CHAPTER II

REVIEW OF LITERATURE(S)

Oxidative Reactions

1. Nature of Oxidation

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:

$$\text{reduced form} \longleftrightarrow \text{oxidized form} + ne^- \qquad (1)$$

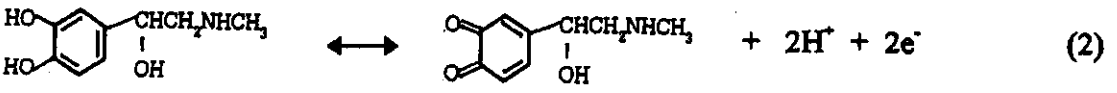
where e^- represents an electron and n is the number of electrons being transferred (Connors et al., 1986).

2. Mechanisms of Oxidation

Two basic mechanisms of oxidative reactions exist as follows (Stewart and Tucker, 1985).

2.1 Classical Oxidation

The classical oxidation occurs by the reversible loss of electrons without an addition of oxygen. For example, adrenaline degrades as follows:



(2)

adrenaline (A) adrenaline-o-quinone (AQ)

The equation shows the oxidation of adrenaline with the resultant production of electrons and takes the form of a half- cell equation. The presence of an oxidation half-cell implies the existence of a corresponding reduction half-cell of the form:

$$\text{oxidized form} + ne^- \longleftrightarrow \text{reduced form} \qquad (3)$$

This specific reduction half-cell reaction depends on the materials in the formulation but can be associated with the reduction of oxygen which is present in the solution as described:

$$1/2O_2 + H_2O + 2e^- \longleftrightarrow 2OH^- \qquad (4)$$

Thus, the total cell reaction for adrenaline degradation can therefore be constructed:

$$A + 1/2O_2 + H_2O \longleftrightarrow AQ + 2H^+ + 2OH^- \qquad (5)$$

An electro-chemical potential can be assigned to this cell reaction using the Nernst equation:

$$E = E^\circ - (RT/nF) \ln (a_{ox} / a_{red}) \qquad (6)$$

where E is the oxidation potential for the half-cell, E° is the standard oxidation potential, R is the gas content, T is the absolute temperature, F is the Faraday constant, n is the number of electrons involved in the reaction, and a_{ox} / a_{red} is the activity of the oxidized and reduced forms, respectively.

The standard oxidation potential of the cell is

$$E^\circ_{cell} = E^\circ_{ox} - E^\circ_{red} \qquad (7)$$

where E°_{ox} and E°_{red} are the standard oxidation potentials of oxidation and reduction half-cells. The greater the standard oxidation potential of the cell, the greater the difference between the oxidation and reduction half-cell potential, the more readily will oxidation occur. Thus, if the standard oxidation potential is known, it is therefor possible to predict the relative susceptibility of a drug to the oxidation of this type.

2.2 Auto-oxidation

Auto-oxidation is the oxidation that takes place spontaneously under mild conditions and the majority of the reactions involve reactions with molecular oxygen, chain reaction and formation of free radicals; organic peroxides are often the intermediates or the final products. The free radicals are chemical species that possess an unpaired valence electron. The auto-oxidation occurs in three phases: initiation propagation and termination (Connors et al., 1986).

Initiation Phase

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 period; the length of the induction period depends on the reaction and the conditions in the solution.

Propagation Phase

The propagation phase, often called the acceleration phase or logarithmic period, involves the consuming of oxygen by free radicals formed in the induction period. Oxygen that is in its ground state is a diradical (Figure 1) and would like to fill its outer electron shell to produce O_2^{2-}, the peroxy dianion. To do so, 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. The rate of oxidation increases rapidly and the highest rate occurs at about 50 % of drug remaining.

Figure 1 The ground state of oxygen molecule (Connors et al., 1986).

Termination Phase

The termination phase, which is dominant during the final step of oxidation, may take place by coupling of one radical with a free radical inhibitor or with another radical in the system to form a non-radical. This causes a decrease in the oxidation rate.

The kinetic behavior in the three phases are described as follows:

Initiation : $RH \rightarrow R^{\bullet} + H^{\bullet}$ (8)

Propagation : $R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$ (9)

$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$ (10)

Termination : $ROO^{\bullet} + X \rightarrow$ non-reactive products (11)

$R^{\bullet} + R^{\bullet} \rightarrow R\text{-}R$ (12)

3. Factors Influencing Oxidation

3.1 Degree of Saturation

Free radical formation has been shown to occur on the methylene group which is in the α position to a double bond.

$$-CH_2-CH=CH-CH_2- \longrightarrow -\dot{C}H-CH=CH-CH_2- \quad (13)$$

α position

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

$-CH_2-CH=CH-CH_2-CH=CH-CH_2-$

↑ α position to both double bond

For example, two kinds of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, simvastatin and L-647,318, of which structures are known to be highly effective cholesterol-lowering agents are depicted in Figure 2.

Simvastatin L-647,318

Figure 2 Chemical structures of simvastatin and compound L-647,318 (Kaufman, 1990).

Simvastatin and L-647,318 are identical in structure except for the diene system in simvastatin. Kaufman (1990) investigated the compounds in 2% sodium dodecyl sulfate (SDS). He found that the oxidation rate constant of simvastatin was higher than that of compound L-647,318. His explanation was that the unsaturated double bonds in simvastatin structure made it more sensitive to produce the free radicals than L-647,318.

3.2 Presence of Heavy Metals

Heavy metals particularly those possessing two or more valency state with a suitable oxidation-reduction potential between them such as copper and iron which requires one more electron to complete the electron pair can act as radical initiators and then cause the catalytic effect of oxidative reactions in a number of ways.

They can react directly with oxygen molecules to produce oxygen radicals which subsequently form peroxy radicals and sometimes they can react with the drug itself to form drug radicals. These radicals are able to enter into a propagation cycle as follows:

$$M^{n+} + RH \rightarrow M^{(n-1)} + H^{+} + R^{\bullet} \quad (14)$$

In the other way, they can also react with a hydroperoxide of a drug or of some other components in the formulation to induce forward reactions:

$$M^{n+} + R'OOH \rightarrow M^{(n-1)} + H^{+} + R'O_2^{\bullet} \quad (15)$$

Because of its initiator property and ability to increase the formation rate of many free radicals, metal ion can reduce the length of the induction period and increase the maximum rate of drug oxidation (Connors et al., 1986; Kumar, Sunder and Potdar, 1992).

The efficiency of various metal ions on the increment of oxidation extent of some aliphatic hydroxylamines in aqueous solutions was evaluated in 1976. A large variation in the effectiveness of different metal ions as catalysts was observed. There were rapid increases in the extent of oxidation when only a small amount of copper ions was added. Manganese, lead, nickel and ferric ions were less effective than copper ions in the order as listed, respectively; magnesium, zinc, silver and ferrous ions produced only minor increases in the levels of oxidation (Beckett, Purkaystha and Morgan, 1976). Like the aliphatic hydroxylamine compounds, the study of promethazine indicated that its oxidation could also be affected by copper and iron ions (Underberg, 1978). Proteins and peptides are known to be susceptible towards transition metal catalyzed oxidation. One of their biotechnological therapeutic products, methionine, degraded faster in a buffer system containing 0.02 mM $FeCl_3$. However, the oxidation of methionine still occured in the absence of $FeCl_3$. This indicated that the buffer and the peptide used had a potential of contaminants of trace amounts of iron or other transition metals in a sufficient amount to drive the oxidation process (Li et al., 1993).

3.3 Presence of Oxygen

Oxygen is an abundant element. It makes up about 46 % of the earth' s crust and of course, 89 % of the water and over 20 % of the air. The auto-oxidation process 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. Thus, it is no wonder that oxidative reactions, both completed and potential, are omnipresent (Connors et al., 1986).

A lot of significant influence of oxygen on the oxidative degradation of certain drugs has been discussed. For example, the oxidation rate of flupenthixol dihydrochloride solutions stored under oxygen is greater than those stored under air (Enever, Po and Shotton, 1979). Amphotericin B, the single most important antibiotic for the treatment of systemic mycoses has the oxidative rate constant under aerobic condition two times greater than that under anaerobic condition (Dicken et al., 1988). Similarly, 5-aminosalicylic acid an anti-inflammatory drug primarily employed in the treatment of inflammatory bowel disease, is more rapidly decomposed in the oxygenated solution compared with the deoxygenated solution (Palsmeier, Radzik and Lunte, 1992). In additions, the oxidation of human insulin-like growth factor I (hIGF-I) in solution with two different amounts of dissolved oxygen at 25 °C was reported. The rate of oxidation is dependent on the amount of oxygen available in the solution (Fransson et al., 1996).

3.4 Effect of the Physical State of the Oxidizable Material

Solid materials are less permeable to air than liquids; so, the oxidative reaction is confined to the surface layers of these particles. Therefore, a change in particle size with a subsequent change in surface area will influence the degree of auto-oxidation. Excessive particle size reduction of solids in a preparation of suspensions can increase the extent of decomposition (Stewart and Tucker, 1985).

3.5 Effect of Humidity

Because oxygen and metal ions are soluble in water, humidity is an important oxidation factor of a dry product. Water in the air can be absorbed and present as a bulk phase surrounding the drug particles in the solid dosage forms (Figure 3). The drug dissolves and a saturated solution is formed. Then the decomposition can occur by the acceleration of oxygen or trace metal ions in the water (Carstensen, 1984).

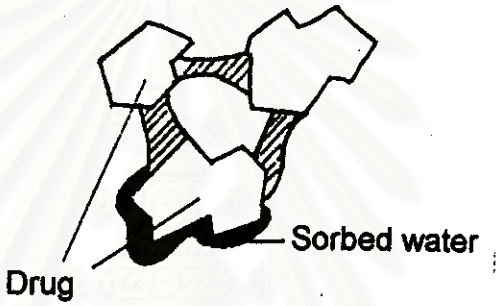


Figure 3 Bound moisture of starch granulated meterial (Carstensen, 1984).

The humidity or moisture effect on the oxidation of drugs in the solid state has been reported. The losses of ascorbic acid content increase progressively with increasing moisture content in the tablets (Ritter et al., 1970). For dialuric acid in the solid state, the rate of oxidation increases when the relative humidity is increased. (Clay, Knevel and Byrn, 1982).

3.6 Effect of pH

As it was described previously, the oxidative reaction involves the reversible loss of electron; this is equivalent to the loss of a molecule of hydrogen that occurs in a multistep process. Increasing of hydrogen ion in the solution causes the solution 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 degrade more rapidly since the hydrogen ion concentration is low. 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).

The effect of high pH on the oxidative degradation of drugs has been reported. The formation rate of degraded products of flupenthixol dihydrochloride increases with increasing pH (Enever et al., 1979). Procaterol, a sympathomimetic amine with potent bronchodilatory activity, has also shown capricious patterns of oxidative decomposition in pharmaceutical preparations. The pH rate profiles of procaterol is likewise indicated that the rate of auto-oxidation of procaterol is inversely proportional to the concentration of hydrogen ion in the medium (Chen and Chafetz, 1987).

A decomposition pathway of 5-aminosalicylic acid (5-ASA) is the oxidative one, due to its p-aminophenol structure. The decomposition of 5-ASA in solution is found to be highly pH dependent. At low pH values, 5-ASA is stable. As the solution pH is increased, the rate of decomposition of 5-ASA increases (Palsmeier et al., 1992).

3.7 Effect of Temperature

The relationship between chemical reactivity and temperature is described by the Arrhenius Equation. In auto-oxidation, an increase in temperature not only affects the primary processes of oxidation but also accelerates the decomposition of the hydroperoxide to aldehydes, ketones and fatty acids (Stewart and Tucker, 1985). But, the interpretation of temperature effects on oxidative reactions is difficult because oxygen solubility in water and other solvents is temperature dependent. In a high temperature condition, the oxygen solubility is low. Thus, an increase in temperature may have a slight effect on the rate of oxidation because of the decreasing of oxygen concentration in the solution. Theoretically, under this circumstances, the reaction rate-temperature relationship according to the Arrhenius Equation will be breakdown. Table 1 shows the oxygen content in water at various temperatures after water has been saturated by air or by pure oxygen (Connors et al., 1986). However,

the decrease in solubility of oxygen with increasing temperature is partially compensated for by the increase in solubility by increasing oxygen pressure above the solution as temperature is raised (Chen and Chafetz, 1987).

Table 1 Oxygen content in water under air and pure oxygen at atmospheric pressure and various temperatures (Connors et al., 1986).

Temperature (°C)	Oxygen content (mM / ml)	
	From air	From pure 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}

In 1977, There was a report of the influence of temperature on the degradation rate of phenothiazine which produced many degraded products; the higher rate of degraded products occurred at the higher temperatures (Roseboom and Perrin, 1977). A report shows that the higher temperature leads to the higher rate of procaterol oxidation (Chen and Chafetz, 1987). Similarly, ethyl icosapentate (EPA) prepared from fish oil and used as an antithrombotic agent for arteriosclerosis was investigated for the auto-oxidation rate of the drug at various temperatures in 1992. The percentage of remaining EPA determined by HPLC analysis decreased exponentially over time after it had been stored for 20 hr at 25, 35 and 45 °C, respectively. The degradation of EPA was accelerated with increasing temperature (Teraoka, Otsuka and Matsuda, 1992).

3.8 Effect of Light

Light or radiation, namely, certain component of the electromagnetic spectrum has a triggering force that may initiate or promote an oxidative breakdown of a drug molecule. Pharmaceuticals may be exposed to the electromagnetic radiation from a number of sources, ranging from direct sunlight through filtered sunlight to a variety of artificial light conditions. Electromagnetic spectrum has been divided into ultraviolet (UV) region (50-400 nm), visible ragion (400-750 nm) and infrared (IR) region (750-10,000 nm) (Connors et al., 1986). Especially, UV region (UV-R) can be divided into three subbands: UV-C (200-290 nm), UV-B (280-320 nm), and UV-A (320-400 nm) (Moore, 1996).

Some drug substances and formulation exipients are colored meaning that they absorb light in the visible region. The color they display is complementary to the light they absorb, e.g., a red powder is absorbing blue light. A great majority of therapeutic substances are white in appearance meaning that they do not absorb light in the visible region, but they absorb light in the UV region as a consequence of their chemical structure. The presence of aromatic residues and conjugated double bonds containing N, S, or O is usually associated with the absorption (Moore, 1996).

3.8.1 Light Absorption Mechanism of Drugs

There are two kinds of degradation mechanism of a drug following light absorption. Firstly, it occurs when the drug molecule itself absorbs energy from the radiation source. Whether or not the drug will absorb energy is evident from its ultraviolet or visible spectrum. Secondly, it occurs when the energy is absorbed by another component of the formulation which imparts their increased energy to the drug molecule with subsequent degradation. The molecules absorbing the radiant energy are called photosensitizes and act as catalysts for the drug photo-oxidative decomposition (Stewart and Tucker, 1985).

In 1969 the stability study of the tetracycline group antibiotic: chlortetracycline, tetracycline and oxytetracycline in solutions containing vitamin B complex, demonstrated that the photosensitizing property of riboflavin was the important factor affecting their significant potency lost by photo-oxidation in only two to four hours (Leeson and Weidenheimer, 1969).

3.8.2 Photophysical and Photochemical Processes

The radiation energies absorbed by drug molecules produce an unstable excited state species and can be lost either by a radiative mechanism in which the energy is given off in the form of fluorescence or phosphorescence or by a radiationless mechanism. The radiationless mechanism can be physical or chemical in nature. The physical decay results in the loss of energy as heat or by collision with other molecules. The net effect of the chemical decay is that sufficient energy is concentrated in some bonds that cause the molecule to decompose chemically into a new species. This whole process can be described as follows (Connors et al., 1986):

$$A \longrightarrow A^* \quad (16)$$

$$A^* \longrightarrow A + h\nu \quad (17)$$

$$A^* \longrightarrow A + \text{heat} \quad (18)$$

$$A^* \longrightarrow \text{product(s)} \quad (19)$$

$$A^* + A \longrightarrow 2A \quad (20)$$

3.8.3 Quantum Yield of a Photochemical Reaction

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, a quantum yield, ϕ, for any one of these processes is defined by

$$\phi = \frac{\text{number of molecules reacted / volume / time}}{\text{number of photons absorbed / volume / time}} \quad (21)$$

For a pure photochemical reaction, the quantum yield has a value in the range 0-1. However, in the case of oxidation, the absorption of energy simply initiates the reaction, ϕ may appear to be greater than 1 (Connors et al., 1986; Moore, 1996).

3.8.4 Planck's Equation

The energy per quantum of electromagnetic radiation which relates to wavelength is given by Planck's equation:

$$E = h\nu \quad (22)$$

where E is the energy expressed in the unit of Joules m^{-2} sec^{-1} or watts m^{-2}, h is the Planck's constant (6.2625×10^{-27} erg.s), and ν is the frequency of the radiation in Hz or sec^{-1}. According to Planck's theory, the shorter the wavelength (λ) or the higher the frequency (ν), the greater is the energy per photon of light absorbed since $\nu = c/\lambda$, where c is the velocity of light (Connors et al., 1986; Moore, 1996).

3.8.5 Spectral Energy Distribution of Solar Radiation

An approximate visualization of the effect of energy involved here is to consider that the IR region is sufficiently energetic to stimulate molecular translation and rotation. This means that the molecules are moved from place to place and are caused to undergo some twisting and turning; the energy needed in this movement is 1000 cal / mole or less. The energy imparted by the near-IR to the edge of the visible causes oscillation and swinging of the atoms in the molecules or vibrations; the energy requirements in the movement lie in the range of 1,000-36,000 cal / mole. As the wavelengths become shorter, i.e., in the visible and UV portions of the spectrum, the effect on the molecules and their constituent atoms is much more marked. Displacement of outer electron and vibratory forces occurred in this state are sufficient to break chemical bond in the molecule. The energies are ranged from about 36,000-72,000 cal / mole in the visible short wavelength region to 72,000-286,000 cal / mole

in the UV region. The energies of light are capable of causing chemical reaction which may be oxidation and make the great stability problems to many drugs and pharmaceuticals (Connors et al., 1986).

Photo-oxidation of nifedipine, an orally active calcium blocking agent, is reported that the oxidation rate decreases exponentially as the light intensity decreases or when irradiation distance increases (Majeed et al., 1987). The UV light can accelerate the oxidation of nimodipine more than the artificial daylight can (Zanocco et al., 1992). The oxidation rate constant of ethyl icosapentate prepared from fish oil under UV light increases with an increase in the irradiation intensity; the effect of visible light is less (Teraoka, Otsuka and Matsuda, 1994).

3.8.6 The Global Solar Radiation behind Window Glass

The spectral distribution and irradiance of the solar radiation at the earth surface, as shown in Figure 4, depends on the location and is subjected to seasonal and diurnal variations. The sum of direct and diffuse solar radiation on a horizontal plane at the earth surface, called global solar radiation, in the emission range between 300 nm and 450 nm is given as 1090 w/m^2. The spectral energy distribution of global solar radiation behind the window depends on the type and thickness of the window glass. The total irradiance transmiting is reduced or cut-off in the UV range as well as shifted towards longer wavelengths (Moore, 1996).

3.8.7 Accelerated Photo-oxidation Tests

In principle, photo-oxidation studies which are based on the photo-degradation tests can be performed by exposing a sample to a light source for a fixed period of time. If any changes in the physical or chemical properties of the sample are observed compared with a reference sample, the results of the study may recommend that the drug should be protected from light. The light source may be natural sunlight. However, the intensity of sunlight, particularly the UV component, varies according

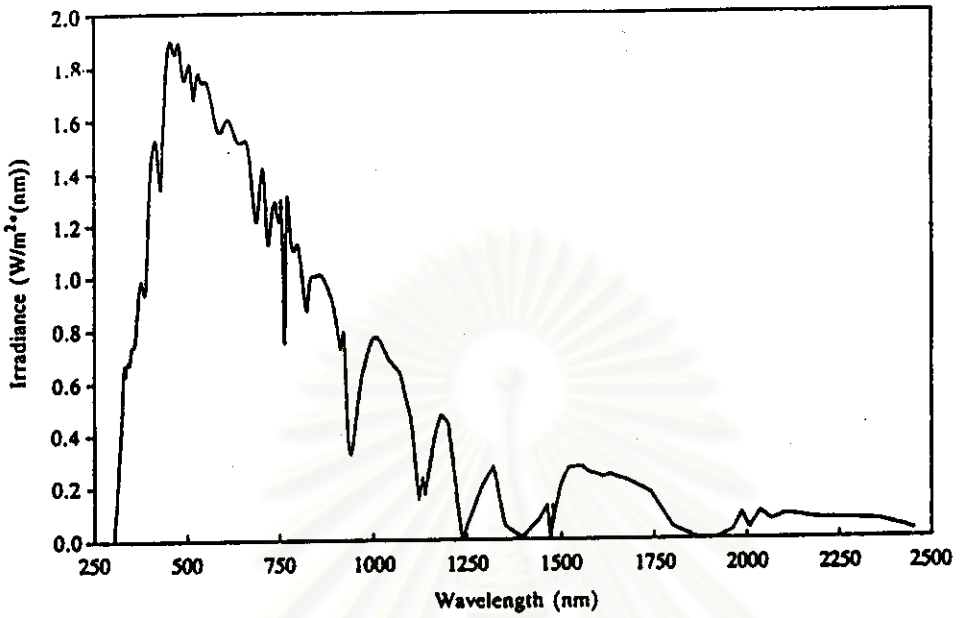


Figure 4 Spectral energy distribution of global solar radiation (Moore, 1996).

to the weather, the latitude, the time of the day, and the season of the year. 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. Nevertheless, the use of natural sunlight is not a viable option (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 the cabinet is well ventilated so that the temperature of the samples dose not rise significantly (Carstensen, 1984). There have been various kinds of artificial light sources used in the past and still in use as follows.

3.8.7.1 Arc Lamps

There are two main types of arc lamps which are a photon source, namely, a mercury arc lamp and a xenon arc lamp. The mercury arc lamp can be constructed in three ways: with the mercury vapor at low, medium and high pressure; each variance has specific characteristics of the mercury emission spectrum line. The low-pressure arc emits the energy as a line at 254 nm. The medium-pressure arc is

also a line source producing greater intensities at 302, 313, 334, 366 and 405 nm. While the high-pressure arc emits across the solar UV and visible regions. Because of the moderate in cost, the long life use, and a good representation of emission in the region, the mercury arc lamp has been widely used in drug photostability studies. The xenon arc lamp, a new development of metal-halide lamps is the lamp having the best resemblance to sunlight. It has a smooth continuous output spectrum with some line emissions in the region of 450-500 nm. There are two disadvantages of the two arc lamps. One disadvantage of all the arc lamps is the high heat output, but this can be dissipated by the use of a heat filter, usually containing water. The other disadvantage is that they have short life span of 750 hr for the metal-halide and 1500-2000 hr for the xenon arc lamp (Moore,1996).

3.8.7.2 Fluorescent Lamps

The operating principle of fluorescent lamps is based on mercury vapor discharged at a very low pressure. The lamp produces the 254 nm emission which is converted to higher wavelengths by 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. Besides, these fluorescent lamps have long lifetimes in excess of 20,000 hr and do not cause a problem with respect to heat output. However, the fluorescent lamps are deficient in the infrared regions of the spectrum. Thus, it is not possible to achieve a sunlight-simulating spectrum with just one type of fluorescent lamp; a combination of a black-light UV-A source must be used (Moore, 1996).

Although artificial light is somewhat similar to sunlight, most of them are less in intensity (Stewart and Tucker, 1985). Moreover, the output from the artificial light source may be filtered by, for example, glass or plastic, as required to simulate the type of packaging. Therefore, the exposure of pharmaceuticals to light is a more difficult situation to control and quantify. What must be considered are the wavelength range involved, and how intense the light and temperature is at the surface

of sample being tested. Thus, the prerequisites for running tests must include the correlation to the tests under natural condition as well as the repeatability and reproducibility of the accelerated tests (Moore, 1996).

Factors tending to decrease the degree of nature sunlight and artificial light correlation are the use of UV radiation having shorter wavelengths than those occurring in natural exposure which may provoke reactions which require higher energies than encountered in a pharmaceutical practice (Carstensen, 1984), the use of a spectral distribution that differs widely from that of daylight, the use of a very high light-flux, and the use of high specimen temperature, particularly with materials which readily undergo changes from thermal effects alone. According to this, the different lamp technologies used and the size of exposure area with a satisfactory uniformity of irradiances required may be realized. Thus, the intensity measurement of visible light and UV irradiation at the place of the samples are always necessary for controlling the variations. 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).

4. Inhibitions of Oxidation

The extent of drug oxidation in pharmaceutical formulations can be minimized by several ways. However, two main important techniques can be described as the following.

4.1 Protection of Factors Influencing Oxidation

4.1.1 Controlling of Temperature

A decrease in temperature will decrease the reaction rate in accordance with the Arrhenius theory stated previously. Most oxidative reactions have energies of activation around 100 J / mole. Hence, the storage of formulations susceptible to oxidation in a place with low temperature such as in a refrigerator instead of at room temperature would decrease the reaction rate by about a quarter

(Stewart and Tucker, 1985). However, the oxygen solubility and the air space in the container must be considered.

4.1.2 Adjustment of pH

As it has already been discussed, the oxidation of many drugs is pH dependent. Most of them degrade more rapidly in neutral to alkaline pH conditions. Increasing of hydrogen ion concentration or decreasing of pH value of drug solutions can decrease the tendency of drug oxidation. A pH range of three to four is generally found to be most useful in retarding the oxidation. However, this technique is not suitable for drugs that precipitate out at lower pH values (Connors et al., 1986; Stewart and Tucker, 1985).

4.1.3 Prevention of Oxygen

Controlling the gas bubble incorporating during a number of manufacturing process such as mixing, blending, jetting or pouring of liquid dosage forms is the first step to prevent oxidation from oxygen. Because oxygen in trapped air bubbles can sometimes encourage undesirable oxidation of drug preparations (Kostenbauder, 1971). Drug preparations should be stored in full, well-stoppered containers. The seal of their containers must be perfect because it ensures the impermeability of air and oxygen (Kumar et al., 1992). Parenteral drugs should be packaged in glass ampoules that are heat sealed under an inert atmosphere. For tablets, capsules, and so on, packaging of the formulations in hermetic strips may be useful for retardation of oxidation (Connors et al., 1986). However, many drugs need more processes to protect them from oxygen. Removing oxygen from a formulation is one of the obvious way to prevent oxidation. This can be done by several ways. Oxygen may be expelled from aqueous preparations by boiling water beforehand, although some oxygen would redissolve during the cooling process. A better way is to bubble nitrogen gas through the solvent to flush the oxygen out of the solution. Another way is to flush the headspace of the container with nitrogen just prior to filling and sealing or capping. This is done mostly with ampoules and bulk powders that are reconstituted

at the time of dispensing. Carbon dioxide is sometimes used but and it will lower the pH of unbuffered solutions if it is present in appreciable amount in water (Connors et al., 1986). The oxygen content in water treated by different ways is shown in Table 2 (Stewart and Tucker, 1985).

The effect of reducing molecular oxygen level in vehicle on the drug oxidation was presented in 1978. The reduced dissolved oxygen levels were produced by deaeration via gas permeation. Dissolved oxygen levels were determined using a dropping mercury electrode polarograph. Pyrogallol which generally underwent rapid oxidation was used as the model drug. The results of this study illustrated that decreasing the level of dissolved oxygen in the system could stabilize pyrogallol in an aqueous solution (Palmieri, 1978).

Table 2 Oxygen content in water (Stewart and Tucker, 1985).

Water	O_2 content (ml/l)	Temperature (°C)
water in equilibrium with oxygen in air	9.14	4
	5.75	25
	0.00	100
water stored at 4°C in closed containers	1.50	4
water boiled and purged with N_2 or CO_2	0.45	20

4.1.4 Protection of light

As mentioned earlier, many compounds undergo physicochemical changes on receiving and absorbing radiant energy or light and a triggering force of light can also promote oxidation (Connors et al., 1986). The degree of oxidation degradation depends on several factors of light such as the type of light, the intensity of light, the distance of illumination and the time of irradiation (Moore, 1996). According to this, the oxidative decomposition can be decreased by minimizing the light factors accelerating the oxidation rate as the following.

Inhibition of the lower wavelengths

The inhibition of the lower wavelengths is very important because the lower wavelengths such as lower visible and ultraviolet radiations which give more energy can produce greater photo-oxidative degradation (Stewart and Tucker, 1985).

Decreasing of radiation intensity

The intensity of light is defined as the number of photons crossing a plane which is placed perpandicular to the radiation per unit area per unit time, for example, the energy flux per unit area. The decrease in the radiation intensity will decrease the degree of photolytic decomposition, particularly the lower wavelength (Stewart and Tucker, 1985). The decrease in radiation intensity can be achieved by increasing the distance between the radiation source and drug, decreasing the number of photons, reducing the time of drug exposure to the radiation, and decreasing the surface area of the drug or drug formulation exposed to the radiation (Stewart and Tucker, 1985). However, many drugs require more than one protection ways. A lot of techniques used for exclusion of light can be accomplished, for example, wrapping around labels, using various cartoning procedures, using coating container which some may incorporate ultraviolet absorbing materials, using the pigmented glass capable of excluding the damaging wavelengths, and using opaque container that all light is excluded (Connors et al., 1986).

Amber glass bottles are known to be capable of blocking ultraviolet light completely. However, highly light-sensitive products should be filled and packed in completely opaque light-resistant containers made of high-density polyethylene material, opaque glass, or glass that is rendered opaque by a special coating. Paper cartons used for packing clear or amber glass ampoules, vials, or bottles can impart resistance to light for certain light-sensitive pharmaceutical products (Kumar et al., 1992). In 1994, the effect of fluorescent light exposure on human epidermal growth factor 1-48 (hEGF 1-48) was reported. The rank order of oxidation rate of drug solutions packed in three kinds of ampoules was as follows: clear ampoules > amber

ampoules > foil-covered ampoules. The result indicated that hEGF 1-48 was more stable by protecting from light (Senderroff et al., 1994).

The Pharmaceutical Codex defines light resistant containers as those which do not transmit more than 10 % incident radiation at any wavelength between 290 and 450 nm. The United States Pharmacopoeia (USP) provides more information concerning containers for parenteral use made of glass types I, II and III and plastic type I-VI. The light-transmission limits of glass and plastic containers are given as the maximum percent transmission for any wavelengths, the same range as the Pharmaceutical Codex. Light transmission for containers of type NP glass and for plastic containers for products intended for oral or topical administrations are also similar to the Pharmaceutical Codex (Connors et al., 1986; Stewart and Tucker, 1985).

A beam of light falling on a flat transparent surface perpendicularly loses some of its energy by reflection (Figure 5). If the beam impinges at an angle other than 90°, the loss is even greater. The transparent material then absorbs part of the energy entering it and transmits the remainder to the second surface, where a second loss by reflection takes place. The fraction of the light transmitted is the ratio of the intensity of the light emerging to the incident intensity. The amount of light energy absorbed is a function of the physicochemical nature and depth of the glass (Connors et al., 1986; Stewart and Tucker, 1985).

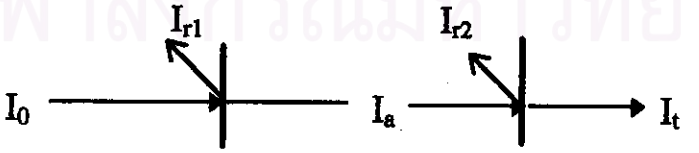


Figure 5 Transmission of light through glass surfaces (Stewart and Tucker, 1985).

The transmitted light intensity (I_t) can be calculated by

$$I_t = I_0 - I_{r1} - I_a - I_{r2} \quad (23)$$

where, I_0 is the intensity of the incident radiation, I_{r1} and I_{r2} are the intensity of reflected radiations at the two glass interfaces and I_a is the intensity of radiation absorbed in the glass (Stewart and Tucker, 1985). The losses from reflection depend on the reflective index of the glass. The fraction of light energy lost due to reflection, R, from a single surface is given by the expression:

$$R = (n - 1)^2 / (n + 1)^2 \quad (24)$$

where n is the index of reflection of the glass in the spectral region being considered. Glass containers have reflective indices of approximately 1.5. Thus R = 0.04 for one or 0.08 for two surfaces. This means that 8 % is lost by reflection, and the maximal transmission is about 92 %. Samples of glass can be spectrophotometrically scanned using the same way as a solution of an organic compound; the procedure is given in the USP. Thus, the actual transmission values for a specific piece of glass depend on its thickness and the absorption coefficient of the glass (Connors et al., 1986). Figure 6 shows the detailed character of the transmission of various glasses and illustrates that the glass wall thicknesses of less than about 2 mm are not suitable for use as light resistant container (Stewart and Tucker, 1985).

The careful selection of a light resistant glass container is therefore important in minimizing the risk of drug photo-oxidation (Stewart and Tucker, 1985). Beside these, theoretically, the use of plastic containers is essentially analogous to that of glass. There is one difference, however, in that oxygen can penetrate many plastic containers, so packaging must have sufficient wall thickness (Connors et al., 1986).

In addition, the development of pharmaceutical technologies is another way to exclude light from the products. In tablet dosage form, opacifiers such as titanium dioxide plays an important role in protecting active ingredients in the cores of sugar-coated or film-coated tablets from exposure to light (Kumar et al., 1992).

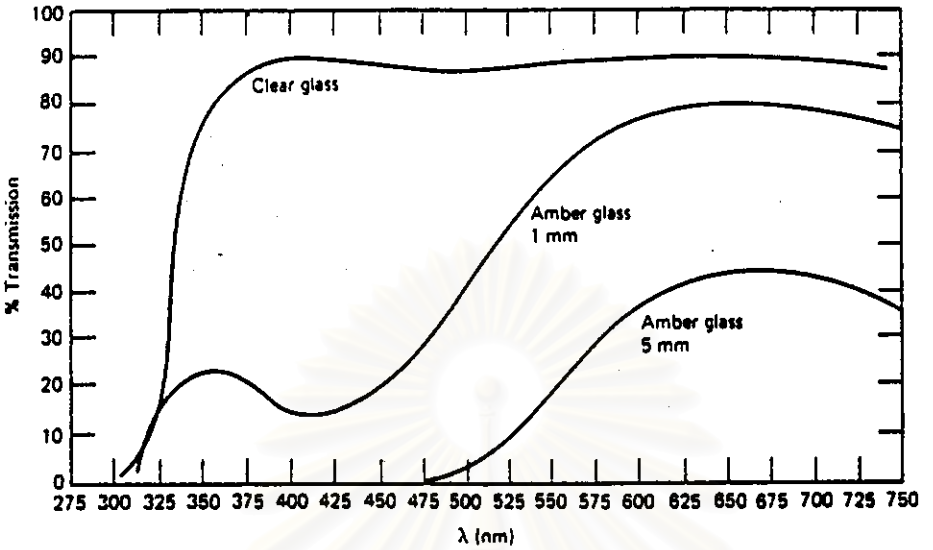


Figure 6 Spectral transmitance characteristics of clear and amber glass and the effect of glass thickness (Connors et al., 1986).

4.2 Addition of Antioxidants

The retardation of oxidative reaction by adding minute quantities of certain compounds was observed as early as 1797 by Berthollet and again by Davy in 1817. This phenomenon was explained at first as "negative catalysis" or as a "poisoning" of a catalyst. About one hundred years after these findings, Lumiere, Lumiere and Seyewetz called these compounds "antioxidant". At the beginning of 1880, the use of antioxidants became more frequent. Literally hundreds of compounds have been proposed for retarding oxidation. The requirements of an antioxidant are the following.

1. It should be safe to use. The compound and its oxidation products must be nontoxic.
2. It imparts no foreign flavor, odor, or color to the drug product, even after prolonged storage or heating.
3. It should be active at extremely low concentrations.
4. It should be easily incorporated into the formulation.
5. It should be easily detected, identified and measured.
6. It should be easily available and low cost.

Although certain antioxidant substances are considered harmless, they should not be used in concentrations greater than required to achieve the desired stabilization of drug (Chipault, 1962).

Antioxidants can conveniently be classified as primary antioxidants, reducing agents and chelating agents (Connors et al., 1986; Stewart and Tucker, 1985).

4.2.1 Primary Antioxidants

Primary antioxidants or free radical inhibitors are the substances that can donate a hydrogen radicals or an electrons while itself form radicals that are stable and incapable of continuing the propagation chain cycle. This retardation effect can increase the induction period of oxidation. Thus, they are sometimes called chain terminators. However, they are consumed during its use and the addition of these compounds to partially decomposed materials is not recommended. Two primary antioxidants are only useful to protect drugs degrading by an auto-oxidation (Connors et al., 1986; Stewart and Tucker, 1985). Besides, they are only effective at extremely low concentrations and the effectiveness of several primary antioxidants decreases as their concentrations are increased. At higher 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). They are usually used in nonaqueous solutions; certain surfactants must be used as co-solvents in aqueous solutions. The maximum concentration of surfactants or solubilizers generally used in parenteral preparations is not more than 0.5 % w/w. Example of these antioxidants are presented as follows (Deluca and Boylan, 1992).

Butylated Hydroxytoluene

This compound is commonly known as BHT (Figure 7) It is one of the synthetic antioxidant. It was originally developed for use in petroleum products and

rubber, and it has been adopted for use in other products of food and drug. Like butylated hydroxyanisole, BHT belongs to a group of compounds known as "hindered phenols" in which the reactivity of the phenolic group is decreased by the ortho or para substituents in their aromatic ring (Deluca and Boylan, 1992).

Purified BHT is a white, crystalline product which is essentially odorless. It melts at 70 °C and is insoluble in water but is soluble in organic solvents and in fats. The antioxidant effect of BHT dose not have an optimum concentration. When its concentration is increased, the antioxidant effect increases; although the rate of increase is less at higher levels (Chipault, 1962). Its LD_{50} (mouse, IV) is 0.18 g / kg (Wade and Welly, 1994). The maximum concentrations of BHT always used in parenteral solutions is 0.02% w / v (Deluca and Boylan, 1992).

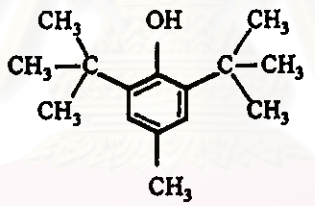


Figure 7 Chemical structure of Butylated hydroxytoluene (BHT).

Tocopherols and Related Compounds

These compounds known as alpha, beta and gramma tocopherols are isomers or homologs formed by methylation of a substituted chroman nucleus called tocol. (Figure 8)

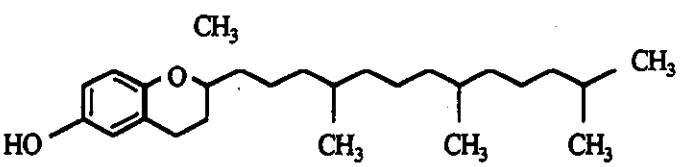


Figure 8 Chemical structure of alpha tocopherol.

At room temperature, all these compounds are slightly yellowish oils and completely soluble in fats. The antioxidant efficiency of the tocopherols is best at low levels and these compounds show an optimum concentration which when exceeded results in decreased stability. The antioxidant activities of the alpha, beta and gamma compounds have been studied most often. Some workers have shown that the antioxidant activities of the tocopherols increase from the alpha compound to the gamma isomer. Moreover, their activities also depend upon temperature (Chipault, 1962). The maximum concentrations used in parenteral solutions is 0.5 % w/w (Deluca and Boylan, 1992).

The effect of primary antioxidants such as alpha tocopherol on the oxidation of lovastatin, a hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, in a solution phase was investigated in 1990. The results showed that alpha tocopherol could inhibit the oxidation of lovastatin and also indicated that the inhibition of lovastatin against oxidation was more than other primary antioxidants investigated (Kaufman, 1990).

4.2.2 Reducing Agents

Reducing agents are compounds that can be oxidized more readily than the drugs that are to protect. Their mechanisms of oxidation can be either reversible 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 $E°_{cell}$ and its concentration (Connors et al., 1986; Stewart and Tucker, 1985).

Sulphurous Acid Salts

Sulphurous acid salts such as sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$) are well-known as reducing agents in aqueous solutions. Because of their low oxidation potentials, they can be preferentially oxidized. They react easier with oxygen according to the equation (Chipault, 1962):

$$SO_3^{2-} + 1/2O_2 \longrightarrow SO_4^{2-} \quad (25)$$

They may sometimes be called oxygen scavengers. In a closed system, they may consume essentially all of the oxygen present and thereby protect the drug. Open systems may require higher concentrations than closed systems (Connors et al., 1986). The metabisulfite and bisulfite salts are used in, acidic and intermediate pH conditions and the sulfite in slightly alkaline or unbuffered solutions. These sulphurous acid salts, in particular, sodium metabisulfite have been extensively used as reducing agents in pharmaceutical formulations. Its concentrations in the range of 0.01-1% w / v have been used, although the most usual concentration is 0.1% w / v. In the development of a formulation, the metabisulfite concentration used needs to be validated experimentally (Chipault, 1962). Where as the concentration of sodium bisulfite usually used in the parenteral solutions is not more than 0.1 - 0.01 % w/v (Deluca and Boylan, 1992) and its LD_{50} (rat, IV) is 0.12 g / kg (Wade and Welly, 1994).

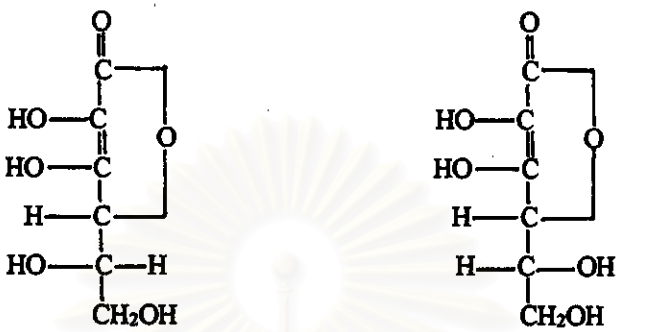
Although this group of antioxidants has been widely used, a number of potential problems exist. In the process of acting as antioxidants, they yield acid sulfates which cause a drop in pH of solutions; a small amount of barium or calcium can be extracted from glass type I. The antioxidation effect of sulfite may be inhibited by inactive addition to the alkenes, alkyl halides, and aromatic nitro and carbonyl compounds (Stewart and Tucker, 1985).

Several researches have been reported about the problem of these reducing agents when they are used as antioxidants. An example is sodium metabisulfite which was found to be ineffective in stabilizing antipyline hydrochloride in aqueous solution. In a contrary, it showed an acceleration effect on the drug decomposition rate (Enerer et al., 1977).

Ascorbic Acid

Ascorbic acid or vitamin C (Figure 9) is a crystalline compound melting with decomposition near 160 °C. It is insoluble in fats, but fatty acid esters of

ascorbic and isoascorbic acid are readily fat soluble and, apparently, are as effective as the free acids.



L-ascorbic acid

D-araboascorbic acid
(Isoascorbic acid)

Figure 9 Chemical structures of ascorbic Acid.

Also, because of their low oxidation potentials, they can be used as the reducing antioxidants. The reversible loss of electrons of ascorbic acid is described by the following (Stewart and Tucker, 1985):

$$\text{Ascorbic acid} \longrightarrow \text{dehydroascorbic acid} + 2H^{+} + 2e^{-} \qquad (26)$$

However, it is destroyed by heat and under certain conditions, it can act as a strong pro-oxidant (Chipault, 1962). LD_{50} (mouse, IV) of ascorbic acid is 0.52 g/kg (Wade and Welly, 1994). The range of concentration used in parenteral solutions is 0.01 - 0.075 % w/v (Deluca and Boylan, 1992).

An antiasthmatic agent, 2-[(4-hydroxyphenyl) amino]-5-methoxy-benzenemethanol (compound 1) which turns red in a solution due to its oxidation to 4-[[2-(hydroxymethyl)-4-methoxyphenyl]imino]-2,5-cyclohexadiene-1-one (compound 2) is one of several drugs that shows the success of ascorbic acid used as an antioxidant. No change in compound 1 was observed in the presence of 0.1 % ascorbic acid because ascorbic acid not only protected compound 1 from oxidation but also reduced compound 2 back to compound 1 again (Yu and Portmann,1990).

4.2.3 Chelating Agents

Chelating agents act as antioxidants by binding to metal ions which are known to be one of the initiator of oxidation. The most effective chelating agents used pharmaceutically are ethylenediaminetetraacetic acid (EDTA), citric acid, phosphoric acid, tartaric acid and many amino acids. 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 (Connors et al., 1986).

Ethylenediaminetetraacetic Acid

Ethylenediaminetetraacetic acid (EDTA) (Figure 10) occurs as an odorless white crystalline powder with a slightly acid taste. It can be soluble in water (1 in 11). High temperature can cause its decomposition. Its LD_{50} (mouse, IV) and LD_{50} (rabbit, IV) are 0.056 and 0.047 g / kg, respectively (Wade and Welly, 1994). Its concentration commonly used in parenteral preparations is 0.01-0.075 % w/v (Deluca and Boylan, 1992).

Many researchers have studied the effect of EDTA when it is used as a chelating agent. EDTA could completely negate the catalytic effect of cupric ions on the oxidation of aliphatic hydroxylamine drugs during storage in pH 7.4 phosphate buffer at room temperature (Beckett et al., 1976). The decreasing of oxidation rate of captopril in a solution stored at 50 °C when 0.1 % w/v disodium edetate was added was reported (Timmins, Jackson and Wang, 1982). By formation of iron complex, EDTA could reduce the rate of oxidation and increase the shelf life of sodium nitroprusside in solutions from 13 days to over 39 days (Asker and Canady, 1984).

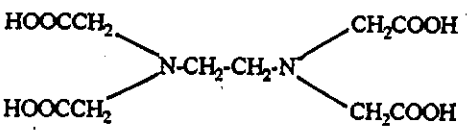


Figure 10 Chemical structure of EDTA.

Citric Acid

Similar to EDTA, citric acid (Figure 11) is highly soluble in water and almost insoluble in fats. Their mono-esters and mono-sodium salts are active while the di- and tri-citrates are completely ineffective; this indicates that at least two free carboxylic groups are necessary for antioxidative potency.

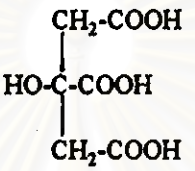


Figure 11 Chemical structure of citric acid (Chipault, 1962).

Citric acid is readily decomposed by heat, but its thermal decomposition products are also good synergists. Beside these, citric acid and the citrates are not toxic and impart no undesirable odor or flavor to drug products (Connors et al., 1986). The concentration of citric acid used in parenteral solutions is 0.3 - 4.0 % w / v (Deluca and Boylan, 1992).

The oxidative degradation of many drugs can be reduced by addition of citric acid. Sodium nitroprusside is an example. There is no change in concentration of sodium nitroprosside for up to 6 months when citric acid is added to the solution of which the pH is adjusted to 4.65 and is stored in the dark; its shelf life is increased from 13 days to over 800 days (Asker and Canady, 1984). Citric acid can also act as the chelaing agent to reduce the oxidation rate of epinephrine by complexing with catalysts. The epineprine solutions are more stable when citric acid is added (Fyhr and Brodin, 1987).

To enhance the effectiveness of antioxidants, it is sometimes useful to use more than one antioxidants. It has been found that a combination of two antioxidants along with a chelating agent to complex metals always works well. This enhanced effectiveness is often referred to as synergism. The best studied examples of

synergism are the mixtures of a chelating agent with chain terminators. The superior antioxidant action of these mixture are obvious (Connors et al., 1986).

As mentioned previously, the oxidation process is a complicate series of reactions which is not easy to understand and can be influenced by several environmental factors which are difficult to control. A lot of drugs and pharmaceuticals are destroyed by this reaction. Many workers have tried to protect the oxidative degradation and most of them have found that one technique used may insufficient to protect or inhibit this reaction. The combination of several methods described earlier may be needed for better protection.