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STUDIES ON VITAMIN-A IN SOLUTION. PART V. AQUEOUS SYSTEM

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Studies on the behaviour of vitamin-A, when dispersed in aqueous medium have been made. The vitamin-A is less stable at acidic p_H of 3.0 than at p_H of about 6.0 The hydroxy antioxidants, like propyl gallate, show no antioxygenic property. The acetate form of vitamin-A is less stable than the alcohol form in aqueous solution.

In previous communications (Basu and Bhattacharya, this *Journal*, 1949, **26**, 419, 459 ; 1950, **27**, 169) the stability of vitamin-A has been studied in glyceride or ester substrates, both natural and synthetic. It has been found that vitamin-A in the form of an ester (acetate) is more stable than the vitamin-A alcohol. The vitamin may be stabilised by the incorporation of some hydroxy antioxidants and the function of these antioxidants is better manifested in the presence of traces of hydrogen donating substances.

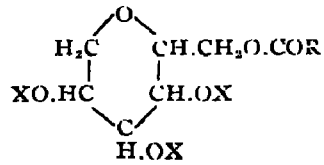
The vitamin-A, generally used for medicinal purpose, is often dispensed in oily menstrum, but it has recently been reported (Esh and Bhattacharya, *Indian J. Physiol. Alli. Sci.*, 1951, **5**, 15; Sobel *et al.*, *Federation Proc.*, 1949, **8**, No.1; *Am. J. Dis. Child.*, 1947, **73**, 543 ; 1949, **77**, 576) that a better utilization is effected if it be dispersed in an aqueous phase. As such our study on the relative stability of vitamin-A alcohol and that of a vitamin-A ester (acetate) was continued with preparations in aqueous phase. At the outset of these investigations two questions arise and these are whether any change in the hydrogen-ion concentration may have any effect on the keeping properties of the vitamin molecule, and whether the hydroxy antioxidant with its greater solubility in aqueous medium will exert any specific antioxygenic function in retarding the oxidative changes in the vitamin molecule. Experimental results recorded in this paper tend to support both the above hypotheses. The vitamin is being found to be more stable when the p_H of the medium is shifted from the acid range and propyl gallate exerts no special effect on the vitamin when dispersed in aqueous phase. Another observation recorded is that the vitamin seems to be less potent when present in the form of an ester (acetate) than when it is in the alcohol form in aqueous medium.

Experimental

Vitamin-A alcohol used was vitamin-A concentrate (Neo Chemical Corporation, U.S.A.) containing one million I.U. per g. Vitamin-A acetate was in pure crystalline form obtained from Distillation Products Inc., U.S.A.

Special distilled water was ordinary double distilled water, redistilled from an all-glass still constructed of Jena glass and equipped with ground glass joints. In operating the still, one drop of phosphoric acid to each litre of water was added. The first portion of the distillate was discarded. The water thus obtained had a specific conductivity of about $1 - 1.5 \times 10^{-6}$ and was free from heavy metals.

Surface active agent was polyoxyethylene sorbitan monolaurate, commercially known as Tween 20, and has the following formula,



where R represents the lauryl radical, and X, the polyoxyethylene groups (total approximately 20). Tween 20 was generously supplied by Atlas Powder Company, U.S.A.

Antioxidant used was pure *n*-propyl gallate having m.p. 146-48°. All other chemicals used in the course of this experiment were of analytical reagent quality.

Procedure: *Vitamin-A Alcohol Solution.*—Tween 20 was added to vitamin-A concentrate so that the latter dissolved and then water was added in such a way that the final concentration of Tween 20 was 20%.

Vitamin-A Acetate Solution.—The crystals of vitamin-A acetate was triturated in an agate mortar and pestle with Tween 20 and when the acetate crystals dissolved, water was added to make the concentration of Tween 20 as 20% of the final solution.

The activity of vitamin-A in both the solutions was determined by the previous process (*loc. cit.*) by hydrolysing with alcoholic potash, extracting the unsaponifiable matter with peroxide-free ether and finally using the alcoholic solution of the unsaponifiable matter in the vitameter-A.

Both these solutions had p_H of 5.7. A portion of each of these solutions was then adjusted to p_H 3.0 with 0.1*N*-hydrochloric acid and another portion to 8.0 with 0.1 *N*-sodium hydroxide. Each of the above preparations at different p_H was then divided into two parts and to one part was added 0.075% propyl gallate, the other, kept as it was. All these solutions were then transferred to 100 c.c. amber coloured bottles fitted with corks having two perforations to allow two glass tubings, one of which reached just above the surface of the solution and the other, at the mouth of the bottle to allow inlet and outlet of air. Bubbling of air through the solution caused frothing beyond control, and as such air was allowed in just on the surface of the liquid. Air was saturated with water vapour prior to entry in the vitamin system by bubbling through four bottles of water in series, the first of these being kept at a temperature of about 50°. All the containers with vitamin-A solution was kept in series and air at the rate of 6 c.c. per second was passed under negative pressure.

The samples adjusted to p_H 8, on keeping overnight, showed a p_H of 6.3, which when adjusted once again to p_H 8 and kept overnight, again changed to 6.3. Representative samples were withdrawn at intervals from each system and analysed for their vitamin-A potency. The results of the experiment are recorded in Tables I-III and in Fig. 1.

TABLE I

Stability of vitamin-A at p_H 3.0.

	Vitamin -A alcohol.				Vitamin- A acetate.			
	Without antioxidant.		With 0.075% propyl gallate.		Without antioxidant.		With 0.075% propyl gallate.	
Aerated for.	Vitamin-A (I.U./g)	Loss of vitamin-A.	Vitamin-A (I.U./g.)	Loss of vitamin-A	Vitamin-A (I.U./g.)	Loss of vitamin-A.	Vitamin-A (I.U /g.)	Loss of vitamin-A.
0 hr.	1722	0%	1722	0 %	2157	0 %	2157	0 %
15	...	...	1458	15.3	1730	19.8	...	...
23	1545	10.3	...	...	...	...	1634	24.2
31	...	...	1373	20.3	1653	23.3	...	...
47	1507	12.5	...	...	1600	25.8	1618	24.5
63	...	...	1329	22.8	...	...	1521	29.5
86	1260	26 8	1244	27.8	1456	32.5	...	...
102	1195	30 6	...	...	1260	41.6	1423	34.0
118	...	...	1042	39.5	...	...	1267	41.2
134	1039	39.7	883	48.7	884	59.0	...	...
174	850	50.6	...	...	...	...	673	68.8
198	600	65 2	620	64.0	580	73.1	571	73.5

TABLE II

Stability of vitamin-A at p_H 5.7.

	Vitamin-A alcohol.				Vitamin-A acetate.			
	Without antioxidant.		With 0.075% propyl gallate.		With antioxidant.		With 0.075% propyl gallate.	
Aerated for.	Vitamin-A (I.U./g.)	Loss of vitamin-A.	Vitamin-A (I.U./g.)	Loss of vitamin-A	Vitamin-A (I.U./g.)	Loss of vitamin-A	Vitamin-A (I.U./g.)	Loss of vitamin-A.
0 hr.	1722	0 %	1722	0 %	2132	0 %	2132	0 %
15	...	...	1648	4.3	2040	4.3	...	...
23	1710	0.7	...	...	...	...	1992	6.6
31	1654	3 9	...	...	1864	12.8	...	...
47	...	...	1637	4.9	...	...	1718	19.4
63	1623	5 7	...	...	1756	17.6	...	...
86	...	...	1626	5.6	1695	20.5	1562	26 7
102	1608	6.6	...	...	1672	21.6	1486	30.3
126	...	...	1503	12.7	...	...	...	...
158	1390	19.3	1256	27.0	...	...	1290	39.5
182	...	...	1200	30.3	1072	49.7	1103	48.3
198	1140	33.8	1181	31.3	1024	52.0	1092	48.8

TABLE III

*Stability of vitamin-A at p_H 6.3.**

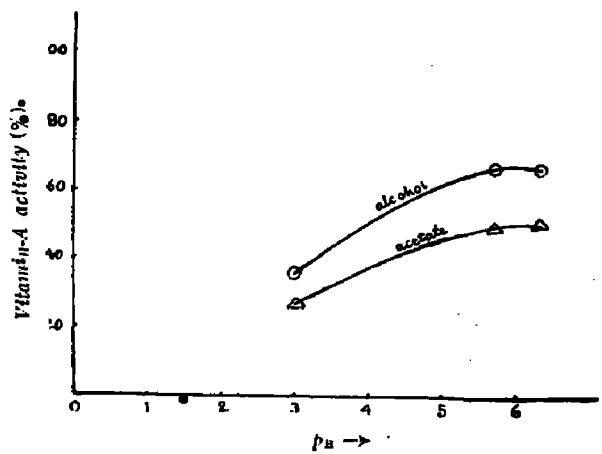
	Vitamin-A alcohol.				Vitamin-A acetate.			
	Without antioxidant.		With 0.075% propyl gallate.		Without antioxidant.		With 0.075% propyl gallate.	
Aerated for.	Vitamin-A (I.U./g.)	Loss of vitamin-A.	Vitamin-A (I.U./g.)	Loss of vitamin-A.	Vitamin-A (I.U./g.)	Loss of vitamin-A.	Vitamin-A (I.U./g.)	Loss of vitamin-A.
0 hr.	1722	0 %	1722	0 %	2114	0 %	2114	0%
15	...	...	1683	2.3	1980	6.3	...	...
23	1687	2.0	...	...	...	...	1968	6.9
31	...	...	1462	15.1	1927	8.8	...	...
47	1642	4.6	...	...	1801	14.8	1778	15.9
63	...	...	1417	17.7	...	...	1702	19.5
86	...	...	1320	23.3	1607	24.0	...	...
102	1627	5.5	...	...	...	...	1596	24.5
118	...	...	1266	26.5	1395	34.0	...	...
134	1561	9.3	1254	27.2	1386	34.4	1256	40 6
174	1300	24.5	1200	30.3	1190	43.7	1174	44.5
198	1130	34.4	1137	34.0	1044	50.6	1071	49 4

* p_H was adjusted to 8.0; but on keeping overnight the p_H changed to 6.3.

DISCUSSION

Effect of p_H.—From the results which have been tabulated according to p_H it may be seen that the vitamin-A in the form of alcohol lost after 198 hours of aeration 65.2%, 33.8% and 34.4% of its activity without the incorporation of antioxidant, and in the form of acetate, 73.1%, 52.0% and 50.6% at p_H values of 3.0, 5.7 and 6.3 respectively. Almost the same order is followed when the systems are adjuvated with 0.075% propyl gallate (cf. Tables I-III.) Thus, it seems that the p_H of the solution has got considerable influence on the stability of vitamin-A in aqueous medium (cf. Kern and Antoshkiw, *Ind. Eng. Chem.*, 1950, **42**, 70.) and that it is less stable in acidic p_H and more stable at p_H of about 6.0 (cf. Fig 1.). The difference between p_H values 5.7 and 6.3 is not marked.

FIG. 1



Influence of Hydroxyphenolic Antioxidant.—The vitamin-A alcohol at p_H 3.0 loses 65.2% without the presence of antioxidant and 64.0% with propyl gallate in 198 hours and the acetate form loses 73.1% in absence and 73.5% in presence of antioxidant in the same period, as may be seen from Table I. Table II shows 33.8% against 31.3% in the case of alcohol form, while vitamin-A acetate has lost 52.0% against 48.8% of vitamin-A potency in absence and in presence of antioxidant respectively. Figures of Table III are almost the same as that of Table II. Thus, it appears that the hydroxyphenolic antioxidant of the type of propyl gallate does not exert any antioxygenic property in stabilising the potency of vitamin-A in water medium.

Effect of the Chemical form of the Vitamin.—The average loss of vitamin-A is about 73% for the acetate form, while it is 64.5% for the alcohol form at p_H 3.0 when aerated for 198 hours (cf. Table I). The losses for acetate and alcohol forms in the same period at p_H values of 5.7 and 6.3 are about 50% and 33% respectively, as may be seen from Tables II and III, as also from Fig. 1. These results tend to indicate that the alcohol form of vitamin-A is more stable than the vitamin-A acetate.

Similar observations have been noted by Lehman of Distillation Products, U. S. A, (*private communication*). It may be mentioned here that the vitamin-A alcohol in aqueous medium at p_H of about 6.0 behaves similarly as the vitamin-A alcohol dissolved in esters or glycerides in the presence of antioxidant, and as the vitamin-A acetate in oils or esters in absence of antioxidant after about 200 hours of aeration so far as the stability of vitamin-A activity is concerned (cf. Basu and Bhattacharya, *loc. cit.*).

The hydroxy-antioxidant, propyl gallate, used in the course of this investigation was 0.075% and has been found to exert no antioxygenic property. The solubility of propyl gallate in water is 0.2 at 25° *. Due to its greater solubility in water and owing to the question of partition coefficient, the antioxidant might not be present in the vitamin-A phase, and thus may be the cause of its failure to exert any special effect in keeping the vitamin-A potency.

Further work is in progress.

CONCLUSION

Vitamin-A in the form of alcohol or acetate, when dispersed in aqueous medium with Tween 20, behaves in a different way from that when dissolved in glycerides or esters.

Vitamin-A acetate, which is more stable in oils and esters, is less stable than the alcohol when dispersed in aqueous menstrum.

The p_H of the aqueous solution determines the stability of vitamin-A irrespective of its chemical form. It is more stable at p_H about 6.0 than at p_H 3.0.

The hydroxy-antioxidant, propyl gallate, having a concentration of 0.075% does not show any antioxygenic property in keeping the vitamin-A potency in aqueous medium.

*The solubility of propyl gallate in arachis oil is less than 0.05 at 25°.

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