CHAPTER I

INTRODUCTION

Chemical stability is one of the most important properties to indicate and assure drug integrity of the strength, identity, quality, bioavailability and purity of pharmaceutical products. It is well known that stability of a drug depends on several factors including physical, chemical and biological factors which resulted in product difference between manufacturers, dosage forms and formulations. It is invalid to use the stability data of raw material or of one preparation to assure the others. Therefore, it is required for all manufactures to conduct stability testing program and place appropriate expiration dating on certain labile drugs. Nevertheless, some local pharmaceutical manufacturers do not conduct appropriate stability testing of their own products on actual marketing conditions of storage. Products that should bear expiration date are sometimes omitted or dated as raw material which is unacceptable.

In 1978, Saisorn, et al. (1) reported a survey on stability of vitamin C in oral liquid preparations obtained from retailed drug store in Thailand. They showed that vitamin C stability in most products had very short half-life but carried no expiration

date on the label. Besides, there were significant differences in specific rate constants among several formulations.

The purpose of this investigation was to demonstrate a systematic kinetic approach to the formulation and development of stable products. Stabilization of multivitamin in oral liquid preparations has been often encountered by pharmacists. This problem was then selected as a model for the study. It has been demonstrated that vitamin C is most labile among all other ingredients in the product and, thus its stability would indicate the product's shelf-life (2).

The approach was outlined as follows :

1 . Selection of a suitable combination of vitamins in the formula

2 . Preformulation studies on the effects of various vehicles, antioxidants, chelating agents, suspending agents, flavors, preservatives and pH on both chemical stability and physical appearance of vitamin C in the oral liquid multivitamin formulations.

3 . Based on information from 2 , several possible formulations would be derived and evaluated for their stability.

4 . Evaluation of each formulation by accelerated stability testing method. The theoretical predicted shelf-life would be compared to the value obtained for the corresponding preparation under actual room temperature conditions of storage.

It is anticipated that such approach would yield a stable multivitamin formula and the accelerated stability testing may be proved valuable as a simple method for shelf-life prediction. It is also hoped to provide pharmacists (primarily in the pharmaceutical industry) the application of theoretical chemical kinetics to assess and control drug and dosage form stability for the design and development of their products.

Literature Review

1. Stability

Stability is defined as the extent to which a product retains the same properties and characteristics within specified limits throughout its period of storage and use (3).

The stability characteristics of drugs are basically classified into five types with certain criteria for accepted stability as shown in Table 1 (3).

Bibart (4) suggested that either chemical or physical stability might be the limiting factor of the shelf-life. Any slight changes in physical appearance such as color fading, odor, or cloudy can cause the patient or consumer lose confidence in the product (5). Unfortunately most physical measurable method is less accurate and precise.

Table 1 Criteria for accepted stability

Type of stability	Conditions maintained throughout the shelf-life of the drug product
Chemical	Each active ingredient retains its chemical integrity and labeled potency within the limits specified in the monograph definition
Physical	The original physical properties, including appearances, palatability, uniformity, dissolution, and suspendability are retained
Microbiological	Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retained effectiveness within the specified limits
Therapeutic	The therapeutic effect remains unchanged.
Toxicological	No significant increase in toxicity occurs.

When the drug goes through chemical changes, generally by oxidation, reduction, hydrolysis and racemization (6), sensitive analytical methodology could be developed to follow both the drug and its degradation products. Therefore, chemical stability is an acceptable method to assess the drug products shelf-life.

Although therapeutic and toxicological stability are most desired for the product to assure its efficacy and safety, there are biological variations and analytical problems that make it impractical (7).

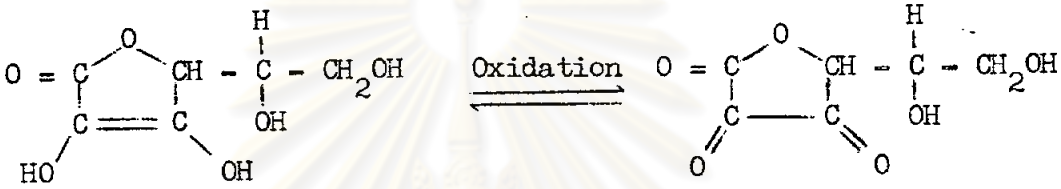
2. Vitamin C

2.1 Stability of Vitamin C

Vitamin C is an unsaturated cyclic lactone. It is stable in dried state, but unstable in aqueous solution. The degradation of vitamin C occurs under both aerobic and anaerobic conditions giving different decomposition products. Under aerobic conditions, vitamin C is oxidized to dehydroascorbic acid, followed by hydrolysis and oxidation to give diketogulonic acid and oxalic acid (8-10). Under anaerobic conditions, it undergoes dehydration and hydrolysis to give furfural and carbondioxide. The pH-rate profiles for both aerobic and anaerobic degradation show maxima around pH 4 (near pK_1). Maximum stability occurs near pH 3 and pH 6. The rate of anaerobic decomposition at pH 6 is about 20 times faster than aerobic conditions (11).

Aerobic decomposition

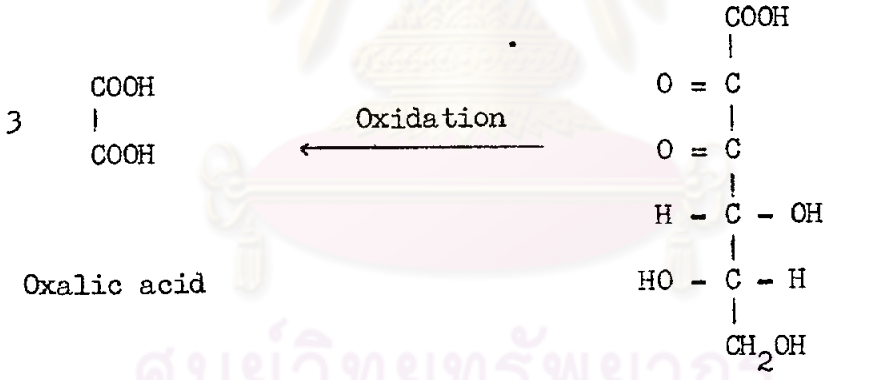
Oxidation rate is dependent on pH, heat, light, oxidative enzymes, metal ions especially by Cu^{+2} and Fe^{+3} and some of oxidative products (11)



L - Ascorbic acid

Dehydroascorbic acid

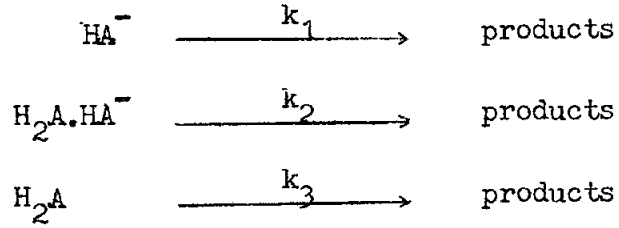
Hydrolysis



Oxalic acid

diketogulonic acid

The kinetic scheme has been postulated (12) to explain the observed pH-rate profile



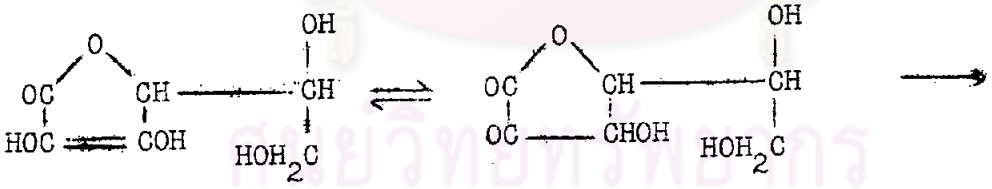
where H_2A and HA^- are undissociated and monodissociated ascorbic acid, and $H_2A.HA^-$ is a complex of undissociated ascorbic acid and monohydrogen ascorbate.

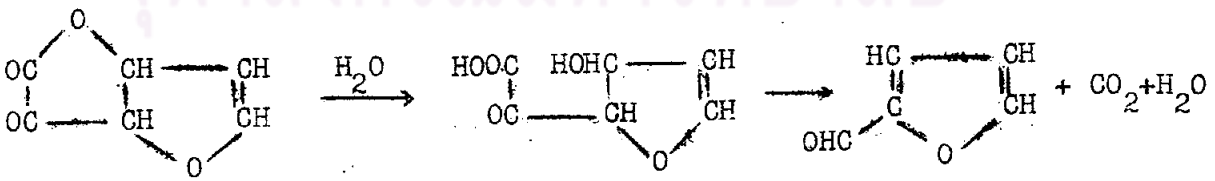
The rate equation for the proceeding scheme is

$$\text{rate} = k_1 [HA^-] + k_2 [H_2A.HA^-] + k_3 [H_2A]$$

Anaerobic decomposition

The dehydration reaction in acidic media is faster than in basic media due to hydrogen ion catalysis (11). Vitamin C is decarboxylated when heated in aqueous solution under anaerobic condition (13-16) and metal ions may be catalysts(17-21).





Furfuraldehyde

$+ 2H_2O$

The kinetic scheme has been postulated (11, 14) below

$$H_2A + H^+ \xrightarrow{k_1} \text{products}$$

$$H_2A \xrightarrow{k_2} \text{products}$$

$$H_2A.HA^- \xrightarrow{k_3} \text{products}$$

$$HA^- + H_2A \xrightarrow{k_4} \text{products}$$

$$HA^- \xrightarrow{k_5} \text{products}$$

$$A^- \xrightarrow{k_6} \text{products}$$

The rate equation is

$$\text{rate} = k_1 [H_2A] [H^+] + k_2 [H_2A] + k_3 [H_2A.HA^-] + k_4 [HA^-] [H_2A] + k_5 [HA^-] + k_6 [A^-]$$

The experimental results of degradation of vitamin C under both aerobic and anaerobic conditions are apparent first-order reaction and the rate equation is

$$\text{rate} = k [A_T]$$

where $[A_T]$ is the total concentration of vitamin C.

It has been reported that the rate of anaerobic decomposition of vitamin C was faster than the aerobic degradation and in many liquid pharmaceutical preparations appeared to follow largely the non-oxidative route (22).

2.2 Factors effecting vitamin C stability

2.2.1 Concentration

The initial concentration of vitamin C in solution has affected the rate of decomposition. Bandelin and Tuschhoff (23), and Finholt, et al. (14) found that higher initial concentration of vitamin C was degraded faster than lower, which followed a first-order reaction.

2.2.2 Vehicle

Vitamin C is very unstable in aqueous solution, its shelf-life can be prolonged by appropriate choice of vehicle (11). Bandelin and Tuschhoff (23, 24), Bartilucci and Foss (25) and Giral (26) studied effects of several vehicles on the stability of vitamin C. It was found that addition of sugar and certain polyhydric alcohol compounds such as glycerin, propylene glycol, sorbitol and alcohol resulted in a definite stabilizing effect on vitamin C. The protective action of all compounds afforded in the same amount of approximately 50 % concentration. The mixture of vehicle containing some of glycerin and/or sorbitol, propylene glycol was better in retarding the decomposition of vitamin C than the single vehicle. Sudeb, et al. (27) found that in all the formulations containing ascorbic acid alone or combined with other vitamins, the vehicle mixture of 60 % sorbitol, 20 % propylene glycol and 20 % glycerin was superior to the other mixture vehicle.

Uprety (28, 29) suggested some fruit juice of apple, lime and pine apple to be used in vehicle mixture of glycerol and sorbitol that prefered natural palatable and protection effect even with or without antioxidant.

2.2.3 Metal ions

Sudeb, et al. (27) and Gupta (30) reported that the maximum catalytic effects of metal ions on the degradation of vitamin C was produced by Cu^{+2} followed by Fe^{+2}, K^{+1} while Mg^{+2}, Na^{+1}, Co^{+2} and Ca^{+2} had little effect.

Nixon and Chawla (31) showed that the rate of oxidation of vitamin C increased rapidly with increasing catalyst concentration but not linearity proportion was observed.

Finholt, et al. (22) reported that all active metals had the highest catalytic effect at pH 4-6 and the difference in catalytic activity of metallic ion depended on their relative tendencies to form complex with vitamin C.

2.2.4 Chelating agents

Kassem, et al. (32, 33) studied the effect of chelating agents and autoclaving on vitamin C injection. It was reported that the stabilizing effect of chelating agents was in the following decreasing order : · N-hydroxyl ethyl ethylene diamine tetraacetic acid (HEDTA) > diethylene triamine pentaacetic

acid (DTPA) > sodium diethyldithiocarbamate > disodium EDTA > dimercaptopropanol > 8-hydroxyquinoline > propyl gallate.

Gladkikh, et al. (34) found that unithiol (2,3-dimercapto propane sodium sulfonate), an antioxidant and chelating agent, was a better stabilizer for vitamin C than metabisulfite, cysteine and disodium EDTA.

2.2.5 Antioxidants

Uprety, et al. (28, 29) pointed that cysteine hydrochloride had stabilizing effect on vitamin C while potassium metabisulfite gave slightly protection.

2.2.6 Surfactants

Aqueous solutions of multivitamin preparations containing oil-soluble vitamin A and D have been prepared in recent years by using nonionic surfactants as solubilizer. Therefore, the effect of various types of non-ionic surfactants on the stability of vitamin C was extensively studied.

Blaug and Hajratwala (35) observed that polysorbate 80 and polyoxalkal had different stability effect on vitamin C. It might be related to difference in aggregating number and size of the micelles formed, making vitamin C adsorbed on the surfactant molecules. Therefore, it was more susceptible to oxidation attack through surface catalysis or formation of associated complex.

Nixon and chawla (31) found that large increase in viscosity of polysorbate 20 has a negligible effect on the oxidation rate of vitamin C.

2.2.7 Suspending agents

Synthetic and natural suspending agents including methylcellulose, carboxymethylcellulose, pectin, and tragacanth were shown to have no protective effect but seemed to accelerate degradation of vitamin C (23). Increased viscosity by adding syspending agent did not retard the rate of decomposition.

2.2.8 Flavoring agents

Trivedi and Patal (36) reported the effect of flavoring agents on the stability of vitamin C. The following decomposition order was observed :

vanillin > raspberry > cherry > chocolate > pine apple > banana

The destruction rate increased with increasing in concentration of flavor

2.2.9 Ionic strength

Finholt, et al. (14) observed no primary salt effect at pH range 1.1 to 10.2 on the stability of vitamin C.

2.2.10 Amino acids

Sudeb, et al. (27) found that the amino acids methionine, tryptophan, leucine, valine, phenyl alanine, asparagine, lysine, histidine, arginine, cysteine, glutamic acid and glutathion had no appreciable influences on stabilizing vitamin C.

2.2.11 Other vitamins

Sudeb, et al. (27) reported that thiamine hydrochloride, panthenol, cyanocobalamine, folic acid, pyridoxine hydrochloride , vitamin A palmitate, riboflavin, inositol and calciferol had no appreciable effect on the degradation of vitamin C while calcium pantothenate, nicotinamide, menadione, thiamine mononitrate and riboflavine 5' phosphate decreased its degradation rate.

Giral (26) found that B-vitamins had also protected vitamin C even in the presence of destructive effect of metals.

Fox and Paterson (37) suggested that the addition of B-complex vitamin to syrups of vitamin C seemed to produce a stabilizing effect, which might be due to interaction of vitamin C with some of the vitamin such as niacinamide to form undissociated compound.

2.2.12 pH

The pH-rate profile of vitamin C in various conditions was investigated. The degradation in both aerobic and anaerobic showed maximum around pH 4 but minimum around pH 6 and pH 3 (11). Uprety, et al. (28) suggested that vitamin C could be stabilized at approximately pH 3 in the presence of some antioxidants and metal binders.

2.2.13 Buffer

The oxidation of vitamin C is both specific and general acid-base catalyses. Finholt, et al. (14) studied the catalytic effects of various buffers at different pH in anaerobic conditions and found that phosphate buffer has catalytic effect at pH 2 to 3, oxalate buffer at pH 3.7 to 4, acetate buffer at pH 4 to 6 and borate buffer at pH 8 to 8.5. Giral (26) recommended that citric acid should be used to protect vitamin C at all pH.

3. Formulation

Vitamin C in oral liquid multivitamin preparation has been found to be easily degraded (1). However, its shelf-life can be prolonged by appropriate formulation (11). In order to modify or improve the formulation, many pharmaceutical additives should be considered by appropriate selection of vehicle, stabilizer, preservative, antioxidant, chelating agent and flavoring agent etc.

Ismaiel and Ismaiel (38) reported that the formulation containing sorbitol (42 % W/V), saccharin sodium (0.25 % W/V), citric acid (0.1 - 0.2 % W/V), sodium edetate and essence of banana or apple was palatable and had only slight discoloration and negligible loss in vitamin C.

Zoni and Lazzeretti (39) reported that the disagreeable odor of multivitamin preparation developed during storage could be eliminated by adding polyvinylpyrrolidinone. In addition, it was also a satisfactory chemical stabilizer for the active ingredients.

4. Stability study

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Prior to 1950, physical changes was used to indicate the stability of pharmaceutical products. By this mean, it had to use much experience to justify the stability, but the methodology was deficient scientific processes. Higuchi, et al. (40,41) have recommended the scientifically designed and well-planned kinetic study of the stability including reaction rate constants and the heat of activation. Recently, computer has been introduced to analyze the resulting data which speed up data processing for establishing a reliable expiration date.

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

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loss and eventually loss competitive marketing products. Therefore, Higuchi and Garrett (40-43) have brought 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. This method has been proved to be reliable and entirely appropriate for assessment and control of drug stability in formulation and dosage forms (40-45).

The heats of activation of vitamin C in various conditions of pH, temperatures, and metal catalysis, was found to fall in the range of 10-30 kcal/mol (11, 12, 13, 15, 35, 46, 47) which is suitable for stability studies by using accelerated thermodegradation method (50, 51).

Theoretical Concept

Order of reaction and specific rate constant

Order of reaction is determined by graphical method of concentration-time profile. The characteristic plot of a zero-order reaction is a straight line and of a first order is exponentially decline curve which can be linearized by logarithm concentration-time plot (50, 51).

In a zero order reaction, the rate of decreasing concentration is independent of concentration, i.e.

$$-\frac{dC}{dt} = k \qquad \text{(Eq. 1)}$$

and upon integration,

$$C_t = C_o - kt$$

In a first order reaction, the rate of decreasing concentration is dependent on concentration of one component, i.e.

$$-\frac{dC}{dt} = kC \qquad \text{(Eq. 2)}$$

$$\int_{C_o}^{C_t} \frac{dC}{C} = -k \int_{o}^{t} dt$$

$$\ln C_t - \ln C_o = -kt$$

$$\ln C_t = \ln C_o - kt \qquad \text{(Eq. 3)}$$

Where C_t is the concentration remaining at time t

C_o is the initial concentration

k is the specific rate constant, in a unit $time^{-1}$

t is the degraded time

The specific rate constant (k) is determined from the slope of the straight line.

Arrhenius relationship

The influence of temperature on reaction rate was proposed by Arrhenius as :

$$k = Ae^{-\frac{\Delta Ha}{RT}} \qquad \text{(Eq. 4)}$$

Where k is the specific rate constant of degradation

A is the frequency factor as a constant

Δ Ha is the heat of activation

R is the gas constant (1.987 calories degree^{-1} mol^{-1})

T is the degree kelvin (^{o}C + 273)

$$\ln k = - \left(\frac{\Delta Ha}{R}\right) \cdot \frac{1}{T} + \ln A \qquad \text{(Eq. 5)}$$

A plot of ln k versus $\frac{1}{T}$ yields a straight line and slope is $- \frac{\Delta Ha}{R}$. The heat of activation is calculated from the product of slope and the gas constant. It represents the energy that reacting molecules must acquire in order to undergo reaction. The higher the value for the heat of activation, the more temperature-dependent is the reaction. Since the Arrhenius plot is linear, it is possible to predict the rate (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 6).

$$\frac{d \ln k}{dt} = \frac{\Delta Ha}{RT^2}$$

Integration between the limits k_1 and k_2 and T_1 and T_2

$$\ln \frac{k_2}{k_1} = \frac{\Delta Ha}{R} \left(\frac{T_2 - T_1}{T_2 \cdot T_1}\right) \qquad \text{(Eq. 6)}$$

where k_1 is the rate constant at T_1

k_2 is the rate constant at T_2

Shelf-life

Shelf-life (t_{90}) is defined as the time required for the product to decrease its concentration from 100 % to 90 % of the labeled amount at normal storage temperature (51). The shelf-life of a first-order reaction can be calculated by rearranging equation (Eq.3).

$$t = \frac{\ln C_o - \ln C_t}{k}$$

and,

$$t_{90} = \frac{\ln 100 - \ln 90}{k} = \frac{0.1054}{k} \qquad \text{(Eq. 7)}$$

The 95 % confidence limit of t_{90} obtained from the 95 % confidence interval of k.

In order to prolong the shelf-life of vitamin C, an increasing initial concentration is considered. The initial concentration over a hundred percent of labeled amount, is called overage which has been generally permitted not more than 30 % of the label claimed.

The shelf-life of an overage can be calculated by :

$$\text{Shelf-life} = \frac{\ln 130 - \ln 90}{k} = \frac{0.3677}{k} \qquad \text{(Eq. 8)}$$