Elevated Temperature Studies on Stability of Ascorbic Acid in Certain Fruit Juice and Aqueous Vehicles

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The stability of ascorbic acid was investigated in certain fruit juice and aqueous vehicles between pH 2.5–6.5 at 37 and 55° in the presence of 0.2 per cent citric acid, 0.01 per cent EDTA, 0.01 per cent propyl gallate, 0.2 per cent ferrous gluconate, 0.2 per cent cysteine hydrochloride, and 0.2 per cent potassium *m*-bisulfite. Lime juice–sorbitol–glycerol (2:2:1) retained 70 per cent ascorbic acid at pH 6.5 after 120 days. Citric acid also protected ascorbic acid. EDTA gave little protection to ascorbic acid. The loss of ascorbic acid in aqueous solution at some pH values was counteracted by 0.9 per cent sodium chloride. Dihydrosteptomycin sulfate in the concentration of 0.33 mg./ml. gave protection to ascorbic acid at all pH levels, except at pH 6.5 (37°) and at pH 5.5 (55°), where a destructive effect was noticed. Stability of ascorbic acid was a function of pH, temperature, vehicle, and head space in the container.

SINCE THE pharmaceutical industry is concerned more with the shelf life of a liquid vitamin mixture, the stability of ascorbic acid was studied at 37 and 55° employing certain fruit juice bases, some of which have been used earlier (1) together with some other formulations. The study was conducted at higher temperatures because any liquid vitamin mixture stable at a higher temperature keeps its quality for a longer time at a correspondingly lower temperature (2). This observation of Garrett has been confirmed by McLeod *et al.* (3), Uprety *et al.* (4), recently by Agrawal *et al.* (5). These higher-temperature tests would also be useful in predicting the stability at room temperature, thus shortening the time normally taken by such storage studies at room temperature.

A separate experiment was run with an aqueous solution of ascorbic acid at room temperature, 37°, and 55° to study the rate of destruction of ascorbic acid and to discover whether a formula could be evolved to correlate the stability of ascorbic acid with temperature and pH. Studies were continued for 3 months or until the retention of the vitamin dropped below 30%.

EXPERIMENTAL

Vitamin (Ascorbic Acid).—This was obtained from F. Hoffmann-La Roche and Company, Ltd., Basel, Switzerland.

Vehicle.—Fruit juices, sorbitol (E. Merck), and glycerol (BDH) were used. The formulations employed are given in Table I. A 5.0 mg./ml. quantity of ascorbic acid was added to the vehicle containing 0.2% (w/v) methyl parahydroxybenzoate (Rhodia, France). After adjusting to the required pH, the preparations were stored in 2-oz. amber-colored, glass-stoppered bottles in incubators at 37 and 55°.

Ascorbic acid was estimated by the 2,6-dichlorophenol-indophenol method.

Antioxidants.—The antioxidants examined for their effects on the stability of ascorbic acid in an aqueous solution were propyl gallate (Ward Blenkinsop, and Company, Ltd., London, England), ethylenediaminetetraacetate and potassium metabisulfite (E. Merck, here referred to as EDTA and KMS, respectively) cysteine hydrochloride (E. Merck), ferrous gluconate (Nila Products Ltd., Bombay, India), and citric acid (BDH). The concentrations of these antioxidants and other formulations are given in the Table I.

After adjusting the pH, a 0.5% solution of ascorbic acid was dispensed into Pyrex test tubes ($6 \times {}^{3}/_{4}$ in.) and kept at room temperature, 37° and 55°. The test tubes were closed with rubber stoppers. Samples were drawn periodically for recording the changes in the retention of ascorbic acid. A similar experiment was conducted using physiological saline. The effect of 0.33 mg./ml. of dihydrostreptomycin sulfate (Pfizer Dumex, Bombay, India) was also examined in an aqueous solution at 37 and 55° between the pH range 2.5 to 6.5.

RESULTS

Ascorbic acid in glass distilled water was lost completely at the end of 60 days both at 37 and 55° in the control and in the presence of 0.02% EDTA, 0.01% propyl gallate, 0.02% ferrous gluconate, 0.02% cysteine hydrochloride, and 0.2% citric acid at all pH values between 2.5 to 6.5, but 0.2% KMS protected ascorbic acid at pH 3.5 (37°) to the extent of 65%, followed by 0.02% EDTA which gave a slight protection up to 30% at pH 2.5. After a storage period of 120 days, KMS in the concentration used had no protective effect on ascorbic acid. (A tabulation of data is not given.)

Table I shows that at 55° and a storage period of 60 days all ascorbic acid added in the different vehicles was oxidized, except for lime juice – sorbitol – glycerol (2:2:1), in which there was a 30% retention at pH 2.5, 3.5, and 5.5 and 40% at pH 6.5. Samples kept at this temperature changed color turning toward deep brown. Inclusion of antioxidants (concentration shown in Table I) failed to improve the stability of ascorbic acid. In apple juice at the end of 60 days, practically all the ascorbic acid was degraded at 37° between the pH range 2.5 to 5.5, but a 35% retention was observed at pH 6.5. EDTA (0.01%) stabilized ascorbic acid at pH 3.5 and 4.5 to the extent of 66 and 48%, respectively. At other pH values, no such protection was noticed. Propyl gallate (0.1%) also protected ascorbic acid at pH 3.5, 4.5, and 6.5. Generally, citric acid protected vitamin C in apple juice at 37° in the concentration used. Ferrous gluconate did not seem to have an influence on the retention of ascorbic acid in apple juice at 37° within the experimental pH range.

Apple juice – glycerol (1:1) was a better vehicle than apple juice up to pH 5.5. The addition of

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TABLE I.—PER CENT RETENTION OF ASCORBIC ACID

No.	Vehicle	Antioxidants or Metal Binder	Days	pH Values 2.5	3.5	4.5	5.5	6.5
		Temperature, 37° C.						
1	Pineapple juice	Control	60	60.00	28.9	2.9	49.3	52.5
2	Apple juice	Control	60	1.43	1.40	1.43	1.45	35.0
3	Lime juice	Control	30	38.3	24.0	8.3	3.0	17.2
4	Lime juice–sorbitol–glycerol (2:2:1)	Control	72	29.4	61.4	75.4	75.4	89.6
			120	20.3	44.2	47.4	45.2	70.0
5	Apple juice 75% (w/v) glycerol (1:1)	Control	48	44.8	59.2	53.5	43.6	21.8
			79	37.0	32.0	20.0	15.4	2.6
6	Apple juice	0.01% EDTA	72	Nil	66.6	48.1	Nil	Nil
7	Apple juice	0.01% Propyl gallate	72	Nil	68.74	79.3	Nil	88.4
			120	Nil	Nil	Nil	Nil	53.5
8	Apple juice	0.2% Citric acid	64	34.2	41.04	34.2	26.2	95.2
			105	Nil	20.6	17.5	17.5	36.1
9	Apple juice	0.2% Ferrous gluconate	60	2.3	2.3	2.3	7.0	22.4
10	Apple juice 75% (w/v) glycerol (1:1)	0.02% EDTA	49	67.7	58.2	42.0	42.8	61.1
			79	53.7	33.3	17.0	29.4	44.9
11	Apple juice 75% (w/v) glycerol (1:1)	0.02% Propyl gallate	46	58.3	56.8	33.5	36.1	28.1
			63	43.2	43.3	20.4	25.4	10.2
		Temperature, 55° C.						
1	Pineapple juice	Control	60	2.5	4.8	3.2	3.8	5.0
2	Apple juice	Control	60	3.0	3.0	3.0	3.0	1.5
3	Lime juice	Control	30	10.6	6.7	2.7	3.0	2.8
4	Lime juice–sorbitol–glycerol (2:2:1)	Control	72	27.3	36.8	Nil	30.1	40.2
			120	6.4	5.7	Nil	5.1	Nil
5	Apple juice 75% (w/v) glycerol	Control	48	9.4	16.4	2.3	2.3	12.2
6	Apple juice	0.01% EDTA	72	Nil	10.6	Nil	Nil	34.2
7	Apple juice	0.01% Propyl gallate	72	Nil	Nil	Nil	41.6	Nil
8	Apple juice	0.2% Citric acid	64	2.7	2.7	2.7	2.7	5.5
9	Apple juice	0.2% Ferrous gluconate	60	2.3	2.3	4.7	4.7	4.8
10	Apple juice 75% (w/v) glycerol	0.02% EDTA	49	36.0	13.6	22.6	2.3	2.3
11	Apple juice 75% (w/v) glycerol	0.2% Propyl gallate	46	5.16	5.16	2.58	2.58	2.58

EDTA to the above vehicle showed better stability at pH 2.5, 5.5, and 6.5. Propyl gallate (0.02%) had no effect on the stability of ascorbic acid in vehicle *5*.

Pineapple juice was better than apple or lime juice as a carrier of ascorbic acid. At the end of 120 days at pH 6.5, lime juice – sorbitol – glycerol (2:2:1) showed 70% retention of ascorbic acid at 37°, whereas at pH 3.5, 4.5, and 5.5 the retention was about 45%. A pH value of 2.5 showed a poor retention in this vehicle.

Room Temperature.—Examination of the data in Table II shows that the best retention of ascorbic acid was at pH 3.5, and the maximum degradation was at pH 5.5 at the end of 50 days. At other pH levels, about 60% destruction occurred. The decomposition of ascorbic acid in physiological saline followed the same pattern as that in distilled water at all pH levels except 5.5, where a protective action was noticed.

At 37°.—About 75% of ascorbic acid was lost after 40 days in distilled water at all pH levels except 3.5. The maximum destruction was at pH 2.5 and the minimum at 3.5. The maximum destruction was at pH 5.5 and the minimum at pH 3.5 in physiological saline. At pH 2.5, NaCl (0.9%) promoted the stability. At other pH values, physiological saline had no influence on the stability within the range of experimental error. The addition of 0.33 mg./ml. of dihydrostreptomycin sulfate to the aqueous solution of ascorbic acid had a stabilizing tendency up to pH 5.5; but at pH 6.5 this concentration of dihydrostreptomycin sulfate exhibited a deleterious effect.

At 55°.—About 70% ascorbic acid was lost at the end of 30 days in distilled water. The destruction was more with the rise in pH up to 4.5; however, at pH 5.5, which appears to be a critical value, the retention was fairly good to the extent of about 52%. At pH 6.5, a complete loss of ascorbic acid occurred at the end of 20 days. In saline, under similar conditions, a greater stability of ascorbic acid was observed at pH 6.5. There seemed to be no influence at other pH values. Dihydrostreptomycin sulfate, in the concentration used, had a stabilizing effect on ascorbic acid at all pH values except 5.5, where a destructive effect was noticed after a period of 40 days in distilled water.

At the end of the experimental period no change in pH was noticed, although a buffer was not used. All the samples containing ascorbic acid developed color. A greater degree of coloration was shown by the samples containing citric acid, the intensity of color was proportional to the degradation of ascorbic acid.

TABLE II.—PER CENT RETENTION OF ASCORBIC ACID IN AQUEOUS SOLUTION

Vehicle	pH	Days 4	10	20	30	40	50
		Room Temperature					
Distilled water	2.5	97.0	93.4	80.0	67.4	57.8	48.5
	3.5	99.79	96.5	87.9	75.1	65.9	54.8
	4.5	94.93	91.2	71.2	64.1	50.0	44.5
	5.5	94.9	86.4	71.2	61.7	41.2	23.6
	6.5	92.11	88.5	75.5	65.7	51.9	36.3
Physiological saline	2.5	49.79	94.6	78.0	73.0	58.6	51.2
	3.5	94.93	91.2	83.2	73.2	65.2	54.8
	4.5	92.30	96.3	78.5	69.0	45.9	44.5
	5.5	100.0	100.0	85.2	75.1	58.9	51.2
	6.5	97.51	96.05	73.0	68.2	47.0	40.8
		Temperature, 37° C.					
Distilled water	2.5	97.6	80.4	57.4	37.4	14.4	...
	3.5	99.8	88.5	65.7	50.2	35.2	...
	4.5	92.3	78.9	61.7	42.7	24.2	...
	5.5	89.87	81.3	61.7	45.1	25.3	...
	6.5	94.79	80.9	68.2	53.9	25.9	...
Physiological saline	2.5	97.51	91.07	73.0	53.5	37.5	...
	3.5	89.87	88.8	76.1	61.7	43.3	...
	4.5	87.45	81.3	71.7	69.0	25.0	...
	5.5	92.1	85.8	65.7	48.7	19.9	...
	6.5	94.6	88.5	68.2	53.5	33.3	...
Distilled water dihydrostreptomycin (0.33 mg./ml.)	2.5	...	82.2	62.4	45.2	22.8	...
	3.5	...	91.2	73.2	55.8	39.3	...
	4.5	...	79.8	63.7	48.2	31.7	...
	5.5	...	99.7	77.0	62.5	37.0	...
	6.5	...	80.4	51.6	36.6	12.4	...
		Temperature, 55° C.					
Distilled water	2.5	85.1	54.2	30.0	4.9	Nil	...
	3.5	86.0	58.1	17.0	2.3	Nil	...
	4.5	80.9	44.3	9.5	1.1	Nil	...
	5.5	93.6	78.9	52.2	30.7	Nil	...
	6.5	77.8	32.7	Nil	Nil	Nil	...
Physiological saline	2.5	85.5	65.7	36.5	17.0	...	...
	3.5	86.0	62.1	23.6	4.6	...	...
	4.5	83.9	62.1	23.6	2.3	...	..
	5.5	83.0	68.2	39.0	24.2	...	
	6.5	90.7	78.4	51.2	29.2	...	...
Distilled water dihydrostreptomycin (0.33 mg./ml.)	2.5	...	71.4	30.7	17.2	...	...
	3.5	...	73.6	47.2	25.8	...	...
	4.5	...	61.5	7.8	2.1	...	...
	5.5	...	79.9	36.0	11.1	...	...
	6.5	...	87.1	68.9	38.6	...	...

DISCUSSION

The stability of ascorbic acid, evidenced from this study, is a function of pH and temperature. At pH 2.5, the stability is markedly decreased. This finding is in support of Ganguly (6), who showed that at low pH air and copper ions decreased stability of an aqueous solution of ascorbic acid.

It is interesting that KMS stabilizes ascorbic acid to a fair extent. This finding confirms our earlier finding (1) and also lends support to the view expressed by Schroeter (7). It is essential that a proper concentration of this antioxidant be added because the mechanism of the protection of ascorbic acid depends upon the availability of the S ions.

EDTA in a concentration of 0.2% also gave some protection to ascorbic acid in an aqueous solution. This finding is in agreement with that of Bartilucci and Foss (8) and also of Ch'en (9). Probably the concentration used in the present investigation was rather low, a factor which accounts for the reduced stability.

Other antioxidants used did not offer protection to ascorbic acid in distilled water, either because the concentrations were insufficient or because the samples were analyzed after storing them for a longer period.

At 55°, most of the ascorbic acid was easily destroyed. This discovery clearly shows that at such a high temperature the oxidation is rapid. A preparation which can be made stable at 55° for 1 month would theoretically be stable for about 5–6 months at 25°. This may shorten the time usually taken in the analytical study at room temperature by one-fifth if attempts are made to stabilize liquid vitamin mixtures at 55°.

Lime juice – sorbitol – glycerol (2:2:1) at pH 6.5 showed a retention of about 40% at 55°. Therefore, this vehicle affords maximum protection among all the vehicles tried, even without the aid of an antioxidant. Our earlier finding (1) that lime juice – sorbitol (1:1) and lime juice – glycerol (1:1) are good vehicles for externally added ascorbic acid has been confirmed and modified with the inclusion of glycerol in this study. The retention of

TABLE III.—PER CENT RATE OF DESTRUCTION OF ASCORBIC ACID IN AN AQUEOUS SOLUTION FOR 24 HOURS

pH	Room Temp.	37° C.	55° C.
2.5	1.05	2.14	3.5
3.5	1.096	1.62	4.15
4.5	1.11	1.894	4.525
5.5	1.53	1.87	2.39
6.5	1.274	1.85	6.73

ascorbic acid in this vehicle at 55° would roughly be equivalent to about 1 year at room temperature.

In apple juice the ascorbic acid degraded completely up to pH 5.5 at 37°. This may be explained with the presumption that apple juice may contain a high concentration of polyphenol-oxidase to catalyze the oxidation of ascorbic acid. The addition of 0.01% EDTA protected ascorbic acid at pH 3.5 and 4.5. This protection can be explained on the basis of the Cu ions being arrested by this metal binder. Propyl gallate also exhibited a protective action at pH 3.5, 4.5, and 6.5, in conformity with the finding of Ch'en (9).

Citric acid in the concentration used protected ascorbic acid in apple juice at all the pH values. Presumably there may exist a sort of competitive inhibition, which may explain the mechanism by which citric acid could stabilize ascorbic acid.

The substitute of 50% of the apple juice by glycerol improved the stability of ascorbic acid at 37° up to pH 5.5. This finding is in agreement with that of Bandelin and Tuschhoff (10), who showed that the addition of polyhydric alcohols tends to produce a stabilizing effect on ascorbic acid.

Pineapple juice showed a good stability at 37° compared to lime juice and apple juice, probably because pineapple juice may contain less ascorbic acid oxidase or other metallic ions injurious to ascorbic acid.

Ascorbic acid showed a 70% retention in vehicle 4 after a period of 4 months at pH 6.5. This is in conformity with the finding of Bartilucci and Foss (8), who showed that optimum pH for the stability of ascorbic acid in solution lies between 6.0 and 7.0.

An aqueous solution of ascorbic acid at room temperature showed the best retention at pH 3.5 and maximum degradation at pH 5.5. This deteriorating action of pH was counteracted by the addition of 0.9% NaCl to the medium. A similar pattern was followed at 37°. The destructive action at pH 3.5 and 37° was further counteracted by physiological saline. This shows that 0.9% NaCl is effective in counteracting the deleterious effect of pH and temperature in an aqueous solution —which may have something to do with the ionic equilibrium in an aqueous solution.

At 55°, the destruction in an aqueous solution was more with the rise in pH. This is in agreement with Bendelin and Tuschhoff (10), who showed that the rate of oxidative decomposition of an ascorbic acid solution increases with the rise in pH values. But a 30.7% retention of ascorbic acid at 55° after a period of 30 days could not offer an explanation. Again, at pH 6.5 NaCl in a concentration of 0.9% was effective in counteracting the oxidation of ascorbic acid.

Dihydrostreptomycin sulfate, in the concentration of 0.33 mg./ml. had a tendency to stabilize ascorbic acid at 37 and 55° at all pH values, except at pH 5.5 and 55°.

Citric acid, in the concentration used, turned the vehicle containing ascorbic acid brown. This has also been shown by Lamden and Harris (11), who stated that the water of crystallization of citric acid promoted browning at 49°. In fact, all the samples turned brown, but the intensity differed and the maximum was observed in those containing citric acid.

The oxidation of ascorbic acid is reported to follow two paths: (*a*) polymerization to furfural and (*b*) oxidation to diketogulonic acid and finally to oxalic acid. The color development is probably lacking in (*b*). Various workers (1, 4) have shown that sulfur, in the form of cysteine, methionine, and KMS, exercised a stabilizing action on ascorbic acid. The stability is likely to be due to sulfur. The mechanism involved is probably formed through a loose bond between S and the hydrogen ions of ascorbic acid. However, this loose bond appears susceptible to changes in temperature and pH due to molecular agitation.

Table III shows the rate of destruction of ascorbic acid in an aqueous solution at three temperatures. The rate of destruction at pH 6.5 is six times more at 55° than at room temperature; at other pH values the rate of destruction varies from two to four times, depending upon the pH. At 37°, the rate of destruction is roughly one and one-half times more than at room temperature. Thus, an idea about the prediction of stability at lower temperature by conducting studies at higher temperatures is given.

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