CHAPTER III

RESULTS

Order of reaction of vitamin C

Various liquid multivitamin formulations were incubated at $60^{\circ} \pm 0.5^{\circ}C$. The concentration of vitamin C remaining at time of sample taken were determined. Table 8 is a typical data obtained from formulation 1 (F-1). Typical plots of concentration against time and logarithm of concentration against time are shown in Figure 1 and 2, respectively. Linearity of the graphs were inspected visually and the slope of linear regression line, the correlation coefficient and F-Test at 95 % confidence limit were calculated by using a wang programable calculator model 2200. It can be seen that a logarithm of concentration against time plot gives the best straight line (53,54) which indicates a first-order reaction (50).

Specific rate constant of vitamin C degradation

The specific rate constant (k) was calculated from the slope of linear regression line (53,54). It is a meaningful indicator for the comparison of vitamin C stability in various formulations. Higher k values would result in more rapid degradation (50). The k value and its 95 % confidence limit was also shown in Table 8.

Effect of vehicles

Sixteen liquid multivitamin preparations were used in the study of the effect of vehicles on stability of vitamin C at 60°C. Table 2 shows the composition of each preparation and the specific reaction rate constant of the formula.

The effect of single vehicle on the stability of vitamin C can be seen from Formula 1 to 8 (F-1 to F-8) in Table 2 and Figure 3. The stability of vitamin C in various vehicles was in the following decreasing order : propylene glycol (F-8) > sorbitol (F-7) > corn syrup (F-6) > distilled water (F-5) > glycerin (F-4) > syrup USP (F-1).

Since syrup USP is one of the most widely used vehicle in vitamin preparations, it is surprising that degradation of vitamin C in syrup is even greater than in distilled water. It was suspected that some impurities in commercial cane sugar might accelerate the degradation of vitamin C. Therefore, analytical grade sucrose was used in formula F-2. The results show no significant difference ($P > 0.05$) between degradation of vitamin C in commercial cane sugar syrup (F-1) and in sucrose AR. syrup (F-2). In formula F-1 containing 40 % of syrup, the degradation was significantly faster than formula F-3 which contains 20 % of syrup.

The effect of mixture of vehicles on the stability of vitamin C was evaluated in formula F-9 to F-16 and summerized in Table 2 and

Figure 4. The presence of 10 % alcohol in 40 % syrup USP (F-12) exerted a noticeable stabilizing effect on vitamin C over that in 40 % syrup without alcohol (F-1).

Various combinations of syrup USP and polyhydric alcohol vehicles of propylene glycol (F-13 and F-16), sorbitol (F-10 and F-15) and glycerin (F-11 and F- 14) had less stabilizing effect than the single components of polyhydric alcohol vehicle as shown in Table 9. The polyhydric alcohols in water ($k = 2.965 \times 10^{-2}$) had greater stabilizing effect than in 20 % syrup USP ($k = 3.836 \times 10^{-2}$) and in 40 % syrup USP ($k = 4.954 \times 10^{-2}$). The presence of the following vehicle enhanced the stability of vitamin C : propylene glycol ($k = 3.028 \times 10^{-2}$) $>$ sorbitol ($k = 4.216 \times 10^{-2}$) $>$ glycerin ($k = 4.230 \times 10^{-2}$) $>$ corn syrup ($k = 4.382 \times 10^{-2}$).

The effects of vehicles on palatability of the preparation were also tested. The order of palatability was found to be as follows : sorbitol $>$ glycerin $>$ corn syrup $>$ simple syrup $>$ propylene glycol.

Color and odor changes of the preparation were qualitatively observed. Fresh preparations have a light yellow color. It gradually darken to dark yellow, brown and dark brown colors. Upon prolonged standing, the preparations showed unpleasant odor. The browning of formulations containing propylene glycol, sorbitol, glycerin and corn syrup was comparatively less than the formulation containing simple syrup (Table 2).

Effect of antioxidants and chelating agent

Formulas F-17 to F-25 were prepared in order to evaluate the effect of antioxidants and chelating agent on the stability of vitamin C. The specific rate constants were determined at $60^{\circ} \pm 0.5^{\circ}C$ and were summarized in Table 3. Cysteine hydrochloride (F-18) showed significant stabilizing effect on vitamin C while sodium metabisulfite (F-17) failed to improve its stability over the control formulation (F-1). Sodium ethylenediamine tetraacetate (sod. EDTA) (F-19) at 0.02 % was also shown to have some protective effect.

The effect of combined antioxidant and chelating agent on the stability of vitamin C in vehicle of 40 % syrup USP and in the mixture of polyhydric alcohol in simple syrup was also investigated and compared to the control formulation. It can be seen from Table 3 that the combination of cysteine hydrochloride and sodium edetate in all vehicle systems (F-21, F-25) showed stabilizing effect (compare to F-1, F-11, respectively in Table 2) while the mixture of sodium metabisulfite and sodium edetate (F-20, F-22, F-23, F-24) showed practically no effect on stability of vitamin C (compare to F-1, F-11, F-10, F-13 respectivety in Table 2).

The following order of protective effect may be concluded from Table 10 that cysteine hydrochloride ($k = 4.762 \times 10^{-2}$) > cysteine hydrochloride + EDTA ($k = 5.007 \times 10^{-2}$) > EDTA ($k = 5.550 \times 10^{-2}$) sodium metabisulfite ($k = 5.858 \times 10^{-2}$) > sodium metabisulfite + EDTA

($k = 5.913 \times 10^{-2}$). However, cysteine hydrochloride has some disadvantages of sulfur smell, acid taste and rapid color change after prolonged storage which is undesirable in the product.

Effect of suspending agent, preservative and flavoring agents

Since the formulas containing core formula and vehicle were unacceptable for consummers, modification of such formulas were made by adding suspending agents, preservatives and flavor. The presence of these agents may alter the stability of vitamin C in the product. To test this hypothesis, formula F-26 to F-35 with various combinations of these agents (see Table 4) were prepared and evaluated for vitamin C stability at 60°C. Table 4 shows the effect of suspending agent, preservative and flavoring agent on stability of vitamin C.

When sodium carboxymethylcellulose (CMC) was used (F-35), the product seemed to be more stable, though not significant than without it (F-31) (see Table 11).

The effect of preservative on vitamin C stability was shown in Table 12. Although, there was no significant effect of methylparaben and sodium benzoate on vitamin C stability, methylparaben ($k = 4.425 \times 10^{-2}$) had more tendency of stabilization of vitamin C than sodium benzoate ($k = 4.755 \times 10^{-2}$).

Flavors such as vanillin with strawberry and banana had no significant stability effect on vitamin C (Table 13). The specific rate effected by vanillin and strawberry ($k = 4.110 \times 10^{-2}$) was not different from banana ($k = 4.009 \times 10^{-2}$).

Effect of pH and buffer

The effect of pH on stability of vitamin C was studied in the pH range 2.5 to 4 in formulas F-36 to F-44 (Table 5). Acetate and citrate buffers were used in the preparations and the ionic strength was adjusted to 0.5 by sodium chloride. The results in Table 5 and Figure 5 show no significant pH effect in citrate buffer. In acetate buffer, an increasing in pH would increase in degradation of vitamin C. However, citrate buffer had more browning effect than acetate buffer and the color was more intense in both buffer when the pH was decreased.

Formulation

In the previous sections, the effects of various possible pharmaceutical additives on the stability of vitamin C in liquid multivitamin preparation were thoroughly investigated. Suitable ingredients and conditions were assembled in order to obtain acceptable products in this section.

Formulations of various types of mixed vehicles, F-45 to F-51, were prepared and determined their specific rate constant as shown in Table 6 and Figure 6. The result showed confirmatively to the effect of vehicle on the stability of vitamin C in previous section that vehicle containing of syrup USP had the deterioration effect on vitamin C (F-46). The mixture of polyhydric alcohol vehicles were effective stabilizer for vitamin C. Propylene glycol at 5 % concentration had no bitter taste but enhanced the stabilizing effect (F-47 to F-49). The mixture

of sorbitol, glycerin and corn syrup showed the most stabilizing effect (F-51). Single polyhydric alcohol vehicle of sorbitol and glycerin showed slight effect on the stability of vitamin C (F-45, F-50). Sodium edetate had slight protective effect on vitamin C (F-47, F-48). Corn syrup used to increase the consistency of product would be satisfactory with the physical appearance and stabilizing effect on vitamin C.

Kinetic studies on the stability of vitamin C in liquid multivitamin formulations

Five formulations were developed based on informations obtained in the previous sections. The combinations of ingredients in these formulas (F-53 to F-57 in Table 7) were expected to yield acceptable formula from both chemical and physical viewpoints. In the purpose of chemical evaluation of vitamin C stability, kinetic parameters were also determined and the validity and reliability of Arrhenius method for prediction of shelf-life was assessed.

The Arrhenius Relationship

The stability of vitamin C in five formulations (F-53 to F-57) and a commercial product (F-52) were determined by accelerated stability testing method and compared to the results obtained from usual room temperature ($30^{\circ} \pm 3^{\circ}C$) and air-conditioned room ($20^{\circ} \pm 1^{\circ}C$) storage. All formulations tested were incubated at 20°, 30°, 40°, 50°, 60° and $70^{\circ}C$. Once the desired temperature was attained, the first samples

were taken at zero time of storage and analyzed for their vitamin C contents. The concentration at zero time was refered as 100 percent of initial concentration. The concentration of vitamin C remaining in subsequent samples were calculated as percentage of initial concentrations. Table 14 showed the concentration of vitamin C remaining at time of sampling. Typical plot of logarithm of the remaining concentration against sampling time are shown in Figure 7. The results of these plots were all linear which indicate a first order degradation for vitamin C. The specific rate constants (k) were calculated from the slope of linear regression line for each product at specified temperature and are summerized in Table 15.

The Arrhenius plot of logarithm of specific rate constant (ln k) versus the corresponding reciprocal of degree kelvin $(\frac{1}{T})$ was also linear as shown in Figure 8. The vertical line at each temperature represent the 95 % confidence limits of individual standard error of apparent apecific rate constant. The Arrhenius plot was extrapolated to temperature 30° and $20^{\circ}C$. The verticle line at these temperatures represent the 95 % confidence limits of individual standard error of prediction, the dash lines is the 95 % confidence interval for the standard error of prediction at any other temperature.

In all formulations studied, the Arrhenius plot were all linear (Table 15, 16).

Heat of Activation

The apparent heat of activation (Δ Ha) of each formulation was calculated from the slope of linear regression of its Arrhenius plot (50,51). The heat of activation and its 95 % confidence limits of standard error were shown in Table 17 and Figure 10. It was found that the heat of activations of five formulations developed were in the range 13-15 kcal/mol, while the commercial product (F-52) had a value of 18 kcal/mol.

Predicted rate and observed rate

Predicted rates were obtained from Arrhenius plot at higher temperature and extrapolated to room temperature at 30°C ($\frac{1}{T}$ = 3.300 x 10^{-3}K) and at 20°C ($\frac{1}{T}$ = 3.413 x 10^{-3}K) as shown in a typical plot in Figure 8. The height of rectangles at 30° and 20°C represent the standard error of prediction, the bars at these temperatures and the dash line represent the 95 % confidence interval for the standard error of prediction at any temperature. It was shown that the longer extrapolation, the larger the error and less accurate in prediction. The results of predicted rate at 30° and 20°C of all formulations were shown in Table 17 and Figure 9.

The observed rate at room temperature (30°C) and at air-conditioned temperature (20°C) were determined according to first-order rate. The predicted rate and the observed rate of each formulation at 30°C and 20°C were shown comparatively in Table 18 and

19 and in Figure 12. It can be seen that no significant difference in the degradation rate obtained from prediction and actual normal storage. These results have proved the reliability and validity of the accelerated stability study method for prediction of the stability of vitamin C in various liquid multivitamin formulations at actual normal storage conditions. (52, 55) (see Figure 11).

Vitamin C in five developed formulations (F-53 to F-57) were more stable than the commercial product (F-52). Table 22 shows the stability of vitamin C in various formulas was in the following decreasing order : F-54 (1.714×10^{-3}) > F-57 (1.882×10^{-3}) > F-55 (1.969×10^{-3}) > F-56 (2.081×10^{-3}) > F-53 (2.655×10^{-3}) > F-52 (3.840×10^{-3}).

Finally, the liquid multivitamin formula of the most stable vitamin C and good appearance is yielded. The formula of each 5 ml contains : vitamin A 5000 I.U. , vitamin D 1000 I.U. , thiamine hydrochloride 2 mg, riboflavin 2 mg, pyridoxine hydrochloride 2 mg, vitamin C 75 mg, vitamin B_{12} 3 mcg, nicotinamide 20 mg, saccharin sodium 0.1 %, sorbitol 40 %, propylene glycol 5 %, corn syrup 40 %, EDTA 0.02 %, MP 0.1 %, vanillin and strawberry flavor 0.1 %.

Shelf-life

The degradation of vitamin C was previously determined of a first-order rate. Therefore, its shelf-life (t_{90}) can be calculated from the first-order reaction at 100 % concentration degraded to reach 90 % (50,51). The shelf-life of vitamin C in all formulations, calculated

from predicted rate and actual normal rate was also no significant difference at 95 % confidence interval (see Table 20, Figure 13). The shelf-life at 20°C was longer than at 30°C and the five developed formulations (F-53 to F-57) had also longer shelf-life than the commercial product (F-52).