

Effect of sweetening agents on the light stability of aqueous solutions of L-ascorbic acid

A.H.L. Ho, A. Puri, J.K. Sugden *

Department of Pharmacy, School of Applied Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

(Received 29 October 1993; Accepted 17 January 1994)

Abstract

Aqueous solutions of L-ascorbic acid were irradiated with simulated sunlight in the presence of mannitol, sorbitol, sucrose, dextrose and 'Canderel', and the residual L-ascorbic acid was measured spectroscopically. The photostability of L-ascorbic acid was enhanced by all of the sweetening agents at 5% w/v concentration. However, the addition of stoichiometric amounts of hydrogen peroxide to provide a source of hydroxyl radicals and 2,2'-azobis(2-amidopropane) to provide hydroperoxyl radicals resulted in diminished stability of L-ascorbic acid solutions. The possibility of the diminished stability of the L-ascorbic acid solutions being due to the action of hydroperoxyl radicals in the presence of hydroxyl radical scavengers is discussed.

Key words: 2,2'-Azobis(2-amidinopropane) dihydrochloride; Ascorbic acid; Canderel; Dextrose; Hydrogen peroxide; Hydroxyl radical; Mannitol; Photostability; Sorbitol; Sucrose

1. Introduction

Ascorbic acid is used as both a therapeutic agent (vitamin C) and as an antioxidant (Lachman, 1968a,b). In view of the current belief that vitamin C supplementation of human diet is beneficial there is a widespread use of this material by the food and soft drinks industries. It is also common practice for foods and soft drinks to have sweetening agents incorporated; these materials can be sugars or artificial sweetening agents.

In view of the fact that most soft drinks, foods and some pharmaceutical products are packaged in such a manner that the product is exposed to both sunlight and artificial light during storage and use, there is a risk of the ascorbic acid undergoing photochemical degradation. The objective of the present work is to examine the effect on the photostability of aqueous solutions of ascorbic acid by the incorporation, individually, of a range of sweetening agents. The sweetening agents chosen were 'Canderel, mannitol, dextrose and sucrose. The investigation was carried out by irradiating the samples with simulated sunlight (Evans et al., 1975) and measuring the

* Corresponding author.

amount of ascorbic acid remaining in the solution.

2. Materials and methods

2.1. Materials

The following materials were obtained from the indicated sources: ABAP (2,2'-azobis(2-amidinopropane) dihydrochloride) (Polyscience Inc. U.S.A.); L-(–)-ascorbic acid (Fisons Scientific Apparatus Ltd, Loughborough, U.K., batch no. 11208110, 99.7% purity); dextrose, B.P. (BDH batch no. 2602); Canderel (maltodextrin, aspartamine, phenylalanine) (Gateway Foods Ltd); hydrochloric acid (specific gravity 1.16) (Fisons Scientific Apparatus Ltd, batch no. 15503030, 32.23% HCl); D-(+)-mannitol (Hopkins & Williams Ltd, batch no. 77763B 61529); phosphotungstic acid (Hopkins & Williams Ltd, batch no. 943/1W65333); sodium acetate (anhydrous) (Hopkins & Williams Ltd, batch no. 0869330891); sodium metavanadate (Aldrich Chemical Co. Ltd, batch no. 13718–26–8); D-(+)-sorbitol (Hopkins & Williams, batch no. 53970W57614); sucrose (BDH, batch no. 1376110L); hydrogen peroxide (100 volume) Boots plc.

2.2. Apparatus

A Beckman DU 70 UV/visible spectrophotometer with matched silica cells of 1 cm path length was used. A Pye Unicam Model 292 pH meter was employed for the pH measurements.

2.3. Methods

The assay of L-ascorbic acid was carried according to the method of Muralikrishna and Murty (1989) with the following modifications:

All volumes were scaled up by a factor of 20. Phosphotungstic acid and sodium metavanadate solutions were prepared in double-distilled water and buffer (pH 1.5) was made from sodium acetate and hydrochloric acid. Ascorbic acid standard solutions (0.1, 0.2, 0.4, 0.8 and 1.0 mg/ml) were prepared in double-distilled water. 25 μ l of each of the L-ascorbic acid standard solutions were added to 5 ml of the complex solution. The contents of the tubes were mixed thoroughly and absorbance readings (A_t) taken at 360 nm against double-distilled water as a blank. The absorbance of the complex solution was also taken at the same wavelength against the same blank and a reading of A_r was obtained (correlation coefficient = 0.994; $p = 0.001$).

Table 1
Photodegradation of aqueous L-ascorbic acid solution (0.8 mg/ml)

Irradiation time (h)	A_t	A_r	$A_r - A_t$	% drug remaining	log % drug remaining	1/% drug remaining
0	1.336	1.650	0.314	100.00	2.0000	0.01000
17	1.370	1.600	0.230	73.24	1.8647	0.01365
18	1.390	1.596	0.206	65.61	1.8169	0.01534
21	1.396	1.602	0.202	64.33	1.8084	0.01554
22	1.406	1.598	0.196	62.42	1.7953	0.01602
23	1.404	1.598	0.194	61.78	1.7908	0.01618
24	1.398	1.584	0.186	59.23	1.7725	0.01688
45	1.370	1.480	0.110	35.03	1.5444	0.02854
48	1.356	1.466	0.110	35.03	1.5444	0.02854
Correlation coefficients				-0.9854	-0.9941	0.9630
Rate constant					2.34×10^{-2}	

The reaction is deemed to follow first order kinetics. A_r , absorbance of reagent; A_t , absorbance of test solution at 360 nm.

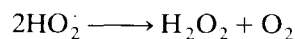
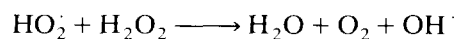
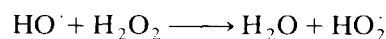
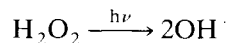
Solutions of L-ascorbic acid (0.8 mg/ml) alone and those containing the sweetening agents each at 5% w/v were irradiated by the method of Evans et al. (1975). The L-ascorbic acid content was measured at time zero and at time intervals up to 48 h.

2.4. Treatment of results

The reaction order was determined by carrying out a linear regression analysis of plots of the percentage L-ascorbic acid remaining against time, log percentage of L-ascorbic acid remaining against time and 1/percentage of L-ascorbic acid remaining against time. The plot yielding the best straight line as determined by the method of Patel and Sugden (1992) was deemed to represent the order of reaction.

3. Discussion

Examination of Table 2 shows that the photodegradation of ascorbic acid solution follows first order reaction kinetics and has a rate constant of 2.34×10^{-2} . On addition of an equimolar amount of hydrogen peroxide the reaction continues to follow first order kinetics but the rate constant rises to 4.92×10^{-2} . This rise in rate constant was expected since hydrogen peroxide on irradiation with light yields mainly hydroxyl radicals. Czapski (1971) reported that the photolysis of hydrogen peroxide proceeds as follows:



There are possible reactions between the radicals formed. Hochanadel et al (1980), Sridharan et al. (1981) and Kurylo et al. (1981) have all demonstrated that hydroxyl and hydroperoxy (HO_2^\cdot) radicals react together. Ascorbic acid has been shown to react with hydroperoxy radicals to form hydrogen peroxide and an ascorbic radical

Table 2

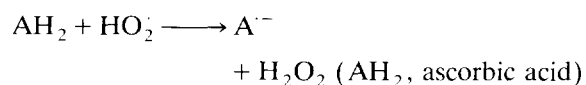
Summary of the photodegradation of aqueous L-ascorbic acid solutions with additives

Compound and additive	Order of reaction	Mean rate constant ^a
L-(–)-Ascorbic Acid	1st	2.34×10^{-2}
L-(–)-Ascorbic acid + hydrogen peroxide (1 ml, 0.3%)	1st	4.92×10^{-2}
L-(–)-Ascorbic acid + sucrose (5%)	1st	1.38×10^{-2}
L-(–)-Ascorbic acid + sucrose (5%) + hydrogen peroxide (1 ml, 0.3%)	1st	5.65×10^{-2}
L-Ascorbic acid + sorbitol (5%)	1st	1.25×10^{-2}
L-Ascorbic acid + sorbitol (5%) + hydrogen peroxide (1 ml, 0.3%)	1st	15.64×10^{-2}
L-Ascorbic acid + mannitol (5%)	1st	1.75×10^{-2}
L-Ascorbic acid + mannitol (5%) + hydrogen peroxide (1 ml, 0.3%)	1st	6.99×10^{-2}
L-Ascorbic acid + Candarel (5%)	1st	1.48×10^{-2}
L-Ascorbic acid + Candarel (5%) + hydrogen peroxide (1 ml 0.3%)	1st	8.92×10^{-2}
L-Ascorbic acid + ABAP (equimolar) ^b	2nd	3.06×10^{-3}
L-Ascorbic acid + ABAP + dextrose (5%) ^b	2nd	2.48×10^{-3}
L-Ascorbic acid + ABAP + sucrose (5%) ^b	2nd	2.90×10^{-3}
L-Ascorbic acid + ABAP + acetaldehyde (0.1 ml) ^b	2nd	2.26×10^{-3}
L-Ascorbic acid + H_2O_2 + acetaldehyde (0.1 ml) ^b	2nd	8.81×10^{-4}

^a Represents a mean of three determinations.

^b Equimolar amounts of L-(–)-ascorbic acid and ABAP/de-ionised water.

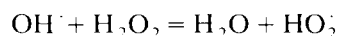
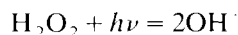
(Nadezhdin and Dunford, 1979):



Clearly, there is a possibility of the (HO_2^\cdot) radical promoting the decomposition of ascorbic acid. Examination of Table 2 shows that all of the sweeteners tested gave reduced rate constants as compared to that of the ascorbic acid control. This suggests that the sweeteners had some effects on the normal photolysis of ascorbic acid which, according to Barr and King (1956), is hydroxyl and hydrogen free radical mediated. The addition of hydrogen peroxide produces a 2-fold increase in the rate constant of the control sample of ascorbic acid. The addition of the sweetening agents tested in every case resulted in an increase in the rate constant above that recorded

for the ascorbic acid with added hydrogen peroxide. However, the addition of ABAP to create a source of hydroperoxy radicals (Niki et al., 1988) results in a change of order from first to second. The rate constants for sucrose and dextrose (2.90 and 2.48×10^{-3}), respectively, are slightly less than that of the control (3.06×10^{-3}), indicating that the presence of the sugars tested has a very small scavenging action on the hydroperoxy radical.

A possible explanation for these results is that the hydrogen peroxide decomposes in the presence of light to give both hydroxyl and hydroperoxy radicals:



The hydroxyl radicals may react with the sweetening agents and the much less reactive HO_2^\cdot radicals are free to react with the ascorbic acid as has been demonstrated by Nadezhdin and Dunford (1979), Cabelli and Bielski (1983), and Fitchett and Gilbert (1986).

It is of interest to note that the photodegradation reaction of ascorbic acid in the presence of ABAP and acetaldehyde gives a rate constant of 2.26×10^{-3} ('control' without acetaldehyde gives a rate constant of 3.06×10^{-3}) and in the absence of ABAP but with added water, the rate constant falls to 8.81×10^{-4} . This result shows that acetaldehyde acts as a quenching agent for the HO_2^\cdot radical. The very low value of the rate constant for the experiments carried out with only ascorbic acid and acetaldehyde suggests that the latter stabilises the former and quenches the radicals formed on irradiation of water in the test system. Kochi (1973) reported that acetaldehyde is a triplet quenching agent. Czapski (1972) points out that the hydroperoxyl radical can be formed in a photolysis reaction where H radicals or e_{aq}^- are formed in the presence of oxygen. This type of photochemical reaction could easily take place in a system where one or more components of a formulation acted as a sensitizer and reduced the energy requirement for the reaction to proceed. Thus, there is a possibility that photochemical degradation of an aqueous formulation may pro-

ceed in the presence of suitable hydroxyl radical scavengers, since these additives are incapable of preventing the degradative effect of the hydroperoxyl radical. This could provide an explanation for the results reported by Kendall et al. (1989) on the effects of hydroxyl and other radicals on the stability of metronidazole in buffer solutions at pH 9.2.

References

- Barr, N.F. and King, C.G., The γ -ray induced oxidations of ascorbic acid and ferrous ion. *J. Am. Chem. Soc.*, 78 (1956) 303–305.
- Cabelli, D.E. and Bielski, B.H.J., Kinetics and mechanism for the oxidation of ascorbic acid/ascorbate by $\text{HO}_2^\cdot/\text{O}_2^\cdot$ (hydroperoxy/superoxide radicals): A pulse radiolysis and stopped-flow photolysis study. *J. Phys. Chem.*, 87 (1983) 1809–1812.
- Czapski, G., Nature of oxygen containing radicals in radiation chemistry and photolysis of aqueous solutions. *Nucl. Sci. Abstr.*, 25 (1971) 26290.
- Czapski, G., The hydroperoxy radical in aqueous solution. *Isr. J. Chem.*, 10 (1972) 987–997.
- Evans, P.G.E., Sugden, J.K. and Van Abbe, N.J., Aspects of the photochemical behaviour of 1-hydroxypyridine-2-thione. *Pharm. Acta. Helv.*, 50 (1975) 94–99.
- Fitchett, M and Gilbert, B.C., Radical reactions of carbohydrates, Part 6: An electron spin resonance study of the hydroxyl radical with some lactones derived from sugars and with ascorbic acid. *J. Chem. Soc., Perkin Trans.*, 2 (1986) 1169–1177.
- Hochanadel, C.J., Sworski, T.J. and Ogren, P.J., Rate constants for the reaction of H_2O with OH^\cdot and with HO_2^\cdot . *J. Phys. Chem.*, 84 (1980) 3274–3277.
- Kendall, A.T., Stark, E. and Sugden, J.K., Effect of hydroxyl radicals on the stability of metronidazole in buffer solution at pH 9.2. *Int. J. Pharm.*, 57 (1989) 217–221.
- Kurylo, M.J., Klais, O. and Laufer, A.H., Mechanistic investigation of the OH and HO_2^\cdot reaction. *J. Phys. Chem.*, 85 (1981) 3674–3678.
- Kochi, J.K. *Free Radicals*, Vol.1, Wiley, New York, 1973.
- Lachman, L., Antioxidants and chelating agents as stabilisers in liquid dosage forms: II. *Drug Cosmet. Ind.*, 102 (1968a) 43–45, 148–149.
- Lachman, L., Antioxidants and chelating agents as stabilisers in liquid dosage forms. *Drug Cosmet. Ind.*, 102 (1968b) 36–38, 40, 146–148.
- Muralikrishna, U. and Murty, J.A., Spectrophotometric determination of ascorbic acid in pharmaceutical preparations and fruit juices. *Analyst*, 114 (1989) 407–408.
- Nadezhdin, A.D. and Dunford, H.B., On the role of perhydroxyl radical as an oxidising agent. *Microsomes. Drug*

- Oxid. Chem Carcinog. (4th Int. Symp. Microsomes Drug Oxid.)*, Vol.1, 1979, pp. 399–402.
- Niki, E., Yamamoto, Y., Takahashi, M., Yamamoto, K., Yamamoto, Yuichi., Komuro, E., Miki, M., Yasuda, H. and Mino, M., Free radical-mediated damage of blood and its inhibition by antioxidants. *J. Nutr. Sci. Vitaminol.*, 34 (1988) 507–512.
- Patel, R. and Sugden, J.K., Photodegradation of aqueous solutions of dequalinium chloride. *Pharmazie*, 47 (1992) 113–115.
- Sridharan, U.C., Qui, L.X. and Kaufman, F., Kinetics of the reactions $\text{OH}^\cdot + \text{HO}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2^\cdot$ at 296 K. *J. Phys. Chem.*, 85 (1981) 3361–3363.