IMPROVED N-BROMOSUCCINIMIDE METHOD FOR ESTIMATION OF ASCORBIC ACID IN FOOD PRODUCTS

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The titrimetric method of ascorbic acid determination by *N*-bromosuccinimide is unreliable in the presence of sulphites. During the titration, the sulphites get oxidised by *N*-bromosuccinimide along with ascorbic acid with the result that higher values for the vitamin are obtained. A method for the elimination of sulphite by means of formaldehyde is described.

Introduction

N-Bromosuccinimide method[1] of Barakat, El-Wahab and El-Sadr for the estimation of ascorbic acid (vitamin C) is based upon the selective oxidation of the vitamin by *N*-bromosuccinimide. Unlike 2,6-dichlorophenolindophenol method[2,3] determination of ascorbic acid by this method is not limited by the presence of interfering substances such as reductones and reductic acid. Substances, including diketogluconic acid, glucose, urea, uric acid, alcohols, esters, citrates and ferrous or ferric salts are also without any influence on the titration process. However, like indophenol method, this titrimetric method is not reliable for the estimation of ascorbic acid in food products or samples containing sodium or potassium sulphites as preservative. Under the experimental conditions of the method, sulphite and sulphide are also oxidized by *N*-bromosuccinimide before iodine is liberated from potassium iodide, with the consequence that higher values for the vitamin are obtained. Determination of ascorbic acid with *N*-bromosuccinimide, therefore, is not entirely specific for the vitamin unless special technique for eliminating the interference by sulphites are applied. A technique which eliminates the interference and gives most satisfactory results is presented in this paper. In designing the technique advantage was taken of the observation made first by Lugg[4] and later by Mapson[5] that HCHO, at low pH, combines rapidly with sulphites and sulphides and only very slowly with ascorbic acid. Based on their observation, Lugg[4] and Mapson[5] also proposed methods which permit estimation of ascorbic acid by indophenol titration in the presence of interfering sulphur compounds.

Experimental

Reagents.—Acetic acid 5% (v/v); metaphosphoric acid 6% (w/v); formaldehyde 40% (v/v); starch solution 1% (w/v), prepared by dissolving 1 g of soluble starch in 10 ml of boiling water and making volume to 100 ml with saturated sodium chloride solution; potassium iodide 4% (w/v); ascorbic acid solution 0.1% (w/v); and *N*-bromosuccinimide 0.1% (w/v). Solutions of *N*-bromosuccinimide and ascorbic acid are freshly made and diluted as required.

Method.—If the sample to be a analysed is a fluid-like fruit juice or squash, a known volume of it is first diluted with distilled water so that 5 ml of the diluted sample would contain 0.5–1.0 mg ascorbic acid, and then an equal volume of metaphosphoric acid is added. The acidulated juice is filtered and determination carried out on aliquots.

Assay.—One ml formaldehyde solution is added to 5 ml sample taken into 50-ml Erlenmeyer flask, which is shaken intermittantly for 1 min. 5 ml potassium iodide, 2 ml acetic acid and 2 drops starch solution are added. Titration is then carried out against *N*-bromosuccinimide solution and ascorbic acid content estimated as described by Barakat *et al.*[1]

Results

Estimation of Pure Ascorbic Acid Solution.—Experiments were carried out for studying the effect of added $Na_2S_2O_5$, on the estimation of ascorbic acid in pure ascorbic acid solution. Titration was carried out in the presence of formaldehyde solution as already described as well as without adding formaldehyde solution in the sample. The results presented in Table 1 clearly reveal interference of sulphite ion in the estimation of ascorbic acid by the method of Barakat *et al.*[1] (NBS). The value of the vitamin content in the sample containing sulphite ion was more than double the amount actually present, when titration was carried out without prior addition of formaldehyde to the sample. This interference was completely eliminated when titration was carried out by the modified method (MNBS) after adding formaldehyde to the sample. Addition of formaldehyde to the sample containing no sulphite caused a decrease in the value of ascorbic acid content of the sample only when it was not acidu-

TABLE 1.—ESTIMATION OF PURE ASCORBIC ACID SOLUTIONS WITH AND WITHOUT ADDED SULPHITE AND PRIOR TREATMENT WITH HCHO BY *N*-BROMOSUCCINIMIDE.

5 ml ascorbic acid solution containing 100 mg ascorbic acid per 100 ml solution was taken.

HPO_3 solution (ml)	TCA solution (ml)	* $Na_2S_2O_5$	HCHO solution (ml)	Ascorbic acid by NBS** (mg/100 ml)
—	—	—	—	98.0
—	—	Present	—	224.0
5	—	Present	1.0	98.2
—	—	—	1.0	60.0
5	—	—	1.0	96.5
—	5	—	1.0	96.5

* 0.08% of sample Solution.
***N*-Bromosuccinimide.

lated with either trichloroacetic acid or metaphosphoric acid.

Effect of Different Concentrations of Sulphites on Estimation of Ascorbic Acid.—Experiments were performed in which concentrations of sulphites, $Na_2S_2O_5$ and Na_2SO_3, in orange juice were caused to vary between 0.04 and 0.2%. Juice, obtained by squeezing orange halves, was filtered through muslin cloth. The juice was divided into several lots and sulphites added to them in the proportion shown in Table 2. Aliquot from each lot was diluted with equal volume of 20% trichloroacetic acid to stabilize the vitamin and precipitate the interfering substances.[1] The acidulated juice was filtered and ascorbic acid estimated in the clear filtrate (Table 2). The vitamin content in the sample without sulphite as estimated by NBS method was 60.0 mg/100 ml. But in the samples containing the sulphites, amounts of ascorbic acid increased with the increase in sulphite contents when estimations were carried out by NBS method. No such discrepancy was observed when samples containing sulphite were assayed by MNBS method. The amount of ascorbic acid estimated by this method was always the same, i.e. 60 mg/100 ml (±1.5%), irrespective of the amount of sulphites present in the sample.

Recovery of Added Ascorbic Acid to Orange Juice.—Some recovery experiments were performed in which pure ascorbic acid was added to orange juice concentrate in the presence and in the absence of sulphite. Orange juice concentrate containing sodium benzoate as preservative was obtained from Shezan International, Lahore $Na_2S_2O_5$ 0.08% (w/v) was added to a portion of the concentrate. For assay, 5 ml concentrate, with or without sulphite, was diluted to 25 ml with meta-

TABLE 2.—EFFECT OF DIFFERENT CONCENTRATIONS OF SULPHITES ON THE ESTIMATION OF ASCORBIC ACID IN ORANGE JUICE BY NBS* AND MODIFIED NBS METHOD (MNBS).

Preservative	%	Ascorbic acid, mg/100 ml, determined by NBS*	MNBS
Nil		60.0	60.00
$Na_2S_2O_5$	0.04	102.0	60.50
	0.08	141.0	61.00
	0.16	224.0	60.00
	0.20	271.0	59.80
Na_2SO_3	0.04	70.0	60.80
	0.08	120.0	60.00
	0.16	182.0	61.00
	0.20	210.0	59.60

**N*-Bromosuccinimide.

TABLE 3.—RECOVERY OF ADDED ASCORBIC ACID TO ORANGE JUICE.

Orange juice Original vitamin content, mg/100 ml by NBS*	Ascorbic acid added mg/100 ml	Total ascorbic acid mg/100 ml	$Na_2S_2O_5$	Total ascorbic acid content, mg/100 ml., by NBS*	MNBS
52.5	—	52.5	Present	141.0	52.4
,,	40.0	92.5	—	93.0	—
,,	40.0	92.5	Present	180.0	90.5
,,	20.0	72.5	—	72.5	—
,,	20.0	72.5	Present	160.0	71.5

**N*-Bromosuccinimide.

phosphoric acid solution and ascorbic acid estimated after filtration. Pure ascorbic acid was added to the filtrate in the form of freshly prepared solution containing 4.0 mg ascorbic acid/ml of the filtrate. The vitamin content in the sulphite-free sample with added pure ascorbic acid was estimated by NBS method and those of samples containing sulphite by both NBS and MNBS methods (Table 3). It is evident from the results that added ascorbic acid was completely recovered from the sulphite-free samples by estimation by NBS method and in sulphite-treated samples by MNBS method. The values of ascorbic acid as estimated by NBS method were higher in samples containing sulphite; added ascorbic acid increased the values by its equivalent amount.

Discussion

According to Barakat *et al.*[1] ascorbic acid is selectively oxidized by *N*-bromosuccinimide to dehydro-ascorbic acid. It is, however, seen from

our results that depending upon the amount of sulphite present in a sample the amount of ascorbic acid estimated by NBS method is always higher than the amount of the vitamin actually present. This shows that sulphite ion, if present, also gets oxidized to sulphite simuultaneously, thereby affecting the accuracy of the method. The error due to sulphite is eliminated when samples containing the preservative are treated with HCHO prior to titration. HCHO under the condition would form condensation product with sulphite, which is then not oxidized by *N*-bromosuccinimide.

Table 1 shows that when samples without added sulphite are treated with HCHO, low values for ascorbic acid are obtained when the stabilizer, HPO_3 or TCA is not used. This is to be expected because ascorbic acid combines with HCHO at different rates according to the pH of the reacting solution and the combination results in the loss of the reducing properties of the ascorbic acid.[6] At pH 1.5 ascorbic acid combines only very slowly with HCHO, whereas at pH 3.5 the combination is rapid.[4,7] According to Mapson[5] there is no significant condensation of HCHO with ascorbic acid in solutions of pH 0.6. Sulphites and sulphides combine readily with HCHO under the condition. It is, thus, important that the recommended procedure of treating samples with either HPO_3 or TCA is strictly followed. The treatment, as would be seen from the results, is adequate enough to ensure accuracy of the modified method even if a sample is free from added sulphites.

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