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RESEARCH DEPT. OF THE CHEMICAL & PHARMACEUTICAL LABORATORIES,
E. R. SQUIBB & SONS, BROOKLYN, N. Y.

DETECTION OF DEXTROSE AND SUCROSE IN LACTOSE.

BY JOSEPH ROSIN AND F. C. HITCHCOCK.

The U. S. P. test for absence of dextrose and sucrose in Lactose is based on the greater solubility of the first two sugars in 70% alcohol, than of the latter. The test is as follows:

Add 20 cc. of 70 per cent (by volume) alcohol to 2 Gm. of finely powdered lactose, shake the mixture frequently during half an hour at 15° C., and filter. A 10-cc. portion of the filtrate remains clear after mixing with an equal volume of dehydrated alcohol (dextrin), and this liquid upon evaporation on a water-bath leaves not more than 0.03 Gm. of residue (sucrose or glucose).

The 0.03 Gm. of residue permitted represents the quantity of lactose dissolved under the condition of the test. If dextrose or sucrose is present to any appreciable extent, the residue will be much greater.

Lactose was first introduced in the 1860 revision of the U. S. P. In the 1880 revision a charring test, with sulphuric acid, for sucrose was included. The alcohol solubility test for sucrose was first introduced in the 8th revision of the Pharmacopœia and has been continued in the subsequent revisions. The British, Netherlands, Swiss and some of the other Pharmacopœias also use, although with some modifications, the alcohol solubility in testing for sucrose. This test, up to a few years ago, worked very well. It is quite simple and, with one stroke, eliminates both sucrose and dextrose. Within recent years, however, objections have been voiced against this test. It is maintained that, owing to the application in the milk sugar industry, of modern manufacturing methods, such as spray drying, some amorphous lactose is produced. This form of lactose is more soluble in alcohol than the crystalline product and, consequently, yields more residue in the test. It has also been claimed, although it has not been proved yet, that some beta-lactose may be formed which has even a greater solubility in 70% alcohol than the amorphous variety. The amount of these varieties of lactose dissolved by 10 cc. of 70% alcohol are, approximately, of the following order:

Lactose, crystalline.....	0.03 Gm.
Lactose, dehydrated at 120° C.....	0.095 Gm.
Lactose, amorphous.....	0.105 Gm.
Beta-lactose, crystalline.....	0.125 Gm.

From this it is evident that the presence of even small quantities of the other forms of lactose would yield more residue in the test, and thus give an erroneous indication of the presence of dextrose or sucrose.

To meet this condition the producers of lactose recommended to the present Revision Committee of the Pharmacopœia that the test now official be replaced by tests specific for dextrose and sucrose.

Barfoed's Reagent forms a very delicate test for dextrose. Hinkel & Sherman (*J. Am. Chem. Soc.*, 29 (1907), 1744) have studied the use of this reagent for the detection of dextrose in the presence of other sugars. They found that, by using 5 cc. of the reagent, the presence of 0.0004 Gm. of dextrose can be shown, either alone or when mixed with di-saccharide, provided that the total weight of di-saccharide does not exceed 0.02 Gm., and the time of heating, at the boiling water temperature, be not greater than about 3 minutes. Based on this work, F. P. Nabenhauer has proposed for the consideration of the Revision Committee the following test for dextrose:

Two Gm. of the sugar (lactose) are treated with 20 cc. of 70% alcohol and shaken frequently for 30 minutes at room temperature, then filtered. Part of the filtrate may be tested for dextrin according to the U. S. P. X. Ten cc. are evaporated to dryness on a water-bath and redissolved in 5 cc. of water. This is mixed with 5 cc. of Barfoed's reagent (13.3 Gm. copper acetate dissolved in 200 cc. 1 per cent acetic acid) and heated in a test-tube in boiling water for three minutes. The presence of as little as 0.1% of glucose is indicated by a precipitate of cuprous oxide.

The sixth edition of the German Pharmacopœia makes use of the Seliwanoff reaction for the detection of sucrose in milk sugar. This test is based on the formation of a red or orange-red color by the action of resorcin on levulose in the presence of hydrochloric acid. Sucrose when treated with hydrochloric acid hydrolyzes into levulose and dextrose. Mr. Nabenhauer has accordingly suggested the German Pharmacopœia test for sucrose. The test is made as follows:

One Gm. of lactose is dissolved in 9 cc. of water. Add 0.1 Gm. resorcinol and 1 cc. of concentrated HCl. Boil for 5 minutes. A yellow color may form, but a red coloration indicates the presence of sucrose.

We investigated both of these tests. We found the method for dextrose to work very smoothly, and our experiments confirm the sensitiveness claimed for it. We found it, however, preferable to let the liquid stand for 20-25 minutes after the heating, before examining for cuprous oxide. When only a small quantity of cuprous oxide is formed it remains suspended for some time, and although it is recognizable by the color of the liquid the indication is not as certain as when it has subsided. Samples of pure lactose, U. S. P. lactose and of lactose containing 0.15% sucrose did not deposit any cuprous oxide when subjected to this test and allowed to stand for one hour.

The German Pharmacopœia's procedure for sucrose did not prove entirely satisfactory. The color produced after heating the mixture was not uniform. In experiments with mixtures of lactose and sucrose, the increase in the color with increased amounts of sucrose was so gradual that it was difficult to determine with certainty just where a positive test was unmistakable. Sometimes 0.5% sucrose seemed to be readily detected, while at other times it took one per cent or more to give a positive reaction. To be at all reasonably sure of the results it is necessary to run a control with pure lactose. This, however, would hardly be practical for the U. S. P.

It soon became apparent that the chief factor interfering with a sharp definition of the test is the lactose itself. Extraction of the sucrose from the lactose with alcohol, thereby eliminating the greater part of the lactose, naturally suggested itself. Two grams of the finely powdered lactose (100 mesh or finer) were

shaken frequently during one-half hour, at room temperature, with 20 cc. of 95% alcohol, filtered and 10 cc. of the filtrate evaporated on the steam-bath. The residue was dissolved in 9 cc. of water, transferred to a test-tube and the test completed as in the German Pharmacopœia. Lactose containing 1% sucrose gave a sharp positive reaction—a reddish yellow color. With 0.5%, however, the reaction was not decisive.

Further experiments have shown that 70% alcohol sufficiently excluded lactose so that no interference from it was encountered, and at the same time the sucrose was extracted in sufficient amount to make 5 to 4 mg. (0.5–0.4%) of sucrose readily detectable. Further, when lactose with one per cent of sucrose was extracted with 70% alcohol, using 10 cc. of the alcohol per Gm. of the sugar, and the residual lactose re-extracted etc., as before, no reaction for sucrose was obtained from the second extraction, showing that 70% alcohol dissolved all, or substantially all, of the sucrose in the first extraction.

Pure lactose, or lactose with added 1% of dextrose or galactose, extracted with 70% alcohol (2 Gm. + 20 cc.) and then treated as described before gave, at most, only a slight yellow color.

The threshold of the sensitiveness of the Seliwanoff test for sucrose in lactose by the method described we found to be at about 2.5 mg.; also that 5 cc. of 70% alcohol per Gm. of lactose will extract, from lactose containing 0.5% sucrose, sufficient of the latter to give as sharp a reaction as when using 10 cc. alcohol per Gm. It follows that by using larger quantities of lactose the test can be made to detect much smaller *percentages* of sucrose.

Samples of pure lactose, and of the lactose admixed with dextrose, sucrose or dextrin, were then submitted to a chemist in another of our laboratories with the directions for testing. The composition of these samples was, of course, not revealed to him.

DIRECTIONS.

Add 50 cc. of 70% alcohol (by volume) to 10 Gm. of the finely powdered lactose. Shake the mixture frequently during half an hour, filter through a small filter (about 9 cm.).

1. Evaporate to dryness 10 cc. of the alcoholic filtrate, dissolve the residue in 5 cc. water, add 5 cc. Barfoed's reagent (13.3 Gm. crystallized copper acetate, 5 cc. 36% acetic acid, water to make 200 cc.), heat in a thin-walled test-tube in boiling water or actively flowing steam exactly three minutes and let stand at room temperature ten minutes. No deposit of red cuprous oxide should be observed (*dextrose*).

2. Evaporate to dryness 25 cc. of the filtrate, dissolve the residue in 9 cc. of water, add 1 cc. 25% HCl and 0.1 Gm. resorcin. Transfer to a thin-walled test-tube and heat for five minutes by immersing the tube in boiling water or actively flowing steam. The liquid should remain colorless or nearly so. A distinct yellow to orange color indicates *sucrose*. He reported:

TABLE I.

	Dextrose.	Sucrose.	Dextrin.	Remarks.
Sample No. 1 (pure lactose + 0.066% sucrose)	No precipitate	Distinctly yellow	Does not show	The residue, after evaporation of the alcohol, from numbers 1, 3 and 5, gave a cloudy solution with water which interfered somewhat with the test for dextrose
Sample No. 2 (pure lactose + 0.5% dextrin)	No precipitate	Not detectable	
Sample No. 3 (pure lactose + 0.04% dextrose)	Barely visible slight red precipitate obscured by whitish flocks	Not detectable	
Sample No. 4 (U. S. P. grade of lactose)	Not detectable	Not detectable	

Sample No. 5 (pure lactose + 0.05% dextrose + 0.05% sucrose)	Barely visible red precipitate obscured by whitish flocks	Doubtful
Sample No. 6 (U. S. P. grade of lactose)	No precipitate	Not detectable
Sample No. 7 (pure lactose + 0.025% dextrose)	No precipitate	Not detectable

Further experiments have shown that heating for a few minutes longer, 8 minutes instead of 5, brought out the color given by sucrose more sharply. In control tests made with sucrose-free lactose the longer heating did not appreciably increase the color of the solution. This makes it also possible to detect with this test smaller quantities of sucrose.

Another set of "unknowns" was submitted to the same chemist to be tested for dextrose and sucrose with the following directions:

Shake frequently, during one-half hour, 10 Gm. of the sample with 50 cc. of 70% volume alcohol and filter.

Dextrose.—Apply the same test as before to 5 cc. and to 10 cc. of the filtrate and observe for precipitation of cuprous oxide after 10 and after 20 minutes standing.

Sucrose.—Apply the resorcin test as before to 10 cc. and to 15 cc. of the filtrate, and heat for 8 minutes instead of 5 minutes.

TABLE II.

Sample.	Dextrose.				Sucrose.	
	5 cc. after 10 min.	5 cc. after 25 min.	10 cc. after 10 min.	10 cc. after 20 min.	10 cc.	15 cc.
"D"						
U. S. P. grade of lactose	No precip.	No precip.	No precip.	No precip.	Almost colorless	Very slightly yellow
"E"						
Same lactose as "D" + 0.1 per cent sucrose	No precip.	No precip.	No precip.	No precip.	Slight yellow	Distinctly yellow
"F"						
Same lactose as "D" + 0.1 per cent dextrose	Very slight precip. of Cu ₂ O	Distinct precip. of Cu ₂ O	Distinct precip. of Cu ₂ O	Distinct precip. of Cu ₂ O	Very slightly yellow	Very slightly yellow

These results practically confirm the threshold limits we found for these tests and those indicated by Table I—about 0.7 mg. and possibly a little less, for dextrose, and about 2 mg. for sucrose. For the purpose of the U. S. P. tests capable of detecting about 0.1% of dextrose and about 0.15% of sucrose should certainly suffice.

The following tests for dextrose and sucrose are accordingly recommended:

Add 25 cc. of 70% alcohol (by volume) to 5 Gm. of lactose powdered to 100 mesh or finer. Shake the mixture frequently during 30 minutes and filter through a dry filter of 7 to 9 cm. diameter. Evaporate 5 cc. of the filtrate on the steam-bath and dissolve the residue in 5 cc. of water. Transfer the solution into a test-tube, filtering if necessary, add 5 cc. of Barfoed's reagent,¹ immerse the test-tube in boiling water or in actively flowing steam for 3 minutes and allow to stand at room temperature for 25 minutes. No red precipitate is produced (dextrose).

¹ Barfoed's reagent is made by dissolving 13.3 Gm. cupric acetate in a mixture of 195 cc. of water and 5 cc. of acetic acid to make 200 cc.

Evaporate another portion of 10 cc. of the alcoholic filtrate on the steam-bath, dissolve the residue in 9 cc. of water, add 1 cc. of 25% hydrochloric acid (made by mixing 22 cc. of hydrochloric acid with 7 cc. of water) and 0.1 Gm. of resorcin. Transfer the mixture to a thin-walled test-tube of 16 to 19 mm. internal diameter and immerse the test-tube in boiling water or, preferably, in actively flowing steam, for 8 minutes. At the end of this time the liquid is colorless or not more than slightly yellow. A distinct yellow or reddish yellow color indicates sucrose.

Dextrin.—It is noted from the results in Table I, that the U. S. P. test failed to reveal 0.5% dextrin. At first we were surprised at this, but upon second thought we realized that, to the contrary, it would be surprising if it did work. Pure dextrin is quite insoluble in 95% alcohol, even in hot, and it could hardly be expected that it would dissolve under the condition of the test to any appreciable extent in 70% alcohol. In fact when one Gm. of the same dextrin that was used for mixing with lactose was shaken with 20 cc. of 70% alcohol, filtered, the filtrate evaporated, the residue when dissolved in water gave only a reddish yellow color with a drop of iodine T.S., showing that only a trace of dextrin dissolved in the alcohol.

Since iodine gives characteristic colors with aqueous solutions of dextrin, we applied it for the detection of dextrin in lactose and found it eminently satisfactory. One gram of the lactose was dissolved in 10 cc. of water, the solution heated for one minute, cooled and a drop of iodine T.S. added. Several U. S. P. grade samples examined in this manner gave no color other than the yellow due to iodine, while lactose mixed with 0.1% dextrin produced a decided violet to blue color.

Smaller proportions of dextrin, 0.5 to 0.3 mg., are also readily detectable by this method, provided the iodine solution is more dilute, otherwise the yellow color of the unconsumed iodine obscures the brilliancy of color given by the dextrin. One drop of an iodine solution, made by diluting 1 volume of iodine T.S. with 4 volumes of water, gives a distinct violet to blue color with 0.5 mg. dextrin in the presence of one Gm. of lactose. Starch will, of course, also give a blue color with the diluted iodine. The U. S. P. test for starch in lactose serves, therefore, for dextrin as well and to embody the foregoing observation of the obscurity of the dextrin-iodide color with small quantities of dextrin, it is recommended that the U. S. P. test for starch be rewritten as follows:

Dissolve one Gm. of lactose in 10 cc. of water, boil for one minute, cool to room temperature and add one drop of iodine solution made by diluting one volume of iodine T.S. with 4 volumes of water. No red, violet or blue is produced (dextrin, starch).

We are indebted to Dr. W. Schnellbach for valuable assistance in this work.

MERCK & Co., INC.,
RAHWAY, NEW JERSEY.

THE RESISTANCE OF MINERAL OILS TO DECOMPOSITION BY SUNLIGHT.*

BY L. W. GREEN AND R. E. SCHOETZOW.

Observations of the behavior of many samples of mineral oils, over a number of years, have given evidence that a variation exists in different samples in regard to susceptibility to spoiling, when bottled oil is exposed to sunlight, and lead to the belief that the geographical source of the oil has influenced its stability.

* Scientific Section, A. P. H. A., Toronto meeting, 1932.