REVIEW ARTICLE

Stabilization of Pharmaceuticals to Oxidative Degradation

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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, counter-ions 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

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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.
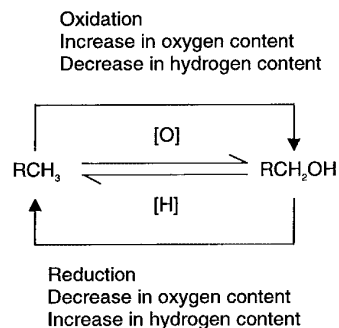
When a compound is oxidized, another compound must be reduced. Hydration and dehydration are not oxidation/reduction reactions, though they add oxygen, since the reaction is essentially an internal oxidation and reduction: one carbon atom is oxidized while another is reduced. The net change to the oxidation 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.

$$H_2C = CH_2 \xrightarrow{H_2O} CH_3CH_2OH$$
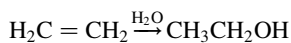
If there is any doubt as to the oxidation status of a molecule, the following procedure helps to identify the oxidation state of a given compound (3):

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:
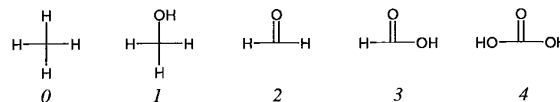
Listed below are the general structures and names of some common pharmaceutically relevant species in the order of increasing oxidation states with the oxidation numbers listed below each species:
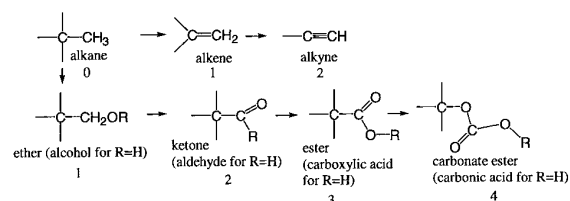
To study an oxidation reaction mechanism, it is important to understand the electron transfer between the species involved by tracking the fate of electrons (using Lewis structures and arrows). For single-electron transfers, single-headed arrows are used, while for two-electron processes, double-headed arrows are used. As an example of tracking an electron transfer, disproportionation of two radicals is shown with arrows indicating the "flow" of electrons:
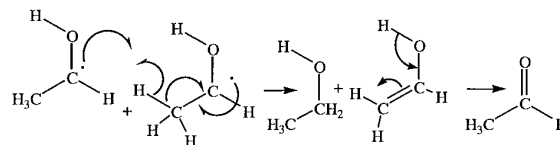
Preliminary Screening for Oxidative Instability

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 O_2 (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, $FeCl_3$ or $CuCl_2$ 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 O_2 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

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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(N,N′-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-N-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)

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