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IMPROVED STABILITY OF LIPID COATED VITAMIN A IN ANIMAL FEED ADDITIVES

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ABSTRACT

Lipids have been studied as methods of encapsulation, permeation enhancers, and as drug delivery systems. A lipid coating containing lecithin,cholesterol and functionalized stearyls was utilized in this study to inhibit the mineral catalyzed Vitamin A degradation in a dry flowable animal feed additive. Results indicate much improved stability.

INTRODUCTION

Vitamin A is very susceptable to oxidation, heat, light, moisture and metal catalysis. (1) (2). Oxidation and hydrolysis are accelerated at high temperatures. Solid formulations are as unstable as liquid Vitamin A products due to the large surface area present for reaction (3). In feed mixtures, the presence of water, peroxides, minerals and peroxidized unsaturated fats all add to the instability of Vitamin A.

Mineral mixtures ordinarily are used to supply calcium, phosphorus and trace minerals to animals and can catalyze the oxidative degradation of Vitamin A.

Vitamin A undergoes both pseudo-zero and first order reactions in liquid media (2). Carstensen has found a log-linear ratio between the first order rate constant and water-vapor pressure (5). Controlling the humidity is one method of improving Vitamin A stability in the solid matrix. Vitamin A solid preparations have shown increased stability when Vitamin A was encapsulated in gelatin (2).

The purpose of this study is to investigate the use of lipid coatings on a powder containing absorbed Vitamin A. The formulation is specifically a vitamin/mineral feed supplement for use in animal nutrition.

By coating a vitamin A dispersion in the components of a lipid bilayer, two results may be realized:

1. Stability from mineral catalyzed degradation.
   The lipid bilayer may halt the catalytic decomposition reaction by physically separating Vitamin A fron the mineral components.

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2. Stability from hydrolytic degradation. If water permeates the bilayer it may be encapsulated by so-called liposome formation and not be available to interact with vitamin A.

MATERIALS AND METHODS

The following materials were used in this study: Vitamin A Palmitate 1,000,000 I.U./ gram was a gift of Hoffman-LaRoche. Lecithin (dry) was food grade and purchased from Central Soya (Decatur, Ill.). Stearylamine was purchased from Aldrich Chemical and Cholesterol U.S.P. , Stearic Acid N.F. and Stearyl Alcohol N.F. were purchased from Ruger Chemical. The Lambert-Kay division of Carter-Wallace gave the vitamin/mineral premix used in this study.

The vitamin/mineral premix used in the study is a nutritionally complete mixture of vitamins, amino acids and the following minerals: calcium,phosphorus, potassium, sodium, magnesium, iron, copper, zinc, manganese, and cobalt, as pharmaceutically acceptable salts.

Formulations were prepared in a 5 Kg. Hobart blender at ambient temperature. The Vitamin A Palmitate was adsorbed onto a dry carrier such as Potassium Phosphate Monobasic (Anhydrous). The lipid composition was then melted in a seperate vessel (neat) and poured onto the agitating Vitamin A coated carrier. After agitation and cooling (30 minutes), the vitamin/mineral premix was added. Agitation continued for 15 minutes . Total mixing time was 45 minutes. The lab prep was done at ambient temperature and relative humidity. No requirements for an inert atmosphere were utilized.

ANALYTICAL METHOD

Vitamin A stability of the powder was analyzed by HPLC using a 0.4 X 30 cm. Porasil column at 313 nm. using a mobile phase of 98:2 Isooctane : Ethyl Ether . Stock and working solutions of Vitamin A palmitiate were prepared in hexane and a calibration curve was prepared. Sample preparation consisted of weighing 50-60 mg of sample into 5 ml DMSO. Extraction of the Vitamin A was done with heat and agitation. 25 ml of hexane was added and agitation and centrfugation followed. 5 mls of the supernatent was pipetted into a 50 ml volumetric flask and was diluted with hexane. The injection volume was 7 microliters.

STABILITY TESTING

Formulations 1 through 4 were analyzed in accordance with the preceeding HPLC method. Storage stability samples of the formulations were analyzed initially, at 1 month, 3 months and 6 months. The samples were stored in the following conditions: ambient temperature (approx.25 degrees), and 37 degrees centigrade in dark cabinets or ovens. Analyses were run in duplicate and averaged for data analysis.

The finished formulae were split into 300 gram amber tinted polystyrene wide mouth bottles, filled to the top and sealed with a torque of 10 to 20 foot-pounds and stored at the indicated conditions. Intial analysis was done to verify initial concentration and recovery. Each bottle was considered a sample volume and duplicate weighings were done for analysis.

Samples were analyzed by HPLC as previously indicated. The results were averaged and normalized to percent of initial assay (100%) (Table 2). All samples were analyzed for moisture content by Karl Fischer method. In all samples, moisture content was below 1 %.

DISCUSSION OF RESULTS

Table 3 is a comparision of zero order and (pseudo) first order constants and their R-squared values. These results were developed from the stability data of Table 2. Once the

TABLE 1
Formulations of Lipid Coated Powders

FORMULATIONS NUMBER:	1.	2.	3.	4.
LECITHIN	-	1.69	1.69	1.69
CHOLESTEROL	-	0.83	0.83	0.83
STEARIC ACID	-	-	0.28	-
STEARYLAMINE	-	0.28	-	-
STEARYL ALCOHOL	-	-	-	0.28
VITAMIN A PALMITATE	0.61	0.61	0.61	0.61
POTASSIUM PHOSPHATE MONOBASIC (ANH.)	57.68	54.98	54.98	54.98
VIT./MINERAL PREMIX	41.71	41.61	41.61	41.61

TABLE 2
Storage Stability Results
% OF Initial Vitamin A Concentration

FORMULA :	1 (CONTROL)		2		3		4	
TEMPERATURE :	R.T.	37	R.T.	37	RT	37	R.T.	37
1 MONTH	85.2	69.0	92.2	75.0	102	92.1	101.1	90.9
3 MONTHS	93.6	63.0	96.1	34.7	93.9	74.8	100.3	77.9
6 MONTHS	43.3	42.0	75.0	26.0	90.2	56.1	97.0	60.1

R.T. is room temperature
37 is 37 degrees centigrade

TABLE 3

Comparision Of Slopes and Regression Coefficients
Of Zero Order and Pseudo-First Order Degradation Profiles

FORMULATION	TEMP	ZERO ORDER		PSEUDO-FIRST ORDER	
		SLOPE	R	SLOPE	R
1 (CONTROL)	R.T.	-5.23	0.7	-10.81	0.61
	37	-11.13	0.69	-11.27	0.97
2	R.T.	-4.16	0.93	-7.51	0.58
	37	-22.12	0.99	-17.92	0.89
3	R.T	-1.95	0.88	-2.31	0.58
	37	-7.38	0.99	-9.81	0.82
4	R.T	-0.58	0.72	-0.50	0.25
	37	-6.60	0.99	-0.86	0.84

TABLE 4
Summary Of Lipid Formulae Reaction Kinetics

PRODUCT	TEMP	REACTION ORDER	%LOSS/DAY	VITAMIN A LOSS/DAY
1 (CONTROL)	R.T.	1	0.172	258
	37 DEGREES	1	0.366	549
2	R.T.	0	0.137	205.5
	37 DEGREES	0	0.727	1090.5
3	R.T.	0	0.064	96.0
	37 DEGREES	0	0.243	394.5
4	R.T.	0	0.019	28.5
	37 DEGREES	0	0.217	325.5

Vitamin A loss per day is based on a normalized initial dose of 150,000 I.U.

lipid coating is applied to the Vitamin A powder, it is interesting to note that the reaction order seems top shift from (pseudo) first order to zero order. This shift is most obvious in the 37 degree data. This may indicate that the decomposition pathway may have changed. This will be the subject of future investigation.

The evaluated temperature data in all cases indicates a substantial increase in degradation of 37 degrees over room temperature for each system. This is due to the low melting range of the lipid coating. In all cases the lipid coating begins its phase transition at 35 degrees. This must be taken into consideration for purposes of commercial utility.

The addition of the lipid coatings to a dispersion of Vitamin A powders definitely increases stability at room temperature (TABLE 4). The decomposition of Vitamin A is retarded in each experimental system as follows:

EXPERIMENT	SYSTEM	FACTOR OF STABILITY IMPROVEMENT
1	CONTROL	1
2	LECITHIN/CHOLESTEROL/ STEARYLAMINE	2.63
3	LECITHIN/CHOLESTEROL/ STEARIC ACID	5.55
4	LECITHIN/CHOLESTEROL/ STEARYL ALCOHOL	20.0

This data indicates that a lipid coating deposited on the substrate containing absorbed Vitamin A retards degradation of the vitamin while in the presence of minerals which would otherwise catalyze degradation.

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