Reliability of Arrhenius Equation in Predicting Vitamin A Stability in Multivitamin Tablets

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Abstract □ Shelflife predictions of vitamin A stability in multivitamin tablets were calculated based on data obtained from the analyses of six multivitamin tablet preparations by the classical Arrhenius treatment. This approach gave excessive variation in the predictions. The same data were treated by a modified Arrhenius method, which reduced the variation. Long-term room temperature data show that incorrect slopes were predicted in three of the six sets of data with the modified approach. The data indicate that erroneous predictions can result when applying the Arrhenius treatment to accelerated data and that this approach should be used with extreme caution in establishing the expiration date of a multivitamin tablet product, especially where the limiting ingredient for expiration dating is a preprocessed raw material and not a single-entity chemical.

Keyphrases □ Vitamin A—stability in multivitamin tablets, predicted using Arrhenius equation □ Stability—vitamin A in multivitamin tablets, predicted using Arrhenius equation □ Arrhenius equation—use in predicting stability of vitamin A in multivitamin tablets

The prediction of chemical stability of pharmaceutical dosage forms is important to the pharmaceutical industry in view of impending governmental proposals on expiration dating. The technique usually used to predict shelflife has been application of the Arrhenius equation. This technique was applied first (1) to the color stability of a liquid multisulfa preparation and later to the stability of certain vitamins in liquid multivitamin preparations (2, 3).

The Arrhenius technique was used to predict expiration dates of liquid multiple-vitamin preparations (4) and vitamin A, thiamine, and ascorbic acid stability in sugar-coated multiple-vitamin tablets (5). The latter study validated the room temperature-predicted degradation rates by assaying, at various intervals, tablets that were stored at room temperature for 3 years. All of these studies were conducted under very tight controls. They all used constant-temperature baths containing oil or water to maintain their accelerated temperature-controlled conditions.

This paper presents the results of the classical and of a modified application of the Arrhenius equation in predicting vitamin A stability in several multivitamin tablet products. Since vitamin A stability in multiple-vitamin tablets is important to the total product stability (5), it is desirable to have a reliable method for its prediction. The purpose of this study was to determine the accuracy of the Arrhenius predictions for vitamin A stability in multivitamin products.

Commercially available dry vitamin A in itself is a formulated product. Its stability in bulk form and when included in a multicomponent system is largely dependent on the purity and integrity of the crystalline vitamin A, the components of the "beading" formula, and the method of achieving the final product. Thus, it is not a single, pure chemical entity to begin with, and it will vary from supplier to supplier.

EXPERIMENTAL

The two sugar-coated tablet formulations, Products A and B, had the following active ingredient composition per tablet: vitamin A, 6850 IU; vitamin D, 875 IU; ascorbic acid, 55.0 mg; thiamine, 2.5 mg; riboflavin, 2.575 mg; niacinamide, 20.4 mg; pyridoxine, 1.05 mg; cyanocobalamin, 1.35 μg; and pantothenic acid, 1.77 mg.

Four chewable tablet formulations, Products C–F, were used. Product C had the following active ingredient composition per tablet: vitamin A, 5720 IU; vitamin D, 500 IU; ascorbic acid, 57.5 mg; thiamine, 2.06 mg; riboflavin, 2.5 mg; niacinamide, 20.0 mg; pyridoxine, 1.05 mg; and cyanocobalamin, 1.15 μg. Products D–F had the same active ingredient composition as Product C but contained 6250 IU of vitamin A/tablet.

All of these compositions are theoretical levels based on bulk label and do not include supplier overages.

The official USP UV absorption method (6) was used to assay for vitamin A content in these tablets. Random samples were taken from the population of each of the six products. One sample was set aside for initial testing of vitamin A content while the other samples were packaged in screw-capped glass containers and placed in temperature-controlled conditions at 50[1], 40[1], 25[1], 55[2], or 45[2] ± 1°.

Products A–D were placed at 50, 45, 40, and 25°. For Products A and B, samples at 50° were withdrawn and assayed every 2nd week for 8 weeks; those at 45° were withdrawn every 3rd week for 12 weeks; those at 40° were withdrawn every 4th week for 16 weeks; and those at 25° were assayed at 26, 52, 95, and 156 weeks. For Products C and D, samples at 50, 45, and 40° were withdrawn and assayed every 4th week for 16 weeks; those at 25° were assayed at 26, 52, 78, 104, and 156 weeks.

Products E and F were placed at 55, 50, 45, 40, and 25°. Samples at 55° were assayed every week for 6 weeks; those at 50° were assayed every 2nd week for 10 weeks; those at 45 and 40° were assayed every 4th week for 16 weeks; and those at 25° were assayed at 13, 26, 35, 52, and 104 weeks (Product E) and at 13, 26, 52, 104, and 156 weeks (Product F).

RESULTS

Figure 1 shows a typical plot of the thermodegradation of vitamin A in these products. The open circle (at time zero) is the average of six assays of five tablets each run by two individuals in triplicate. The other points on the graph are averages of four assays of five tablets each run by two individuals in duplicate. This general format was followed for the six products.

Table I contains the thermodegradation data for the six products. Standard statistical techniques (7–10) were used to calculate the table

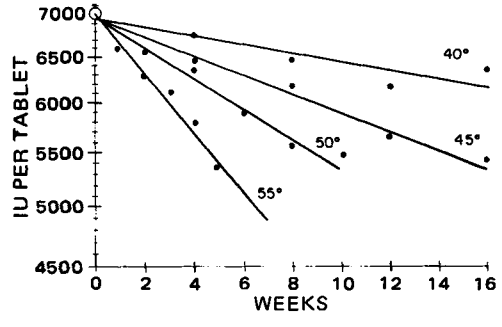

Figure 1—*Typical pseudo-first-order plots of the thermodegradation of vitamin A.*

[1] Walk-in storage areas, Tyler Refrigeration Corp.
[2] Thelco model 4 bench top unit, Precision Scientific Co.

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Table I—Initial Vitamin A and Values of Calculated Rate Constants and Intercepts with 95% Confidence Limits for Each Thermodegradation Curve

Product	ln (Average Initial Potency)	40°		45°		50°		55°	
		k, weeks^{-1}	b, ln potency	k, weeks^{-1}	b, ln potency	k, weeks^{-1}	b, ln potency	k, weeks^{-1}	b, ln potency
A	8.83	0.0353 ± 0.00415	8.82 ± 0.0371	0.0723 ± 0.00495	8.84 ± 0.0332	0.132 ± 0.00924	8.81 ± 0.0413	—	—
B	8.93	0.0570 ± 0.00418	8.95 ± 0.0374	0.132 ± 0.00608	8.96 ± 0.0408	0.247 ± 0.0218	8.85 ± 0.0976	—	—
C	8.70	0.0136 ± 0.00227	8.70 ± 0.0212	0.0188 ± 0.00246	8.70 ± 0.0230	0.0282 ± 0.00399	8.69 ± 0.0373	—	—
D	8.82	0.00680 ± 0.00134	8.82 ± 0.0125	0.0112 ± 0.00207	8.82 ± 0.0191	0.0194 ± 0.00210	8.81 ± 0.0196	—	—
E	8.83	0.00597 ± 0.00119	8.82 ± 0.0111	0.0141 ± 0.00180	8.83 ± 0.0168	0.0259 ± 0.00306	8.83 ± 0.0178	0.0459 ± 0.00708	8.81 ± 0.0237
F	8.85	0.00729 ± 0.00232	8.84 ± 0.0217	0.0164 ± 0.00196	8.85 ± 0.0183	0.0268 ± 0.00285	8.85 ± 0.0152	0.0520 ± 0.00493	8.85 ± 0.0172

Table II—Range of Room Temperature k Values from Confidence Limits of Arrhenius Plot, Predicted Range of Vitamin A Potency from k Values and Room Temperature Intercept at t = 156, k Values and Intercepts with Confidence Limits from Room Temperature Data, 95% Confidence Limits, Range of Vitamin A Potency at t = 156 Weeks, and Overlap Data

Product	Arrhenius Plot Data: Range of Predicted k Values, weeks^{-1}	Arrhenius Plot Data: Range of Predicted Vitamin A Potency, IU/tablet	25° Data: k, weeks^{-1}	25° Data: b, ln Intercept ±95% Confidence Limits	25° Data: 95% Confidence Limits, IU/tablet	Overlap
A	0.000844-0.0208	273-6100	0.00129	8.85 ± 0.0346	5410-5990	Over entire range
B	0.000221-0.134	0-7390	0.00153	8.94 ± 0.0298	5770-6290	Over entire range
C	0.000943-0.0177	380-5130	0.00164	8.69 ± 0.0385	4380-4840	Over entire range
D	0.000494-0.00304	4200-6240	0.000560	8.82 ± 0.0171	6050-6300	Over entire range
E	0.000282-0.00169	5280-6580	0.00214	8.84 ± 0.0283	4600-5270	0-45.8 weeks and after 162.6 weeks
F	0.000402-0.00214	5020-6590	0.00150	8.86 ± 0.0120	5460-5640	Over entire range

values. The intercepts and the average of the initial assay values agreed well; in fact, the initial values are in the range of the limits of the intercept, which indicates that the pseudo-first-order degradation model is correct.

The Arrhenius equation data were plotted by Arrhenius plotting techniques and are typically represented by Product E in Fig. 2. This figure was generated using the least-squares fitting technique (7–10). After the Arrhenius equations were determined, a range of predicted room temperature rate constants were determined from the confidence limit curves. This range is shown by broken lines in Fig. 3 for Product E when inserted into the room temperature degradation equation (11). The statistically treated room temperature data for Product E are represented by the solid line and curves in Fig. 3.

To determine accurately the overlap between y values found from predicted k values and the values found from the 95% confidence limits about the room temperature data, the point or points of intersection must be determined. Algebraic manipulation of the equations gives rise to a quadratic equation to solve for x (12). The range of k values obtained from the confidence limits about the Arrhenius plot regression line, the predicted range of vitamin A potency at t = 156 weeks, the 25° data equations as determined by least-squares fitting, the range of vitamin A potency from the 95% confidence limits about the room temperature regression line at t = 156 weeks, and the overlap values for the six products are listed in Table II.

The potency of the vitamin A remaining was obtained by substituting the predicted k values into the room temperature degradation equation, setting t = 156 weeks, setting ln C_0 equal to the 25° data equation intercept, and taking the antilogarithm. Inspection of Table II reveals that the predicted k values and 95% confidence limits overlapped over the entire 156 weeks for all products except E. It also can be concluded that the classical application of the Arrhenius techniques gives such a wide range of k values and subsequent potency at 3 years as to be of little value. For instance, the prediction for Product B is from 0 to 7390 IU/tablet, which is meaningless.

It was, therefore, decided to use a modified application of the Arrhenius technique[3].

The degradation rate constants, k, were calculated by the formula (13):

$$m = \frac{y_1 - y_2}{x_1 - x_2} \qquad \text{(Eq. 1)}$$

where y_1 = ln [Σ initial vitamin A potency/n], y_2 = ln [Σ vitamin A potency at time x_2/n], $x_1 = 0$, and x_2 = time in weeks at y_2. For comparison to the classical method, an average k at each temperature and the 95% confidence limits on k were calculated (14, 15). These values for all six products at each temperature are given in Table III. A comparison of Tables I and III reveals that the range of k values overlapped in all cases,

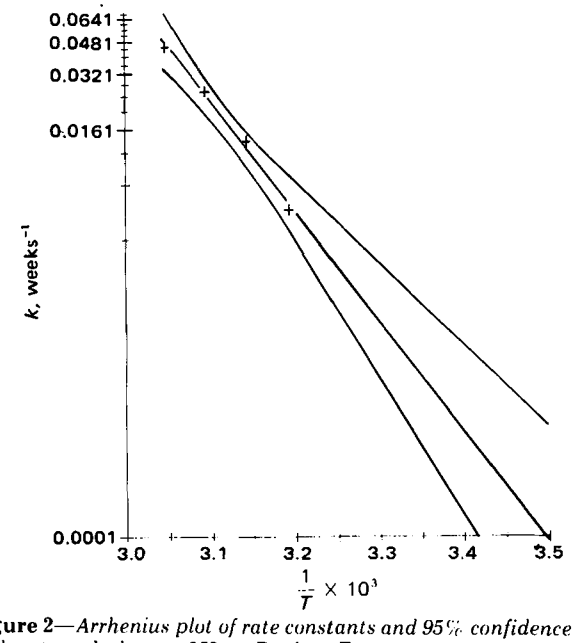

Figure 2—*Arrhenius plot of rate constants and 95% confidence limits with extrapolations to 25° on Product E.*

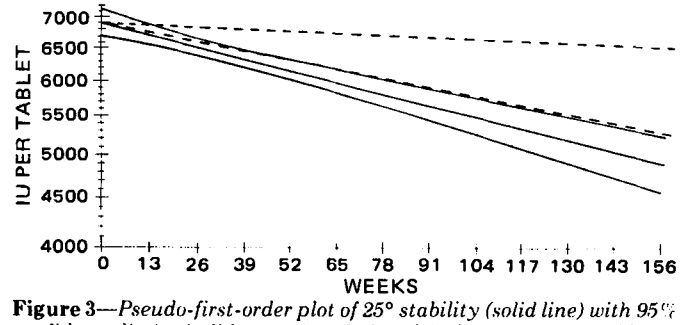

Figure 3—*Pseudo-first-order plot of 25° stability (solid line) with 95% confidence limits (solid curves) and plot of Arrhenius upper and lower predicted* k *values (broken lines) on Product E.*

[3] Suggested by J. T. Carstensen to B. T. Palermo, June 16, 1976.

Table III—Average k Values and 95% Confidence Limits for Each Thermodegradation Curve

Product	40°	45°	50°	55°
A	0.0370 ± 0.00455	0.0746 ± 0.0120	0.145 ± 0.0205	—
B	0.0521 ± 0.00790	0.123 ± 0.0151	0.285 ± 0.0626	—
C	0.0138 ± 0.00341	0.0197 ± 0.00652	0.0315 ± 0.00932	—
D	0.00703 ± 0.00305	0.0117 ± 0.00229	0.0214 ± 0.00330	—
E	0.00722 ± 0.00199	0.0128 ± 0.00306	0.0275 ± 0.00333	0.0560 ± 0.0177
F	0.00965 ± 0.00416	0.0180 ± 0.00424	0.0280 ± 0.00354	0.0527 ± 0.00506

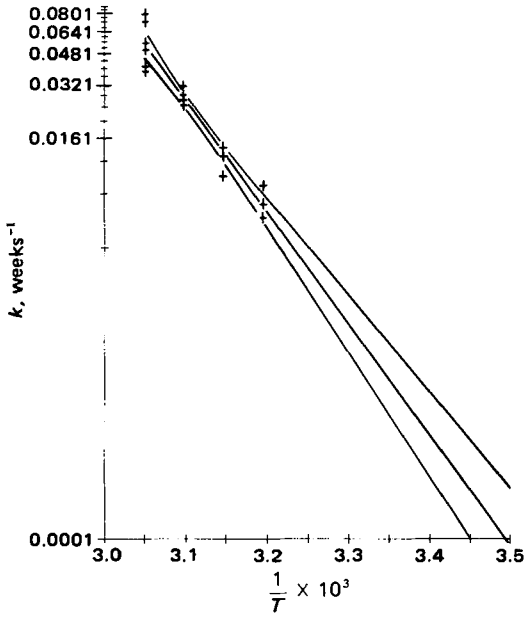

Figure 4—*Arrhenius plot of rate constants derived from modified application and 95% confidence limits with extrapolations to 25° on Product E.*

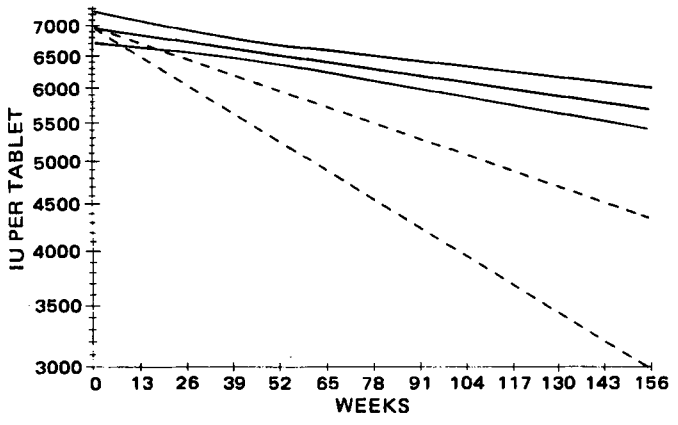

Figure 5—*Pseudo-first-order plot of 25° stability (solid line) with 95% confidence limits (solid curves) and plot of Arrhenius upper and lower predicted* k *values (broken lines) from modified application on Product A.*

indicating that the k values were not altered significantly from the classical approach.

The modified Arrhenius equation plot is typically represented by Product E in Fig. 4. These data were treated as were the previous Arrhenius plot data to give a range of k values and are shown by broken lines in Fig. 5 for Product A when inserted into the room temperature degradation equation. The actual room temperature data found for Product A also are represented in Fig. 5 by the solid line and curves. The range of k values, predicted vitamin A potency at t = 156 weeks, and overlap data are given in Table IV for all six products.

Comparison of the lower and upper k values in Tables II and IV reveals that this procedure predicts k values within the range predicted by the classical procedure, which indicates that the Arrhenius equation plot was not changed significantly. This comparison also reveals that the modified procedure results in a much smaller range of k values and, consequently, a tighter limit of vitamin A potency at 3 years. This result is due to the difference in the degrees of freedom (two for the classical application and 17 for the modified application for Product E), with a resulting smaller t value for the modified application of the Arrhenius plot. Table IV also shows that the Arrhenius plot did not accurately predict the room temperature degradation pattern in three of the six products.

Table IV—Range of k Values Obtained from Modified Application of the Arrhenius Technique, Predicted Range of Vitamin A Potency from k Values and Room Temperature Intercept at t = 156, and Overlap Data

Product	Range of Predicted k Values, weeks^{-1}	Range of Predicted Vitamin A Potency, IU/tablet	Overlap
A	0.00302–0.00539	3010–4350	0–17.0 weeks
B	0.00233–0.00469	3670–5310	0–28.7 weeks
C	0.00193–0.00639	2190–4400	Over entire range
D	0.000571–0.00195	4990–6190	Over entire range
E	0.000451–0.00109	5830–6440	0–20.7 weeks
F	0.00102–0.00211	5070–6010	Over entire range

DISCUSSION

The shelflife of a product is determined by the stability of its most unstable ingredient. In the multiple-vitamin products, vitamin A is the limiting ingredient. The problem is to find a method that will establish accurately the stability pattern of vitamin A in these products at room temperature. When vitamin A data generated at elevated temperatures are treated by the classical application of the Arrhenius plot, the resulting predicted range of room temperature k values is very wide. The modified application simply increases the degrees of freedom by determining a greater number of rate constants from the available data, which decreases the range of predicted k values without altering the Arrhenius equation.

The predicted room temperature degradation lines as determined by the k values should be inside the actual room temperature confidence limits or encompass those limits. Since three of the six products tested did not meet these criteria, it was concluded that the Arrhenius equation did not give the same room temperature k value as was calculated from the room temperature data. Therefore, 25° stability data must be generated to determine the shelflife accurately.

This study did not corroborate Tardif's (5) findings that the room temperature stability data validate the predicted degradation rates of vitamin A. Tardif used constant-temperature oil baths and different temperatures than were used in this study. Both studies indicated that the pseudo-first-order reaction rate is the correct model; however, this study generated an incorrect predicted k in three out of six cases.

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Formulation and Evaluation of Ethiodized Oil Emulsion for Intravenous Hepatography

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Received April 28, 1978, from the **Pharmacy Department and the* ‡*Diagnostic Radiology Department, The Clinical Center, National Institutes of Health, Bethesda, MD 20014.* Accepted for publication June 12, 1978.

Abstract □ A study was conducted to prepare and evaluate an ethiodized oil emulsion for intravenous administration that would selectively opacify the liver. Several formulations with differing globule size were prepared and compared for their *in vivo* activity (degree of liver opacification obtained) and stability. Results of studies conducted in rabbits and monkeys revealed that the oil globules that concentrate most in liver were 2.0–3.0 μm in diameter. The formulation of choice was stable for 6 months at refrigeration temperature (2–6°). In monkeys, this formulation produced an improved diagnostic image of the liver using computerized tomography at a dose of 0.2 ml/kg. The potential use of such emulsions in diagnostic radiology is briefly discussed.

Keyphrases □ Ethiodized oil—various emulsions for intravenous administration prepared, effect of oil globule size on liver opacification in rabbits and monkeys □ Radiopaque media—ethiodized oil, various emulsions for intravenous administration prepared, effect of oil globule size on liver opacification in rabbits and monkeys □ Hepatography, intravenous—various emulsions of ethiodized oil prepared, effect of oil globule size on liver opacification in rabbits and monkeys

There have been numerous previous attempts to opacify the liver and spleen by intravenously administered contrast material. As early as 1930, Keith and Briggs (1) used intravenously injected emulsified oil to opacify the liver and spleen in rats. Degkwitz (2) produced an iodine-containing oily contrast material[1] (I), which was used in clinical studies (3) following experiments in animals.

Experimental work (4) with I in mice, rabbits, and guinea pigs indicated that intravenous injection did opacify the spleen, liver, and placenta. This same contrast material was used in nine patients but was too toxic for routine clinical examinations (5). Emulsified ethyl diiodostearate was injected in 10 patients, most of whom had severe toxic reactions (6). Experimental work (7) in dogs used an emulsified oily contrast medium of ethiodized oil[2]. The average globule size of the emulsion (7) was 0.3 μm, and it opacified the liver and spleen.

Recently, two experimental emulsified forms of iodized oil[3], II*a* and II*b*, were produced. Their basic difference was the average globule size of the emulsion, II*a* (emulsion grossiere) having the larger globule size and II*b* (emulsion fine) the smaller. Emulsion II*b*, mode diameter of 1.3 μm (range 0.16–7 μm), was used intravenously in two clinical studies (8, 9), but up to 75% of the patients had disturbing reactions such as fever, chill, anorexia, nausea, vomiting, and a 10–20% drop in platelet counts. These reactions appeared to be dose related and almost constant above the 1.2-ml/kg dose level. In another study (10) with 32 rhesus monkeys, a 2.0-ml/kg iv dose of the same emulsion produced an opacified diagnostic image of the liver and spleen on X-ray examination.

A recent revolutionary discovery in diagnostic roentgenology, computerized tomography (CT), with its improved contrast resolution, raised hopes that a fraction of the previously utilized dose would opacify the liver sufficiently for diagnostic evaluation without undesirable side effects. With this new equipment, Vermess *et al.* (11) opacified the liver and demonstrated carcinogen-induced hepatic tumor in the rhesus monkey with an intravenous dose as low as 0.1–0.2 ml of II*b*/kg.

The possible use of such small doses in human subjects renewed interest in the ethiodized oil emulsion for clinical hepatography. Unfortunately, II*b* was no longer available. Therefore, a study was initiated to prepare an ethiodized oil emulsion for intravenous hepatography. In addition, since none of the mentioned reports attempted to correlate the size of the oil globules with the opacifying ability and since no quantitative data related the dose to the density of the liver scans, a more systematic study of the subject appeared to be warranted.

The size of the oil globules is of primary importance in the opacification of the various tissues, in accordance with Degkwitz's theory that different tissues absorb oil globules of different sizes (2). This fact compounded the problem

[1] Jodsol.
[2] Ethiodol, iodinated ester of poppyseed oil, iodine content 37% (w/v), is routinely used for lymphography; Savage Laboratories, Houston, TX 77036.
[3] II*a* is AG-52-315 and II*b* is AG 60-99; Lipidol UF, Laboratoires Andre Guerbet, Aulnay sur Bois, France.

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