RP247

A STUDY OF THE METHOD FOR TITRATING ALDOSE SUGARS WITH STANDARD IODINE AND ALKALI

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ABSTRACT

Modification of the iodine-alkali method is recommended, based on the observation that alkali added slowly to a solution of an aldose sugar containing a small amount of iodine will tend not to form sodium iodate, but to react very rapidly with the sugar, viz:

$$RCHO+I_2+3\ NaOH \rightarrow RCOONa+NaI+2\ H_2O$$

By addition of the iodine and alkali successively in small portions, the concentration of sugar relative to the iodine-alkali or sodium hypoiodite is kept at a level favoring the sugar oxidation reaction. The formation of iodate, therefore, indicates the complete oxidation of the aldose to the monobasic acid. By this procedure only 2 ml excess of tenth normal iodine is necessary. This eliminates the 100 to 200 per cent excess iodine required by other methods, which has been found to cause overoxidation. The determination can be carried out in 10 minutes. Iodate formation, moreover, takes place more rapidly than the very slow oxidation of ketoses and nonreducing sugars. The oxidation of the nonaldehydic sugars consumes iodine and alkali in approximately the 4:5 ratio required for the conversion of a primary alcoholic group to a carboxyl. It has further been demonstrated that the percentage of aldose calculated from the alkali used in the oxidation reaction checks the iodine value. By the use of an excess of pure dextrose, therefore, this reaction can be used to standardize the iodine solution against the alkali solution or vice versa.

CONTENTS

I. INTRODUCTION

A number of attempts have been made to establish a quantitative relationship between various aldose sugars and the iodine consumed by their oxidation in alkaline solution. The postulated reactions for this oxidation are:

$$I_2+2\ NaOH \leftrightarrows NaIO+NaI+H_2O \quad (1)$$
$$RCHO+NaIO+NaOH \rightarrow RCOONa+NaI+H_2O \quad (2)$$

Equations (1) and (2) may be combined to give the reaction as usually written.

$$RCHO+I_2+3\ NaOH \rightarrow RCOONa+2\ NaI+2\ H_2O \quad (3)$$

It was early recognized that the sodium hypoiodite may also react to form sodium iodate:

$$3\ NaIO \rightarrow NaIO_3+2\ NaI \quad (4)$$

or combining (1) and (4)

$$3\ I_2+6\ NaOH \rightarrow NaIO_3+5\ NaI+3\ H_2O \quad (5)$$

This removes some of the iodine from the sugar oxidation reaction. After the completion of the sugar oxidation, the system is made acid to liberate the iodine from sodium hypoiodite and sodium iodate:

$$NaIO+NaI+2\ HCl \rightarrow I_2+2\ NaCl+H_2O \quad (6)$$
$$NaIO_3+5\ NaI+6\ HCl \rightarrow 3\ I_2+6\ NaCl+3\ H_2O \quad (7)$$

This liberated iodine, together with any free iodine present, before acidification is titrated with sodium thiosulphate solution:

$$I_2+2\ Na_2S_2O_3 \rightarrow 2\ NaI+Na_2S_4O_6 \quad (8)$$

The difference between the total iodine added and the excess iodine as found by the thiosulphate titration represents the amount of iodine used up in the oxidation of the sugar according to equation (3). It has recently been shown by Slater and Acree [1] that a check on this iodine value may be obtained by titrating with standard alkali the free acid left after completing the iodine titration.

$$HCl+NaOH \rightarrow NaCl+H_2O \quad (9)$$
$$\text{Sugar acid lactone}+H_2O \rightleftarrows RCOOH \quad (10)$$
$$RCOOH+NaOH \rightarrow RCOONa+H_2O \quad (11)$$

This permits a calculation of the amount of aldose sugar on the basis of the alkali used up in reaction (3).

The method was first expounded by Romijn [2] who found that the use of strong alkalis, such as sodium hydroxide caused the oxidation to proceed beyond the monobasic acid stage, represented in

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[1] Analytical edition, Ind. Eng. Chem., **2**, p. 274; 1930.

[2] Zeit. Anal. Chem., **36**, pp. 18, 349; 1897.

reaction (3); he, therefore, preferred the use of buffer salts, such as sodium and ammonium carbonate, potassium bicarbonate, trisodium phosphate, and borax to control the hydroxyl ion concentration. The procedures studied and recommended by Bougault,[3] Colin and Lievin,[4] and Cajori,[5] and recently by Pirrone and Irrera,[6] MacLeod and Robison,[7] and Englis and Byer[8] also involve the use of these buffer salts. However, extended time periods are necessary for the complete oxidation of the sugar in these weakly alkaline mediums, and furthermore consistent results are not always obtainable. Bland and Lloyd[9] used a so-called neutral hypoiodite solution, made by mixing equivalent amounts of $N/10$ sodium hydroxide and iodine immediately preceding the addition of the sugar solution. Willstatter and Schudel[10] recommended that the iodine be mixed with the sugar solution and that the alkali be added drop by drop to the mixture. They state that the amount of alkali added must be sufficient to neutralize the monobasic acid formed and that the reaction goes well if the theoretical amounts of iodine and alkali are used. However, in their description of the experimental procedure, they prescribe double the theoretical amount of iodine and one and a half times the necessary alkali. This procedure was also studied by Judd,[11] Baker and Hulton,[12] Kolthoff,[13] Hinton and Macara,[14] Voorhies and Alvarado,[15] and Goebel.[16] In all instances upwards of 100 to 200 per cent excesses of iodine and alkali were used. The results varied, some workers reporting quantitative results, while others found the results inconsistent, obtaining both low and high values.

Goebel emphasized the fact that too rapid addition of the alkali to the sugar-iodine mixture or previous admixture of the alkali and iodine as in Bland and Lloyd's method, would favor the formation of iodate, reaction (5), and so exhaust the iodine before the complete oxidation of the sugar. This would account for the low results obtained by some workers. The high results are readily ascribable to overoxidation in the presence of excess iodine and alkali which Romijn first noted and which many later workers have failed to take into consideration. The term overoxidation as used throughout this article refers to a further oxidizing action of the alkaline iodine reagent on the aldose sugar molecule after the conversion of the aldehyde group to a carboxyl. It is evidently necessary, therefore, to modify the method so that an excess of the reagents will be avoided. The new method and some data obtained by its use are given in this article.

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[3] Compt. rend., **164**, pp. 949, 1008; 1917.
[4] Bull. Soc. Chim. (4), **23**, p. 403; 1918.
[5] J. Biol. Chem., **54**, p. 617; 1922.
[6] L'industria Chimica, **4**, p. 105; 1929.
[7] Biochem. J., **23**, p. 517; 1929.
[8] Analytical edition, Ind. Eng. chem., **2**, p. 121; 1930.
[9] J. Soc. Chem. Ind., **33**, p. 949; 1914.
[10] Ber., **51**, p. 780; 1918.
[11] Biochem. J., **14**, p. 255; 1920.
[12] Biochem. J., **44**, p. 754; 1920.
[13] Pharm. Weekblad., **60**, p. 362; 1923.
[14] Analyst, **49**, p. 2; 1924.
[15] Ind. Eng. Chem., **19**, p. 848; 1927.
[16] J. Biol. Chem., **72**, p. 801; 1927.

II. EXPERIMENTAL WORK

1. STUDY OF THE FORMATION OF IODATE DURING THE OXIDATION OF ALDOSES

The customary procedure in the determination as outlined by Willstatter and Schudel and generally followed by subsequent workers was the addition of double the amount of necessary iodine to the sugar solution and then the slow addition of an amount of alkali corresponding to the theoretical 2:3 ratio. This technique, involving the presence of a molecular excess of iodine over sugar, allows the formation of iodate to take place simultaneously with the oxidation of sugar and thereby in part counteracts the excesses of reagents used. To show experimentally this simultaneous formation of iodate and oxidation of sugar a slight excess of *N*/10 iodine solution (21 ml) was added to 50 ml of xylose solution containing 1 millimol (0.1500 g), and 25 ml *N*/10 NaOH were added over varying time periods. This amount of alkali is insufficient to oxidize the sugar completely and, therefore, insures the presence of free iodine and sugar to compete for the alkali through the intermediate action of hypoiodite throughout the entire experiment. After a definite interval had elapsed following the addition of the alkali, the free iodine[17] left was titrated with *N*/10 thiosulphate solution. The iodine combined as iodate was then liberated by the addition of *N*/5 HCl and titrated with *N*/10 thiosulphate solution. The results obtained are shown in Table 1. An increase in the rate of addition of the alkali is seen to accelerate the formation of iodate to a marked degree. This would indicate that the iodate reaction goes at a much slower rate than the oxidation of the aldose sugar. The data presented in Tables 2 and 6 give further proof that the iodate reaction is slower than the sugar oxidation.

If the sugar were present in excess of the iodine, the high concentration of sugar relative to the iodine would favor the oxidation reaction. This is readily accomplished by adding the iodine and alkali alternately in small portions, taking special precautions to add the alkali drop by drop with constant stirring. When the 25 ml of alkali were added in 4 portions to the iodine-sugar mixture described above, the iodine liberated from iodate required only one or two drops of the thiosulphate reagent to titrate it, showing that the oxidation reaction used up the alkali practically quantitatively.

If this same predominance of the aldehyde oxidation reaction over the iodate formation should hold true until the sugar had been completely converted to the monobasic acid, a rapid jump in the iodate content would indicate the end point of the oxidation reaction. This, of course, would depend on the velocity of the iodate formation being greater than that of the overoxidation reaction. This has been experimentally observed to hold true. The results of experiments in which both deficiencies and excesses of the reagents were used while adding the reagents in 4 portions as described above, are presented in Table 2. They show that in the presence of an aldose sugar very little iodate is formed, and that after the aldehyde group of the sugar has

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[17] In experiments in which there is considerable free iodine an error would be introduced due to the direct reaction of hypoiodite with thiosulphate instead of the titration of hypoiodite as iodine. This error is discussed at further length below. It should be stated that this procedure was used only in the iodate experiments described in Tables 1 and 2. In the other data the solution was acidified before titrating with thiosulphate unless noted otherwise.

been completely oxidized, the amount of iodate found increases rapidly. This provides us, therefore, with a reliable index of the completion of oxidation and permits the modification of the iodine-alkali method so that the aldose sugars can be determined in the presence of only slight excesses of the reagents and so avoid undue overoxidation.

TABLE 1.—*Effect of rate of addition of alkali on iodate formation*

[0.1500 g xylose, 21 ml N/10 I and 25 ml N/10 NaOH used in each experiment]

Time of addition of alkali	Interval before titration with thoisulphate	Iodate found	Remarks
Min.	*Min.*	*ml N/10 I*	
0	0	3.24	Iodine added in 1 portion.
0	5	3.97	Do.
¾	0	.67	Do.
¾	0	.62	Do.
¾	5	.78	Do.
¾	10	.81	Do.
5	0	.39	Do.
5	0	.29	Do.
5	5	.19	Do.
5	5	.34	Do.
5	0	.08	I and NaOH added in 4 portions.
5	5	.07	Do.

TABLE 2.—*Relation of iodate formation to completion of sugar oxidation*

[Aliquot containing 1 millimol or 0.1500 g xylose taken for each experiment. Theory requires 20 ml iodine and 30 ml alkali for sugar oxidation]

ml *N*/10 I added	ml *N*/10 NaOH added	ml *N*/10 I found combined as iodate	Remarks
10.12	20.11	0.05	Deficiency of both reagents.
17.62	30.67	0.13	Deficiency of iodine.
19.62	30.23	0.30	Do.
21.10	25.32	0.08	Deficiency of alkali.
20.80	28.41	0.16	Do.
20.54	30.74	0.27	Slight excess of both reagents.
20.02	30.24	0.28	Do.
22.04	35.28	1.14	Excess of both reagents.
24.64	38.10	3.67	Do.
25.06	39.51	4.20	Do.
30.05	45.41	8.57	Do.
40.44	60.10	16.89	Do.

2. DETERMINATION OF OPTIMUM EXPERIMENTAL CONDITIONS

In order to establish the experimental procedure necessary for obtaining accurate results, it was necessary to determine the effect of varying the mode of addition of reagents, the time necessary for the completion of the reaction, and the excess of reagents which will give an iodate value indicative of completion of the reaction and yet be insufficient to cause objectionable overoxidation.

(a) EFFECT OF VARYING THE MODE OF ADDITION OF REAGENTS

In regard to the addition of reagents, Willstatter and Schudel recommended the dropwise addition of alkali to the iodine-sugar mixture with constant stirring. They stated that the reaction goes well if theoretical amounts of iodine and alkali are used, but prescribed the addition of one and one-half to four times the theoretical amount of iodine and a similar quantity of alkali. However, Table 3 shows that the use of only theoretical amounts of the reagents in this manner gives low results (experiment 1), whereas the excesses of the reagents cause considerable overoxidation, roughly 2 per cent (experiments 5 and 6). Experiments 2, 3, and 4 in Table 3 indicate that when the alkali is added dropwise to the iodine-sugar mixture more nearly quantitative results are obtained by using small excesses of the reagents and reducting the time of oxidation. The addition of the alkali rapidly from a burette (25 ml per minute) did not differ materially from the dropwise addition in the results obtained (experiments 7 and 8). On the other hand, when the reagents are added according to the method generally used by Slater and Acree; that is, the rapid addition of iodine to the alkali-sugar mixture, the relative concentrations of alkali and sugar allow the iodate reaction to proceed simultaneously with the sugar oxidation. With slight excesses of the reagents low results are, therefore, obtained (experiment 13); even when the iodine is added dropwise to the alkali-sugar mixture, the sugar oxidation is incomplete (experiment 11). This mode of titration, therefore, not only necessitates using excesses of the reagents, resulting in possibility of overoxidation (experiments 14 and 12), but also masks a rapid jump in iodate formation at the completion of the aldehyde oxidation. This same effect has already been noted in Table 1 for the rapid addition of alkali to the iodine-sugar mixture.

TABLE 3.—*Effect of variations in the mode of addition of the reagents on the completeness of oxidation*

[Aliquot containing 1 millimol of dextrose taken for each experiment; 15-minute interval before acidification and titration with thiosulphate, unless otherwise noted. Complete oxidation to the monobasic acid requires 20 ml $N/10$ I and 30 ml $N/10$ NaOH]

Number of experiment	Reagent added first	Manner of addition of reagents		ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ I consumed in oxidation	ml $N/10$ NaOH consumed in oxidation
		I	NaOH				
1	Iodine	1 portion rapidly.	Dropwise	20.05	30.09	19.51	29.20
2	do	do	do	21.60	35.53	20.27	30.39
3	do	do	do.[1]	21.61	35.40	19.98	29.94
4	do	do	do.[1]	23.00	35.37	20.09	30.19
5	do	do	do	30.15	45.40	20.39	30.56
6	do	do	do.[1]	30.21	46.06	20.27	30.42
7	do	do	Rapidly [1]	21.55	35.48	19.99	30.06
8	do	do	do	30.33	45.81	20.32	30.36
9	do	4 portions rapidly.	4 portions dropwise.	21.52	35.43	20.19	30.12
10	do	do	do.[1]	21.45	35.43	20.08	29.99
11	Alkali	Dropwise	1 portion rapidly.	21.63	35.38	19.55	29.57
12	do	do	do	30.36	45.65	20.58	31.16
13	do	Rapidly	do	21.68	35.34	17.98	26.95
14	do	do	do	30.33	45.69	20.34	30.56
15	do	4 portions dropwise.	4 portions rapidly.	21.57	35.44	20.12	30.20

[1] An interval of only two minutes before acidification and titration with thiosulphate was allowed in these experiments.

If the addition of the alkali and iodine is made in four portions and the iodine is added drop by drop to the alkali-sugar mixture (experiment 15) the consumption of iodine and alkali by the sugar is quite comparable to that obtained by the reverse process, namely, the addition of alkali dropwise to the iodine-sugar mixture (experiments 9 and 10). In the further experimental work the mode of addition of the reagents adopted was the dropwise addition of alkali to the iodine-sugar mixture, splitting the addition of the reagent into several portions.

(b) TIME FACTOR

The time factor in this reaction has always been very important and quite empirical. Some investigators have recommended 5 minutes and others have prescribed 30 minutes for the completion of the oxidation after the addition of the reagents. When weak buffer salts have been substituted for the sodium hydroxide, the time has been extended from 30 minutes to 24 hours with temperature control becoming an important factor. These extended time periods render the use of buffer salts impractical for ordinary determination of aldoses. Using the modified procedure described herein, the time interval necessary before making the back titrations was determined. The results of these experiments (Table 4) indicate that in the presence of 1.5 to 2.0 ml excess of iodine and 1.6 to 5 ml excess of alkali the oxidation reaction proceeds to completion in about two minutes and that longer time periods result in overoxidation. On consideration it will be seen that this is the logical outcome, since the addition of iodine and alkali has been performed at such a rate over a period of five to six minutes that the aldehyde sugar group is constantly exhausting the added reagents and that, therefore, only a short time is required to oxidize the last traces of aldehyde groups. This observation is in agreement with the results of Baker and Hulton who found that three to five minutes sufficed for the completion of the reaction. Instead of a purely empirical time interval, therefore, we have a reaction that consumes the reagents quantitatively as they are added and may be considered as complete two minutes after the last of the reagents is added.

(c) EXCESS OF REAGENTS REQUIRED

Investigation of the amount of reagents to be added in excess involved three points; first, the determination of that excess which will give complete oxidation of the sugar to the monobasic acid; second, the determination of whether the amounts of one or both reagents must be controlled; and third, a study of the overoxidation that might be expected with excesses of reagents. The data in Tables 4, 5, and 6 furnish a solution of the first of these problems. Thus it is seen that when the reagents are added in four portions, running in the alkali dropwise, and a 2-minute interval is allowed before acidifying and titrating with thiosulphate, 1.5 to 2.0 ml excess of iodine and 5 ml excess of alkali give complete oxidation. Larger excesses of the reagents result in a jump in the thiosulphate titer as shown in the last two columns of Table 6. It would be possible to use either this slight excess of each reagent or to use a slight excess of one reagent and have the other present in a much greater excess. A study of Tables 4 and 5 will readily show such to be the case. However, it is more convenient and simplifies the method if an excess of 5 ml of alkali is added, keeping the iodine to about 2.0 ml excess.

This results in all but a trace of the iodine being used up in the oxidation reaction or iodate formation, and so enables one to judge the excess of iodine that has been added by the thiosulphate titer. Finally there must be considered the overoxidation that takes place with excesses of iodine and alkali. Fortunately, as we have seen, the overoxidation proceeds at a much lower rate than the formation of iodate. However, on a pure sugar sample for which 20 ml of *N*/10 iodine is required for one millimol, 0.1 ml of the iodine reagent is equal to 0.5 per cent of the sugar. This means that if 0.1 ml of iodine were to be used up in further oxidation of the sugar molecule, the result based on a 2 : 3 ratio of iodine : alkali consumed would be 0.5 per cent high. Table 6 shows that with 20 ml excesses of iodine and alkali an overoxidation is obtained which consumes on the average about 0.4 ml of iodine.[18] The error due to overoxidation in such cases is therefore about 2 per cent. In general, previous workers have used such an excess of reagents. With the slight excesses of reagents recommended in this paper, the error in the determination of a millimol of aldose is about plus or minus 0.3 per cent. This is equivalent to about a drop of *N*/10 iodine and is therefore all that can be expected of a volumetric procedure.

TABLE 4.—*Effect of varying the oxidation period on the consumption of iodine*

[Aliquot containing 1 millimol of xylose taken for each experiment. Iodine and alkali added alternately in four portions, adding the alkali dropwise. Complete oxidation to the monobasic acid requires 20 ml *N*/10 I and 30 ml *N*/10 NaOH]

ml *N*/10 I	ml *N*/10 NaOH added	Interval before acidification and titration with thiosulphate	ml *N*/10 I consumed in oxidation	ml *N*/10 NaOH consumed in oxidation
		Min.		
21. 57	35. 46	0	19. 81	29. 67
21. 56	35. 22	1	19. 98	29. 99
21. 55	35. 40	2	19. 98	30. 08
21. 54	35. 40	5	20. 10	30. 12
21. 55	35. 32	10	20. 15	30. 20
21. 57	35. 44	15	20. 12	30. 20
21. 54	35. 42	20	20. 19	30. 33
[1] 22. 14	35. 47	1	19. 88	29. 91
[1] 22. 05	35. 56	2	20. 02	30. 02
[1] 22. 05	35. 63	3	20. 07	30. 09
24. 61	31. 61	1	20. 09	30. 04
25. 14	31. 63	2	20. 15	30. 08
25. 07	31. 62	5	20. 23	30. 33
21. 59	31. 61	1	19. 87	29. 77
21. 66	31. 62	2	20. 01	30. 00
21. 52	31. 62	5	20. 11	30. 11

[1] One millimol of dextrose used in these experiments.

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[18] The consumption of iodine and alkali in those experiments in Tables 6, 8, and 9, in which free iodine was determined is too high due to the simultaneous oxidation of the thiosulphate reagent by the iodine in the alkaline solution. These experiments will be discussed below.

TABLE 5.—*Effect of varying the excess of each reagent on the oxidation of sugar*

[Aliquot containing 1 millimol of xylose or dextrose used for each experiment. Iodine and alkali were added alternately in four portions; 2-minute interval before acidification and titration with thiosulphate; complete oxidation to the monobasic acid requires 20 ml *N*/10 I and 30 ml *N*/10 NaOH]

ml *N*/10 I added	ml *N*/10 NaOH added	ml *N*/10 I consumed in oxidation	ml *N*/10 NaOH consumed in oxidation	Reagent in excess
[1] 20.36	35.06	19.64	29.43	Alkali.
20.52	35.09	19.89	29.80	Do.
21.33	33.78	19.99	30.01	Do.
[1] 21.51	35.34	19.96	29.89	Do.
21.55	35.40	19.98	30.08	Do.
[1] 22.05	35.56	20.02	30.02	Do.
22.07	35.18	20.07	30.13	Do.
[1] 23.00	35.44	20.06	30.11	Do.
23.11	35.24	20.07	30.08	Do.
25.31	39.14	20.12	30.26	Do.
[1] 21.66	31.62	20.01	30.00	Slight excess of each reagent.
25.14	31.63	20.15	30.08	Iodine.

[1] One millimol of dextrose used in these experiments.

TABLE 6.—*"Overoxidation" of xylose*

[Aliquot containing 1 millimol of xylose taken for each experiment. This xylose was converted to xylonic acid by alternate slow addition of iodine and alkali. Data in this table presents effect of further addition of these reagents]

ml *N*/10 I added	ml *N*/10 NaOH added	ml *N*/10 I consumed in oxidation	ml *N*/10 NaOH consumed in oxidation	ml *N*/10 I left free	ml *N*/10 I combined as iodate
3.11	5.24	0.07	0.08	(1)	3.04
5.31	9.14	.12	.26	(1)	5.19
10.35	19.62	.26	.39	(1)	10.09
[2] 20.08	30.08	.45	.81	(1)	19.63
[3] 20.39	22.05	.36	.36	(1)	20.03
[4] 20.36	22.36	.36	.60	(1)	20.00
5.08	10.04	1.20	1.76	0.36	3.52
10.05	15.41	1.18	1.65	.30	8.57
[2] 20.18	34.22	1.76	2.26	.35	18.07
[2] 20.28	33.37	2.53	3.52	.37	17.38
[2] 20.25	30.24	1.97	2.72	.50	17.78
[3] 20.19	21.51	2.80	3.85	1.71	15 68
[3] 20.33	22.41	2.63	3.65	1.34	16.36

[1] Free iodine was not titrated separately in these experiments, but was included with iodine obtained from iodate by acidifying.

[2] Iodine and alkali were added in alternate portions of 5 and 7.5 ml, respectively, running in the alkali drop by drop.

[3] Iodine and alkali were added rapidly in alternate portions of 5 ml each, with 1-minute interval after each pair.

[4] Alkali was added first in one portion, then the iodine was run in drop by drop.

(d) SLOW OXIDATION EXPERIMENTS

A series of slow oxidation experiments was performed in which the iodine and alkali were added in 0.5 to 2 ml portions to determine the minimum excess of reagents that would be required under these conditions. The results are shown in Table 7. When 0.22 to 0.62 ml excess of iodine and slight excess of alkali were added, and acidified and titrated after two minutes interval, the oxidation was not complete (experiments 1, 2, 3). When an interval of 15 minutes was allowed before titrating the iodine with thiosulphate the oxidation was more nearly complete (experiment 4), indicating that under these conditions the small amount of free iodine present just after the reagents had been added is utilized in the oxidation of the balance of the sugar rather than forming iodate. By adding an excess of alkali, drop by drop, the oxidation reaction is brought more nearly to completion (experiments 5 and 6). These experiments indicate that the slight excess of iodine found necessary for complete oxidation, namely, 2 ml $N/10$ I, is required to offset the free iodine involved in the hypoiodite equilibrium reaction (equation (1)).

3. INVESTIGATION OF THE ACTION OF THE REAGENTS ON NONALDEHYDIC SUGARS

Before applying this method to various sugar-containing materials, it is necessary to know whether sugars other than aldoses would be attacked. For a study of this phase of the problem, levulose and sucrose[19] were employed as examples of ketoses and nonreducing sugars, respectively. Here again it is necessary to determine whether the formation of iodate proceeds more rapidly than the oxidation of these carbohydrates. Tables 8 and 9 show that this is experimentally observed. Furthermore, it will be seen that levulose is somewhat more reactive than sucrose toward these reagents. This is clearly shown in Table 10, which gives the apparent iodine values assigned to dextrose, levulose, and sucrose by various authors. The values taken from this paper are those in which the sugars were oxidized by 20 ml $N/10$ I and 30 ml $N/10$ NaOH using the recommended procedure.

TABLE 7.—*Slow oxidation of dextrose*

[Aliquot containing 1 millimol of dextrose was taken for each experiment. Iodine and alkali were added alternately in portions of 2 and 3 ml, respectively, until 18 ml iodine and 27 ml alkali had been added; then the iodine and alkali were added alternately in portions of 0.5 and 0.75 ml, respectively; the excess of the alkali was added with the last portion. The iodine was run in rapidly from a burette; the alkali was added dropwise with vigorous stirring. Addition of the reagents required 13 minutes; a two-minute interval was allowed before acidification and titration with thiosulphate. Complete oxidation of the sugar to the monobasic acid requires 20 ml $N/10$ I and 30 ml $N/10$ NaOH]

Number of experiment	ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ I consumed in oxidation	ml $N/10$ NaOH consumed in oxidation	ml $N/10$ $Na_2S_2O_3$ required
1	20.22	30.35	19.40	29.14	0.82
2	20.34	32.16	19.64	29.56	.70
3	20.62	31.02	19.65	29.62	.97
4	[1] 20.16	30.45	19.82	29.70	.34
5	20.06	37.64	19.62	29.59	.44
6	20.65	37.73	19.83	29.76	.82

[1] Fifteen-minute interval before acidification and titration with thiosulphate was allowed in this experiment.

Table 11 presents the results of some typical analyses of mixtures of these sugars and dextrose. It will be observed that the results are nearly quantitative for the aldose despite the presence of considerable levulose or sucrose. In the presence of this slight excess of iodine, 2 ml, these sugars would be very little attacked. Furthermore, the presence of excess iodide salt, both that added with the iodine reagent and that formed during the oxidation of the aldose, would reduce the amount of hypoiodite present at any time (equation (1)) and, therefore, would result in less oxidation of the sugar.

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[19] We are indebted to Dr. R. F. Jackson, of the polarimetry section, for a supply of pure levulose. Bureau of Standards Standard Samples No. 17 of sucrose and No. 41 of dextrose were used in the experiments described in this paper. The xylose used was specially prepared by several recrystallizations from concentrated water solutions.

The amount of iodide salt introduced in the solution by the oxidation of a millimol of aldose with 20 ml N/10 I is roughly equivalent to 0.8 g potassium iodide. The effect of this salt on the oxidation of levulose and sucrose is shown in Table 12. The values for sucrose are reduced to about the same order as those obtained for the overoxidation of xylose. The oxidation of levulose is markedly inhibited by the addition of KI, but with 20 ml N/10 I it is still of a greater order than the oxidation of sucrose or overoxidation of xylose. With 2 ml N/10 I there is very slight oxidation of either sucrose or levulose in the presence of 0.8 g potassium iodide; Table 11 indicates that in the presence of aldose the amount of oxidation of these nonaldehydic sugars, although definite, is practically negligible. The method recommended in this paper, therefore, has the advantage in routine analysis of eliminating the necessity of applying corrections for oxidation of ketoses and nonreducing sugars, such as are required in the presences of large excesses of reagents. Table 11 also illustrates the applicability of the method to small amounts of aldose sugars, since quantities as low as 0.036 g dextrose were titrated practically quantitatively.

TABLE 8.—*Oxidation of levulose*

[Aliquot containing 1 millimol or 0.1800 g of levulose taken for each experiment unless otherwise noted]

ml N/10 I added	ml N/10 NaOH added	ml N/10 I consumed in oxidation	ml N/10 NaOH consumed in oxidation	ml N/10 I left free	ml N/10 I combined as iodate
0.57	2.18	0.12	0.22	0.00	0.45
.57	3.11	.20	.19	.00	.37
.57	2.63	.07	.21	.00	.50
.60	3.05	.09	.17	.00	.51
.60	2.65	.18	.32	.00	.42
.71	2.79	.10	.18	.00	.61
1.14	4.04	.30	.47	.00	.84
1.22	3.75	.20	.38	.00	1.02
5.18	9.12	.36	.51	(1)	4.82
6.29	9.88	.73	1.08	(1)	5.56
10.24	19.59	.65	1.09	(1)	9.59
[2] 20.52	31.77	1.47	2.22	(1)	19.05
[3] 20.20	22.99	.43	.79	(1)	19.77
[4] 20.43	22.99	1.46	2.07	(1)	18.97
[5] 49.97	45.65	16.88	22.18	(1)	33.09
[6] 113,94	137.02	97.76	124.55	(1)	16.18
5.13	8.96	.82	1.07	.16	4.15
10.03	17.45	1.42	1.90	.04	8.57
10.04	17.15	1.30	1.74	.08	8.66
[2] 20.18	32.46	2.58	3.57	.24	17.36
[2] 20.32	32.13	3.03	4.14	.13	17.16
[2] 20.37	31.85	3.63	4.87	.11	16.63
[3] 20.07	22.91	1.94	2.67	.81	17.32
[3] 20.21	22.71	1.93	2.75	.91	17.37
[6] 113.75	136.86	102.91	130.79	7.46	3.38

[1] Free iodine was not titrated separately in these experiments, but was included with iodine obtained from iodate by acidifying.

[2] Iodine and alkali were added in alternate portions of 5 and 7.5 ml, respectively, running in the alkali drop by drop.

[3] Iodine and alkali were added rapidly in alternate portions of 5 ml each with one minute interval after each pair.

[4] Alkali was added first in one portion, then the iodine was run in drop by drop.

[5] Five grams of levulose were used in this experiment. Alkali and iodine were added rapidly in alternate portions of 5 ml each with one minute interval after each pair.

[6] Twenty-one grams of levulose were used in this experiment. The reagents were added as in (5).

Table 9.—*Oxidation of sucrose*

[Aliquot containing 1 millimol or 0.3422 g of sucrose taken for each experiment unless otherwise noted]

ml *N*/10 I added	ml *N*/10 NaOH added	ml *N*/10 I consumed in oxidation	ml *N*/10 NaOH consumed in oxidation	ml *N*/10 I left free	ml *N*/10 I combined as iodate
0.56	2.18	0.11	0.15	0.00	0.45
.57	2.19	.06	.10	.00	.51
.58	2.35	.06	.08	.00	.52
.59	2.73	.09	.17	.00	.50
.60	2.48	.04	.09	.00	.56
1.19	3.65	.14	.25	.00	1.05
5.14	9.10	.24	.35	(1)	4.90
10.18	19.57	.60	.72	(1)	9.58
[2] 20.50	31.72	.75	1.09	(1)	19.85
[3] 20.61	31.79	1.39	2.12	(1)	19.22
[4] 20.19	22.98	.41	.61	(1)	19.78
[5] 20.25	22.99	.41	.75	(1)	19.84
[6] 20.30	22.98	.53	.71	(1)	19.77
[7] 20.24	22.98	.49	.83	(1)	19.75
[8] 20.18	23.01	.29	.46	(1)	19.89
[9] 20.28	23.03	.39	.66	(1)	19.89
[10] 20.21	23.06	.61	.91	(1)	19.60
[11] 20.42	23.00	1.39	2.02	(1)	19.03
[7] 49.98	45.50	1.04	1.48	(1)	48.94
[9] 49.67	45.67	1.05	1.55	(1)	48.62
[12] 49.65	45.35	14.31	18.03	(1)	35.34
[13] 99.74	137.11	71.07	90.68	(1)	28.67
5.12	9.09	.67	.93	.15	4.30
10.15	19.52	1.32	1.95	.32	8.51
[2] 20.22	31.93	2.31	3.20	.45	17.46
[4] 20.19	22.98	1.72	2.45	.62	17.85
[6] 49.74	45.68	2.99	4.17	5.23	41.52
[7] 49.97	45.72	2.89	4.13	5.38	41.70
[9] 49.77	45.65	2.47	3.48	5.04	42.26
[12] 49.67	45.30	9.18	11.89	7.01	33.48
[14] 47.43	45.59	19.52	25.21	10.93	16.98
[15] 43.41	45.64	27.11	34.32	10.69	5.61
[13] 99.73	136.25	78.40	99.17	2.85	18.48

[1] Free iodine was not titrated separately in these experiments, but was included with iodine obtained from iodate by acidifying.

[2] Iodine and alkali were added in alternate portions of 5 and 7.5 ml, respectively, running in the alkali drop by drop.

[3] Same as 2, except that the alkali was added first.

[4] Iodine and alkali were added rapidly in alternate portions of 5 ml each with 1-minute interval after each pair.

[5] Same as 4, except that the alkali was added first.

[6] Same as 4, except that the alkali was added drop by drop.

[7] Same as 4, except that the alkali was added first and then the iodine drop by drop.

[8] Iodine and alkali were added rapidly and a 5-minute interval allowed before the thiosulphate titration.

[9] Same as 8, except that the alkali was added first.

[10] Same as 8, except that the alkali was added drop by drop.

[11] Same as 8, except that the alkali was added first and then the iodine drop by drop.

[12] Five grams of sucrose were used; reagents were added as in 5.

[13] Thirty grams of sucrose were used; reagents were added as in 5.

[14] Twenty grams of sucrose were used; reagents were added as in 5.

[15] Fifty grams of sucrose were used; reagents were added as in 5.

Table 10.—*Apparent iodine values assigned to sugars by various authors*

[Values are given in grams of iodine per gram of sugar. Size of sugar sample used for determination is given in parentheses after the value]

Sugar	Judd	Baker and Hulton	Hinton and Macara	Comparable values found in this paper
Dextrose	1.315 (0.1 g)	1.41 (0.1 g)	1.410 (0.08 g)	1.410 (0.18 g)
Levulose	.103 (.1 g)	.10 (.1 g)	.0065 (2 g)	.104 (.18 g)
Sucrose	.01 (.1 g)	.02 (.1 g)	.0032 (10 g)	.028 (.34 g)

TABLE 11.—*Oxidation of mixtures of dextrose with levulose and sucrose*

[Aliquot containing 2 millimol equivalents total sugar taken for each experiment]

Composition (in per cent)			ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ I consumed in oxidation	ml $N/10$ NaOH consumed in oxidation	Per cent aldose sugar found	
Dextrose	Levulose	Sucrose					Calculated from I	Calculated from NaOH
10	90		6.10	11.09	4.06	6.05	10.15	10.08
20	80		10.08	17.02	8.00	12.06	20.00	20.10
40	60		17.97	29.19	15.99	24.05	39.98	40.08
50	50		22.06	35.16	19.99	30.02	49.98	50.04
10		90	6.02	11.06	4.02	6.06	10.05	10.10
20		80	10.00	18.91	8.04	12.08	20.10	20.13
40		60	18.07	31.36	15.99	24.07	39.98	40.12
50		50	21.98	35.55	20.00	30.03	50.00	50.05

TABLE 12.—*Effect of addition of potassium iodide on the oxidation of levulose and sucrose*

[Aliquot containing 1 millimol of sugar used for each experiment]

LEVULOSE

Amount of KI added	ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ I consumed in oxidation	ml $N/10$ NaOH consumed in oxidation
None.	2.07	4.86	0.22	0.28
0.8 g	2.07	5.03	.10	.11
None.	20.52	31.77	1.47	2.22
0.8 g	20.60	31.41	.94	1.46
SUCROSE				
None.	2.04	5.02	0.06	0.14
0.8 g	2.05	5.42	.08	.09
None.	20.50	31.72	.75	1.09
0.8 g	20.57	31.19	.34	.62

In order to obtain further information on the relationship of the oxidation of these nonaldehydic sugar molecules to the oxidation of aldose sugars 20 ml $N/10$ iodine and sufficient amounts of alkali to utilize this iodine completely were added under various conditions to solutions containing 1 millimol of sucrose. The effects of the order of addition of reagents, the speed of addition, and the amount of the reagents added at one time were investigated. (Table 9.) Although these experiments were not very successful in obtaining a marked increase in the consumption of iodine and alkali, they illustrate further the effect of varying the mode of addition of the reagents. It was found that the greatest consumption of the reagents in the oxidation of a millimol of sucrose was obtained by adding the alkali first and then the iodine, either running in each reagent rapidly in one portion or adding them alternately in 7.5 and 5 ml portions, respectively, with 1-minute intervals between each pair and adding the iodine drop by drop. A greater consumption of iodine and alkali in the oxidation of levulose and sucrose was obtained by using more concentrated solutions of these sugars. Table 13 shows that the ratio of iodine to alkali consumed is predominantly that required by the oxidation of a primary alcohol group to a carboxyl, namely, 4 : 5. Inasmuch as more vigorous oxidizing agents attack first the aldehyde group and then the end CH_2OH group to produce a dibasic acid, the reaction with iodine-alkali would be expected to proceed in that fashion.

4. STUDY OF THE TITRATION OF IODINE IN ALKALINE SOLUTION

It was noted above that the titration of free iodine with the thiosulphate reagent in the presence of alkali results in an error due to the oxidation of thiosulphate to sodium sulphate. The investigations of this reaction by Topf [20] and Bougault [21] are particularly noteworthy.

TABLE 13.—*Study of equivalent ratio of iodine and alkali consumed in the oxidation of sucrose and levulose*

[CHO group requires 2:3 ratio; CHOH, 3:4 ratio; CH_2OH, 4:5 ratio]

ml N/10 I consumed in oxidation	ml N/10 NaOH consumed in oxidation	Ratio found
9.18	11.89	4:5.18
14.31	18.03	4:5.04
19.52	25.21	4:5.17
27.11	34.32	4:5.06
71.07	90.68	4:5.10
78.40	99.17	4:5.06
[1] 16.88	22.18	4:5.26
[1] 97.76	124.55	4:5.10
[1] 102.91	130.79	4:5.08

[1] Levulose used in these experiments; sucrose used in remainder.

The equation is as follows:

$$Na_2S_2O_3 + 4\ I_2 + 10\ NaOH \rightarrow 2\ Na_2SO_4 + 8\ NaI + 5\ H_2O \qquad (12)$$

In this reaction 1 mol of thiosulphate is equivalent to 8 atoms of iodine whereas in the ordinary titration of iodine with thiosulphate 1 mol is equivalent to 1 atom of iodine (equation (8)). It is readily seen that such a difference in equivalence can produce large errors in the experimental results. Reference to Tables 6, 8, and 9 will show the magnitude of the error obtained. The apparent consumption of iodine by the overoxidation of xylose with 20 ml excess of iodine and 30 ml excess of alkali when the free iodine was titrated in alkaline medium is approximately 2 ml, equivalent to 10 per cent overoxidation, but the true value as determined by acidifying before titrating with thiosulphate, is 0.45 ml, or 2.25 per cent overoxidation. Similarly the oxidation of levulose and sucrose apparently consumed 3 and 2.31 ml of N/10 iodine, respectively, when the free iodine was titrated in alkaline medium, but the true values were found to be 1.47 and 0.75 ml of N/10 iodine, respectively. When these latter values for the true consumption of iodine in oxidation are calculated to per cent of oxidation based on four equivalents of iodine required per mol of these sugars, as found experimentally (Table 13) it is found that 3.68 per cent of levulose and 1.88 per cent of sucrose were oxidized.

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[20] Zeit. anal. Chem., **26**, p. 137; 1887.

[21] Compt. rend., **164**, p. 949; 1917.

If the per cent of oxidation is calculated on the basis of two equivalents of iodine as required for aldoses, the values are twice as high as they should be. The preliminary oxidation data reported by Slater and Acree in a previous article [22] are too high, due to these two factors, namely, the titration of free iodine in alkaline solution and the calculation of the per cent oxidation of sucrose and levulose on the basis of two equivalents of iodine required per mol of sugar instead of four equivalents.

The work of Topf indicated that the free iodine and iodine combined as hypoiodite could be measured with about 1 per cent error by saturating the alkaline solution with carbon dioxide. Some experiments were, therefore, carried out to determine the utility of that procedure in the present work. Experiment 1 in Table 14 shows that with small amounts of free iodine the error due to oxidation of the thiosulphate reagent in the solution saturated with carbon dioxide is negligible. In this experiment the alkali was added to the iodine before saturating with carbon dioxide and so most of the iodine had reacted to form iodate. In experiment 2 the alkali was saturated with carbon dioxide before adding the iodine. The pH of such a solution was found to be 6.4. The iodine did not react to form iodate at that pH. Moreover it was found that about 1 per cent of the 25 ml of iodine added was consumed in the oxidation of the thiosulphate reagent, confirming Topf's results. Experiments 3 and 4 indicate that at pH 6.4 the oxidation of dextrose takes place very slowly, and is only about one-third complete after 18 hours. In experiments 5, 6, and 7 the addition of the reagents to the sugar solution was carried out by the procedure recommended in this paper. After a 2-minute interval the solution was saturated with CO_2 and the free iodine titrated with thiosulphate. Acidification and further titration with thiosulphate gave the amounts of iodine combined as iodate or rather the amount of iodine reacting according to equation (5). Under these conditions the values for the consumption of iodine in the oxidation of the sugar checked closely the values obtained by direct acidification. (Tables 5 and 6.) The amounts of free iodine in these experiments, namely 1 to 2 ml, were too small to show the probable 1 per cent error noted above. The iodate values obtained by this procedure are somewhat lower than those noted when the free iodine is first titrated in alkaline solution. (Table 2.) This is probably due to small amounts of the iodine or hypoiodite liberated from iodate by acid reacting with the tetrathionate before the solution becomes sufficiently acid to prevent the oxidation. It will be observed that the alkali consumption values for this series of experiments are low, indicating that boiling the solution for 10 to 20 minutes was not sufficient to eliminate the carbon dioxide. Treatment of these solutions with a stream of carbon-dioxide-free air would evidently be required to obtain better alkali oxidation values. These experiments indicate that for purposes of ordinary sugar analysis it is preferable to acidify before titrating with thiosulphate.

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[22] See footnote 1, p. 1064.

TABLE 14.—*Effect of carbon dioxide on titration of free iodine*

[Aliquot containing 1 millimol of dextrose taken for each experiment unless otherwise noted. Free iodine titrated with $N/10$ thiosulphate in CO_2 saturated solution. Iodine combined as iodate was liberated by acidification and was titrated with the thiosulphate reagent. The solution was boiled to drive off CO_2 and the excess acid titrated with standard alkali using phenolphthalein as indicator]

Experiment No.	ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ I consumed in oxidation	ml $N/10$ NaOH consumed in oxidation	ml $N/10$ I left free	ml $N/10$ I combined as iodate	Remarks
1	19.93	29.87	----------	----------	0.72	19.25	Dextrose omitted; reagents mixed and then saturated with CO_2.
2	25.54	30.61	0.25	0.27	25.29	.00	Dextrose omitted; alkali saturated with CO_2 before mixing with I.
3	22.26	36.18	.43	.52	21.83	.00	Alkali added to sugar and saturated with CO_2 before adding I. 15-minute oxidation period.
4	22.16	35.43	6.85	10.17	15.31	----------	Same as 3, except for 18 hour's oxidation period. Acidified before titrating with thiosulphate.
5	22.04	35.20	20.00	29.77	1.11	.93	Oxidation carried out in usual manner; 2-minute interval before saturation with CO_2.
6	22.02	35.51	19.98	29.44	1.08	.96	Same as 5.
7	30.47	45.26	20.31	30.18	2.22	7.94	Do.

They show, furthermore, that the iodine present in the free state or combined as hypoiodite can be determined separately from iodine which has reacted to form iodate by saturating the solution with carbon dioxide and titrating with thiosulphate before acidifying to decompose the iodate. The values for free iodine thus obtained are, in general, about 1 per cent low.

5. USE OF THE METHOD FOR STANDARDIZATION OF IODINE OR ALKALI SOLUTIONS

This iodine-alkali titration of aldose sugars provides a new method for standardizing either iodine or alkali. For inasmuch as the aldehyde group uses up iodine and alkali quantitatively in a 2 : 3 ratio, if we keep the sugar present in excess and use either iodine or alkali of known strength, the strength of the other reagent can be calculated. Table 15 indicates the accuracy that may be attained in such standardization. Thus in the first experiment in the table 20.49 ml of the iodine reagent were consumed in the oxidation. The 30.66 ml $N/10$ alkali consumed are equivalent to 20.44 ml $N/10$ I on the basis of the 2 : 3 ratio of iodine to alkali required. This would, therefore, mean that the calculated strength of the iodine reagent is $0.1N\times 20.44/20.49$ or 0.0998 N, or only 0.2 per cent under the true value 0.1 N given by careful standardization against arsenite solutions. Bureau of Standards standard dextrose was used in these experiments. Formaldehyde was found to give equally satisfactory results. This application of the iodine-alkali method should be especially useful in providing occasional checks on the strength of the reagents as it is only necessary to have an excess of an aldose sugar present. Thus an aliquot of the sugar solution being analyzed could be used to check the reagents provided there were no impurities present containing other groups active towards the iodine, such as unsaturated or phenolic groups.

TABLE 15.—*Quantitative relationship of alkali and iodine consumed in oxidation of aldehydes*

Sample	ml $N/10$ I added	ml $N/10$ NaOH added	ml $N/10$ NaOH consumed in oxidation	ml $N/10$ I required for oxidation: calculated from NaOH consumed	ml $N/10$ I consumed in oxidation: observed value
Excess dextrose	24. 30	30. 71	30. 66	20. 44	20. 49
Do	20. 10	32. 07	30. 02	20. 01	20. 06
Do	20. 30	32. 01	30. 34	20. 23	20. 25
Do	20. 14	32. 12	30. 18	20. 12	20. 09
Excess formaldehyde	20. 04	30. 92	30. 00	20. 00	20. 03

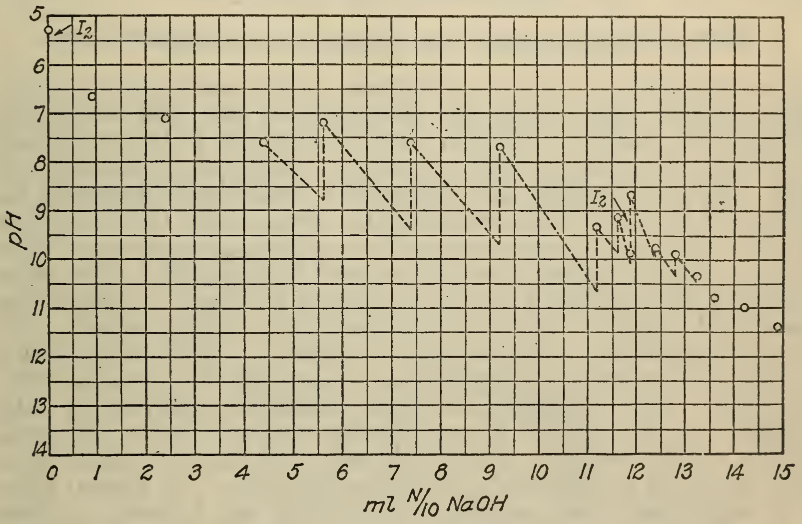

FIGURE 1.—*Titration of xylose with I_2 and NaOH using a glass electrode*

6. EFFECT OF pH ON THE REACTION VELOCITY

In order to obtain information on the pH changes taking place in the mixture during the oxidation, a titration was made with a glass electrode,[23] to determine the hydrogen ion concentration. Figure 1 presents the curve so obtained with 0.06 g xylose, requiring 8 ml $N/10$ I and 12 ml $N/10$ NaOH for complete oxidation. The iodine was added in two portions, an initial portion of 8.5 ml and another 0.8 ml after 11.9 ml of NaOH had been added. This second addition of iodine was necessitated by losses due to volatilization during the 3-hour period required for the experiment. The broken line shows the rise in pH that occurs upon the addition of alkali followed by a drop as the alkali is used up in the reaction. This drop takes place

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[23] The authors wish to acknowledge the generous cooperation of M. R. Thompson, of the chemistry division, for carrying out this experiment with his apparatus.

very slowly and probably had not reached the equilibrium point during the 10 to 15 minute intervals between additions of alkali. The rapid rise in pH was not measured for the first three portions of alkali added. The last portions of alkali cause a steady rise in pH, signifying the exhaustion of the iodine in the solution. This experiment and also experiments 3 and 4 in Table 14 show that at about pH 7.0 or lower the oxidation of the sugar proceeds very slowly and that in order to use the reaction for practical analysis it is necessary to raise the pH to between 9 and 10. The regulation of the pH is therefore of prime importance in both the analytical method and in the practical application of these reactions in the production of sugar acids from the sugars by the use of chlorine and bromine. A further study of the mechanism of the reaction and the effect of the concentration of hydrogen ion and other reactive constituents on the reaction velocity is contemplated.

III. DISCUSSION OF RESULTS

1. EXPERIMENTAL EVIDENCE REGARDING OVEROXIDATION

The necessity for the recommended modification of the iodine-alkali method hinges upon the overoxidation that will occur with excess of the reagents. Romijn clearly states that in the presence of free alkali the decomposition proceeds beyond the monobasic acid stage. Notwithstanding this, Willstatter and Schudel prescribe the mixing of 1½ to 4 times or generally about twice the theoretical amount of iodine with the sugar solution and the subsequent addition, drop by drop with shaking of 1½ parts of *N*/10 NaOH. This latter statement may be taken as either meaning 1½ times the theoretical amount of alkali or 1½ times the amount of iodine added. The latter interpretation has been usually applied. Taken either way it is clearly an excess of reagents and, in general, will result in overoxidation. This procedure has been essentially followed by later workers and has resulted in considerable confusion as to the applicability of the method. Thus Judd reported that quantitative results could not be obtained, the values being low. A typical series of 12 experiments was published by Voorhies and Alvarado. Three of them, or 25 per cent, gave low results; three gave results within 0.5 per cent of 100 per cent; and six, or 50 per cent, were high, due to overoxidation. The low results as pointed out above are ascribable to exhaustion of the iodine by iodate formation before the completion of the sugar oxidation. Attention must be called to the fact that some investigators have diverged from the use of *N*/10 NaOH and have used stronger alkali. This would favor the formation of iodate and, therefore, give low results unless great excesses of reagents were added. The same effect would be obtained if the alkali were added rapidly instead of slowly with constant stirring. A somewhat similar effect is noted in the experiments of Hinton and Macara who found that the apparent iodine value of dextrose diminished when the amount of alkali added was increased. The greater excess of alkali would naturally combine with a larger proportion of the iodine to form iodate, and thus leave a deficiency of iodine for the sugar.

2. OXIDATION OF KETOSES AND NONREDUCING SUGARS

An equally confusing situation exists as to the effect of these reagents on sucrose and levulose. Romijn also noted an attack on these sugars and was later confirmed by Bougault and others who found that this so-called secondary oxidation was characteristic of all polyatomic alcohols. Some investigators, however, including Willstatter and Schudel, found no evidence of any action on the ketoses and nonreducing sugars. There is considerable variation in the iodine values assigned to these sugars by those investigators who noted their oxidation, as Table 10 shows. This is readily understood from consideration of the experimental studies reported herein. Such values would depend on the amount of sugar present, the amount of reagents added and the mode of addition, and are of use only when empirical conditions of sugar concentration, reagent concentration, mode and rate of addition, time of oxidation, etc., are kept constant. Our studies have reaffirmed Bougault's conclusion that this secondary oxidation is the result of a characteristic action on the polyhydroxy molecule.

IV. RECOMMENDED PROCEDURE

For the determination of the aldose present it is preferable to take an aliquot of the sugar solution or a weighed amount of the solid substance, which will react with approximately 20 ml of $N/10$ iodine. Titrate this solution with $N/10$ NaOH or HCl until it is exactly neutral to phenolphthalein. Add the phenolphthalein at this point only when it is necessary to bring the solution to neutrality and use only one drop as the alcohol introduces a potential source for loss of iodine. A water solution of this indicator or of phenol red or thymol blue might be used for this titration. Add 5 ml of $N/10$ iodine from a burette; then add drop by drop from a burette 7.5 ml of $N/10$ NaOH. Repeat this process until 22 ml of iodine and 35 ml of alkali solution have been run in. This operation will take about five to six minutes. Allow a two-minute interval for the completion of the oxidation. Acidify with $N/10$ (or $N/5$) HCl to free the iodine from any sodium iodate present and titrate the liberated iodine with $N/10$ thiosulphate. Starch indicator may be used. Add two to three drops of phenolphthalein solution and titrate the excess acid with $N/10$ NaOH.

If the iodine liberated by acidification requires more than 2 to 3 ml of $N/10$ thiosulphate, too much iodine has been added, resulting in overoxidation. If a very accurate determination is desired, the experiment is repeated, and the amount of the thiosulphate titration less 2 ml is deducted from the amount of iodine to be added to the unknown. On the other hand, if the thiosulphate titration after acidification is less than 1.5 ml, a sufficient amount of iodine has not been added. In such a case it is necessary to repeat the experiment adding more iodine and alkali.

The number of ml $N/10$ I minus the number of ml $N/10$ $Na_2S_2O_3$, and the number of ml $N/10$ NaOH minus the number of ml $N/10$ HCl give the quantities of $N/10$ I and $N/10$ NaOH used in the oxidation of the sugar. One millimol of the sugar (0.15 g xylose) requires for oxidation 20 ml of $N/10$ I and 30 ml of $N/10$ NaOH. Either iodine or alkali consumed, or both as checks, may be used to calculate the amount of aldose present.

The ketose and nonreducing sugars are not oxidized sufficiently to interfere with the precision of the method unless iodine and alkali are used in excess of the above amounts. Lignins and other compounds oxidized by iodine and alkali generally react differently from sugars and disturb the 2:3 ratio for iodine and alkali.

The following suggestions may be found helpful:

1. In all cases a trial analysis for the sugar content will help in a more accurate control of a suitable excess of the reagents in the final analysis. The excess of reagents that have been added can be estimated from the back titer with $N/10$ thiosulphate and the amount of $N/10$ I and $N/10$ NaOH added can be correspondingly reduced. Thus if the thiosulphate titer in a trial analysis is approximately 10 ml, the amount of $N/10$ iodine added in the final analysis would be decreased by about 8 ml.

2. For ordinary analysis it is only necessary to determine accurately the amounts of $N/10$ I and $N/10$ thiosulphate used. This would eliminate the necessity of reading and recording the acid and alkali figures. In such cases, therefore, it is only necessary to add the iodine and alkali reagents as set forth above, allow a 2-minute interval, then acidify and titrate the liberated iodine with thiosulphate. The thiosulphate titer will then indicate whether sufficient iodine has been added or show the approximate degree of overoxidation if an excess has been used.

3. When adding the portions of $N/10$ alkali to the solution containing iodine, one should stir it vigorously to avoid localized concentration of alkali.

4. An excess of iodine in colored solutions can be readily detected with starch. However, a small amount of free iodine persists in the solution even in the presence of unoxidized sugar and can not be taken as indicating the end point of the oxidation. A rapid jump in the amount of iodate formed seems to be the only reliable index of the end point.

5. When acidifying to liberate the iodine from iodate, the best procedure is to add sufficient acid to neutralize the excess alkali and to free the iodine from the iodate present, titrate the iodine, then add more acid, and so on until no more iodine is liberated by the acid. The use of this step from time to time during the trial analysis will indicate when the end point has been reached. Such results, however, can be used only to determine the approximate amount of reagents needed, for after the first titration with thiosulphate, $Na_2S_4O_6$ is introduced into the mixture which is also oxidized by iodine in alkaline solution and, therefore, causes the figures for iodine and alkali consumption to be high. For the same reason titration of the free iodine with thiosulphate before acidification should be avoided.

6. It should be emphasized that the previous methods have not provided any indication whether sufficient reagents had been added, except repeating the experiment using larger amount of iodine and alkali. However, by this modified procedure a thiosulphate titer equivalent to 2 ml $N/10$ thiosulphate solution or higher is evidence that the end point has been reached, and vice versa, a very low thiosulphate titer indicates that sufficient reagents have not been added.

7. There is a small amount of the lactone of the sugar acid formed after acidification with HCl which will result in a fading end point

with phenolphthalein. However, when the titration is carried out in a glass-stoppered flask and the alkali is added slowly a pink color which persists for one minute or longer may be taken as the end point. The fading due to the lactone can be further differentiated from the effect of carbon dioxide by conducting the titration in the presence of a stream of carbon dioxide-free air.

The following example will serve to illustrate the determination and calculation of the percentage of sugar present.

Weight of xylose (dry) 1.5291 g; dissolve and dilute to 500 ml volume with distilled water; 50 ml aliquot taken for titration.

The solution was found to be neutral to phenolphthalein, then 22.41 ml *N*/10 I and 35.19 ml *N*/10 NaOH were added alternately in four portions of 5 and 7.5 ml, respectively, the excess of each reagent being added with the final portion. The iodine was run in rapidly from a burette, the alkali drop by drop with constant stirring. After a two-minute interval 13.69 ml *N*/10 HCl were added to free the iodine combined as $NaIO_3$, and 2.06 ml *N*/10 thiosulphate were required to titrate the liberated iodine. It was then found that 9.07 ml *N*/10 NaOH were used up in bringing the solution back to the neutral point.

Summing up these volumes we find that the following amounts of reagents were used: 22.41 ml *N*/10 I, 2.06 ml *N*/10 thiosulphate, 44.26 ml *N*/10 NaOH and 13.69 ml *N*/10 HCl. Subtracting the thiosulphate titer from the iodine titer and the acid titer from the alkaline titer we find that 20.35 ml *N*/10 I and 30.57 ml *N*/10 NaOH have been consumed in oxidizing the xylose.

Since 20 ml *N*/10 iodine and 30 ml *N*/10 NaOH are required for 1 millimol (or 10 ml of *N*/10) xylose the amount of xylose actually present in the sample can be calculated as follows:

$$\frac{\text{Wt. of millimol}}{\text{Wt. of sample}} \times \frac{\text{ml reagent used}}{\text{ml reagent required per millimol}} \times 100 = \text{per cent xylose}$$

$$\frac{0.1500}{0.1529} \times \frac{20.35}{20.00} \times 100 = 99.81 \text{ per cent xylose based on iodine consumed.}$$

$$\frac{0.1500}{0.1529} \times \frac{30.57}{30.00} \times 100 = 99.96 \text{ per cent xylose based on alkali consumed.}$$

V. SUMMARY AND CONCLUSIONS

Experimental evidence is presented which shows that the customary procedure of adding excess iodine and alkali in the titration of aldose sugars results in overoxidation and, therefore, high values.

The slow addition of small portions of iodine and alkali alternately to the sugar solution results in the nearly quantitative utilization of the reagents in the oxidation of the sugar.

The completion of the sugar oxidation is indicated by a rapid jump in iodate content.

Ketoses and nonreducing sugars are only relatively slightly attacked; they do not interfere with the routine determination of aldoses in mixed sugar samples.

The oxidation of ketoses and nonreducing sugars consumes iodine and alkali in approximately the ratio required for the conversion of a primary alcoholic group to a carboxylic group, namely 4 : 5.

The method provides a convenient and accurate means of standardizing the iodine solution against the alkali solution or vice versa, or of rapidly checking the reagents by using an excess of pure dextrose.

Details of the modified method and a typical experiment with calculations are presented.

WASHINGTON, July 23, 1930.