SOME PHYSICAL PROPERTIES OF LEVULOSE AND ITS ESTIMATION BY COPPER REDUCTION METHODS

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ABSTRACT

Fundamental physical properties of levulose have been determined in order to facilitate its quantitative determination. Densities of aqueous solutions are given at 20° and 25° C. for concentrations 0 to 70 per cent. Expansions have been measured over a 50° temperature interval. The density of crystalline levulose is shown to be 1.598. Refractive indices of levulose solutions have been measured at 20° and 25° C. between 0 and 90 per cent concentration. The saccharimetric rotations of levulose solutions have been measured at 20° and 25° and the change of rotation over a 50° interval of temperature determined.

Copper reduction methods for the determination of levulose have been studied. Nyns's selective method for the estimation of levulose has been modified by specifying a period of 75 minutes' digestion at 55° C. (instead of 150 minutes at 49° C.). An electrometric method for the estimation of cuprous oxide is described. The errors of analysis have been reduced by attention to details.

Simplified methods of computation have been devised which permit the rapid calculation of the levulose content of sugar mixtures.

CONTENTS

I. INTRODUCTORY

The ketose sugar, levulose, or *d*-fructose, promises in the future to assume an importance in some degree commensurate with its abundance and intrinsic value. It is widely distributed in natural products in which it may occur existing in a free state, or combined with other hexoses to form compound sugars, or condensed to form polysaccharides of high molecular weight. It has been shown in a previous article[1] that crystalline levulose can be prepared from potentially abundant sources by a process capable of industrial development. Such a process requires accurate and rapid methods of analytical control. Numerous foodstuffs, many of them long-established articles of diet, contain levulose whose content has hitherto been but roughly gaged.

The accurate assay of all these materials requires a quantitative knowledge of the fundamental properties of pure levulose and its aqueous solutions. The present communication is intended to contribute data on the physical properties of levulose and on reduction methods for its estimation. While the investigation can not be considered exhaustive, each step has been carried out with the intention of meeting the needs of ordinary analytical precision.

II. PURIFICATION OF LEVULOSE

The levulose which served as the main source of supply for the experiments described in the present article had previously been prepared from the juices of the Jerusalem artichoke by hydrolysis with sulphuric acid, defecation and precipitation with lime, and crystallization from aqueous solution by the method described by Jackson, Silsbee, and Proffitt.[2] It consisted of relatively large well-formed tilted cubes and was already approximately 99.5 per cent pure. Additional supplies of levulose were derived from inulin obtained from dahlia juices. From the inulin the levulose was crystallized either directly after hydrolysis or after hydrolysis and precipitation with lime.

For further purification the sugar was dissolved in about an equal weight of water, treated with carbon, filtered, and evaporated in a vacuum to a thick sirup. The sirup was transferred to a glass jar, admixed with alcohol, seeded, and the jar tumbled end over end in a motor-driven apparatus until crystallization was complete. The crystals were purged in a centrifugal machine and thoroughly washed with alcohol. Characteristic experimental results are assembled in Table 1.

TABLE 1.—*Recrystallization of levulose from alcoholic solution*

n_D^{20} thick sirup	Levulose	Weight of sirup	Calculated weight of levulose	Strength of alcohol (by volume)	Weight of alcohol	Yield of dry crystals	
						Weight	Per cent
	Per cent	*g*	*g*	*Per cent*	*g*	*g*	
1.508	89.2	897	800	95	346	482	60
1.508	89.2	1,078	962	100	353	735	76
1.5133	91.3	359	328	95	144	211	64

[1] Jackson, Silsbee, Proffitt, B. S. Sci. Papers No. 519, vol. 20, p. 519, 1926.
[2] See footnote 1.

The method of purification was so effective that a single recrystallization of levulose, already fairly pure, produced samples which in general could not be distinguished by our measurements from those recrystallized two or three times. All samples which were used for the measurements of physical properties were recrystallized at least twice.

After a period of air drying the crystals were further dried at about 50° C. and were stored in a desiccator. Although levulose is somewhat hygroscopic, the pure crystalline substance can apparently be completely dried by simple measures. Samples prepared as described above usually contained no more than 0.02 per cent of residual moisture. Extensive experimentation showed that this could be eliminated by warming to 50° C. in a thin layer for about two hours. Such a sample was further heated at 70° C. for about 30 hours without further loss. It was then heated at 60° to 70° C. in a vacuum chamber without loss of weight. If heated to much higher temperatures the substance suffers loss of weight as a result of decomposition.[3]

III. DENSIMETRIC MEASUREMENTS

1. DENSITIES OF LEVULOSE SOLUTIONS

For a relatively short range of concentrations the densities of aqueous solutions of levulose have previously been determined by a number of investigators,[4] usually incidentally to measurements of its rotatory power. For the purpose of extending the range of concentrations and of aiding in the critical selection of valid existing data additional density measurements have been made and are reported below.

Densities were measured in the present investigation by means of flasks whose necks, 6 mm inside diameter, were graduated in 10 divisions at intervals of 0.02 ml. The volume reading could be made to one-tenth of one of these divisions thus permitting a precision of two units in the fifth decimal of density for a flask holding 100 ml. The largest of these flasks contained at the lowest graduation mark 126.333 ml at 20° C. For concentrations up to 24 per cent this flask was used exclusively. For concentrations from 30 to 60 per cent at least one measurement was made with this flask at 10 per cent intervals, while additional measurements were made with flasks containing 48.461 and 25.303 ml, respectively. The flasks were calibrated frequently at 20° and 25° C. and were found to have kept a constant volume within 0.002 ml during the course of the investigation.

The volumes of the smaller flasks could be observed with the same precision as that of the larger flask, but the percentage errors of observation became three and five times as great, respectively. It was, therefore, necessary to weight the observations in accordance with their respective accuracies. This was done by summing up the weights of levulose and of solution and the volumes of solutions which had approximately the same concentration, thus obtaining in effect one large solution for computation of data. In these averaged

[3] The conclusions respecting the drying of levulose are in harmony with previous work on this subject. For summary of previous work see Browne's Handbook of Sugar Analysis, p. 20. John Wiley & Sons, New York, 1912.

[4] Landolt u. Börnstein Tabellen, vol. 1, p. 468, 1923.

values (Table 3) the data obtained with the larger flasks predominated in proportion to their volumes.

The levulose, prepared and dried as previously described, was introduced into the weighed flask and weighed. The sugar was dissolved and the air removed from the solution by warming slightly and applying a vacuum, care being exercised to avoid loss by spattering. Water was then added to some point within the graduated scale and after mixing by a rotary motion the flask was placed in a thermostat for at least a half hour before the final observation of volume. The neck of the flask was dried by a stream of filtered air. Since the manipulation was time-consuming, mutarotation was complete before the final observation of volume.

The volumes of all solutions below 20 per cent concentration were measured at 20° and 25° C. Solutions of higher concentrations were measured at 20° and at 25° C. or at some neighboring temperature which was carefully observed.

At the time of each weighing the density of the air in the balance case was determined by weighing a glass bulb whose true mass had been determined by A. T. Pienkowsky, of this bureau. All weights were reduced to vacuum, the density of levulose being assumed 1.598 in accordance with a determination described below.

The data are assembled in Tables 2 and 3. Sylvia M. Goergen has made a least square adjustment of the data in Table 2 and computed the formulas (1) and (2).

$$D_4^{20} = 0.99823 + 0.0038893\ p + 0.0000140\ p^2 \qquad (1)$$

$$D_4^{25} = 0.99708 + 0.0038557\ p + 0.0000139\ p^2 \qquad (2)$$

which are valid between 0 and 20 per cent levulose.

The observed densities of the dilute solutions in Table 2 show a mean deviation from the formulas of two units in the fifth decimal for both temperatures. We, therefore, believe that the formulas are valid to some units in the fifth decimal and have solved them for 1 per cent intervals between 0 and 20 per cent levulose for purposes of tabulation. These calculated densities are given in Table 18, p. 437.

The data for the higher concentrations are given in Table 3. An adjustment was made by the method of least squares, yielding the formula,

$$D_4^{20} = 0.99936 + 0.0037842\ \mathrm{p} + 0.00001636\ p^2 \qquad (3)$$

which is valid between 24 and 70 per cent. A comparison of the densities calculated from the formula with the experimental data in Table 3 discloses a mean deviation of 6 units in the fifth decimal. In our judgment the fourth decimal of the adjusted densities is reliable within about one-half a unit and we have, therefore, solved the formula between 24 and 71 per cent for 1 per cent intervals to the fourth decimal for tabulation in Table 18, p. 437. Formulas (1) and (3) yielded essentially identical values between 20 and 24 per cent.

TABLE 2.—*Densities of dilute levulose solutions at 20° and 25° C.*

Weight levulose (in air, brass weights)	Weight levulose (vacuum)	Weight solution (vacuum)	Concentration (vacuum)	Volume of solution $t=$ 20.0° C.	D_4^{20} observed	D_4^{20} calculated formula (1) [1]	Deviation from formula (1) [1]	Volume of solution $t=$ 25.00° C.	D_4^{25} observed	D_4^{25} calculated formula (2) [2]	Deviation from formula (2) [2]
g	*g*	*g*	*Per cent*	*ml*				*ml*			
3.3542	3.3561	127.463	2.633	126.377	1.00859	1.00857	+0.00002	126.532	1.00736	1.00733	+0.00003
7.2042	7.2082	128.924	5.591	126.345	1.02041	1.02041	.00000	126.509	1.01909	1.01907	+.00002
11.7513	11.7583	130.683	8.998	126.335	1.03437	1.03436	+.00001	126.521	1.03285	1.03289	−.00004
12.4818	12.4894	130,991	9.534	126.371	1.03656	1.03658	−.00002	126.550	1.03509	1.03510	−.00001
17.9369	17.9475	133.093	13.485	126.372	1.05318	1.05322	−.00004	126.557	1.05164	1.05160	+.00004
20.5601	20.5723	134.068	15.345	126.339	1.06119	1.06121	−.00002	126.540	1.05949	1.05951	−.00002
23.2543	23.2682	135.104	17.223	126.339	1.06937	1.06937	.00000	126.549	1.06759	1.06760	−.00001
23.2629	23.2767	135.100	17.229	126.328	1.06944	1.06940	+.00004				
23.3536	23.3687	135.159	17.290	126.358	1.06965	1.06966	−.00001	126.564	1.06790	1.06789	+.00001
24.3190	24.3331	135.542	17.952	126.369	1.07259	1.07257	+.00002				

[1] (1) $D_4^{20}=0.99823+0.0038893\ p+0.0000140\ p^2$.

[2] (2) $D_4^{25}=0.99708+0.0038557\ p+0.0000139\ p^2$.

TABLE 3.—*Densities at 20° C. of levulose solutions of higher concentrations*

Number of determinations	Weight levulose (vacuum)	Weight solution (vacuum)	Concentration	Volume solution at 20.0° C.	D_4^{20} observed	D_4^{20} calculated formula (3)[1]	Residual Obs.−Calcd.
	g	*g*	*Per cent*	*ml.*			
1	34.0572	139.282	24.452	126.418	1.10176	1.10167	+0.00009
2	61.9334	198.156	31.255	174.813	1.13353	1.13361	−.00008
3	145.2537	355.746	40.831	301.193	1.18112	1.18114	−.00002
3	173.6622	342.833	50.655	278.040	1.23303	1.23302	+.00001
2	117.1697	195.000	60.087	151.643	1.28591	1.28579	+.00012
1	23.4329	33.886	69.152	25.303	1.33921	1.33927	−.00006

[1] (3) $D_4^{20}=0.99936+0.0037842\ \mathrm{p}+0.00001636\ p^2$.

2. MEAN EXPANSION OF LEVULOSE SOLUTIONS BETWEEN 20° AND 25° C.

By subtracting formula (1) from formula (2) and dividing by the difference in temperature we obtain formula (4), which is the mean change of density per degree between 20° and 25° C.

$$\frac{\Delta D}{\Delta t}=-(0.000231+0.00000672\ \mathrm{p}-0.0000000224\ p^2) \qquad (4)$$

valid between 0 and 20 per cent levulose.

The solutions of concentration higher than 20 per cent were made up primarily for the determination of density at 20°, but their densities were also measured at 25° C. or some neighboring temperature which was in each case accurately observed. The data are shown in Table 4. In order to adjust these density measurements to 25° the mean change of density per degree between 20 and the temperature of observation was computed for each solution. Since the change of density with temperature is not exactly linear, it was necessary to apply a correction to the measurements of those solutions whose densities were determined at temperatures other than 25° C. This was computed by assuming that the deviation of levulose density at other temperatures was the same percentage of the mean change between 20° and 25° C. as the deviation of densities of sucrose solutions of similar concentration. This assumption was justified by the agreement shown between the corrected density coefficients of the same levulose solutions observed at different temperatures. The corrected coefficients given in column 12 of Table 4 were averaged for groups of similar concentrations in columns 13 and 14. These data were adjusted by the method of least squares yielding the formula,

$$\frac{\Delta D}{\Delta t}=-(0.0002145+0.00000795\ \mathrm{p}-0.0000000136\ p^2) \qquad (5)$$

which is valid between 20 and 70 per cent levulose and 20° and 25° C. By application of this formula to the densities at 20° C. the data at 25° C. were computed to four decimals and are included in Table 18, p. 437.

TABLE 4.—*Density-temperature coefficient of levulose solutions of higher concentrations*

Weight levulose (air, brass weights)	Weight levulose (vacuum)	Weight solution (vacuum)	Per cent concentration (vacuum)	Temperature	Volume at $t°$ C.	D_4^t	D_4^{20} Formula (3)	Δt (t-20)	$-\Delta D$	$-\Delta D/\Delta t$	$-\Delta D/\Delta t$ Corrected to 25° C.	Averages		
												Concentration	$-\Delta D/\Delta t$ Observed	$-\Delta/\Delta t$ Formula (5) [1]
g	*g*	*g*		° *C.*	*ml*				$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	*Per cent*	$\times 10^{-5}$	$\times 10^{-5}$
34.0366	34.0572	138.981	24.505	25.52	126.380	1.09971	1.10193	5.52	222	402	401			
34.0366	34.0572	138.832	24.531	29.93	126.456	1.09787	1.10205	9.93	418	431	414	24.518	41	40
17.1008	17.1109	54.914	31.159	25.00	48.554	1.13099	1.13315	5.00	216	402	402			
44.7967	44.8225	142.952	31.355	29.24	126.542	1.12968	1.13410	9.24	442	478	463	31.257	43	45
60.3388	60.3746	148.764	40.584	25.71	126.407	1.17687	1.17988	5.71	301	527	525			
23.4678	23.4816	57.285	40.991	25.00	48.568	1.17948	1.18197	5.00	249	498	498			
61.3617	61.3975	149.236	41.127	25.00	126.527	1.17987	1.18266	5.00	279	558	558	40.901	53	52
15.6291	15.6383	31.150	50.202	25.00	25.373	1.22768	1.23056	5.00	288	576	576			
78.3394	78.3858	155.150	50.523	28.25	126.401	1.22744	1.23231	8.25	487	590	580	50.363	58	58
79.5899	79.6381	155.610	51.177	27.26	126.359	1.23149	1.23587	7.26	438	603	596			
79.5899	79.6381	155.606	51.179	30.67	126.576	1.22935	1.23588	10.67	653	612	594	51.178	59	59
18.7291	18.7401	32.247	58.114	25.00	25.363	1.27142	1.27453	5.00	311	622	622	58.114	62	63
98.3703	98.4296	162.390	60.613	26.60	126.418	1.28455	1.28884	6.60	429	650	645	60.613	65	65
23.4191	23.4329	33.884	69.156	25.00	25.366	1.33580	1.33929	5.00	349	698	698	69.156	70	70

[1] $(5)\frac{\Delta D}{\Delta t} = -(0.0002145 + 0.000007947\ p + 0.00000001358\ p^2)$.

The change of volume with temperature is related to the change of density with temperature by

$$\frac{dV}{dt} = -\frac{dD}{dt} \times \frac{V}{D} = -\frac{dD}{dt} \times \frac{1}{D}, \text{ since } V = 1$$

Expansion coefficients calculated from the density-temperature coefficients by this equation have been included in Table 18, p. 437.

3. CORRECTIONS TO BE APPLIED TO BRIX HYDROMETERS IMMERSED IN LEVULOSE SOLUTIONS

The densities of levulose solutions deviate appreciably from those of sucrose solutions of the same percentage composition. In order to calculate the correction at 20° C. to be applied to hydrometers which are standard for sucrose solutions at 20°, it is merely necessary to compute the "Brix" of a levulose solution of known concentration by referring its density to the sucrose density table. The departure of the percentage of sucrose found from the percentage of levulose in the given solution is the correction to be applied to the reading of a Brix hydrometer.

To determine the corrections for temperatures other than 20° C. we have available the density-temperature coefficients described in the previous section. The handbooks on cane sugar analysis [5] have, in conveniently tabulated form, the corrections to be applied to the Brix hydrometer for changes of temperature of sucrose solutions. The corrections for levulose solutions would differ from these in the ratio of the respective expansion coefficients of levulose and sucrose solutions. The mean expansion coefficients of sucrose between 20° and 25° C. were calculated from the sucrose density tables [6] and compared with the coefficients of levulose. Levulose solutions have higher expansion coefficients than sucrose solutions. At a concentration of 10 per cent the ratio of the coefficients is 1.12; at 20 per cent, 1.20; at 30 per cent, 1.22; at 40 per cent, 1.19; at 50 per cent, 1.23; at 60 per cent, 1.28; and at 70 per cent, 1.33. The corrections for sucrose were multiplied by these respective ratios to give the corrections for levulose for the change of temperature only. These corrections were then added algebraically to the corrections at 20° to give the total corrections to be applied to Brix hydrometer readings. The resulting corrections are tabulated in Table 19, p. 438.

From the data at hand it would be feasible to construct a Baumé scale of levulose densities by application of the formula [7]

$$\text{Degrees Baumé} = 145 - \frac{145}{\text{Specific gravity } (20°/20°)}$$

4. EXPANSION OF LEVULOSE SOLUTIONS AT WIDE TEMPERATURE INTERVALS

In the analysis of solutions containing levulose it is frequently necessary to determine polariscopic readings at widely divergent temperatures. These readings must be corrected for the expansion of

[5] For example, B. S. Circular No. 44, p. 128.
[6] Landolt u. Börnstein Tabellen, vol. 1, p. 463, 1923.
[7] Bates and Bearce, B. S. Tech. Paper No. 115.

the solution in order that both readings may be referred to the same volume concentration of solute.

A convenient volume-measuring apparatus was made by sealing a 5 ml graduated pipette to a long narrow glass bulb. This was carefully calibrated by weighing it filled with distilled water and reading the change of volume at various temperature intervals up to 100° C. The accuracy of the calibration was attested by the fact that the glass itself showed a constant cubical expansion coefficient.

For analytical methods it is convenient to use the temperature interval 20° to 70° C. As indicated in Table 5 a mean change of 0.044 ml per 100 ml per ° C. may be taken for solutions of about 5 per cent concentration and 0.045 for about 18 per cent concentration.

TABLE 5.—*Expansion of solutions between wide temperature limits*

	Concentration of solids	Temperature interval	Mean $\frac{\Delta v}{\Delta t}$ for 100 ml
	g/100 ml	*°C.*	*ml*
Pure levulose	18.4	20 – 69	0.045
	4.7	20.4– 70	.044
	3.5	20.6– 69	.043
Water		20 – 70	.042
Artichoke sample [1]		22 – 50	.038
		22 – 66.3	.042
		22 –100	.053

[1] An 80 g sample of ground fresh artichokes hydrolyzed with HCl, defecated, made to 325 ml, and filtered in preparation for analysis.

5. DENSITY OF CRYSTALLINE LEVULOSE

Since many of the calculations in the present investigation required a reduction of the weights of crystalline levulose to a vacuum, a determination of the density of the cubelike crystal form of levulose was considered necessary.[8]

For the measurement of density a flask was used of about 20 ml volume with a neck 3 mm in diameter graduated in 10 divisions of 0.0056 ml each. The volume could be observed to a precision of 0.001 ml.

Pure dry levulose was weighed in the calibrated flask, and the flask was filled with dry toluene saturated with levulose at 20° C. Air trapped in the crystals was removed by applying gentle suction while rotating the flask. The mixture was made to volume at 20.0° C. and reweighed. Three independent determinations of the density of levulose and of the toluene saturated with levulose were made. All weights were corrected to vacuum. The density (d_4^{20}) was found to be 1.598.

[8] Previously announced values: $d_4^{17.5}$, 1.669 (crystal form not specified), Int. Critical Tables, vol. 1, p. 203, Index No. 1674; d_4^0, 1.555 (needles), Handbook of Chemistry and Physics, Hodgman and Lange, Chem. Rubber Co., 13 ed., p. 356, 1928.

TABLE 6.—*Density of crystalline levulose*

Weight pure toluene (vacuum)	Volume toluene	$D^{25°}_{4°}$ toluene	Weight levulose (vacuum)	Weight toluene (vacuum)	Total volume	Volume toluene	Volume levulose	$D^{25°}_{4°}$ levulose
g	*m*		*g*	*g*	*ml*	*ml*	*ml*	
18.0143	20.794	0.86632	10.7459	12.1948	20.801	14.075	6.726	1.5977
18.0330	20.814	.86639	3.8529	15.9482	20.820	18.408	2.412	1.5974
18.0075	20.783	.86645	6.7969	14.3284	20.790	16.538	4.252	1.5984
Average		.86639						1.598

IV. REFRACTIVE INDICES OF LEVULOSE SOLUTIONS

The convenience and precision of the modern refractometer make it desirable that this instrument become available for the determination of levulose concentrations. For the measurements recorded below, three instruments were employed, namely, a Zeiss immersion refractometer, a Valentine refractometer of the Abbe type, which permitted an estimation of the fifth decimal of index, and for the highest concentrations an Abbe instrument, permitting an estimate of the fourth decimal.

The indices of solutions ranging from zero to about 20 per cent were measured by means of a Zeiss immersion refractometer. This instrument had previously been carefully calibrated at five points by L. W. Tilton, of this bureau, by means of salt solutions whose indices were determined on a precision spectrometer. It was our privilege to consult Mr. Tilton frequently during the course of these experiments and his assistance is gratefully acknowledged.

The measurements were made in a large thermostat containing about 200 liters in which temperature was maintained within 0.01° of constancy. Preliminary experiments showed that unless the temperature of the room was closely the same as that of the bath the flow of heat through the barrel of the instrument affected slightly the temperature of the small body of solution under observation. It was deemed necessary then to control the temperature of the whole refractometer as far as feasible. For the final readings the levulose solution was contained in the metal cup provided with the refractometer. The instrument itself was inserted in a large glass tube 77 mm in diameter and 28 cm long containing enough water to immerse the metal cup. The glass tube was immersed in the water of the large thermostat nearly to its upper rim and the mouth was stuffed with cotton cloth. Thermometers placed in the thermostat, in the water surrounding the cup, and in the air about the barrel of the refractometer showed essentially identical temperatures. Under these favorable conditions of temperature regulation the line of total reflection was exceedingly sharp and permitted a precision of setting within 0.1 or 0.2 of a drumhead division, or 0.01 or 0.02 of a scale division.

The instrument was illuminated by a frosted lamp placed in a large test tube immersed in the water of the thermostat, the light being reflected at the proper angle by a submerged mirror.

Concentrations of levulose were selected to coincide with the calibrated points on the scale. Before or after each determination, readings were made with distilled water. Throughout the whole

series this "zero point" remained constant at 14.42 ± 0.01 at 20.00° C. All readings were corrected to correspond to a reading of 14.50 at 20° C. or 13.25 at 25° C. for distilled water as defined by the arbitrary scale of the instrument. These readings are equivalent to indices of 1.33300 at 20.00° and of 1.33252 at 25°.

The arbitrary scale readings were translated into indices of refraction by means of the table of equivalents supplied by the manufacturer. Since the scale of the instrument had been calibrated by measurement of solutions whose indices were known, and since the instrument corrections were small, the indices of levulose solutions here presented are probably closely equivalent to absolute measurements, although admittedly they do not have the validity of measurements made with a spectrometer. It is the purpose of this bureau to undertake absolute measurements at some future time.

While the precision of setting of the line of total reflection was usually 0.01 or 0.02 scale division, corresponding to some units in the sixth decimal of refractive index, confidence is placed only in 0.1 scale division or 3 or 4 units in the fifth decimal. There are apparently some factors beside precision of setting which are as yet imperfectly controlled.

Great caution must be exercised in the use of the arbitrary scale. The data presented here refer solely to readings made on instruments graduated according to the scale as originally proposed by Pulfrich.[9] On this scale, 14.5 is equivalent to 1.33300; 50.0 to 1.34650; and 100.0 to 1.36464. If instruments are used which do not conform to the original system, the arbitrary scale readings must first be converted to refractive indices before reference is made to the index tables presented below.

In Table 7(*a*) are presented the observed data at 20° and 25° C. Sylvia M. Goergen has computed by the method of least squares interpolation formulas (6) and (7) which are valid between 0 and 20 per cent levulose, and has ascertained the agreement between the calculated and observed data.

$$n_D^{20} = 1.33300 + 0.0014159\,p + 0.00000491\,p^2 \qquad (6)$$

$$n_D^{25} = 1.33252 + 0.0014059\,p + 0.00000487\,p^2 \qquad (7)$$

The indices and scale readings at integral percentage concentrations are given in Table 20, page 438.

The measurements for the range 18 to 86 per cent levulose (Table 7(*b*)) were made with the Valentine refractometer of the Abbe type in a thermostated room maintained at 20° C. The temperature of the prisms was further controlled by water circulated from a thermostat. The readings at 25° were made in a laboratory whose temperature was uncontrolled, but the circulating water was carefully controlled at approximately 25° C. Temperature corrections resulting from deviations of the circulation water from the standard temperatures were small and could be applied with but little uncertainty.

Indices for the range 86 to 89 per cent (Table 7(*c*)) were measured with the Abbe refractometer. Both of these Abbe type refractometers had been calibrated at several scale points.

[9] Z. f. angew. Chemie, p. 1186, 1899.

All solutions were made up by weighing the pure dry levulose and adding the calculated amount of water. The supersaturated solutions were prepared by dissolving the sugar in hot water, but the final weighing was not made until the resulting solutions were cooled. All percentages are expressed in terms of weights in air with brass weights.

Least square adjustment yielded formulas

$$n_D^{20} = 1.33344 + 0.0013625\ p + 0.000006645\ p^2 \quad (8)$$

$$n_D^{25} = 1.33312 + 0.0013415\ p + 0.000006762\ p^2 \quad (9)$$

which are valid between 20 and 63 per cent levulose, and

$$n_D^{20} = 1.33377 + 0.0013570\ p + 0.000006680\ p^2 \quad (10)$$

$$n_D^{25} = 1.33345 + 0.0013360\ p + 0.000006800\ p^2 \quad (11)$$

valid between 63 and 90 per cent levulose. The experimental and calculated data are included in Table 7 and the computed indices for 1 per cent intervals in Table 20, page 438.

TABLE 7.—*Refractive indices of levulose solutions*

(*a*) MEASURED ON IMMERSION REFRACTOMETER

Weight levulose, air, brass weights	Weight solution, air, brass weights	Concentration of levulose	Zeiss immersion scale reading $t=20.0°$ [1]	n_D^{20} [2]	Deviation from formula (6)	Zeiss immersion scale reading $t=25.0°$ [1]	n_D^{25} [2]	Deviation from formula (7)
g	*g*	*Per cent*						
		0	14.50	1.33300	0	13.25	1.33252	0
0.4381	9.0108	4.862	32.77	1.34001	0	31.39	1.33949	+.00002
.8312	16.7541	4.961	33.15	1.34016	0			
.7440	8.8401	8.416	46.76	1.34528	+.00002			
1.5447	17.9232	8.618	47.60	1.34560	0	46.01	1.34500	0
1.9047	16.0668	11.855	60.60	1.35043	−.00005	59.02	1.34985	−.00002
1.2075	7.9699	15.151	74.66	1.35557	−.00001	72.92	1.35494	0
1.6200	8.4827	19.098	92.11	1.36185	+.00002	90.16	1.36115	0

[1] The arbitrary scale units employed here conform solely to the system as originally proposed for the immersion refractometer; thus, for example, $14.50=n_D$ 1.33300; 100.0=1.36464. (Pulfrich, Zeit. f. angew. Chemie, p. 1186, 1899.)

[2] Derived from table of equivalents which convert arbitrary scale readings into indices of refraction.

TABLE 7.—*Refractive indices of levulose solutions*—Continued

(*b*) MEASURED ON VALENTINE-ABBE REFRACTOMETER

Weight levulose, air, brass weights	Weight solution, air, brass weights	Concentration of levulose	n_D^{20} observed	Deviation from formula (8)	n_D^{25} observed	Deviation from formula (9)
g	*g*	*Per cent*				
1.0850	5.7274	18.944	1.36157	−0.00006	1.36094	+0.00001
1.0688	4.6561	22.955	1.36822	0	1.36748	0
1.1919	4.4228	26.948	1.37503	+.00005		
1.4402	4.6590	30.912	1.38197	+.00006	1.38104	−.00001
17.1008	54.8660	31.168	1.38239	+.00003	1.38162	+.00012
44.7960	143.1180	31.300	1.38259	−.00001		
2.6925	6.5822	40.906	1.40035	+.00005	1.39932	0
23.4678	57.2366	41.001	1.40046	−.00002	1.39950	+.00001
61.3617	149.3230	41.093	1.40059	−.00006	1.39958	−.00008
15.6291	31.1260	50.212	1.41856	−.00005	1.41758	+.00005
79.5900	155.9480	51.036	1.42030	+.00001		
18.7291	32.2222	58.125	1.43510	+.00001	1.43398	+.00003
98.3700	162.6270	60.488	1.44013	−.00004	1.43894	−.00007

Weight levulose, air, brass weights	Weight solution, air, brass weights	Concentration of levulose	n_D^{20} observed	Deviation from formula (10)	n_D^{25} observed	Deviation from formula (11)
g	*g*	*Per cent*				
23.4191	33.8590	69.166	1.45964	+0.00006	1.45845	−.00005
5.8569	7.7837	75.246	1.47383	+.00013	1.47261	+.00009
8.5520	11.2766	75.838	1.47524	+.00014	1.47399	+.00008
4.8635	6.1826	78.664	1.48188	+.00003	1.48046	−.00015
4.8365	5.8752	82.321	1.49062	−.00012	1.48945	+.00001
12.0706	14.6504	82.390	1.49093	+.00002	1.48967	+.00006
4.6539	5.4159	85.930	1.49954	−.00016	1.49823	−.00001

(*c*) MEASURED ON ABBE REFRACTOMETER

5.5476	6.3893	86.826	1.5019	0		
10.2809	11.6725	88.073	1.5052	+.0001	1.5038	+0.0001
10.7568	12.1401	88.601	1.5065	+.0001	1.5051	+.0001
10.8767	12.2217	88.991	1.5073	−.0001	1.5060	0
10.9897	12.3035	89.321	1.5082	−.0001	1.5069	+.0001

V. SACCHARIMETRIC NORMAL WEIGHT OF LEVULOSE

1. MEASUREMENTS OF SACCHARIMETRIC ROTATIONS

No measurements have previously been published of the rotatory power of levulose in terms of the white light of the quartz-wedge saccharimeter. Since this instrument is the most convenient means of carrying out polariscopic analysis, the experiments here described were made to determine the normal weight of crystalline levulose (*Wo*) which in 100 ml of solution reads −100° S. and the normal weights (*W*) of aqueous solutions which, when diluted to 100 ml, show rotations in negative sugar degrees equal to their respective percentage concentrations. The distinction between *Wo* and the variable weights *W* is necessarily introduced because the rotation of levulose does not vary strictly linearly with concentration. Let it be noted that in the following discussion *Wo* alone represents the normal weight of pure crystalline levulose, while the *W*'s represent

weights of solution of pure levulose in water. The latter will yield polariscopic readings equal to their percentage concentrations.

In the absence of direct measurements it has previously been customary to assume that the normal weights of sucrose and levulose were in the inverse ratio of their respective specific rotations. This involves the assumption that the rotatory dispersions of levulose and sucrose are identical. Since the latter assumption has not been verified experimentally, and since the specific rotation itself is known with little certainty, any computed value of the normal weight is but an approximation.

The present measurements were made with a Bates type Fric saccharimeter which had been used in previous similar investigations. Its negative scale was calibrated against the quartz plates which serve as primary standards of this bureau, and whose absolute rotations have been measured many times on a high precision polarimeter. The sugar values of these plates were expressed in terms of the normal quartz plate as measured by Bates and Jackson.[10] According to this standardization the normal quartz plate rotates 40.690° for $\lambda = 5{,}461$ A, and 34.620° for $\lambda = 5{,}892.5$ A at 20° C.

The solutions whose rotations were measured were those whose concentrations and densimetric data are tabulated in Table 2. The readings were made in water-jacketed polariscope tubes whose lengths were accurately known. Temperatures were observed frequently by means of calibrated thermometers and were, in general, constant for long periods of time within 0.02° to 0.05° C. The saccharimeter was illuminated by a 100-watt lamp, the light from which passed through a 15 mm column of 6 per cent potassium dichromate before entering the instrument. Before observation the solutions usually had stood for at least two hours, and therefore, mutarotation was in every instance complete.[11]

All temperature corrections were reduced to a minimum by careful temperature control. The corrections for variations in temperature of the quartz wedges were calculated by assuming a temperature coefficient of quartz of 0.000144.[12]

In order to apply proper temperature corrections for the levulose solutions, rotations were observed at 20° and at 25° C. Since the concentrations had also been previously determined at both temperatures, this procedure served the purpose of determining the normal weights at both temperatures as well as providing a reliable means of correcting for small deviations from the two standard temperatures.

For computing the rotations, all operations and apparatus, including quartz wedges, were adjusted or corrected to the two respective standard temperatures.

The observations are recorded in Tables 8 and 9. The normal weights at the respective concentrations were computed by dividing the number of grams of levulose (c) in 100 ml $\times$ 100 by the observed rotation.

$$\frac{100 \times c}{\text{Obs. pol.}} = W \tag{12}$$

[10] B. S. Sci. Paper No. 268, vol. 13, p. 67, 1916.

[11] According to Hudson and Yanovsky (B. S. Sci. Papers No. 533, vol. 21, p. 271, 1926) the mutarotation velocity constant is 0.082 at 20° C.

[12] Bates, Bull. B. S., vol. 2, p. 245, 1906.

These values vary with concentration in the same manner as the specific rotation. The observed normal weights were correlated with the concentrations by the method of least squares, yielding the formulas

$$W_{t=20} = 18.803 - 0.01801\,c - 0.000191c^2 \quad (13)$$
$$W_{t=25} = 19.446 - 0.02061\,c - 0.000141c^2 \quad (14)$$

in which c is the number of grams of levulose in 100 ml weighed in air with brass weights.

From the adjusted normal weights the adjusted polarizations were computed by dividing each concentration by the respective adjusted normal weight. The deviations of the observed polariscopic readings from the computed readings are shown in columns 6 of Tables 8 and 9.

The normal weight of the normal solution or the concentration of levulose required to read exactly −100° S. was calculated from formulas (13) and (14) by imposing the condition that $c = Wo$.[13] Upon solution of the resulting equations we obtain 18.4067 g for the normal weight (*Wo*) of levulose at 20°, and 19.0030 g at 25° C. We shall accept the values 18.407 and 19.003, respectively, weighed in air with brass weights.

TABLE 8.—*Saccharimetric rotations of levulose solutions at 20° C.*

Levulose (vacuum)	Weight levulose in 100 ml (air, brass weights)	D_4^{20}	Observed polarization at 20° C. ×(−1)	Calculated polarization ×(−1)	Deviation obs.− calc. polarization ×(−1)	Observed normal weight	Calculated normal weight formula (13)
Per cent	*g*		°*S*	°*S*	°*S*	*g*	*g*
5.5909	5.7018	1.02041	30.49	30.50	−0.01	18.701	18.694
8.9977	9.3017	1.03437	49.98	49.96	+.02	18.611	18.619
9.5341	9.8771	1.03656	53.11	53.09	+.02	18.597	18.606
13.4846	14.1916	1.05318	76.63	76.59	+.04	18.519	18.509
15.3442	16.2737	1.06119	88.11	88.16	−.05	18.471	18.459
17.222	18.4063	1.06937	100.02	100.00	+.02	18.404	18.407
17.229	18.4146	1.06944	100.01	99.96	+.05	18.397	18.406
17.289	18.4821	1.06965	100.44	100.46	−.02	18.400	18.405
------	18.5672	----------	100.87	100.89	−.02	18.406	18.403
17.952	19.2444	1.07259	104.66	104.67	−.01	18.388	18.385

TABLE 9.—*Saccharimetric rotations of levulose solutions at 25° C.*

Levulose (vacuum)	Weight levulose in 100 ml (air, brass weights)	D_4^{25}	Observed polarization at 25° C. × (−1)	Calculated polarization ×(−1)	Deviation obs.− calc. polarization ×(−1)	Observed normal weight	Calculated normal weight formula (14)
Per cent	*g*		°*S*.	°*S*.	°*S*.	*g*	*g*
5.5909	5.6944	1.01909	29.47	29.47	0.00	19.321	19.324
8.9977	9.2880	1.03285	48.28	48.27	+.01	19.249	19.242
13.4846	14.1709	1.05165	74.12	74.09	+.03	19.118	19.125
15.3442	16.2479	1.05949	85.18	85.19	−.01	19.075	19.074
17.2218	18.3758	1.06759	96.56	96.62	−.06	19.031	19.019
17.229	18.3834	1.06762	96.69	96.66	+.03	19.014	19.019
17.289	18.4520	1.06790	97.06	97.03	+.03	19.012	19.017
17.952	19.2104	----------	101.12	101.12	.00	18.998	18.998

[13] This yields the equations

$$c_{t=20} = 18.803 - 0.01801\,c - 0.000191\,c^2$$
$$c_{t=25} = 19.446 - 0.02061\,c - 0.000141\,c^2$$

The c^2 terms were determined numerically with sufficient accuracy by interpolation between two calculated values for solutions approximately normal.

If the polarization were to indicate directly the percentage of levulose in the sample taken for analysis, it would be necessary to take a different normal weight for each concentration. It is more convenient to employ a constant normal weight and apply corrections for the deviations of rotation from constant proportionality. To determine these corrections, formulas (13) and (14) were solved for each integral value of c from 1 to 20 g yielding the respective normal weights. Each value of c multiplied by 100 and divided by its respective normal weight yields a numerical value equal to the actual rotation for each concentration. But each value of c divided by the constant normal weights 18.407 and 19.003 yields the percentage of levulose in the sample taken for analysis. The deviation of the actual rotation from the percentage of levulose is a measure of the correction to be applied to the polarization in order to give the true percentage composition of the sample. These corrections are given in Table 21, page 439.

2. CHANGE OF POLARIZATION OF LEVULOSE BETWEEN WIDELY SEPARATED TEMPERATURES

The high temperature coefficient of the rotatory power of levulose suggests that polariscopic readings at widely separated temperatures may serve as a measure of the concentration of the sugar.[14] The measurement is in a high degree dependent upon the efficiency of the apparatus used for temperature control. The measurements reported below were made in water-jacketed continuous polariscope tubes, 400 mm in length, constructed of Monel metal. Within the water jacket was placed a spiral baffle which imparted a rotary motion to the water stream, thus greatly increasing the velocity of water over the surface of the tube. We acknowledge gratefully the assistance of M. J. Proffitt and J. A. Bogan in the designing of these tubes.

Water was supplied by centrifugal pumps operating in thermostats held at the high and low temperatures, respectively. The temperatures of polarization were observed by means of thermometers placed in the circulating water immediately before its entrance into and after its emergence from the water jacket. In general, the water stream was so abundant and rapid that the two thermometers showed closely agreeing temperatures. The mean of the readings of the two thermometers was assumed to be the true temperature of the solution. The stem corrections of the thermometers were carefully calculated for the high temperature observation.

The observations for concentrations of levulose from 3 to 9 g in 100 ml were made in a 400 mm Bates-Fric saccharimeter. The remaining measurements were made in a Schmidt and Haensch 600 mm instrument with a quartz plate of +100.05° sugar value placed in series with the tubes.

Corrections were made for the deviation of the temperature of the quartz wedges and the quartz plate from the standard temperatures. We suggest as a matter of definition that the high temperature polarization be measured with the quartz wedges at the same temperature as that of the low temperature measurement. The expansion of the solution at the high temperature was corrected for by using the expansion coefficients given in Table 5.

[14] For a review of this subject see Browne's Handbook of Sugar Analysis, p. 297, 1912, John Wiley & Sons, New York.

The experimental data shown in Table 10 indicate that between room temperature and about 70° to 75° C. 1 g of levulose in 100 ml changes 0.0344° S. for each degree change in temperature . Contrary to Vosburgh's[15] conclusions, our data show no systematic deviation of the coefficient with concentration of sugar. The measurements indicate that levulose can be determined by this method with a mean precision of about one-third of 1 per cent of the quantity measured. This degree of precision has, however, been secured only by great attention to detail and by averaging a considerable number of observations.

TABLE 10.—*Change of polarization of levulose between wide temperature intervals*

Weight levulose in 100 ml at 20° C.	Number of observations	Polarization[1] at $t°_1$ C.	Temperature $t°_1$ C.	Polarization[1] at t_2	Temperature t_2° C.	ΔP	ΔT	$\frac{\Delta P}{\Delta T \times g}$	Levulose calculated	Error
g		°S.		°S.		°S.	°C.		*g*	*Per cent*
3.000	7	15.94	20.10	10.84	69.54	5.10	49.44	0.03439	2.998	−0.07
6.000	8	32.21	19.59	21.56	70.95	10.65	51.36	.03456	6.026	+.43
9.000	6	48.46	19.70	32.39	71.78	16.07	52.08	.03428	8.967	−.41
12.000	3	62.90	24.13	41.77	74.83	21.13	50.70	.03473	12.111	+.93
15.000	3	78.78	24.13	52.57	75.16	26.21	51.03	.03424	14.927	−.52
18.000	3	95.02	24.00	63.12	75.70	31.90	51.70	.03428	17.934	−.35
Average								.03441		.35

[1] Polarizations are corrected for length of tube and temperature of quartz wedges.

VI. COPPER REDUCTION METHODS FOR TOTAL REDUCING SUGAR

1. METHOD OF MUNSON AND WALKER

The method of Munson and Walker[16] is well adapted to the determination of total reducing sugar over a wide range of concentrations. In the articles in which the method was originally described the authors gave the copper equivalents for dextrose and invert sugar but no equivalents for levulose. In order that this widely employed method might become available for sugar mixtures having a high ratio of levulose, we have made analyses of pure levulose for the purpose of determining the respective copper equivalents.

The details[17] of the analyses were carried out rigorously in accordance with Munson and Walker's specifications, except that copper was determined by thiosulphate titration instead of by direct weighing of the copper precipitate. The titration method is less tedious and eliminates many of the uncertainties inherent in gravimetric analysis.

Immediately preceding the analysis of each levulose solution a similar analysis was conducted with a standard dextrose solution of such sugar content that both precipitated approximately the same weight of copper.

In Table 11 are shown the data obtained. Columns 2 and 3 show that the present experiments are in essential agreement with Munson and Walker's data on dextrose. Column 6 shows the weight of levulose (calculated from columns 4 and 5) which yields the same weight of copper (column 2) as the dextrose in column 1, and column 7 the ratio of weights of the sugars. These observed ratios were plotted against the weight of dextrose and from the smoothed curve the values in column 8 were read. The reciprocals of these values are given in column 11.

[15] J. Am. Chem. Soc., vol. 42, p. 1696, 1920.
[16] J. Am. Chem. Soc., vol. 28, p. 663, 1906; vol. 29, p. 541, 1907; vol. 34, p. 202, 1912.
[17] Quoted paragraphs are reprinted from the Report on Chemical Methods for Reducing Sugars, by R. F. Jackson, associate referee (J. Assoc. Official Agri. Chem., vol. 13, p. 199, 1930).

From column 8 it is apparent that the reducing ratio is a function of the concentration of sugar. Evidently the practice of employing a single-valued ratio, regardless of concentration of sugar, is hazardous, unless it has been demonstrated that the ratio is constant.

In column 10 are the ratios of weights of invert sugar to dextrose computed from Munson and Walker's table. If it is assumed that the reducing power of a sugar mixture is an additive property of the constituents, the ratios of invert sugar to dextrose can be extrapolated to those of levulose to dextrose, as is done in column 9. A comparison of these extrapolated with the experimental ratios (column 8) reveals a serious discrepancy. Either the rule of mixtures is inaccurate or some error exists in the experimental data. Such error may occur either in the present data for levulose or in Munson and Walker's values for invert sugar.

TABLE 11.—*Copper-reducing equivalents of dextrose and levulose by Munson and Walker's method*

Weight dextrose taken	Copper found	Copper from Munson and Walker's table	Weight levulose taken	Copper found	Weight levulose yielding same copper as dextrose	Ratio of weights of levulose to dextrose observed	Ratio from curve	Munson and Walker's table extrapolated to levulose	Ratio: Weights of invert sugar to dextrose (Munson and Walker)	Reducing power of levulose (reciprocal) of column 8
1	2	3	4	5	6	7	8	9	10	11
mg	*mg*	*mg*	*mg*	*mg*	*mg*					
200	375.1	374.7	213.1	374.9	213.1	1.066	1.067	1.056	1.028	0.937
200	375.9	374.7	212.5	374.1	213.5	1.068	1.067	1.056	1.028	.937
160	307.0	306.8	170.5	303.1	172.7	1.079	1.075	1.060	1.030	.930
120	234.6	235.4	127.9	231.0	129.8	1.082	1.082	1.065	1.033	.924
100	197.7	198.3	106.6	194.4	108.4	1.084	1.086	1.067	1.034	.921
85.75	173.0	171.5	94.03	173.6	93.7	1.093	1.089	1.070	1.035	.918
64.31	129.9	130.2	70.52	130.8	70.0	1.089	1.093	1.074	1.037	.915
42.88	88.8	87.9	47.01	88.8	47.0	1.097	1.097	1.079	1.040	.912

2. LANE AND EYNON'S VOLUMETRIC METHOD

More expeditious and in most instances more precise than the gravimetric estimation of total reducing sugar is the volumetric method of Lane and Eynon.[18] The method is described in the references cited and may be used without modification for the analysis of sugar mixtures containing levulose.

In their tabulation of empirical factors Lane and Eynon give the values for dextrose, invert sugar, and levulose. Inasmuch as levulose in natural products is almost invariably accompanied by dextrose in widely varying ratio, it has been found desirable to tabulate factors interpolated at 10 per cent intervals between pure dextrose and pure levulose. The interpolated factors are given in Table 22, page 439.

The degree of precision attainable by this method is to a considerable extent dependent upon the skill and experience of the analyst. As a corollary of this fact individual procedure and possible variations in the composition of the reagents may cause variations in the standard factors. It is therefore important that the analyst standardize his own analysis by titrating pure solutions of the respective sugars, thus ascertaining the correction to be applied to Lane and Eynon's tables. The correction can be applied uniformly to the tabulated factors or to the burette reading.

[18] J. Soc. Chem. Ind., vol. 42, p. 32, 1923. J. Assoc. Official Agri. Chem., vol. 9, p. 35, 1926.

The calculation of levulose in a levulose-dextrose mixture determined by methods to be described below is necessarily based upon the uncorrected factors interpolated from Lane and Eynon's data. For this purpose it is necessary to compute what the burette reading would be if Lane and Eynon's factors applied to the titration without correction. The correction to the burette reading (for 25 ml of Soxhlet reagent) is most conveniently determined by reference to the last three columns of Table 22, page 439.

The method of derivation of the quantities in this table is as follows: The factor correction found as described above by titrating 25 ml of Soxhlet solution with a standard reducing sugar solution is

$$c = \frac{S\,T}{100} - F \tag{15}$$

in which c = factor correction (difference between determined and tabulated factors), S = mg of reducing sugar per 100 ml in solution taken, T = observed titration, and F = factor in Lane and Eynon's table corresponding to the observed titration. Lane and Eynon's factors satisfy the equation

$$F_0 = (119.36 + 0.0471\ T_0 + 7.3\ R) \tag{16}$$

where T_0 = titration in ml and R = ratio of levulose to total reducing sugar. The factor for any titration is

$$F_1 = 119.36 + 0.0471\ T_1 + 7.3\ R + c$$

where c is the factor correction from equation (15). Since

$$100\frac{F}{T} = \text{mg sugar}$$

$$100\frac{F_0}{T_0} = 100\frac{F_1}{T_1}$$

or

$$\frac{119.36 + 0.0471\ T_0 + 7.3\ R}{T_0} = \frac{119.36 + 0.0471\ T_1 + 7.3R + c}{T_1}$$

hence

$$T_1 - T_0 = \frac{cT_0}{119.36 + 7.3\ R} \tag{17}$$

To solve equation (17) we must know the ratio of levulose to total reducing sugar as well as the titer and factor correction. Since in an unknown solution we do not know the ratio, R, and as T_0 will differ from T_1 by a very small amount, we can write as an approximation

$$T_1 - T_0 = \frac{cT_1}{119.36 + (7.3 \times 0.50)} = \frac{cT_1}{123.0} \tag{18}$$

in which we assume that the ratio is 0.50. The introduction of the approximation influences the value of the correction by a negligible amount, as the following example shows.

Assume that the observed titration of a pure levulose solution was 25.00 ml and the factor correction was 2.0. From equation (18) we obtain

$$T_1 - T_0 = 0.41 \text{ ml}$$

and

$$T_0 = 24.59 \text{ ml}$$

whereas from the more rigorous equation (17)

$$T_0 = 24.61 \text{ ml}$$

The difference between the two calculated values is within the experimental error of the titration.

The corrections in Table 22 were calculated from equation (18). It should be noted that when the factor correction, c, is positive, the titer correction is to be subtracted from the observed titer and vice versa.

VII. BIOURGE AND NYNS'S SELECTIVE DETERMINATION OF LEVULOSE

1. INTRODUCTORY

In an effort to distinguish between levulose and dextrose by reduction methods Biourge [19] made the important observation that at 50° C. the quantity of copper reduced in Ost's reagent by levulose was ten times as great as that reduced by dextrose. At a later period Nyns [20] elaborated the principle and determined the copper-levulose equivalents for a wide range of sugar concentrations. Notwithstanding Biourge's observation that dextrose had an appreciable reducing action under the conditions of the analysis, Nyns stated that neither dextrose nor other hexoses reduced even traces of copper. In a preliminary study of the method Jackson [21] found that dextrose exerted a reducing action equivalent to about one-thirteenth of the reducing power of levulose. This ratio appeared to be constant regardless of the relative concentrations of levulose and dextrose.

Important contributions to the method have been made by Zerban and Sattler [22] and by Schuette and Terrill.[23]

Nyns's method proved extremely serviceable for the estimation of levulose in numerous products. For immediate purposes it was used in unmodified form, but the long period of digestion (2½ hours) made it tedious and time consuming. In order to render the method more convenient and reliable we suggest the modifications described below which permit a shorter time of digestion, an accurate and rapid method of copper determination, occasional agitation during reduction, and higher concentrations of copper sulphate (25.3 g) [24] in Ost's reagent. The agitation of the solution during reduction was

[19] Bull. assoc. école sup. brasserie Louvain, January, 1898.
[20] Sucr. Belge, vol. 44, p. 210, 1924. Bull. assoc. école brasserie Louvain, vol. 25, p. 63, 1925. C. A., vol. 19, p. 1236, 1925.
[21] J. Assoc. Official Agri. Chem., vol. 9, p. 178, 1926.
[22] Ind. Eng. Chem., vol. 2, p. 307, 1930.
[23] J. Am. Chem. Soc., vol. 52, p. 4960, 1930.
Although the articles by Zerban and Sattler, and Schuette and Terrill, have a direct bearing on the present article, lack of available space prevents a detailed discussion of their conclusions.
[24] Nyns specified 15 g of copper sulphate. For a discussion of the concentration of copper see article by Zerban and Sattler, footnote 22.

suggested by Zerban and Sattler in order to obviate the unequal distribution of copper, an occurrence which we also had observed in analyzing samples of high levulose concentration.

2. EFFECT OF TIME AND TEMPERATURE UPON THE REDUCTION REACTION

In an effort to shorten the 2½-hour period of digestion specified by Nyns the reduction was carried out at various temperatures, the amount of copper reduced being determined at appropriate intervals of time. Parallel experiments were conducted in which the samples contained either pure levulose or levulose admixed with known weights of dextrose. The temperatures selected were 48.6°, 54.8°, and 58.8° C. The experimental results are shown diagrammatically in Figure 1. The reduction occurs relatively rapidly in the early stages

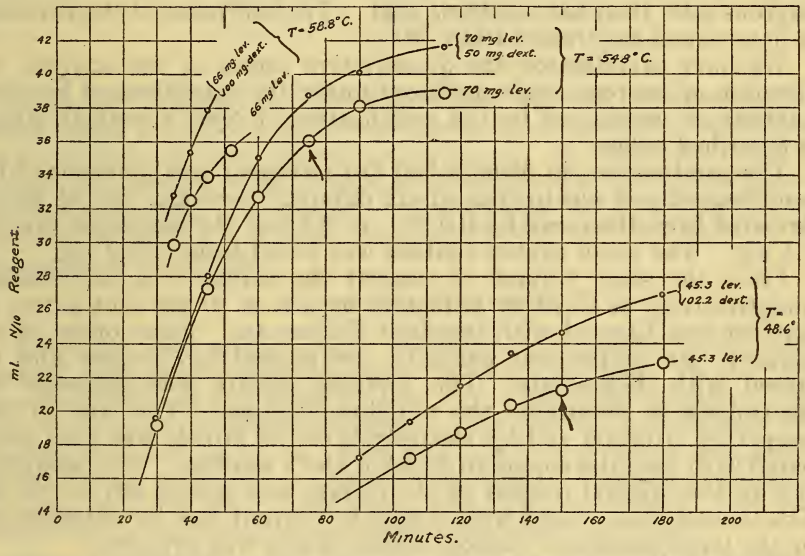

FIGURE 1.—*Rate of reduction of copper in Ost's solution by levulose at various temperatures*

of the reaction and continues more slowly for an indefinite period. The terminal point of 2½ hours selected by Nyns is evidently arbitrary and does not represent a definite end point. The filtrate from the reduced copper continues to reduce more copper even upon standing at room temperature.

The curves at 54.8° and even at 58.8° show a retardation in the rate of precipitation in the later stages of the reaction and permit the arbitrary selection of a terminal point. For the reduction at 54.8° the reaction approaches completion in about 75 minutes, or one-half the time specified by Nyns. This period was deemed sufficiently convenient for regular operation, and moreover, the reducing effect of dextrose was found to be about one-thirteenth of that of levulose, as had proved to be the case with Nyns's original method. For further investigation, therefore, the temperature of 55° C. and the time of 75 minutes were selected.

3. AN ELECTROMETRIC METHOD FOR THE DETERMINATION OF REDUCED COPPER

In Nyns's process for the selective determination of levulose the most tedious or most uncertain step has in the authors' experience been the determination of reduced copper. The most expeditious method is the permanganate titration process originally devised by Mohr and adopted by Nyns for his method. The permanganate method has in practice proved less reliable and convenient than theoretical considerations would lead one to expect and has, in general, had but limited application.

A priori considerations led to the belief that if potassium dichromate could be substituted for permanganate the defects inherent in processes which required the use of the latter reagent could be eliminated. Of greatest importance for the present purposes it permits the use of hydrochloric acid which has a greater solvent effect on cuprous salts than has sulphuric acid. The end point of the titration is determined electrometrically. [25]

We have investigated the quantitative aspect of the analysis by titration of cuprous oxide produced under the conditions of levulose analysis as carried out by the modification of Nyns's method, which is described below.

The total copper in 50.00 ml of Ost's reagent was determined by acidification and electrolysis of six different portions, five of which deviated from the mean by 0.0, 0.1, or 0.2 mg, the remaining one by 0.4 mg. The mean copper content was found to be 320.2 mg.

From the same volume of reagent the copper was precipitated quantitatively as Cu_2O by reduction by a 5 or 10 per cent excess of levulose and titrated with standard dichromate. From other 50 ml portions the copper was partially precipitated by levulose and titrated with dichromate. The acidified filtrate was subjected to electrolysis to determine the unreduced copper. The sum of the copper by titration and by electrolysis of the filtrate was then compared with the total copper in 50 ml of Ost's solution. The acidification of the original reagent or the filtrate was carried out in 500 ml Erlenmeyer flasks fitted with a trap to prevent loss by entrainment in the large volume of carbon dioxide which was evolved.

Electrolysis was carried out in a volume of about 200 ml containing 3 ml of sulphuric acid and 1.5 ml of nitric acid in excess of the acid required to neutralize the carbonate. Copper was deposited on platinum gauze electrodes by a current of about 0.18 ampere flowing for a period of about 17 hours. The absence of copper from the electrolyzed solutions and from the completely reduced Ost's solutions was assured by the absence of discoloration after addition of hydrogen sulphide. Experimental results are shown in Table 12.

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[25] G. S. Forbes and E. P. Bartlett, J. Am. Chem. Soc., vol. 35, p. 1527, 1913.

TABLE 12.—*Determinations of reduced copper in 50 ml of Ost's solution by electrometric dichromate titration*

[Total copper taken, 320.2 mg. 1 ml of dichromate equals 10 mg of copper]

Dichromate 0.1573 *N*	Cu in filtrate	Cu recovered	Deviation	Error
ml	*mg*	*mg*	*mg*	*Per cent*
32.06	0	320.6	+0.4	+0.13
32.01	0	320.1	−.1	−.03
32.05	0	320.5	+.3	+.09
32.05	0	320.5	+.3	+.09
32.04	0	320.4	+.2	+.06
32.03	0	320.3	+.1	+.03
32.01	0	320.1	−.1	−.03
30.18	18.1	319.9	−.3	−.09
30.23	17.9	320.2	0	0
30.32	17.1	320.3	+.1	+.03
27.18	48.2	320.0	−.2	−.06
23.43	85.9	320.2	0	0
Average	.....	320.26	±0.17	±0.05

Evidently the electrometric dichromate titration of cuprous oxide precipitated from Ost's solution is about as accurate as the electrolytic method and incomparably more rapid. An effort was made to extend the method to the determination of copper precipitated from Soxhlet's solution. The results were, however, less satisfactory than those cited above. Further experimentation will be required to determine its reliability.

4. STANDARD SOLUTIONS

Potassium dichromate.—This substance is available from commercial sources in very pure form. A quantity of the "C. P." salt was recrystallized and dried at 150° C. A standard solution, $N\times$-0.15730 was prepared by dissolving 7.7135 g of the purified salt and making to 1 liter at 22° C. One ml of such a solution is equivalent to 10.00 mg of copper. During subsequent titrations changes of volume resulting from deviations from this temperature were corrected for by assuming an expansion coefficient of 0.00020.

Ferrous ammonium sulphate.—A solution, $N\times 0.1573$, was prepared by dissolving 61.8 g of the commercial C. P. hexahydrate, adding 5 ml of concentrated H_2SO_4 and making to 1 liter. This solution lost about 0.3 per cent of its reducing power per day. The practice during the present investigation was to titrate it against the dichromate solution at the beginning and at the end of each series of analyses. During the few hours lapse of time no appreciable change was observable.

Ost's solution.—Dissolve 250 g of K_2CO_3 (anhydrous) in about 700 ml of hot water and add 100 g of pulverized $KHCO_3$. Agitate until completely dissolved. Cool and add with very vigorous agitation a solution of 25.3 g of pure $CuSO_4.5H_2O$ in 100 to 150 ml of water. Make to 1 liter and filter.

5. ANALYTICAL PROCEDURE

Transfer 50 ml of Ost's reagent to a 150 ml Erlenmeyer flask and add by means of an accurately graduated pipette a volume of the solution to be analyzed which contains not more than 92 mg of

levulose or its equivalent of a levulose-dextrose mixture, remembering that dextrose has about one-twelfth of the reducing power of levulose. Add enough water to make the total volume 70 ml. Immerse in a water bath regulated preferably within 0.1° C. at 55° C. Digest for exactly 75 minutes, agitating with a rotary motion at intervals of 10 or 15 minutes.

At the expiration of the prescribed time filter the precipitated copper on a closely packed Gooch crucible and wash flask and filter thoroughly without attempting to transfer the precipitate quantitatively. It is well, however, to transfer all of the loose-lying cuprous oxide, leaving in the flask only the small portion which adheres to the walls. Remove the asbestos mat by means of a glass rod and transfer to a 400 ml beaker. Add 5 or 10 ml of water and disintegrate the asbestos mat. Add a carefully measured volume of standard potassium dichromate ($N \times 0.1573$) in excess of the volume required to oxidize the cuprous oxide. In many cases the expected amount of precipitated copper will be roughly known and it will be possible to gage the volume of dichromate which will supply a 3 to 4 ml excess. If the amount of precipitated copper is not even roughly known it is preferable to add an amount which will supply an assured excess, since a very large excess introduces no uncertainty, provided its volume is accurately measured. Of this volume about 1 ml is added to the original reaction flask in order that the residual cuprous oxide may be dissolved and subsequently added to the remainder of the solution. Add to the Erlenmeyer flask by means of a graduated cylinder about 50 ml of 1:1 HCl. Pour slowly into the 400 ml beaker with constant stirring and continue to stir until the cuprous oxide is completely dissolved. Wash the Erlenmeyer with a jet from the wash bottle, receiving the rinsings in the beaker. Examine the asbestos critically by looking through the bottom of the beaker, which is held above the eye. If any undissolved cuprous oxide remains it can be clearly discerned as dark-colored particles. Immerse the crucible in the acidified solution to dissolve such cuprous oxide as remained in it. Remove the crucible with a glass rod, washing it free from solution. Dilute the solution as thus prepared to about 250 ml and titrate the excess of dichromate with ferrous sulphate electrometrically.

If a large number of samples require analysis the solutions may for convenience be allowed to await the titration after the addition of dichromate and hydrochloric acid.

6. DETERMINATION OF COPPER-LEVULOSE EQUIVALENTS

On each of six days a series of analyses was made to determine the copper reduced by varying amounts of levulose. The samples were introduced into the water bath at intervals of six or seven minutes. At the time each sample was immersed or removed all those previously introduced were momentarily agitated by a rotary motion to insure the uniform distribution of cupric copper. At the expiration of 75 minutes the solutions were filtered, washed, and prepared for titration by one worker, while the other performed the titration and prepared additional samples for analysis. By proceeding in this manner we were enabled to perform conveniently about 25 analyses in a period of about four hours. An analyst working alone can perform about 17 analyses in the same period of time.

The experimental results are assembled in Table 13 and plotted in Figure 2. The experiments were arranged in such a manner as to show the reproducibility of duplicate experiments performed, on the same day, on different days, and after an extended period of time. Three different preparations of Ost's solution were used for the analyses. One solution was used on March 26, 1931, and preserved for the final series on April 28 in order to be assured that no change in results occurred after an extended period of aging.

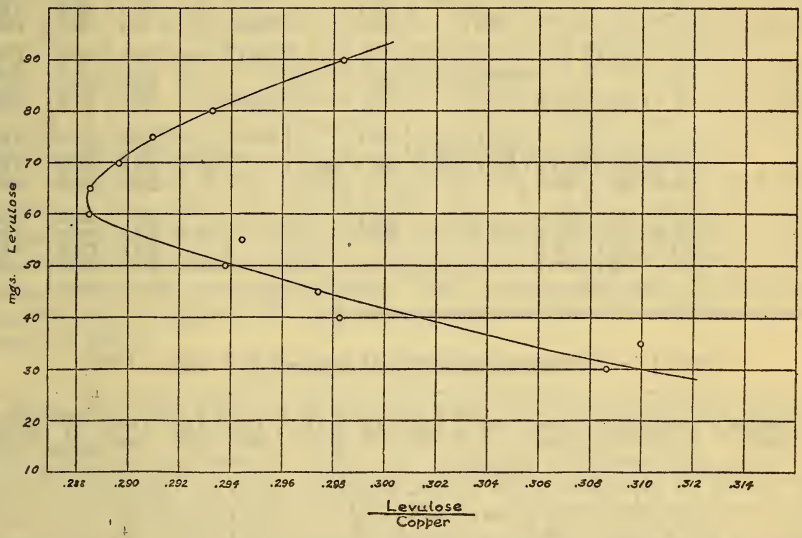

FIGURE 3.—*Showing the variation of the levulose-to-copper ratio with varied concentrations of levulose*

The averaged values of the copper precipitated by each quantity of levulose are given in column 11. The weights of levulose divided by the respective weights of copper yielded the factors which were serviceable for conversion of copper into its levulose equivalent. These factors are plotted as a function of copper in Figure 3.

Preliminary calculations made to adjust the experimental data by the method of least squares failed to yield an equation which represented the data accurately. The adjustment was accordingly made by drawing Figure 3 on a large scale. In determining the curve the points were weighted according to the number of determinations included in each average. The equivalents for integral weights of copper are tabulated in Table 23, page 440.

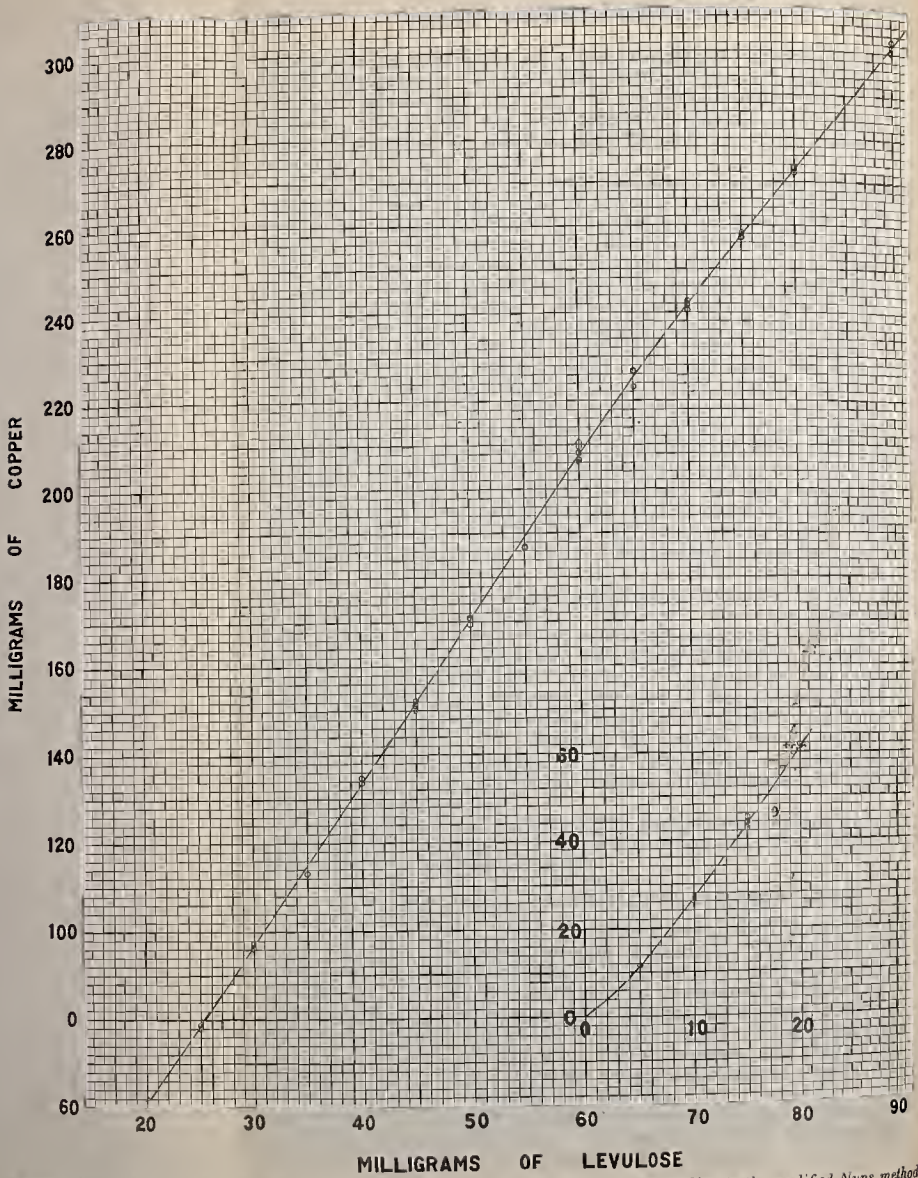

FIGURE 2.—*Copper in Ost's solution reduced by levulose in 75 minutes at 55° C. according to the modified Nyns method*

99675-32. (Face p. 427.)

TABLE 13.—*Copper-levulose equivalents*

[Milligrams of copper reduced by varying amounts of levulose]

Levulose (mg)	Mar. 26	Mar. 27	Mar. 31	Apr. 3	Apr. 3	Apr. 8	Apr. 8	Apr. 28	Apr. 28 [1]	Average	Adjusted	Ratio: Levulose / Copper
5						11.6				11.6	11.7	4.310
10				27.3	27.0	26.1		26.6		26.7	26.7	3.738
15	43.7		44.0			42.1		42.6	45.4	43.6	43.7	3.444
20						61.7				61.7	61.0	3.242
25				78.0		77.8		78.9		78.2	78.7	3.197
30	97.2	96.1	97.4					97.2	97.4	97.1	96.7	3.086
35				112.9						112.9	114.7	3.100
40				134.7		133.5				134.1	132.5	2.983
45	152.3	151.8	149.9					151.0	151.5	151.3	151.0	2.974
50				170.4		169.3		170.8		170.2	170.0	2.938
55				187.0	186.6					186.8	189.3	2.944
60	209.6	208.2	210.6	206.7	206.1	208.0	208.0		206.6	208.0	208.0	2.885
65	223.0	226.4	226.6							225.3	225.3	2.885
70	242.0	242.6	241.6			240.4				241.7	242.0	2.896
75	257.7	258.3	257.9	257.8		257.2		257.8	258.1	257.8	257.8	2.909
80	272.1	273.1	273.3							272.8	273.0	2.933
90	301.5	302.7	300.1						302.2	301.6	301.5	2.984

[1] Analyses performed with same Ost's solution as on Mar. 26.

TABLE 14.—*Deviations of individual analyses from adjusted table*

Levulose (mg)	Mar. 26	Mar. 27	Mar. 31	Apr. 3	Apr. 3	Apr. 8	Apr. 8	Apr. 28	Apr. 28 [1]	Average	Sum of errors	Mean deviation
5						−0.1				−0.1		
10				+0.6	+0.3	−.6		−0.1		0		
15	0		+0.3			−1.6		−1.1	+1.7	−.1		
20						+.7				+.7		
25				−.7		−.9		+.2		−.5	+6.2	
30	+0.5	−0.6	+.7					+.5	+.7	−.6	−5.7	0.63
35				−1.8						−1.8		
40				+2.2		+1.0				+1.6		
45	+1.3	+.8	−1.1						+.5	+.3		
50				+.4		−.7		+.8		+.2		
55				−2.3	−2.7					−2.5	+11.4	
60	+1.6	+.2	+2.6	−1.3	−1.9	0	0		−1.4	0	−13.2	1.2
65	−2.3	+1.1	+1.3							0		
70	0	+.6	−.4			−1.6				−.3		
75	0	+.5	+.1	0		−.5		0	+.4	0		
80	−.9	+.1	+.3							−.2	+6.3	
90	0	+1.2	−1.4						+.7	+.1	−7.1	.6

[1] Analyses performed with same Ost's solution as on Mar. 26.

7. DISCUSSION OF ERRORS

In Table 14 are shown the deviations of the individual analyses from the adjusted table of equivalents. For convenience of critical study these experiments have been divided into three groups representing, respectively, high, medium, and low concentrations of levulose. The essential agreement of positive with negative residuals in each group shows that the adjusted table represents fairly the experimental data. For the whole series the average deviation of a single experiment is 0.8 mg of copper. In the group of high levulose concentrations (65 to 90 mg) the mean error of a single determination is 0.6 mg of copper. The average weight of copper reduced in this group of analyses was

273 mg. The mean error of analysis was therefore but 0.2 per cent of the quantity measured.

In the median range of levulose concentrations (35 to 60 mg) the average deviation from the adjusted copper equivalents is 1.2 mg of copper. This error is twice as great as that in either of the two other groups. The reason for this is not far to seek, for an inspection of Figure 3 shows that this is the region of greatest curvature of the levulose-copper ratio, and relatively large variations occur with slight alterations of conditions. The average weight of copper reduced in this group of analyses was 176 mg. The average error of a single experiment was, therefore, 0.7 per cent of the quantity measured.

In the range of low levulose concentrations (5 to 30 mg) the mean error of a single determination was 0.63 mg of copper. The average weight of copper reduced was 58.8 mg. The average error of a single analysis was, therefore, 1.1 per cent of the quantity measured.

In recapitulation the mean errors in the high, median, and low groups of analyses were, respectively, 0.2, 0.7, and 1.1 per cent of the quantities measured. This result corroborates the prediction made by Jackson [26] that the higher concentrations of copper in Ost's solution, permitting correspondingly high concentrations of levulose, yielded analytical results of greater precision than the Ost's reagent used by Nyns.

8. EFFECT OF DEXTROSE

In conjunction with the analyses of pure levulose solutions described above similar analyses were made on levulose-dextrose mixtures. The experimental results which are arranged in descending order of ratio of levulose to total reducing sugar are assembled in Table 15. The total copper precipitated (column 4) was converted into its levulose equivalent by reference to Table 23, page 440. The result is designated in column 5 of Table 15, "apparent levulose." The difference between apparent levulose and the true levulose taken represents in terms of milligrams of levulose the reducing effect of the weight of dextrose taken. The quotient of the weight of dextrose divided by the excess of apparent over the true levulose is the number of milligrams of dextrose which is equivalent in reducing power to 1 mg of levulose. These values are given in column 6 of Table 15 and also in Table 16, which permits a more discriminating analysis of the results. It is at once apparent from Table 16 that even for extreme variations of levulose and dextrose concentrations no considerable systematic deviations from a constant dextrose equivalent of levulose occur. The experiments include a range of ratios of levulose to total reducing sugar (column 1 of Table 15) varying from 2.9 to 75 per cent. No attempt was made to determine the reducing action of dextrose for mixtures of higher levulose ratio, since for such mixtures the total effect of dextrose amounts to a very small correction, and any probable deviation from a constant reducing power would be negligible.

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[26] J. Assoc. Official Agri. Chem., vol. 13, p. 200, 1930.

TABLE 15.—*Reduction of levulose–dextrose mixtures*

[Modified Nyns method]

Ratio: Levulose / Total sugar	Levulose taken	Dextrose taken	Copper precipitated	Apparent levulose found	Mg Dextrose ≅ 1 mg levulose	Dextrose taken ÷12.4	Corrected levulose found [1]	Error of levulose taken
Per cent	*mg*	*mg*	*mg*	*mg*		*mg*	*mg*	*Per cent*
100.0	75	0	257.8	75.0				
75.0	75	25	264.9	77.4	10.4	2.0	75.4	+0.5
75.0	50	16.7	174.7	51.3	12.8	1.35	49.95	−.1
61.6	80	50	284.3	83.9	12.8	4.0	79.9	−.1
58.4	70	50	255.6	74.3	11.6	4.1	70.2	+.3
54.5	60	50	223.7	64.5	11.1	4.1	60.4	+.7
50.0	75	75	276.3	81.1	12.3	6.1	75.0	0
50.0	50	50	184.6	53.8	12.8	4.0	49.8	−.2
47.4	45	50	164.7	48.7	13.5	4.0	44.7	−.7
45.5	25	30	87.6	27.5	12.0	2.4	25.1	+.4
44.2	80	100	295.2	87.7	13.0	8.1	79.6	−.5
41.2	70	100	268.0	78.4	11.9	8.1	70.3	+.4
Mean error								0.35
37.5	60	100	240.3	69.5	10.5	8.1	61.4	+2.3
37.5	30	50	113.0	34.6	10.9	4.05	30.55	+1.8
33.3	75	150	293.6	87.1	12.3	12.1	75.0	0
31.0	45	100	179.9	52.6	13.1	8.1	44.5	−1.1
25.0	50	150	211.3	61.0	13.7	12.1	48.9	−2.2
25.0	25	75	99.3	30.9	13.3	6.1	24.8	−.6
23.1	30	100	126.5	38.3	12.0	8.1	30.2	−.7
23.1	15	50	59.6	19.6	10.9	4.1	15.5	+3.3
16.7	60	300	286.3	84.5	12.2	24.25	60.25	+.4
15.4	50	275	244.9	71.0	13.1	22.2	48.8	−2.4
Mean error								1.5
13.0	15	100	74.9	24.0	11.1	8.1	15.9	+6.0
10.4	40	350	225.0	64.9	14.0	28.3	36.7	−8.2
9.8	30	275	180.8	52.8	12.1	22.2	30.6	+2.0
8.3	45	500	282.9	83.4	13.0	40.4	43.4	−3.6
5.7	30	500	238.1	68.8	12.9	40.4	28.4	−5.3
4.8	25	500	207.7	60.0	14.3	40.4	19.6	−21.6
2.9	15	500	178.7	52.3	13.4	40.4	11.9	−20.6
Mean error								9.8

[1] Column 5 minus column 7.

The mean of all the values given in Table 16 is 12.4. The most generally useful part of the table will be that which is applicable to samples whose compositions lie in the lower left quadrant bounded by levulose, 45 to 80 mg, and dextrose, to 150 mg. Such samples may range from 20 to 100 per cent in ratio of levulose to total reducing sugar. Within this quadrant the mean value for the reducing action of dextrose is 12.3 in essential agreement with the average of all determinations made. If we take the upper left-hand quadrant bounded by levulose, 15 to 50 mg, and dextrose, to 150 mg (overlapping the quadrant previously considered), the mean value is 12.3. The upper right-hand quadrant bounded by levulose, 15 to 50 mg, and dextrose, 250 to 500 mg, has the mean value 13.1.

TABLE 16.—*Reducing action of dextrose by the modified Nyns method*

[Tabulated figures indicate the number of milligrams of dextrose which is equivalent in reducing power to 1 mg of levulose]

Dextrose / Levulose	16.7	25	30	50	75	100	150	250	275	300	350	500
15				10.9		11.0						13.4
25			12.0		13.3							14.3
30				10.9		12.0			12.0			12.9
40											14.0	
45				13.5		13.7						13.0
50	12.8			12.8			13.7		13.1			
60				11.1		10.5				12.2		
70				11.6		11.9		13.2				
75		10.4			12.3		12.3					
80				12.8		13.0						

Viewed as a whole the data in Table 16 seem to indicate that for constant amounts of dextrose, its reducing effect is practically independent of the concentration of levulose. For greatly increased concentrations of dextrose (for example, 350 to 500 mg) its reducing power seems to decrease slightly. The most disturbing feature is the variability of individual analyses. These variations will, however, seriously affect the precision of the levulose analysis only when the dextrose content of the sample is relatively high.

In order to ascertain the degree of precision which may be expected in the analysis of levulose-dextrose mixtures, we have divided Table 15 into three sections, in each of which the mean error of analysis has been computed. The first group of analyses indicates that sugar mixtures ranging from 40 to 100 per cent levulose can be analyzed with a mean error of about 0.35 per cent, which is but slightly greater than the error of analysis of pure levulose solutions. Samples ranging from about 15 to 40 per cent ratio of levulose to total sugar can be analyzed with a mean error of about 1.5 per cent.

Below a 15 per cent ratio the dextrose corrections become very large, and the errors of analysis increase greatly. Even within this range, however, the analytical results have some significance. One could, for example, determine the ratio of levulose to total sugar within one-half of 1 unit. For such low ratios of levulose it is probable that a direct determination of dextrose [27] would yield more reliable results.

9. EFFECT OF SUCROSE

A limited number of analyses were made of levulose-sucrose mixtures in order that the modified method described above might be available for the determination of levulose in the products of the cane-sugar industry. In harmony with the conclusions of Zerban and Sattler we find that the effect of sucrose is small. The analytical results are given in Table 17. The weight of copper precipitated by sucrose in the presence of levulose can be represented by the formula:

$$\text{mg Cu} = 3.32\,S - 0.31\,S^2 + 0.27 \qquad (19)$$

in which S is the number of grams of sucrose. The equation is valid only between 1 and 5 g of sucrose.

[27] Kline and Acree, B. S. Jour. Research, vol. 5 (RP247), p. 1063, 1930.

TABLE 17.—*Reducing effect of sucrose in the presence of levulose by the modified Nyns method*

Levulose	Sucrose	Copper precipitated	Copper [1] precipitated by levulose	Copper precipitated by sucrose	Average	Calculated copper [2]
mg	*g*	*mg*	*mg*	*mg*	*mg*	
10	1	30.5	26.7	3.8		
25	1	82.1	78.7	3.4	3.3	3.3
50	1	172.6	170.0	2.6		
75	1	261.1	257.8	3.3		
50	2	177.2	170.0	7.2	5.65	5.65
75	2	261.9	257.8	4.1		
10	5	35.3	26.7	8.6		
25	5	87.8	78.7	9.1	9.0	9.0
50	5	179.3	170.0	9.3		
0	5	2.4				

CALCULATED COPPER PRECIPITATED BY INTEGRAL WEIGHTS OF SUCROSE [2]

Sucrose.....g..	1	2	3	4	5
Copper.....mg..	3.3	5.7	7.4	8.5	9.0

[1] From Table 23, p. 440.
[2] Cu (mg)=3.32 S−0.31 S^2+0.27.

VIII. ANALYSIS OF SUGAR MIXTURES CONTAINING LEVULOSE

1. INTRODUCTORY

There are six general methods of analysis from which it is theoretically possible to select three in order to determine the three quantities—total solids, levulose, and dextrose. If, in addition, sucrose is present a Clerget analysis must be performed. Aside from the Clerget analysis the methods to be considered are: (1) Total dry substance by density, refractive index, or desiccation; (2) total reducing sugar by Lane and Eynon titration or other method; (3) Nyns's levulose analysis; (4) aldose by the method of Kline and Acree; (5) direct polarization; and (6) levulose by temperature coefficient.

It is well known that desiccation processes for the determination of total solids are difficult if the sample contains levulose. The difficulty is greatly increased when levulose becomes the predominating substance. We have, therefore, in the early sections of the present article given particular attention to the determination by densimetric and refractometric methods. While these methods yield only apparent dry substance it is probable that the values (especially those derived from refractive index) will be more serviceable than those derived from desiccation methods.

From a mixture containing no optically active or reducing substance but levulose and dextrose, a combination of any two of the methods 2 to 6 (except the combination 3 and 6) will yield a complete sugar analysis. In the presence of other optically active substances only combinations 2 and 3, 2 and 4, and 3 and 4 will yield correct results. Combinations 2 and 3, and 2 and 5 will be considered in detail. Other combinations, and in particular the question of purity determination, will be discussed in subsequent articles.

2. DETERMINATION OF LEVULOSE AND DEXTROSE IN MIXTURES BY POLARIZATION AND LANE AND EYNON TITRATION. THE MATHEWS FORMULA

This combination of analytical processes and the method of calculation described below are valid under the assumptions that the only optically active or reducing substances present in the sample are dextrose and levulose and that the rotation of a sugar mixture is the algebraic sum of the rotations of the constituents whose specific rotations are referred to the concentration of total sugar rather than to the partial concentration of each.[28] The calculations below are also based upon the assumption that the titrations are made with reagents and procedure to which Lane and Eynon's factors apply without correction. If necessary, the burette readings should be corrected in the manner described on p. 439 (Table 22).

While the method of determination applies strictly only to pure mixtures of levulose and dextrose, it may sometimes be applied to crude mixtures to yield a proximate analysis by neglecting the errors due to optically active impurities. If the sample in question has previously been subjected to acid hydrolysis the error introduced in the analysis of plant juices is usually small.

For a constant ratio of levulose to total reducing sugar the polarization (P) will vary with the concentration of total sugar, while the titration (T) will vary inversely as the concentration. Since for the reducing sugar analysis we usually must dilute the sample, the product, $P \times T$, will vary with the dilution (D), but the product, $\frac{P \times T}{D}$, will vary but slightly with dilution. It is therefore possible to tabulate in a brief space the ratios of levulose to total sugar as functions of the quantities $\frac{P \times T}{D}$ and T. The tabulation is given in Table 24, page 442.

For convenience this method of solution will be referred to in subsequent articles as the Mathews formula.[29]

The rotation of any mixture in saccharimeter degrees at 20° C. is given by

$$P_{(t=20°)} = -[5.31154 + 0.0064928(x+y)]x + [3.03537 + 0.0020837(x+y)]y, \quad (20)$$

where x and y are the concentrations of levulose and dextrose, respectively, in grams per 100 ml.

The total reducing sugar, S, as determined by Lane and Eynon's method is given by

$$S = \frac{100F}{T} \quad (21)$$

T is the titer in ml and is corrected to correspond to the factor F (see p. 421). F is given approximately by the relation

$$F = 119.36 + 0.0471\ T + 7.3\ R \quad (16)$$

where $R = \frac{x}{x+y}$.

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[28] Vosburgh, J. Am. Chem. Soc., vol. 43, p. 219, 1921.

[29] This ingenious method of calculation was devised by the junior author. (R. F. J.)

S in equation (21) is related to the total reducing sugar by

$$DS=x+y \tag{22}$$

where D is the number of volumes to which one volume of the solution polarized is diluted for the Lane and Eynon titration.

In constructing Table 24, page 442, the quantity $\frac{P\times T}{D}$ was computed for selected values of T, D, and R from equations (20), (21), and (22). Since $\frac{P\times T}{D}$ is not exactly linear with RD as varies, a small correction, $c=\frac{f\times D}{T}$, must be applied in which f is a factor given in a supplementary table. An example illustrating the operation of the table is given on page 440.

3. CALCULATION OF LEVULOSE IN A LEVULOSE-DEXTROSE MIXTURE FROM ANY METHOD OF TOTAL REDUCING SUGAR ANALYSIS AND THE MODIFIED NYNS'S METHOD

If a sample has been analyzed for total reducing sugar by any selected method and for levulose by the modification of Nyns's method described above, the resulting data are sufficient for a calculation of levulose and dextrose by a general method of successive approximations. Compute from the reducing sugar analysis the total reducing sugar in a given volume or weight expressed as dextrose, unless the approximate composition of the sample is known. In the latter case refer the copper to the sugar mixture of that composition. From the Nyns's analysis compute the apparent levulose in the same volume or weight of sample. Subtract the apparent levulose from the total sugar to obtain the apparent dextrose. Divide the apparent dextrose by 12.4 and subtract the quotient from the apparent levulose to obtain a new approximation to the levulose content. Subtract the approximate levulose from the total sugar to obtain a truer value for the dextrose, again divide by 12.4, continue the approximation in the same manner until successive calculations show a negligible change in the levulose. For a final calculation the copper reduced in the total sugar analysis must be referred to the sugar mixture of the finally determined composition.

If the sample under examination contains sucrose which has been determined by Clerget analysis the copper precipitated in the Nyns's analysis is first corrected for the sucrose by Table 17 before the method of approximations is applied.

If the total sugar is determined by Lane and Eynon titration and levulose by the modified Nyns's method the tedious calculation described above can be avoided by the method described below.

4. CALCULATION OF LEVULOSE IN A LEVULOSE-DEXTROSE MIXTURE FROM LANE AND EYNON TITRATION AND THE MODIFIED NYNS'S METHOD

In order to avoid the tedious procedure described in the previous paragraph a convenient method of solution was devised applicable when reducing sugar is determined by Lane and Eynon's method.

Lane and Eynon's factors for levulose-dextrose mixtures (Table 22, p. 439) may be expressed by

$$F = 119.36 + 0.0471\ T + 7.3\ R \tag{16}$$

in which F is the factor, T the titer, and R the ratio of levulose to total reducing sugar. The total reducing sugar (S) in a sample is

$$\frac{100\ F}{T} = S \tag{21}$$

whence

$$100\ \frac{119.36 + 0.0471\ T + 7.3\ R}{T} = S \tag{23}$$

The true levulose is

$$L = SR$$

and the apparent levulose is

$$\begin{aligned} l &= L + 0.0808\ S(1-R) \\ &= 0.9192\ SR + 0.0808\ S \end{aligned}$$

whence

$$S = \frac{l}{0.9192\ R + 0.0808} \tag{24}$$

Combining equations (23) and (24)

$$\frac{T \times l}{100} = (119.36 + 0.0471\ T + 7.3\ R)\ (0.9192\ R + 0.0808) \tag{25}$$

Equation (25) was solved for varying R and T and plotted in a series of curves in each of which T was constant. These curves were found to be very nearly straight lines, and even for widely varying values of T were but slightly displaced from each other. It was, therefore, possible to tabulate a complete series of values of R for relatively few values of T. In solving the analytical data it is merely necessary to multiply the burette reading for the Lane and Eynon titration by the milligrams of apparent levulose in 100 ml and refer the product divided by 100 to Table 25, page 444. Under the proper titration, T (or interpolated between two adjacent columns), the ratio of levulose to total sugar is read directly. An example showing the method of operation is given on page 444.

IX. SUMMARY

1. The densities of aqueous levulose solutions are expressed by

$$D_4^{20} = 0.99823 + 0.0038893\ p + 0.0000140\ p^2$$
$$D_4^{25} = 0.99708 + 0.0038557\ p + 0.0000139\ p^2$$

which are valid between 0 and 20 per cent

$$D_4^{20} = 0.99936 + 0.0037842\ p + 0.0000164\ p^2$$

valid between 20 and 70 per cent.

2. The mean expansion coefficient between 20° and 25° C. is

$$\frac{\Delta D}{\Delta t} = -(0.000231 + 0.00000672\ p + 0.0000000224\ p^2)$$

valid between 0 and 20 per cent, and

$$\frac{\Delta D}{\Delta t} = -(0.0002145 + 0.00000795\ p + 0.0000000136\ p^2)$$

valid between 20 and 70 per cent.

3. Corrections to convert the readings of Brix hydrometers standard for sucrose solutions to percentage of levulose have been computed and tabulated.

4. The density of crystalline levulose $\left(\frac{20°}{4°}\right)$ has been found to be 1.598.

5. Refractive indices of levulose solutions are represented by the formulas:

$$n_D^{20} = 1.33300 + 0.0014159\ p + 0.00000491\ p^2$$

$$n_D^{25} = 1.33252 + 0.0014059\ p + 0.00000487\ p^2$$

which are valid between 0 and 20 per cent levulose, and

$$n_D^{20} = 1.33344 + 0.0013625\ p + 0.000006645\ p^2$$

$$n_D^{25} = 1.33312 + 0.0013415\ p + 0.000006762\ p^2$$

which are valid between 20 and 63 per cent levulose, and

$$n_D^{20} = 1.33377 + 0.0013570\ p + 0.000006680\ p^2$$

$$n_D^{25} = 1.33345 + 0.0013360\ p + 0.000006800\ p^2$$

valid between 63 and 90 per cent levulose.

6. The saccharimetric normal weight of crystalline levulose is 18.407 at 20° C. and 19.003 at 25° C.

7. The normal weights of dilute solutions are represented by

$$W_{(t=20)} = 18.803 - 0.01801\ c - 0.000191\ c^2$$

$$W_{(t=25)} = 19.446 - 0.02061\ c - 0.000141\ c^2$$

in which c = grams of levulose in 100 ml.

8. A table of concentration corrections for constant normal weights has been computed.

9. For the temperature interval 20° to 70° C. each gram of levulose in 100 ml decreases 0.0344° S in rotation for each degree rise in temperature. This value appears to be independent of concentration of sugar.

10. Determinations of the copper reduced by levulose in Munson and Walker's method have been made. It is shown that the ratios of the reducing powers of dextrose to levulose are not constant, but are a function of concentration of sugar.

11. A modification of the Nyns selective reduction method for levulose has been suggested which permits a performance of the analysis in about one-half the time previously specified.

12. An accurate and rapid electrometric method of cuprous oxide analysis by dichromate titration is described.

13. The copper reduced by pure levulose by the modified Nyns method has been determined and tabulated.

14. The reducing power of dextrose has been found to be essentially independent of the concentration of dextrose or levulose, 12.4 mg of dextrose being equivalent in reducing power to 1 mg of levulose under the conditions prescribed for the modified Nyns's method.

15. The reducing effect of sucrose has been found to be

$$mg\ Cu = 3.32\ S - 0.31\ S^2 + 0.27$$

in which S is the number of grams of sucrose. The formula is valid only between 1 and 5 g of sucrose.

16. Methods of calculation of the concentration of levulose in samples analyzed by optical and chemical methods are described.

X. APPENDIX

TABLE 18.—*Densities of levulose solutions and mean density and expansion coefficients between 20° and 25° C.*

[All weights corrected to vacuum]

Levulose	D_4^{20}	D_4^{25}	$\frac{-\Delta D}{\Delta t}$	$\frac{\Delta v}{\Delta t}$	Levulose	D_4^{20}	D_4^{25}	$\frac{-\Delta D}{\Delta t}$	$\frac{\Delta v}{\Delta t}$
Per cent			$\times 10^{-6}$	$\times 10^{-6}$	*Per cent*			$\times 10^{-5}$	$\times 10^{-5}$
0	0.99823	0.99708	231	231	36	1.1568	1.1544	48	42
1	1.00214	1.00095	238	237	37	1.1618	1.1593	49	42
2	1.00607	1.00484	245	243	38	1.1668	1.1643	50	43
3	1.01003	1.00877	252	249	39	1.1718	1.1693	50	43
4	1.01402	1.01272	259	255	40	1.1769	1.17435	51	43
5	1.01803	1.01670	266	261	41	1.1820	1.1794	52	44
6	1.02207	1.02071	273	267	42	1.1872	1.1845	53	44
7	1.02614	1.02475	280	273	43	1.1923	1.1897	53	45
8	1.03024	1.02881	287	278	44	1.1975	1.19485	54	45
					45	1.2028	1.20005	55	45
9	1.03437	1.03290	294	284					
10	1.03853	1.03702	301	290	46	1.20805	1.2053	55	46
11	1.04271	1.04118	308	295	47	1.2134	1.2106	56	46
12	1.04692	1.04535	315	300	48	1.2187	1.2159	57	46
					49	1.2241	1.2212	57	47
13	1.05116	1.04955	323	307	50	1.2295	1.2266	58	47
14	1.05543	1.05378	330	313					
15	1.05972	1.05804	337	318	51	1.2349	1.2320	59	47
16	1.06405	1.06233	345	324	52	1.2404	1.2374	59	48
					53	1.2459	1.2429	60	48
17	1.06840	1.06664	352	329	54	1.2514	1.2484	60	48
18	1.07278	1.07098	360	336	55	1.2570	1.2539	61	49
19	1.07719	1.07535	367	341					
20	1.08162	1.07975	375	347	56	1.2626	1.2595	62	49
			$\times 10^{-5}$	$\times 10^{-5}$	57	1.2682	1.2651	62	49
21	1.08606	1.0842	38	35	58	1.2739	1.2707	63	50
22	1.09055	1.0886	38	35	59	1.2796	1.2764	64	50
23	1.09507	1.0931	39	36					
24	1.09962	1.0976	40	36	60	1.2853	1.2821	64	50
25	1.10420	1.1022	41	37	61	1.2911	1.2878	65	50
					62	1.2969	1.2936	66	51
26	1.1088	1.10675	41	37	63	1.3027	1.2994	66	51
27	1.11345	1.11135	42	38					
28	1.1181	1.1160	43	38	64	1.3086	1.3052	67	51
29	1.1229	1.1207	43	39	65	1.3145	1.3111	67	51
30	1.1276	1.1254	44	39	66	1.3204	1.3170	68	51
					67	1.3263	1.3229	69	52
31	1.1324	1.13015	45	40					
32	1.1372	1.1349	46	40	68	1.3323	1.3289	69	52
33	1.14205	1.1397	46	40	69	1.3384	1.3349	70	52
34	1.1469	1.1446	47	41	70	1.3444	1.3409	70	52
35	1.15185	1.1495	48	41	71	1.3505	1.3470	71	53

TABLE 19.—*Corrections to readings of Brix hydrometers immersed in levulose solutions*

[The corrections at 20° C. were determined by referring the densities of levulose solutions of known concentration to the sucrose density tables of Plato. The corrections at other temperatures are the algebraic sum of the corrections at 20° C. and the temperature corrections for sucrose multiplied by the ratios of the expansion coefficients of levulose and sucrose. The hydrometer is assumed to be constructed of Jena 16 III glass. Compare B. S. Circular No. 19, p. 23.]

[Standardized for sucrose solutions at 20° C.]

Temperature ° C.	Observed Brix														
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70
	Corrections														
18	−0.09	−0.16	−0.20	−0.26	−0.29	−0.30	−0.29	−0.29	−0.26	−0.22	−0.21	−0.09	+0.01	+0.12	+0.23
19	−.05	−.10	−.15	−.20	−.22	−.21	−.22	−.20	−.17	−.13	−.08	.00	+.10	+.21	+.32
20	.00	−.05	−.09	−.13	−.15	−.14	−.13	−.12	−.09	−.05	+.02	+.10	+.20	+.31	+.44
21	+.04	.00	−.02	−.06	−.08	−.05	−.04	−.04	−.01	+.05	+.08	+.20	+.30	+.42	+.56
22	+.10	+.06	+.03	+.01	−.01	+.02	+.04	+.05	+.06	+.13	+.22	+.30	+.40	+.52	+.65
23	+.16	+.12	+.10	+.07	+.08	+.10	+.13	+.13	+.17	+.23	+.32	+.40	+.51	+.62	+.76
24	+.21	+.19	+.17	+.15	+.16	+.19	+.21	+.23	+.27	+.33	+.42	+.50	+.61	+.73	+.87
25	+.27	+.25	+.25	+.23	+.23	+.27	+.30	+.32	+.36	+.41	+.50	+.59	+.70	+.82	+.96
26	+.33	+.32	+.31	+.30	+.33	+.35	+.38	+.41	+.46	+.52	+.60	+.70	+.81	+.93	+1.08
27	+.40	+.39	+.38	+.38	+.40	+.45	+.48	+.51	+.55	+.60	+.70	+.80	+.92	+1.04	+1.18
28	+.46	+.45	+.46	+.46	+.50	+.54	+.57	+.61	+.64	+.70	+.80	+.90	+1.02	+1.14	+1.29

TABLE 20.—*Refractive indices of levulose solutions*

[All data computed from weights in air with brass weights. Immersion readings are referable solely to the scale of arbitrary units proposed by Pulfrich (Zeit. angew. Chemie, p. 1186; 1899). According to this scale, 14.5=1.33300; 50.0=1.34650; and 100.0=1.36464]

Per cent	$n^{20°}_D$	Zeiss immersion reading 20°	$n^{25°}_D$	Zeiss immersion reading 25°	$\frac{-\Delta n}{\Delta t°}$	Per cent	$n^{20°}_D$	$n^{25°}_D$	$\frac{-\Delta n}{\Delta t°}$	Per cent	$n^{20°}_D$	$n^{25°}_D$	$\frac{-\Delta n}{\Delta t°}$
					$\times 10^{-6}$				$\times 10^{-6}$				$\times 10^{-5}$
0	1.33300	14.50	1.33252	13.25	96	32	1.38385	1.38297	175	64	1.4479	1.4467	24
1	3442	18.18	3393	16.90	98	33	8564	8476	177	65	501	489	24
2	3585	21.87	3535	20.58	100	34	8745	8655	180	66	524	512	24
3	3729	25.63	3678	24.29	102	35	8927	8836	183	67	547	535	24
4	3874	29.42	3822	28.05	104	36	9111	9018	185	68	569	557	24
5	4020	33.26	3967	31.87	106	37	9295	9201	188	69	592	580	24
6	4167	37.14	4113	35.71	108	38	9481	9386	190	70	615	602	24
7	4315	41.05	4260	39.61	110	39	9669	9573	192	71	638	625	24
8	4464	45.03	4408	43.53	112	40	9858	9760	195	72	661	648	24
9	4614	49.05	4557	47.53	115	41	1.40048	9949	197	73	684	672	25
10	4765	53.11	4707	51.54	117	42	0239	1.40140	199	74	708	695	25
11	4917	57.19	4857	55.57	119	43	0432	0331	202	75	731	719	25
12	5070	61.32	5009	59.68	121	44	0625	0524	204	76	755	742	25
13	5224	65.51	5162	63.81	123	45	0821	0718	206	77	779	766	25
14	5379	69.75	5316	68.00	125	46	1018	0914	208	78	803	790	25
15	5534	74.03	5470	72.25	127	47	1216	1111	210	79	827	814	25
16	5691	78.36	5626	76.56	129	48	1415	1309	213	80	851	838	25
17	5849	82.75	5783	80.92	132	49	1616	1509	214	81	875	862	25
18	6008	87.17	5942	85.28	135	50	1818	1710	216	82	900	887	25
19	6169	91.64	6102	89.71	137	51	2021	1912	218	83	924	912	25
20	6332	96.14	6262	94.17	139	52	2226	2117	219	84	949	936	25
21	6496	100.72	6425	98.71	142	53	2432	2322	221	85	974	961	25
22	6659	105.37	6588	103.31	146	54	2640	2528	223	86	999	986	25
23	6827	-------	6753	-------	148	55	2848	2736	224	87	1.5024	1.5011	25
24	6996	-------	6921	-------	151	56	3058	2945	226	88	049	036	25
25	7166	-------	7088	-------	154	57	3270	3156	228	89	074	062	25
26	7336	-------	7258	-------	157	58	3482	3368	229	90	100	087	25
27	7506	-------	7426	-------	161	59	3696	3581	231	91	126	113	25
28	7680	-------	7598	-------	164	60	3913	3797	232	92	151	139	25
29	7854	-------	7771	-------	166	61	4130	4014	233	93	177	165	25
30	8030	-------	7945	-------	169	62	4348	4232	235	94	203	191	25
31	8207	-------	8121	-------	172	63	4569	4451	236	95	230	217	25

TABLE 21.—*Corrections to be applied to saccharimetric readings of levulose solutions when a constant normal weight is used*

[Normal weight at 20° C.=18.407 gm; normal weight at 25° C.=19.003 gm]

Polarization	Correction		Polarization	Correction		Polarization	Correction	
	20° C.	25° C.		20° C.	25° C.		20° C.	25° C.
0	0.00	0.00	40	+0.54	+0.57	75	+0.44	+0.47
5	+.10	+.11	45	.56	.60	80	.38	.40
10	.19	.21	50	.57	.61	85	.30	.33
15	.28	.30	52	.58	.61	90	.21	.23
20	.35	.39	55	.57	.61	95	.11	.12
25	.42	.45	60	.56	.59	100	.00	.00
30	.47	.50	65	.53	.56	105	−.11	−.14
35	.51	.55	70	.50	.52	110	−.20	−.26

NOTE.—In order to avoid constant repetition of the negative sign, the polarizations of levulose are considered positive. The positive signs in the above table indicate that the negative polarizations of levulose are to be increased to higher negative values.

TABLE 22.—*Table of factors for Lane and Eynon's volumetric reducing sugar method*

The factor represents the number of mg sugar required to reduce 25 ml of Soxhlet reagent.

$$100 \times \frac{\text{factor}}{\text{titer}} = \text{mg sugar in 100 ml.}$$

[In the last three columns are titer corrections corresponding to experimentally determined factors which differ from the tabulated factors by 1, 2, and 3 units. When the experimental factor is greater than the tabulated, the correction is to be subtracted from the observed titer, and vice versa. This corrected titer is to be used with the tabulated factor]

Titer	Dextrose	10	20	30	40	Invert Sugar	60	70	80	90	Levulose	Titer corrections		
												1	2	3
15	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.1	125.9	126.6	127.4	0.12	0.24	0.37
16	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.1	125.9	126.6	127.4	.13	.26	.39
17	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.2	125.9	126.7	127.5	.14	.28	.41
18	120.2	120.9	121.6	122.3	123.0	123.7	124.5	125.2	126.0	126.7	127.5	.15	.30	.44
19	120.3	121.0	121.7	122.3	123.0	123.7	124.5	125.3	126.0	126.8	127.6	.15	.31	.46
20	120.3	121.0	121.7	122.4	123.1	123.8	124.6	125.3	126.1	126.8	127.6	.16	.33	.49
21	120.3	121.0	121.7	122.4	123.1	123.8	124.6	125.4	126.1	126.9	127.7	.17	.34	.51
22	120.4	121.1	121.8	122.5	123.2	123.9	124.7	125.4	126.2	126.9	127.7	.18	.36	.54
23	120.4	121.1	121.8	122.5	123.2	123.9	124.7	125.5	126.2	127.0	127.8	.19	.37	.56
24	120.5	121.2	121.9	122.6	123.3	124.0	124.8	125.5	126.3	127.0	127.8	.20	.39	.59
25	120.5	121.2	121.9	122.6	123.3	124.0	124.8	125.6	126.3	127.1	127.9	.20	.41	.61
26	120.6	121.3	122.0	122.7	123.4	124.1	124.9	125.6	126.4	127.1	127.9	.21	.42	.63
27	120.6	121.3	122.0	122.7	123.4	124.1	124.9	125.7	126.4	127.2	128.0	.22	.44	.66
28	120.7	121.4	122.1	122.8	123.5	124.2	125.0	125.7	126.5	127.2	128.0	.23	.45	.68
29	120.7	121.4	122.1	122.8	123.5	124.2	125.0	125.8	126.5	127.3	128.1	.24	.47	.71
30	120.8	121.5	122.2	122.9	123.6	124.3	125.1	125.8	126.6	127.3	128.1	.25	.49	.73
31	120.8	121.5	122.2	122.9	123.6	124.3	125.1	125.8	126.6	127.3	128.1	.25	.50	.76
32	120.8	121.5	122.2	123.0	123.7	124.4	125.2	125.9	126.7	127.4	128.2	.26	.52	.78
33	120.9	121.6	122.3	123.0	123.7	124.4	125.2	125.9	126.7	127.4	128.2	.27	.54	.80
34	120.9	121.6	122.3	123.1	123.8	124.5	125.3	126.0	126.8	127.5	128.3	.28	.55	.83
35	121.0	121.7	122.4	123.1	123.8	124.5	125.3	126.0	126.8	127.5	128.3	.29	.57	.85
36	121.0	121.7	122.4	123.2	123.9	124.6	125.4	126.1	126.9	127.6	128.4	.29	.59	.88
37	121.1	121.8	122.5	123.2	123.9	124.6	125.4	126.1	126.9	127.6	128.4	.30	.60	.90
38	121.2	121.9	122.6	123.3	124.0	124.7	125.5	126.2	127.0	127.7	128.5	.31	.62	.93
39	121.2	121.9	122.6	123.3	124.0	124.7	125.5	126.2	127.0	127.7	128.5	.32	.63	.95
40	121.2	121.9	122.6	123.4	124.1	124.8	125.6	126.3	127.1	127.8	128.6	.33	.65	.98
41	121.3	122.0	122.7	123.4	124.1	124.8	125.6	126.3	127.1	127.8	128.6	.33	.67	1.00
42	121.4	122.1	122.8	123.5	124.2	124.9	125.6	126.4	127.1	127.9	128.6	.34	.68	1.02
43	121.4	122.1	122.8	123.5	124.2	124.9	125.7	126.4	127.2	127.9	128.7	.35	.70	1.05
44	121.5	122.2	122.9	123.6	124.3	125.0	125.7	126.5	127.2	128.0	128.7	.36	.72	1.07
45	121.5	122.2	122.9	123.6	124.3	125.0	125.8	126.5	127.3	128.0	128.8	.37	.73	1.10
46	121.6	122.3	123.0	123.7	124.4	125.1	125.8	126.6	127.3	128.1	128.8	.37	.75	1.12
47	121.6	122.3	123.0	123.7	124.4	125.1	125.9	126.6	127.4	128.1	128.9	.38	.76	1.15
48	121.7	122.4	123.1	123.8	124.5	125.2	125.9	126.7	127.4	128.2	128.9	.39	.78	1.17
49	121.7	122.4	123.1	123.8	124.5	125.2	126.0	126.7	127.5	128.2	129.0	.40	.80	1.20
50	121.8	122.5	123.2	123.9	124.6	125.3	126.0	126.8	127.5	128.3	129.0	.41	.81	1.22

TABLE 23.—*Copper-levulose equivalents according to Jackson and Mathews's modification of Nyns's selective method for levulose*

[All data expressed in milligrams]

Cu	Levulose	Cu	Levulose	Cu	Levulose	Cu	Levulose	Cu	Levulose	Cu	Levulose	Cu	Levulose	Cu	Levulose
1	0.6	40	13.9	79	25.1	118	36.0	157	46.6	196	56.8	235	67.9	274	80.4
2	1.1	41	14.2	80	25.4	119	36.2	158	46.9	197	57.1	236	68.2	275	80.7
3	1.6	42	14.5	81	25.7	120	36.5	159	47.1	198	57.3	237	68.5	276	81.0
4	2.1	43	14.8	82	25.9	121	36.8	160	47.4	199	57.6	238	68.8	277	81.4
5	2.5	44	15.1	83	26.2	122	37.1	161	47.7	200	57.9	239	69.1	278	81.7
6	2.9	45	15.4	84	26.5	123	37.3	162	47.9	201	58.1	240	69.4	279	82.0
7	3.3	46	15.7	85	26.8	124	37.6	163	48.2	202	58.4	241	69.7	280	82.4
8	3.7	47	16.0	86	27.0	125	37.9	164	48.4	203	58.7	242	70.0	281	82.7
9	4.1	48	16.3	87	27.3	126	38.2	165	48.7	204	58.9	243	70.3	282	83.1
10	4.5	49	16.6	88	27.6	127	38.5	166	49.0	205	59.2	244	70.7	283	83.4
11	4.8	50	16.8	89	27.9	128	38.7	167	49.2	206	59.4	245	71.0	284	83.8
12	5.1	51	17.1	90	28.1	129	39.0	168	49.5	207	59.7	246	71.3	285	84.1
13	5.5	52	17.4	91	28.4	130	39.3	169	49.7	208	60.0	247	71.6	286	84.4
14	5.9	53	17.7	92	28.7	131	39.6	170	50.0	209	60.3	248	71.9	287	84.8
15	6.2	54	18.0	93	29.0	132	39.9	171	50.2	210	60.6	249	72.2	288	85.1
16	6.5	55	18.3	94	29.2	133	40.1	172	50.5	211	60.9	250	72.5	289	85.5
17	6.9	56	18.6	95	29.5	134	40.4	173	50.8	212	61.1	251	72.8	290	85.9
18	7.2	57	18.9	96	29.8	135	40.7	174	51.0	213	61.4	252	73.1	291	86.2
19	7.6	58	19.1	97	30.1	136	40.9	175	51.3	214	61.7	253	73.5	292	86.6
20	7.9	59	19.4	98	30.4	137	41.2	176	51.5	215	62.0	254	73.8	293	86.9
21	8.2	60	19.7	99	30.7	138	41.5	177	51.8	216	62.3	255	74.1	294	87.3
22	8.5	61	20.0	100	30.9	139	41.7	178	52.1	217	62.6	256	74.4	295	87.6
23	8.9	62	20.3	101	31.2	140	42.0	179	52.3	218	62.9	257	74.7	296	88.0
24	9.2	63	20.6	102	31.5	141	42.3	180	52.6	219	63.2	258	75.1	297	88.4
25	9.5	64	20.9	103	31.8	142	42.6	181	52.8	220	63.4	259	75.4	298	88.7
26	9.8	65	21.2	104	32.1	143	42.8	182	53.1	221	63.7	260	75.7	299	89.1
27	10.1	66	21.4	105	32.3	144	43.1	183	53.4	222	64.0	261	76.0	300	89.5
28	10.4	67	21.7	106	32.6	145	43.4	184	53.6	223	64.3	262	76.4	301	89.8
29	10.7	68	22.0	107	32.9	146	43.7	185	53.9	224	64.6	263	76.7	302	90.2
30	11.0	69	22.2	108	33.2	147	43.9	186	54.2	225	64.9	264	77.0	303	90.5
31	11.3	70	22.5	109	33.5	148	44.2	187	54.4	226	65.2	265	77.4	304	90.9
32	11.6	71	22.8	110	33.7	149	44.5	188	54.7	227	65.5	266	77.7	305	91.3
33	11.9	72	23.1	111	34.0	150	44.7	189	54.9	228	65.8	267	78.1	306	91.7
34	12.2	73	23.4	112	34.3	151	45.0	190	55.2	229	66.1	268	78.4	307	92.0
35	12.5	74	23.7	113	34.6	152	45.3	191	55.5	230	66.4	269	78.7	308	92.4
36	12.8	75	24.0	114	34.8	153	45.6	192	55.7	231	66.7	270	79.0	309	92.8
37	13.1	76	24.2	115	35.1	154	45.8	193	56.0	232	67.0	271	79.4	310	93.2
38	13.4	77	24.5	116	35.4	155	46.1	194	56.3	233	67.3	272	79.7	311	93.5
39	13.7	78	24.8	117	35.7	156	46.4	195	56.5	234	67.6	273	80.0	312	93.9

Example illustrating the use of Table 24 (*see discussion, p. 433*).—Assume that a solution of levulose and dextrose polarized at 20° C. −43.8° S., and that 5 ml of this solution diluted to 100 ml gave a Lane and Eynon titration of 26.18 ml when the correction for the titration factor is zero. Then

$$D = 100/5 = 20$$

and

$$\frac{PT}{D} = -\frac{43.8 \times 26.18}{20} = -57.3.$$

By Table 24 the approximate ratio is 89.8 per cent, and the correction factor, f, is −0.80. The correction is

$$\frac{f \times D}{T} = -\frac{0.80 \times 20}{26.18} = -0.6$$

and the true ratio is

$$89.8 - 0.6 = 89.2$$

The concentration of total sugar is calculated in the usual way from the titer

$$\frac{100\ F}{T} = \frac{100 \times 127.0}{26.18} = 485.1 \text{ mg per 100 ml of solution titrated}$$

The concentration in the polarized solution is

$$0.4851 \times 20 = 9.702 \text{ g per 100 ml}$$

and levulose is

$$9.702 \times 89.2 \text{ per cent} = 8.654 \text{ g per 100 ml.}$$

TABLE 24.—*Ratio of levulose to total sugar from Lane and Eynon titration and polarization by Mathews formula*

[The table gives the per cent ratio (R) of levulose to total reducing sugar calculated from the Lane and Eynon titration and the direct polarization at 20° C. P is the polarization in °S; T is the corrected Lane and Eynon titer; D is the number of volumes to which one volume of the solution polarized was diluted for the titration; and f is the factor used for correcting the per cent ratio found in the table. This correction is given by

$$\frac{f \times D}{T},$$

and is to be added algebraically.

The table can be used when the polarization is made at a temperature other than 20° C. by using the temperature coefficient, $\Delta R/\Delta t°$. When the temperature is $t°$, the correction is $\frac{\Delta R}{\Delta t°}(t°-20°)$, and is to be added algebraically to the ratio.]

$\frac{P \cdot T}{D}$	T=15	T=25	T=35	T=45	f	$\frac{\Delta R}{\Delta t°}$	$\frac{P \cdot T}{D}$	T=15	T=25	T=35	T=45	f	$\frac{\Delta R}{\Delta t°}$	$\frac{P \cdot T}{D}$	T=15	T=25	T=35	T=45	f	$\frac{\Delta R}{\Delta t°}$
37	−0.6	−0.4	−0.3	−0.2	0.29	0	1	34.0	34.0	34.0	34.0	−0.13	0.15	−35	68.6	68.4	68.3	68.2	−0.54	0.30
36	+0.4	+0.5	+0.7	+0.8	.28	0	0	35.0	35.0	35.0	35.0	−.14	.15	−36	69.5	69.4	69.3	69.2	−.56	.30
35	1.4	1.5	1.6	1.7	.27	0.01	−1	35.9	35.9	35.9	35.9	−.15	.16	−37	70.5	70.3	70.2	70.1	−.57	.31
34	2.3	2.4	2.6	2.7	.26	.01	−2	36.9	36.9	36.9	36.9	−.16	.16	−38	71.4	71.3	71.2	71.1	−.58	.31
33	3.3	3.4	3.5	3.6	.24	.02	−3	37.8	37.8	37.8	37.8	−.17	.17	−39	72.4	72.3	72.4	72.0	−.59	.32
32	4.2	4.4	4.5	4.6	.23	.02	−4	38.8	38.8	38.8	38.8	−.18	.17	−40	73.4	73.2	73.1	73.0	−.60	.32
31	5.2	5.3	5.4	5.5	.22	.02	−5	39.8	39.8	39.7	39.7	−.20	.17	−41	74.3	74.2	74.1	73.9	−.61	.32
30	6.2	6.3	6.4	6.5	.21	.03	−6	40.7	40.7	40.7	40.7	−.21	.18	−42	75.3	75.1	75.0	74.9	−.63	.33
29	7.1	7.2	7.3	7.4	.20	.03	−7	41.7	41.7	41.6	41.6	−.22	.18	−43	76.2	76.1	76.0	75.8	−.64	.33
28	8.1	8.2	8.3	8.4	.19	.04	−8	42.6	42.6	42.6	42.6	−.23	.19	−44	77.2	77.0	76.9	76.8	−.65	.34
27	9.0	9.1	9.2	9.3	.18	.04	−9	43.6	43.6	43.6	43.5	−.24	.19	−45	78.2	78.0	77.9	77.7	−.66	.34
26	10.0	10.1	10.2	10.3	.16	.04	−10	44.6	44.5	44.5	44.5	−.25	.19	−46	79.1	79.0	78.8	78.7	−.67	.34
25	11.0	11.1	11.1	11.2	.15	.05	−11	45.5	45.5	45.5	45.4	−.27	.20	−47	80.1	79.9	79.8	79.6	−.68	.35
24	11.9	12.0	12.1	12.2	.14	.05	−12	46.5	46.4	46.4	46.4	−.28	.20	−48	81.0	80.9	80.7	80.6	−.69	.35
23	12.9	13.0	13.1	13.1	.13	.06	−13	47.4	47.4	47.4	47.3	−.29	.21	−49	82.0	81.8	81.7	81.5	−.71	.36
22	13.8	13.9	14.0	14.1	.12	.06	−14	48.4	48.4	48.3	48.3	−.30	.21	−50	83.0	82.8	82.6	82.5	−.72	.36
21	14.8	14.9	15.0	15.0	.11	.07	−15	49.4	49.3	49.3	49.2	−.31	.22	−51	83.9	83.7	83.6	83.4	−.73	.37
20	15.8	15.8	15.9	16.0	.09	.07	−16	50.3	50.3	50.2	50.2	−.32	.22	−52	84.9	84.7	84.5	84.4	−.74	.37
19	16.7	16.8	16.9	16.9	.08	.07	−17	51.3	51.2	51.2	51.1	−.34	.22	−53	85.8	85.7	85.5	85.3	−.75	.37
18	17.7	17.7	17.8	17.9	.07	.08	−18	52.2	52.2	52.1	52.1	−.35	.23	−54	86.8	86.6	86.4	86.3	−.76	.38
17	18.6	18.7	18.8	18.8	.06	.08	−19	53.2	53.1	53.1	53.0	−.36	.23	−55	87.8	87.6	87.4	87.2	−.78	.38
16	19.6	19.6	19.7	19.8	.05	.09	−20	54.2	54.1	54.0	54.0	−.37	.24	−56	88.7	88.5	88.3	88.2	−.79	.39
15	20.6	20.6	20.7	20.7	.04	.09	−21	55.1	55.0	55.0	54.9	−.38	.24	−57	89.7	89.5	89.3	89.1	−.80	.39
14	21.5	21.6	21.6	21.7	.02	.10	−22	56.1	56.0	56.0	55.9	−.39	.24	−58	90.6	90.4	90.3	90.1	−.81	.39
13	22.5	22.5	22.6	22.6	.01	.10	−23	57.0	57.0	56.9	56.8	−.40	.25	−59	91.6	91.4	91.2	91.0	−.82	.40
12	23.4	23.5	23.5	23.6	.00	.10	−24	58.0	57.9	57.9	57.8	−.42	.25	−60	92.6	92.3	92.2	92.0	−.83	.40
11	24.4	24.4	24.5	24.5	−.01	.11	−25	59.0	58.9	58.8	58.7	−.43	.26	−61	93.5	93.3	93.1	92.9	−.85	.41
10	25.4	25.4	25.4	25.5	−.02	.11	−26	59.9	59.8	59.8	59.7	−.44	.26	−62	94.5	94.3	94.1	93.9	−.86	.41

9	26.3	26.4	26.4	26.4	−.03	.12	−27	60.9	60.8	60.7	60.6	−.45	.27	−63	95.4	95.2	95.0	94.8	−.87	.42
8	27.3	27.3	27.3	27.4	−.05	.12	−28	61.8	61.7	61.7	61.6	−.46	.27	−64	96.4	96.2	96.0	95.8	−.88	.42
7	28.2	28.3	28.3	28.3	−.06	.12	−29	62.8	62.7	62.6	62.5	−.47	.27	−65	97.4	97.1	96.9	96.7	−.89	.43
6	29.2	29.2	29.3	29.3	−.07	.13	−30	63.8	63.7	63.6	63.5	−.49	.28	−66	98.3	98.1	97.9	97.7	−.90	
5	30.2	30.2	30.2	30.2	−.08	.13	−31	64.7	64.6	64.5	64.4	−.50	.28	−67	99.3	99.0	98.8	98.6	−.92	.43
4	31.1	31.1	31.2	31.2	−.09	.14	−32	65.7	65.6	65.5	65.4	−.51	.29	−68	100.2	100.0	99.8	99.6	−.93	.44
3	32.1	32.1	32.1	32.1	−.10	.14	−33	66.6	66.5	66.4	66.3	−.52	.29	−69	101.2	101.0	100.7	100.5	−.94	.44
2	33.0	33.0	33.1	33.1	−.11	.14	−34	67.6	67.5	67.4	67.3	−.53	.29	−70	102.2	101.9	101.7	101.5	−.95	.44

Example illustrating the use of Table 25 (see discussion, p. 434).—Assume that a solution of levulose and dextrose gave a Lane and Eynon titer of 25.89 ml and that 20 ml of the same solution precipitated 247.3 mg of copper by the modified Nyns method. The original solution then contained by Table 23 $\frac{100\times71.7}{20}=358.5$ mg of apparent levulose per 100 ml. Then

$$\frac{T\times l}{100}=\frac{25.89\times358.5}{100}=92.8$$

Referring to Table 25 we find opposite this product and under $T=25$ the true ratio of levulose to total sugar to be 71.5 per cent. Lane and Eynon's factor is 125.7 and the total sugar per 100 ml is $\frac{125.7}{25.89}=485.5$ mg, of which 71.5 per cent = 347.1 mg is levulose and 138.4 mg is dextrose.

TABLE 25.—*Ratio of levulose to total sugar from Lane and Eynon titration and Nyns "apparent" levulose*

$\frac{T\times l}{100}$	T=15	T=25	T=35	T=45	$\frac{T\times l}{100}$	T=15	T=25	T=35	T=45	$\frac{T\times l}{100}$	T=15	T=25	T=35	T=45
11	1.2	1.2	1.1	1.1	50	35.6	35.4	35.2	35.0	89	68.5	68.3	68.1	67.8
12	2.1	2.1	2.0	2.0	51	36.5	36.3	36.1	35.9	90	69.3	69.1	68.9	68.6
13	3.0	3.0	2.9	2.9	52	37.3	37.2	36.9	36.7	91	70.2	70.0	69.7	69.4
14	3.9	3.8	3.8	3.7	53	38.2	38.0	37.8	37.6	92	71.0	70.8	70.6	70.3
15	4.8	4.7	4.7	4.6						93	71.9	71.7	71.4	71.1
					54	39.1	38.9	38.6	38.4					
16	5.7	5.6	5.6	5.5	55	39.9	39.7	39.5	39.3	94	72.7	72.5	72.3	72.0
17	6.5	6.5	6.4	6.4	56	40.8	40.6	40.3	40.1	95	73.5	73.3	73.1	72.8
18	7.4	7.4	7.3	7.3	57	41.7	41.5	41.2	40.9	96	74.3	74.2	73.9	73.6
19	8.3	8.2	8.2	8.1	58	42.5	42.3	42.1	41.8	97	75.2	75.0	74.7	74.4
20	9.2	9.1	9.1	9.0						98	76.0	75.8	75.6	75.3
					59	43.4	43.2	42.9	42.6					
21	10.1	10.0	10.0	9.9	60	44.2	44.0	43.8	43.5	99	76.8	76.6	76.4	76.1
22	11.0	10.9	10.9	10.8	61	45.0	44.8	44.6	44.3	100	77.6	77.4	77.2	76.9
23	11.9	11.8	11.8	11.7	62	45.9	45.7	45.5	45.2	101	78.5	78.2	78.0	77.7
24	12.8	12.7	12.6	12.5	63	46.7	46.5	46.3	46.0	102	79.3	79.0	78.8	78.5
										103	80.1	79.9	79.6	79.3
25	13.7	13.6	13.5	13.4	64	47.6	47.4	47.2	46.9					
26	14.5	14.4	14.3	14.2	65	48.4	48.2	48.0	47.7	104	81.0	80.7	80.4	80.1
27	15.4	15.3	15.2	15.1	66	49.3	49.0	48.8	48.5	105	81.8	81.5	81.2	80.9
28	16.3	16.2	16.1	16.0	67	50.1	49.9	49.7	49.4	106	82.6	82.3	82.0	81.7
					68	51.0	50.8	50.6	50.2	107	83.5	83.2	82.8	82.5
29	17.2	17.1	17.0	16.9						108	84.3	84.0	83.6	83.3
30	18.1	18.0	17.9	17.8	69	51.8	51.6	51.4	51.1					
31	19.0	18.9	18.7	18.6	70	52.7	52.5	52.2	51.9	109	85.1	84.8	84.4	84.1
32	19.9	19.8	19.6	19.5	71	53.5	53.3	53.1	52.8	110	85.9	85.6	85.2	84.9
33	20.8	20.6	20.5	20.4	72	54.3	54.1	53.9	53.6	111	86.7	86.4	86.0	85.7
					73	55.2	55.0	54.7	54.4	112	87.5	87.2	86.8	86.5
34	21.6	21.5	21.4	21.3						113	88.3	88.0	87.6	87.3
35	22.5	22.4	22.3	22.2	74	56.0	55.8	55.6	55.3					
36	23.4	23.3	23.2	23.1	75	56.8	56.6	56.4	56.1	114	89.1	88.8	88.4	88.1
37	24.3	24.2	24.0	23.9	76	57.7	57.5	57.2	56.9	115	90.0	89.6	89.3	88.9
38	25.2	25.1	24.9	24.8	77	58.5	58.3	58.1	57.8	116	90.8	90.4	90.1	89.7
					78	59.4	59.2	58.9	58.6	117	91.6	91.3	91.0	90.6
39	26.1	26.0	25.8	25.7						118	92.4	92.1	92.8	91.4
40	26.9	26.8	26.6	26.5	79	60.2	60.0	59.8	59.5					
41	27.8	27.7	27.5	27.4	80	61.1	60.9	60.6	60.3	119	93.2	92.9	92.6	92.2
42	28.7	28.6	28.4	28.3	81	61.9	61.7	61.4	61.1	120	94.0	93.7	93.4	93.0
43	29.5	29.4	29.2	29.1	82	62.8	62.5	62.3	62.0	121	94.8	94.5	94.2	93.8
					83	63.6	63.3	63.1	62.8	122	95.6	95.3	95.0	94.6
44	30.4	30.3	30.1	30.0										
45	31.3	31.2	31.0	30.8	84	64.4	64.2	63.9	63.6	123	96.4	96.1	95.8	95.4
46	32.2	32.0	31.9	31.7	85	65.2	65.0	64.8	64.5	124	97.3	97.0	96.6	96.2
47	33.0	32.9	32.7	32.5	86	66.0	65.8	65.6	65.3	125	98.1	97.8	97.4	97.0
48	33.9	33.8	33.6	33.4	87	66.9	66.7	66.4	66.1	126	98.9	98.6	98.2	97.8
					88	67.7	67.5	67.2	66.9	127	99.7	99.4	99.0	98.6
49	34.8	34.6	34.4	34.2										

WASHINGTON, January 27, 1932.

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