Moisture Stress Tests in Stability Programs

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Although the effect of moisture in solid dosage forms is qualitatively recognized, the severity of this parameter is often much greater than anticipated. Quantization of interaction of moisture with active components is often possible and an apparent order of reaction can be established rather easily. The higher the order the more severe the effect of moisture content and the closer this variable must be controlled in stability programs. Vitamin A is used to exemplify these points.

TARDIF in a recent publication (1) touched on the effect of moisture on vitamin A stability in solid dosage forms. The moisture sensitivity of vitamin A is greatly affected by the presence of antioxidants, and as will be demonstrated below, can be exaggerated considerably depending on (*a*) the presence of antioxidants in the vitamin A beadlet and (*b*) the tablet base used. The sucrose base employed by Tardif is moderately severe. In our investigations we have found lactose to be a severe interacting substance that lends itself well to artificial testing of (*a*) the quantitative nature of the interaction of vitamin A with moisture and (*b*) as a screening agent for vitamin A beadlets of different matrix composition.

Higuchi and Reinstein (2) at an early date studied the order of reaction of vitamin A with water in hydroalcoholic systems and found the reaction order to be second order with respect to ethanol at high ethanol concentration, slowly approaching first order as the ethanol concentration was decreased. The order with respect to water was found to be complex, changing from much less than 1 in solvent containing 2% water, increasing to 1 at 5% water content, and then to 2 at 20% water content.

The authors have reported their findings in the lactose solid state system, which is a much cruder system than the systems employed by Higuchi and Reinstein.

Theory.—If vitamin A degrades by functional dependence of water content, then a $(1 + a)$ order reaction rate may be expressed as

$$dC_A/dt = -K \cdot C_A \cdot C^a_{H_2O} \qquad \text{(Eq. 1)}$$

where C denotes concentration and the subscripts denote vitamin A acetate and water. If the water is in sufficient excess, Eq. 1 will degenerate to the pseudo first order:

$$dC_A/dt = -K' \cdot C_A \qquad \text{(Eq. 2)}$$

or log (% retained) $= -K' \cdot$ (time) $+ 2$, where

$$K' = K \cdot C^a_{H_2O} \text{ or}$$
$$\log K' = \log K + a \cdot \log C_{H_2O} \qquad \text{(Eq. 3)}$$

"a" will appear as the slope of K' *versus* log C_{H_2O} plots regardless of whether Naperian or non-Naperian rate constants and logarithms are used. Non-Naperian data will be employed in the following.

EXPERIMENTAL

Samples of tablets were made of the following composition: 427.5 mg. of lactose, 5.0 mg. of calcium stearate, and 17.5 mg. of vitamin A acetate beadlets (containing no antioxidant).

The materials were predried at 37° for 24 hr. and various amounts of moisture were added to subparts of the granulation which was then compressed on a Stokes E machine using $^{13}/_{32}$ in. standard concave punches, the thickness being about 10.3 mm. The tableting of the wetter samples was not particularly satisfactory from a mechanical point of view.

The samples were stored in well-sealed bottles at 5, 25, 55, 70, and 85° and assayed at the periods shown in Table I.

Samples of vitamin A palmitate beadlets were incorporated in the same base. The vitamin A palmitate beadlets all contained antioxidants as opposed to the experimental lots of vitamin A acetate beadlets used. The results from storage testing of these are shown in Table III.

RESULTS

Table I yields storage results in terms of units of vitamin A/Gm. of tablet weight.

The table purposely omits longer term data to illustrate the rapidity and utility of the procedure. The use of longer periods is also questionable in view of recent findings (5) regarding equilibrium aspects of vitamin A beadlets, in particular in dryer formulations.

Extracting the K' values (in units of days^{-1}) from Table I yields the data in Table II. The $\log_{10}$ of K' is shown rather than K' to allow treatment by Eq. 3.

The data in Table II are shown graphically in Fig. 1, and analysis by the method of least squares show the lines (*a*) not to differ significantly with respect to slope and (*b*) to yield a best estimate of $a = 2.0 - 3.0$.

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TABLE I.—VITAMIN A ACETATE CONTENTS OF LACTOSE BASE TABLETS AT VARIOUS TEMPERATURES

Journal No. % Moisture	55-12A 0	55-12B 0.5	55-12C 1	55-12D 1.5	55-12E 2
Init.-Ref.	17,550	17,470	17,380	17,300	17,200
85°, 2 days	10,600	9,800	6,300	2,430	1,130
85°, 4 days	6,100	8,900	2,750	980	0
70°, 4 days	12,800	14,950	9,700	5,450	1,550
70°, 8 days	9,400	9,650	6,800	2,250	600
55°, 15 days	15,300	15,250	8,800	2,700	0
25°, 15 days	17,550	16,950	13,950	8,050	5,800
5°, 30 days			14,850	9,080	3,070

TABLE II.—LOG_{10} K'-VALUES AT VARIOUS TEMPERATURES

Journal No.	% H_2O Added	85° Log K'	70° Log K'	55° Log K'	25° Log K'	5° Log K'	Log (H_2O)[a]
55-12A	0	...	...	...	...	...	...
B	0.5	2.982–4	2.362–4	1.602–4	0.938–4		1.7–1
C	1	3.323–4	2.754–4	2.290–4	1.811–4	1.375–4	0.0–1
D	1.5	3.558–4	3.069–4	2.725–4	2.340–4	1.976–4	1.175–1
E	2	3.762–4	3.348–4		2.497–4	2.394–4	1.3–1

[a] It is assumed that the moisture present prior to water addition is bound; the water "concentration" is taken as moisture added.

The assays from 3 different lots of vitamin A palmitate with different beadlet composition are shown in Table III at the 2% moisture level, as an example of the screening aspects of the procedure. It should be noted that the beadlets referred to in Table III are all vitamin A *palmitate* beadlets containing antioxidants, whereas the beadlets referred to in Table I are vitamin A *acetate* beadlets containing no antioxidants.

DISCUSSION

It is apparent from the data that lactose plus moisture is a severe test for vitamin A. It hence may serve as a good agent for evaluating vitamin A beadlets made by various processes and compositions.

The data of Table II have been plotted graphically in Fig. 1 and yield the information that the over-all apparent interaction order is 4 ($a = 3$).

This is a relatively high order. For stability programs, therefore, there appears to be a need for preliminary determination of interaction order since the stability must be viewed in light of the moisture content. Such information should be secured early in the program.

Determination of the interaction order is rapidly obtained. Examplewise, data in Table I (which suffice for this purpose) were secured in about 1 month. The actual calculation of the a-value can be done graphically simply by determining the slope of the plot of log K' *versus* log (H_2O). For instance, the 25° data of Table II, taking points B and D would yield a slope (a-value) of $(2.340 - 0.938)/(1.175 - 0.700) = 2.96$.

Most of the data may be treated by Arrhenius plotting although in the case of the samples in Table I with high moisture contents, elevated temperatures give unreasonably high degradation rates and would have to be extrapolated differently (3) if such extrapolating were needed. The utility of the procedure outlined in stability programs and evaluations is quite apparent.

If the apparent order of the reaction is known it can be established whether losses are solely due to moisture or whether other factors are involved. If, for instance, one tablet formulation contained 2% moisture, another 1%, and the K-values depended on, *e.g.*, $K' = K \cdot C_{H_2O}^2$ then it may be con-

TABLE III.—VITAMIN A PALMITATE IN LACTOSE BASE TABLETS CONTAINING 2% MOISTURE

Tablet	A	B	C
Storage			
Initial	9890[a]	9300	7960
2 days at 85°	2480	2800	1420
k (days^{-1})	0.300	0.261	0.372
4 days at 70°	8300	7230	5840
k (days^{-1})	0.0190	0.0270	0.0342
15 days at 55°	7570	6420	3200
k (days^{-1})	0.00757	0.0107	0.0264
45 days at 45°	5660	6780	5000
k (days^{-1})	0.00543	0.00305	0.00446
180 days at 37°	7750	5560	1850
k (days^{-1})	0.000600	0.00124	0.00355
360 days at 25°	7755	7240	5715
k (days^{-1})	0.000300	0.000300	0.000396

[a] Assays are listed in units per tablet.

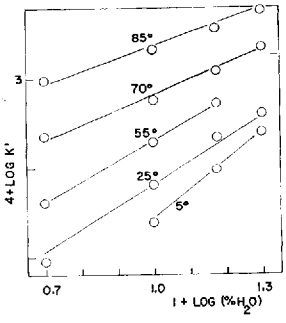

Fig. 1.—Vitamin A acetate-lactose tablets. Plot of the logarithm of the apparent first-order rate constant, K', as a function of amount of water added.

cluded that if K' (2%) = 4 K' (1%) the difference in K' values is solely due to moisture. Such a conclusion could not have been reached without knowledge of the apparent (third) order of reaction.

SUMMARY

1. Vitamin A acetate has been used as an example to demonstrate "order of interaction" in solid dosage forms.

2. Since moisture is a variable seldomly controlled within extremely narrow limits in stability programs, it may prove useful, in many cases, to establish the "order of interaction" between moisture and active component(s).

3. Screening bases for such studies can be selected by choosing the worst offenders in preliminary compatibility programs (4).

4. If a high-order interaction is established, stringent moisture control data should be obtained in the stability program.

5. The study of "order of interaction" may be carried out in a relatively short span of time (30 days).

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New Pharmacologic Aspects of β-Diethyl-aminoethyl 2,2-Diphenylpentanoate

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In a comparison with atropine and structurally related adiphenine using a gross *in vivo* screen in rats, β-diethyl-aminoethyl 2,2-diphenylpentanoate (SKF 525-A) appeared to act peripherally as a parasympatholytic and/or sympathomimetic. SKF 525-A apparently has some selective activity on the central nervous system (blepharoptosis, hypothermia) indicating a capacity to cross the blood-brain barrier. Drug–receptor interactions were studied on the isolated rat jejunum using furtrethonium as the agonist. SKF 525-A was primarily a noncompetitive antagonist with a competitive component and qualitatively different from the activities of atropine, adiphenine, and papaverine. The respective pA_2 and pD'_2 values are reported. The SKF 525-A receptor appears composed of the cholinergic receptor plus another spasmogen receptor. SKF 525-A did not inhibit the action of acetylcholinesterase, but was a potent inhibitor of monoamine oxidase at physiological concentrations.

MUCH OF the work previously reported on β - diethyl - aminoethyl 2,2 - diphenyl pentanoate (SKF 525-A) has been concerned with its ability to act as a multipotent inhibitor of various liver microsomal degradation reactions (1). The original observations made for SKF 525-A concerning potentiation of barbiturates and other central nervous system depressants (2–5) suggested possible potentiation by CNS mediation; however, Brodie (1) demonstrated that this agent was able also to prolong the activity of the central stimulant, amphetamine. This study was prompted by the chemical similarity between SKF 525-A and adiphenine, and also by the lack of comprehensive screening in the literature.

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EXPERIMENTAL

In Vivo Hippocratic Screening.—In accordance with the method of Malone and Robichaud (6), nonfasted albino rats (Wistar strain) in the weight range of 150–250 Gm. were injected intraperitoneally with 5 logarithmetically spaced doses of each drug tested (1 lethal, 1 essentially ineffective dose, and 3 effective log-dosages between those two). Observations were made using the standard worksheet (6) at 5, 10, 15, 30, and 60 min. postinjection, 2, 4, and 24 hr. postinjection, and 2, 4, and 7 days postinjection.

Mechanism of Drug-Receptor Interaction.—Using the methods of Ariëns (7), van Rossum (8), and van Rossum and van den Brink (9), cumulative dose-response curves were made utilizing rat jejunum and furtrethonium iodide as the reference agonist. The bath solution was standard Tyrode's oxygenated with 95% oxygen and 5% CO_2 and containing the calcium disodium salt of ethylenediaminetetraacetic acid in a concentration of 1×10^{-5} Gm./L. The jejunum was mounted in the bath (37.5°) using a modified Magnus technique. All drug concentrations were calculated in terms of drug base. When an antagonist was tested it was