RESEARCH PAPERS

THE MEASUREMENT OF THE STABILITY OF VITAMIN A IN HIGH POTENCY CONCENTRATES

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The vitamin A industry has developed considerably during recent years. Fish-liver oils of low potency (less than 50,000 I.U./g.) and of poor organoleptic quality have been progressively converted to concentrates of steadily increasing strength (up to 1,500,000 I.U./g.) and odour and taste have been greatly improved. For the last two years synthetic concentrates have been able to compete with natural products.

Whatever the origin and the form of presentation of the vitamin A, it still remains a substance particularly susceptible to the action of air, light and heat. Of all these modifying factors, oxidation is in practice, by far the most important. The stability of vitamin A, from this point of view, has been studied by numerous authors who have proposed various methods of measurement; in particular can be mentioned Buxton[1], Chevallier and co-workers[2], Bose and Banerjee[3], Kern *et al.*[4], and Swain[5]. In preference to the methods used by these authors (direct measurement of destroyed vitamin A, examination of peroxide index, manometric determination of oxygen absorbed by the oil in a thin layer) we recommend, like Sanford *et al.*[6], and Bolomey[7], an application of the so-called "Swift method" or "active oxygen method"[8,9] commonly used for the rapid determination of the stability of oils with respect to rancidity. It consists in bubbling air through the oil, maintained at a temperature of 100°C., and determining the time necessary for the destruction of 50 per cent. of the vitamin originally present. With the modifications which we have added, this method has been found to be very practicable, and has always given us reproducible results. In the present work we shall first describe the method of operation, then the modifications used, and finally the results obtained in the course of an examination of commercial preparations of vitamin A, of natural and synthetic origin, which are commercially available.

1. The Method

The preparation to be examined is diluted to a uniform level of 10,000 I.U./g. with squalane (hexamethyltetracosane) and 12 to 15 g. of this solution is introduced into a bubbler similar to that described by Sanford[6]; this quantity should occupy a height of 8·0 cm. in the tube. Several of these tubes are placed simultaneously in a water-bath at 96°C. and

pure oxygen is bubbled through at a pressure of 4 to 5 cm. of mercury, the flow being regulated for each tube by passing through a calibrated orifice of 0·4 mm. diameter. The destruction of vitamin A was followed every 30 or 60 minutes by determining the content of vitamin A of samples taken by means of a fine glass rod left permanently in the tubes. The actual determination was made by dilution of a weighed sample in *iso*propanol followed by spectrophotometric examination at 328 mμ. The index of stability is the time, expressed in hours, required for the destruction of 50 per cent. of the vitamin A originally present.

2. Discussion of the Method

(a) *Dilution.* Sanford[6] and Bolomey[7] worked with non-diluted oils. Actual commercial products are very variable in content and range from 10,000 to 1,000,000 I.U./g. It is fairly evident that this difference in concentration will influence the results, thus making it difficult to compare the "stability" of a poor oil with a rich one. The experiments reported below demonstrate the correctness of this view.

Pure crystalline vitamin A acetate (lot H 65)* was dissolved in squalane at concentrations from 2,000 to 250,000 I.U./g. and the resulting solutions were submitted to tests for stability. The observed stability indices are given in Table I.

TABLE I

Influence of concentration of vitamin A on the index of stability

Initial concentration	Index of stability
I.U./g. 2,000	15·5
5,000	7·85
10,000	4·25
25,000	1·75
50,000	0·6
100,000	0·5
250,000	<0·5

These figures show the importance which must be attached to the dilution level and explain why we have diluted all further samples to a uniform level with a suitable solvent. The level chosen was 10,000 I.U./g. —the lower limit of concentration of natural oils and of vitamin preparations used at present in the pharmaceutical and food industries; at the same time it is sufficiently high to lend itself to spectrophotometric measurement.

(b) *Nature of solvent used.* Having accepted the principle of dilution, it is essential to choose a diluent which has as little effect as possible on the oxidation of the vitamin, or at least only a limited and constant effect.

* The spectrum characteristics of this sample have been published by Chatain and Debodard (*C. R. Acad. Sci., Paris,* 1950, **251,** 1102).

In order to obtain a preliminary idea of the behaviour of possible solvents, we have examined a number of them after having added 10,000 I.U. of vitamin A per g. in the form of a concentrate (600,000 I.U./g.) obtained by molecular distillation.

Table II summarises the results obtained; together with the stability indices we have given the iodine values, peroxide values, and acidities—all factors which play a role in the oxidation of vitamin A.

TABLE II

INFLUENCE OF THE NATURE OF THE SOLVENT ON THE INDEX OF STABILITY OF VITAMIN A

Nature of solvent	Stability index	Iodine value	Peroxide value	Acidity	Notes
Arachis oil M.	3·5	84	21	neutral	
„ „ L.C.A.	17	60	5	„	(1)
Oil of *Centrophorus granulosus*	3	285	2·5	„	(2)
Cetyl alcohol	20	0	4	„	
Stearic acid	1	0	2	„	
Whale oil, m.pt. 40°	21	51	5	„	(3)
Hydrogenated whale oil, m.pt. 41°	32·55	29	0·5	„	
Spermaceti	20	0	18	„	
Squalane	31·5	5	0	„	
Soft paraffin	2·25	0	16	„	(4)
Paraffin, French Codex	18·15	0	0	„	

(1.) Fresh pressed oil, not refined.
(2.) Containing 70 to 80 per cent. of squalane and about 1,000 I.U./g. of vitamin A.
(3.) Sample kept in the laboratory for more than 6 months.
(4.) Old sample.

These results, as well as numerous others, which it is not necessary to report here, show that the ideal diluent should comply with the following conditions.

(1) It should be as stable as possible in presence of oxygen at 100°C. (2) It should be neutral and remain so during the heating, since any acidity produces rapid destruction of vitamin A (see stearic acid). This condition excludes glycerides in general, and most of the esters of fatty acids. (3) It should be always the same so that a reference stock may be kept. This condition excludes natural oils, always of uncertain stability, and paraffin oils of variable origin. (4) It should be fluid at ordinary temperatures, non-volatile at 100°C., and easily obtainable.

For all these reasons squalane, obtained by hydrogenation of squalene, was chosen; the latter is easily obtained in a high state of purity by molecular distillation at 100°/120°C. of the liver oil of *Centrophorus granulosus*, after preliminary steam distillation. When so prepared it is a colourless oil, perfectly stable at 100°C. in presence of oxygen, does not hydrolyse, and has the following chemical characters: iodine value, less than 5; saponification value, 0; peroxide value, 0; acidity, 0. Further, it is transparent in the ultra-violet and does not interfere with the determination of vitamin A. The adoption of this diluent has allowed us to

obtain reproducible results on products of varying concentration, and has enabled us to make progress in the evaluation of the stability of numerous oils with an improvement of the methods used.

(c) *Secondary factors.* The work was performed at 96°C. so as to shorten the tests (Bolomey[7]); for the same reason oxygen was used instead of air. The effect of variation of temperature on the speed of oxidation has been determined by Sanford[6] and Bolomey[7], who found that it approximately doubled for a rise of 10°C.

The results below (Table III) obtained simultaneously on 3 different concentrates at 96°C. and 60°C. confirms this observation and allows the comparison of results obtained at different temperatures.

The temperature factor (a) here is: (SI=Stability Index)

$$SI_{96} = SI_{60} \times a\ 0{\cdot}1(96-60) = SI_{60} \times a^{3{\cdot}6}$$

$$a^{3{\cdot}6} = \frac{SI_{96}}{SI_{60}} = 11{\cdot}8$$

$$a = 1{\cdot}98$$

TABLE III

INFLUENCE OF TEMPERATURE ON STABILITY INDEX

Concentrate	Initial content of vitamin A I.U./g.	Stability index at 96°C. in hours	Stability index at 60°C. in hours	Ratio $\frac{SI_{60}}{SI_{96}}$
E 334	340,000	5·75	65·75	11·5
46107	395,000	10·5	123	11·7
H 398	1,000,000	7	86	12·3
			Mean... ...	11·8

3. EXAMINATION OF VARIOUS PREPARATIONS OF VITAMIN A

The determination of vitamin A stability has been extended to the concentrates given in Table IV, chosen as representative of present-day production. Table V and Figure I summarise the results obtained. Under the conditions described, vitamin A of natural origin showed a distinct superiority. Thus in this category, concentrates obtained by molecular distillation (5,6,7,8) were by far the most stable, their index of stability varying from 12·4 to 17. It can be seen (Fig. 1) that the first phase of oxidation, namely the induction period of these samples is on an average 8 hours; the final drop is more or less rapid according to the product. The induction period is lacking on the other hand, with samples obtained by saponification or by chromatography or with synthetic products (except 12).

Sample 4, obtained by solvent extraction, showed a moderate stability. Among the products of synthesis which were examined, sample 12 (stability index 9·1) was distinguished from all the others by an increased stability which was comparable with the results obtained on molecularly distilled natural concentrates of relatively poor stability.

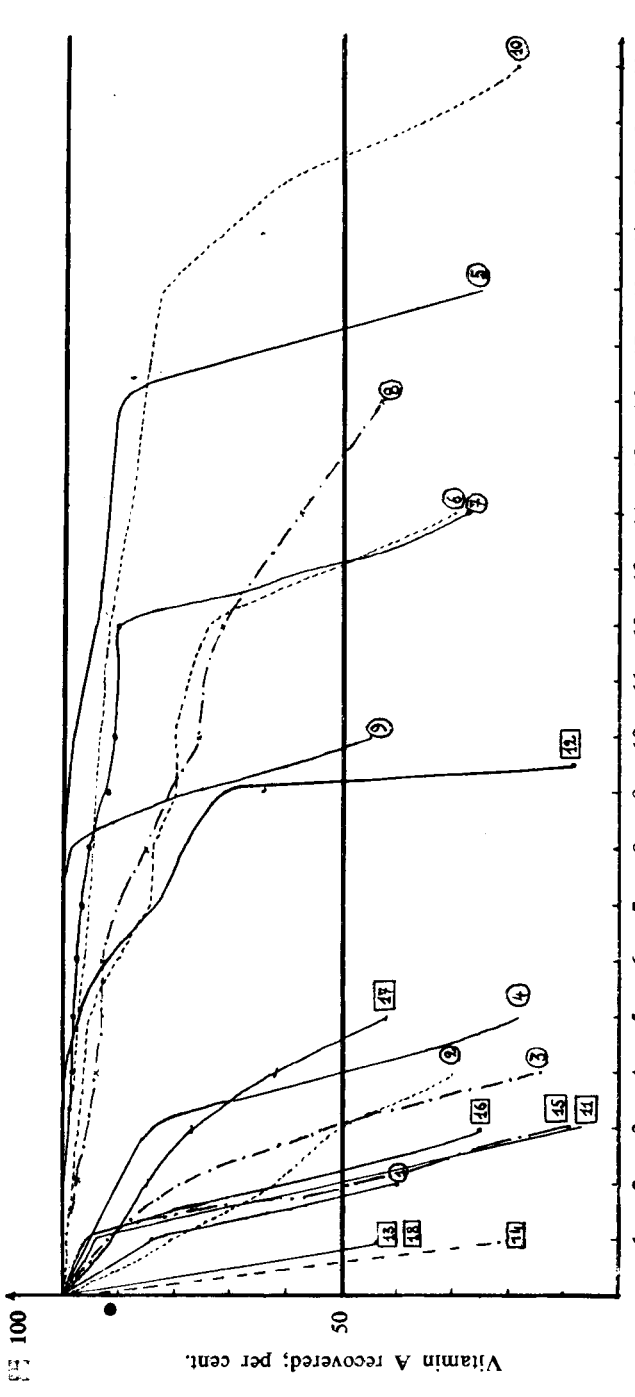

FIG. 1. Percentage of vitamin A recovered, plotted against time. The numbers refer to the products enumerated in Table IV. The numbers of natural products are in circles (1) those of synthetic products are in squares [11]

TABLE IV

PRODUCTS EXAMINED

No.	Concentration in thousands I.U. g.							Origin
	a	b	c	d	e	f	g	
1 ...	560							Norway
2 ...	820							U.S.A.
3 ...		650						France
4 ...			200					S. Africa
5 ...				150				France
6 ...				400				France
7 ...				600				U.S.A.
8 ...				200				U.S.A.
9 ...					1·500			France
10 ...					110			France
11 ...						1·100		U.S.A. manf. 3
12 ...						932		U.S.A. ,, 1
13 ...						1·00		U.S.A. ,, 3
14 ...							900	U.S.A. ,, 2
15 ...							1·025	U.S.A. ,, 3
16 ...							1·135	U.S.A. ,, 2
17 ...							1·025	U.S.A. ,, 1
18 ...							1·075	U.S.A. ,, 3

(a) Concentrates obtained by saponification of liver oils, which contain all their vitamin in the form of alcohol.
(b) Concentrates obtained by chromatography.
(c) Concentrates obtained by selective extraction of vitamin A.
(d) Concentrates obtained by molecular distillation.
(e) Concentrates of natural vitamin A, prepared by molecular distillation of fish liver oils, followed by saponification and acetylation.
(f) Concentrates of synthetic vitamin A.
(g) Concentrates of synthetic vitamin A palmitate.

DISCUSSION

The question arises as to whether or not it is possible to explain the considerable differences of stability observed during the tests, although it must be recognised that any explanations are limited to the experimental results obtained.

Regarding products obtained by saponification and containing vitamin A alcohol, it is generally admitted that this form is less stable than vitamin A as an ester. Our results in fact confirm this.

It is also known that the resistance of vitamin A to oxidation depends to a large extent on the antioxidants present[1,4,10,11,12,13]. The high degree of stability shown by concentrates obtained by molecular distillation may be explained by the fact that the antioxidants initially present in the natural oils are found after distillation in the same fractions as the vitamin A. The addition of a supplementary amount of antioxidants is also possible, and it might be supposed that the less stable products merely differ from the more stable ones by the level of antioxidants present.

TABLE V

PERCENTAGE OF VITAMIN A RECOVERED, AS FUNCTION OF TIME OF OXIDATION

Time hours	Number of samples																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	84	81	92	95	100	100	100	100	100	100	94	100	44	21	96	96	91	36
2	40	62	79	89	100	98	100	98	100	100	50	100			49	60	85	
3		51	53	85	100	97	99	96	100	100	6·9	100			8·5	25	77	
4		30	14	49	100	96	98	94	100	98		100					62	
5				18	100	95	98	93	100	97		97					42	
6					100	90	98	93	100	96		92						
7					100	84	97	90	100	96		83						
8					100	84	95	85	99	95		79						
9					99	80	92	80	78	94		74						
10					98	80	91	76	45	93		8						
12					94	74	90	71		92		9·5*h*						
14					92	30	27	57		88								
16					90			43		86								
18					25					82								
20										60								
21										19								
Stability index	1·8	3·0	3·1	4·0	17·3	13·1	13·2	15·0	9·8	20·3	2·0	9·1	0·9	1	2·0	2·3	4·5	1

In order to clear up this point, we have attempted to improve the less stable products obtained by saponification (1), by chromatography (3) or by synthesis (11,15.18) by adding to them various antioxidants such as DL-α-tocopherol, nordihydroguaiaretic acid, ional, mixture of hydroquinone-citric acid. A solution of crystalline vitamin A acetate in squalane was treated similarly. Table VI shows some of the results obtained by the addition of hydroquinone and citric acid.

It may be noted that, while solutions of vitamin A acetate can be stabilised satisfactorily and may then be compared with natural con-

TABLE VI

EFFECT OF ADDED ANTIOXIDANT ON THE STABILITY INDEX

Concentrate No.	Stability index	
	Concentrate alone	Plus antioxidant
1...	1·75	5
3...	3·05	4
11...	2·0	4·25
15...	2·0	4·0
18...	0·6	0·9
Crystalline Vitamin A acetate...	4·25	10·6

centrates, the results are very different for the other products, particularly of synthetic origin; with these products it is exceptional to exceed a stability index of 4. It seems, therefore, that the poor stability of such forms of vitamin A is not due to a lack of antioxidants, but rather to the presence of unknown substances which facilitate the oxidation of the vitamin A and thus play the part of pro-oxidants. Trials which are now being undertaken indicate that in certain cases it may be possible to remove these substances from the oils, thus greatly improving their stability.

In conclusion it may be noted that our observations regarding the comparative stability of concentrates of natural origin and those of synthetic origin are in contradiction with those of Lindholm and Terp[14] published in 1950. These authors, however, used a simple exposure of the oils in an oven at 45°C., and they used synthetic concentrates stated to be 200,000 I.U./g.; such products are not at present commercially available.

SUMMARY

1. This paper gives a standardised procedure based on the Swift method for the determination of the resistance of vitamin A to oxidation, and characterised particularly by the uniform dilution of all samples with a special diluent.

2. Applied to a series of commercial vitamin A products of natural or synthetic origin, the method showed the greater stability of natural concentrates obtained by molecular distillation.

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