

THE RELIABILITY OF THE SORENSEN-ORCINOL METHOD
FOR ESTIMATING SUGARS

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1937

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
PROCEDURE	2
REAGENTS	4
CHOICE OF WAVE-LENGTH	5
RESULTS	7
SUMMARY	10
ACKNOWLEDGMENT	12
REFERENCES	13

INTRODUCTION

A method adapted to the determination of individual sugars in mixtures is much needed. Many methods have been devised using as their basis the various chemical and physical properties of the sugars. Browne (1) described methods involving the difference in rotatory power of different sugars. The rotations are usually small and in some cases uncertain so that this method of identification upon the whole is not satisfactory where more than one sugar is likely to be present or where their concentrations are low. Kline and Acree (2) describe a method for titrating aldose sugars with standard iodine and alkali in the presence of ketoses. While this method gives a quantitative value for the aldose sugars present it is not generally applicable for the determination of individual sugars.

The reducing power of the sugars is the basis of a method developed by Whitmoyer (3). The method depends upon the reduction of ferricyanide by sugars and the subsequent oxidation of the potassium ferrocyanide to potassium ferricyanide by ceric sulfate. The usefulness of this method, as well as other methods based on the reducing power of the sugars, is limited due to the interference of impurities

which are likely to be present in many test solutions.

The color reactions of the sugars with various reagents have been studied by Dehn and associates (4) as a means of identification of individual sugars. Single tests are given which are specific for a few of the sugars but do not include many of the more common ones. These reactions are limited in practice since the same color may be produced by any one of several different sugars.

PROCEDURE

The Sorensen-orceinol (5) method was chosen as a possible means of determining the sugars present in hydrolyzed casein. The method was developed from two older methods for the colorimetric determination of sugars published by Dische and Popper (6), and by Tillmans and Philippi (7). It is based on the difference in the rate at which color is developed by different sugars when heated with orceinol and sulfuric acid.

One cc. of the sugar solution to be tested was mixed with 2 cc. of 2 per cent orceinol solution and 15 cc. sulfuric acid. The solution was heated in a constant temperature water bath at 80°C. for a predetermined length of time. The solution was then removed and cooled in crushed

ice and the extinction coefficient read in a spectrophotometer immediately after cooling, using a solution depth of 1 cm.

The readings were made against distilled water or against blanks consisting of 1 cc. distilled water, 2 cc. orcinol solution, and 15 cc. sulfuric acid. The blank was heated and chilled exactly as the test solution with which it was to be used. In order to simplify the procedure, water was used in most cases as the standard, since it was found that solutions read against it gave curves of the same general shape as those read against an orcinol blank.

The extinction coefficients were plotted against the time of heating for samples handled in this manner which had been heated for five, ten, fifteen, twenty, twenty-five, and thirty minutes. Samples of known sugars having known concentrations were also run in the same way and their curves also plotted. If the curves were the same the sugars present in the unknown were said to be the same as the sugars with which it was compared.

In order to determine the reliability of this method pure solutions of known concentrations were tested. In order to select the concentration best adapted to the method, solutions of varying concentrations were tried. Since Sorensen gave data for .01 to .05 per cent solutions

it was necessary to keep the concentrations within those limits so as to have the results comparable.

It was found that the general shape of the curves did not change when using concentrations varying between .01 and .05 per cent. The .03 per cent solutions were found to give readings sufficiently high to be easily and accurately read, and this concentration was chosen for these comparative tests.

REAGENTS

To insure that differences between the results in this laboratory and those reported by Sorensen were not due to accidental differences in materials, the following variations were introduced: acid concentration, sample of orcinol, sample of sulfuric acid, and sample of sugar.

Samples of sulfuric acid having concentrations of 50, 65, 70 and 74 per cent were used with .03 per cent lactose solutions. There was an increase in the optical density when the concentration was increased from 65 to 74 per cent. However, the 50 per cent acid failed to produce any red coloration. The optical density produced by the different concentrations of acid are shown in table 1.

Table 1. Optical densities developed by lactose, orcinol and sulfuric acid.

Heating time	Optical density		
	65%	70%	74%
5	.11	.25	.33
10	.25	.40	.53
15	.27	.41	.56
20	.25	.39	.53
25	.22	.35	.50
30	.25	.34	.50

The general shapes of the curves produced were the same for the three concentrations of acid compared. The increase in density with an increase in sulfuric acid concentration between 65 and 74 per cent was in agreement with the findings of Sorensen. No variation was caused by any of the other changes.

CHOICE OF WAVE-LENGTH

In order to choose the wave-length at which to read the optical density of the solutions, Sorensen plotted the absorption curves of test solutions which had been heated five, fifteen, and twenty-five minutes. Eight filters which she designated as S 75, S 72, S 61, S 57, S 53, S 50, S 47, and S 43 were used. The five and fifteen minute curves showed maxima at S 53, but the twenty-five minute

curve did not give a sharp maximum. From these findings the S 53 filter was selected as the proper one to use in making readings for the purpose of plotting time--optical density curves.

In this laboratory the absorption curves were plotted for test solutions of sugars which had been heated for five, ten, fifteen, twenty, twenty-five, and thirty minutes. The readings were made in a spectrophotometer at wave-lengths from 450 m μ to 650 m μ at ten m μ intervals against a blank of distilled water. The curves for lactose, glucose, and galactose plotted in this manner all showed maxima at 520 m μ for solutions heated five, ten, fifteen, and twenty minutes. The twenty-five and thirty minute curves did not show definite maxima. All of the curves rose sharply toward the shorter wave-lengths between 490 m μ and 450 m μ . Table 2 gives a comparison of the optical density of orcinol-sugar solutions read at 520 m μ and 530 m μ .

Table 2. Comparison of optical density of oreinol-sugar solutions read at 520 m μ and 530 m μ .

Heating time	Lactose		Glucose		Galactose	
	520 m μ	530 m μ	520 m μ	530 m μ	520 m μ	530 m μ
5	.20	.20	.12	.12	.40	.39
10	.39	.35	.27	.24	.49	.45
15	.35	.31	.36	.33	.42	.39
20	.33	.29	.38	.34	.36	.34
25	.30	.27	.40	.35	.34	.30
30	.30	.28	.45	.40	.30	.26

As shown by the data recorded in table 2, a comparison of the curves showing time against density read at 520 m μ , with those read at 530 m μ , shows no difference in the general shape. However, there was a tendency for the curves read at 520 m μ to be more sensitive than those read at 530 m μ .

RESULTS

The values given in table 3 were obtained from .03 per cent solutions of lactose, galactose, and glucose. They are representative values obtained from at least ten trials. The values given by Sorensen when using a blank were scaled from a curve given by her. They may be used as a means of comparing results.

The results, recorded in table 3, show that the values obtained with glucose are in agreement with those reported by Sorensen for readings made against a blank. These values give a curve increasing in optical density with an increase in heating time. The corrected values reported by Sorensen differ from both those found in this laboratory and also the values read against a blank by her, in that they give a curve with a definite maximum at the fifteen minute point. Sorensen's uncorrected results which agree with the results in this laboratory cannot be used in place of her corrected results for the estimation of individual sugars in a mixture.

The results for galactose were in agreement with those reported by Sorensen. Both showed a definite maximum at the ten minute point.

The results obtained with lactose are not in agreement with those reported by Sorensen. The curves obtained in this laboratory have a maximum between the ten and fifteen minute points, both for those read against water and those read against a blank. The values given by Sorensen for readings against a blank show a steady increase in optical density with an increase in heating time, and do not have a maximum or minimum. The corrected values given show a slight maximum at the ten minute point and a sharp maximum

at the twenty minute point. This difference between the corrected and uncorrected values cannot be accounted for by applying a correction factor as she describes in another part of her work. The correction described was determined by reading the optical density of a mixture of the orcinol solution and sulfuric acid without the sugar. This value was subtracted from the density readings of the solutions containing sugar. Since this value was a constant, the general shape of the curves should not be changed.

SUMMARY

One cc. of the sugar solution to be tested was mixed with two cc. of 2 per cent orcinol solution and 15 cc. sulfuric acid. The solution was heated in a constant temperature water bath at 80°C. for a predetermined length of time. The extinction coefficient was read in a spectrophotometer immediately after cooling, using a solution depth of one cm.

The values for glucose found in this laboratory differed from Sorensen's corrected values in that they showed a maximum at the fifteen minute point. The values for lactose differed from all of Sorensen's values. They showed a maximum at twenty minutes heating. These curves are not suitable for Sorensen's method of estimating sugars

in a mixture. This failure was not avoided by changing the concentration of acid or by changing the sample of orcinol, sugar, or acid.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to his major instructor, Dr. C. H. Whitnah, for the constant assistance and advice given during the course of this work. He also wishes to thank Dr. J. E. Hedrick, of the Chemical Engineering Division, for the use of apparatus which aided greatly in the experimental work on this problem.

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