

Measurement of Turbidity with a Spectrophotometer^{1,2}

With Especial Reference to Sugarhouse Products

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FROM the early days of the sugar industry it has been known that suspended solids in sugar juices and sirups have detrimental effect upon the quality of the sugar produced therefrom. In raw sugar manufacture it has a bearing on the cleanliness and filterability of the sugars, and in the manufacture of direct consumption and refined sugars suspended material may be responsible for the production of off-colored or dull-appearing sugars.

It is therefore frequently desirable to introduce a turbidity control of various clarification processes, but owing to the lack of adequate methods or standards, such a control has not been widely adopted. The principal objection to the methods recommended for use in sugar technology (4, 5, 8) is that usually no provision is made, or it is inadequately made, to compensate for the color of the product, which may introduce considerable error. Some of the methods for determining turbidity require, too, the use of turbidity standards which are frequently unstable and cannot, therefore, be highly recommended. The ideal method is one which will permit the determination of turbidity in either colored or colorless solutions with equal accuracy, and in which the results may be expressed in numerical values with or, preferably, without reference to synthetic turbidity standards. The method which is to be described is believed to approach this ideal more closely than any yet proposed.

Theory and Brief Description of Methods Used for Determining Turbidity

When a turbid solution is illuminated by a beam of light, a portion of the incident light is reflected, a portion is absorbed by the suspended particles and by the solution, particularly if colored, and a portion of the light is transmitted through the solution. One group of methods measures the intensity of the scattered light or the Tyndall beam in relation to the incident or standard light. Another group determines the masking effect or absorption of light by the particles (and color), the so-called extinction methods, or, inversely, the intensity or the percentage of the incident light that is transmitted. There are so many factors such as the number, size, shape, and other physical characteristics, of the particles which affect the intensity of either the Tyndall or the transmitted light, that the mathematical expressions which have been developed (11) relating some of these

As it is frequently desirable to measure the turbidity of sugar liquors during the course of sugar manufacture or refining, a number of methods have previously been proposed. Adoption of these methods has not, however, been extensive because, it is believed, too large errors are introduced owing to lack of color compensation. In the method described, transmission of light at a definite wave length is taken as a measure of turbidity, and color is compensated by using as a standard a portion of the same sugar solution from which suspended solids are removed. For the preparation of this standard, filtration through paper with the aid of a slow-filtering grade of commercial kieselguhr is recommended in place of asbestos or sand. Turbidities are expressed in $-\log t$ values as being the most convenient method, in spite of the fact that $-\log T$ values are not strictly proportional to the concentration or the depth of the suspension.

factors cannot be applied except under rather ideal conditions. In nephelometric analysis (12), for instance, it is possible to control conditions sufficiently to obtain a considerable degree of precision, but such is not the case when attempting to determine the quantity of suspended material in a sugar liquor from a measure of its turbidity. The most disturbing factors are the varying composition and the varying size of particles, which covers the entire range from coarse to molecular dispersion.

This is, perhaps, not as serious as it appears since in practice the comparative turbidities and not the actual quantity of material present is the chief concern. It does not matter greatly whether the particles are relatively large or small, heavy or light in density, except to suggest means for their removal. It is desirable, however, to have a method which gives comparable values—that is, one which gives with sufficient accuracy the relative turbidities of sirups or juices of the same general type.

As already indicated, one of the principal difficulties with existing methods is the lack of color compensation. Since so many shades and intensities of color are encountered, it is not very practicable to attempt the preparation and use of synthetically colored solutions or glass screens. In the method which the writer proposes, color compensation is accomplished by using a portion of the sugar solution to be tested from which material responsible for visible turbidity has been removed. This immediately brings up the question whether it is possible to remove suspended material without altering the color and, if so, the best method by which this may be accomplished.

In accurate colorimetric work, particularly in spectrophotometric analysis, it is required that all material causing turbidity be removed from the solution before making the color determination. For this purpose, various methods have been proposed. Peters and Phelps (?) recommend filtration through specially prepared asbestos, whereas Lunden (6) claims that ultracentrifuging or filtration through sand are the only proper methods, and Spengler and Landt (10) that special filters of other types are required. Honig and Bogstra (3) also recommend sand filtration but apparently adopt filtration through kieselguhr in spite of the fact that various investigators claim that this agent has a selective adsorption action on color.

It is very doubtful whether it is actually possible to remove all the material causing turbidity, if judged by the Tyndall beam, without changing the color of the solution. It is well known that small particles within the range of colloid dimensions (5 to 200 $m\mu$) and even those which exceed this

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size to some extent exhibit color phenomena by both reflected and transmitted light. Thus, if the clarification process is sufficiently efficient to remove suspended particles within this range of size, the apparent color of the solution would, without question, be changed to some extent. This fact accounts, it is believed, for the diversity of opinions as to the best method for clarifying sugar solutions for colorimetric analysis and the belief of some investigators that kieselguhr selectively adsorbs color. It is the author's opinion that this agent removes only by mechanical means, and not by true adsorption, particles within the range of size which affects color to a slight extent. These particles are not completely removed by either asbestos or sand under the procedures generally recommended for preparing sugar solutions for spectrophotometric analysis.

The results of some preliminary experiments conducted by the author substantiate this view. For example, a raw sugar solution, clarified with kieselguhr, had the same $-\log t$ value regardless of whether 1, 2, or 3 per cent of filter aid (based on sugar solids) was used or whether the solution was filtered immediately or after it had stood for some time. From our knowledge of adsorption, considered in its broader sense, one would expect differences in the color of the solution treated in these different ways unless it were assumed that maximum adsorption occurred with the smallest quantity filter aid used and in the minimum time period, which are very doubtful assumptions to make. Kieselguhr unquestionably removes particles of smaller size than is possible by filtration through asbestos or sand. However, the minimum size of particles which are removed by these various agents has never been determined. The experiments conducted by the writer indicate that true adsorption of color is lacking or extremely slight at the most, so that it is permissible to use kieselguhr for the preparation of sugar solutions for either colorimetric analysis or as a standard in compensating for color in turbidity measurements.

Description of Spectrophotometric Method for Measuring Turbidity

The method proposed for measuring turbidity is based upon the principle of measuring the transmission of light through the turbid solution, using as a standard to compensate for color a portion of the same sugar solution from which material causing turbidity has been removed. The principles involved and the apparatus used are the same as for spectrophotometric color analysis (7, 9).

Lambert and Beer's law, which is used so extensively in spectrophotometric analysis, correlating transmission of light and concentration of color and thickness of solution, unfortunately does not hold exactly for turbid solutions. Until it is possible to develop more adequate relationships it is proposed to assume the validity of Lambert and Beer's law and to express turbidities as $-\log t$ values in accordance with the following equation:

$$-\log t = \frac{1}{cb} (-\log T)$$

where t = transmittancy reduced to unit conditions as regards concentration and thickness of solution, c = concentration of the solution expressed as grams of dry substance per 1 cc.; b = thickness of solution expressed in cm.; and T = the transmitted fraction of the incident light.

In order to determine how closely this relationship might be expected to hold in the case of suspensions, a series of bentonite suspensions were prepared whose concentrations were made to range from 0.25 to 2.00 grams per 100 cc. (coarse particles included) by aliquoting from a 5 per cent suspension. The $-\log t$ values of these suspensions were then determined at 560 $m\mu$ in cells of different length with

a Keuffel and Esser color analyzer. Between the high and the low concentrations, a maximum variation of 15 per cent in the $-\log t$ value was observed, whereas with different length tubes the maximum variation in $-\log t$ values was about 10 per cent. These variations are probably somewhat exaggerated by the inability to obtain a correct aliquot of the stock suspension owing to the rapid settling of the larger particles. In spite of these divergences from Lambert and Beer's law, it is considered advisable to express turbidities as $-\log t$ values, as this affords a fairly close index of relative turbidities with sugar liquors of most types and undoubtedly better than by any other method so far proposed.

In order to ascertain whether the method of color compensation is satisfactory and whether turbidities can be measured in colored solutions as accurately as in colorless solutions, two series of bentonite suspensions in sugar sirups were prepared. One sirup was colorless and the other was colored strongly with caramel to simulate a sirup prepared from a dark raw sugar. The concentration of bentonite in each series ranged from 0.25 to 2.00 grams per 100 cc. of final solution. The transmission of light was measured at 560 $m\mu$ through a 1-cm. cell, again with a Keuffel and Esser color analyzer. The standards for the two solutions were, of course, a portion of the colorless sirup for the one series of determinations, and a portion of the colored sirup for the other series. The data given in Table I show conclusively, it is believed, that when color is properly compensated, the transmission of light gives a very satisfactory measure of turbidity regardless of the color of the solution.

Table I—Transmission of Light through Bentonite Suspensions
(At 560 $m\mu$ in 1-cm. cell)

BENTONITE CONCENTRATION	TRANSMISSION THROUGH BENTONITE	
	Colorless sirup	Colored sirup ^a
Grams/100 cc.	%	%
0.00	100.0	100.0
0.25	84.3	84.5
0.50	70.5	70.3
0.75	58.3	58.4
1.00	48.7	49.1
1.50	33.1	33.6
2.00	22.9	22.6

^a Colored with caramel.

Preparation of Samples for Turbidity Determination

It is well known that dilution of many impure sugar solutions causes an increase in turbidity; hence it is important that the procedure for preparing the solutions for turbidity measurements be carefully standardized. Juices and factory sirups can, as a rule, be filtered with kieselguhr without dilution, a procedure which is recommended by all means. Difficulties exist, however, when dealing with solid and semi-solid products, and undoubtedly differences of opinion will arise as to the selection of the density to which dilutions should be made. Since it is necessary to filter a portion of the sugar solution, it will probably not be practicable to exceed 60 per cent solids to any material extent except for a very few high-purity products; but for comparative purposes it is believed to be permissible to prepare a more dilute solution such as one of a 50 per cent solids concentration. Such details will have to be worked out by the person making the tests, keeping in mind, however, that whatever concentration is decided upon, all similar products must be diluted to the same concentration if comparative values are to be obtained. This is a rather unsatisfactory procedure to recommend, but it cannot very well be avoided.

Clarification of that portion of the sample to be used for color compensation is accomplished by filtration with the aid of kieselguhr. Since it is desired to remove as much of the suspended matter causing turbidity as is possible, the