfer process. The electron acceptor can be a peroxy radical (giving peroxide, HOO^-) or oxygen (giving superoxide, O_2^-). These species themselves can be reactive intermediates. Importantly, they are potentially mobile in solid dosage forms; 2. Electron transfer can occur from a Lewis base to an oxidant in the system. In general, this type of electron transfer will occur if the radical formed in the electron transfer is relatively stable and the donor has a low electron affinity (see "Redox Potential"). Amines, thiols, and phenolate ions are particularly prone to this type of electron transfer. Primary and secondary amines can oxidize to hydroxylamines or imines, while tertiary amines and pyridines can go to amine-N-oxides. In the process shown below, metal mediated electron transfer of an amine to oxygen forms superoxide, which in turn can abstract a hydrogen atom to give an imine:



Phenols can undergo metal-catalyzed electron transfer from the phenolate ion followed by hydrogen-atom



abstraction from the environment to eventually give dienones, as shown below:



Note that the superoxide generated in each case is capable of further oxidation reactions by itself abstracting hydrogen atoms (40). Solution results have indicated that uncatalyzed direct electron transfer to oxygen is unlikely (41).

Electron-transfer processes may be especially significant oxidation mechanisms in condensed states since chain initiation and propagation processes of the drugs and excipients are hindered by lack of mobility. Electron transfer generates mobile oxidizing species, which may play a significant role in solid dosage form oxidation.

Peroxides and Other Oxidizing Agents

Formation and Presence of Oxidants in Excipients

Peroxide impurities in excipients, especially polymeric excipients, are a major source of oxidation in pharmaceutical formulations (42). Other common oxidant impurities include superoxide, hypochlorites, and formic acid. For a given excipient, oxidant levels will likely vary according to the manufacturer, the molecular weight (in the case of polymers), the particular lot, and the storage conditions. A discussion of detection of peroxides can be found in "Detecting and Controlling Impurities". The source of the peroxides and other oxidants in excipients is sample dependent, but falls into two classes:

1. *Impurities from excipient manufacturing.* In some cases, peroxides and other oxidants are introduced during the polymer manufacturing process including the polymer initiation and purification. Peroxides are used sometimes as initiators in polymerizations and are difficult to remove completely during the purification processes. This is the case with the polyethylene glycols, polysorbates (e.g., Tweens), and polyvinylpyrrolidone (43). With some cellulose, a peroxide or hypochlorite bleaching process is used that can

allow trace peroxides or hypochlorite to remain in the excipient. In the case of polyethylene oxides, high molecular weight material is prepared and then oxidatively degraded to give the desired molecular weight range (44). This degradation leads to peroxides and other low molecular weight species (such as formate ions). Aldehyde impurities can induce drug oxidation in some cases (45).

2. *Products of polymer degradation.* Peroxides can also be formed through autoxidative degradation processes in both polymeric and low molecular weight excipients (see "Autoxidation—Chain Processes"). Autoxidation with polymers is problematic due to both initiation and propagation. Initiation can occur with high molecular weight polymers due to homolytic strand scission. As the backbone length increases, there is an increased likelihood of conformations that have high strain, facilitating thermal bond cleavage. Propagation occurs with polymers having easily extractable hydrogen atoms (see for example the degradation reaction of polyethers shown in "Oxidation Effects on Controlled Release Dosage Forms"). For those polymers for which these degradation pathways are possible, careful attention should be paid to the conditions that accelerate degradation and result in increased peroxide levels. In particular, polymeric excipients will often degrade considerably faster at temperatures above their glass-transition temperatures (T_g) (46). Superoxide can also be formed as an autoxidation ("Autoxidation—Chain Processes") or electron-transfer ("Electron Transfer") by-product.

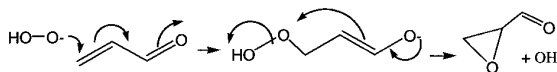
It is often helpful to contact the manufacturer of an excipient to obtain certificates of analysis (COAs) and information on manufacturing processes and purification protocols to track down possible sources of oxidation. With all excipients, manufacturing conditions, purification processes, and storage conditions may play a role in the formation of oxidative impurities, such that lot-to-lot variability may be observed.

Peroxide Reactions with Drugs

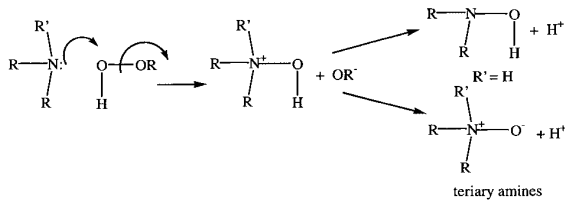
Peroxides can undergo three types of oxidative chemical reactions with drugs (2):



1. *Nucleophilic addition reactions.* When a hydroperoxide is in an alkaline environment, the peroxide anion can act as a nucleophile to add to drugs. In particular, a Michael addition to an α,β -unsaturated ketone followed by loss of hydroxide leads to an epoxide:



2. *Electrophilic displacement reactions.* With drugs having good nucleophilic centers, such as amines or thiols, hydroperoxides can act as electrophiles. The product is ultimately an amine-N-oxide (for tertiary amines), a hydroxylamine (for primary and secondary amines), or a sulfoxide:



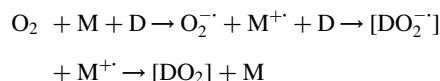
3. *Radical reactions.* Peroxide can be reduced to the more reactive hydroxyl radical in the presence of metals (see "Catalysis"). Hydroxyl will extract a hydrogen from most organic species resulting in chain processes as shown in "Autoxidation—Chain Processes". Radical chain processes initiated by peroxides generally implicate metals.

Catalysis

Direct two-electron reaction of molecular oxygen with organic compounds, although very often thermodynamically favorable, is spin forbidden. Because the ground state of molecular oxygen is a triplet diradical, reaction with the singlet ground state of most organic compounds is forbidden, which imposes a severe kinetic hindrance on this reaction pathway. However, oxygen's reduced free-radical species, such as superoxide and hydroxyl radical, are not spin forbidden to react with organic molecules (47). Therefore, though weaker oxidants thermodynamically, they are stronger oxidizing agents kinetically than molecular oxygen.

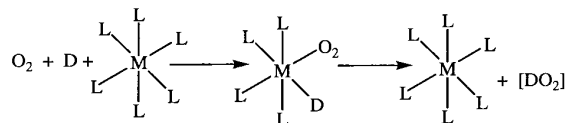
One of the roles that metal catalysts can play is to facilitate the reduction of molecular oxygen to more kinetically reactive reduced species. An example of such

a process is shown below:



Here M is a metal catalyst, which reduces oxygen to superoxide. Superoxide in turn reacts with the drug D to form an unstable adduct $[\text{DO}_2^{\cdot-}]$, which donates an electron to the oxidized metal M^{++} regenerating the catalytic metal and forming the drug decomposition product $[\text{DO}_2]$. The net result is two-electron oxidation of the drug using the metal to overcome the spin barrier.

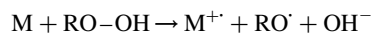
A second role for metal catalysts is to bind (ligate) drug and oxygen simultaneously, and in the process, allow the two to combine as they dissociate from the metal. This process is possible for metals with relatively weak ligands (L) and accessible oxidation states:



Again, the metal effects a two-electron oxidation by molecular oxygen.

Because many transition metal ions have two or more relatively stable oxidation states differing by one electron, they can be efficient catalysts for both of the above oxidation mechanisms (48). In pharmaceutical systems, the key metal ions associated with these processes are iron and copper, though other metal ions may play a role. Iron and copper ions are routinely found in drug substances and formulation excipients. Metal ions also exist in low concentrations in almost every buffer system (see "Detecting and Controlling Impurities") (49).

Another example of metal catalysis is the Fenton-type reaction (50,51). Transition metal ions can reduce peroxide to generate hydroxyl radical, the most reactive oxidative species that can exist in aqueous solution:



When peroxide is present in the formulation, either brought in by added excipients, or formed through molecular oxygen reduction, the presence of transition metal ions can alter the reaction mechanism and significantly accelerate the oxidative degradation of drugs. Both iron and copper salts can effect this peroxide reduction. In the presence of a reducing agent, the metal



salt can be regenerated. This makes the reaction catalytic in metal. Effectively, the metal catalyzes peroxide reduction by the reducing agent, thereby making peroxide a more potent oxidizing species.

Light can also cause oxidation reactions (52). Light absorbed by a drug can induce cleavages to produce radicals, which react rapidly with molecular oxygen (no spin barrier). Similarly, light absorbed by photosensitizers can react with oxygen to form more reactive oxidizing agents such as peroxides. In addition, some light absorbers will undergo intersystem crossing between the excited singlet state and a triplet state. Quenching of the triplet state of the absorber by molecular oxygen results in the formation of singlet oxygen (i.e., spin-paired oxygen). This species will react rapidly with many organic molecules since there is no spin barrier to reaction. In some cases, light absorption will result in the formation of radicals, which in turn initiate autoxidation reactions (see "Autoxidation—Chain Processes").

Acidity and pH Effects

It is in general more difficult to remove an electron from a drug when it is more positively charged. For this reason, drug stability against oxidation is often greater under lower pH conditions, which promote protonation of drugs if protonation is possible. In the converse, higher pH conditions, which deprotonate a drug, generally make that drug more susceptible to oxidation. The pH of a solution containing drug will affect oxidation depending on the pK_a of the drug, assuming the protonation site is conjugated with the oxidation site on the molecule.

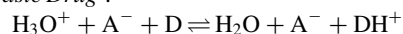
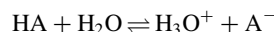
Typically, drug dissociation constants are measured in water or combinations of water with other solvents. These data are generally reported in terms of pK_a values of the drug for cases where the drug gives up a proton in water (an acidic drug), or of the conjugate acid of the drug in cases where the drug accepts a proton in water (a basic drug). In dosage forms, the actual dissociation constant for a drug may differ significantly from that in aqueous solution, as discussed below.

Effects of Solvent on Dissociation

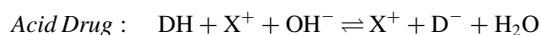
In aqueous solution, the charged species (protonated basic drug or deprotonated acidic drug plus the corresponding counterions) are generally dissociated to give free ions with the equilibria being essentially proton

exchanges between water and drug molecules. This process is shown in the following equilibria for a basic drug:

Basic Drug :



where A^- is the counterion of a protonating acid (or counterion of the drug salt form) and D is the drug. Likewise, the following equilibrium represents the action in water of an acidic drug:



where DH is the drug (protonated form) and X^+ is a cation, which can be a hydronium ion. As can be seen in these equilibria, the extent to which the drug will be in its deprotonated form will depend on the intrinsic basicity (acidity) of the drug and the hydronium ion concentration in the water (the pH). What can also be seen is that the counterions for acidic or basic drugs will only affect the equilibria to the extent they ion-pair with the drug and to the degree the counterions affect the pH (acting as buffers).

With nonaqueous solutions, the polarity and dielectric constants can be significantly lower than with water-based solutions. Since the energy of separating opposite charges is proportional to the distance between the charges and the dielectric constant of the medium (Coulomb's law), as one goes to lower dielectric constant solvents, there is a greater tendency for the ion pairs to be in direct contact rather than freely solvated (53). The result of this ion pairing is that the counterion can significantly affect significantly the equilibrium position and properties of the ion pair. Likewise, the dipole moment and hydrogen bonding ability of water stabilize the ionic species. In solvents with lower dipole moments and tendencies to hydrogen bond, equilibrium constants for ionization will shift to favor the neutral species, i.e., a lower pK_a for a basic drug and a higher pK_a for an acidic drug. In Table 5, the dielectric constants and dipole moments for a number of solvents are listed. It should be noted that hydrogen bonding and polarizability can also play a role in determining the dissociation constant for a drug in a nonaqueous medium. Solvents in the same general class will generally show similar tendencies to stabilize ions. Solvent mixtures will behave to a first approximation as a combination of component



properties. Dissolved solutes can also have a significant impact on the ion pairing of drugs. In particular, dissolved salts can raise the dielectric constant of the medium and stabilize ions (as well as destabilizing solubilization of lower polarity substances). Since for solids in the amorphous state the system essentially behaves as a solution, one can treat the situation in the same fashion. In other words, for solid excipients, liquid solvents can be used to determine the approximate tendency for dissociation. This is most easily seen for polymeric excipients where the organic functional groups can be compared to monomeric solvents.

Counterion Effects on Dissociation

Whereas water acts as an acid and base to equilibrate the drug based on the pH of the aqueous environment, with nonaqueous solvents, there is sometimes little proton transfer between the drug and the solvent. Instead, proton transfer can occur between the counterion itself and the drug or between the drug and any environmental bases present (discussed below). For basic drugs, this situation is shown in the following equilibrium:



As can be seen in this equilibrium, the extent to which the drug will be in its deprotonated form depends on the proton affinities of both the drug and the counterion. In this case, the strength of the acid HA (basicity of A^-) will have a profound effect on the equilibrium position. We therefore expect that the weaker the basicity of the counterion of the salt of the drug, the less deprotonated

Table 5

Some Solvent Factors Affecting Acidity (54)

Solvent	Dielectric Constant/ T ($^{\circ}C$)	Dipole Moment /Debye
Water	78.5/25	1.85
Formamide	111.0/20	3.37
Methanol	32.7/25	2.87
Ethanol	24.6/25	1.66
Acetone	20.7/25	2.69
Acetic acid	6.15/20	1.68
Diethyl ether	4.34/20	1.15
Dimethylformamide	3.67/20	3.86
Tetrahydrofuran	7.58/25	1.75
Phenol	9.78/60	1.45

Table 6

Basic Drug Counterion Acidities

Acid	pK_a
Hydrochloric acid (55)	− 6 to − 7
Benzene sulfonic acid (56)	0.7
Aspartic acid (57)	1.99, 3.90, 10.00
Glutamic acid (57)	2.13, 4.32, 9.95
Tartaric acid (58)	2.93, 4.23
Fumaric acid (58)	3.03, 4.54
Citric acid (58)	3.13, 4.76, 6.40
Malic acid (58)	3.40, 5.05
Lactic acid (58)	4.14
Ascorbic acid (58)	4.17, 11.57
Benzoic acid (58)	4.19
Acetic acid (56)	4.75
Sorbic acid (58)	4.76

drug will be present (counterion effects are discussed further in “Drug Substance: Counterions (for Solid Dosage Forms)”). In Table 6 the pK_a values for the conjugate acids of a number of common counterions are shown. Although these values are for aqueous equilibria, they should provide a relative guide to counterion choices for improved oxidation performance. In other words, one expects greater basic drug oxidation stability in nonaqueous environments with the salt of drugs made with acids higher on the list in Table 6.

Adventitious Bases

With acidic drugs starting in the neutral form, deprotonation hinges on what can accept the proton in the environment and the stabilization of the drug ion pair. Since most acidic drugs are either carboxylic acids (pK_a 4–5) or sulfonic acids (pK_a about 1), functional groups of solvents or excipients capable of accepting protons under some circumstances are listed in Table 7. The lower a functional group is located on this table (i.e., its corresponding conjugate base), the more likely it can remove a proton from the drug. Besides the pK_a , the ratio of excipient basic/acidic sites to drug will affect the amount of proton transfer and hence the drug stability. This factor becomes important when the pK_a values for the drug and excipients in the environment are within about 2 pK_a units.



Table 7
Functional Groups of Excipients That Can Deprotonate Protonated Drugs (55)

Excipient Conjugate Acids of Proton Acceptors	pK_a
$R-OH^+-R$ (ether)	-2 to -5
ROH_2^+ (alcohol)	-2 to -5
H_3O^+ (water)	-1.74
$ArNH_3^+$ (amines)	-10 to +5

Solid-State Effects on Dissociation

In the solid state, several new factors are introduced, especially in the case of crystalline drugs (see "Oxidation in Solid Dosage Forms" for a discussion of oxidation in solid dosage forms). For a crystalline drug, the energetics of deprotonation can be quite different from those in the solution state due to lattice energy effects. Removal of a proton from a crystalline basic drug salt form can cost the crystal considerable energy depending on whether molecules of neutral drug can pack in the salt crystal. Often molecules of neutral drugs cannot easily fit into the salt lattice since the packing in ionic crystals generally involves ion-pair interactions typical of salt crystals while the neutral form will associate based on other interactions of the molecules. In other words, removing the proton from the drug involves not only the solution effects, which sets the pK_a value, but additional factors favoring the pure crystal. Removing the proton can also involve a significant activation barrier. Both factors tend to make the salt of a basic drug more stable than an aqueous solution pK_a value would suggest. Likewise, deprotonation of an acidic drug crystal can result in a large energy barrier due to disruption of the crystal lattice. In practical terms, one of the best ways of stabilizing a drug to oxidation in the solid state is to form and maintain good crystals of the drug. Unfortunately, during the tableting process, there is a tendency for some of the drug to go into an amorphous form. In addition, some drugs may form a solid solution with compatible excipients. In either case, the stability of the drug may suffer.

For drugs destabilized in the dosage form due to deprotonation, lowering of the pH in solution can often help the situation. With solid dosage forms, this can be problematic. One must generally use a nonvolatile acid

to avoid loss during processing and storage. In addition, since most nongaseous molecules do not migrate at significant rates across solid-solid interfaces, it is anticipated that the acid should be placed within the reactive (amorphous) phase of the drug. In practical terms, acid stabilization, when effective, typically performs best when intimate chemical contact is possible by addition from solution either as part of a wet granulation or as a fluid-bed process. In many cases, acid addition does not improve stability. Suitable acids are listed in Table 12.

Oxidation in Solid Dosage Forms

Oxidation of organic crystals has been the subject of active research for several years. Investigators have often studied simple, pure systems (i.e., single crystals) or performed experiments on mixed systems at extreme conditions (e.g., 7°K) (59). Though much was learned from these simple systems, the relevance to more complex pharmaceutical systems has been uncertain (60). Pharmaceutical solids comprise organic molecules that vary in physical form from crystalline to totally amorphous. In addition to the drug substance, solid excipients are added to the formulation, which are necessary for manufacture and performance of the dosage form. Excipients also vary in physical form; consequently, the final solid dosage form is a complex matrix of organic, polymeric, and inorganic solids each with vastly different physicochemical properties. This mixture is then triturated, mixed, blended, densified, or compressed, which undoubtedly induces more changes to the physical make-up of the dosage form. For these reasons, oxidation mechanisms in pharmaceutical solids are difficult to study, which may account for the paucity of reported research in this area.

Oxidation of Solid Drug Substances

As in solution, oxidation in pure solid requires that an oxidizing agent be present in the system for reaction to occur. In most cases, it is difficult to determine if oxidation occurs within the bulk of a crystal or at defect or surface sites, where crystal-lattice energies are weaker and exposure to oxidants is greater. Molecular oxygen from the atmosphere has been shown to react with organic crystals (59). This reactivity depends on the crystal form (morphology), which governs the permeability and solubility of oxygen in the crystal matrix.



In some cases, reactivity decreases with increasing melting point suggesting that stronger crystal-lattice energies inhibit oxygen diffusion. Desolvation may precede oxidation due to the formation of tunnels within the crystal lattice, which permit oxygen permeation. Free radicals are known to form in crystalline solids (61), which can react with molecular oxygen to form reactive and diffusible oxygen species such as superoxide anion or peroxy radical. These reactive species can abstract hydrogen atoms at reactive positions on drugs (see "Identifying Oxidative Reactivity Based on Structure") or induce electron transfer from a drug (see "Electron Transfer"). In most cases, however, reactions of crystalline materials will not produce products that can fit into the crystalline lattice; therefore, the reaction is hindered energetically. Exceptions to this rule can occur if the product is similar in crystal structure to the starting material (62).

Oxidation in Solid Drug Products

In many cases, the pure drug substance is quite stable, while the dosage form shows significant drug oxidation. This difference in reactivity is generally attributable to one of the following three causes:

1. *Amorphous drug.* During the processing of drug to form a solid dosage form, it is possible to mechanically generate amorphous drug. This need not involve more than a small percentage of the drug to cause a stability issue since we are generally concerned with far less than one percent drug degradation. Amorphous drug regions have greater mobility and lack crystal-lattice stabilization energy, and as a result, oxygen permeability and solubility will be higher. Any fortuitous radical-pair initiation is more likely to lead to free radicals, which can react with oxygen in a chain process (see "Autoxidation—Chain Processes"). Greater mobility and higher oxygen concentrations present in amorphous drug also facilitate electron transfer to oxygen (see "Electron Transfer"). In addition, since the amorphous form of a compound is higher in energy, the rate of a reaction to a common product will be faster.
2. *Solid solution.* Excipients can potentially solvate some of the drug either directly or by bringing in low levels of moisture. In a solid solution form,

the drug will be amorphous with all the corresponding reactivity discussed above. In addition, the excipient solvation can result in drug deprotonation (see "Acidity and pH Effects"), which can make a drug more reactive. Since increased drug mobility is often correlated with drug instability, an abrupt decrease in stability at temperatures above the T_g of the solid solution may be anticipated (46,63). In some cases, examining the thermal behavior (differential scanning calorimetry) of drug plus excipient can provide insight into the mechanism of drug substance degradation by indicating both the presence of a compatible phase and transition temperatures associated with that phase.

3. *Excipient reactivity.* Excipients can be a source of oxidants (see "Formation and Presence of Oxidants in Excipients") and metals (see "Catalysis"). For more discussion on detection and control of impurities in excipients, see "Detecting and Controlling Impurities". Excipients can also be involved in generating mobile oxidative species such as peroxy radicals, superoxide, and hydroxyl radicals. This will depend on the hydrogen bond strength of the excipients and whether there are good electron donating sites (e.g., amines).

Effects of Sterilization on Oxidation

Thermal sterilization of liquid formulations can often accelerate the rate of drug oxidation. Predicting the degree to which heat will accelerate oxidation is difficult due to the inverse relationship between the amount of dissolved oxygen and the temperature. That is, at higher temperatures, there is less dissolved oxygen but a faster intrinsic reaction rate (64–67) and potentially more initiation. When using antioxidants in parenteral formulations, one must be wary of the possibility of antioxidants not surviving the intense heat of terminal sterilization. The initial screening of antioxidants for a formulation, therefore, should include a test of the terminal sterilization cycle to ensure minimal inactivation of the antioxidant.

Although radiation sterilization (68,69) may be carried out using either an electron beam or gamma-ray photons from a radioisotope source, the latter is more common. Today, there are a number of drugs on the market that are radiation sterilized. These include



ophthalmic ointments and drops, topical ointments, injectables, and veterinary products. The major advantage in using gamma irradiation as a terminal sterilization procedure is its high penetration combined with a minute temperature rise (usually less than 5°C) (70). Therefore, gamma radiation can be applied to the packaged product, and is applicable to heat-sensitive dosage forms.

As with other methods of sterilization, irradiation involves a compromise between inactivation of microorganisms and degradation of the drug product. For aqueous solutions and suspensions, the effects of ionizing radiation on the water are more relevant than direct radiolysis of the drug. Water, upon irradiation, can have an oxidizing effect on solutes with lower redox potentials (71). The primary radiolysis products of water are the hydrated electron, and hydroxyl and hydrogen radicals. These products can indirectly lead to drug oxidation. None of the radical species is stable; the resultant termination products (in addition to H₂O) are H₂O₂ and H₂ as well as products stemming from hydrogen abstractions and radical additions. In the case of a drug, these termination products give a radical of the drug, which can undergo addition of oxygen to form a peroxide, addition of hydroxyl radical to give an alcohol or ketone, or elimination of another hydrogen atom to give a double bond. All these processes are formal oxidations. The water by-product of radiolysis, hydrogen peroxide, is itself a strong oxidant (see "Peroxides and Other Oxidizing Agents"), which can react with a number of drugs. Since the peroxide generated can be slow to react, long-term stability studies should be performed after irradiation in order to monitor the possibility of slow oxidation.

Past studies have shown that incorporation of antioxidants can often prevent irradiation-induced oxidation of drugs in aqueous solutions when undergoing terminal sterilization (72). The free radicals formed can be scavenged to protect the drugs. It is possible that some antioxidants themselves undergo direct or indirect degradation by sterilization, rendering them ineffective at oxidation protection. This is another reason to confirm the stability by aging the samples after sterilization.

Although a great amount of information has been accumulated on the radiation stability of many pharmaceutical compounds, it is still necessary to examine each new compound to assess the feasibility of irradiation for sterilization without degradation. In any formulation, the stability of an individual component

may be altered when irradiated as part of the whole formulation. Because of this, testing of actual formulation performance and stability after irradiation is imperative. Some of these degradation processes can be minimized by irradiating at low temperatures or in the absence of oxygen.

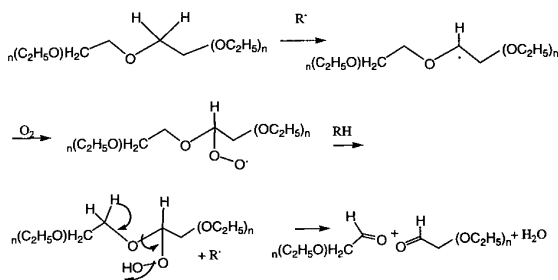
Oxidation Effects on Controlled Release Dosage Forms

Often extended release or controlled delivery of a drug is achieved by using polymeric excipients to control the rate that an active drug is introduced to the targeted delivery site. The exact mechanism by which a polymer controls the delivery of the drug is dependent on the formulation type or platform. For example, in a matrix tablet formulation, drug releases from the polymer-based matrix upon hydration either by diffusion or by erosion of the matrix. The release rate depends on the molecular weight or viscosity of the hydrated polymer-drug matrix, the degree of chain entanglement or swelling, and also interactions between the drug and the polymer (73). In most applications that use polymeric excipients to control the rate of drug delivery, the ability of the polymer to create a viscous medium or barrier for diffusion of drug is critical. The property of the polymer that is most crucial to its ability to have such an effect is its molecular weight. Therefore, any process that significantly reduces the molecular weight of the polymer is likely to affect its ability to control the drug delivery.

Oxidative degradation can lead to a loss in molecular weight for several polymers commonly used in controlled release applications. In addition to loss in molecular weight, oxidative degradation in polymers can produce reactive impurities and end groups, which can compromise the chemical stability of drugs. Polymer degradation that does not cleave the polymer backbone will not in general be as significant for controlled release applications. An example of a class of controlled release polymers that can degrade to compromise the drug release rate is the polyoxyethylenes, including poly(ethylene oxides) (PEO), poly(ethylene glycols), and poly(oxyethylene) alkyl ethers (74). The oxyethylene polymers tend to be very reactive and can undergo oxidative degradation via a number of pathways. The reactivity of these polymers is greater in solution than in the solid state and is dependent on temperature, light, and the presence of catalysts (e.g., peroxides). In the presence



of free radicals, the polyether groups are highly susceptible to oxidative degradation via a chain process analogous to autoxidation (see "Propagation") (50).



This process reduces the polymer molecular weight, and therefore, its effectiveness in controlled release applications. To prevent this degradation pathway, the vendor for PEO (Union Carbide) typically adds 100–1000 ppm of butylated hydroxy toluene (BHT) (44). This antioxidant is quite effective; however, it is volatile and can be lost during any heating steps. One should use caution that the level of BHT does not drop too low after processing, or add additional BHT as part of a granulation step (see "Chain Terminators" for use of antioxidants). The vendor also recommends tocopherols for stabilizing PEOs. The rate of polymer degradation with these types of polymers can increase dramatically above their softening temperatures (55–60°C).

Oxidation of Protein Pharmaceuticals

Oxidation is one of the major degradation routes for proteins, although many times it is convoluted with, or even superseded by one or more of the other major protein degradation routes (deamidation, aggregation/precipitation, and adsorption). Oxidation can be significant in both liquid and solid formulations (75–77), and commonly has a complex dependence on both the chemical structure of the protein of interest and the formulation and storage conditions. Initiators such as singlet oxygen, superoxide radicals, alkyl or hydrogen peroxide, hydroxyl radicals, or halide oxygen (75,78) typically catalyze the protein oxidation. Uncatalyzed oxidation of proteins is usually found to proceed too slowly to be of concern in formulations, and so is normally preempted by catalyzed oxidation or nonoxidative degradation. The most important factors to control in an effort to minimize catalyzed oxidation are the formulation conditions (pH, buffer), the presence of

impurities such as trace metals and peroxides in excipients, exposure to light, and the physical state of the formulation (liquid vs. solid). In the solid state (i.e., lyophile), the water content can also be an important factor in oxidative stability. Chemical structure (primary sequence) is particularly influential on protein oxidative stability, but is also the one factor that is usually not in the control of the formulation scientist.

There are five naturally occurring amino acids that contain side chains with a high electron densities, which therefore tend to oxidize under practical formulation and storage conditions: histidine (His), methionine (Met), cysteine (Cys), tryptophan (Trp), and tyrosine (Tyr). There are a number of different mechanisms by which each of these amino acids oxidizes in the presence of different catalysts. Without going into these specific mechanisms in detail, the formulation and storage conditions appropriate to address these reactions are discussed below.

Formulation Conditions (pH and Buffer)

Formulation pH can greatly affect the oxidative stability of proteins. Cysteine oxidation is usually accelerated at higher pH, where the thiol is deprotonated (free amino acid $pK_a = 8.3$). Peroxide-catalyzed oxidation of Met is also pH-dependent, but decreases with increasing pH. This can be useful in that the difference in pH dependence can sometimes be used to distinguish between Met and Cys oxidation (75,79). Solution pH and solid-state effective pH can also strongly influence the propensity of His residues to oxidize via catalysis by singlet oxygen because at low pH His is protonated (free amino acid $pK_a = 6.0$) causing it to be relatively unreactive (80–82). As another example of the effect of pH, the photo-oxidation of Trp and His residues at neutral pH proceeds faster than for other amino acids since at lower pH, Trp and Met are found to be the more easily photo-oxidized (82,83). Although much less frequently cited, there are also reports in which the buffer (e.g., glycine) has been implicated as the catalyst for oxidation during storage (84). However, generally the effect of the buffer on protein oxidative stability manifests itself only through control of the solution pH.

The compactness and stability of the folded state of a protein is very sensitive to pH due to changes in the ionization states of side chains and of the amino acid



backbone (75,85–87). Even subtle changes in the higher-order structure of proteins may significantly change the degree of solvent exposure and the local chemical environment of a given amino acid, leading to either promotion or retardation of oxidation. This phenomenon is documented for deamidation of proteins (87), and has been used to explain the reaction rates of different histidine groups in α -crystallin (85), in which residues that have greater solvent exposure show greater reactivity. However, it remains difficult to predict *a priori* whether the protein conformation will play a significant role in determining oxidative reactivity of a given protein or of the specific amino acid units that make up the protein.

Excipient Impurities

It is important to realize that impurities such as trace metals and peroxides may be present at sufficient levels in commercial excipients to cause appreciable oxidation (see “Catalysis” and “Detecting and Controlling Impurities”). Cys, Met, and His are all sensitive to metal-catalyzed oxidation (75). With His, this is complicated by a dependence on the identity of the amino acids that occupy nearby positions on the protein backbone. In addition, protein conformation appears to play a role in metal-catalyzed oxidation (75,88). In addition, metals may bind to proteins, leading to high effective local metal concentrations, which may exacerbate oxidation issues with proteins (89,90).

Peroxide impurities, commonly found in polysorbates such as Tween 80 and Tween 20, as well as commercial polyethylene glycol (PEG), are typically at sufficient levels to cause appreciable degradation, especially at elevated temperatures (see “Peroxides and Other Oxidizing Agents” and “Detecting and Controlling Impurities”). Peroxides can prevent an excipient’s use in protein formulations or lead to the use of specially processed excipients in cases where the excipient is an essential formulation component. This illustrates an important point in protein formulation: the variety of routes by which proteins degrade often leads to situations where a particular excipient or formulation/storage condition is optimal to prevent one route of degradation (e.g., aggregation or adsorption) but accelerates another route of degradation (e.g., oxidation or microbial growth). Unfortunately, there is no general means to address these situations and they must be treated on a case-by-case basis, factoring in

not only the physico-chemical properties of the protein, but also the desired product profile and the storage and shipping restrictions.

Photo-Oxidation

Photo-oxidation is a common route of oxidation for proteins, particularly for those containing Trp residues. As with other routes of oxidation, photo-oxidation depends on additional factors such as solution pH. For example, His is less prone to photo-oxidation under acidic conditions, presumably because it is protonated (80–82). Photo-oxidation can be significant in both solid and liquid dosage forms, and may or may not be significantly attenuated in lyophiles, depending on the particular molecular motions involved in the rate-determining step. Protecting the dosage form from light with either amber bottles or some form of secondary packaging (e.g., foil pouches) can prevent photo-oxidation. The latter is usually preferred with liquids to avoid leaching of trace metals (usually iron) from the amber glass.

Physical State

The physical state of the formulation (solution vs. lyophile) can influence oxidative stability of proteins, much as it can the stability of small molecules. In terms of stability, the advantages of liquid vs. solid dosage forms for proteins are often analogous to the situation with small molecules. However, the choice of liquid vs. solid dosage form is in general not a straightforward one. The typical motivation for choosing a lyophile as a means of minimizing oxidation is either based on a desire to attain low water contents or to limit molecular mobility (or both). In some cases intermediate moisture contents are optimal (91,92) due to the different roles that water plays both in the oxidation mechanism (where water may slow the process) and as a plasticizer of the amorphous matrix (where water may exacerbate oxidation). In addition, the freezing step in the lyophilization process may cause dramatic changes in the local protein environment (e.g., concentrating of salts, pH changes), leading to conformational changes and unfolding, or adsorption to the ice–liquid interface. These processes ultimately lead to accelerated oxidation of the protein in the resulting product. Thus, a lyophilized formulation is recommended only after other factors such as formulation pH, light exposure, and excipient impurities have been addressed.



Recommendations for Formulation Design

To prevent or at least minimize protein oxidation, the following steps are recommended in the order listed:

1. Store under refrigerated conditions and protect from light, if feasible;
2. Optimize the formulation pH guided by the considerations outlined in "Formulation Conditions (pH and Buffer)", and reduce the headspace oxygen in the container (see "Procedure for Applying a Nitrogen Headspace to Liquids");
3. Remove or replace excipients that are suspected of introducing transition metals or peroxides into the formulation, or obtain specially purified excipients with minimal contaminants;
4. Add antioxidants to the formulation (see "Antioxidants"); and
5. Lyophilize the protein formulation.

Due to the complexities induced by the many potential oxidation sites present in typical pharmaceutical proteins, each of which has different intrinsic chemical properties and a different local chemical environment as well as different sensitivities to local and global protein conformation, only general guidelines such as those above can be given with confidence. In addition, traditional approaches of accelerated stability testing such as elevated temperature are not expected to be broadly applicable for many of the same reasons.

Oxidation of DNA Pharmaceuticals

Common strategies for DNA-based drug delivery include ionically binding the anionic DNA to cationic carrier molecules such as cationic lipids, polymers, and peptides (93), or direct injection of uncomplexed "naked" DNA into the target tissue (94). While oxidative degradation of DNA is a major issue in the formulation design, hydrolytic and physical degradations are equally formidable stability challenges that must be dealt with in the formulation (95–97), often with conflicting tendencies.

In the presence of transition metals and oxygen, free-radical catalyzed oxidation is often a major degradation pathway for DNA (98). Due to the anionic phosphate surface charge of DNA, metal ions tend to associate on the surface leading to proximate generation of a relatively high concentration of free radicals (see

"Catalysis") that can readily damage the DNA. Oxidation of DNA can lead to extensive damage of all the bases [guanine (G), cytosine (C), adenine (A), and thymine (T)], resulting in mutations and strand breakage along the phosphate backbone; however, the major oxidation product is 8-hydroxyguanine (99). Guanine is most susceptible to oxidative damage because its oxidation potential of 1.29 V vs. normal hydrogen electrode (NHE) is the lowest of the four common bases measured using pulse radiolysis (A, 1.42 V; C, 1.6 V; T, 1.7 V) (100,101). Base sequence also plays an important role in determining oxidative susceptibility. Guanine doublets (5'-GG-3') are more readily oxidized on the 5'- rather than the 3'-end, which helps to make the 5'-guanine on a -GG- doublet one of the most reactive oxidative sites on DNA (100). This relatively high propensity for oxidation occurs because of the high charge density on the 5'-end. The oxidation product of guanine (8-hydroxyguanine) pairs preferentially with adenine (instead of its normal pair with cytosine), and may result in transcriptional mutations of the expressed mRNA, and ultimately in mutant proteins (102). Another deleterious effect of 8-hydroxyguanine formation is the reduced promoter clearance observed when the mutation occurs in or near the promoter region (102). Oxidation often produces formamidopyrimidines, which are ring-opened imidazole moieties of purines. These altered purine residues may also result in mismatch mutations that can affect the gene product (98). Oxidation is also reported to cause massive helical distortion due to lesions formed by dimerization of purines (98).

Photo-oxidation may occur if DNA is exposed to light, especially in the presence of sensitizers (103) (see "Catalysis"). Again, the major site of oxidation occurs at guanine residues.

Methods to analyze for oxidative degradation include HPLC, LC-MS, capillary electrophoresis, fluorometric assays, and gel electrophoresis (104). The most easily detected manifestation of the degradation is strand breakage that converts supercoiled DNA into the circular form, and eventually linearized and fragmented forms of the DNA. These various topoisomers of the DNA are visualized easily by agarose electrophoresis, and may be quantified using densitometry, although it is impossible to differentiate whether damage is due to hydrolysis or oxidation. Alternatively, HPLC methods utilizing ion-exchange or size-exclusion columns have been developed to resolve the various topoisomers in lieu of agarose gel



electrophoresis (105). Sensitive capillary electrophoresis techniques have been developed to resolve plasmid DNA, with reports citing an ability to measure changes in the linking number of the plasmid (106). Mass spectrometry remains the most powerful technique to identify the oxidized bases that are generated, although it must be coupled with effective enzymatic digestion techniques. Recently, HPLC methodology using an electrochemical detector has been developed to identify oxidized bases (99). Fluorescent reagents that selectively bind 8-hydroxyguanine can be used to measure the amount of this oxidized base in conjunction with a fluorescence activated cell sorter. The analytical methodology selected depends on many factors with consideration of the sensitivity vs. the effort involved being critical.

Strategies for overcoming oxidative degradation include the following:

1. *Dosage form.* Since the primary dosage form is parenteral, the selection of a ready-to-use liquid or a lyophilized dosage form is critical for oxidative stability. The ready-to-use solution is the easiest to produce and administer, but suffers from the most oxidative instability (as well as hydrolytic and mechanical) due to generation of hydroxyl free-radicals in the presence of trace transition metals. Lyophilization minimizes the formation of hydroxyl free-radicals, but results in higher production costs, more elaborate administration, and a more complicated formulation involving cryoprotectants.
2. *Addition of excipients.* Excipients can be used to inhibit oxidative degradation. Typically, a combination of a hydrogen atom donor and metal chelator are added to synergistically diminish the effects of oxidative degradation. Some examples are ethanol and editic acid (EDTA), or citrate and triethanolamine (95–97,107).
3. *Packaging.* The choice of packaging (see “Packaging”) is a critical factor for protecting the DNA. It is important to protect the DNA from light. Inhibiting the amount of oxygen that can permeate the vial is of utmost importance, for this reason glass vials tend to be preferred over plastic. It is also advisable to apply a nitrogen headspace over the sample (see “Procedure for Applying a Nitrogen Headspace to Liquids”).

MECHANISM OF FIXES

Formulation

Dilution Effects

If drug reactivity is associated with peroxide or metal impurities in the excipients (see “Peroxides and Other Oxidizing Agents”, “Catalysis”, and “Detecting and Controlling Impurities”), the oxidative degradation rate can be affected greatly by the drug concentration. Under dilute drug conditions, there is proportionately more impurity per drug, which can lead to higher reaction rates. One should therefore work with as concentrated a drug (in solution or solid dosage forms) as possible when impurities are suspected.

Detecting and Controlling Impurities

Common contaminants that contribute significantly to drug oxidation in dosage forms include metals, peroxides, and formic acid (see “Peroxides and Other Oxidizing Agents” and “Catalysis”). Trace metals are almost ubiquitous in dosage forms, and since they are often catalysts rather than consumed, they can affect rates even at low levels. Typically, iron and copper are the two metals of most concern. They can be detected with high sensitivity using atomic absorption (AA) or inductively coupled plasma (ICP) methodologies. Since controlling metal contamination often can be challenging, it is worthwhile to determine if these metals are indeed catalysts for the drug oxidation. This can sometimes be accomplished by spiking experiments with FeCl_3 and CuCl_2 . Greater oxidation in these samples supports (but does not prove) a role for metals in the oxidation. Mitigating metal contamination involves the following possibilities:

1. *Adding of chelating agents to the formulation.* These include citric acid (number 7 in Table 12), EDTA (number 9), fumaric acid (number 11), and malic acid (number 13). Though it is difficult to predict when a chelator will be effective when used with solid dosage forms, the chelator will often perform better when added as part of a wet granulation process.
2. *Reducing or eliminating metal contact during processing particularly welded metal and non-passivated stainless steel.* This is especially applicable to liquid formulations. With metal contamination in the drug substance, synthetic process changes may reduce the issue. In some cases solvents used in the production of the drug



substance introduce metal contaminants. At times, alternative grades or vendors for solvents can alleviate this issue.

3. *Working with excipient vendors on setting specifications for metals. In some cases, special high-purity excipients can be provided.*
4. *Checking the applicability of alternative vendors for suspect excipients.*

Peroxide contamination is generally associated with polymeric excipients (see "Formation and Presence of Oxidants in Excipients"). Sensitive qualitative and quantitative detection of peroxides can be accomplished with assays reported in the literature (108,109).

In cases where peroxides are believed to be the problem, the following solutions are recommended.

1. Check to see if peroxides are present in the drug substance. If so, make appropriate changes to the process or solvents to minimize peroxides.
2. Use an alternative excipient. In some cases, differences in molecular weight can have a significant impact on the peroxide levels in polymeric excipients.
3. Keep the concentration of the suspect excipient low.
4. Work with the vendor to obtain lots with lower levels of peroxides. This could ultimately lead to setting peroxide level specifications. In some cases, different vendors may provide similar materials with different peroxide levels.
5. Use antioxidants. Antioxidants can reduce formation of peroxides, but may be less effective at eliminating peroxides already present in a dosage form (see "Peroxide Quenchers")

Acids/Buffers

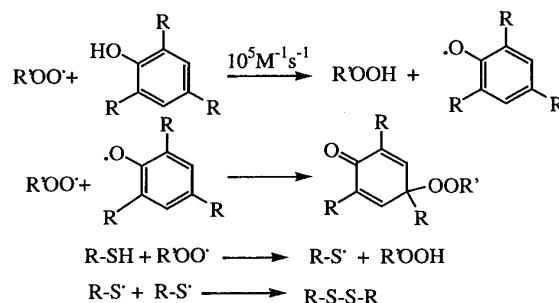
As discussed in "Acidity and pH Effects", the environmental pH can play a significant role in the stabilization of drugs to oxidation. For liquid dosage forms, adjustment of the pH is often straightforward with the use of buffers (though caution must be exercised such that buffers do not introduce metal contamination (see "Catalysis" and "Detecting and Controlling Impurities") or react with the drug). With solid dosage forms, the matter can be quite a bit more complex. In general, acids are expected to be more effective if they are in the same phase as the drug, though it is hard to predict if they will be effective for any given system. For most systems, this

means the acid should be added from solution. This can be accomplished as part of a wet granulation or by coating of particles using fluid-bed technology. Unlike in solution, acids added in solid dosage forms must be nonvolatile to remain within the dosage form upon processing and storage. This restricts the choices considerably. In particular, strong acids capable of protonating drugs with relatively low pK_a values are limited. Most of the choices available are carboxylic acids with pK_a values in the range of 2–5 (see Table 12, items 2, 3, 7, 11, 12, 13, 19). The only pharmaceutically acceptable, nonvolatile strong acid available today is toluene sulfonic acid (see Table 12, item 23). When using acids in solid dosage forms, such factors as the solubility of the acid in the drug phase (amorphous region or solid solution) can be more significant than the pK_a . For polar drugs, there is a likely correspondence between the acid's water solubility and its tendency to be soluble in the drug phase, and conversely, more hydrophobic drugs may need less water-soluble acids.

Antioxidants

Chain Terminators (110–113)

The most widely used class of antioxidants can be classified broadly as chain terminators. In the autoxidation process (see "Autoxidation—Chain Processes"), propagation of radical chains with consumption of oxygen allows a single initiation event to lead to a large number of drug oxidations. Chain terminators are molecules with weak bonds to hydrogen atoms such that they are attacked in the propagation steps. However, importantly, they form stable radicals, which do not propagate chains. Such species include thiols, which donate a hydrogen atom and dimerize to form disulfides; and phenols, which donate a hydrogen atom followed by further oxidation to enones, as shown below:



Because these species disrupt a chain process, they can be very efficient; i.e., they can often work at very low levels (114). In addition, most are stable in air such that they are not consumed in the absence of other degradation processes.

In solid dosage forms, this type of antioxidant is expected to work best when in intimate contact with the drug and/or reactive excipients to disrupt the chain process. Because the chain lengths are generally shorter in solid dosage forms than in liquids (lower mobility), the concentration of antioxidant may need to be higher to intercept radicals. It should be noted that these types of antioxidants could also terminate electron-transfer based oxidants such as superoxide by hydrogen-atom donation.

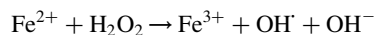
Sacrificial Reductants

Sacrificial reductants are compounds that are oxidized more readily than the drug. They effectively scavenge oxygen while they are themselves consumed. This strategy can be especially effective in systems where the amount of oxygen present is limited due to packaging. For example, in a glass sealed system, the amount of oxygen is limited to the headspace and dissolved oxygen. Examples of such antioxidants include sulfites and ascorbic acid (20). In some cases, this class of antioxidants can be problematic in the presence of

metals since the reducing agents can sometimes reduce metals to more reactive forms. In solid dosage forms, antioxidants of this type can be effective without intimate contact with the drug since they lower the oxygen level in the environment; however, with permeable packaging, these antioxidants can be consumed rapidly. Caution should be taken that accelerated stability measurements do not provide a misleading prediction of long-term aging since once the antioxidant is consumed, the system instability will increase.

Peroxide Quenchers

Ferrous (Fe^{2+}) ions can degrade peroxides as shown in the following reaction (50,51,115–117):



For the ferrous ion to not be consumed there needs to be a reducing agent present capable of donating an electron to the ferric ion. The hydroxyl radical formed in the Fenton process is very reactive and will be consumed by free-radical quenchers, such as mannitol, but also can react with drugs directly. Therefore, though ferrous ions can be effective peroxide quenchers, the hydroxyl radical formed in the Fenton process can also be an initiator for free-radical reactions and drug degradation. This limits the use of ferrous ions as peroxide quenchers.

Table 8

Antioxidant Stabilization of Fats/Oils. All Antioxidants Are at 0.02% (118)

Antioxidant	Days to 20 meq Peroxide Value PV/kg Fat (Chicken, Pork, Beef) ^a	Days to 70 meq PV (Soybean Oil) ^a	PV (ppm) After 3 days (Butterfat/80°C)
None (control)	7	5	265
α -Tocopherol	17	6	10
γ -Tocopherol	35	7	^b
Butylated hydroxyanisole (BHA)	28	8	^b
Butylated hydroxytoluene (BHT)	19	9	^b
Ascorbyl palmitate	10	11	74
Propyl gallate	^b	15	^b
α -Tocopherol + ascorbyl palmitate	31	^b	^b
γ -Tocopherol + ascorbyl palmitate	63	^b	^b
Ascorbyl palmitate (500 ppm) + lecithin (500 ppm) + α -tocopherol (100 ppm)	^b	^b	1

^a Using thin layer analysis.

^b Data not reported.

Copyright © Marcel Dekker, Inc. All rights reserved.



Ranking of Effectiveness of Antioxidants in Oils

Although the effectiveness of antioxidants can vary considerably depending on the system, a ranking of antioxidants in oils can be used as a general guide (Table 8). As can be seen, there are strong synergistic effects with certain antioxidant combinations. In particular, ascorbyl palmitate and tocopherols act much more strongly together than independently (118).

Cyclodextrins

Cyclodextrins are complexing agents that have pockets able to enclose some molecules. Under some conditions, cyclodextrins have been found to stabilize drugs to oxidation by making the susceptible site on the drug difficult to approach (119–121). For a cyclodextrin to complex a drug, the drug must be an appropriate size and shape to fit in the cyclodextrin cavity. In addition, dosage size limitations or cost of goods can limit this approach.

Drug Substance: Counterions (for Solid Dosage Forms)

As discussed in “Acid and pH Effects” counterions can have effects on the stability of drugs to oxidation (in the solid state). These effects can be divided into four categories:

1. *Ratio of free-base (for basic drugs) to protonated drug.* In general, the more acidic the acid form of the counterion, the more drug will be protonated

(see “Acidity and pH Effects”) and the greater the corresponding drug stability will be.

2. *Drug solubility.* Counterions can affect drug solubility, which can lead to a greater amount of drug dissolved in adventitious moisture or in excipients. Since drug instability in the solid state is correlated strongly with the amount of noncrystalline drug (see “Oxidation in Solid Dosage Forms”), counterions can impact stability in this manner.
3. *Crystal-lattice energy.* A lower crystal-lattice energy may lead to formation of crystal defects and amorphous regions in the crystal. These sites are often the positions of greatest oxidative reactivity (see “Oxidation in Solid Dosage Forms”). The lattice energy will in general correlate with the melting point of the crystal. It is therefore advisable to select counterions that give drug substances with the highest melting point.
4. *Counterion antioxidant activity.* In some cases, the counterion can oxidize preferentially to the drug, thereby helping to stabilize the drug. Counterions such as citrate and ascorbate can be useful in this regard.

Because of the sometimes-conflicting tendencies for the above factors, it is difficult to predict *a priori*, which counterions will provide the greatest stability for a given drug. Though undoubtedly counterions have been studied with regard to drug stability for numerous drug candidates, there is little published on the issue. At this

Table 9

Oxygen Permeability (Transmission Rate) for Packaging Materials (122,123)

Material	Oxygen Transmission [cm ³ mm/(m ² day atm)] [ASTM D1434]
Low density polyethylene (LDPE)	241
High density polyethylene (HDPE)	102 [26.3 at 0°C, Ref. (123)]
Polypropylene (PP)	89
Polystyrene (PS)	127
Polyvinylchloride (PVC)	4
Polycarbonate (PC)	114
Unoriented polyethylene terephthalate (PET)	2.5
Oriented PET	2



point, one must rely on trial and error to find the best counterion for oxidative stability.

Packaging

Solid Dosage Forms

For autoxidation instability processes where atmospheric oxygen is the source of oxidant, packaging can represent a reasonable option for stabilization of the drug. In using packaging, one must contend both with the oxygen available in the headspace and oxygen permeation through the container walls and caps. Whereas glass bottles offer a good barrier to oxygen permeability, they are often undesirable due to shipping and handling issues. For most plastic containers, the rate of oxygen permeation can be significant. In Table 9, the oxygen permeation rates for a number of packaging materials are shown.

The amount of oxygen that permeates into a 30 cm³ square HDPE bottle in a year can be estimated assuming no leakage from the seal and the maximum pressure differential.

$$\text{Surface area of bottle} = 62 \text{ cm}^2$$

$$\begin{aligned} \text{Oxygen pressure} &= (\text{mole fraction O}_2)(\text{total pressure}) \\ &= 0.18 \text{ atm} \end{aligned}$$

$$\begin{aligned} \text{Volume of oxygen} &= (\text{transmission})(\text{surface area}) \\ &\quad \times (\text{time})(\text{pressure})/(\text{thickness}) \\ &= 102 \text{ cm}^3 \text{ mm}/(\text{m}^2 \text{ day atm})(6.2 \\ &\quad \times 10^{-3} \text{ m}^2)(365 \text{ days}) \\ &\quad \times (0.18 \text{ atm})/1.0 \text{ mm} \\ &= 41.5 \text{ cm}^3 \text{ O}_2/\text{year} (0.2 \text{ mmol} \\ &\quad \text{oxygen}) \end{aligned}$$

In addition to permeation through the bottle walls, the rate of oxygen ingress can be affected greatly by the container top. With bottle caps sealed by screwing, leakage can be significant sometimes. It is therefore generally recommended that if possible, solid dosages be packaged with some type of oxygen impermeable seal. A particularly useful closure system involves the use of heat induction sealing (HIS) where a foil is transferred from a special cap to form an airtight seal.

In addition to oxygen permeation, there is significant oxygen in the headspace of bottles. Packaging under nitrogen can reduce significantly the headspace oxygen, though this process can be cumbersome for solid dosage forms.

Both controlling the oxygen permeability and headspace oxygen in bottles assumes adequate stability of the dosage form once the bottle is opened. These approaches are appropriate for shelf stability, but may not be appropriate for drugs requiring significant stability after consumer opening.

An option for solid dosage forms that addresses this limitation is blister packaging (124–126). In general, better oxygen barrier properties result in higher cost blister packaging and/or involve opaque blisters (foil–foil), which may be less desirable from a customer standpoint. It is sometimes feasible to blister package under nitrogen or argon to reduce the headspace oxygen.

Liquid Dosage Forms

For many liquid dosage forms, glass containers are the standard. Though they have low permeability, glass containers can be a source of metal ion leachables (127) [in HDPE and PP containers used for pharmaceutical products, there are generally no trace metal contaminants (128)]. The USP Type I glass, which is made of borosilicate, has the highest chemical durability out of the different types of glass containers available for pharmaceutical packaging due to high levels of aluminum oxide. To promote melting and fining, the alkali level is also increased with increasing aluminum oxide level. Type I amber vials have manganese and iron oxide, which act as coloring agents to give the desired color. Typical levels of iron oxide in Type I molded amber vials are 1.0–1.24%. Typical levels of iron oxide in Type I molded clear vials are <0.05%. Levels of manganese oxide in amber vials are 5–7% (129). Other oxides that are present in Type I glass include zinc and barium oxide. Therefore, one needs to consider metal ion leachables from glass containers as a source of catalysts for oxidation, especially iron and manganese. Leaching of alkaline materials from the glass surface may also enhance oxidation of a drug by altering the formulation pH. A common practice is to remove the surface alkali and improve the chemical durability of glass containers by surface treatments. The most common method to remove alkali from the interior



Table 10

Additives and Other Factors for Stabilizing Liquid Dosage Forms

Aqueous	Oxygen dependent	pH < 5: 1, 3, 8, 9, 10, 15, 16, 20, 21 pH 5–6.5: 1, 3, 8–13, 15–17, 20, 21 pH > 6.5: 3, 7, 9, 10–13, 15, 17, 18, 21, 22
	Oxygen independent	pH < 7: excipient without peroxides, 16 pH > 7: excipient without peroxides, 17, 18
Oil	Oxygen dependent: 4, 5, 6, 14, 22	
	Oxygen independent: excipient without peroxides	

The recommendations for additive use are labeled according to the number for the additives found in Table 12. Softgels are considered liquid dosage forms. Lyophiles are also considered as liquids; however, acids to be added to lyophiles will generally need to be nonvolatile as with the solid dosage forms. Suspensions are considered as liquids with the appropriate solvent being the suspending liquid. In addition to the suggestions in this table, one should always consider options such as changing the formulation or special packaging.

surface of glass containers is to sulfur-treat the glass with ammonium sulfate (130). Using sulfur-treated glass containers may improve the overall stability of the drug formulation.

In liquid products, many packaging options such as vials, pre-filled syringes, and multi-component syringes use rubber closures to seal the drug formulation from the outside environment. These closures require particular

attention since the permeability of the containers (usually glass) is often low, making the closure the likely path for oxygen ingress. Choosing a closure for any of the aforementioned packaging systems requires evaluation of the closure material in terms of both its oxygen permeability, and its chemical and physical stability when in contact with the liquid pharmaceutical formulation. In liquid dosage forms, the choice of

Table 11

Additives and Other Factors for Stabilizing Solid Dosage Forms

Acidic drug	Oxygen dependent		$pK_a < \text{approximate pH}$: 1–24 $pK_a \geq \text{approximate pH}$: 1, 3–10, 14–18, 20–22
	Oxygen independent		$pK_a < \text{approximate pH}$: 2, 3, 7, 11–13, 16–19, 23 $pK_a \geq \text{approximate pH}$: 16–18
Basic drug	Counterion weak base	Oxygen dependent	$pK_a < \text{approximate pH}$: 1–24 $pK_a \geq \text{approximate pH}$: 1, 3–10, 14–18, 20–22
		Oxygen independent	$pK_a < \text{approximate pH}$: 2, 3, 7, 11–13, 16–19, 23 $pK_a \geq \text{approximate pH}$: 16–18
	Counterion not a weak base	Oxygen dependent	$pK_a < \text{approximate pH}$: change counterion to weaker base, 1–24 $pK_a \geq \text{approximate pH}$: change counterion to weaker base, 1, 3–10, 14–18, 20–22
		Oxygen independent	$pK_a < \text{approximate pH}$: change counterion to weaker base, 2, 3, 7, 11–13, 16–19, 23 $pK_a \geq \text{approximate pH}$: change counterion to weaker base, 16–18

The additives may perform more effectively when added from solution. This can be accomplished by wet granulations or fluid-bed processes. In addition to the suggestions in this table, one should always consider options such as changing the formulation or special packaging. The numbers refer to the additives in Table 12.



Table 12
Additives for Reducing Oxidation in Pharmaceutical Dosage Forms (58,114,115,135)

Additive	CAS #	pK _a	H ₂ O Sol. (mg/mL)	Alc. Sol. (mg/mL)	wf%, Liquids	Synergists	Caveats	Mechanism	Drug Precedence
1 Acetyl cysteine	616-91-1	About 2	Soluble	Soluble	0.1–0.5	Ascorbic acid	Light sensitive, use in acidic environment	H-atom donor	
2 Adipic acid	124-04-9	4.4, 5.28	45	Very soluble	0.02–0.5	Acetyl cysteine	Light sensitive; discolors on oxidation; can increase metal catalysis	Acid	Vivifit Berna, Children's Sulfad Chewables, Gabitril Film Tab, Soriatane
3 Ascorbic acid (Vitamin C)	50-81-7	4.17, 11.57	333	20				Acid, preferentially oxidized	
4 Ascorbyl palmitate	137-66-6		0.002	125	0.01–0.02	Tocopherols	Can increase metal catalysis	H-atom donor	Modril
5 BHA	25013-16-5	About 10	Insoluble	Freely soluble	0.005–0.02	Citric acid, BHT, propyl gallate	Light sensitive, especially with metals	H-atom donor	Mevacor, Vesunoid, Acetane, Zacor, Stromectol, Rocaltrol, Vantin
6 BHT	128-37-0	About 10	Insoluble	Freely soluble	0.005–0.02	Maleic acid, BHA, propyl gallate	Light, heat, humidity sensitive; can turn yellow	H-atom donor	Covera-HS, Ditropan XL, Rocaltrol
7 Citric acid	77-92-9 (5949-29-1 for hydrate)	3.128, 4.761, 6.396	>2000	667	0.3–2.0		Use above pH 6.5 for chelation	Acid, chelator	Dinestapp Extentabs, Droxia Capsules, Eleptry Capsules, Estratest, Pinate, Carbazol Capsules, Ticlid Tablets, Tylenol with Codeine, Stromectol Tablets, Aldoril, Aldomet, Aldoclor, Neprelan Tablets, Hydrea Capsules, Bufferin, Tylenol Flu, Lactaid Caplets, E.E.S. 400 Film Tabs, Enex PSE, Excedrin PM, Erythrocin Stearate, Ery-Tab
8 Cysteine	52-90-4	1.92, 8.33, 10.78	Soluble	Soluble	0.1–0.5		More stable in acid; light sensitive	H-atom donor	Zyban SR, Wellbutrin SR, Achret, Stephan, Nephramine
9 Disodium eddic acid (EDTA)	6381-92-6 (60-00-4 acid, 85715-60-2 trisodium salt)	2.00, 2.67, 6.16, 10.26	2		0.005–0.1	True antioxidants	Unstable above 150°C	Chelator	Pritin, Excedrin Extra Strength Gelatins, Maximum Strength Midol PMS and Menstrual, Aldomet, Trilisate, Depen Titratable, Vesunoid Capsules, Acetane Capsules, Aleve, Mexid Capsules, Diprivan, Nesacane, Mepergan, Netromycin, Onak
10 Dihydrothreitol	27565-41-9	12	Soluble	Soluble			pH > 5 for chelation	H-atom donor	Verelan, Cardizen CD
11 Fumaric acid	110-17-8	3.03, 4.54	5	36				Acid, chelator	Copaxone
12 Glutamic acid	56-86-0	2.10, 4.07, 9.47	12	Insoluble			pH > 5 for chelation	Acid, chelator	RYNA-CX, Cimitor
13 Malic acid	97-67-6	3.4, 5.05	1750	455		BHT	Light sensitive, darkens with Fe	H-atom donor	Advil Gel Caplets, Excedrin Extra Strength Gelatins, Lexxel, Benylin, Terramycin
14 Propyl gallate	121-79-9	8.11	1	333	0.05–0.1	BHT, BHA	Light sensitive, store < 40°C	Reduction of O ₂	Mepergan, Terramycin
15 Sodium formaldehyde sulfoxylate	870-72-4	<2	Soluble	Insoluble	0.005–0.15		Light sensitive; less stable with dextrose; reacts with hydroxy benzylic alcohols; best < pH 7	Reduction of O ₂	Soma with Codeine, Talacen Caplets, Tylox, Tylenol with Codeine, Amilacin, Bactrim, Corlorum, Duranest, EpiPen Jr., Mepergan, Moban, Netromycin, Normillo, Phenergan, Propofol, Sencorane with Epinephrine, Septra, Streptomycin, Vibramycin, Xylocane with Epinephrine
16 Sodium metabisulfite	7681-57-4	1.82, 7.00	526	Slight	0.01–1.0			Reduction of O ₂	Minecin, Netromycin, Orgaran, Tension
17 Sodium sulfite	7757-83-7	1.82, 7.00	12.5	Slight	0.01–0.2		Best > pH 7	Reduction of O ₂	Aggrenox Capsules, Nycamint, Risperdal
18 Sodium bisulfate	7772-98-7		50	Soluble in isopropanol	0.1–0.5		Best > pH 7 (unstable in acids)	Reduction of O ₂	
19 Tartaric acid	87-69-4	2.93, 4.23	1560	400			Unstable in alkaline solution	Acid	
20 Thio glycerol	96-27-5							H-atom donor	
21 Thiourea	62-56-6	About 10	90	Soluble	0.001–0.05	Ascorbic acid	Unstable to heat, light, alkali, Fe, Ag, B, γ, δ better as antioxidant than α	H-atom donor	Neoral for Microemulsion, Fortovase, Miricette, Desogen, Ortho-Cept 28, K-Tab, Ambisome
22 Tocopherols (Vitamin E)	10191-41-0; 148-03-8; 7616-22-0; 119-13-1		Insoluble		0.05–0.075	Ascorbyl palmitate		H-atom donor	
23 Toluene sulfonic acid	6192-52-5	<1	670	Soluble			Strong acid	Acid	

The numbers are used in reference to the preferred additives shown in Tables 10 and 11.

Copyright © Marcel Dekker, Inc. All rights reserved.



packaging closures is critical since there is such close contact between the formulation and the closure, and since there can be increased chemical reactivity of rubber when in contact with moisture. The type of rubber and the degree of saturation (i.e., number of double bonds) for the elastomer both affect the physical and chemical stability of the rubber formulation, which in turn can affect the oxidative stability of a liquid formulation (131).

In addition to the elastomer itself there are numerous other ingredients used to make rubber, including vulcanizing agents, accelerators, activators, antioxidants, fillers, lubricating agents, and pigments. Previous studies have shown that the residues from some of these additives can be leached out of the rubber closures into formulations (132–134). Therefore, in choosing an elastomeric closure for a formulation sensitive to oxidative degradation one must acquire all the technical information on that particular rubber to determine if any of the possible extractables could catalyze oxidation of the drug. Examples of such extractables include peroxides, antioxidants, and metal ions. Understanding the degradation mechanism of the drug allows one to consult with various vendors in order to choose the appropriate rubber closure.

Procedure for Applying a Nitrogen Headspace to Liquids

For oxygen-sensitive compounds in liquid formulations, including a nitrogen sparge during the manufacturing process and a nitrogen headspace in the final package can suppress oxidation of the drug. This can be accomplished by heating the liquid (to approximately 55°C), then sparging with nitrogen until the desired dissolved oxygen content is reached. After cooling, the compounding can be carried out under positive (2–3 psi) nitrogen pressure. An oxygen level between 3 and 8% in the headspace is generally attainable at most manufacturing sites. However, if these levels are not attainable, using argon (which is a heavier gas) instead of nitrogen is an option to explore, though this may entail a higher manufacturing cost. In laboratory settings, the above process can be simplified by nitrogen sparging for 15 min (without heating or sampling). Adding a nitrogen headspace to filled vials in the lab can take place in a glove bag or glove box. Compressed mixed gas tanks containing a range of oxygen/nitrogen levels are

purchased easily and can be useful in determining manufacturing specifications.

SUMMARY

In the preceding chapters, procedures were discussed for determining if oxidation is a problem in a dosage form. The types of mechanisms seen for oxidation in pharmaceutical systems are covered with some of the experimental and theoretical procedures for establishing the specific mechanisms discussed. Finally, recommended methods of reducing oxidation are covered. In Tables 10–12, procedures for fixing oxidation issues are shown for different types of oxidation instabilities with different dosage forms. In particular, antioxidants and acids are listed with recommendations for their use. The fixes are listed in terms of option numbers for additives found in Table 12. In the end, every drug presents a unique situation. It is hoped that the information provided in this review will allow for the more rapid and efficient handling of oxidation problems with most drug products.

ACKNOWLEDGMENTS

The authors wish to thank Dan Arenson, Amy Antipas, Joi Carter, Eva Lee, Hai Wang, Cheryl Reiber, Scott Smith, and Sheri Shamblin of Pfizer for helpful suggestions, help in writing and editing sections of this review. We would also like to thank Professors Ned Porter, Brad Anderson, Miguel Garcia-Garibay, and Daniel Kemp for helpful suggestions, discussions, and providing key references.

REFERENCES

1. Solomons, T.W.G. *Organic Chemistry*, 5th Ed.; John Wiley and Sons: New York, 1992; 449.
2. March, J. *Advanced Organic Chemistry*, 4th Ed.; John Wiley and Sons: New York, 1992; 1158.
3. Menger, F.M.; Mandell, L. *Electronic Interpretation of Organic Chemistry*; Plenum Press: New York, 1980; 27.
4. Brittain, H. Personal communication.
5. Singh, S.; Bakshi, M. Guidance on Conduct of Stress Tests to Determine Inherent Stability of Drugs. *Pharmacol. Technol.* **2000**; 1–14 (General references to stress tests, <http://www.pharmaportal.com/articles>).



6. Alsante, K.M.; Friedmann, R.C.; Hatajik, T.D.; Lohr, L.L.; Sharp, T.S.; Snyder, K.D.; Szczesny, E.J. Degradation and Impurity Analysis for Pharmaceutical Drug Candidates. In *Handbook of Modern Pharmaceutical Analysis*; Ahuja, S., Scypinski, S., Eds.; (General references to stress tests, Chapter 4) Academic Press: Boston, 2002, in press.
7. Carstensen, J.T. *Drug Stability Principles and Practices*, 2nd Ed.; Marcel Dekker, Inc.: New York, 1995; Vol. 68.
8. Hudlicky, M. *Oxidations in Organic Chemistry—186*; American Chemical Society: Washington, DC, 1990.
9. Boccardi, G. Autoxidation of Drugs: Prediction of Degradation Impurities from Results with Radical Chain Initiators. *Farmaco* **1994**, 49 (6), 431–435.
10. Howard, J.A. *Free Radicals*; John Wiley and Sons: New York, 1973; Vol. 2.
11. Porter, N.A. *Membrane Lipid Oxidation*; CRC Press: Boca Raton, 1990; Vol. 1.
12. Huyser, E.S. *Free Radical Chain Reactions*; Wiley–Interscience: New York, 1970.
13. Bard, A.J.; Faulkner, L. *Electrochemical Methods: Fundamentals and Applications*, 2nd Ed.; John Wiley and Sons: New York, 2000; 479–480.
14. Wilson, T.D. Structural Influences on the Amperometric Detection of Opiates in High Performance Liquid Chromatography. *J. Chromatogr.* **1984**, 301, 39–46 (Note that this potential is a polarographic half-wave potential).
15. Ryan, E.; Gamache, P.; Acworth, I.; ESA, Inc. Poster presented at the 43rd Pittsburgh Conference and Exposition, New Orleans, March 1992.
16. Blessel, K.W.; Rudy, B.C.; Senkowski, B.Z. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1974; Vol. 3, 127–147.
17. Risley, D.S.; Bopp, R.J. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1990; Vol. 19, 193–219.
18. Rudy, B.C.; Senkowski, B.Z. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1974; Vol. 3, 307–331.
19. Ranta, V.-P.; Urtti, A.; Auriola, S. Gradient High-Performance Liquid Chromatography Analysis of Enkephalin Peptides, Their Metabolites and Enzyme Inhibitors Using Combined Ultraviolet and Electrochemical Detection. *J. Chromatogr. Anal.* **1997**, 766, 85–97.
20. Connors, K.A.; Amidon, G.L.; Stella, V.J. *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists*, 2nd Ed.; John Wiley and Sons: New York, 1986; 93–94, see also pp 161–808.
21. Lombardo, F.; Campos, G.; Caldwell, C.J. Pfizer Corporation, unpublished results.
22. Chan, H.K.; Fogg, A.G. Voltammetric Determination of Phenylbutazone and Oxyphenylbutazone at a Glassy Carbon Electrode. *Anal. Chim. Acta* **1979**, 109, 341–349.
23. Oungpipat, W.; Southwell-Keely, P.; Alexander, P.W. Flow Injection Detection of Tetracyclines by Electrocatalytic Oxidation at a Nickel-Modified Glassy Carbon Electrode. *Analyst* **1995**, 120, 1559–1565.
24. Leeson, L.J.; Weidenheimer, J.F. Stability of Tetracycline and Riboflavin. *J. Pharm. Sci.* **1969**, 58, 355–357.
25. Kerr, J.A. Bond Dissociation Energies by Kinetic Methods. *Chem. Rev.* **1966**, 66 (5), 465–500.
26. Lowry, T.H.; Richardson, K.S. *Mechanism and Theory in Organic Chemistry*, 3rd Ed.; Harper and Row: New York, 1987; 161–162.
27. Hoellering, R.; Gasteiger, J.; Steinhauer, L.; Schultz, P.-P.; Herwig, A. Simulation of Organic Reactions from the Degradation of Chemicals to Combinatorial Synthesis. *J. Chem. Inf. Comput. Sci.* **2000**, 40, 482–494.
28. Ihlenfeldt, W.D.; Gasteiger, J. Computer Assisted Planning of Organic Synthesis: The Second Generation of Programs. *Angew. Chem., Int. Ed. Engl.* **1996**, 34 (23/24), 2613–2633.
29. Judson, P.N.; Lea, H. Accessing Knowledge About Chemical Synthesis by Computer. *Chim. Oggi* **1996**, 14 (9), 21–24.
30. Available at <http://www.epa.gov/oppt/exposure/docs/episuited/.htm> <DLForm.htm>.
31. Vogue, P. Syracuse Research Corporation's Environmental Fate Data Bases. *J. Chem. Inf. Comput. Sci.* **1996**, 36 (3), 615.
32. Jorgensen, W.L.; Laird, E.R.; Gushurst, A.J.; Fleischer, J.M.; Gothe, S.A.; Helson, H.E.; Pedersen, G.D.; Sinclair, S. CAMEO: A Program for the Logical Prediction of Products of Organic Reactions. *Pure Appl. Chem.* **1990**, 62, 1921–1932.
33. Walling, C. Some Properties of Radical Reactions in Synthesis. *Tetrahedron* **1985**, 41 (19), 3887–3900.
34. Howard, J.A. Homogeneous Liquid Phase Autoxidations. *Free Radic.* **1973**, 2, 3–62.
35. Maillard, B.; Ingold, K.U.; Scaiano, J.C. Rate Constants for the Reactions of Free Radicals with Oxygen in Solution. *J. Am. Chem. Soc.* **1983**, 105, 5095–5099.
36. Von Sonntag, C.; Schuchmann, H.-P. Peroxyl Radicals in Aqueous Solutions. In *Peroxyl Radicals*; Alfassi, Z.B., Ed.; John Wiley and Sons: New York, 1997; 173–234.
37. Porter, N. Personal communication.
38. Ingold, K.U. Peroxy Radicals. *Acc. Chem. Res.* **1969**, 2 (1), 1–9.



39. Russell, G.A. Deuterium-Isotope Effects in the Autoxidation of Alkyl Hydrocarbons. Mechanisms of the Interaction of Peroxy Radicals. *J. Am. Chem. Soc.* **1957**, 79, 3871–3877.
40. Cabelli, D.E. The Reactions of HO_2/O_2^- Radicals in Aqueous Solution. In *Peroxy Radicals*; Wiley: Chichester, 1997; 407–437.
41. Rigo, A.; Scarpa, M. Generation of Superoxide in the Autoxidation of Ascorbate and Glutathione. In *Oxygen Radicals in Chemistry and Biology*; Walter de Gruyter and Co.: Berlin, 1984; 171–176.
42. Hartauer, K.J.; Arbuthnot, G.N.; Baetschi, S.W.; Johnson, R.A.; Luke, W.D.; Pearson, N.G.; Rickard, E.C.; Tingle, C.A.; Tsang, P.K.S.; Wiens, R.E. Influence of Peroxide Impurities in Povidone and Crospovidone on the Stability of Raloxifene Hydrochloride in Tablets: Identification and Control of an Oxidative Degradation Product. *Pharm. Dev. Technol.* **2000**, 5, 303–310.
43. Buhler, V. *Kollidone*; 2nd Ed.; BASF Aktiengesellschaft: Ludwigshafen, Germany, 1993.
44. Union Carbide Customer Support Information.
45. Dubost, D.C.; Kaufman, M.J.; Zimmerman, J.A.; Bogusky, M.J.; Coddington, A.B.; Pitzenberger, S.M. Characterization of a Solid State Reaction Product from a Lyophilized Formulation of a Cyclic Heptapeptide. A Novel Example of an Excipient-Induced Oxidation. *Pharm. Res.* **1996**, 13 (12), 1811–1814.
46. Hancock, B.C.; Zografi, G. Characteristics and Significance of the Amorphous State in Pharmaceutical Systems. *J. Pharm. Sci.* **1997**, 86 (1), 1–12.
47. Miller, D.M.; Buettner, G.R.; Aust, S.D. Transition Metals as Catalysts for “Autoxidation” Reactions. *Free Radic. Biol. Med.* **1990**, 8 (1), 95–108.
48. Koppenol, W.H. Free Radical Damage and Its Control. In *New Comprehensive Biochemistry*; Rice-Evans, C.A., Burdon, R.H., Eds.; Elsevier: Amsterdam, 1994; 3–24.
49. Buettner, G.R.; Jurkiewicz, B.A. Catalytic Metals, Ascorbate and Free Radicals: A Combination to Avoid. *Radiat. Res.* **1996**, 145 (5), 532–541.
50. Taqui Khan, M.M.; Matell, A.E. *Homogeneous Catalysis by Metal Complexes*; Academic Press, New York, 1974, vol 1, 143–149.
51. MacFaul, P.A.; Wayner, D.D.M. Ingold, K.U. A Radical Account of “Oxygenated Fenton Chemistry”. *Acc. Chem. Res.* **1998**, 31 (4), 159–162.
52. Roth, H.J. Photochemistry and Pharmaceuticals. *Farm Glas.* **1968**, 24 (8–9), 349–359.
53. Szwarc, M.; Streitwieser, A.; Mowery, P.C. *Ions and Ion Pairs in Organic Reactions*; John Wiley and Sons: New York, 1974; Vol. 2, Chapter 2.
54. Lowry, T.H.; Richardson, K.S. *Mechanism and Theory in Organic Chemistry*, 3rd Ed.; Harper and Row: New York, 1987; 177–180.
55. Lowry, T.H.; Richardson, K.S. *Mechanism and Theory in Organic Chemistry*, 3rd Ed.; Harper and Row: New York, 1987; 297–298.
56. Weast, R.C., (Ed.) *Handbook of Chemistry and Physics*, 52nd Ed.; The Chemical Rubber Co.: Ohio, 1971.
57. Streitwieser, A., Jr; Heathcock, C.H. *Introduction to Organic Chemistry*, 2nd Ed.; Macmillan Publishing Co.: New York, 1981; 938.
58. Kibbe, A.H., (Ed.) *Handbook of Pharmaceutical Excipients*, 3rd Ed.; American Pharmaceutical Association: Washington, DC, 2000.
59. Byrn, S.R.; Pfeiffer, R.R.; Stowell, J.G. *Solid-State Chemistry of Drugs*, 2nd Ed.; SSCI: West Lafayette, 1999.
60. Crowley, P.J. Excipients as Stabilizers. *Pharm. Sci. Technol. Today* **1999**, 2, 237–243.
61. Bennett, J.E.; Root, K.D.J. Free Radicals in the Solid State. *Electron Spin Reson.* **1972**, 1, 118–155.
62. Garcia-Garibay, M. Personal communication.
63. Duddu, S.P.; Weller, K. Importance of Glass Transition Temperature in Accelerated Stability Testing of Amorphous Solids: Case Study Using a Lyophilized Aspirin Formulation. *J. Pharm. Sci.* **1996**, 85, 345–347.
64. Akers, M.J. Preformulation Screening of Antioxidant Efficiency in Parenteral Solutions. *J. Parenter. Drug Assoc.* **1979**, 33 (6), 346–356.
65. Parasrampur, J.; Li, L.; Dudleston, A.; Zhang, H. The Impact of an Autoclave Cycle on the Chemical Stability of Parenteral Products. *J. Parenter. Sci. Technol.* **1993**, 47 (4), 177–179.
66. Akers, J.; Agalloco, J. Sterility and Sterility Assurance PDA. *J. Pharm. Sci. Technol.* **1997**, 51 (2), 27–77.
67. Duncan, M.R.; Wang, P.L.; Moldenhauer, J.E. Determining the Capability of a Drug Product to Be Terminally Sterilized: A Case Study Involving a Heat-Sensitive, Oxygen Sensitive Drug Product. *Pharm. Dev. Technol.* **1998**, 3 (4), 527–534.
68. Jacobs, G.P. A Review of the Effects of Gamma Radiation on Pharmaceutical Materials. *J. Biomater. Appl.* **1995**, 10 (7), 59–83.
69. Reid, B.R. Gamma Processing Technology: An Alternative Technology for Terminal Sterilization of Parenterals. *J. Pharm. Sci. Technol.* **1995**, 49 (2), 83–88.
70. Jacobs, G.P. Gamma Radiation Sterilization. In *Encyclopedia of Pharmaceutical Technology*; Marcel Dekker, Inc.: New York, 1992; 305–328.
71. Gopal, N.G.S. Radiation Sterilization of Pharmaceuticals and Polymers. *Radiat. Phys. Chem.* **1978**, 12, 35–50.



72. Pandula, E.; Farkas, E. Elimination of Gamma-Induced Radiation in Aqueous Pharmaceutical Preparations. *Acta Pharm. Hung.* **1969**, *40*, 165–169.
73. am Ende, M.T.; Peppas, N.A. FTIR Spectroscopic Investigation and Modeling of Solute/Polymer Interactions in the Hydrated State. *J. Biomater. Sci. Polym. Edn.* **1999**, *10*, 1289–1302.
74. Donbrow, M. Stability of the Polyoxyethylene Chain. *Surfactant Sci. Ser.* **1987**, *23* (Nonionic Surfactants), 1011–1073.
75. Cleland, J.L.; Powell, M.F.; Shire, S.J. The Development of Stable Protein Formulations: A Close Look at Protein Aggregation, Deamidation, and Oxidation. *Crit. Rev. Ther. Drug Carrier Sys.* **1993**, *10*, 307–377.
76. Lai, M.C.; Topp, E.M. Solid-State Chemical Stability of Proteins and Peptides. *J. Pharm. Sci.* **1999**, *88*, 489–500.
77. McNally, E.J., (Ed.) *Protein Formulation and Delivery*; Marcel Dekker, Inc.: New York, 2000; 111–200.
78. Halliwell, B.; Gutteridge, M.C. Role of Free Radicals and Catalytic Metal Ions in Human Disease: An Overview. In *Methods in Enzymology: Free Radicals and Metal Ions in Human Disease*; Academic Press: New York, 1990; Vol. 186, 1–85.
79. Means, G.E.; Feeney, R.E. *Chemical Modification of Proteins*; Holden-Day, Inc.: San Francisco, 1971; 1–254.
80. Foote, C.S. Mechanisms of Photosensitized Oxidation. *Science* **1968**, *162* (3857), 963–970.
81. Matheson, I.B.C.; Lee, J. Chemical Reaction Rates of Amino Acids with Singlet Oxygen. *Photochem. Photobiol.* **1979**, *29* (5), 879–881.
82. Sluyterman, L.A.Æ. Photooxidation, Sensitized by Proflavine, of a Number of Protein Constituents. *Biochim. Biophys. Acta* **1962**, *60*, 557–561.
83. Spikes, J.D.; Straight, R. Sensitized Photochemical Processes in Biological Systems. *Ann. Rev. Phys. Chem.* **1967**, *18*, 409–436.
84. Pikal, M.J.; Dellerman, K.; Roy, M.L. Formulation and Stability of Freeze-Dried Proteins: Effects of Moisture and Oxygen on the Stability of Freeze-Dried Formulations of Human Growth Hormone. *Dev. Biol. Stand.* **1992**, *74*, 21–38.
85. McDermott, M.; Chiesa, R.; Roberts, J.E.; Dillon, J. Photooxidation of Specific Residues in α -Crystallin Polypeptides. *Biochemistry* **1991**, *30* (35), 8653–8660.
86. Rietveld, A.W.M.; Ferreira, S.T. Kinetics and Energetics of Subunit Dissociation/Unfolding of TIM: The Importance of Oligomerization for Conformational Persistence and Chemical Stability of Proteins. *Biochemistry* **1998**, *37* (3), 933–937.
87. Xie, M.; Schowen, R.L. Secondary Structure and Protein Deamidation. *J. Pharm. Sci.* **1999**, *88* (1), 8–13.
88. Farber, J.M.; Levine, R.L. Sequence of Peptide Susceptible to Mixed Function Oxidation. Probable Cation Binding Site in Glutamine Synthetase. *J. Biol. Chem.* **1986**, *261* (10), 4574–4578.
89. Hovorka, S.W.; Hong, J.; Cleland, J.L.; Schoeneich, C. Metal-Catalyzed Oxidation of Human Growth Hormone: Modulation by Solvent-Induced Changes of Protein Conformations. *J. Pharm. Sci.* **2001**, *90* (1), 58–69.
90. Stadtman, E.R. Oxidation of Free Amino Acids and Amino Acid Residues in Proteins by Radiolysis and by Metal-Catalyzed Reactions. *Ann. Rev. Biochem.* **1993**, *62*, 797–821.
91. Sanches, R.; Melo, W.L.B.; Columbo, M.F. Effects of Hydration in the Oxygenation and Autoxidation of Carbonmonoxyhemoglobin Powder. *Biochim. Biophys. Acta* **1996**, *874* (1), 19–22.
92. Karel, M. Free Radicals in Low Moisture Systems. In *Water Relations in Foods, Proceedings of International Symposium*; Duckworth, R.B., Ed.; Academic Press: New York, 1975; 435–453.
93. Smith, J.; Zhang, Y.; Niven, R. Toward Development of a Non-viral Gene Therapeutic. *Adv. Drug Deliv. Rev.* **1997**, *26* (2,3), 135–150.
94. Wolff, J.A. Naked DNA Gene Transfer in Mammalian Cells. *The Development of Human Gene Therapy*; Cold Spring Harbor Monogram Series 36, 1999; 279–307.
95. Evans, R.K.; Xu, Z.; Bohannon, K.E.; Wang, B.; Bruner, M.W.; Volkin, D.B. Evaluation of Degradation Pathways for Plasmid DNA in Pharmaceutical Formulations via Accelerated Stability Studies. *J. Pharm. Sci.* **2000**, *89* (1), 76–87.
96. Anchordoquy, T.J.; Koe, G.S. Physical Stability of Nonviral Plasmid-Based Therapeutics. *J. Pharm. Sci.* **2000**, *89* (3), 289–296.
97. Adami, R.C.; Collard, W.T.; Gupta, S.A.; Kwok, K.Y.; Bonadio, J.; Rice, K.G. Stability of Peptide-Condensed Plasmid DNA Formulations. *J. Pharm. Sci.* **1998**, *87* (6), 678–683.
98. Lindahl, T. Instability and Decay of the Primary Structure of DNA. *Nature (Lond.)* **1993**, *362* (6422), 709–715.
99. Aust, A.E.; Eveleigh, J.F. Mechanisms of DNA Oxidation. *Soc. Exp. Biol. Med.* **1999**, *222* (3), 246–251.
100. Núñez, M.E.; Hall, D.B.; Barton, J.K. Long-Range Oxidative Damage to DNA: Effects of Distance and Sequence. *Chem. Biol.* **1999**, *6* (2), 85–97.
101. Steenken, S.; Javanovic, S.V. How Easily Oxidizable Is DNA? One-Electron Reductive Potentials of Adenosine and Guanosine Radicals in Aqueous Solution. *J. Am. Chem. Soc.* **1997**, *119*, 617–618.



102. Viswanathan, A.; Doetsch, P.W. Effects of Nonbulky DNA Base Damages on *Escherichia coli* RNA Polymerase-Mediated Elongation and Promoter Clearance. *J. Biol. Chem.* **1998**, *273* (33), 21276–21281.
103. Araki, K.; Silva, C.A.; Toma, H.E.; Catalani, L.H.; Medeiros, M.H.; Mascio, P.D. Zinc Tetraethynylated Porphyrin Binding and Photoinduced Oxidation of Calf Thymus DNA. *J. Inorg. Biochem.* **2000**, *78* (4), 269–273.
104. Middaugh, C.R.; Evans, R.K.; Montgomery, D.L.; Casimiro, D.R. Analysis of Plasmid DNA from a Pharmaceutical Perspective. *J. Pharm. Sci.* **1998**, *87* (2), 130–146.
105. Thompson, J.A. A Review of High-Performance Liquid Chromatography in Nucleic Acids Research III. Isolation, Purification and Analysis of Supercoiled Plasmid DNA. *Biochromatography* **1986**, *1* (2), 68–71 (see also pp 74–80).
106. de Carmejane, O.; Schweinfus, J.J.; Morris, M.D. Plasmid Topoisomer Separation by Capillary Gel Electrophoresis. *Proc. SPIE-Int. Soc. Opt. Eng.* **1999**, *3602* (Advances in Fluorescence Sensing Technology IV), 346–354.
107. Billany, M.R.; Khatib, K.; Gordon, M.; Sugden, J.K. Alcohols and Ethanolamines as Hydroxyl Radical Scavengers. *Int. J. Pharm.* **1996**, *137*, 143–147.
108. Gordon, A.J.; Ford, R.A. *The Chemists' Companion*; John Wiley and Sons: New York, 1972.
109. Wolff, S.P. Ferrous Ion Oxidation in Presence of Ferric Ion Indicator Xylenol Orange for Measurement of Hydroperoxides. *Methods Enzymol.* **1994**, *233*, 182–189.
110. Bruton, G.W.; Ingold, K.U. Autoxidation of Biological Molecules. Antioxidant Activity of Vitamin E and Related Chain-Breaking Phenolic Antioxidants In Vitro. *J. Am. Chem. Soc.* **1981**, *103* (21), 6472–6477.
111. Doba, T.; Burton, G.W.; Ingold, K.U. EPR Spectra of Some α -Tocopherol Model Compounds. Polar and Conformational Effect and Their Relation to Antioxidant Activities. *J. Am. Chem. Soc.* **1983**, *105* (21), 6505–6506.
112. Burton, G.W.; Doba, T.; Gabe, E.; Hughes, L.; Lee, F.L.; Prasad, L.; Ingold, K.U. Autoxidation of Biological Molecules. Maximizing the Antioxidant Activity of Phenols. *J. Am. Chem. Soc.* **1985**, *107* (24), 7053–7065.
113. Porter, N.A. Mechanisms for the Autoxidation of Polyunsaturated Lipids. *Acc. Chem. Res.* **1986**, *19* (9), 262–268.
114. Akers, M.J. Antioxidants in Pharmaceutical Products. *J. Parenter. Sci. Technol.* **1982**, *36* (5), 222–228.
115. Buettner, G.R. Spin Trapping: ESR Parameters of Spin Adducts. *Free Radic. Biol. Med.* **1987**, *3* (4), 259–303.
116. Walling, C. Fenton's Reagent Revisited. *Acc. Chem. Res.* **1975**, *8* (4), 125–131.
117. Rush, J.D.; Koppenol, W.H. Oxidative Intermediates in the Reaction of Ferrous EDTA with Hydrogen Peroxide. Reactions with Organic Molecules and Ferrocyclochrome c. *J. Biol. Chem.* **1986**, *261*, 6730–6733.
118. Klaui, H. Tocopherol, Carotene and Ascorbyl Palmitate. *Int. Flavors Food Addit.* **1976**, *7*, 165–171.
119. Acarturk, F. The Effect of Cyclodextrins on the Physical and Chemical Stability of Drugs. Effect on Solid State Stability. *FABAD J. Pharm. Sci.* **1993**, *18* (2), 77–85.
120. Lutz, B.; Volker, M. Cyclodextrins—Molecular Capsules with Useful Cavities. *Prax. Naturwiss. Chem.* **1990**, *39*, 10–17.
121. Duchene, D.; Debrueres, B.; Vaution, C. Improvement of Drug Stability by Cyclodextrin Inclusion Complexation. *S.T.P. Pharma* **1985**, *1*, 37–43.
122. Eastman Chemical Company product information on bottle polymer materials.
123. Wang, Y.; Eastal, A.J.; Chen, X.D. Ethylene and Oxygen Permeability Through Polyethylene Packaging Films. *Packag. Technol. Sci.* **1998**, *11*, 169–178.
124. Forcinio, H. Choosing a Blister Material. *Pharm. Technol.* **2000**, *May*, 26–30.
125. Taborsky, C.J.; Foster, M.G.; Lockhart, H.; Polgar, B. Permeation of Unit-Dose Blister Market Containers Under USP and ICH Conditions. *Pharm. Technol.* **2000**, *August*, 38–42.
126. Pilchik, R. Pharmaceutical Blister Packaging, Part 1, Rationale and Materials. *Pharm. Technol.* **2000**, *November*, 68–77.
127. Pavanetto, F.; Gento, I.; Conti, B.; Modena, T.; Montanori, L. Investigation on the Contribution of Raw Materials and of the Primary Container on Aluminum, Cadmium and Lead Contamination of Large-Volume Parenteral Solutions. *Int. J. Pharm.* **1989**, *54* (2), 143–148.
128. Avis, K.E.; Levchuk, J.W. *Remington: Science and Practice of Pharmacy*, 19th Ed.; Gennaro, A.R., Ed.; Mack Publishing Co.: Easton, PA, 1995; Vol. 2, 1531.
129. Sanga, S.V. Review of Glass Types Available for Packaging Parenteral Solutions. *J. Parenter. Drug Assoc.* **1979**, *33* (2), 61–67.
130. Roseman, T.J.; Brown, J.A.; Scothorn, W.W. Glass for Parenteral Products: A Surface View Using the Scanning Electron Microscope. *J. Pharm. Sci.* **1976**, *65* (1), 22–29.
131. Bontempo, J.A. Considerations for Elastomeric Closures for Parenteral Biopharmaceutical Drugs. *Drugs Pharm. Sci.* **1997**, *85* (Development of Biopharmaceutical Parenteral Dosage Forms), 223–240.





132. Airaud, C.B.; Gayte-Sorbier, A.; Memburg, R.; Laurent, P. Leaching of Antioxidants and Vulcanization Accelerators from Rubber Closures into Drug Preparations. *J. Biomater. Sci. Polym. Ed.* **1990**, *1* (4), 231–241.
133. Danielson, J.W.; Oxborrow, G.S.; Placencia, A.M. Quantitative Determination of Chemicals Leached from Rubber Stoppers into Parenteral Solutions. *J. Parenter. Sci. Technol.* **1984**, *38* (3), 90–93.
134. Danielson, J.N.; Oxborrow, G.S.; Placencia, A.M. Chemical Leaching of Rubber Stoppers into Parenteral Solutions. *J. Parenter. Sci. Technol.* **1983**, *37* (3), 89–92.
135. *Physicians' Desk Reference (Online)*. Medical Economics Co.: Montvale, NJ, 2000.

Received April 23, 2001

Accepted June 9, 2001

