THE STABILITY OF VITAMIN A ALCOHOL IN AQUEOUS AND OILY MEDIA

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There have been numerous investigations[1,2,3,4,5,6,7,8,9,10] into the stability of vitamin A, generally in oil solution. However, research over the past 10 years into the development of synthetic non-ionic surface-active agents has now made it possible to prepare aqueous dispersions of vitamin A; to the naked eye they look like true solutions. As a consequence, interest now centres upon the comparative stability of this vitamin in aqueous dispersions and in oily solutions.

The preparation of aqueous dispersions is the subject of numerous patents. In particular, British Patent 588,298 (1947) has received considerable attention. It describes the use of polyoxyethylene-sorbitan monolaurate and mono-oleate as dispersing agents. The stabilities of vitamin A alcohol in aqueous dispersions containing these agents and in cottonseed oil have been studied by Kern and Antoshkiw.[11] In accelerated temperature tests they showed that vitamin A alcohol in aqueous dispersions containing polyoxyethylene sorbitan monolaurate is significantly more stable than when in solution in cottonseed oil.

Considerable search and experiment have gone to discover other non-ionic surface-active agents that will permit preparation of stable aqueous vitamin A dispersions, and several have been found suitable by us. These substances are condensation products of polymerised ethylene oxide with higher fatty alcohols. Two of them are commercially available —"Brij 35" (Atlas Powder Co.), which is described as polyoxyethylene lauryl alcohol, and "Lubrol W" (I.C.I.), which is described as consisting substantially of polyoxyethylene cetyl-stearyl alcohol. The aqueous dispersion of vitamin A alcohol that is the subject of this paper was prepared with "Lubrol W."

Experimental

We have found that the amount of "Lubrol W" required to prepare aqueous dispersions of vitamin A is a direct function of the weight of the substance being dispersed and is of the order of 10 times its amount. This is in agreement with British Patent 588,298 (1947). However, incorporating glycerol to the extent of 30 per cent. of the final volume of the dispersion makes it possible to halve the amount of dispersing agent. Accordingly, our aqueous preparations have been made at a potency of approximately 12,000 I.U. of vitamin A in each g. by using 5·0 per cent. w/v of "Lubrol W" and 30·0 per cent. v/v of glycerol.

The vitamin A alcohol used for this work was prepared by saponification of a rich concentrate of vitamin A acetate. The potency of the resultant

vitamin A alcohol was $1{\cdot}6 \times 10^6$ I.U./g. determined spectrophotometrically. The B.P. arachis oil used in preparing the oily solution of vitamin A alcohol had a peroxide value of less than 1·0.

To evaluate the stability of vitamin A alcohol in aqueous dispersion and in oily solution, we have made tests of two kinds. First, the stability has been determined over a long period under conditions approximating to commercial storage. The test samples were stored for alternate periods of 14 days at room temperature (15° to 22° C.) and at 37° C. The samples of the oily solution and the aqueous dispersion were sealed in ampoules under air, the free air space in the ampoule occupying approximately 75 per cent. of the ampoule capacity. Initially and intermittently over a period of 20 months one ampoule has been withdrawn and the vitamin A content determined spectrophotometrically. The initial potency of the vitamin A in both of the test preparations was approximately 12,000 I.U./g.

Secondly, accelerated heat tests have been performed on identical preparations. In a similar manner an aqueous dispersion or an oily

TABLE I

VITAMIN A ALCOHOL IN ARACHIS OIL

Initial potency—12,000 I.U./g.

Exposed in ampoules for alternate 14 days at room temperature (15° to 22° C.) and 37° C.

	Age of sample in months					
	0	1	3	11	15	20
Percentage of vitamin A remaining	100	87	68	67	53	34

TABLE II

VITAMIN A ALCOHOL IN AQUEOUS DISPERSION

Initial potency—12,000 I.U./g.

Exposed in ampoules for alternate 14 days at room temperature (15° to 22° C.) and 37° C.

	Age of sample in months						
	0	1½	4	5	7	9	20
Percentage of vitamin A remaining	100	102	98	101	96	96	92

TABLE III

VITAMIN A ALCOHOL IN AQUEOUS DISPERSION AND OILY SOLUTION

(Accelerated test)

Initial potency—12,000 I.U./g.

Samples exposed in ampoules at 100° C.

	Time of exposure in hours			
	0	2	4	8
Vitamin A alcohols in arachis oil	100	87	70	57
Vitamin A alcohol in aqueous dispersion	100	94	86	70

NOTE: Figures are for percentages of Vitamin A remaining.

solution of vitamin A alcohol was sealed in ampoules under air, with a free air space equal to 75 per cent. of the ampoule capacity. The samples were assayed initially and then exposed to a temperature of 100° C. ± 1° in a thermostatically-controlled oven for a period of 8 hours. At the end of 2, 4 and 8 hours ampoule contents were assayed spectrophotometrically.

Results are recorded in Tables I and II (long-term tests) and Table III (accelerated tests).

Discussion

It is apparent from these results that vitamin A alcohol is substantially more stable in an aqueous dispersion than in an arachis oil solution. These results are in agreement with the conclusion recorded by Kern and Antoshkiw.[11] From their results it might have seemed that polyoxyethylene sorbitan monolaurate was exerting a specific stabilising effect upon the vitamin. However, taken into consideration along with the present work, the superior stability of the aqueous dispersions over the oily solutions would appear to be a function of the physical state in which the vitamin A is present. The solubilisation of hydrophobic lipoid substances requires the presence of micelles. These micelles are of colloidal dimensions and are believed to consist of loose spherical aggregates of the hydrocarbon chain ions, with the polar heads turned towards the aqueous phase. It is considered that lipophilic substances such as vitamin A alcohol are taken up into the interior of the micelles. It is postulated by Kern and Antoshkiw that such micelles are impermeable to oxygen and their evidence supports this view. The greater stability exhibited by aqueous dispersions may also be explained by the instability of peroxides. In oily solutions the formation of peroxides is an important preliminary step in the destruction of vitamin A. In an aqueous medium at elevated temperatures, however, one would not expect peroxides to be stable, and any peroxides that might be present in the vitamin A concentrate would subsequently be destroyed.

Summary

1. The stability of vitamin A alcohol in aqueous dispersion with "Lubrol W" as the dispersing agent, and in oily solution have been compared over a period of 20 months.

2. Accelerated stability tests have been performed on an aqueous dispersion of vitamin A alcohol and an oily solution.

3. It was found that an aqueous dispersion of vitamin A alcohol, when "Lubrol W" was used as the dispersing agent, exhibited greater stability than a solution in arachis oil.

Our thanks are due to Miss R. P. Russell, B.Sc., of the Analytical Department, for carrying out the assays.

References

1. Bolomey, *J. biol. Chem.*, 1947, **169**, 323.
2. Buxton, *Indust. Engng. Chem., Anal. Ed.*, 1947, **39**, 225.
3. Caspe and Hadjopoules, *Amer. J. Pharm.*, 1938, **110**, 533.

4. Feigenbaum, *Nature, Lond.*, 1946, **157**, 770.
5. Kern *et al.*, *Indust. Engng. Chem., Anal. Ed.*, 1949, **41**, 2849.
6. Stern *et al.*, *J. Amer. chem. Soc.*, 1947, **69**, 689.
7. Marcus, *J. biol. Chem.*, 1928, **80**, 9.
8. Guerrant, *et al.*, *Anal. Chem.*, 1948, **20**, 465.
9. Sen Gupta, *J. Ind. chem. Soc.*, 1946, **23**, 233.
10. Chilcot, Guerrant and Ellenberger, *Anal. Chem.*, 1949, **21**, 960.
11. Kern and Antoshkiw, *Indust. Engng. Chem., Anal. Ed.*, 1950, **42**, 709.
12. British patent No. 588,298, 1947.

DISCUSSION

The three papers on vitamin A were discussed together. The first was presented by MR. E. HAYES, the second by DR. N. EVERS, and the third by MR. C. L. J. COLES.

DR. R. E. STUCKEY (London) said that results obtained by molecular distillation indicated that the amount of vitamin A alcohol in fish-liver oils was negligible. He asked to what percentage the authors would suggest limiting the amount of vitamin A alcohol in the more concentrated products such as halibut-liver oil. The estimation of vitamin A was becoming more and more complex. The 3-point correction procedure was rarely satisfactory with raw cod-liver oil. He did not entirely agree with Mr. Swann's work. Cod-liver oils fell into two categories. In the first the oil could readily be saponified, the curve obtained was fairly free from irrelevant absorption, and it was possible to use the 3-point correction procedure. In the second, which unfortunately included many B.P. oils, the curve even after 30 minutes' saponification was unlike that of pure vitamin A. In his view it was not satisfactory either to use the factor of 1600 or to apply the 3-point correction procedure, the magnitude of the correction being too large. A satisfactory procedure for such oils was to carry out a chromatographic separation after saponification. It was surprising to note in the paper by Coles and Thomas the lack of stability of a solution in oil of vitamin A alcohol stored under room conditions. He asked whether they had any comparative figures for other forms of vitamin A or natural oils.

MR. J. H. OAKLEY (London) said the use of the agents described by Coles and Thomas for dispersing oils seemed to have a wide application. It had been shown in papers relating to soaps that various materials affected the micellar structure. Had the authors any information on the effect of glycerol and ethanol on the micellar structure? Did the use of those substances enable the proportion of dispersing agent to be further reduced? Had the authors tried the addition of antioxidants, e.g., ascorbic acid, in the aqueous phase, and what effect had they had on the stability of the vitamin A?

MR. D. N. GORE (Dorking) asked Mr. Coles about the effect of dilution on the stability of the aqueous dispersion? Was there any critical concentration above or below which the stability fell?

DR. G. E. FOSTER (Dartford) supported Mr Swann in his preference for the gross absorption in the assay of cod-liver oil. Until something better was available, in his view the conversion factor of 1600 was the

best for ensuring agreement between laboratories. It would be interesting if the authors could give some information concerning the assay of vitamin A in malt preparations.

DR. R. M. SAVAGE (Barnet) asked Mr. Coles if there was any correlation between the biological and spectrophotometric results on the aqueous dispersion. If the micellar structure turned out to be so stable in retaining vitamin A, the assay results by physical methods might not be related to the biological results.

MR. C. J. EASTLAND (London) asked whether there was any information regarding the chronic toxicity of the dispersing agent.

DR. F. WOKES (King's Langley) observed that Mr. Swann used for detection of the end-point the fluorescence in ultra-violet light, and asked how that compared with antimony trichloride for sensitivity. It would be useful if some indication could be given of the proportion of irrelevant absorption occurring in the various preparations to which the Morton and Stubbs correction was applied.

MR. E. HAYES (London) said that it was very difficult to get a true estimation of vitamin A potency in cod-liver oil. Probably everybody in the industry would like to see a reversion to the 1600 times gross *E* factor. The difficulty about the Morton and Stubbs correction was the assumption that the irrelevant absorption was linear at three fixation points. Many people did three estimations and put the solutions in the spectrophotometer at the same time. That did not provide a good estimate of reproducibility, and he had found that differences between laboratories of 10 per cent. were common, and differences of 5 per cent. very common. Demands for precision of 2 per cent. were, in his opinion, impracticable. The biological assay for cod-liver oil appeared to have fallen into disuse, and he was of the opinion that it was a little too early for that to have happened. The World Health Organisation produced an international standard in 1949 which was still being issued. However, it was difficult to see the purpose of the standard because it had been shown that it was not up to specification; the vitamin A percentage was a little low. Spectrophotometry was not specific. It was possible to get many materials which had an absorption curve close to that of vitamin A but which had no biological activity. Much work on the biological side was necessary before estimating the true potency of preparations.

DR. N. EVERS (Hertford) asked Mr. Hayes why he desired to limit the amount of vitamin A alcohol in fish oils. Was vitamin A alcohol inactive or harmful?

MR. C. L. J. COLES, in reply, said that the alcohols were solubilised more easily than esters, and in an aqueous dispersion the vitamin A alcohol was more stable than the ester. Antioxidants had been used and tocopherol had been found to be the most satisfactory. Vitamin A in aqueous dispersion was utilised two or three times more efficiently than vitamin A from an oily vehicle, and if it were to be assayed biologically there was the difficulty of defining a standard. On dilution with water or milk the product was stable for 4 to 5 hours. He did not think that Lubrol W had previously been used in medicine. Each batch of the

substance should be examined individually for toxicity. By the addition of glycerol it was possible to reduce the amount of Lubrol, and by the introduction of alcohol it could be reduced still further.

MR. E. HAYES (London), in reply, said that in high potency preparations a limit of 7 per cent. for the vitamin A alcohol content would be adequate. There were two reasons for desiring the exclusion of vitamin A alcohol. First, it interfered with the Morton and Stubbs correction which was based on the assumption that vitamin A was present as an ester, and if there were two forms with two different absorption spectra it complicated matters. Second, it was generally assumed that vitamin A alcohol was less stable than vitamin A esters.

DR. N. EVERS, in reply, said he thought that Mr. Swann would agree that on the whole the chromatographic separation gave the most reliable results. The B.P. 1948 method was empirical in their view, but it did give results comparable with the vitamin A present. With malt preparations no difficulty was experienced with the absorption method. On the question of detecting vitamin A in the eluate by the fluorescence method, Mr. Swann would probably say that it was more sensitive than the antimony trichloride test and could be observed continuously. He did not think that Mr. Swann stated that the E correction multiplied by 1600 gave results comparable with biological figures.