

# THE EVOLUTION OF HYDROGEN SULPHIDE IN SYRUPS CONTAINING HYPOPHOSPHOROUS ACID

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DURING the course of normal analytical control on pharmaceutical syrups, it was found that an objectionable odour was frequently associated with syrups containing hypophosphorous acid, especially syrup of calcium hypophosphite, B.P.C. 1934, syrup of ferrous iodide B.P.C. and syrup of hydriodic acid B.P.C. Other syrups containing hypophosphorous acid are compound syrup of hypophosphites B.P.C. and triple syrup B.P.C.

This development of odour in preparations made with raw materials which had passed official standards for purity necessitated an investigation. The odour of the syrups in bulk was suggestive of hydrogen sulphide, and to prove that this was indeed the responsible factor, the following test was used. It is a modification of the sulphide stain method of Mann<sup>1</sup>. 50 ml. of each syrup which had developed an odour was placed in the apparatus shown in Figure I and kept at 45°C. for 24 hours. A black stain on the lead acetate wool was obtained in each case, confirming the presence of hydrogen sulphide.

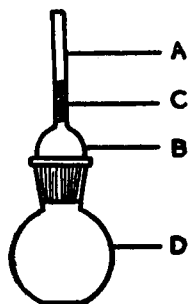


Fig. 1

- A. 90 mm. length of glass tubing, 6 mm. outside diameter and 4 mm. inside diameter fused into
- B. B.24 cone.
- C. Plug of lead acetate wool (absorbent cotton B.P.C. soaked in solution of lead acetate B.P. and dried).
- D. 100 ml. flask with B.24 socket.

To establish which ingredient of the syrups was responsible for the production of hydrogen sulphide a series of batches of syrup of calcium hypophosphite B.P.C. 1934 was made from batches of raw materials, omitting from each a single ingredient. These experiments were submitted to both an odour test and the stain test described. By a process of elimination it was found that the sugar was the constituent responsible for the presence of a hydrogen sulphide progenitor. This latter substance reacted with the hypophosphorous acid giving rise to hydrogen sulphide. That the reaction was not a simple sulphide/acid decomposition was shown by the non-evolution of hydrogen sulphide with hydrochloric acid, sulphuric acid and phosphoric acid. It appeared, then, that the mechanism was essentially a reduction by the hypophosphorous acid of a sulphur compound present in the sugar, with the consequent formation of hydrogen sulphide. Ultramarine, an inorganic colour, index number 1290, probable formula  $\text{Na}_4\text{Al}_3\text{Si}_3\text{S}_2\text{O}_{12}$ , is

reputed to be used in the sugar industry to mask the yellowness of sugar which has not been completely decolorised during refining. The British Pharmacopœia 1948, page 495, gives the following test for the presence of ultramarine in sucrose:—"10 g. dissolved in 20 ml. of hot water form a clear, colourless and odourless solution, which on the subsequent addi-

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tion of 1 ml. of dilute hypophosphorous acid does not develop an unpleasant odour in 1 hour." The test is based on the reduction of the sulphur complex contained in ultramarine to hydrogen sulphide and the detection of the gas by its odour. No specification as to a closed vessel is given.

A sample of sugar known to evolve hydrogen sulphide in a production batch of syrup of calcium hypophosphite B.P.C. 1934 was tested: (a) By the B.P. method for ultramarine using an open flask; (b) as (a) using a closed flask; (c) by the sulphide stain test using 10 g. of sucrose, 20 ml. of hot water and 1 ml. of dilute hypophosphorous acid.

The results of these tests are given in Table I.

TABLE I

B.P. ultramarine test (one hour)		Sulphide stain test	
Open flask	Closed flask	Room temp. 18°C.	45°C.
No odour	No odour	1 hr. no reaction 3 hrs. no reaction 6 hrs. faint reaction 16 hrs. distinct reaction	no reaction faint reaction distinct reaction distinct reaction

If the production of hydrogen sulphide were due to the presence of ultramarine these results suggested that the conditions of the British Pharmacopœia test for ultramarine were not sufficiently sensitive. They also showed that a reaction could be obtained fairly quickly by using the sulphide stain test and heating at 45°C. Although it was later shown that the production of hydrogen sulphide was not due to ultramarine, the experiments conducted to determine the limit of sensitivity of the British Pharmacopœia test for ultramarine are worth recording. These indicated that 16 p.p.m. of ultramarine is the limit at which the odour becomes definitely unpleasant when tested by the method of the British Pharmacopœia, below this figure the odour is tenuous and sucrose producing such an odour could not be deemed to fail the British Pharmacopœia test for ultramarine. As all these odours are rather fugitive a closed flask is to be preferred. It should be emphasised that reliance on the sense of smell to detect hydrogen sulphide in low concentrations is not to be recommended as the ability to detect the gas differs considerably in individuals, some being quite insensitive. It was also shown that by increasing the amount of sugar to 40 g. 1 p.p.m. of ultramarine is just detectable by the stain test after 24 hours. The test is rather less sensitive to aqueous suspension of ultramarine, due perhaps to higher solubility and rate of oxidation of small amounts of hydrogen sulphide.

Ultramarine is insoluble in water and experiment showed that 0.001 g. in 1,000 g. of control sugar, when made into a syrup and filtered through a No. 30 Whatman filter paper left a distinct blue residue easily discerned by a low power microscope. When 1,000 g. of sugar which was known to evolve hydrogen sulphide was submitted to this test no blue residue

was apparent either visually or microscopically. Hence ultramarine is not the cause of the objectionable odour.

One of the stages in the refining of sucrose is bleaching with sulphur dioxide in the "thin juice" stage. The Public Health (Preservatives, etc.) in Food Regulations<sup>2</sup> permit up to 70 p.p.m. of sulphur dioxide in sugar, but this statutory limit is not mentioned in the monograph on sucrose in the British Pharmacopœia.

As it has been shown that ultramarine was not the source of hydrogen sulphide in the samples examined, combined sulphur dioxide was thought to be a possible precursor. 1 mg. quantities of the following inorganic compounds of sulphur were reacted with hypophosphorous acid:—sodium metabisulphite, calcium sulphite, sodium sulphite and sodium thiosulphate, using the technique of the sulphide stain test described below.

### THE SULPHIDE STAIN TEST

The apparatus used is shown in Figure I.

*Method.* Dissolve 40 g. of sucrose in 19 ml. of hot water, cool, add 1 ml. of hypophosphorous acid, close the flask and maintain at 45°C. for 24 hours.

A positive reaction for hydrogen sulphide was obtained in 24 hours from traces of those inorganic salts derived from sulphur dioxide which are likely to be residual in sucrose as a result of the bleaching process.

A series of samples of sucrose containing various amounts of sulphur dioxide was prepared and tested by the ultramarine method of the British Pharmacopœia. A distinct odour of hydrogen sulphide was produced in 1 hour by ultramarine-free sugar containing between 30 and 50 p.p.m.

TABLE II  
SULPHIDE STAIN TESTS AND SULPHUR DIOXIDE CONTENT OF NORMAL  
MANUFACTURING BATCHES OF SUGAR

				After 24 hours at 45°C.	After 24 hours at room temperature	Parts per million of sulphur dioxide determined by the B.P. method
1.	...	...	Sugar, refined	Very black	Black	29
2.	...	...	Sugar, refined	Black	Faint black	18
3.	...	...	Sugar, refined	No reaction	No reaction	3
4.	...	...	Sugar, refined	Very black	Black	26
5.	...	...	Cane sugar	No reaction	No reaction	0
6.	...	...	Sugar, refined	No reaction	No reaction	0
7.	...	...	Sugar, refined	Very black	Black	13
8.	...	...	Sugar, refined	Black	Faint black	8
9.	...	...	Sugar, refined	No reaction	No reaction	3
10.	...	...	Sugar, refined	Very black	Black	29
11.	...	...	Sugar, refined	Black	Faint black	22
12.	...	...	Cane sugar	No reaction	No reaction	0
13.	...	...	Beet sugar	No reaction	No reaction	0
14.	...	...	Sugar, refined	Very black	Black	29
15.	...	...	Sugar, refined	Very black	Black	18
16.	...	...	Sugar, refined	Very black	Black	29
17.	...	...	Sugar, refined	Very black	Black	29
18.	...	...	Sugar, refined	Black	Black	13
19.	...	...	Sugar, refined	Very black	Black	29
20.	...	...	Sugar, refined	No reaction	Black	6
21.	...	...	Sugar, refined	Black	Black	13
22.	...	...	Sugar, refined	Very black	Black	19
23.	...	...	Sugar, refined	Very black	Black	19
24.	...	...	Sugar, refined	Very black	Black	26
25.	...	...	Sugar, refined	Very black	Black	26

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of sulphur dioxide. In order to increase the sensitivity of the test, 40 g. of sucrose was examined by the stain test. The results showed that the limit of detection of sulphur dioxide falls between 10 and 15 p.p.m. after 1 hour and between 7 and 10 p.p.m. after 24 hours at room temperature. With heating at 45°C., 7 to 10 p.p.m. are detectable after 1 hour and 5 to 7 p.p.m. after 24 hours.

Attention was now directed to the sulphur dioxide content of sucrose and all batches subsequently received were tested by the stain method using 40 g. and heating at 45°C. for 24 hours. The stains produced were compared with the figures obtained by the method of the British Pharmacopœia for the determination of sulphur dioxide. The degree of blackening obtained showed a relationship to the sulphur dioxide content, as will be seen from the results given in Table II. Attention is drawn to the fact that batches of sucrose with a very low sulphur dioxide content have been supplied.

Experiments were continued to find the maximum limit of sulphur dioxide permissible in sucrose, below which the objectionable odour would not develop when used in the preparation of syrups containing hypophosphorous acid. The syrups investigated were syrup of calcium hypophosphite B.P.C. 1934, syrup of ferrous iodide B.P.C., compound syrup of hypophosphites B.P.C., syrup of hydriodic acid B.P.C. and syrup triplex B.P.C. The results are recorded in Tables III, IV, V and VI.

**TABLE III**  
**TESTS ON SYRUP OF CALCIUM HYPOPHOSPHITE B.P.C. 1934 CONTAINING**  
**KNOWN AMOUNTS OF SULPHUR DIOXIDE**

Quantities Taken	Sulphide Stain Test				Odour after Standing at Room Temperature for	
	At 45° C.		At Room Temperature		7 Days	8 Weeks
	1 Hour	2 Hours	1 Hour	24 Hours		
Syrup, 50 ml. containing Sulphur Dioxide, parts per million, based on Sucrose content						
30 ... ..	Very distinct reaction	Very distinct reaction	—	—	Very distinct odour of hydrogen sulphide	Very distinct odour of hydrogen sulphide
20 ... ..	Very distinct reaction	Very distinct reaction	—	—	Very distinct odour of hydrogen sulphide	Distinct odour of hydrogen sulphide
15 ... ..	Very distinct reaction	Very distinct reaction	—	—	Odour of hydrogen sulphide	Slight unpleasant odour
10 ... ..	Distinct reaction	Very distinct reaction	No reaction	Distinct reaction	Faint unpleasant odour	No odour
7.5 ... ..	No reaction	Distinct reaction	No reaction	Faint reaction	Very faint unpleasant odour	—
5 ... ..	No reaction	No reaction	No reaction	No reaction	No odour	—

TABLE IV

TESTS ON SYRUP OF FERROUS IODIDE B.P.C. CONTAINING KNOWN AMOUNTS OF SULPHUR DIOXIDE

Quantities Taken	Sulphide Stain Test			Odour after Standing at Room Temperature for		
	At 45° C.		At Room Temperature after	24 Hours	7 Days	8 Weeks
	1 Hour	2 Hours	7 Days			
Syrup, 50 ml. containing Sulphur Dioxide, parts per million, based on Sucrose content						
30 ... ..	No reaction	Very distinct reaction	—	Very distinct odour of hydrogen sulphide	Faint odour of hydrogen sulphide	None
25 ... ..	No reaction	Very distinct reaction	—	Very distinct odour of hydrogen sulphide	Faint unpleasant odour	None
20 ... ..	No reaction	Distinct reaction	No reaction	Distinct unpleasant odour	Very faint unpleasant odour	None
15 ... ..	No reaction	Faint reaction	No reaction	Faint unpleasant odour	No odour	None
10 ... ..	No reaction	No reaction	No reaction	No odour	No odour	None

TABLE V

TESTS ON COMPOUND SYRUP OF HYPOPHOSPHITES B.P.C. CONTAINING KNOWN AMOUNTS OF SULPHUR DIOXIDE

Quantities Taken	Sulphide Stain Test			Odour After Standing at Room Temperature for	
	At 45° C.		At Room Temperature after	7 Days	8 Weeks
	1 Hour	24 Hours	7 Days		
Syrup, 50 ml. containing Sulphur Dioxide parts per million, based on Sucrose content					
70 ... ..	No reaction	Distinct reaction	No reaction	None detectable	None detectable
50 ... ..	No reaction	No reaction	No reaction	None detectable	None detectable
40 ... ..	No reaction	No reaction	No reaction	None detectable	None detectable
30 ... ..	No reaction	No reaction	No reaction	None detectable	None detectable

Consideration of the results presented in these Tables will show that (1) Syrup of calcium hypophosphite B.P.C. 1934 retained the odour of hydrogen sulphide for a considerable period. A syrup prepared with sucrose containing 10 p.p.m. of sulphur dioxide developed a faint unpleasant odour which persisted for longer than 7 days but gradually disappeared. The odour developed from sucrose containing 20 p.p.m. of sulphur dioxide was most apparent after a period of 8 weeks.

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### TABLE VI

TESTS ON SYRUP OF HYDRIODIC ACID B.P.C. CONTAINING KNOWN AMOUNTS OF SULPHUR DIOXIDE

Quantities Taken Syrup, 50 ml. containing Sulphur Dioxide, parts per million, based on Sucrose content	Sulphide Stain Test at 45° C.		Odour After Standing at Room Temperature for 24 Hours
	1 Hour	24 Hours	
30 ... ..	Very distinct reaction	Very distinct reaction	—
25 ... ..	Very distinct reaction	Very distinct reaction	—
20 ... ..	Very distinct reaction	Very distinct reaction	—
15 ... ..	Very distinct reaction	Very distinct reaction	—
10 ... ..	Distinct reaction	Very distinct reaction	Very strong odour of hydrogen sulphide
8 ... ..	Faint reaction	Very distinct reaction	—
6 ... ..	Very faint reaction	Very distinct reaction	Distinct odour of hydrogen sulphide
4 ... ..	Very faint reaction	Very distinct reaction	—
3 ... ..	Very faint reaction	Distinct reaction	—
2 ... ..	Barely detectable	Distinct reaction	—
1 ... ..	No reaction	Distinct reaction	—

(2) Syrup of ferrous iodide B.P.C. also retained the odour of hydrogen sulphide but for a shorter period than syrup of calcium hypophosphite B.P.C. 1934. A syrup prepared with sucrose containing 25 p.p.m. of sulphur dioxide gave an odour of hydrogen sulphide detectable at the end of 7 days but all samples examined had lost their odour at the end of 8 weeks.

(3) Compound syrup of hypophosphites B.P.C. prepared with sucrose containing the statutory limit of 70 p.p.m. of sulphur dioxide did not develop a noticeable odour of hydrogen sulphide, although its presence has been detected by the sulphide stain test. The odour of hydrogen sulphide is possibly masked by the odour of chloroform in this preparation.

(4) Triple syrup B.P.C. produced a slight odour of hydrogen sulphide when prepared with sucrose containing the statutory maximum limit of 70 p.p.m. of sulphur dioxide. The odour is somewhat masked by the aromatics and the chloroform present but a very distinct sulphide stain is obtained.

(5) Syrup of hydriodic acid B.P.C. developed a distinct odour of hydrogen sulphide when prepared with sucrose containing 5 or more p.p.m. of sulphur dioxide. The odour persisted after a period of 7 days.

### CONCLUSIONS

It has been shown that various pharmaceutical syrups containing hypophosphorous acid develop an odour of hydrogen sulphide after manufacture. The cause of this odour is the reduction by the hypophosphorous acid of sulphur compounds in the sucrose employed. Although the Public Health (Preservatives, etc.) in Food Regulations<sup>2</sup> allow a statutory maximum of 70 p.p.m. of sulphur dioxide in sugar, it has been found that quantities very much below this figure will cause the development of an odour of hydrogen sulphide. This odour persists for varying lengths of time in the different syrups. The sucrose from which all these syrups were prepared complied with the British Pharmacopœia test for ultramarine. The reduction of the sulphur complex of ultramarine to hydrogen sulphide by hypophosphorous acid is more rapid than the reduction of other sulphur compounds to hydrogen sulphide by the same acid thus sugars which comply with the B.P. test for ultramarine may subsequently develop an odour of hydrogen sulphide when used in the preparation of certain syrups which contain hypophosphorous acid.

### SUMMARY

1. The sensitivity of the method for the detection of the presence of ultramarine in sucrose described in the British Pharmacopœia has been examined.

2. Sulphur dioxide in sucrose used in the preparation of certain syrups containing hypophosphorous acid has been shown to be responsible for the subsequent evolution of hydrogen sulphide and the opinion is expressed that the presence of sulphur dioxide in sucrose B.P. should be prohibited.

3. A method has been proposed for the detection of traces of ultramarine and other sulphur compounds in sucrose.

### REFERENCES

1. Ogilvie and Mann. *Int. Sugar J.*, 1926, **28**, 644.
2. *The Public Health (Preservatives, etc., in Food) Regulations*. S.R.O. 1925 No. 775 as amended by 1926 No. 1557 and 1927 No. 577.

### DISCUSSION

The paper was presented by MR. W. H. STEPHENSON.

The CHAIRMAN asked whether the impurity of the sucrose was due to the use of beet sugar. What would be the effect on supplies of imposing a more stringent limit on the sulphur dioxide content? It would be interesting to know whether syrups could be formulated to prevent the reaction which the author described.

PROFESSOR H. BRINDLE (Manchester) said he considered that there must be some loss of sulphur dioxide in carrying out the test, because although only 1 p.p.m. of ultramarine was required to give a positive reaction, at least 6 to 7 p.p.m. of sulphur dioxide, with its much greater sulphur content, was required to produce the same result. He asked

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whether the authors considered that the air space above the reacting liquid caused oxidation of sulphur dioxide. The loss could possibly be due to the slow reduction of sulphur dioxide, because in syrup of hydriodic acid 1 p.p.m. of sulphur dioxide could be detected, whereas in the absence of hydriodic acid, as in compound syrup of hypophosphites, not less than 7 p.p.m. of sulphur dioxide were required to give a positive reaction. He suggested that the addition of some reducing agent to give quick reduction of the sulphur dioxide would give a more sensitive test. Lead acetate paper might be a more sensitive indicator of sulphuretted hydrogen than plumbised wool.

DR. W. MITCHELL (London) asked the author whether, in conducting his experiments with calcium hypophosphite, he had eliminated hypophosphorous acid as a source of hydrogen sulphide. He asked whether sugar manufacturers still used ultramarine or was it replaced by a water soluble aniline blue? The authors showed that whereas no trouble was encountered with compound syrup of hypophosphites, in triple syrup hydrogen sulphide was formed; more information on that point would be welcomed. A requirement of no sulphur dioxide in sugar would be rather exacting, 5 p.p.m. would be more reasonable.

DR. D. C. GARRATT (Nottingham) said that according to a manufacturer ultramarine was not used in sugar refining.

MR. R. L. STEPHENS (London) said that on the contrary, he had received a report from a manufacturer that ultramarine was still in use. He quoted the following figures for sulphur dioxide content: lump sugar 20 to 40 p.p.m., granulated sugar 20 p.p.m., mineral water sugar not more than 2 p.p.m. He saw no reason why sulphate should not reduce to hydrogen sulphide in the same way as sulphites. Had the use of the B.P. arsenic limit apparatus for determining reducible sulphur compounds, possibly using a stream of nitrogen, been considered? In view of the statement that syrups lost their unpleasant odour in from 1 to 8 weeks, it would be interesting to know under what conditions they had been stored.

MR. A. W. BULL (Nottingham) drew attention to certain syrups which had been suggested as constituents of children's mixtures and which might be preserved with sulphur dioxide. On occasion he had observed unpleasant odours in preparations containing such syrups. There was no such objection to preservatives such as benzoic acid and sodium benzoate.

MR. J. H. OAKLEY (London) said that in his experience, mineral water sugar was the most suitable for these preparations. More detailed information from the sugar manufacturers regarding the type of sugar, cane or beet, supplied would be helpful. In his opinion, iron B.P. was more likely to offend than sulphur dioxide. Could the author comment on the toluene-like odour which sometimes developed in syrup of tolu?

DR. G. E. FOSTER (Dartford) drew attention to another possible source of contamination—micro-organisms which were known to be capable of reducing sulphur compounds to hydrogen sulphide.

MR. STEPHENSON, in reply, said that the crux of the whole matter appeared to be beet sugar. Sugar had, on occasion, been supplied which



gave no sulphur dioxide reaction according to the B.P. test. It was fairly well known that sugar tended to hold sulphur dioxide as such for considerable periods. According to his information ultramarine was only used in cube sugar and at a concentration of 0.5 p.p.m. It was probably advisable to retain the ultramarine limit in the B.P. He agreed that hydriodic acid catalysed the reduction of sulphite by hypophosphorous acid. Plumbised wool moulded in the shape of a cone was quite satisfactory and sensitive for the detection of small quantities of hydrogen sulphide. He was satisfied that the hypophosphorous acid was not the source of the sulphide. Tests for sulphate had not been carried out as it was not regarded as a possible source of the odour. The syrups were stored at room temperature in daylight and at the end of 8 weeks the odour was present in some and not in others. He was aware that sulphur-producing organisms might be responsible for the development of odour but he considered it unlikely in view of the low pH of the preparations.

NOTE: At a subsequent session DR. D. C. GARRATT (Nottingham) stated that he had again communicated with the sugar manufacturers and it appeared that one included ultramarine in refined cube sugar only, whereas others add it to other forms of sugar. The sulphur dioxide content also varies, one manufacturer adding 5 p.p.m. and others including greater amounts.