The Determination of Ascorbic Acid in the Presence of Sulphur Dioxide

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The determination of ascorbic acid with aqueous 2:6-dichlorophenolindophenol is not entirely specific for this vitamin unless special techniques for eliminating interfering substances are applied in each case. SO_2 has a particularly strong reducing action on indophenol, and, when used for the preservation of fruit juices and cordials or in the processing of dried fruits and vegetables, should be removed before the ascorbic acid content of these materials can be accurately determined.

Three methods for eliminating free SO_2 have been described: (1) By boiling the extract and distilling off SO_2 [Kirkpatrick, 1941*a*; Downer, 1942]. (2) By passing a stream of O_2 -free N_2 through the extract [Mapson, 1942]. (3) By formation of the non-reducing bisulphite compound of SO_2 with acetone [Mapson, 1942]. SO_2 in fruit juices sweetened with glucose or invert sugar forms the bisulphite compound with the hexose. In amounts of SO_2 up to 1300 parts per million (p.p.m.), indophenol titration is not affected unless the sugar concentration falls below 20% [Kirkpatrick, 1941*b*].

The first two methods are lengthy, and only the third would be practicable as a routine method when many samples are to be examined. In an attempt to find a method by which accurate results may be obtained without the necessity of a fixed rate of titration, the effect of adding H_2O_2 , which rapidly converts SO_2 quantitatively into non-interfering H_2SO_4 , has now been investigated. Initial experiments showed that the addition of an equal volume of 3% H_2O_2 to an ascorbic acid solution acidified with one drop of concentrated HCl had no appreciable effect on the value obtained by indophenol titration, when the dye was added from the burette. The ascorbic acid was thus unaffected by the H_2O_2 , and a diminution in titration value was experienced only when Fe or Cu salts were added to the ascorbic acid, and the solution was allowed to stand for some time before titration.

EXPERIMENTAL

In a detailed study of the effect of adding SO_2 and varying quantities of H_2O_2 to ascorbic acid extracts from various sources, the experiments summarized in Table 1 were carried out. The end-point in each case was reached within 1 min.

Solutions and reagents employed

Ascorbic acid solution: 6.25 mg. dissolved in 100 ml. distilled water. For each titration 10 ml. were acidified with one drop of concentrated HCl.

Orange juice solution: 20% (v/v) in distilled water.

Cauliflower leaf: extract made with 2% HPO_3 .

Parsley: extract made with 2% HPO_3 .

0.05% aqueous 2:6-dichlorophenolindophenol: 1 ml. was equivalent to 0.25 mg. ascorbic acid.

Hydrogen peroxide: a 3% solution of B.P. quality.

KHSO₃ solution: a 0.934% (w/v) solution in distilled water. 1 ml. added to 10 ml. ascorbic acid solution produced a SO_2 concentration of 450 p.p.m.

DISCUSSION

Table 1 shows that H_2O_2 does not appreciably oxidize ascorbic acid, whether the latter is contained in pure aqueous solution (Exp. 2), orange juice (Exp. 8), or HPO_3 extract of vegetables (Exps. 16 and 19), even after standing for 6 min. The use of HPO_3 as extractant has been reported to guard against the danger of oxidation of ascorbic acid by Fe or Cu, and this probably explains why cauliflower and parsley extracts give good values after H_2O_2 addition, in spite of traces of dissolved mineral salts. The addition of more Fe or Cu salt, however, causes slow oxidation (Exp. 21). H_2O_2 has no effect on unreduced indophenol (Exp. 6).

In all experiments the addition of KHSO_3 gives rise to very high titration values, but H_2O_2 neutralizes the effect of the SO_2 , and the original titration figure is obtained.

In all experiments in which H_2O_2 has been added to ascorbic acid solutions, the end-point with indophenol is a permanent pink. This colour deepens at varying speeds due to reoxidation of the reduced and decolorized indophenol (leuco-compound) to the original coloured form. The rate of reformation of indophenol is very slow with a concentration of 1.5–2% H_2O_2 (Exps. 10 and 11) and is more rapid with 0.075% H_2O_2 (Exp. 14). The reoxidation of reduced indophenol is still more rapidly catalyzed with added Fe and Cu salts (Exp. 21). The diminution in the titration value with lower concentrations of H_2O_2 (e.g. 0.075%) is most probably due to

Table 1. *Experiments showing the effect of H₂O₂ on the indophenol titration of sulphited and unsulphited ascorbic acid extracts*

Exp. no.	Solution used (ml.)	KHSO ₅ added (ml.)	3% H ₂ O ₂ added (ml.)	Indophenol titration value (ml.)	End-point and remarks (A=pink; permanent 1 min. B=pink; colour deepens very slowly on standing)
	Ascorbic acid				
1	10	—	—	2.5	A
2	10	—	10	2.45	B
3	10	1	—	>4	Strongly reducing
4	10	1	10	2.45	B
5	10	—	10 (stood 6 min.)	2.3	B
6	Nil	—	10 + 10 ml. water and 1 drop conc. HCl	<0.05	A
	Orange juice				
7	10	—	—	3.9	A
8	10	—	10	3.9	B
9	10	1	—	>7	Strongly reducing
10	10	1	20	3.8	B
11	10	1	10	3.8	B
12	10	1	5	3.8	Pink colour deepens slightly more rapidly
13	10	1	1	3.7	Colour deepens more rapidly
14	10	1	0.5	3.6	Colour deepens rapidly
	Cauliflower leaf				
15	10	—	—	1.52	A
16	10	—	10	1.52	B
17	10	1	10	1.52	B
	Parsley				
18	10	—	—	6.65	A
19	10	—	10 (stood 6 min.)	6.6	B
20	10	1	10	6.65	B
21	10	1 + traces FeCl ₃ and CuSO ₄	10	6.1	Pink colour deepens very rapidly

reoxidation of reduced indophenol during the titration, and reduction of the reformed indophenol by ascorbic acid still present. A great advantage of the H₂O₂ procedure is that the pink end-point does not fade.

General method for estimation of ascorbic acid in solutions containing sulphite

The acidity of a measured portion of the solution is brought below pH 3.5 by the addition of HCl, if necessary, and a volume of 3% H₂O₂ equal to that

of the whole is added. Indophenol is run in from a burette until a permanent pink colour is obtained. The end-point should be reached within 1 min.

SUMMARY

1. A method for the elimination, by means of H₂O₂, of the interference of SO₂ in ascorbic acid titrations is described.

2. Under the conditions used, H₂O₂ has no appreciable effect on ascorbic acid, unless Fe or Cu salts are present.

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The Determination of Ascorbic Acid in the Presence of Interfering Substances, Notably Reductones

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Reductone, reductic acid and other compounds formed in hot alkaline sugar solutions reduce 2:6-dichlorophenolindophenol in the same way as does

ascorbic acid. In fact Kertesz [1934] suggested a method of determining the ascorbic acid equivalent of indophenol solutions by titrating the gluco-