Equilibrium Phenomena in Solid Dosage Forms

By J. THURØ CARSTENSEN*, J. B. JOHNSON, D. C. SPERA, and M. J. FRANK

Certain aspects of the mechanics of drug decay in solid dosage forms are discussed. Apparent equilibration often takes place in solid dosage forms. In order to obtain data that are amenable to detection of equilibria and can be subjected to mathematical analysis, it is necessary, in the case of fairly stable drugs, to assay for degradation products. Van't Hoff plots should be used in this case.

THE AUTHORS are presently engaged in the study of stability behavior in solid dosage forms.

Theoretical treatment of reaction through moisture interfaces should lead to zero-order behavior (1). To our knowledge only Leeson and Mattocks (2) and Lachman (3) have touched on this in a quantitative manner. Garrett (4, 5) has paved the way for use of extrapolatory procedures and Higuchi (6), Tardif (7), and Carstensen (8, 9) have reported on first- and zero-order behavior of vitamin A in solid dosage forms.

Nevertheless, the literature is poor in reports on this important aspect of stability evaluation, and some of the authors' findings are presented with special emphasis on (*a*) the occurrence of apparent equilibria and (*b*) the importance of following degradation product formation rather than evaluating only percent active drug retained.

THEORETICAL

It is a general practice to evaluate stability by percent active component retained.[1] For short term evaluation, however, it is more meaningful, and in general much more instructive, to gauge degradation by the amount of decomposition products formed.

In general, a good analytical procedure for the intact drug exists at the time pharmaceutical product development is initiated, but breakdown products may be either unknown or poorly quantitated.

It has been the authors' experience that application of TLC in evaluating compatibility (9) as well as stability, gives insight far beyond simply testing for percent intact drug retained, even though analytical procedures for the latter may be good to ± 2% and the former only to ± 20%.

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[1] It is also popular practice to treat log linear relations by the method of least squares directly. This, however, is quite fallacious if the data are very scattered (10).

EXPERIMENTAL

Vitamin A tablets were made by mixing vitamin A acetate beadlets (17.5 mg. per tablet of 500 unit/mg. beadlets), spray-dried lactose (427.5 mg. per tablet), and calcium stearate (5 mg. per tablet), and compressing the tablets on a conventional tablet machine using a $^3/_8$-in. deep concave punch. The tablets were stored at 25, 37, 45, 55, 70, and 85° in sealed containers, and samples were removed at regular intervals. The tablets were assayed by a modified USP method[2] using Morton Stubbs correction factor.

Stability of isopropanolic solutions of vitamin A acetate were studied in the absence of air and oxygen in the following fashion.

A solution of vitamin A acetate in isopropanol was prepared under nitrogen and transferred to the vessel shown in Fig. 3. Nitrogen was bubbled through the vessel through stopcock B, with stopcock A open. Both stopcocks were then closed, and the vessel stored at 25°. Samples were taken at various intervals by opening stopcocks A and B, and applying nitrogen pressure at position A, and collecting the sample at position B.

Vitamin E succinate tablets were made in the same fashion as described for vitamin A acetate tablets. They contained 6 mg. vitamin E succinate, 192 mg. lactose, and 2 mg. magnesium stearate. Storage conditions and sampling procedure paralleled those for vitamin A acetate tablets.

The tablets inherently contain sufficient moisture for complete de-esterification. Even though this would imply a second or higher order reaction, it will be seen that the data lend themselves to first-order kinetics and that meaningful extrapolations can be made.

The melting point of vitamin E succinate is 68°, but addition of additives[3] (lactose, magnesium stearate) sufficiently lowers the melting point so that the physical state of the vitamin E succinate is the same at all temperatures studied.

Vitamin E succinate potency was determined spectrophotometrically by measurement of the absorbance at 285 mμ of an ethanol extract of the tablet mass. The absorptivity, at 285 mμ in 1-cm. cells, is 3.78 for *dl*-α-tocopherol succinate.

A portion of finely ground tablet mass equal to approximately 10 mg. of vitamin E succinate was accurately weighed, transferred to a 100-ml. volu-

[2] USP method employs four washings with ether, whereas the modified method (which in our hands gives identical results) employs two extractions with petroleum ether.

[3] If vitamin E succinate and magnesium stearate are melted together and cooled, the following congealing points may be observed: vitamin E succinate 99%–magnesium stearate 1%, 45°; vitamin E succinate 95%–magnesium stearate 5%, 30°; vitamin E succinate 90%–magnesium stearate 10%, 5°.

metric flask, and 50 ml. of 95% ethanol added. The liquid was shaken for 10 min. and then brought to volume with ethanol, filtered through Whatman No. 12 paper, and absorbance of this solution then was measured at 285 mμ on a suitable spectrophotometer.

The free tocopherol was determined by thin-layer chromatography. An amount of tablet mass equal to approximately 10 mg. of vitamin E succinate was triturated with 1 ml. of chloroform. Fifty microliters of this solution (equal to 500 mcg. of vitamin E succinate) was then applied to a Silica Gel G thin-layer chromatographic plate, and the following thin-layer chromatographic system was used: adsorbent, Silica Gel G; sample, 500 mcg. from chloroform solution; solvent, cyclohexane–diethyl ether (80:20); development, 15 cm. ascending; visualization, 20% phosphomolybdic acid in 95% ethanol.

RESULTS AND DISCUSSION

Sufficient information may be extracted by studying percent retained in the case of lactose tablets containing vitamin A, since in this case, losses are of fair magnitude. Figure 1 shows the percent retained of vitamin A in lactose–magnesium stearate base tablets as a function of time. The assay is modified USP with Morton Stubbs correction, and although data pertaining to degradation products are absent (except for the quantitative Morton Stubbs correction), they show that the assay will level off.

The vitamin A used was vitamin A acetate containing substantial amounts of antioxidants. In the absence of antioxidants the effect is noticeable only after long periods at room temperature. Three batches of vitamin A acetate, containing no antioxidants, when incorporated into uncoated multivitamin tablets, retained vitamin A potency at room temperature in the pattern shown in Fig. 2. Statistical evaluation, as well as visual observation, shows the potency to level off, but long periods at room temperature are necessary to observe the effect.

That equilibration should take place is not unreasonable, since isopropanolic solutions of vitamin A acetate show a similar behavior, if care is taken to exclude air, for example by use of a vessel such as shown in Fig. 3. Figure 4 shows the results of such a study.

The vitamin A beadlets may be viewed as an isolated system in the tablet matrix, and as long as compression of the tablet is kept reasonably gentle the fall-off of potency is fairly independent of the surrounding mass. The reason for introducing vitamin A at this point is to demonstrate that equilibrium conditions do occur in solid dosage forms, and that, if the equilibrium point is sufficiently in favor of the degradation product(s), it is possible to follow the pattern by determining percent retained as a function of time.

Since this is the exception rather than the rule in good tablet formulations, it would be more appropriate to study a compound which would be relatively stable.

It is obvious, however, that in such a case much longer storage times would be necessary to attain meaningful results, if assays of the intact molecule were followed. If individual assay tolerances were ±2%, either a great many assays would have to be

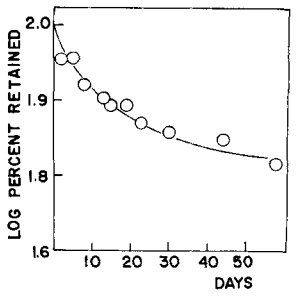

Fig. 1—Stability pattern at 45° of vitamin A acetate beadlets (containing antioxidants) in lactose base tablets.

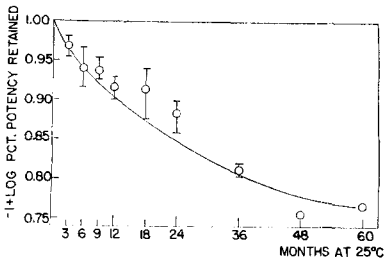

Fig. 2—Long term room temperature stability of vitamin A beadlets (without antioxidants) in a multivitamin tablet formulation.

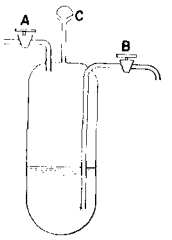

Fig. 3—Storage and sample vessel used for studying stabil ty patterns of vitamin A acetate in isopropanol solutions. The vessel allows sample storage and transfer under nitrogen.

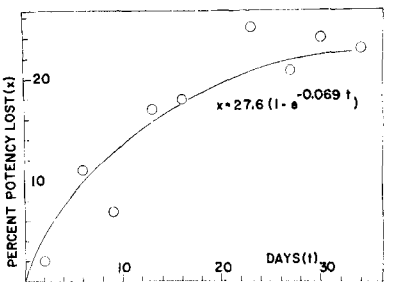

Fig. 4—Stability pattern at 25° of vitamin A acetate dissolved in isopropanol.

performed to distinguish between, *e.g.*, 100% and 99.5% content of active component, or sufficient time would have to elapse in storage to allow the content of active component to drop to a level which would be significantly different from initial content to allow differentiation by triplicate (or other reasonable multiplicate) assays. If the rate of degradation product formation is followed, however, even a crude assay will be more meaningful for this purpose. The example of vitamin E succinate tablets (6 mg.) shown in Table I demonstrates this.

TABLE I—VITAMIN E SUCCINATE DE-ESTERIFICATION AT 55°

Storage Time, 55°	mg./Tablet	% Free Tocopherol[a]
0 days	5.92	0
3 days	...	0.5–1.0
5 days	5.96	1.0
7 days	5.93	1.5
10 days	5.92	1.5
20 days	6.11	2.0

[a] As mentioned, the content of free tocopherol here was evaluated by comparing thin-layer chromatograms of the tablet with standard chromatograms of tocopherol with various amounts of tocopherol applied. In the amounts shown, the assay of "decomposition product" is good to ±25 relative percent in amounts above 1% decomposition product present.

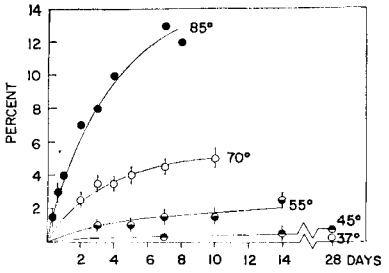

Fig. 5—Stability patterns at various temperatures of vitamin E succinate in lactose base tablet . For estimation of asymptotic values, see text.

It is obvious that the tablet and assay variance here does not allow stability evaluation by column 2 (intact molecule). The data regarding decomposition product formation as they stand are not impressive, but considering the fact that the data concerning percent retained can give no quantitative information at all, and viewed in light of the 85°-data described in the following, they lend themselves to mathematical analysis.

The product formation curve at 85° is shown in Fig. 5 and is indicative of equilibration. Considering the simplest form, $A \rightleftarrows B$, and denoting forward and backward rate constants by k_+ and k_-, and equilibrium concentration by X_e, would lead to the rate equation for content of decomposition product (X):

$$X = X_e[1 - e^{-(k_+ + k_-)t}] = X_e(1 - e^{-kt}) \quad \text{(Eq. 1)}$$

where $k = k_+ + k_-$. X then is the concentration of the component denoted by B above, and $(1 - X)$ is the content of intact molecule A.

Drawing a crude curve through the points of Fig. 5 and recording the X-values at time 2, 4, and 6 yield equations (*i*), (*ii*), and (*iii*) below. It will be noted, that for arithmetic handling any arithmetic series (*e.g.*, $t = 1, 2$, and 3 or $t = 3, 6$, and 9) could have been used equally well. However, the highest numbers, compatible with the data, give the best estimates.

k and X_e are then evaluated by the procedure shown:

$$(i) \quad X_2 = X_e - X_e \cdot e^{-2k}$$

$$(ii) \quad X_4 = X_e - X_e \cdot e^{-4k}$$

$$(iii) \quad X_6 = X_e - X_e \cdot e^{-6k}$$

Subtraction now yields:

$$(ii) - (i) \quad X_e(e^{-2k} - e^{-4k}) = X_4 - X_2$$

$$(iii) - (ii) \quad X_e(e^{-2k} - e^{-6k}) = X_6 - X_2$$

Division of $(ii) - (i)$ by $(iii) - (i)$ then eliminates X_e and yields:

$$(X_4 - X_2)e^{-6k} - (X_6 - X_2)e^{-4k} + \{(X_6 - X_2) - (X_4 - X_2)\}e^{-2k} = 0$$

or, providing that $(X_4 - X_2)$ is different from zero:

$$(iv) \quad (X_4 - X_2)e^{-2k} \times \left\{(e^{-2k})^2 - \frac{X_6 - X_2}{X_4 - X_2}(e^{-2k}) + \frac{X_6 - X_4}{X_4 - X_2}\right\} = 0$$

The equation is satisfied when the quadratic equation in brackets is zero, *i.e.*, when:

$$(v) \quad (e^{-2k})^2 - \frac{X_6 - X_2}{X_4 - X_2}(e^{-2k}) + \frac{X_6 - X_4}{X_4 - X_2} = 0$$

or

$$(vi) \quad (X_4 - X_2)(e^{-2k})^2 - (X_6 - X_2)(e^{-2k}) + (X_6 - X_4) = 0$$

One of the roots of the quadratic equation (*v*) is an estimate of e^{-2k} (and hence an estimate of k) and insertion of this value in, *e.g.*, (*i*), will yield X_e.

It will be noted that the factoring of $(X_4 - X_2)$ in (*iv*) requires it to be different from zero, but $X_4 - X_2 = 0$ only if $k = 0$. In this case $e^{-k} = 1$, and $e^{-kt} = 1$ for all t, and Eq. 1 degenerates to $X = 0$. For this case $e^{-2k} = 1$ and $X_6 = X_4 = X_2 = 0$, and the quadratic equation (*v*) is satisfied by this value.

The equation hence always has the superfluous root $e^{-2k} = 1$, and the other root is the value determining k. Since, in a quadratic equation the two roots ($e^{-2k'}$ and e^{-2k}) add up to the negative coefficient of the first power term, when the equation is normalized, and since, here, $e^{-2k'} = 1$, then:

$$e^{-2k'} + e^{-2k} = 1 + e^{-2k} = \frac{X_6 - X_2}{X_4 - X_2}$$

or

$$e^{-2k} = \frac{X_6 - X_2 - (X_4 - X_2)}{X_4 - X_2} = \frac{X_6 - X_4}{X_4 - X_2}$$

The 85°-data obtained with vitamin E succinate yield the following values (*iv*):

$$0.039\, e^{-2k}\{(e^{-2k})^2 - 1.54\, e^{-2k} + 0.537\} = 0$$

The sum rule gives: $1 + e^{-2k} = 1.54$ or $e^{-2k} = 0.54$. A check on how good the crude curve is, can be obtained by solving for e^{-2k} in conventional fashion and ascertaining that the one root is (or is close to) 1.

In the case above, the roots are $e^{-2k} = 0.523$ and $e^{-2k'} = 1.037$, *i.e.*, the latter is sufficiently close to 1.0 to justify the initial estimate of X_2, X_4, and X_6, and the ensuing k value. Having a first estimate, a best estimate can be found by computer, using Taylor series and iteration as described by Wylie (10).

Inserting $e^{-2k} = 0.54$ in (*i*) yields:

$$X_2 = 0.066 = X_e(1 - 0.54)$$

so

$$X_e = 0.143 \text{ or } X_e = 14.3\%$$

TABLE II—DEGRADATION OF VITAMIN E SUCCINATE IN TABLETS AT VARIOUS TEMPERATURES

Time, Days	Temp., °C.	Free Tocopherol, %
0.33	85	1.5
0.67	...	3.0
1	...	4.0
2	...	7.0
3	...	8.0
4	...	10.0
5	...	12.0
7	...	13.0
10	...	12.0
2	70	2.5
3	...	3.5
4	...	3.5
5	...	4.0
7	...	4.5
10	...	5.0
7	45	0.25
14	...	0.50
28	...	0.75
28	37	0.25

is indicated in this case, since equilibrium values, rather than rate constants are being studied. Stability prediction should indeed be done by this equation rather than by Arrhenius plotting, when equilibrium conditions prevail. The data are presented graphically in Fig. 6. The rate constants, in this case, may or may not follow an Arrhenius relationship. Figure 7, however, shows that they conform fairly well in this case to this type equation. However, for prediction of stability, the rate constants are of no importance if equilibrium is ultimately reached.

The important features then are (*a*) that it becomes apparent that equilibrium is reached and that Van't Hoff rather than Arrhenius plotting is indicated, and (*b*) that use of percent retained data is of no value for extrapolation when compounds have fair stability.

In such cases a crude assay of the degradation product is not only more meaningful, but also more informative and accurate.

TABLE III—VAN'T HOFF CORRELATION OF VITAMIN E SUCCINATE TABLETS

Temp., °C.	$10^3/T$ (°K^{-1})	X_e, %	K (Range)	$\ln_e K$ (Range)	k (Days^{-1}) (Range)	$\ln_e k$ (Range)
85	2.79	13.9–14.3	0.16 –0.17	(−1.83)–(−1.77)	0.27 –0.54	(−1.309)–(−0.616)
70	2.92	5.8– 8.0	0.062–0.087	(−2.78)–(−2.44)	0.15 –0.31	(−1.897)–(−1.171)
55	3.05	2.5– 3.6	0.026–0.037	(−3.65)–(−3.30)	0.11 –0.17	(−2.207)–(−1.772)
45	3.15	1.0– 1.4	0.010–0.014	(−4.61)–(−4.27)	0.049–0.067	(−3.016)–(−2.700)

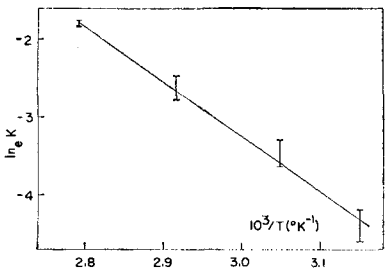

Fig. 6—Van't Hoff plot of apparent equilibrium values derived from asymptotes in Fig. 5.

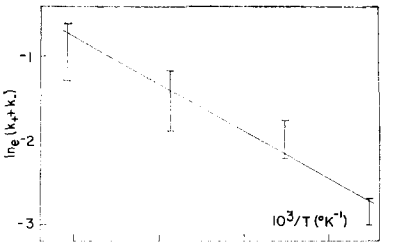

Fig. 7—Arrhenius type plot of the sum of rate constants of vitamin E succinate in lactose base tablets. Values are derived from the curves shown in Fig 5.

The theoretical curve then is $X = 0.143\ (1 - 0.735^t) = 0.143\ (1 - e^{-0.31t})$ as a first estimate.

The theoretical curve can then easily be constructed, and is shown in Fig. 5. Plots at 70° and 55° can similarly be run in a span of 20 days, and fairly good values of X_e may be obtained; longer periods are required for lower temperatures.

Results from storage at other temperatures are shown in Table II and graphically in Fig. 5. The ranges of k and X_e values, as well as of the apparent equilibrium values [$K = X_e/(100 - X_e)$] are shown in Table III. Van't Hoff plotting, *i.e.:*

$$\ln_e K = \frac{-\Delta H}{RT} + \alpha$$

SUMMARY

Cases of equilibrium in solid dosage forms are presented. Such cases should be handled by Van't Hoff plotting rather than Arrhenius plotting.

The virtues of following breakdown product formation as opposed to sole reliance on assays of intact drug are presented.

A rapid method for establishing crude estimates of sum of rate constants and equilibrium value is presented.

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Keyphrases

Drug stability in solid dosage forms
Vitamin A decay
Vitamin E decay
Equilibrium phenomena
UV analysis
TLC determination of free tocopherol

p-Methoxycinnamate and Its Metabolite in Rabbit Serum

By WON SICK WOO

A method of *p*-methoxycinnamate determination in serum is described. *p*-Methoxycinnamate, following intravenous injection, disappeared very rapidly from the serum with a half-life of 0.4 hr. due to metabolic alteration. When orally administered to fasted rabbits, it is rapidly absorbed and maximum concentration is reached within 1 hr. Maximum concentration proportionally increases with increasing dose. The metabolite of *p*-methoxycinnamate in serum was identified as *p*-methoxybenzoate.

SCHROPHULARIAE RADIX, a medicinal plant cultivated in Korea, has long been used as an antipyretic and anti-inflammatory drug. In this laboratory, *p*-methoxycinnamic acid (*p*-MCA) was isolated from this plant, and its antipyretic and analgesic properties have been reported (1, 2). It was also shown that *p*-MCA decreased the ascorbic acid contents of adrenals of rats (3) and in unpublished experiments it has been shown that it inhibits the edema formation by irritants such as yeast, formalin, croton oil, and dextran. When administered to a human and rabbits, it is oxidized to *p*-methoxybenzoic acid (*p*-MBA) which is excreted in the urine as conjugates of glycine and glucuronic acid (4).

The present paper describes the rate of decline of the serum *p*-MCA concentration in rabbits following a single dose, and its metabolite in serum. A rapid and convenient method for the measurement of *p*-MCA in serum is included.

EXPERIMENTAL

Estimation of p-Methoxycinnamate in Serum—The determination method of *p*-MCA has not yet been reported. The following UV spectrophotometry for measurement of *p*-MCA in serum was established.

Serum sample (0.2 ml.) was pipeted into a 5-ml. volumetric flask, brought to exactly 5 ml. by adding a 5% solution of $HClO_4$, and thoroughly mixed. After heating at 60–70° for 10 min., the mixture was cooled and centrifuged at 3000 r.p.m. for 5 min. The clear supernatant was poured into a 1-cm. silica cell and the absorbance was measured at a wavelength of 308 mμ in a Beckman DU spectrophotometer. The test solution was read against a 5% solution of $HClO_4$. The mean absorption of serum was subtracted from the absorbance readings. The mean absorption was determined on a series of sera from normal untreated animals.

Figure 1 shows the standard curve obtained by this method as applied to standard solution of sodium *p*-MCA. Linear function is clearly demonstrated over the range studied.

Table I illustrates the recovery of added amounts of sodium *p*-MCA to rabbit serum. The amounts recovered vary from 96 to 112%, averaging 100.5%.

When serum contained more than 20 mg. % sodium *p*-MCA, the analysis was carried out using a smaller aliquot of sample plus water, and diluting

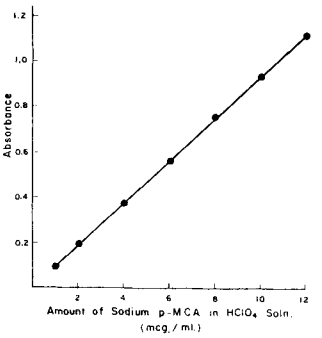

Fig. 1—Calibration curve for Na p-MCA.

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