It would seem in point to study the distribution as a function of cation concentration at constant nitrate concentration and *vice versa*, especially if appropriate activity data become available. However, for a practical extraction the total nitrate concentration is hardly a process variable since it must necessarily be high for a practical extent of extraction into the alcohol.[15] A more pertinent consideration is the variation of the separation factor, α, of two cations with the ratio of their concentrations at constant nitrate concentration.

From the definition of the separation factor, it follows that the ratio of corresponding ordinates in Fig. 1 should be the theoretically attainable separation factor for an aqueous cationic ratio of unity and some particular total solute concentration. For the neodymium/lanthanum case, the ratio values are 1.70 and 1.88, respectively, for values of 0.035 and 0.060 on the abscissa. Thus for a constant neodymium/lanthanum ratio, there seems to be no great change of efficiency with total solute concentration. Templeton and Peterson[5] experimentally obtained $\alpha = 1.5$ under the conditions

$$X'_{\mathrm{Nd}^{3+}} \cong X'_{\mathrm{La}^{3+}} \cong 0.03 \text{ and } X'_{\mathrm{NO}_3^-} \cong 0.18.$$

To test the efficiency at a low neodymium/lanthanum ratio, a new measurement was made

Nd_2O_3, aqueous, % of total oxides	5.50
Nd_2O_3, alcohol, % of total oxides	7.34

$$X'_{\mathrm{Nd}^{3+}} = 0.0028 \qquad X'_{\mathrm{La}^{3+}} = 0.049$$
$$X'_{\mathrm{NO}^{3+}} = 0.156 \qquad \alpha = 1.36$$

Thus α changes only slightly over the range of practical working conditions.

The present data would seem to be sufficient for the planning of a flow separation process. The smallest flow system, if designed for complete separation, would be at least on a semi-pilot-plant scale and would involve considerable effort and amounts of material. However, because of the high concentrations inherently involved, the chief promise of this technique is in the working of large quantities with relative simplicity.

Summary

1. Data have been determined for the distribution of lanthanum, cerous, praseodymium, neodymium and samarium nitrates between water and *n*-hexyl alcohol at 25°.

2. Cerous nitrate is less extractable from water by *n*-hexyl alcohol than is lanthanum nitrate but for the other rare earths investigated there is an increased extractability into the alcohol with increasing atomic number.

3. There appear to be large enough differences in extractability for the development of a practical separation process. The separation factors change only slightly over the range of practical concentrations. Such a process would necessitate the handling of relatively large amounts of material.

ANN ARBOR, MICHIGAN RECEIVED JANUARY 5, 1949

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[15] The yield is easily recovered since even a concentrated alcohol solution will surrender all its nitrate to an equal volume of water.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Reducing Powers of Sugars under Equivalent Conditions in a Carbonate Buffered Cupric Tartrate Micro Reagent of Low *p*H

BY LAWRENCE J. HEIDT, F. WILLIAM SOUTHAM, JEAN D. BENEDICT AND MAYNARD E. SMITH[1]

The method devised by P. A. Shaffer, A. F. Hartmann and M. Somogyi[2] for estimating reducing sugars is one of the most sensitive and reliable ways to follow the rates of hydrolysis of sugars and glycosides.[3] Shaffer and Somogyi have studied the method in great detail and have given many precautions and the reasons for them in order to insure the proper use of the method. But they recommend a copper reagent, SS 50, that is at a *p*H, about 9.3, where the yield of cuprous oxide is sensitive to slight changes in the *p*H of the reagent. This necessitates frequent recalibration of each batch of the reagent and great care in reproducing the experimental conditions in order to obtain reliable results. We have restudied the method and have made certain changes which have improved the yields of cuprous oxide and the reliability of the method especially in inexperienced hands. We have determined the reducing powers of sixteen sugars under equivalent conditions and have found that the moles of cuprous oxide produced per mole of sugar differ in a systematic way with differences in the configuration and structure of the sugars.

Materials.—The sugars were all snow-white crystalline specimens over 99% pure. Most of them were anhydrous crystals, but the L-rhamnose and lactose were monohydrates, the melibiose was a dihydrate, and the D-glucosamine was crystallized as the hydrochloride. Many

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[1] Mr. Southam did most of the experimental work presented in this article. Mrs. Benedict and Mr. Smith did some of the preliminary work. Mrs. Benedict's work is given in her Master's Thesis, September, 1947, Department of Chemistry, M. I. T., Cambridge, Mass.

[2] (a) P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 377 (1920-21); (b) P. A. Shaffer and M. Somogyi, *ibid.*, **100**, 695 (1933); (c) M. Somogyi, *ibid.*, **70**, 599 (1926), **117**, 771 (1927), and **160**, 61 (1945).

[3] See for example the papers by (a) C. B. Purves and C. S. Hudson, THIS JOURNAL, **59**, 49 and 1170 (1937), and (b) L. J. Heidt and C. B. Purves, **66**, 1385 (1944).

of them were purified and recrystallized in this Laboratory by various people associated with the work sponsored by the Sugar Research Foundation. In a few cases, they were the remains of samples previously used as standards in the hydrolytic work.[3b] All of them gave clear colorless solutions whose specific rotations in yellow light agreed within 0.5% with the accepted values. Their melting points also agreed with the accepted values except in the case of D-arabinose, L-rhamnose monohydrate and L-fucose. In these cases, it required extra care in drying the samples to raise the melting points to the accepted values, but this did not alter measurably any of their specific rotations, so we did not bother to give this extra treatment to the major portions of those sugars.

The other reagents were analytical reagent grade or C. P. quality. The Rochelle salt was $KNaC_4H_4O_6{\cdot}4H_2O$, Mallinckrodt Analytical Reagent Material, Accession No. 4201, Control TNX-1. The specific rotation at 24.5° in yellow light was $+22.2°$ at $C = 10$ g. per 100 ml. of water solution; this value also is within 0.5% of the accepted value.

The water was taken from the laboratory supply of chloride-free distilled water.

Apparatus.—The *p*H values were measured to 0.02 *p*H unit by means of a *p*H meter equipped with a glass electrode and saturated calomel half cell. The saturated solution of potassium chloride in the bridge was renewed each day the instrument was used. The scale of the instrument was adjusted and checked by means of buffer tablets, and when properly set at either *p*H 4, 7 or 9 correct readings were obtained over the range 2 to 9.5.

Solutions were stored in the dark in clean Pyrex glass stoppered bottles. The glassware was cleaned by scrubbing it with soapy water followed, after a rinse, by chromic acid, concd. sulfuric acid + a little chromium trioxide and then after washing out most of the acid, by a one per cent. solution of trisodium phosphate at 60 to 100°, and finally by hot distilled water until the rinse water was no longer made alkaline to brom thymol blue.

All filtering was done through sintered glass. The copper solutions especially were not allowed to contact foreign oxidizable material of any kind such as cloth and paper in order to avoid the formation of cuprous oxide by substances other than the sugars.

Volumes were measured with calibrated Normax glassware, especially all volumes measured out with burets or transfer pipets. The 50-ml. burets used in the titrations were graduated to 0.10 ml.

The reproducibility of the volumes delivered by the 1, 2, 3, 4, and 5 ml. transfer pipets was 0.0013 ml. This was accomplished by emptying the pipet while holding the tip against the inside wall of the receptacle until the liquid had drained freely into the tip, then easing the liquid out of the tip by gentle air pressure without blowing air out of the tip or allowing the tip to break contact with the inside wall of the receptacle. Corrections were made for the slightly larger volumes of liquid delivered by this method.

The aliquots of the copper reagents used in the analyses were reproduced to 0.0010 ml. by using the same pipet for measuring out all the aliquots and constricting it at the mark to a capillary about 1 mm. in diameter, and by emptying the pipet in the manner outlined above.

The test-tubes employed to hold the 10 ± 0.02 ml. mixtures of the copper reagent, sugar and water were all the same size, at least six inches long, and each was large enough (25 × 200 mm. or larger) to hold also all of the solutions subsequently used in the analysis, *i. e.*, about 65 ml. The test-tubes were held vertically about 1 cm. apart in batches of up to twenty by metal racks or baskets which allowed the water in the constant temperature baths to circulate freely among them. Each test-tube was capped with a clean empty drying tube or short stem funnel which rested in its mouth and extended about half way to the liquid in the test-tube. The caps retard the escape of water and keep the mixtures blanketed with the carbon dioxide produced by the heat treatment.

One constant temperature bath consisted of vigorously boiling water and the other was freely running tap water. The water level in each bath was maintained constant about one inch above the liquid in the test-tubes.

The *compositions of the reagents* and an *outline of the analytical procedure* are given in Tables I and II.

TABLE I

THE COMPOSITION OF THE COPPER REAGENT

The quantities given are for one liter of water solution at 25°. The composition of the Shaffer and Somogyi Reagent No. 50 is given in the last column.

This research	SS 50
5.0 g. (0.02 mole) $CuSO_4{\cdot}5H_2O$	0.03 mole
17.0 g. (0.06 mole) Rochelle salt	.089 mole
25.0 g. (0.24 mole) Na_2CO_3	.24 mole
0.700 g. (0.0033 mole) KIO_3	(8.35 to 41.7) 10^{-4} mole
1.00 g. (0.006 mole) KI	0.006 to 0.03 mole
Enough $NaHCO_3$ to bring the *p*H to the desired value[4]	.238 mole

The copper reagents were made up by adding the copper sulfate dissolved in 100 ml. of water to the rochelle salt and sodium carbonate dissolved together in 500 ml. of water. The addition was made slowly by means of a separatory funnel whose exit was kept well under the carbonate solution while the latter was vigorously stirred in order to avoid loss of carbon dioxide. The

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(4) The $NaHCO_3$ required to bring the *p*H to 8.90, 9.00, 9.10, 9.20, 9.30 and 9.40 is 0.60, 0.49, 0.42, 0.36, 0.28 and 0.23 mole, respectively, but these quantities of $NaHCO_3$ differ slightly every time the solutions are made up in spite of great care to avoid loss of carbon dioxide.

TABLE II

THE SOLUTIONS EMPLOYED AND PERTINENT INFORMATION

Solution		Composition per liter of solution at 25°	Volume in ml. used in each analysis	Precision in ml.
I	Copper	See Table I	5	±0.001
	Sugar[5]	See Table VI	0, 1, 2, 3, 4 or 5	± .002
	Water[6]	Distilled water	5, 4, 3, 2, 1 or 0	± .01

The total volume of the above mixture was fixed at 10 ± 0.02 ml. Duplicates were always made up except when more samples were required to follow the changes in *p*H. Blanks containing the copper reagent and water but no sugar were included in every batch. Care was taken to introduce the solutions as far down into the test-tube as was practical in order to avoid leaving any part of them upon the wall out of the rest of the mixture. Every mixture was well mixed, heat treated, cooled to 25° and analyzed as soon as possible. The mixing was done by swirling the test-tube.

II	92 g. (0.5 mole) $K_2C_2O_4 \cdot H_2O$, and 50 g. (0.3 mole) KI	1.2	±0.1
III	2 *N* H_2SO_4	3	± .1

The above solutions were thoroughly mixed into the heat treated solution I until a clear solution of I_3^- was obtained. The I_3^- was titrated immediately.

IV[7]	0.002 *M* $Na_2S_2O_3$ (thio), fresh and very slightly alkaline	Up to 50	±0.02
V	1% soln. of water soluble starch, fresh and boiled 3 min.	2 drops (0.1 ml.)	± .02

Solution V was added within 5 to 3 ml. of the end-point and mixed into the solution of I, II, III and IV until the entire solution was deep blue.[8]

iodate, iodide and most of the bicarbonate were then added as solids, the volume was brought up to 970 ml. and the *p*H adjusted to its final value by the stepwise addition of bicarbonate, whereupon the volume was brought up to one liter and the solution thoroughly mixed. The reagents were allowed to age at least three days before they were filtered and ready for use; thereafter the yields of oxide obtained from them could be duplicated within 1% for months as long as they did not deposit a precipitate.

The changes in the *p*H of the copper reagents when diluted and heated are given in Table III. These changes are not measurably different when the sugar is present and cuprous oxide is formed as can be seen by comparing the appropriate entries in Tables III and IV. The results, therefore, support the previous assumption that the buffering is adequate to take care of the sugar and the formation of the oxide. They also show that the buffering is not adequate to prevent changes in the *p*H when the solutions are diluted and heated; so the cut in the concentration of the copper reagent is as important as its initial *p*H in determining the yield of oxide.

The conditions under which the yields of cuprous oxide are most easily reproduced and as high as practical were determined by three sets of experiments on each sugar. The first set established the heat treatment necessary to reach into the plateau where less than 1% of the cuprous oxide is produced in the last five minutes of the heat treatment. The second set established the *p*H of the copper reagent where the yield is least sensitive to small changes in the *p*H. The third set established the range of sugar concentration where the yield is constant, and was carried out with the greatest care.

The first set of experiments was made up of at least two blanks and twelve identical mixtures each consisting of 5 ml. of the copper reagent at *p*H 8.9, 3 ml. of the sugar solution and 2 ml. of water. The test-tubes containing duplicate solutions were held together in pairs by loops at the ends of wires; so that both tubes of a pair could be removed simultaneously from the boiling water-bath and placed simultaneously in the cold water-bath. The transfer was carried out at the end of every five or ten minutes interval until the yield of oxide was found to remain substantially constant. Typical results are presented in Table IV together with the results obtained with copper reagents at other *p*H values. It is seen that prolonged heating does not greatly alter the yield after the plateau has been reached, and that the length of the heat treatment required to reach into the plateau with the copper reagent at *p*H 8.9 will also reach into the plateau with any of the other reagents and can be readily determined within five minutes. Subsequently this set of experiments was carried out only with the reagent at *p*H 8.9 and the changes in *p*H were not followed.

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(5) The concentration of the sugar soln. was adjusted so that the 3-ml. aliquot reduced between forty and fifty per cent. of the copper in the final set of experiments. The thio titers then all differed enough from the blank to make the error in determining the end-point a small percentage error in the thio differences. Different size aliquots of the same sugar solution were used rather than 5-ml. aliquots of five different concentrations of the sugar because the former procedure is easier to use in hydrolytic work and avoids dilution errors. Each sugar solution was made up by weighing out to 0.1 mg. in a dry 100-ml. volumetric flask about 0.0008 mole of the sugar, then adding sufficient water to dissolve the sample and bring the volume up to 100 ml. The solution was thoroughly mixed and diluted five-fold to make the sugar solution added to the copper reagents.

(6) When the sugar solutions contain other ingredients as they do in hydrolytic work, these substances should be present at the same concentration unless it has been proved that they do not affect the yield of cuprous oxide or their presence can be corrected for in another way.

(7) Fresh 0.005 *N* thio and burets graduated to 0.05 ml. are equally satisfactory. The fresh solutions were made up daily from a stock solution of 0.1 *N* thio in 0.01 *N* NaOH. The thio solutions were frequently standardized against KIO_3 or As_2O_3 through I_3^-.

(8) At the end-point of the titration of the I_3^-, the deep blue color of the oxidized starch gave way to the light blue color of cupric copper, the solution was viewed end-on against a background of milk glass or white paper, and the color was compared with that of a similar solution containing a slight excess of thio. A lavender instead of the deep blue color and a vague instead of a sharp end-point indicates that the starch solution has begun to decompose and should be discarded for a fresh one. A vague end-point can also be produced by too little or too much acid or solution II or both. Every solution was thoroughly mixed near the end-point by inverting the test-tube against the thick part of the palm of the hand. N. C. Turner, *Sci.*, **108**, 302 (1948), cites experiments indicating that the deep blue color is produced by starch that has been oxidized by the iodine but does not necessarily contain iodine in any form.

Solution II sometimes changes after long use and produces low and erratic results. This is especially annoying because the end-point remains sharp and one is apt to become discouraged instead of replacing the solution with a fresh clean one.

TABLE III

THE EFFECT OF DILUTION AND HEAT UPON THE *p*H OF THE COPPER REAGENT

The diluted reagent was a solution of the copper reagent and an equal volume (5 ml.) of water. The *p*H values have been smoothed. The numbers in parentheses give the number of expts. upon which the other values are based.

*p*H of copper reagent	*p*H of diluted reagent	Δ*p*H	*p*H of diluted reagent after heating in 100° bath							
			5	10	15	20	30	40	50	60 min.
8.90	9.15 (9)	0.25 ± 0.020	9.17	9.20	9.23	9.25	9.28	9.31	9.33	9.35
9.00	9.24 (11)	.24 ± 0.019	9.26	9.28	9.30	9.32	9.35	9.37	9.39	9.41
9.10	9.33 (11)	.23 ± 0.021	9.35	9.37	9.39	9.41	9.44	9.46	9.48	9.50
9.20	9.43 (12)	.23 ± 0.026	9.46	9.48	9.50	9.52	9.54	9.57	9.59	9.60
9.30	9.53 (1)	.23					9.63			
9.40	9.64 (1)	.24					9.73			

TABLE IV

THE RATE OF REDUCTION OF THE COPPER REAGENT BY D-XYLOSE AND THE EFFECT OF *p*H UPON THE RATE

The heated mixture was made up of 5 ml. of the copper reagent, 3 ml. of 0.00177 *M* D-xylose, and 2 ml. of water. Only the *p*H values have been smoothed. The smoothed analytical results show that in every case twenty-five minutes of heating the mixture in the 100° bath is sufficient to reach into the plateau of nearly constant yield. The % Cu^{II} reduced equals 100 $(B - S)/B$ where B and S are the ml. of (0.002 *N*) thio needed to titrate the blanks and sugar mixtures respectively, since the iodate in the copper reagent is just sufficient with excess iodide and acid to oxidize all the copper from valence one to two.

*p*H of copper reagent	*p*H of mixture in 100° bath after		Δ *p*H	% Cu^{II} reduced at 100°C. in 25 min.	Fraction of the % Cu^{II} reduced in 25 min. after heating for			
	0 min.	30 min.			10	15	20	30 min.
8.90	9.15	9.27	0.12	38.8	0.86	0.98	1.00	1.00
9.00	9.24	9.35	.11	39.0	.87	.96	.98	1.00
9.10	9.33	9.43	.10	38.4	.90	.97	.99	1.00
9.20	9.43	9.52	.09	36.9	.96	.98	.98	1.00
9.30	9.53	9.62	.09	35.2	.97	1.00	1.00	1.00
9.40	9.64	9.72	.08	34.1	.97	.99	1.00	1.00

The second set of experiments was made up of a pair of blanks of each of the copper reagents at *p*H 8.90, 9.00, 9.10, 9.20 and occasionally 9.30 and 9.40. Each of these pairs was accompanied by a similar pair containing 3 ml. of the sugar solution and 2 ml. of water. The whole batch was subjected simultaneously to the heat treatment found in the first set of experiments to be sufficient to reach into the plateau of nearly constant yield with the copper reagent at *p*H 8.90. Typical results are presented in Table IV in the column headed % Cu^{II} reduced at 100° in twenty-five minutes. All the sugars produced the best yields of oxide with the reagent at *p*H 9.00 and under these conditions the yields were also least sensitive to small changes in the *p*H of the reagent and the heated mixtures.

The experimental background is now sufficiently developed to give the basis for the concentration of tartrate used in the copper reagents. The results of the experiments carried out for this purpose are given in Table V; they show that the best yield of oxide is obtained with 0.060 *M* tartrate and that the yields at this concentration are not unduly sensitive to small changes in the *p*H of the reagent and the tartrate concentration. The copper reagent at *p*H 9.00 and 0.06 formal in tartrate remains clear and does not deposit a cupric precipitate for several weeks unless seeded with crystals of the precipitate that eventually forms. The formation of the precipitate lowers the concentration of copper in the reagent and the yield of cuprous oxide the reagent will produce with a sugar. In order to avoid this, one makes up to double strength a large batch of the reagent containing all the ingredients except the bicarbonate and then determines within 0.1% the amount of bicarbonate required to bring a measured aliquot of the batch up to *p*H 9.00 when diluted to the prescribed volume. Thereafter one makes up from the double strength mixture, water and the calculated amount of bicarbonate, a quantity of the copper reagent sufficient to supply one's needs for only the next two weeks. In this way one can reproduce the *p*H of the copper reagent within 0.01 and its oxidizing power within 0.2% after it is three days old and has been filtered subsequently through fine sintered glass. A less precise procedure is to make up each blank and mixture of the copper reagent and sugar by adding together the proper volumes of the double strength mixture, bicarbonate, water and sugar solution. The precipitation of cupric copper at *p*H 9.00 can also be prevented by increasing the concentration of tartrate in the reagent but this lowers the yield of cuprous oxide.

The third set of expts. was made up with the copper reagent at the *p*H producing the best yield as determined in the second set of expts. The mixtures comprised the blanks, and pairs containing 1, 2, 3, 4 and 5 ml. aliquots of the sugar solution and 4, 3, 2, 1 and 0 ml. of water, respectively. The heat treatment was the same as in the second set of expts. A typical plot of the results is given in Fig. 1 which is a copy of a plot in which one cm. of the graph paper represented 0.50 ml. of 0.002 *N* thio and 0.05 ml. of the sugar solution in order to show the deviations in the results.

Evaluation of the Results.—It can be seen in Fig. 1 that the points representing the 1, 2 and 3 ml. aliquots of the sugar solution fall on a straight line within the limits of error. This was true in every case. The best straight lines were determined in the usual manner by the

TABLE V

THE EFFECT OF THE CONCENTRATION OF TARTRATE UPON THE YIELD OF CUPROUS OXIDE

The heated solutions were mixtures of 5 ml. of the copper reagent at the indicated *p*H,[9] 3 ml. of 0.00177 molar D-xylose and 2 ml. of water. The tartrate concentrations are in moles per liter of the copper reagent at 25°. The copper reagents at *p*H values to the left of the heavy line slowly produced a light blue precipitate, but the other copper reagents remained clear.

(Tartrate)	Time mixture was in 100° bath	Per cent. Cu^{II} reduced when the copper reagent was at *p*H 8.30	8.50	8.70	8.90	9.00	9.10	9.20
0.142	40 min.	38.2	36.1	38.5	37.9		36.1	
.060	25 min.				38.8	39.0	38.4	36.9
.043	25 min.				34.5	36.0	35.8	34.9
Δ *p*H by heat		0.7			0.12	0.11	0.10	0.09
From *p*H		8.45			9.15	9.24	9.33	9.43

TABLE VI

THE YIELDS OF CUPROUS OXIDE PRODUCED BY THE SUGARS UNDER EQUIVALENT CONDITIONS[10]

	Sugar	Min. heated at 100°	Moles sugar oxidized = (1.012 to 5.014)x $10^{-6}x$	Lowest % of Cu^{II} reduced	Precision in moles Cu_2O = $10^{-7}x$	Moles Cu_2O lost = $10^{-7}x$	Yield in moles of Cu_2O per mole sugar	Yield in SS 50[11] Min. heated	Un-cor.	Cor.
	Aldopentoses									
1	D-Xylose	25	1.773	12	0.94	4.3 ± 0.9	3.68 ± 0.02	35	3.32	3.42
2	D-Lyxose	20	1.872	12	1.64	9.0 ± 1.8	3.57 ± 0.04			
3	D-Ribose	20	1.847	10	1.01	7.8 ± 1.1	3.12 ± 0.03			
4	D-Arabinose	30	1.816	10	0.78	6.4 ± 0.8	2.99 ± 0.02	35	2.65	2.74
5	L-Arabinose	40	2.056	11	1.18	16.1 ± 1.3	3.42 ± 0.04	35	3.02	3.12
	Aldohexoses									
6	D-Glucose	20	1.583	13	1.09	7.5 ± 1.2	4.41 ± 0.03	15	4.10	4.22
7	D-Glucosamine	15	1.594	12	1.14	7.5 ± 1.6	4.30 ± 0.04			
8	D-Mannose	30	1.698	13	2.32	3.4 ± 2.1	4.04 ± 0.04	35	3.92	4.03
8	D-Mannose	40	1.660	11	1.07	13.0 ± 1.5	4.18 ± 0.03			
8	D-Mannose	50	1.638	11	1.48	15.8 ± 1.6	4.19 ± 0.05			
9	L-Rhamnose	35	1.614	10	0.80	6.3 ± 0.9	3.61 ± 0.02	35	3.10	3.20
10	D-Galactose	25	1.724	12	0.55	3.9 ± 0.6	3.51 ± 0.02	35	3.45	3.57
11	L-Fucose	50	1.738	9	1.08	3.6 ± 0.9	2.81 ± 0.02	35	2.34	2.45
	Ketohexoses									
12	D-Fructose	10	1.644	13	0.75	6.6 ± 0.8	4.29 ± 0.02	15	3.98	4.10
13	L-Sorbose	20	1.600	12	1.98	4.3 ± 3.2	3.89 ± 0.06			
	Disaccharides[12]									
14	Cellobiose	45	1.539	13	1.84	9.0 ± 2.0	4.77 ± 0.06			
15	Lactose	40	1.260	11	0.80	7.3 ± 0.9	4.75 ± 0.03	15	3.76	3.97
16	Melibiose	40	1.483	14	1.39	3.8 ± 1.2	4.86 ± 0.03			

method of least squares. The deviations from these straight lines are given in Fig. 2. A statistical analysis showed that the scattering is random so the best lines through the points are indeed straight. The slopes of the lines, therefore, give the yields of cuprous oxide automatically corrected for the oxide lost, and the values of the intercepts at zero concentration of reducing sugar equal the negative of the amounts of oxide lost before titration. The values are given in Table VI in the appropriate columns.

Comparison of the Configurations and Reducing Powers of the Sugars.—Richtmyer and

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(9) The copper reagents at *p*H 8.30 and 8.50 contained 0.0471 mole of Na_2CO_3 in both cases and 0.476 and 0.298 mole of $NaHCO_3$ respectively per liter. In the case of the copper reagents containing the most tartrate at *p*H 8.70, 8.90 and 9.10, 0.0942 mole of Na_2CO_3 was used in each case, and 0.453, 0.298 and 0.178 mole of $NaHCO_3$ respectively per liter of solution. The other copper reagents contained very nearly the same amounts of Na_2CO_3 and $NaHCO_3$ given in Table I.

(10) Equivalent conditions are those where the yields are least sensitive to changes in the *p*H of the copper reagent, the sugar concentration and the heat treatment. These conditions were determined for each sugar by three sets of expts. described in the text, and were found to prevail when the copper reagent was at *p*H 9.00 and had the composition given in Table I. The precision is the probable error in reproducing the difference between the thio titers for the blanks and sugar solutions: 1 ml. of 0.002 *N* thio is equivalent to 10^{-6} mole of Cu_2O. The average precision was 0.12 ml. of 0.002 *N* thio. The average amount of cuprous oxide lost in each analysis was 0.75×10^{-6} mole.

(11) (a) These results were obtained by N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **58**, 2540 (1936), who used Shaffer and Somogyi's Reagent No. 50 with 1 g. KI. The yields were corrected by adding 0.3 ml. to their 0.005 *N* thio differences; this volume is equivalent to the 0.75×10^{-6} mole of cuprous oxide lost in our expts. under similar conditions. In every analysis they used 5 ml. of the copper reagent and 1.2 mg. of sugar. In most cases, the heat treatment was sufficient to reach the plateau of constant yield of cuprous oxide. Twenty-five to sixty per cent. of the Cu^{II} was reduced. (b) The raw yields obtained by Shaffer and Somogyi under similar conditions for D-xylose, D-arabinose, D-glucose, D-mannose and D-fructose are 3.4, 2.9, 3.80, 4.1 and 4.0, respectively.

(12) The reducing unit of these disaccharides is the glucose part of the molecules.

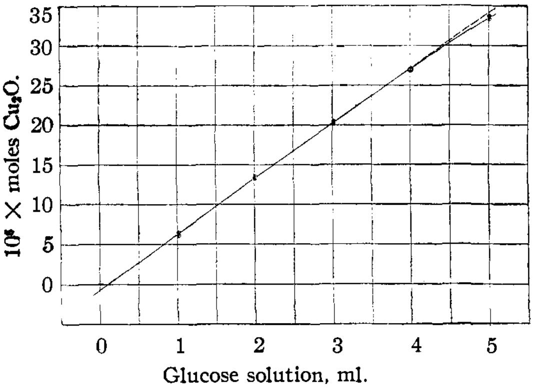

Fig. 1.—The differences between the thio titers for the blanks and sugar mixtures plotted against the amounts of D-glucose in the mixtures. The thio solution was 0.00200 *M* $Na_2S_2O_3$, so one ml. of it is equivalent to 10^{-6} mole of cuprous oxide. The sugar solution contained one-fifth of 0.1425 g. of D-glucose in 100 ml. of distilled water solution, so one ml. of it contained 1.583 × 10^{-6} mole of the sugar. The point of fusion of the straight and curved portions of the line is at 3.8 ml. of the sugar solution. Beyond this point the yields of cuprous oxide fall off because the concentration of Cu^{II} was lower in the final stages of the reaction.

Hudson[11a] have found that when the tartrate in the copper reagent SS 50 is changed from the *d*- to the *l*-form the yield of cuprous oxide also changes. The changes are in the same direction for all sugars whose hydroxyl groups on C atoms 2, 3 and 4 have the same configuration. Also the reducing power of D-altrose in *l*-tartrate is the same as the L-form in *d*-tartrate, and although the yield of oxide is lower for the D-form in *d*-tartrate, it is nevertheless equal to the yield produced by the L-form in *l*-tartrate. This same situation exists in the case of D- and L-arabinose.

Their results also show that the differences between the yields in *d*- and *l*-tartrate are greater when the hydroxyl groups of the sugars on C atoms 3 and 4 are *cis* than when they are *trans*. The sugars with hydroxyls *cis* on C atoms 3 and 4 and the ratios of their reducing powers in *d*- and *l*-tartrate (*d*/*l*) are: D-altrose, 0.620; L-altrose, 1.60; L-allose, 1.60; D-arabinose, 0.865; L-arabinose, 1.16; D-galactose, 1.20, and L-fucose, 0.894. The sugars with hydroxyls *trans* on C atoms 3 and 4 and the ratios of their reducing powers in *d*- and *l*-tartrate (*d*/*l*) are: D-mannose, 0.984 (*cf.* D-altrose); D-xylose, 1.01 (*cf.* D-arabinose); D-glucose, 1.01 and L-rhamnose, 0.965.

The above facts must be kept in mind when our results are compared, so we give after each difference cited below an estimate in parentheses of the difference between the yields when the tartrate is the form giving the higher yield. The estimates were made by applying to our net yields the per cent. change in the yields observed by Richtmyer and Hudson corrected for the cuprous oxide lost.

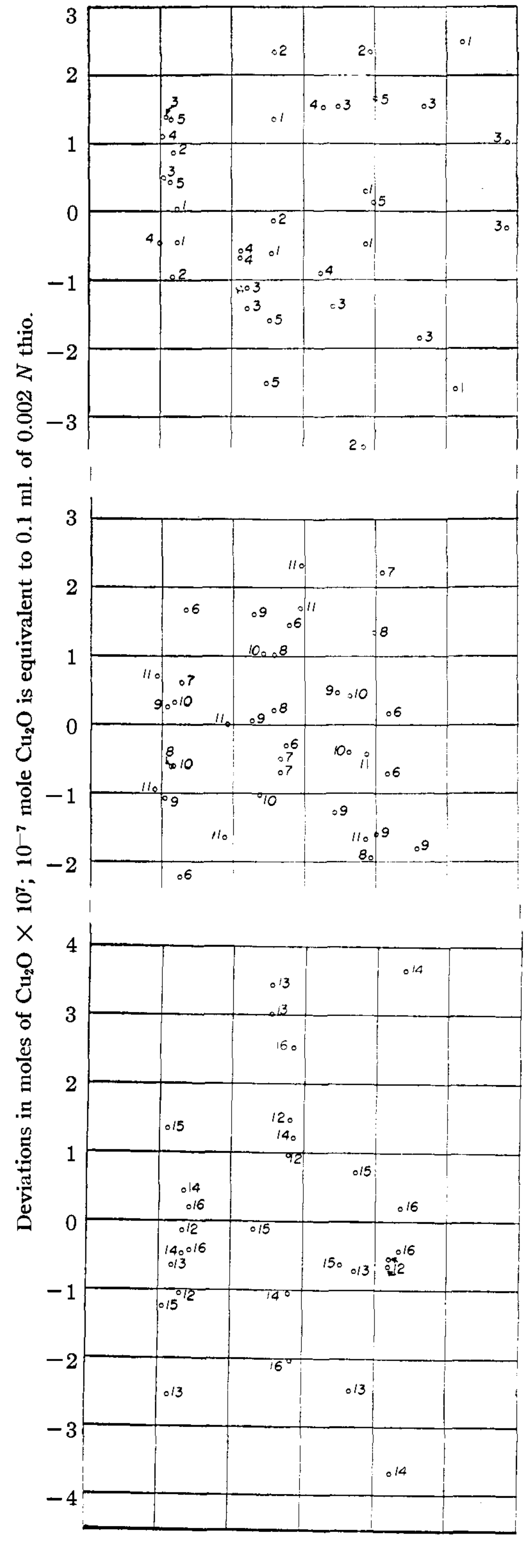

Fig. 2.—Deviations of the results from the best straight lines through them. The numbers in the figure identify the results as belonging to the sugars given the same numbers in Table VI.

The difference between the reducing powers of the epimers are: D-glucose, 4.41 − D-mannose, 4.19 = 0.22 (0.15), D-xylose, 3.68 − D-lyxose, 3.57 = 0.11 (> 0.06) and D-arabinose, 2.99 − D-ribose, 3.12 = −0.13 (> 0.05). The difference between sugars whose hydroxyl groups on C atoms 3 and 4 are *trans vs. cis* is: D-glucose, 3,4 *trans*, 2,3 *trans*, 4.41 − D-galactose, 3,4 *cis*, 2,3 *trans*, 3.51 = 0.90 (0.90). The last two sugars have opposite configurations on C atom 4. These results show that sugars whose neighboring hydroxyl groups are *trans* give higher yields of oxide under corresponding conditions than when these groups are *cis*.

The difference between the reducing powers of corresponding aldohexoses and aldopentoses is: D-glucose, 4.41 − D-xylose, 3.68 = 0.73 (0.69) and D-mannose, 4.19 − D-lyxose, 3.57 = 0.62 (0.62). The difference is about the same as when the hydroxyl group on C atom 6 is replaced by hydrogen, *i. e.*, D-mannose, 4.19 − D-rhamnose, 3.61 (same as L-rhamnose in the *d*-tartrate) = 0.65 (0.69) and D-galactose, 3.51 − D-fucose, 2.81 (same as L-fucose in the *d*-tartrate) = 0.70 (0.70).

The reducing power is only slightly lower, 0.11, when the hydroxyl group on C atom 2 of D-glucose is replaced by the NH_2 group in D-glucosamine. The reducing power is increased, 0.35, when the hydroxyl group on C atom 4 of D-glucose forms either the galactoside, lactose, or the glucoside, cellobiose, and it is increased by 0.55 when the hydroxyl group on C atom 6 of D-glucose forms the glucoside, melibiose. Only the glucose unit serving as the aglycone in these disaccharides reduces the copper reagent because the sugar residue of glycosides is non-reducing in the form of the hemi-acetal.

We are pleased to acknowledge generous financial support from the Sugar Research Foundation and the encouragement and patience of their Scientific Director, Dr. Robert C. Hockett.

Summary

1. The method devised by Shaffer and coworkers for estimating reducing sugars has been modified to give yields of cuprous oxide that are more easily reproduced and are up to 10% larger than formerly. The average reproducibility in each analysis is 1.2×10^{-7} mole of cuprous oxide which corresponds to an uncertainty of 0.048 ml. of 0.005 N thio in the difference between the thio titers for the blanks and sugar solutions.

2. A procedure is given for estimating the yields of cuprous oxide which automatically corrects for the oxide lost.

3. The yields of cuprous oxide produced by fourteen aldoses and two ketoses have been measured under equivalent conditions and the differences are compared with the differences in the configuration and structure of the sugars.

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[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Studies on Lignin and Related Products. I. The Oxidation of Basic Calcium Lignosulfonate with Mercuric Oxide and with Silver Oxide[1,2]

BY IRWIN A. PEARL

Lautch, Plankenhorn and Klink[3] treated isolated "cuproxam" lignin with alkali and oxides of copper, silver, mercury and lead and found that the oxides of these metals did not increase the yields of vanillin obtainable from lignin by the use of alkali alone.

Other work in this laboratory on the oxidation of vanillin to vanillic acid by means of silver oxide[4,5] and mercuric oxide[6] indicated that oxidation of lignin materials in the presence of metal oxides might yield vanillic acid instead of vanillin as the principal oxidation product. Therefore, the reaction of lignin materials with a large number of metallic oxides was reinvestigated. This paper deals with the reaction of basic calcium lignosulfonate (BCLS) with mercuric oxide and with silver oxide.

In preliminary experiments, BCLS was treated at the boiling point with a suspension of a freshly prepared mercuric oxide in alkaline solution, and the reaction product was acidified with sulfuric acid, extracted with ether and fractionated to bisulfite, bicarbonate, and alkali solubles and neutrals. These extracts yielded a total of approximately 60% of mercury-containing, ether-soluble products based on the original lignin. No attempt was made to isolate pure compounds from these mercury-containing fractions.

During the separation of the bisulfite-soluble fraction from the original ether extract, a white crystalline precipitate separated. Upon warming with dilute sulfuric acid, this crystalline precipi-

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(1) Presented before the Division of Cellulose Chemistry at the 114th meeting of the American Chemical Society, Western Session, Portland, Oregon, September 13-17, 1948.

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results. Some of the data reported in this paper have been disclosed in U. S. Patent 2,433,227 (Dec. 23, 1947).

(3) Lautch, Plankenhorn and Klink, *Angew. Chem.*, **53**, 450 (1940).

(4) Pearl, THIS JOURNAL, **68**, 429 (1946).

(5) Pearl, *ibid.*, **68**, 1100 (1946).

(6) Pearl *ibid.*, **70**, 2008 (1947).