CHAPTER II

LITERATURE REVIEWS

Fluconazole is an antifungal agent that has FDA approved labeling for use in the threatment of candidiasis and cryptococcal meningitis. These opportunistic infections frequently occur in immunocompromised persons, such as patients with AIDS, cancers, and organ transplants. Fluconazole is available in both oral (as tablets) and intravenous dosage forms. Oral fluconazole 50-100 mg/day has been reported to be as effective as oral ketoconazole 200-400 mg/day for the treatment of oropharyngeal candidiasis in immunocompromised patients (Wit et al., 1989). In addition, fluconazole treatment has been successful in patients with cryptococcal mentingitis who failed to respond to amphotericin B with or wihtout flucytosine.

An oral liquid formulation of fluconazole may be required for patients who cannot ingest or swallow tablets. Although intravenous fluconazole may be an alternative in this situation, an oral liquid formulation may be preferable because of increased patient compliance and decreased cost.

In pharmaceutical terms, solutions are liquid preparations that contain one or more chemical substances dissolved in a suitable solvent or mixture of mutually miscible solvents (USP. 1995). Because of a particular pharmaceutical solution's use, it may be classified as an oral solution, otic solution, ophthalmic solution, or topical solution. Still other solutions, because of their composition or use, may be

classified as other pharmaceutical dosage forms. For example, aqueous solutions containing a sugar are classified as syrups ; sweetened hydroalcoholic (combinations of water and ethanol) solutions are termed elixirs ; solutions of aromatic materials are termed spirits if the solvent is a alcoholic or aromatic waters if the solvent is aqueous. Solutions prepared by extracting active constituents from crude drugs are termed tinctures or fluidextracts, depending upon their method of preparation and their concentration. Tinctures may also be solutions of chemical substances dissolved in alcohol or in a hydroalcoholic solvent. Certain solutions prepared to be sterile and pyrogen-free and intended for parenteral administration are classified as injections.

Generally, water is always the solvent of choice in pharmaceutical preparations. However, when it is not possible for physical and chemical reasons (such as limited solubility and hydrolytic reactions) to use a whole aqueous system, techniques of solubilization become important. In general, the aqueous solubility of a drug can be increased by a variety of techniques. The choice of a method, however, depends upon the nature of the drug, and degree of solubilization required. Some of these technique are :

1. Complexation

This method utilizes complexing agents (also called ligand e.g. EDTA, citric acid, caffeine, etc.) to associate with a drug. The application of this technique is quite limited with some problems. First, if complexation is rapid and total reversible process, it may result in precipitation upon dilution. A second problem associated with complexation is the necessary presence of the ligand. Since the ligand will normally be present in molar ratios equivalent to and often

much greater than the drug. This may cause some undesirable effects. The final limiting factor is the fact that , the apparent solubility increased by this technique is an order of magnitude or less. When solubility increased of 10^2 or 10^3 are required, other approaches are probably best consideration.

2. Micellar solubilization

By adding of a surfactant, the solubility of insoluble or poorly soluble drug is increased by the presense of surfactant-micelles. Non-ionic surfactants are frequently included in a dosage formulation in different amounts depending on the role they play in this form (Krasowska, 1980). It is generally accepted that a drug solubilized in micelles is not available for absorption. This is because the drug absorption process from micellar solutions is usually explained on the basis of a pseudo-phase-separation model in which two phases are considered ; the dispersed micellar phase and the continuous aqueous phase. The drug is partitioned between these two phases with a constant partition ratio. Therefore, micellar solubilization would have a negative effect on drug absorption. Moreover, the possible short or long term adverse effect of the surfactant on the body, the concomitant solubilization of other ingredients such as preservatives, coloring agent, flavoring agents which may result in alterations in stability and effectiveness of drug product make this technique to be limited. However, this method is better than the previous one, because of its higher degree of increased solubility.

3. Using mixed solvents (cosolvents)

The solubility of a drug can be increased by selecting some nonaqueous solvents to form a binary of temary mixed solvent with water. The solubility of

many drugs have often been interpreted on the basis of polarity differences between solutes and solvents. It results principally from interactions between solute and solvent molecules. Mixed solvent can provide proper polarity of each drug by varying the ratio of aqueous and nonaqueous solvents. Additionally, the selection of appropriate mixed solvents can ensure the solubiltiy of all compounds in the formulation and minimize the potential for precipitation which may result from cooling. However, the selection of nonaqueous solvent for a drug vehicle is a compromise among influencing factors, such as its chemical and physical properties, its pharmacological and physiological properties in the body. The most frequently used mixed solvents are propylene glycol, ethanol, glycerine and polyethylene glycol in water. The major pharmaceutical and pharmacological properties of these and other water-miscible cosolvents have been reviewed by Speigel and Nose worthy (Spiegel and Noseworthy, 1963).

4. Other methods.

By using some chemical reactions such as making the drug in the form of prodrugs or by solid dispersion process, the solubility of a drug may be increased.

Syrups

Syrups are concentrated, aqueous preparations of a sugar or sugar-substitute with or without added flavoring agents and medicinal substances. Syrups containing flavoring agents but not medicinal substances are called nonmedicated or flavored vehicles (syrups). Some official, previously official and examples of commercially available nonmedicated syrups are presented in Table 1.

Table 1 Examples of nonmedicated syrups.

Nonmedicated Syrup	Comments
Cherry Syrup	A sucrose-based syrup containing about 47% by volume of cherry juice. The syrup's tart and fruit flavor is attractive to most patients and the acidic pH of the syrup makes it useful as a vehicle for drugs requiring an acid medium.
Cocoa Syrup	This syrup is a suspension of cocoa powder in an aqueous vehicle sweetened and thickened with sucrose, liquid glucose, and glycerin, and flavored with vanillin and sodium chloride. The syrup is particularly effective in administering bitter tasting drugs to children.
Orange Syrup	This sucrose-based syrup utilizes sweet orange peel tincture, and citric acid as the source of flavor and tartness. The syrup resembles orange juice in taste and is a good vehicle for drugs stable in an acidic medium.
Ora-Sweet and Ora-Sweet SF (Paddock Laboratories)	Commercially available vehicles for the extemporaneous compounding of syrups. Both vehicles have a pH between 4 and 4.5 and are alcohol free.
Raspberry Syrup	A sucrose-based syrup containing about 48% by volume of raspberry juice. It is a pleasantly flavored vehicle used to disguise the salty or sour taste of saline medicaments.
Syrup	This is an 85% solution of sucrose in purified water. This "simple syrup" may be used as the basis for the preparation of syrups.

These syrups are intended to serve as pleasant-tasting vehicles for medicinal substances to be added in the extemporaneous compounding of prescriptions or in the preparation of a standard formula for a medicated syrup, which is a syrup containing a therapeutic agent. Due to the inability of some children and elderly people to swallow solid dosage forms. In doing so, considerations of drug solubility, stability, and bioavailability must be considered case by case (Pesko, 1993). The liquid dosage form selected for compounding may be a solution or a suspension, depending upon the chemical and physical characteristics of the particular drug and its solid dosage from. Vehicles are commercially available for this purpose (Pesko, 1993).

Medicated syrups are commercially prepared from the starting materials ; that is by combining each of the individual components of the syrup, as sucrose, purified water, flavoring agents, coloring agents, the therapeutic agent, and other necessary and desirable ingredients. Naturally, medicated syrups are employed in therapeutics for the value of the medicinal agent present in the syrup.

Syrups provide a pleasant means of administering a liquid form of a disagreeable tasting drug. They are particularly effective in the administration of drugs to youngsters, since their pleasant taste usually dissipates any reluctance on the part of the child to take the medicine. The fact that syrups contain little or no alcohol adds to their favor among parents.

Any water-soluble drug that is stable in aqueous solution may be added to a flavored syrup. However care must be exercised to insure the compatibility between the medicinal drug substance and the other formulative components of the syrup. Also, certain flavored syrups have an acidic medium, whereas others

may be neutral or slightly basic and the proper selection must be made to insure the stability of any added medicinal agent. Perhaps the most frequently found types of medications administered as medicated syrups are antitussive agents and antihistamines. This is not to imply that other types of drugs are not formulated into syrups ; indeed, a wide variety of medicinal substances can be found in syrup form and among the many commercial products.

Components of syrups (Carter, 1975 ; Swinyard and lowenthal, 1990).

1. active ingredient
2. the sugar, usually sucrose, or sugar-substitutes used to provide sweetness and viscosity
3. antimicrobial preservatives
4. flavorants
5. colorants
6. purified water

Many syrups, especially those prepared commercially, contain special solvents, solubilizing agents, thickeners, or stabilizers.

Sweetening agent

Sucrose is the sugar most frequently employed in syrups, although in special circumstances it may be replaced in whole or in part by other sugars, as dextrose, or non-sugars as sorbitol, glycerin and propylene glycol. In some instances, all glycogenetic substances (materials converted to glucose in the body), including those agents mentioned above, are replaced by nonglycogenetic substances such as methycellulose or hydroxyethylcellulose. These two materials are not hydrolyzed and absorbed into the blood stream, and their use results in an

excellent syrup-like vehicle for medications intended for use by diabetic patients and others whose diets must be controlled and restricted to nonglycogenetic substances. The viscosity generally resulting from the use of these cellulose derivatives is much like that of a sucrose syrup. The addition of one or more artificial sweeteners usually produces an excellent facsimile of a true syrup.

Antimicrobial preservatives

The amount of a preservative required to protect a syrup against microbial growth varies with the proportion of water available for growth, the nature and inherent preservative activity of some formulative materials (as many flavoring oils that are inherently sterile and posses antimicrobial activity), and the capability of the preservative itself. Among the preservatives commonly used in the preservation of syrups with the usually effective concentrations are presented in Table 2

Table 2 Preservatives commonly used in syrups.

Preservative	Amount (%)
Benzoic acid	0.1-0.2
Sodium benzoate	0.1-0.2
Butyl paraben	0.02
Propyl paraben	0.05
Methyl paraben	0.1
Sorbic acid	0.05-0.2
Sodium sorbate	0.05-0.2

Frequently alcohol is used in the preparation of syrups to assist in the dissolving of alcohol-soluble ingredients, but normally it is not present in the final product in amounts that would be considered to be adequate for preservation (15-20%).

Flavorant

Most syrups are flavored with synthetic flavorants or with naturally occurring materials as volatile oils (e.g. orange oil), vanillin, and others, to render the syrup pleasant tasting. Because syrups are aqueous preparations, these flavorants must possess sufficient water-solubility. However, sometimes a small amount of alcohol is added to a syrup to ensure the continued solution of a poorly (water) soluble flavorant.

Clorant

To enhance the appeal of the syrup, a coloring agent is generally used which correlates with the flavorant employed (i.e. green with mint, brown with chocolate, etc.). The colorant used is generally water soluble, nonreactive with the other syrup components, and color-stable at the pH range and under the intensity of light that the syrup is likely to encounter during its shelf-life.

Oxidative reactions.

Oxidation is a reaction occurred by the loss of electrons. This reaction is a complementary one ; its partner is reduced by the acceptation of electrons. These two reactions can not happen without the other. Thus, the oxidative-reductive

reaction, which is often called redox reaction, involves the electron transfer process that occurs by a transfer of proton and can be described by :

$$\text{reduce form} \longleftrightarrow \text{oxidized form} + ne^-$$

where e^- represents an electron and n is the number of electrons being transferred (Conners et al., 1986).

Auto-oxidation

Auto-oxidation is the reaction of any material with molecular oxygen. Free radicals are produced by reactions involving homolytic bond fission of a covalent bond, so that each atom or group involved retains one of the electrons of the original covalent bond. This may be depicted as follows :

$$A : B \rightarrow A^{\bullet} + B^{\bullet}$$

These radicals are highly unsaturated and readily take electrons from other substances, causing oxidation. The auto-oxidation of an organic substance RH by a free radical chain process can be simply described as follows :

Initiation phase

$$RH \xrightarrow[\text{light, heat}]{\text{activation}} R^{\bullet} + H^{\bullet}$$

The initiation phase involves the initial formation of free radicals in the solution. There are some factors affecting this phase such as light or trace quantities of metal ions. In this phase the rate of oxidation is very low and unmeasurable. Thus, it is often called the induction preiod ; the length of the induction period depends on the reaction and the conditions in the solution.

Propagation phase

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow ROOH + R\cdot$$

$$ROOH \rightarrow RO\cdot + \cdot OH$$

The propagation phase involves the consuming of oxygen by free radicals formed in the induction period. Oxygen must accept two electrons from a donor molecules or initial free radicals formed and in so doing could in theory generate many other free radical molecules in the solution. The free radicals formed in this phase will react with the drug and chain reaction occurs.

Termination phase

$$RO_2\cdot + X \rightarrow \text{inactive products}$$

$$RO_2\cdot + RO_2\cdot \rightarrow \text{inactive products}$$

$$R\cdot + R\cdot \rightarrow \text{inactive products}$$

The termination of the reaction may take place by combining two $RO_2\cdot$ radicals or by X, a free radical inhibitor. X generally converts the peroxy radical

RO_2• to a hydroperoxide and becomes a resonance stabilized radical incapable of continuing the chain.

Factors influencing oxidation

1. Presense of heavy metals

Heavy metals, particularly those possessing two or more valency states, with a suitable oxidation-reduction potential between them (copper, iron, cobalt, and nickel) generally catalyze oxidative deteriorations. These metals reduce the length of the induction period and increase the maximum rate of oxidation. They can affect the rates of chain initiation, propagation, and termination, as well as the rate of hydroperoxide decomposition. In each case, their major function is to increase the rate of formation of free radicals. They can catalyze oxidative reactions in a number of ways. They can react directly with oxygen to produce an oxygen radical, which can then initiate an autooxidation. The metal ion can form a complex with oxygen and subsequently form a peroxy radical. The metal ion can react with the drug itself to form drug radicals. These radicals are able to enter into a propagation cycle as follows :

$$M^{n+} + RH \longrightarrow M^{(n-1)+} + H^{+} + R\bullet$$

In the other way, they can also react with a hydroperoxide in the formulation to catalyze the breakdown as follows :

$$M^{n+} + R'OOH \longrightarrow M^{(n-1)+} + H^{+} + R'O_2\bullet$$

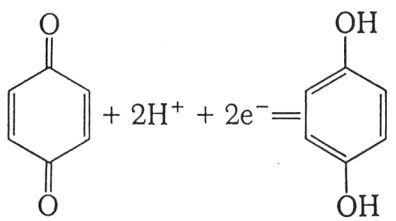
R'OOH could be a hydroperoxide of the drug itself or of some other component of the formulation.

Trace metal impurities in buffer salt caused an accelerated decomposition of prednisolone. The oxidative degradation with and without 0.1% disodium salt of ethylenediamine tetracetic acid at different buffer concentrations showed that the solutions not containing no chelating agent was degraded more rapidly when the buffer concentration was increased, the buffered solutions containing chelating agent showed that the degradation was independent of the concentration of the buffer (Lachman, Deluca and Akers, 1986). Simirarly, 0.05 %w/v EDTA in the formulation prior to freeze-drying retarded RNase degradation dramatically. EDTA was believed to chelate cations which may have been introduced with the buffer salts in trace quantities sufficient to catalyze autooxidation reactions (Townsend, Byron and Deluca, 1990).

2. Effect of pH

Many oxidations are catalyzed by hydrogen and hydroxyl ions. This can partly be ascribed to the fact that the redox potential for many reactions depends on pH. Because of increasing of hydrogen ion in the solution, the solution has a low pH value. The concentration of H^+ or pH value influences the oxidation rate of a compound. In neutral and alkaline pH conditions, many drugs such as ascorbic acid, phenols, and sulfhydryl compounds all degrade more repidly in neutral to alkaline pH conditions. In a contrary, at a low pH value or high hydrogen ion concentration, it is generally found most useful in minimizing oxidation (Connors et al., 1986).

For example, the system quinone/hydroquinone may be taken as a classic example to illustrate this point.



The oxidation potential may be expressed by the following simplified version of the Nernst equation :

$$E = E_o + \frac{0.06}{2} \log \frac{C_{H+}^{2} \cdot C_{quinone}}{C_{hydroquinone}}$$

where E_o is the so-called standard potential, E is the actual potential,2 equals the number of electrons taking place in the change from the oxidation-form to the reduced-form and 0.06 is a calculated approximate constant. It can be seen from this equation that an increase in the concentration of hydrogen ions causes an increase in the value of E. This means that the reduced-form of the system is less readily oxidized when the pH is low. Since pharmaceuticals that undergo deterioration through oxidation are generally in the reduced-form, minimum decomposition is usually found in the pH range of 3-4 (Lachman, Deluca and Akers,1986).

The effect of high pH on the oxidative degradation of drugs has been reported. The degradation of thiorphan is accelerated by basic and neutral pH. Therefore, thiorphan must be preserved at an acid pH (Gimenez et al., 1988). The pH rate profiles for the degradation of cadralazine showed a sigmoid curve superimposed on one branch of a v-graph and revealed a profound degradation

rate increase in the neutral and basic pH region, where cadralazine was in the neutral form. In the acidic pH region, the degradation rate appeared low (Visconti et al., 1984).

3. Effect of solvents

Water is always the solvent in pharmaceutical preparations. However, when it is not possible for physical and chemical reasons such as limited solubility to use a wholly aqueous system. Solvents other than water may be used as the solvent instead of water. They may have a catalyzing effect on oxidation reactions when used in combination with water or alone.

The effect of solvent on the oxidative degradation of drug has been reported. For example, fenprostalene is intrinsically stable toward aerobic oxidation in nonaqueous solution, but is susceptible to oxidation by peroxide intermediates formed during cosolvent (polyetylene glycol400-water) decomposition (Johnson and Taylor, 1983).

4. Presence of oxygen

The most common form of oxidation decomposition occurring in pharmaceutical preparations is auto-oxidation, which involves the reaction of free radicals with molecular oxygen. The amount of oxygen available influences the rate and extent of oxidative degradation of drugs and pharmaceuticals which are so much oxygen around. Only a small amount of oxygen is needed to initiate the reaction (Connors et al., 1986).

The effect of oxygen on the oxidative degradation of drugs has been reported. The formation rate of degraded products of prednisolone increases with aerobic condition (Lachman, Deluca and Akers,1986). The neuroleptic compound flupenthixol dihydrochloride solutions stored under oxygen has the oxidative rate constant greater than those stored under air (Enever, Po and Shotton, 1979). Similarly, a purge with nitrogen should improve the stability of thiorphan in solution (Gimenz et al., 1988). In additions, Pyrogallol is more rapidly decomposed in the oxygenated solution compared with the deoxygenated solution (Palmieri, 1978).

5. Degree of saturation

In initiation phase, free radical formation has been shown to occur on the methylene group which is in the α position to a double bond.

$$\overset{\alpha}{\text{-CH}_2\text{-CH=CH-CH}_2\text{-}} + O_2 \longrightarrow \text{-}\overset{\bullet}{\text{C}}\text{H-CH=CH-CH}_2\text{-} + HO_2\bullet$$

Propagation phase :

$$\text{-}\overset{\bullet}{\text{C}}\text{H-CH=CH-CH}_2\text{-} + O_2 \longrightarrow \text{-}\overset{OO\cdot}{\text{C}}\text{H-CH=CH-CH}_2\text{-}$$

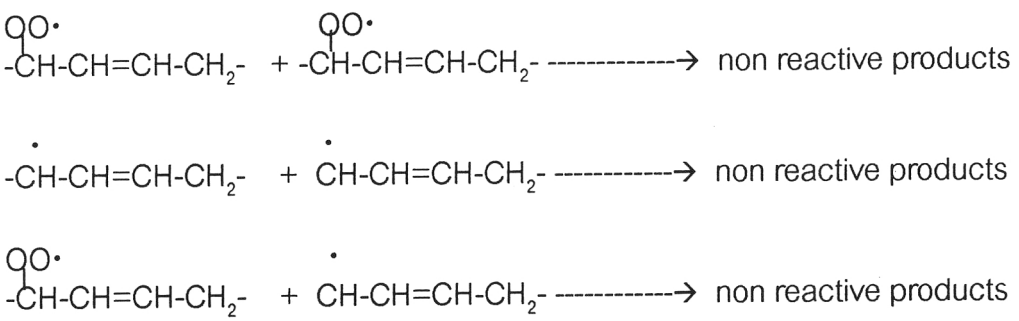
$$\text{-}\overset{OO\cdot}{\text{C}}\text{H-CH=CH-CH}_2\text{-} + \text{-CH}_2\text{-CH=CH-CH}_2 \longrightarrow \text{-}\overset{OOH}{\text{C}}\text{H-CH=CH-CH}_2\text{-} + \text{-}\overset{\bullet}{\text{C}}\text{H-CH=CH-CH}_2\text{-}$$

hydroperoxide

↓

Fatty acid, Aldehyde, Ketone

Termination phase :



The reactivity of the α -methylene can be enhanced by further unsaturation of the molecule producing an unconjugated system and the rate of degradation via auto-oxidation increase. Likewise, the more unsaturated molecule can oxidize faster than the molecules which is less unsaturation (Stewart and Tucker, 1985).

For example, linoleic acid has more unsaturated molecule can oxidize faster than oleic acid.

$[CH_3(CH_2)_4 - CH = CHCH_2CH = CH\ (CH_2)_7\ COOH]$ = Linoleic acid

$[CH_3(CH_2)_7\ CH = CH\ (CH_2)_7\ COOH]$ = oleic acid

Simvastatin and L-647, 318 are identical in structure except for the diene system in simvastatin. The oxidation kinetics clearly reveal that saturation of the diene system as in L-647, 318 completely deacticates the compound toward oxidative degradation, whereas simvastatin is reactive. This result demonstrates that the reactive site of simvastatin is located about the conjugated double bonds (Kaufman, 1990).

6. Effect of temperature

Interpretation of temperature effects on oxidative reactions is made difficult by the multiple steps in many of the reactions and because oxygen solubility in water and other solvents is temperature dependent. Since each reaction in a complex scheme will have its own activation energy, it is possible that as the temperature is changed a different reaction will become rate determining. Theoretically, under such circumstances, the adherence of the reaction-rate/temperature relationship to the Arrhenius equation will break down. Table 3 shows the oxygen content of water at various temperatures if the water is saturated by air or by purge oxygen. As can be seen for the air data, a 20°C change in temperature (5→25°C) results in a 40% decrease in oxygen concentration. (Connors et al., 1986).

Table 3 Oxygen content of water under air and pure oxygen at atmospheric pressure and various temperatures (Connors et al., 1986).

Temperature (°C)	Oxygen content (mM/ml of water)	
	From air	From purge oxygen
0	-	2.18×10^{-3}
5	0.386×10^{-3}	-
10	0.340×10^{-3}	-
15	0.304×10^{-3}	-
20	0.267×10^{-3}	-
25	0.232×10^{-3}	1.29×10^{-3}
50	-	9.28×10^{-4}
100	-	7.51×10^{-4}

7. Effect of light

It is well known that light can change the properties of different materials and products. Light or radiation is component of the electromagnetic spectrum has a triggering force that may initiate or promote an oxidative breakdown of drug molecule (Moore, 1996).

Light absorption mechanism of drugs

There are two Kinds of degradation mechanism of a drug following light absorption. Firstly, primary photochemical reactions. It occurs when the drug molecule itself absorbs light or radiation energy from the radiation source to produce an unstable excited state species, A^*, (eq.1). The absorbed energy can be lost either by a radiative mechanism in which the energy is given off in the form of fluorescence or phosphorescence, (eq.2), or by a radiationless mechanism, (eqs. 3-5). The radiationless mechanism can be physical or chemical in nature. The physical decay results in the loss of energy as heat, (eq.3), or by collision with other molecules (quenching), (eq.4). The net effect of the chemical decay is that sufficient energy is concentrated in some bond that the molecule chemically decomposes (or rearranges) in to a new species, (eq.5). This whole process can be definded by (eqs. 1-5) (Conners, Amidon and Stella, 1986).

$$A \xrightarrow{h\nu} A^* \qquad \text{_____ (eq.1)}$$

$$A^* \xrightarrow{k_1} A + h\nu \qquad \text{_____ (eq.2)}$$

$$A^* \xrightarrow{k_2} A + \text{heat} \qquad \text{_____ (eq.3)}$$

$$A^{\cdot} + A \xrightarrow{k_3} 2A \qquad \text{_____ (eq.4)}$$

$$A^{\cdot} \xrightarrow{k_4} \text{Product (s)} \qquad \text{_____ (eq.5)}$$

Secondary photochemical reactions or secondly photosensitiser, the energy is absorbed by non-drug molecules (B) which impart their increased energy to the drug molecule (A) which subsequent degradation (eqs. 6, 7). The molecules absorbed radiant energy are called photosensitisers and act as catalyst for drug decomposition.

$$B \longrightarrow B^{\cdot} \qquad \text{_____ (eq.6)}$$

$$B^{\cdot} + A \longrightarrow A^{\cdot} + B \qquad \text{_____ (eq.7)}$$

According to the Stark-Einstein Law the absorption of one quantum of radiation results in the formation of one photoexcited molecule. Since the photoexcited molecule may take part in several photochemical processes (eqs. 2-5), a quantum yield, Φ, for any one of these processes is defined by

$$\Phi = \frac{\text{number of molecules undergoing the process}}{\text{number of quanta absorbed}}$$

For a pure photochemical reaction the quantum yield has a value in the range 0-1. However, if $A^{\cdot}$ is a radical or pseudoradical that can take part in an oxidative free-radical chain reaction, so that the absorption of energy simply initiates the reaction, then each quantum of energy may result in many molecules decomposing, in which case Φ may appear to be greater than 1.

The energy per quantum of electromagnetic radiation is given by Eq. (8) :

$$E = h\nu \qquad \text{_____ (eq.8)}$$

$$\nu = c/\lambda \qquad \text{_____ (eq.9)}$$

where E = energy absorbed,

h = Plank's constant (6.625×10^{-27} erg-s),

c = velocity of light,

λ = wavelength

ν = radiation frequency

Thus the shorter the wavelength (λ) of light, the more energy is absorbed. Consequently, the radiations absorbed from the ultraviolet and violet portions of the light spectrum are more active in initiationg chemical reactions than those absorbed from the other longer wavelength portions of the spectrum (Connors et al., 1986).

Spectral characteristics of sunlight and artifical light sources.

Ultraviolet radiation (UV-R) has been divided into three subbands : UV-C, UV-B and UV-A. The UV-C band ranges from 200 nm to 290 nm and is often called shortwave or far-UV because the wavelengths in this region are the shortest UV-R transmitted through air. Although most drugs and all cellular constituents absorb UV-C, sunlight at the earth's surface contains no UV-C because of efficient absorption by molecular oxygen and ozone in the upper atmosphere.

The UV-B spectral region is often defined as encompassing wavelengths from 280 to 320 nm. However, no solar radiation penetrates to the ground at wavelengths between 280 and 290 nm.

UV-A is the long wavelength UV region from 300-400 nm. It is also called near-UV because of its proximity to the visible spectrum.

Sunlight has a very high output in the visible (400-800 nm) and infrared (800-3200 nm) regions.

Artificial light sources can have varying spectral characteristics depending on the particular construction. The glass bulb or tube in an artificial light source can be said to act as the ozone layer does with respect to natural sunlight, limiting the UV-B component to about 300 nm, depending on the glass used (Tonnesen, 1996). There have been various kinds of artificial light sources used in photo-oxidation tests as follows.

Arc lamps

Mercury arc lamps can be constructed in three ways, with the mercury vapour at low, medium or high pressure, each variant having specific characteristics in emphasizing certain aspects of the mercury emission spectrum.

The arc lamp having the best resemblance to sunlight is the xenon arc lamp, although the development of new metal-halide lamps had led to competition for the claim. The xenon arc has a relatively smooth continuous output spectrum with some line emissions superimposed in the region 450-500 nm, whereas the metal-halide lamp is more uniform across the 350 to 550 nm region. One

disadvantage of all the arc lamps is the high heat output, as seen by the continued output above 500 nm, but this can be dissipated by the use of a heat filter, usually containing water. Since photochemical reactions are generally initiated by UV radiation, adjustment of the intensity above 500 nm is most unlikely to lead to erroneous photostability data. On the other hand, overheating of the sample by the lamp may lead to thermal decomposition processes to complicate the issue.

The other principal disadvantage of both xenon and metal-halide sources is their comparatively short life span of 750 hr for the metal-halide and 1500 to 2000 hr for the xenon arc. Their initial cost is high and the focus of their irradiation is such that only a relatively small number of samples can be irradiated at one time (Moore, 1996).

Fluorescent lamps

The operating principle of fluorescent lamps is based on mercury vapour discharge at very low pressure, producing the 254 nm emission which is converted to higher wavelengths by the phosphor coating on the inside surface of the tube. The daylight, cool-white, and near-UV fluorescent tubes are available for drug photostability studies ; all of which have the advantage that they can be set up in large banks at relatively low cost to irradiate large number of samples at one time. It is not possible to achieve a sunlight-simulating spectrum with just one type of fluorescent lamp ; a combination must be used to get the appropriate amounts of UV-A, UV-B and visible components. Other combinations which have been suggested involve the use of a black-light UV-A source with daylight fluorescent lamp (Moore, 1996).

Currently, the luminance exposure of artificial visible daylight can be measured in term of lux.hr while the radiant exposure of the UV part of radiation can be measured in term of $W.hr/m^2$ (Moore, 1996).

Accelerated photo-oxidation tests

In principle, photodegradation studies could be performed by exposing samples to natural sunlight and analysing after varing times. However, the intensity of sunlight, particularly the UV component, varies according to the weather, the latitude, the time of day and the season of the year. Nonetheless, it would be a realistic situation to set samples in a window where direct sunlight could fall on them to varying extents in the course of the day. It the study was continued for a period of at least a year, the conditions would average over all seasons, but would only apply to a particular region. Thus, the quantitative data from experiments on the same formulation performed in different laboratories are unlikely to be in agreement in most cases. The use of natural sunlight is not a viable option given the variability of conditions involved (Moore, 1996). Thus, the photostability of a pharmaceutical product is best checked by using a cabinet of artificial light source which has an output with a spectral power distribution as near as possible to that of sunlight and cabinet is well ventilated so that the temperature of the samples dose not rise significantly (Carstensen, 1984).

Inhibitions of oxidation

The stability of pharmaceutical compounds undergoing oxidative degradation can be increased by several approaches.

1. Prevention of oxygen

Since, in many cases, oxidative degradation of drug take place in aqueous solution, it is helpful to keep the oxygen content of these solutions at a minimum. If the water is cooled in an atmosphere essentially free from atmospheric oxygen, there is no increase in oxygen content. To obtain water that contains a minimum amount of free oxygen, water after it is first boiled is purged with carbon dioxide or nitrogen gas. The oxygen content of water treated with carbon dioxide is reduced to 0.45 ml per liter at 20°C (Lachman, Deluca and Akers, 1986). For parenteral drugs this can be achieved by packaging the drug in glass ampuls that are heat sealed under an inert atmosphere. For tablets, capsules, and so on, packaging of the formulation in a hermetic strip may be useful in preventing the oxidation (Connors et al., 1986).

2. Protection form light

Oxidative breakdown of drugs generally proceeds through the sequence of initiation, propagation, and termination. As mentioned earlier, a triggering force that may promote oxidation is light. Light does initiate or promote an oxidative breakdown of a drug, the exclusion of the particular wavelength range of light responsible for the catalysis will often suppress the oxidation. This can be achieved by the total exclusion of light using an opaque container or the use of pigmented glass capable of excluding the damaging wavelengths (Connors et al., 1986).

3. Alteration of solution pH

As has already been discussed, the oxidation of many drugs is pH sensitive. Acidic drugs such as ascorbic acid, phenols, and sulhydryl compounds all degrade more rapidly in neutral to alkaline pH conditions. For such drugs the pH range 3 to 4 is generally found most useful in minimizing oxidation. Obviously, this pH range would not be useful for acidic drugs that have limited aqueous solubility at low pH values. Amine drugs such as the phenothiazines appear to be most stable in their protonated forms, that is, also at low pH values (Connors et al., 1986).

4. Addition of antioxidants

Antioxidants are added to pharmaceutical formulations as redox systems possessing higher oxidative potential than the drug that they are designed to protect, or as chain inhibitors of radical induced decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Lachman, Deluca and Akers., 1986).

An ideal antioxidant should satisfy the following requirements :

1. It should be active at extremely low concentrations (0.01-0.001%).
2. The compound and its oxidation products must be nontoxic.
3. It should be easily incorporated into the substrate.
4. It should impart no foreign flavor, odor, or color to the food, even after prolonged storage or heating.

5. It should be easily available and low cost.
6. It should be easily detected, identified and measured.

Antioxidants are substances that in small quantities, are able to prevent or greatly retard the oxidation of drugs. They are divided broadly into primary antioxidants, reducing agents and chelating agents (Chipault, 1962).

Primary antioxidants

Primary antioxidants or true antioxidants are substances that interfere with autooxidation by interrupting the chain propagation mechanism. Thus, they are sometimes called chain terminators, that is, agents capable of reacting with radicals in solutions to produce a new species, a chain terminator radical, which does not reenter the radical propagation cycle. The new radical may be intrinsically stable or may dimerize to form an inert molecule (Connors et al., 1986).

$$AH + R\cdot \longrightarrow RH + A^{\cdot} \quad ; \text{ Antioxidant (AH)}$$

$$AH + ROO^{\cdot} \longrightarrow ROOH + A^{\cdot} \quad ; \text{ Free radical } (R^{\cdot}, ROO^{\cdot})$$

$$A^{\cdot} + A^{\cdot} \longrightarrow AA$$

$$A^{\cdot} + R^{\cdot} \longrightarrow AR$$

Gallic acid and the gallates.

Gallic acid is soluble in water but nearly insoluble in fats. Esterification of the carboxyl group with fatty alcohols gives esters which become progressively less soluble in water and more soluble in fats as the molecular weight of the alcohol increases. The lower gallates such as ethyl, propyl, and butyl gallate

remain slightly soluble in both water and fats, but the higher octyl, decyl, and dodecyl esters are practically insoluble in water but easily dissolve in fats and oils. Two primary antioxidants are only useful to protect drugs degrading by an auto-oxidation. Besides, they are only effective at extremely low concentrations and the effectiveness of several primary antioxidants decreases as their concentrations are increased. At highter levels, they may again accelerate the rate of auto-oxidation. In general, the most effective primary antioxidants are highly reactive and are readily destroyed by heat (Chipault, 1962).

Propyl gallate

Propyl gallate is an odorless white crystalline powder with a bitter astringent taste. It can be soluble in water (1 in 286). It can decompose at high temperature. Its LD_{50} (cat, oral) and LD_{50} (rat, oral) are 0.4 g/kg and 3.8 g/kg. It is primarily used, in concentrations of 0.002% w/v to prevent peroxide formation in ether and at 0.01% w/v to prevent the oxidation of paraldehyde (Wade and Welly, 1994).

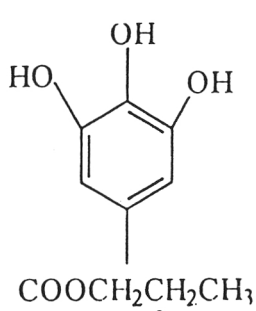


Figure 1 Chemical structure of propyl gallate

The effect of propyl gallate in fatty preparations stability was investigated in 1993. The results showed that propyl gallate could protect the oxidation of the

fatty product (Irache et al., 1993). Similarly, propyl gallate could inhibit the oxidative reaction of lovastatin in solution phase (Kaufman, 1990).

Chelating agents

Chelating agents are compounds that act as antioxidants by binding to metal ions. The most effective chelating agents used pharmaceutically are ethylenediaminetetracetic acid (EDTA), citric acid, many of the amino acids, phosphoric acid and tartaric acid. EDTA and citric acid are the two most useful agents. Their metal-binding capacities are dependent on their state of ionization, both being most effective when their carboxylic acid groups are fully ionized. Thus, they lose their chelating capacity at low pH. Just because an agent is able to chelate metal ions dose not mean that it will reduce the effectiveness of the metal ion to act as a catalyst. There are circumstances in which the metal ion may bind to some functional groups and in this bound capacity actually may be a better catalyst than in the unbond state. The chelating agents mentioned above, however, generally act to lower the catalytic activity of the metal ions towards radical chain reactions (Connors et al., 1986).

Disodium edetate (Disodium ethylenediaminetetracetate)

Disodium edetate is an odorless white crystalline powder with a slightly acid taste. It is soluble in water (1 in 11). It can decompose at high temperature. Its LD_{50} (mouse, oral) is 0.03 g/kg. In pharmaceutical formulations disodium edetate is used as a chelating agent typically at concentrations 0.005-0.1% w/v (Wade and Welly, 1994).

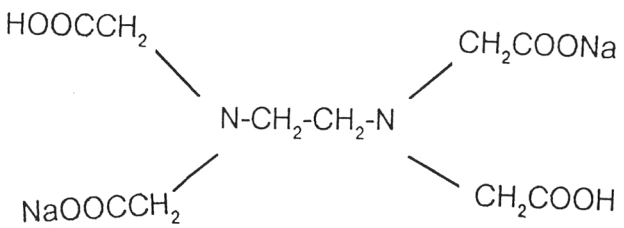


Figure 2 Chemical structure of disodium edetate

The oxidative degradation of many drugs can be reduced by addition of disodium edetate. Amitriptyline hydrochloride in aqueous solution is an example. (Enever, Li wan po and Shotton, 1977). In additions, the decreasing of oxidation rate of captopril in solution stored at $50^{o}C$ when 0.1% w/v disodium edetate was added was reported (Timmin S, Jackson and Wang, 1982).

Reducing agents

Reducing agents are compounds that are more readily oxidized than the agents they are to protect. Their mechanisms can be either revesible loss of electrons or auto-oxidation. The effectiveness of the reducing agent which acts as the reversible loss of electrons always depends on the magnitude of E^{o}_{cell} and its concentration (Connors et al, 1986). Example of these antioxidants are sulphurous acid salts.

Sulphurous acid salts

Example of Sulphurous acid salts are sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$) and sodium metabisulfite (NaS_2O_5). These compounds have low oxidation potentials, they can be preferentially oxidized. They react easier with

oxygen (Chipault, 1962). Sodium sulfite is a good pharmaceutical example of oxygen scavenger. It reacts with oxygen according to Equation (10).

$$SO_3^{2-} + \tfrac{1}{2} O_2 \longrightarrow SO_4^{2-} \quad \text{(eq.10)}$$

The sulfites are very commonly used, and a word of caution is in order. In the process of acting as an antioxidants, sulfites yield acid sulfates, which cause a drop in pH. They react with compounds such as alkenes, alkyl halides, and aromatic nitro and carbonyl compounds (Connor et al., 1986).

Sodium metabisulfite

Sodium metabisulfite occurs as colorless, prismatic crystals or as a white to creamy-white crystalline powder which has the odor of sulfur dioxide and an acid. It can be soluble in water (1 in 1.9). In water, sodium metabisulfite is immediately converted to sodium and bisulfite (HSO_3^-) ions. Aqueous sodium metabisulfite solutions also decompose in air, especially on heating, and solutions which are to be sterilized by autoclaving should therefore be filled into containers in which the air has been replaced with an inert gas, such as nitrogen. The addition of dextrose to aqueous sodium metabisulfite solutions results in a decrease in the stability of the metabisulfite. It should be stored in well-closed container, protected from light. Sodium metabisulfite is used as an antioxidant at concentrations of 0.001-1.0 % w/v. The acceptable daily intake of sodium metabisulfite, and other sulfites, has been set at up to 3.5 mg/kg body-weight (Wade and Welly, 1994).

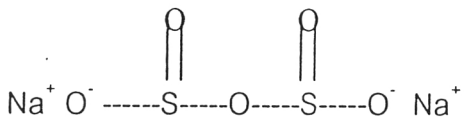


Figure 3 chemical structure of sodium metabisulfite

Sodium sulfite (Na_2SO_3)

Sodium sulfite is an odorless white crystalline powder. It can be soluble in water (1 in 3.2) (Wade and Welly, 1994).

Sodium bisulfite ($NaHSO_3$)

Sodium bisulfite is a white crystalline powder. It can be soluble in water (1 in 3.5) (Wade and Welly,1994).

Many researcher have studied the effect of sodium bisulfite when it is used as a reducing agent. Stability of 5-azacytidine was increased approximately 10-fold over its stability in water or lactated ringer injection by the addition of excess sodium bisulfite and the maintenance of pH at 2.5 (Chatterji and Gallelli, 1979). Similarly, the results showed that sodium bisulfite could inhibit the oxidation of morphine solution (Yeh and Lach, 1971). In additions, sodium bisulfite prevented more than 10% oxidation of apomorphine hydrochloride in water maintained at room temperature (Wilcox et al., 1980).

The effectiveness of bisulfite as an antioxidant in typical pharmaceutical systems depends on the ease of compound which is oxidized in comparison with the drug it is to protect. Substances that inhibit bisulfite oxidation may exert important effects on the overall stability of the product by decreasing the antioxidant effect of bisulfite. It has been postulated that the mechanism by which these substances inhibit sulfite activity is through the formation of coordination compounds between inhibitor and bisulfite. Typical substances that can inhibit the oxidation of bisulfite are mannitol, phenols, inorganic anions, aldehydes, ketones, and alkaloids (Lachman, Deluca and Akers, 1986).

Stability study

Chemical or physical stability might be the limiting factor of the shelf-life (Bibart, 1979). Any slight changes in physical appearance such as color fading, odor, of cloudy can cause the patient or consumer lose confidence in the product. Unfortunately most physical measurable method is less accurate and precise.

The stability study may be determined by classical method or by accelerated thermodegradation method but the classical method, by aging in actual normal storage conditions, has some disadvantages of time consuming, unsuitable for formulation development, economic loss and eventually loss competitive marketing products. The principles of chemical kinetic to evaluate drug stability at higher temperature and extrapolate to actual normal storage or any lower temperature degradation by using Arrhenius relation have been used (Garrett, 1962). This method had been proved to be reliable and entirely appropriate for assessment and control of drug stability in formulation and dosage forms (Garrett, 1962 : Kennon, 1964).

1. Rate of reaction

The rate of reaction can be expressed either as the decrease in concentration per unit time of any of the reacting substances, or as the increase in concentration per unit time of one of the degraclation products. The rate of reaction may be written as –d[D]/dt or d[P]/dt where [D] is drug concentration, [P] is product concentration.

1.1 Order of reaction

Order of a chemical reaction determines the shape of the concentration-time profile of drug or drug product, whereas the rate constant determines its slope. The order of reaction with respect to a single reactant is equal to the power to which the concentration term of the reactant is raised in the experimental rate equation. For example, a chemical reaction can be expressed as :

$$uA + vB + wC \longrightarrow \text{Products} \quad \text{(eq.11)}$$

and its reaction rate is:

$$\text{Rate} = k[A]^u[B]^v[C]^w \quad \text{(eq.12)}$$

Where A, B and C are the reactant and k is a rate constant, u, v and w will be the order of reaction. If u, v and w have values of 2, 1 and 0, respectively, it is said to be second order with respect to A, first order in B and zero order in C. In this instance, the experimentally determined rate is only dependent upon the concentrations of reactants A and B. The overall order of reaction is the sum of

the powers of the concentration terms affecting the experimentally determined rate. In the above example, the reaction would be third-order overall.

1.1.1 First-order reaction

A typical first-order reaction may be written as :

$$D \xrightarrow{k_1} \text{Product} \quad \text{(eq.13)}$$

and its corresponding rate equation as

$$-d[D]/dt = k_1 [D] \quad \text{(eq.14)}$$

This expression defines the rate of the reaction, whereas we actually need to know the concentration-time profile. This is obtained by integrating the rate from $t = 0$ to $t = t$, where $[D]$ at $t = 0$ is $[D]_o$

$$\int_{[D]_o}^{[D]} \frac{d[D]}{[D]} = -\int_o^t k_1 \, dt \quad \text{(eq.15)}$$

$$\ln [D] = \ln [D]_o - k_1 t \quad \text{(eq.16)}$$

Alternative forms of this equation are

$$[D] = [D]_o e^{-kt} \quad \text{(eq.17)}$$

and $$\log [D] = \log [D]_o - (k_1 t/2.303) \quad \text{(eq.18)}$$

A plot of log [drug concentration] against time will be linear with the slope equal to $-k_1/2.303$, yielding the rate constant. The dimension of k_1 is 1/time, for example, s^{-1}. The half-life, $t_{½}$ is the time for [D] to become $[D]_o/2$, that is, one-half the original concentration. An equation for $t_{½}$ is found by substituting $[D] = [D_o]/2$ into equation 18

$$t_{½} = 0.693/k_1 \quad \text{(eq.19)}$$

The shelf-life, t_{90} of a drug is usually taken to be the time for [D] to reach $0.90\ [D]_o$, that is, 10% decomposition, so in a similar way we find

$$t_{90} = 0.105/k_1 \quad \text{(eq.20)}$$

1.1.2 Second-order reaction

A Second-order reaction is one having a rate equation of the form,

$$2D \longrightarrow \text{products}$$

$$\text{Rate} = k[D]^2 = -\frac{1}{2}\frac{d[D]}{dt} \quad \text{(eq.21)}$$

$$\int_{[D]_o}^{[D]} -\frac{d[D]}{[D]^2} = k\int_0^t dt \quad \text{(eq.22)}$$

$$\frac{1}{[D]} - \frac{1}{[D]_o} = kt \quad \text{(eq.23)}$$

$$\frac{1}{[D]} = \frac{1}{[D]_o} + kt \quad \text{(eq.24)}$$

A plot of $\frac{1}{[D]}$ against time will be linear with the slope equal to k, yielding the rate constant. The dimension of k is $\frac{1}{\text{conc.time}}$, for example, $(\text{conc. time})^{-1}$.

When [D] is expressed in molar units. The half-life and shelf-life are, respectively :

$$t_{1/2} = \frac{1}{k\,[D]_o} \qquad \text{(eq.25)}$$

$$t_{90} = \frac{1}{9[D]_o\,k} \qquad \text{(eq.26)}$$

1.1.3 Zero-order reaction

A zero-order reaction is one having a rate equation of the form,

$$-d[D]/dt = k_o \qquad \text{(eq.27)}$$

The zero-order reaction has no concentration dependence. Integrating from t = o to t = t with $[D] = [D]_o$ at t = o, and [D] = [D], at any time .

$$[D] = [D]_o - k_o t \qquad \text{(eq.28)}$$

Hence for a zero-order reaction, a plot of concentration against time is linear, with slope of $-k_o$. The unit of k_o are concentration/time, for example, M/s, when [D] is expressed in molar units. The half-life and shelf-life are, respectively :

$$t_{1/2} = 0.5[D]_o/k_o \qquad \text{(eq.29)}$$

$$t_{90} = 0.1[D]_o/k_o \qquad \text{(eq.30)}$$

Temperature effects

Activation energy calculations

It was noted that reaction rates are expected to be proportional to the number of collisions per unit time. Since the number of collisions increases as the temperature increase, we would expect the reaction rate to increase with increasing temperature. Experimentally, the reaction rate constant is observed to have an exponential dependence on temperature :

$$k = Ae^{(-Ea/RT)} \quad \text{(eq.31)}$$

where k is the reaction rate constant of any order, A and Ea are constants, R is the gas constant (1.987 calories degree^{-1} mol^{-1}) and T is the absolute temperature. Ea is called the activation energy of the chemical reaction or heat of activation. This equation is the Arrhenius equation and can be written in several equivalent forms as follows :

$$\ln k = -(Ea/R)\cdot 1/T + \ln A \quad \text{(eq.32)}$$

$$\log k = \log A - Ea/2.303\ RT \quad \text{(eq.33)}$$

$$\log k_2/k_1 = Ea/2.303R\ [1/T_2 - 1/T_1] \quad \text{(eq.34)}$$

$$\log k_2/k_1 = Ea(T_2 - T_1)/2.303\ RT_1T_2 \quad \text{(eq.35)}$$

where k_2 and k_1 are the rate constants at temperatures T_2 and T_1, respectively. The interpretation of Ea is as follows : as the reaction proceeds from reactants to products, the system must pass through a state whose energy is greater than that of the initial reactants. This "barrier" is what prevents the

reactants from immediately becoming products, the activation energy is a measurement of this barrier.

Equation 33 indicates that a graph of log k against 1/T will be linear with a slope of –Ea/2.303 R. This type of graph is called an Arrhenius plot. From this plot Ea can be determined.

Since the Arrhenius plot is linear, it is possible to predict the rate constant (k) at room temperature or at any lower temperature by extrapolation. Once the k value is obtained, it can be used to estimate the shelf-life. When the heat of activation is known, it is possible to predict the specific rate constant at lower temperature from the rate obtained at one elevated temperature study by using the integrated equation of Arrhenius relation (Equation 35).