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Original Article

EFFECT OF STORAGE CONDITIONS ON THE STABILITY OF ASCORBIC ACID IN SOME FORMULATIONS

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ABSTRACT

Objective: The stability of ascorbic acid is affected by temperature, pH, sunlight and the presence of metals like copper and iron.

The study seeks to investigate the effect of storage conditions on the stability of ascorbic acid in tablets (buccal tablets) and syrups sampled from the Ghanaian market.

Methods: Ascorbic acid tablets were sampled and stored separately at room temperature and under refrigeration (in a fridge) and assayed periodically for 35 d. Ascorbic acid syrups were also sampled and stored at room temperature, in a bowl of water and under refrigeration and also assayed periodically for 35 d. The mode of assay was iodimetry.

Results: For both formulations, storage under refrigeration saw the least breakdown and at room temperature, the breakdown of ascorbic acid was greatest. The syrups stored in a bowl of water were more stable than those stored at room temperature. The % breakdown of ascorbic acid in the syrups and tablets stored at room temperature were statistically significant in comparison to that under refrigeration as determined by a T-test. The % breakdown of ascorbic acid in the syrups stored in a bowl of water was not statistically significant in comparison to that under refrigeration.

Conclusion: Ascorbic acid formulations should be stored under refrigeration or at low temperatures if possible. In the absence of refrigeration, patients should be advised to store syrups of ascorbic acid in a bowl of water and the tablets at cool places in homes.

Keywords: Ascorbic acid, Room temperature, Refrigeration, Bowl of water and iodimetry

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INTRODUCTION

L-ascorbic acid, commonly known as vitamin C, is a very important water-soluble vitamin. It is a low molecular weight antioxidant that has diverse uses in both plants and animals [1].

In plants, ascorbic acid functions as a redox buffer, a cofactor in the process of photosynthesis, biosynthesis of hormones and the regeneration of other antioxidants. It also plays a role in the regulation of cell division, cell growth and signal transduction [2].

In humans, ascorbic acid or vitamin C helps in the synthesis of collagen, acts as an immune system booster by scavenging free radicals in the body, aids in enzyme activation and oxidative stress reduction. There is a lot of evidence that ascorbic acid offers protection against respiratory tract disease, reduces risks for cardiovascular diseases and some cancers [3, 4].

Scurvy is a condition that results when there is a deficiency of ascorbic acid in the body, and its clinical manifestations are lethargy, purpuric lesions, myalgia and bleeding of the gums [5].

Some sources of ascorbic acid are oranges, tomatoes, sweet pepper, vegetable oils like sunflower oil and olive oil, and nuts and seeds [6].

Synthetic ascorbic acid are available as formulations of effervescent tablets, chewable tablets, syrups, drops and injections [7].

The stability of ascorbic acid is affected by a lot of factors; temperature, pH, sunlight and the presence of certain metals like Iron and Copper. Temperature changes have effects on the concentrations or amounts of ascorbic acid in formulations and natural sources like fruits. High storage temperatures cause an increase in the breakdown of ascorbic acid whereas low temperatures cause a reduction in the rate of breakdown [8].

In Ghana and some parts of West Africa and the developing world, most people do not have refrigerators in their homes so when they are given formulations of ascorbic acid to use and administer over a period, they have no means of storage at such low temperatures. Since they are not being stored at recommended low temperatures where the breakdown is minimal, the products could experience appreciable breakdown over the course of usage. The erratic power supply (frequent and prolonged power outages) also means that such products cannot be stored consistently at such low refrigeration temperatures. These issues of storage raise stability concerns of ascorbic acid in formulations.

The study seeks to investigate and compare the effect of storage conditions on the stability of ascorbic acid in syrups, tablets and the pure powder stored under refrigeration (in the fridge), at room temperature and in a bowl of water with a view to recommending the best possible storage condition for ascorbic acid.

MATERIALS AND METHODS

Reagents and chemicals used

Potassium iodide, potassium iodate, iodine crystals, and sulphuric acid (all from Fisher chemicals Ltd, UK), sodium thiosulphate pentahydrate (Fizmerk chemicals Ltd, UK) and starch powder (Merck private Ltd, India). All the chemicals used were of analytical grade.

The pure ascorbic acid powder used was of laboratory grade.

Collection of samples of formulations of ascorbic acid

The brands of ascorbic acid syrups and tablet (buccal tablets were used) used were obtained from pharmacies at Prampram in the Greater Accra region of Ghana. All the primary and secondary packaging of the ascorbic acid syrups and tablets were assessed and did not show any sign of physical or chemical degradation in their packaging.

Storage of samples

Samples of the ascorbic acid syrup in amber coloured bottles were stored at room temperature (33 °C), under refrigeration or in a fridge (4–6 °C) and in a bowl of water (26-27 °C) such that half the height of the bottle was immersed in the water. The samples were stored under these various storage conditions for 35 d.

Samples of the ascorbic acid tablets in blisters were stored at room temperature (33 °C) and in a fridge (4–6 °C). The samples were stored under the various storage conditions for 35 d.

Sufficient amounts of the pure ascorbic acid powder were put into very clean and dry amber coloured bottles and tightly covered. They were then stored at room temperature (33 °C), in a fridge (4–6 °C) and in a bowl of water (26-27 °C) such that half the height of the bottle was immersed in the water. The samples were stored under the various storage conditions for 35 d.

Assay of samples

Each of the samples of ascorbic acid syrups, tablets and powder was assayed on the same days predetermined over 35 d. The mode of the assay was iodiometry [9]. Triplicate assays were performed for each sample and the percentage content of each sample expressed as the mean with standard deviation.

Reaction rate equations

Zero order rate equation

$$C = Co - kt$$

For the zero order graphs, the final concentration, C, was plotted against time of assay, t, and the slope, k, is the zero order rate constant and Co is the initial concentration.

First order rate equation

$$\text{In } C = \text{In } Co - k't$$

For the first order graphs, In C, was plotted against time of assay, t, and the slope,k′, is the first order rate constant. Co is the initial concentration and C is the final concentration after a certain time, t.

Second order rate equation

$$\frac{1}{C} = \frac{1}{Co} + k''t$$

For the second order graphs, $\frac{1}{C}$ was plotted against time of assay, t, and the slope,k′′, is the second order rate constant. Co is the initial concentration and C is the final concentration after a certain time, t [10].

Determination of the order of breakdown of ascorbic acid

The percentage contents of the various samples of ascorbic acid for each formulation (syrups and tablets) were converted to concentration terms needed to plot line graphs for zero order, first order and second order rates of breakdown. The line graphs for a particular order of breakdown for each formulation were obtained by plotting the correct concentration terms against the times of assay [11].

The coefficients of correlation and the slopes were obtained for each line graph.

The line graphs were drawn with Microsoft excel 2013.

Statistical analysis

The significance of the % breakdown of ascorbic acid in tablets and syrups stored at room temperature and in a bowl of water in comparison to storage under refrigeration was determined by a t-test calculated as:

$$t = \frac{(x - \mu)\sqrt{\mathbf{n}}}{s}$$

t is the critical value; μ (true mean) is the mean % content of ascorbic acid in formulation stored in fridge after 35 d; x is the mean % content of ascorbic acid formulation stored at room temperature or in bowl of water; s is the standard deviation of the three determinations that gave x and n is the number of assays per sample [12].

The t-test was carried out at a confidence level of 95%.

RESULTS AND DISCUSSION

The assay results for all the formulations of ascorbic acid including the pure powder under the various storage conditions show that the concentrations of ascorbic acid in the various products reduced with time under the storage conditions investigated (tables 1, 2 and 3).

Table 1: Assay results for ascorbic acid in tablets stored under the different storage conditions for 35 d

Time of assay (d)	Mean concentration (% w/w)	
	Tablets stored at room temperature	Tablets stored in Fridge
0	105.57±0.0572	105.57±0.0572
2	104.75±0.3581	104.91±0.2247
7	102.77±0.2738	104.83±0.0796
14	101.73±0.1392	103.91±0.3018
21	100.60±0.2002	103.41±0.2831
28	100.06±1.2522	102.92±0.2337
35	98.52±0.2133	101.87±0.5137

Mean concentration of ascorbic acid given as mean±SD (n=3).

Table 2: Assay results for pure ascorbic acid powder stored under the different storage conditions for 35 d

Time of assay (d)	Mean concentration (% w/w)		
	Pure Powder stored at room temperature	Pure powder stored in a bowl of water	Pure powder stored in fridge
0	104.13±0.6770	104.13±0.6770	104.13±0.6770
2	102.53±1.1877	102.95±0.0699	103.33±1.3260
7	101.12±2.0541	101.59±1.3052	103.02±0.2572
14	100.11±2.4162	100.73±1.8326	102.84±0.9806
21	99.72±0.8214	100.01±2.4957	101.22±0.9975
28	97.90±1.4671	99.31±0.1252	101.14±0.9268
35	96.31±0.8546	97.67±0.5811	100.35±0.5613

Mean concentration of ascorbic acid given as mean±SD (n=3).

Table 3: Assay results for ascorbic acid in syrups stored under the different storage conditions for 35 d

Time of assay (d)	Mean concentration (% w/w)		
	Syrup stored at room temperature	Syrup stored in a bowl of water	Syrup stored in fridge
0	115.50±0.0000	115.50±0.0000	115.50±0.0000
2	115.50±0.0000	115.50±0.0000	115.50±0.0000
7	114.30±0.5196	114.57±0.8505	115.13±0.4619
14	113.43±0.4619	113.70±0.0000	114.30±0.5196
21	110.80±0.5196	112.83±0.4619	113.40±0.5196
28	105.40±0.0000	110.23±0.4619	112.57±0.4619
35	102.87±0.8505	107.70±1.7776	110.23±1.3204

Mean concentration of ascorbic acid given as mean±SD (n=3)

Also from the experiment, it was observed that the concentrations of ascorbic acid in the various formulations under different storage conditions reduced at different rates with time. For the tablets, the concentrations of ascorbic acid reduced at a faster rate at room temperature than in the fridge (fig. 1).

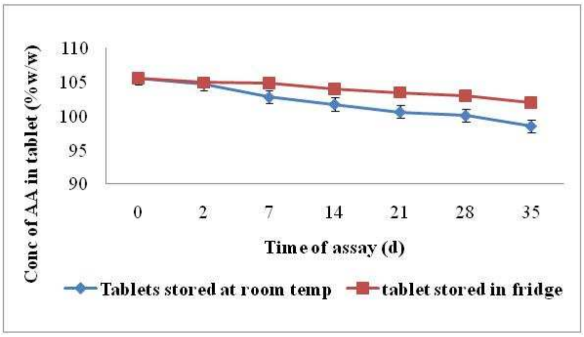


Fig. 1: Rates of degradation of ascorbic acid (AA) in tablets stored under the different storage conditions for 35 d

In both the pure powder and syrups, storage under refrigeration gave the highest stability or experienced the least breakdown while storage at room temperature experienced the most breakdown. The stability of ascorbic acid in the powder and syrup stored in a bowl of water was better than that at room temperature (fig. 2 and 3).

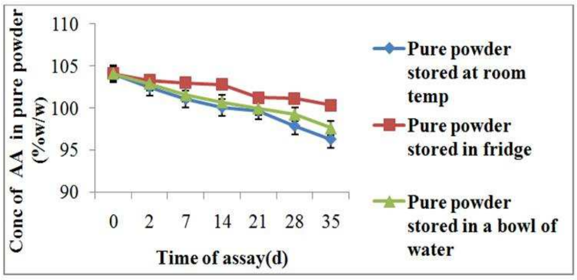


Fig. 2: Rates of degradation of ascorbic acid (AA) in pure powder stored under the different storage conditions for 35 d

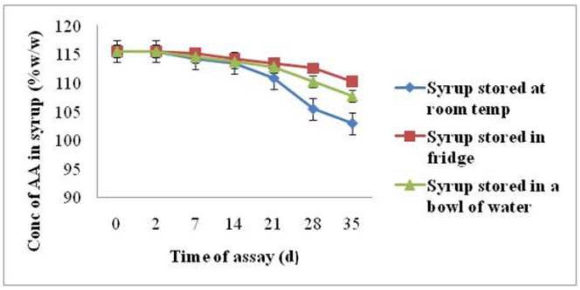


Fig. 3: Rates of degradation of ascorbic acid (AA) in syrup stored under the different storage conditions for 35 d

Table 4: Concentration terms for the various orders of breakdown for ascorbic acid in tablets stored at room temperature

Time of assay (d)	Concentration terms		
	Zero order c (% w/w)	First order $In\ c$	Second order $1/c$
0	105.57	4.659	0.00947
2	104.75	4.652	0.00955
7	102.77	4.632	0.00973
14	101.73	4.622	0.00983
21	100.60	4.611	0.00994
28	100.06	4.605	0.00999
35	98.52	4.590	0.01015

The mean concentrations of ascorbic acid were used for the conversions.

Table 5: Concentration terms for the various orders of breakdown for ascorbic acid in tablets stored in fridge

Time of assay (d)	Concentration terms		
	Zero order c (% w/w)	First order $In\ c$	Second order $1/c$
0	105.57	4.659	0.00947
2	104.91	4.653	0.00953
7	104.83	4.652	0.00954
14	103.91	4.644	0.00962
21	103.41	4.639	0.00967
28	102.92	4.634	0.00972
35	101.87	4.624	0.00982

The mean concentrations of ascorbic acid were used for the conversions.

Table 6: Concentration terms for the various orders of breakdown for ascorbic acid in syrup stored in a fridge

Time of assay (d)	Concentration terms		
	Zero order c (% w/w)	First order $In\ c$	Second order $1/c$
0	115.50	4.749	0.00866
2	115.50	4.749	0.00866
7	115.13	4.746	0.00869
14	114.30	4.739	0.00875
21	113.40	4.731	0.00882
28	112.57	4.724	0.00888
35	110.23	4.703	0.00907

The mean concentrations of ascorbic acid were used for the conversions.

Table 7: Concentration terms for the various orders of breakdown for ascorbic acid in syrup stored at room temperature

Time of assay (d)	Concentration terms		
	Zero order c (% w/w)	First order $In\ c$	Second order $1/c$
0	115.50	4.749	0.00866
2	115.50	4.749	0.00866
7	114.30	4.739	0.00875
14	113.43	4.731	0.00882
21	110.80	4.708	0.00903
28	105.40	4.658	0.00949
35	102.87	4.633	0.00972

The mean concentrations of ascorbic acid were used for the conversions.

Table 8: Concentration terms for the various orders of breakdown for ascorbic acid in syrup stored in a bowl of water

Time of assay (d)	Concentration terms		
	Zero order c (% w/w)	First order $In\ c$	Second order $1/c$
0	115.50	4.749	0.00866
2	115.50	4.749	0.00866
7	114.57	4.741	0.00873
14	113.70	4.734	0.00880
21	112.83	4.726	0.00886
28	110.23	4.703	0.00907
35	107.70	4.679	0.00929

The mean concentrations of ascorbic acid were used for the conversions.

To be able to quantify the amount of ascorbic acid lost or broken down in the formulations under the various storage conditions with time, it was necessary to determine the reaction order of breakdown. The mean concentrations of ascorbic acid in the formulations under the different storage conditions were converted to concentration terms needed to plot line graphs for zero order, first order and second order reaction rates (Tables 4-8). The graphs were obtained by plotting the various concentration terms against their corresponding times of assay [11]. To obtain the order of breakdown for ascorbic acid in any of the formulations, the order whose graph gave the most linearity was taken to be the order of breakdown. The linearity was measured by using the coefficient of correlation, R^2, which shows the strength of correlation between the terms on the y-axis and those on the x-axis. The larger the R^2, the greater the strength of correlation and the more linear the graph [13]. The R^2 were obtained from the line graphs plotted with Microsoft excel.

The breakdown of ascorbic acid in the tablets stored under the conditions studied followed a second order kinetic. The R^2 for their second order graphs were generally higher than the R^2 obtained for their zero order and first order graphs (table 9). This implied that, their second order graphs were more linear. Fig. 4, 5 and 6 respectively show zero order, first order and second order sample line graphs for the breakdown of ascorbic acid in tablets stored at room temperature for 35 d.

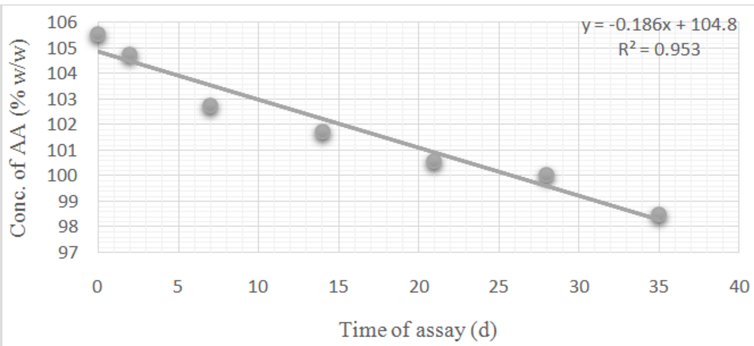


Fig. 4: A zero order graph for ascorbic acid (AA) in tablets stored at room temperature

Table 9: Data from reaction order graphs for ascorbic acid tablets

Storage condition	Zero order		First order		Second order	
	R^2	k	R^2	k'	R^2	k''
Room temp.	0.9539	0.1866	0.9572	0.0018	0.9589	2×10^{-5}
Fridge	0.9779	0.0959	0.9801	0.0009	0.9801	9×10^{-6}

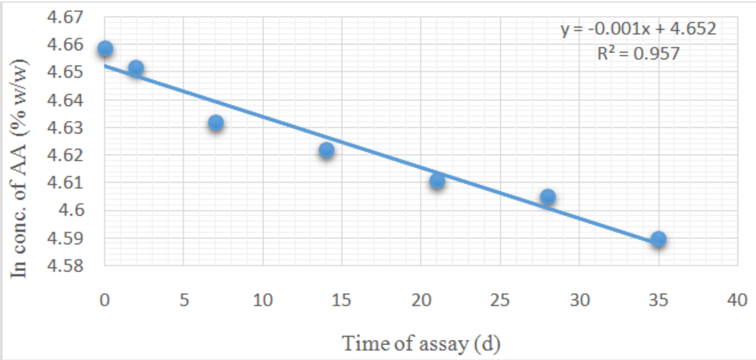


Fig. 5: A first order graph for ascorbic acid (AA) in tablets stored at room temperature

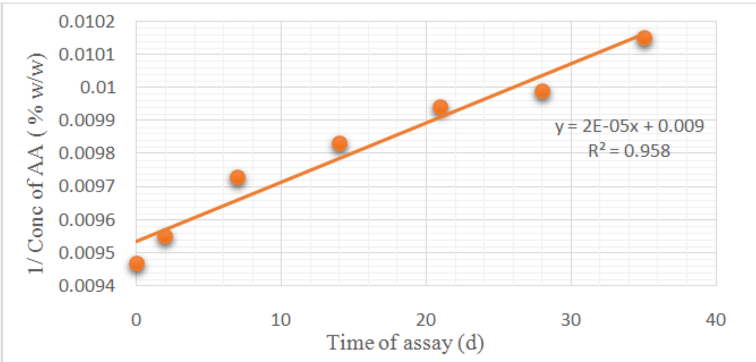


Fig. 6: A second-order graph for ascorbic acid (AA) in tablets stored at room temperature

The breakdown kinetics for ascorbic acid in the syrups stored under the three different conditions followed a zero-order. The R^2 obtained for the zero order line graphs under the three different storage conditions were higher than those obtained for the line graphs for first and second orders.

The extent or degree of breakdown of ascorbic acid in the tablets and syrups under the various conditions of storage can be determined by comparing their rate constants, k and k'' for zero order and second order respectively. The higher the rate constant, the faster the rate of breakdown and the less stable the product whereas a low rate constant is indicative of a slow breakdown and a more stable product [11].

For the tablets, the second order rate constant for ascorbic acid under refrigeration was smaller than that for at room temperature (table 9). This implies that refrigeration offered a more stable condition of storage for ascorbic acid than at room temperature.

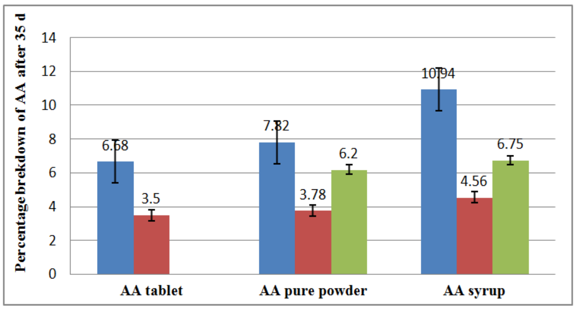
The breakdown of ascorbic acid in the syrups was via a second order kinetic hence the second order rate constant, **k''**, was used. The rate constant for storage under refrigeration (in a fridge) was the smallest, followed by that in a bowl of water and that at room temperature was the biggest (table 10). It follows that the syrup formulations stored in a fridge were the most stable and those stored at room temperature were the least stable. The syrups stored in a bowl of water were, however, more stable than those stored at room temperature.

Table 10: Data from reaction order graphs for ascorbic acid syrup

Storage condition	Zero order		First order		Second order	
	R^2	k	R^2	k'	R^2	k''
Room temp.	0.9324	0.3683	0.9259	0.0034	0.9234	3×10^{-5}
Fridge	0.9339	0.1397	0.9270	0.0012	0.9251	1×10^{-5}
Bowl of water	0.9398	0.2125	0.9307	0.0019	0.9273	2×10^{-5}

In general, storage under refrigeration stored the products more stable as it gave the least percentage breakdown and storage at room temperature gave the most percentage breakdown.

Storage in a bowl of water gave a breakdown percentage better than at room temperature for the syrups and pure powder (fig. 7).



Key: blue-room temperature; red-fridge; green-bowl of water

Fig. 7: Percentage breakdown of ascorbic acid after 35d in the various formulations under the different storage conditions

The significance of the percentage breakdown of ascorbic acid in the formulations stored at room temperature and in a bowl of water was calculated using a T-test in comparison to the percentage breakdown under refrigeration (table 11). The T-test was done at a confidence level of 95%.

Table 11: Statistical significance of the effects of the storage conditions on the stability of ascorbic acid using a T-test

Formulation	T-value (n=3)
Tablets at room temperature	-27.203
Syrup at room temperature	-15.0
Syrup in a bowl of water	-2.465

The T-test was carried out at a confidence level of 95%.

The critical value at a confidence level of 95% is ±4.303. From table 11, the T-values for the % breakdown of ascorbic acid in tablets and in the syrups at room temperature lie outside the range –4.303 to 4.303. These imply that the percentage breakdown of ascorbic acid is very significant in tablets and syrups stored at room temperature when compared to storage in a fridge. Storage at room temperature should therefore not be encouraged. Storage of syrups in a bowl of water gave a % breakdown that was not significant according to the T-test since the calculated T-value of –2.465 was within the range of –4.303 to 4.303. This implies that storage in a bowl of water can be done in the absence of refrigeration.

CONCLUSION

Ascorbic acid breaks down with time in tablets and syrups and even in the pure powder during storage. Storage under refrigeration minimizes breakdown whereas storage at room temperature encourages significant breakdown with time in comparison to breakdown under the other storage conditions studied.

Storage of syrups in a bowl of water should be preferred to storage at room temperature in the absence of refrigeration because the syrups stored in a bowl of water were found to be more stable.

In the absence of refrigeration, ascorbic acid tablets should be stored at cool places in homes to minimize degradation.

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CONFLICT OF INTERESTS

The authors declare they have no competing interest

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