Stability of Ascorbic Acid in a Liquid Multivitamin Emulsion Containing Sodium Fluoride

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The kinetics of the thermal oxidative nonphotolytic degradation of ascorbic acid in a liquid multivitamin emulsion containing sodium fluoride have been studied. The vitamin appears to degrade initially by a pseudo zero-order reaction, but other evidence indicates that the true order of the reaction is closer to one. The reaction rate increases rapidly as the pH rises from 3.2 to 3.8, and it is this pH effect which probably causes the reaction to appear zero-order in its initial stages. By use of the Arrhenius equation, data obtained from high temperature studies have been used to predict shelf life at room temperature. Factors affecting the accuracy of the predictions are discussed. Sodium fluoride does not appreciably affect the stability of ascorbic acid in this preparation, but a reaction between sodium fluoride and glass, producing a precipitate (Na_2SiF_6), has been observed.

IN THE development of new pharmaceutical products, investigators are often asked to pass judgment on the shelf life of a product long before complete room temperature stability data are available. It then becomes necessary to predict the room temperature shelf life using data obtained from accelerated kinetic studies of degradation rates at elevated temperatures. The theory and mechanics of such predictions have been well outlined by E. R. Garrett of these laboratories (1–3). In order to make the best use of these methods, it is necessary to give careful consideration to all factors that might affect the accuracy of the predictions.

The present investigation was undertaken to determine the shelf life of a liquid o/w multivitamin emulsion containing, as active ingredients, sodium fluoride and vitamins A, B_6, D, and C. Results of preliminary experiments indicated that the stability of ascorbic acid would be the limiting factor in the shelf life of this product; consequently, only the stability of that vitamin was followed during the accelerated kinetic studies. Although Garrett (2) had already studied the stability of ascorbic acid in this type of system, it had been shown that many additives significantly affect the stability of ascorbic acid (4–6) and that the reactions by which ascorbic acid degrades are many and complex (7–9). Consequently, it was necessary to conduct kinetic studies on the new product before reasonably accurate stability predictions could be made. The effect of pH and sodium fluoride on the ascorbic acid stability and the nature of the reaction between sodium fluoride and glass were also studied to a limited extent.

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EXPERIMENTAL

Composition of the Product.—The product is a water-polyol based o/w emulsion labeled to contain, in each 0.6 ml., 1.105 mg. sodium fluoride, 1.5 mg. vitamin A palmitate in oil, 1.0 mg. pyridoxine hydrochloride U. S. P., 25 mcg. vitamin D_3 in oil, 50 mg. ascorbic acid U.S.P., and various sweetening agents, preservatives, emulsifiers, *etc.* When necessary, the pH of the product is adjusted to the desired level with 0.1 *N* sodium hydroxide. The pH of this product is very erratic and can vary as much as ±0.2 unit from lot to lot and can vary with time within the same lot. This makes it difficult (*a*) to set up experiments studying variables other than pH, and (*b*) to evaluate results of these experiments.

As usual, the overage on the labeled amounts of the vitamins is that required to attain acceptable room temperature shelf life. However, since this report is concerned with the stability of ascorbic acid only, it will suffice to say that the theoretical initial concentration of ascorbic acid is 82.5 mg./0.6 ml. in one formula (Preparation I, pH adjusted to 3.35) and 70.0 mg./0.6 ml. in the other (Preparation II, pH adjusted to 3.20).

Kinetic Studies on the Effect of Temperature on Ascorbic Acid Stability.—After determining (by careful gross visual examination) that the emulsion was stable over the temperature range of the kinetic study, 30 ml. of the preparation was filled into 38-ml. capacity high-density polyethylene bottles; the bottles were then loosely capped and placed in amber 1-lb. ointment jars. When the bottles were tightly capped, the CO_2 pressure buildup, caused by the degradation of ascorbic acid and by the elevated temperatures, ruptured the plastic containers. Glass containers could not be used here because of a reaction between sodium fluoride and glass. The loss of water (through vaporization) from the loosely capped bottles was found to be negligible. Amber jars were used because the product would be stored in opaque cardboard containers; therefore, only the nonphotolytic degradation was of interest.

The jars were then sealed and immersed in constant temperature baths set at 70.0, 60.0, 47.0, and

40.0 (±0.1°). Samples were periodically removed from the baths and immediately refrigerated. Just prior to being assayed (by the U.S.P. iodometric titration method) the samples were allowed to equilibrate at room temperature. Both Preparation I and Preparation II (see previous section) were subjected to this treatment.

Kinetic Studies on the Effect of pH, Initial Ascorbic Acid Concentration and Sodium Fluoride on Ascorbic Acid Stability.—To determine the effect of pH on ascorbic acid stability, one lot of the product was divided into three parts, and the pH of each part was adjusted to a different level (3.15, 3.40, and 3.80).[1] The samples were stored as outlined above, this time at 70.0° only, and the stability was determined in the usual manner.

To help determine the apparent order of the reaction, the effect of initial ascorbic acid concentration on the apparent degradation rate was studied. One lot of the product was made with an initial ascorbic acid concentration of 40.0 mg./0.6 ml. (Preparation III, pH adjusted to 3.50).[2] Again the samples were stored at 70.0° only, and the stability was followed in the usual way.

In order to study the effect of sodium fluoride on the ascorbic acid stability in this product, part of Preparation III was processed without sodium fluoride (Preparation IV, pH adjusted to 3.30).[2] These samples were stored at 70.0° and the stability was determined in the usual manner.

Studies on the Effect of Sodium Fluoride on Glass.—Initially, it was planned to package this product in glass, the standard container for this type of preparation. However, after only 24 hours storage in ordinary soft glass or in U.S.P. Type I glass, the product developed a precipitate. To determine the nature of this material, it was separated from the emulsion by centrifugation and subjected to X-ray diffraction analysis using a General Electric XRD-5 diffraction unit.

To further elucidate the conditions under which this precipitate would form, sodium fluoride A.R. (1.105 mg./0.6 ml.) and ascorbic acid (50.0 mg./0.6 ml.) were dissolved in deionized water; and the pH was adjusted to various levels (2.78, 3.25, 4.10, 4.79, 5.80, and 6.35) with 0.1 *N* sodium hydroxide. The solutions were stored in ordinary soft glass and in high-density polyethylene bottles. They were observed for 1 week at room temperature, after which they were assayed for sodium fluoride. When a precipitate was present in the bottle the clear supernatant was used for the assay sample.

RESULTS AND DISCUSSION

The reactions by which ascorbic acid degrades are many and complex (7–9). It is not the intent here to present a complete discussion of the subject, but rather to briefly summarize the more important details so that the data presented herein will be more meaningful. A more complete account may be found in the literature.

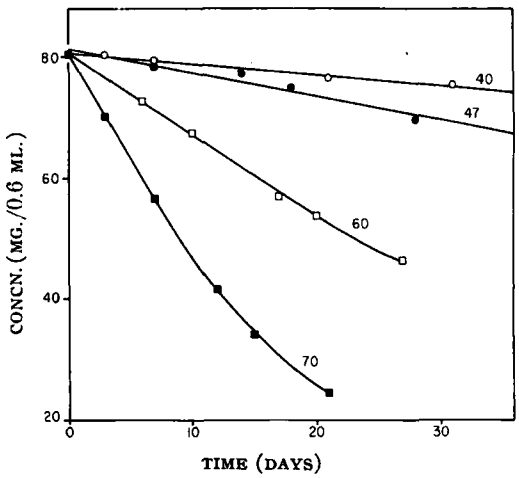

Fig. 1.—Zero-order plots of the thermal oxidative degradation of ascorbic acid in Preparation I; concentration (mg./0.6 ml.) against time (days).

In aqueous solution, ascorbic acid undergoes both a reversible (oxidative) and an irreversible (nonoxidative) degradation. The nonoxidative reaction rate, apparently first-order, decreases with an increase in pH in the 2.2 to 6.0 range; it then increases as the pH rises to 7.0 and above. The products of this anaerobic degradation are furfural, carbon dioxide, and numerous uncharacterized compounds (7). This reaction has not been studied as extensively as the oxidative one.

The oxidative reaction, catalyzed by copper and other trace substances, is 100–1000 times faster than the nonoxidative (7). The aerobic reaction rate increases rapidly as the pH rises from 2.0 to 5.0, then decreases slightly from pH 5.0 to 9.0, then increases rapidly again above pH 9.0. The irregularity of the pH-stability curve seems to be due to the differences in concentrations of the three species of ascorbic acid (undissociated, singly ionized, and doubly ionized) that can exist in aqueous solution at various pH's, and to the different reaction rate constants associated with each of these species (8).

The first step of the oxidation process, in which dehydroascorbic acid is produced, is reversible.[3] However, subsequent oxidation of dehydroascorbic acid is irreversible, and leads to the production of oxalic and threonic acids, carbon dioxide, hydrogen peroxide, and numerous uncharacterized compounds

[1] The pH of the emulsion rises about 0.20 units at room temperature the first one or two days after it is processed; from then on it remains relatively constant at room temperature. The recorded pH's are those measured after the pH has reached this "constant" value. However, at elevated temperatures, the pH continues to rise (0.15 to 0.20 units) during the first part of the kinetic study (how fast it rises depends on the temperature); after that it remains relatively constant. All pH determinations were made on a Beckman Zeromatic pH meter.

[2] In order to eliminate the pH effect on the degradation rate, it was necessary to adjust the pH of these formulas to about the same level as the pH of Preparation I. But since it is extremely difficult to attain the exact desired pH in these systems, there is a measurable pH difference between Preparations I, III, and IV. These differences should be kept in mind when evaluating the data.

[3] The term "reversible," as used here, means that by using appropriate reducing agents, dehydroascorbic acid can be reconverted to ascorbic acid (8). There is a great deal of disagreement as to the optimum pH for the ascorbic acid-dehydroascorbic acid interconversion reaction. Ball (10) says the reaction takes place only below pH 5.0, while Fruton (11) states that it takes place between 5.5 and 7.5. Ghosh and Char (12) have shown that it is possible to reduce dehydroascorbic acid to ascorbic acid in the 2.5 to 7.5 pH range. Levenson (13) says that the irreversible oxidation rate is slowest at pH 3.5. This illustrates that ascorbic acid degradation is extremely complex, and that different results can be obtained by making relatively small changes in the system.

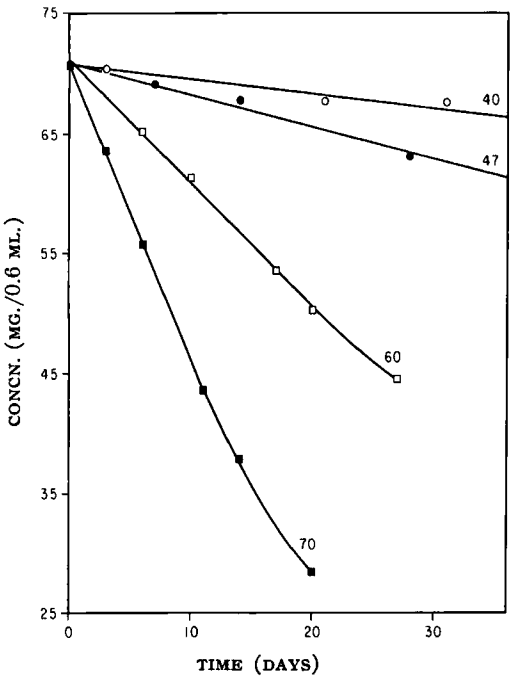

Fig. 2.—Zero-order plots of the thermal oxidative degradation of ascorbic acid in Preparation II; concentration (mg./0.6 ml.) against time (days).

TABLE I.—TABULATION OF SLOPES (k_0),[a] THEIR STANDARD DEVIATIONS (σk_0), AND THEIR 95% CONFIDENCE LIMITS FOR ZERO-ORDER PLOTS

Temp., °C.	k_0 (mg./0.6 ml./day)	σk_0	95% Confidence Limits
Preparation I (pH 3.35 and Initial Ascorbic Acid Concn. 82.5 mg./0.6 ml.)			
70.0	3.411	0.042	2.875–3.946
60.0	1.370	0.024	1.294–1.445
47.0	0.380	0.040	0.254–0.507
40.0	0.176	0.017	0.103–0.250
Preparation II (pH 3.20 and Initial Ascorbic Acid Concn. 70.0 mg./0.6 ml.)			
70.0	2.485	0.029	2.359–2.610
60.0	1.033	0.030	0.939–1.127
47.0	0.271	0.025	0.163–0.379
40.0	0.112	0.021	0.022–0.202

[a] For a plot of concentration *vs.* time, the slope = the zero-order rate constant (k_0).

(8, 9). Some of the oxidation products act as catalysts for the degradation of more ascorbic acid; the drug thus undergoes autoxidation (9).

The literature is in general agreement on the fact that ascorbic acid undergoes apparent first-order degradation in simple drug-buffer systems (8, 9). However, in a more complex pharmaceutical system (containing other vitamins, flavors, sugars, etc.), Garrett (2) found that the degradation of ascorbic acid was pseudo zero-order during the initial phase of the reaction. In the later stages apparent first-order kinetics prevailed.

Effect of Temperature.—The effect of temperature on the degradation rate of ascorbic acid is shown in Figs. 1 and 2 where concentration of ascorbic acid is plotted against time at four different temperatures for Preparations I and II, respectively. The behavior of the points (a straight line can be drawn through the points above 45 mg./0.6 ml.; below that concentration the line is curved) at each temperature indicates that the reaction is pseudo zero-order during its initial stages; however, as the reaction proceeds, first-order kinetics become apparent. These results agree essentially with those of Garrett (2). A summary of the statistical treatment of the zero-order data obtained from Figs. 1 and 2 is given in Table I.

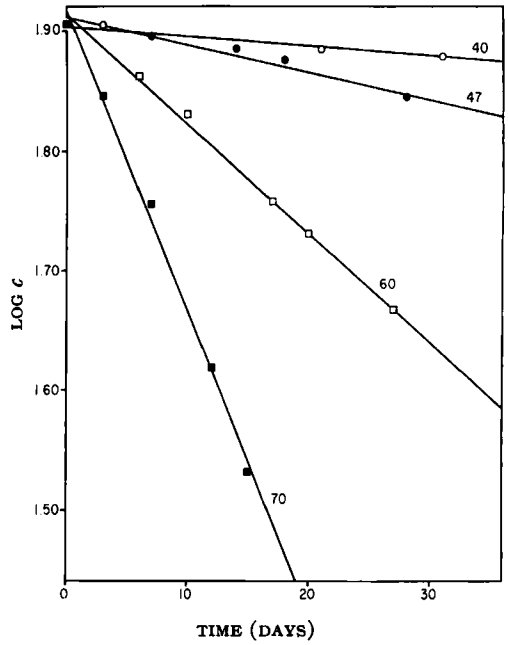

Fig. 3.—First-order plot of the thermal oxidative degradation of ascorbic acid in Preparation I; log concentration (mg./0.6 ml.) against time (days).

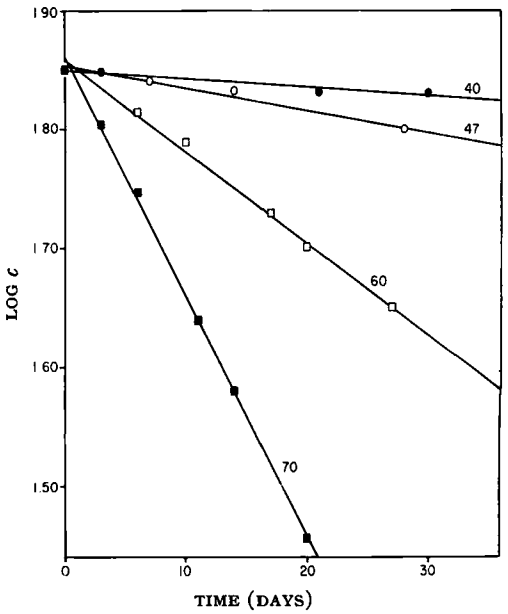

Fig. 4.—First-order plot of the thermal oxidative degradation of ascorbic acid in Preparation II; log concentration (mg./0.6 ml.) against time (days).

TABLE II.—TABULATION OF SLOPES (k_1),[a] THEIR STANDARD DEVIATIONS (σk_1), AND THEIR 95% CONFIDENCE LIMITS FOR FIRST-ORDER PLOTS

Temp., °C.	$k_1 \times 10^2$ (day^{-1})	$\sigma k_1 \times 10^2$	95% Confidence Limits ($\times 10^2$)
Preparation I (pH 3.35 and Initial Ascorbic Acid Concn. 82.5 mg./0.6 ml.)			
70.0	2.532	0.058	2.372–2.692
60.0	0.908	0.025	0.838–0.978
47.0	0.219	0.027	0.135–0.303
40.0	0.098	0.012	0.047–0.148
Preparation II (pH 3.20 and Initial Ascorbic Acid Concn. 70.0 mg./0.6 ml.)			
70.0	2.009	0.042	1.891–2.126
60.0	0.761	0.027	0.686–0.836
47.0	0.176	0.018	0.097–0.255
40.0	0.070	0.013	0.014–0.126

[a] For a plot of logarithm *vs.* time, the slope = k_1 = the first-order rate constant/2.303 = k_1'/2.303.

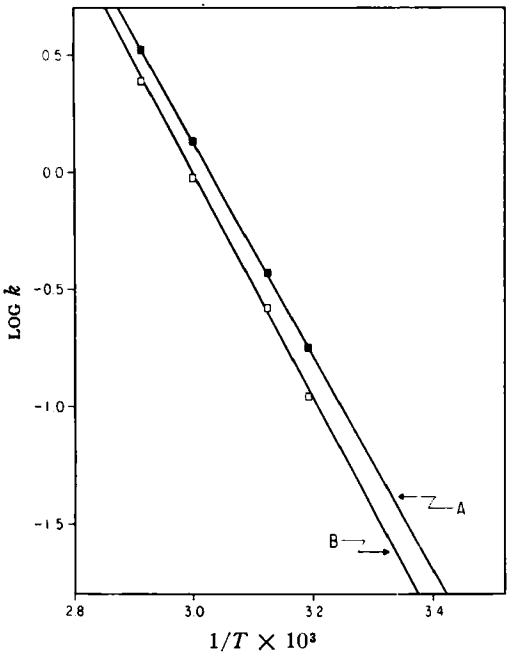

Fig. 5.—Arrhenius plots of log k (zero-order rate constants from Figs. 1 and 2; mg./0.6 ml./day) against the reciprocal of the absolute temperature for Preparation I (plot A) and Preparation II (plot B).

In making predictions of shelf life it is essential to consider the possibility that the reaction might be pseudo first-order from the beginning, and then to consider what the difference is between predictions based on zero-order kinetics and those based on first-order kinetics. Therefore, the logarithm of ascorbic acid concentration was plotted against time (Figs. 3 and 4) for Preparations I and II, respectively. For convenience and ease of calculation, the numerical value of the slopes (k_1) of the lines in Figs. 3 and 4 were used in the Arrhenius plots, calculations of shelf life, etc., rather than the actual first-order rate constant (k_1'). The first-order rate constants could be obtained by plotting logarithm *vs.* time, or by using the following equation: $2.303\ k_1 = k_1'$. A summary of the statistical treatment of these first-order data is given in Table II.

Arrhenius Plots.—In order to best predict the shelf life of this product, Arrhenius plots were made using data obtained from both the zero-order and first-order plots. In Fig. 5 the logarithm of the zero-order rate constants for both preparations (obtained from Figs. 1 and 2) are plotted against the reciprocal of the absolute temperature, while in Fig. 6 the same thing is done for the "first-order" slopes obtained from Figs. 3 and 4. A summary of the statistical treatment of the Arrhenius plot data is given in Table III. It should be noted that the apparent heats of activation for the reaction [these agree very well with those obtained by Garrett (2)] are very similar in all four cases, but that the shelf lives are considerably different, depending on which order is assumed for the reaction. This is due, in part, to the fact that a first-order reaction will slow down as substrate concentration decreases, whereas a zero-order reaction will continue at a constant rate.

Effect of pH.—The effect of pH on the degradation rate of ascorbic acid can be seen in Fig. 7 where a zero-order plot is made for the degradation of ascorbic acid at 70° at three different pH's, and in Fig. 8 where the same data are plotted as first order. The pH dependence of the reaction rate can better be seen in Figs. 9 and 10, where zero-order reaction rates (Fig. 9) and "first-order" slopes (Fig. 10) are plotted against pH. The values for the k's at pH 3.20 and 3.35 were obtained from the slopes of the 70° plots in Figs. 1 and 2. Since

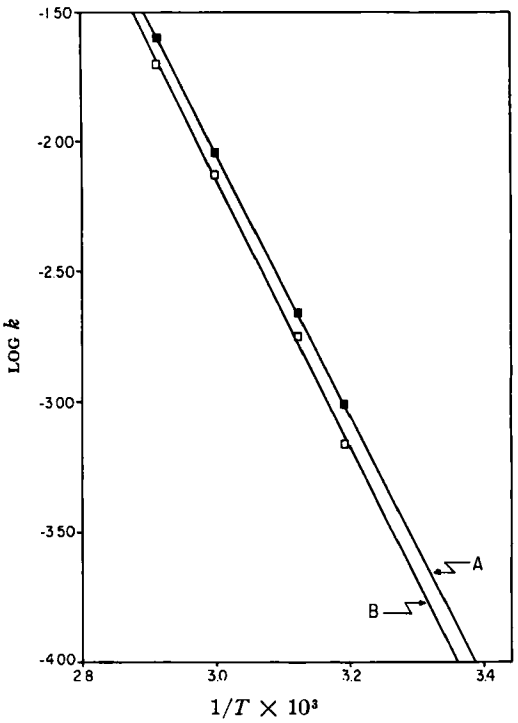

Fig. 6.—Arrhenius plots of log k (slopes of first-order plots from Figs. 3 and 4; day^{-1}) against the reciprocal of the absolute temperature for Preparation I (plot A) and Preparation II (plot B).

TABLE III.—TABULATION OF ARRHENIUS EQUATIONS OF BEST FIT, APPARENT HEATS OF ACTIVATION (ΔH_a), k_0 AND k_1 AT ROOM TEMPERATURE, SHELF LIFE (t_{90})[a] AND 95% CONFIDENCE LIMITS OF SHELF LIFE

Preparation	Equation of Best Fit	ΔH_a (Kcal/mole)	Predicted k_0 and k_1 (25° C.)[b]	t_{90}, Years	95% Confidence Limits of t_{90}
I—Zero-Order	$\log k_o = -4599/T + 13.94$	21.0	0.0326	3.15	2.79–3.56
II—Zero-Order	$\log k_o = -4809/T + 14.43$	22.0	0.0200	3.43	1.90–6.00
I—First-Order	$\log k_1 = -5077/T + 13.20$	23.2	0.000148	4.89	4.29–5.57
II—First-Order	$\log k_1 = -5216/T + 13.52$	23.9	0.000106	4.95	3.16–7.72

[a] The shelf life is defined as the time it takes for the ascorbic acid to degrade to 90% of the labeled potency. Since the labeled potency is 50 mg./0.6 ml., t_{90} for Preparation I is the time it takes for the ascorbic acid concentration to drop from 82.5 mg./0.6 ml. (initial) to 45.0 mg./0.6 ml. (90% of label); and t_{90} for Preparation II is the time it takes for the ascorbic acid concentration to drop from 70.0 mg./0.6 ml. (initial) to 45.0 mg./0.6 ml. (90% of label). [b] Units for k_0 are mg./0.6 ml./day and the units for k_1 are day^{-1}.

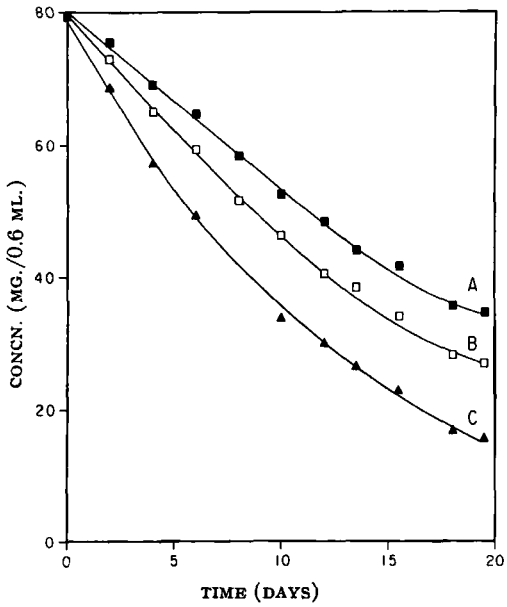

Fig. 7.—Zero-order plot showing the effect of pH on the degradation rate of ascorbic acid; *A*—pH 3.15, *B*—pH 3.40, *C*—pH 3.80; concentration (mg./0.6 ml.) against time (days). Temperature was 70°.

Preparation I and Preparation II have slightly different initial ascorbic acid concentrations, the zero-order reaction rate constants associated with each of these formulas will be slightly different, regardless of pH (see next section). This has not been taken into account in Figs. 9 and 10.

The straight lines in Figs. 9 and 10 are not meant to imply that there is a strict linear relationship between pH and reaction rate in this pH range; rather they are meant to illustrate the significant effect of pH on ascorbic acid stability. Csuros and Petro (8) have represented the pH profile of the reaction as being slightly curved in this pH region. They showed the same significant pH dependence of the reaction rate that is reported here.

Effect of Initial Ascorbic Acid Concentration.—The effect of initial ascorbic acid concentration on its degradation rate is shown in Table IV, where the k's (70°) for Preparation I (82.5 mg./0.6 ml.) are compared with those for Preparation III (40.0 mg./0.6 ml.). The k's for Preparation III were obtained from the *A* plot in Fig. 11 (zero-order) and Fig. 12 (first-order). Note that the pH of Preparation I is lower than that of Preparation III. Considering the pH effects only, a faster rate for Preparation III would be expected. However, the comparison shows that a drop in initial ascorbic acid concentration overcomes the pH effect and slows the rate. The apparent zero-order rate constant is affected much more than is the "first-order" slope; and by using appropriate equations, it can be shown that the true reaction is closer to first-order than to zero-order (14). The cause(s) of the apparent drop in the value of the first-order rate constant when the initial concentration was reduced, even though the pH difference favored a change in the opposite direction, was (were) not investigated.

A possible explanation for the apparent zero-order character of the first part of the reaction is that the initial rise in pH at elevated temperatures (see footnote 3) causes an increase in the reaction rate which offsets the tendency of the first-order reaction to slow down as the substrate concentration decreases. As soon as the pH reaches a constant value, no further deviations from first-order kinetics

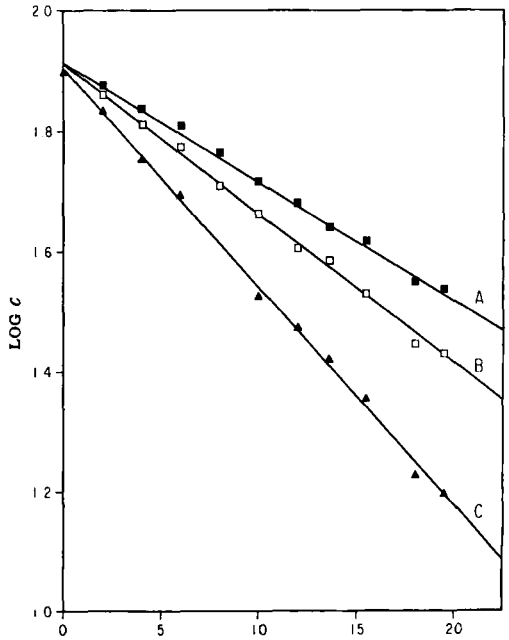

Fig. 8.—First-order plot showing the effect of pH on the degradation rate of ascorbic acid; *A*—pH 3.15, *B*—pH 3.40, *C*—pH 3.80, log concentration (mg./0.6 ml.) against time (days). Temperature was 70°.

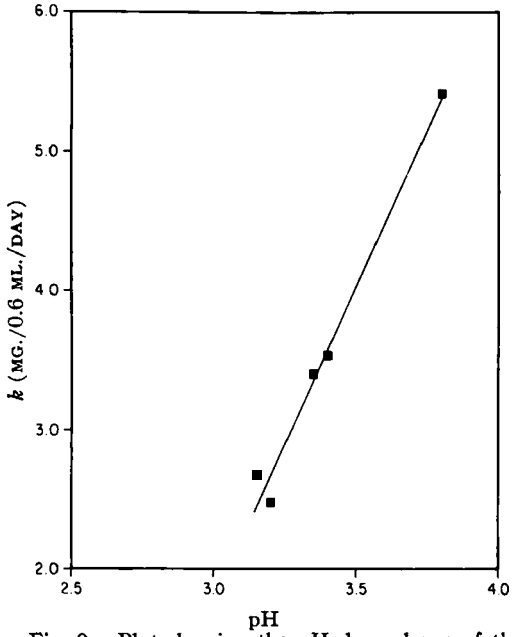

Fig. 9.—Plot showing the pH dependence of the degradation rate; zero-order rate constants (70°; mg./0.6 ml./day) against H.

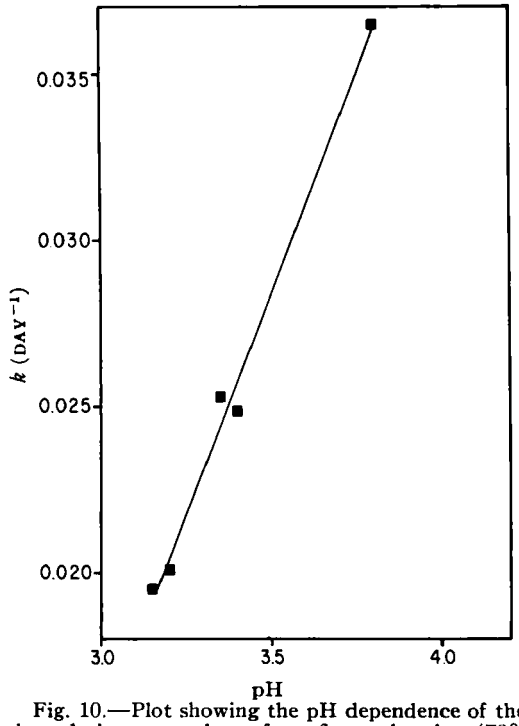

Fig. 10.—Plot showing the pH dependence of the degradation rate; slopes from first-order plots (70°; day^{-1}) against pH.

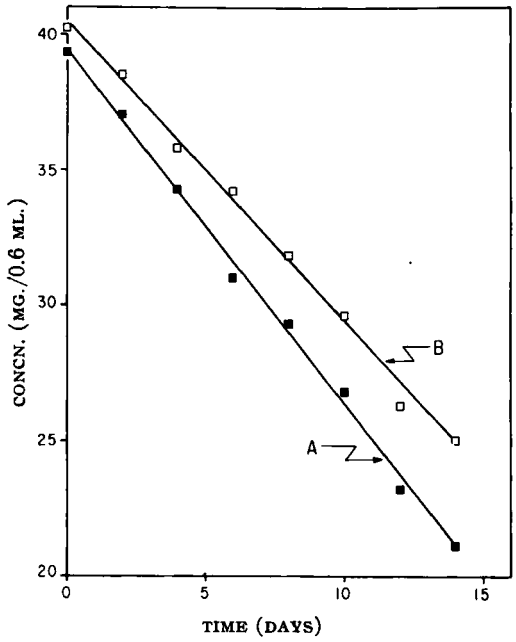

Fig. 11.—Zero-order plot showing the effect of sodium fluoride on ascorbic acid stability at 70°; *A*—Preparation III (with sodium fluoride), *B*—Preparation IV (without sodium fluoride); concentration (mg./0.6 ml.) against time (days).

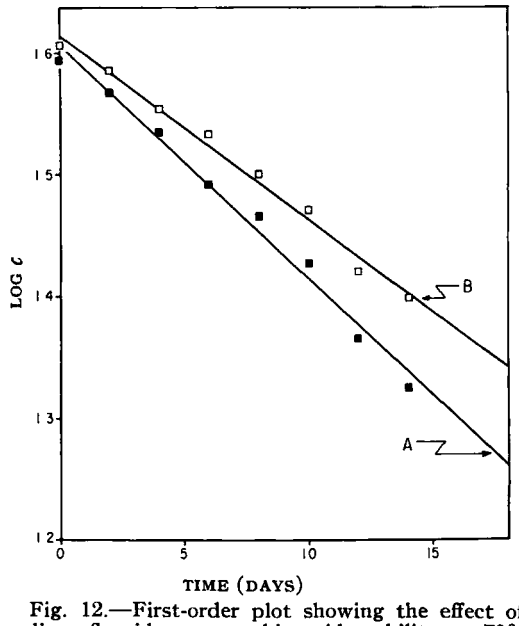

Fig. 12.—First-order plot showing the effect of sodium fluoride on ascorbic acid stability at 70°; *A*—Preparation III (with sodium fluoride), *B*—Preparation IV (without sodium fluoride); log concentration (mg./0.6 ml.) against time (days).

Table IV.—Comparison of Zero-Order Rate Constants (k_0) and "First-Order" Slopes (k_1) for Preparation I (82.5 mg./0.6 ml.) and Preparation III (40.0 mg./0.6 ml.) at 70°C.

Preparation	pH	k_0 (mg./0.6 ml./day)	k_1 (× 10^2 day^{-1})	k_0 (I)/k_0 (III)	k_1 (I)/k_1 (III)
I	3.35	3.411	2.532	2.60	1.31
III	3.50	1.310	1.930		

TABLE V.—COMPARISON OF ZERO-ORDER RATE CONSTANTS (k_0) AND "FIRST-ORDER" SLOPES (k_1) FOR PREPARATION III (WITH SODIUM FLUORIDE) AND PREPARATION IV (WITHOUT SODIUM FLUORIDE)

Preparation	pH	k_0 (mg./0.6 ml./day)	k_1 ($\times 10^2$ day^{-1})	k_0 (III)/k_0 (IV)	k_1 (III)/k_1 (IV)
III	3.5	1.310	1.930	1.17	1.27
IV	3.3	1.120	1.514		

are apparent. The fact that (*a*) the period of pH change corresponds closely with the period during which zero-order kinetics are apparent, and (*b*) the magnitude of the deviations from the first-order character of the reaction is compatible with the magnitude of the pH effect on the degradation rate, supports this explanation.

Effect of Sodium Fluoride.—The effect of sodium fluoride on the stability of ascorbic acid is shown in Figs. 11 and 12. Plot *A* in each figure is the plot of the kinetic data obtained at 70° for Preparation III, while plot *B* is the plot of the kinetic data obtained at 70° for Preparation IV (identical to III except that it contains no fluoride). It can be seen (Table V) that sodium fluoride does not significantly affect the stability of ascorbic acid. The small increase in reaction rate seen with Preparation III can be explained on the basis of its slightly higher pH.

Effect of Sodium Fluoride on Glass.—The precipitate which was isolated from the preparations stored in glass was identified by X-ray diffraction analysis as sodium fluosilicate, Na_2SiF_6 (15). Further studies on the effect of pH on precipitate formation showed that the precipitate forms between pH 2.4 and 4.3 overnight at room temperature, but not between pH 4.8 and 6.5, after 7 days' storage at room temperature. Assays showed no detectable loss of fluoride ion from the system in those preparations in which a precipitate formed. It is possible that a small amount of hydrogen fluoride is liberated at the more acid pH's and that this material attacks the glass. Longer term studies were not carried out because the tests showed that the precipitate forms at the pH's (2.8–3.4) which, because of stability and taste considerations, are optimum for the product. Therefore, it was necessary to package the product in plastic bottles.

Correlation of Predictions with Actual Room Temperature Stability.—The problem in this study was not so much one of predicting shelf life for a product containing a definite amount of ascorbic acid and having a certain pH; rather it was one of deciding what initial ascorbic acid concentration and what pH would allow a shelf life of 2 years for the product. On the basis of the results of this study it was decided to market the product at pH 3.4 or slightly lower with an initial ascorbic acid concentration of 82.5 mg./0.6 ml. These predictions are based on zero-order kinetics because (*a*) the reaction is pseudo zero-order in the concentration range of interest, and (*b*) predictions based on zero-order kinetics give the shortest shelf life; therefore predictions based on zero-order kinetics will err on the safe side, if at all. The first-order treatment of the data has been included for informational purposes. The usefulness of this general approach is demonstrated by the fact that preliminary (6 months) room temperature stability data on the above formulation indicate a shelf life of 2.2–2.8 years.

General Considerations.—An attempt has been made to investigate those problems which could be solved in a reasonable length of time and whose solution might lead to a better product or to a significantly more accurate prediction of shelf life. No attempt has been made to ascertain (*a*) the effect of the polyol concentration and of temperature on the thermodynamic activity of the hydrogen ions and on the dissociation constants of ascorbic acid, (*b*) the effect of ionic strength on the degradation rate, (*c*) the effect of loosening the caps of the polyethylene bottles on the diffusion of oxygen, or (*d*) the effect of temperature on the diffusion rates of oxygen, carbon dioxide, and water vapor through the walls of the polyethylene bottles. These problems were not investigated because (*a*) the system is too complex, and (*b*) the answers to these questions are not likely to lead to a better product or to a significantly more accurate prediction of shelf life. The effect of antioxidants on the ascorbic acid stability was not studied because previous experience had shown that antioxidants are not particularly effective in stabilizing ascorbic acid in this type of system.

SUMMARY

1. The degradation rates of ascorbic acid at elevated temperatures in a liquid, o/w emulsion-type multivitamin preparation have been determined. Arrhenius plots have been made using these kinetic data, and room temperature shelf life has been predicted. Preliminary room temperature studies indicate that these predictions are accurate.

2. The reaction appears to be pseudo zero-order during the initial stages of the reaction; in the latter phases it assumes first-order characteristics. Studies on the effect of initial ascorbic acid concentration on the apparent degradation rate indicate that the true reaction rate is probably closer to first-order than to zero-order.

3. The reaction rate increases rapidly as the pH increases from 3.2 to 3.8. This effect of pH is postulated to be the reason for the zero-order nature of the initial phases of the reaction.

4. Sodium fluoride does not appreciably affect the stability of ascorbic acid in this product.

5. Storing the product in glass results in a precipitate (Na_2SiF_6, sodium fluosilicate) which forms at room temperature within 24 hours between pH 2.4 and 4.3 but not between pH 4.8 and 6.5 after seven days.

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Problems in Chemotaxonomy I

Alkaloids of *Peschiera affinis*

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A phytochemical study of this plant has yielded three indole alkaloids, two of them being of the α-ketoindole type and the third containing a simple nonconjugated indole moiety. One of the α-ketoindole alkaloids has been identified as vobasine, whose recently described structure is found compatible with NMR spectral data. The isolation procedures are described for the second α-ketoindole alkaloid, affinine, and for the indole alkaloid, affinisine.

THE HISTORY of the genus *Tabernaemontana* L. (family *Apocynaceae*) is exceedingly complex. At various times, botanists have split other genera from it (*e.g.*, *Voacanga*, *Ervatamia*, *Gabunia*, *Conopharyngia*) leaving a residue of synonymy of genera and/or species within the group. With regard to South American representatives, botanical opinion concerning their classification has varied widely. Markgraf has separated and readjusted the genus *Tabernaemontana* L. into nine genera, preserving that name for species found in the Antilles, Central America, and parts of northwestern South America, while maintaining *Peschiera* A. DC. for those found in Brazil (1). Woodson does not share this opinion (2).

There is reason to believe that even in closely related genera sufficient differences in alkaloid composition exist to serve as a basis for chemotaxonomic distinctions. In Farnsworth's excellent review of the periwinkles (3) one notes that of the 48 characterized alkaloids isolated from *Vinca* and *Catharanthus* species, only one, akuammine (vincamajoridine) is common to both genera. Obviously, it will be necessary to know more about the actual structures before detailed comparisons can be made between the chemical types found in each genus. As of now, however, it can be stated that the dimeric C_{46} bases have been found only in the genus *Catharanthus*.

From the chemical point of view, the genera in the subtribe Tabernaemontainae have been found to contain two major structural types of alkaloids: the predominant ibogaine, I, type (4, 5) and the tabernaemontanine,[1] II, type (6, 7).

In the course of a broad screening program, about thirty plants which were identified as either *Tabernaemontana* or *Peschiera* species by South American botanists have been encountered. In order to detect any chemical differences which may exist between, and perhaps thus differentiate, these closely related genera, we have chosen a number of them for detailed study. The first of these, reported here, is *Peschiera affinis* (Meull.-Arg.) Miers.

DISCUSSION

A 5.8-Kg. sample of *P. affinis*[2] collected in northeastern Brazil was extracted with alcohol until removal of alkaloidal material was complete (Mayer's reagent). The extract was concentrated under reduced pressure and the residue was treated with dilute phosphoric acid. The filtered, aqueous

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[1] It is of interest to note that the source of tabernaemontanine is said to be *Tabernaemontana coronaria* (8) = *Ervatamia coronaria* (7), possibly synonymous with *E. divaricata* (7).

[2] A specimen of this plant has been deposited with Prof. R. E. Schultes, Curator, Botanical Museum, Harvard University, Cambridge, Mass.