

Anthrone in Estimating Low Concentrations of Sucrose

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ALTHOUGH A NUMBER of methods have been developed for the estimation of low sucrose concentrations, the one which is universally used in sugar processing factories is the Molisch α -naphthol test. This is customarily used when the range of sugar concentration is 10-250 p.p.m., where it cannot be measured accurately by polarimetric means. Inasmuch as the α -naphthol test is a ring test, it cannot be adapted readily to transmittancy measurements and the sucrose content of a solution is usually reported after visual estimation to be a trace, light or heavy. Hand-colored cards have been used as standards, but they are far from being completely satisfactory.

The use of anthrone dissolved in concentrated sulfuric acid was suggested by Dreywood as the reagent in a qualitative test for carbohydrates (1).² The extreme sensitivity of this test plus the fact that it is not a ring test made it appear to offer a promising improvement over the α -naphthol test in the measurement of low concentrations of sucrose.

The Chemical Anthrone

Anthrone is not easily available, at least in this country. The chemical used in this work was synthesized from anthraquinone, using the method in Organic Syntheses (2). Anthraquinone is reduced with tin and hydrochloric acid in a medium of glacial acetic acid, followed by recrystallization from benzene and petroleum ether. It can also be synthesized from *O*-benzoylbenzoic acid.

Whatever method of synthesis is used, it is important to use sintered glass filters to avoid the introduction of bits of filter paper which would cause a positive blank test.

Anthrone is the keto form of a tautomeric pair of which the enol form is anthranol. The equilibrium between the tautomers is represented by the equation in figure 1. The two substances have quite different properties. Thus anthrone is pale yellow in color, is insoluble in cold alkali and is non-fluorescent in dilute solution under ultra-violet light. It is comparatively unreactive, but will form addition compounds with Grignard reagents. Anthranol, on the other hand, is dark golden-yellow in color, is soluble in cold alkali, is highly reactive and fluoresces strongly in dilute solution under ultra-violet light. Anthranol is easily oxidized by bromine and, in fact, the proportion of anthranol in a mixture of the two tautomers can be determined by titrating with bromine in alcoholic solution under ultra-violet light until the fluorescence disappears (3).

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²The numbers in parentheses refer to literature cited.

In various solvents the proportion of anthrone to anthranol differs markedly. Thus in pyridine, tautomerization proceeds nearly completely to the enol form, while in glacial acetic acid, anthrone contains only 1.3 percent of the enol form at equilibrium (3).

Anthranol may be prepared from anthrone by dissolving the latter in alcoholic KOH, precipitating the anthranol with cold, dilute H_2SO_4 , filtering and washing.

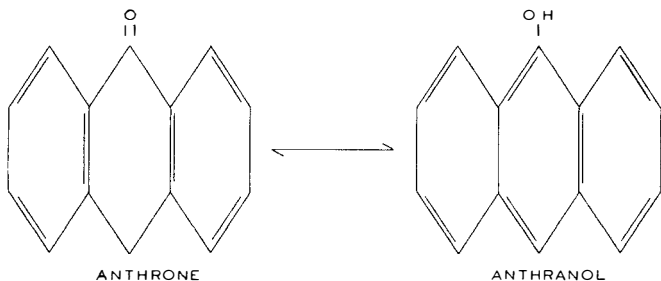


Figure 1.- Anthrone-anthranol tautomerization.

Inasmuch as the anthrone is used in concentrated sulfuric acid solution, it is of interest to know what tautomeric form exists in this solution. No information on this point was found in the literature, but for the following reasons it is believed that the keto form is the predominant substance:

1. 0.05 percent solutions of anthrone in concentrated sulfuric acid do not fluoresce under ultra-violet light. If anthranol solutions of the same concentration are prepared, they also do not fluoresce.

2. Both anthrone and anthranol solutions give practically indistinguishable color tests with dilute sugar solutions. This might indicate that anthranol tautomerizes immediately to anthrone in concentrated sulfuric acid, although it could indicate just the opposite change.

3. Freshly prepared solutions of anthrone in concentrated sulfuric acid give a negative test for enols with aqueous ferric chloride solution. However, as the anthrone solution stands, the bright canary-yellow color darkens to a pale orange and furthermore, a positive test for an enol is obtained. If the anthrone solution is stored at 0° C. in a refrigerator, very little color change is noted and a negative ferric chloride test is observed. This may be interpreted to mean that anthrone slowly tautomerizes to a small extent in sulfuric acid solution to form anthranol, a reaction which is slowed by reduced temperature.

Reagent Strength and Keeping Property

Dreywood (1) recommended that the strength of the reagent be 0.2 percent. Preliminary experiments showed that the reagent strength could be reduced to 0.05 percent and still give tests of sufficient sensitivity to permit distinguishing between solutions containing 10 and 25 p.p.m. sucrose. The more dilute reagent offers an advantage because its bright yellow color is reduced. By using tubes of greater diameter, the strength of the reagent may be still further reduced. This offers no particular gain because an increased volume of reagent is required and furthermore, the larger test tubes are inconvenient to handle in making many routine measurements.

As previously stated, the anthrone used in this work was prepared according to the method in Organic Syntheses (2). Solutions of this material in concentrated sulfuric acid turned a brown-green color overnight. Since the presence of an impurity was suspected, the anthrone was recrystallized from glacial acetic acid to yield material which slowly changed to an orange-green color on long standing. A second recrystallization from the same solvent was of benefit, but not a third. Possible one recrystallization would be sufficient if an excess of solvent were used and the anthrone slowly crystallized and well washed with fresh solvent.

In order to test the keeping property of the anthrone reagent, various samples of anthrone in concentrated sulfuric acid were sealed in 18 mm. (outside diameter) Pyrex tubes and stored at room temperature. One sample was stored at 0 to 1° C. The transmittancies of the tubes relative to distilled water were measured at intervals. In figure 2 they are plotted relative to the initial transmittancy. These measurements were made in a Photovolt Lumetron colorimeter using unfiltered light.

The apparent anomaly that material which was recrystallized three times developed color faster than material which was recrystallized twice is believed to be due to the fact that the room temperature was higher during the test period on the former material.

Even solutions prepared from the purified anthrone and reagent grade sulfuric acid slowly darkened to an orange color on standing, due perhaps to anthranol formation. Other decomposition products are undoubtedly formed also. For that reason, it is suggested that the 0.05 percent reagent be freshly prepared every 3 or 4 days.

Procedure

Because of the fact that temperature has a pronounced effect on the color forming reaction, it is important that the test be performed in a standardized manner. If the reagent is added to the sample slowly and the test tube is kept cool with cold water, a practically colorless solution can be obtained even though 2000 p.p.m. of sugar is present. Furthermore, if the tube is heated externally to boiling, the typical blue-green color is changed to a reddish-brown. By using the same volumes of reagent and sample solution, but varying the size of the test tube or other container

so as to vary its heat capacity, the upper temperature limit after mixing is altered and consequently the color. Precisely duplicable results in a test of this nature can only be secured by using a rigidly standardized procedure, adiabatically performed. The following procedure, however, will yield results which are quite consistent and suitable for the control laboratory:

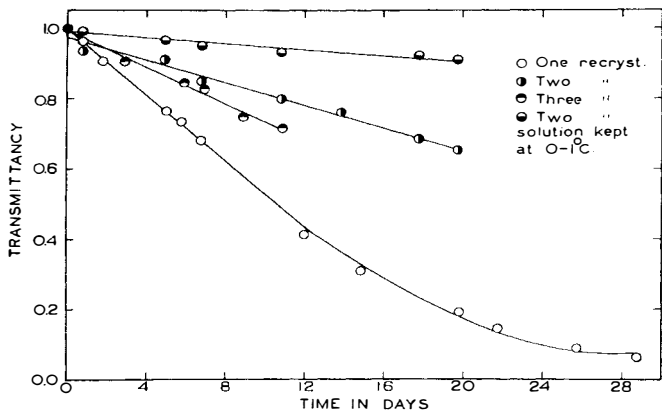


Figure 2. Effect of recrystallization of anthrone on color development in 0.05 percent solutions.

2.0 ml. of the solution to be tested are measured into a clean, dry 15x125 mm. Pyrex test tube and 3.0 ml. of 0.05 percent anthrone dissolved in concentrated sulfuric acid (reagent grade) are added so as to form a bottom layer. The tube is then shaken to effect complete mixing. The transmission of the solution for white light is next measured in a photoelectric colorimeter. Distilled water is used as the standard, with its transmission taken to be 1.000. By reference to a previously prepared calibration curve or table, the concentration of sucrose in the original sample is ascertained. Table 1 and figure 3 present calibration data obtained with a Lumetron photoelectric colorimeter, Model 402E, manufactured by the Photovolt Corporation, New York, N. Y.

Table 1.- Light transmission of sucrose solution-anthrone mixtures.

Distilled water		1.000 ¹	1.000 ²
Anthrone reagent + distilled water		0.791	0.783
Anthrone reagent + 10 p.p.m. sucrose solution		0.659	0.658
Anthrone reagent + 25 p.p.m. sucrose solution		0.493	0.498
Anthrone reagent + 50 p.p.m. sucrose solution		0.317	0.323
Anthrone reagent + 125 p.p.m. sucrose solution		0.115	0.109
Anthrone reagent + 250 p.p.m. sucrose solution		0.024	0.024

¹Anthrone reagent prepared 4 hours previously.

²Anthrone reagent prepared 27 hours previously.

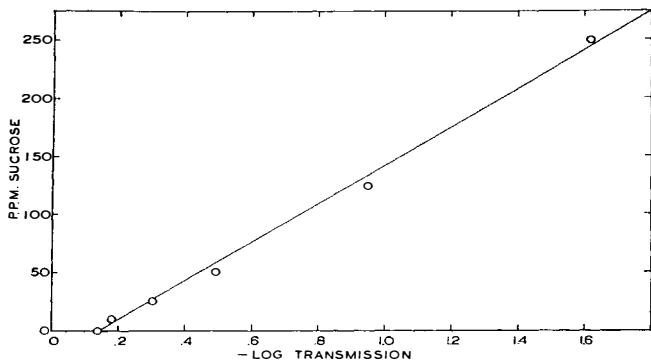


Figure 3.—Light transmission of sucrose solution—anthrone mixtures.

Rate of Color Development

Determinations were made of the time required to reach the maximum color density after mixing the reagent and solutions containing various concentrations of sucrose. In these determinations, the sucrose solution was contained in one test tube and the reagent in another. At zero time the solutions were mixed by pouring the reagent into the sucrose solution and then pouring the mixture back into the first tube. Transmittancy readings were taken at frequent intervals until the reactions were substantially complete. In figure 4 the optical density (-log transmittancy) is plotted against time after mixing. The transmittancies are the values relative to the transmittancy of the blank.

Table 2 shows the time required to reach 90 and 95 percent of the final density. A delay of 2 minutes before measuring the color will insure that 95 percent of the color has been developed, while about 90 percent is developed in 1 minute.

Method of Color Measurement

The photoelectric colorimeter provides the most accurate and convenient method of color measurement. Various types of instruments can be used, depending on the degree of precision required. For the average factory laboratory, a single photocell colorimeter in which the transmittancy is indicated by the deflection of a microammeter is probably suitable. This may be calibrated using the particular test tubes (usually 15-18 mm.) which fit the holder. The data presented in table 1 were taken from two runs

made on successive days with sucrose solutions which were freshly prepared just before the tests were conducted. It is difficult to evaluate the precision of the method with only two runs, but the difference between these runs amounts to about 2 percent, based on the sucrose content.

If it is desired to use a visual comparison method, Lovibond standard color plates may be used in a suitable constructed comparator. It is possible to make close matches between the plates and the coloring matter produced in the anthrone test. This method offers an advantage if the samples are turbid because one can disregard the turbidity to a large extent in matching colors.

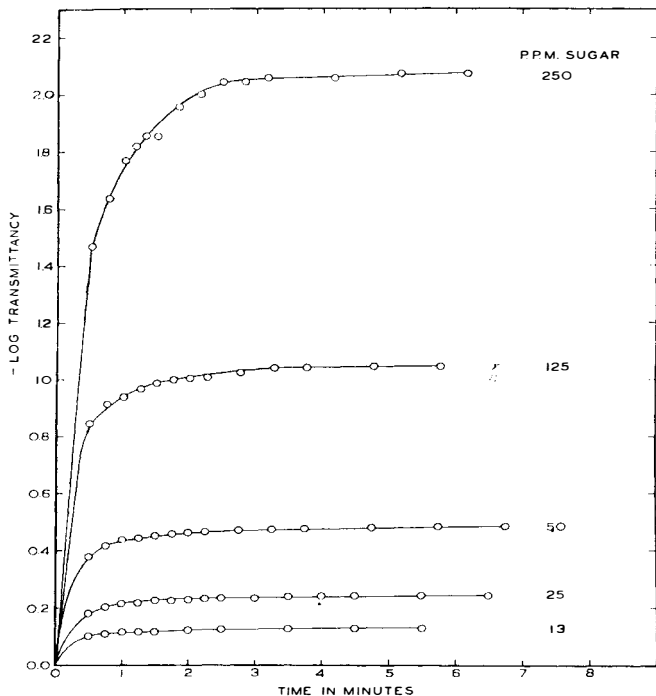


Figure 4.—Rate of color development in solutions of anthrone reagent and sugar.

Table 2.—Rate of color development in anthrone test.

P.P.M. Sucrose	Maximum Density	Time to reach	
		0.95 maximum density (minimum)	0.90 maximum density (minimum)
250	2.07	1.8	1.4
125	1.05	1.5	1.0
50	0.49	2.1	1.1
25	0.24	1.8	1.1
12.5	0.13	1.8	1.1

Primary standards made up from the anthrone reagent and sucrose solutions of known concentration and sealed in Pyrex tubes were not found to be satisfactory because the blue color gradually fades and the solution becomes slightly cloudy. At the higher sugar concentrations, a slight amount of dark-colored material coats the inside of the tubes.

Other color standards which might be used include color transparency film such as Ekatchrome, hand-colored cards or Carbro prints. These have not been found to be satisfactory, inasmuch as the colors in the film are not permanent and the latter two means make use of reflected rather than transmitted light.

Effect of Impurities

The impurities commonly present in the condensed water samples of a beet sugar factory do not interfere seriously in this test. Solutions of diffusion juice, thick juice, molasses and Steffen filtrate were prepared, each containing 200 p.p.m. of sucrose. The blue-green colors formed in the first three solutions were closely the same, but an olive-green color was obtained with the Steffen filtrate sample. This is not surprising when the low sugar to solids ratio of this material is considered. A special calibration would be required for the accurate measurement of the sucrose content of condensed waters containing Steffen filtrate.

Comparison With Alpha-Naphthol Test

The main advantage of anthrone is that it gives a more accurate and more sensitive test than α -naphthol. The coloring matter is distributed throughout the whole body of the solution and is not concentrated in a shallow ring. Furthermore, the extent of agitation of the tube has no effect on the test as it does with α -naphthol. Solutions containing 10 and 25 p.p.m. can be readily distinguished from each other. It is very difficult with the α -naphthol test to make this distinction.

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