

REDUCING SUGARS

PRINCIPLE

Reducing sugars are determined by reaction of a water soluble portion of the sample with an excess of standard copper sulfate in alkaline tartrate (Fehling's) solution under controlled conditions of time, temperature, reagent concentration and composition, so that the amount of copper reduced is proportional to the amount of reducing sugars in the sample analyzed. In this adaptation of Schoorl's method (Note 1) the reducing sugar concentration expressed as dextrose, is estimated by iodometric determination of the unreduced copper remaining after reaction.

SCOPE

This method was designed specifically for water soluble dextrans and maltodextrans and is applicable to other carbohydrates, such as low DE glucose syrups and solids (Note 2). Dextrans are modified starches prepared from starch by heat treatment in the dry state with or without the addition of small quantities of reagents. The method is not recommended for samples above 30 DE, since it tends to give higher results as the relationship of reduced copper with respect to reducing sugars becomes less linear at the higher DE's.

SAFETY

Follow Good Laboratory Practices throughout.

MEDIA AND REGEANTS

1. Fehling's Solution (Note 1)
 - A. Dissolve 34.64 g of reagent grade crystalline copper sulfate pentahydrate in purified water and dilute to 500-mL volume.
 - B. Dissolve 173 g of reagent grade potassium sodium tartrate tetrahydrate and 50 g of reagent grade sodium hydroxide in purified water and dilute to 500-mL volume. Let stand overnight. If precipitation is present, filter through glass wool prior to use.

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2. Potassium Iodide Solution, 30%: Dissolve 150 g of reagent grade potassium iodide in purified water and dilute to 500-mL volume. The reagent should be protected from light during storage, and it should be discarded when the presence of free iodine becomes evident.
3. Sulfuric Acid Solution, 28%: Cautiously pour 85 mL of concentrated sulfuric acid (96% H₂SO₄; sp g 1.84) into 400 mL of purified water while stirring. Cool to room temperature and dilute to 500-mL volume.
4. Sodium Thiosulfate Solution, 0.1 *N*: Standard (commercially available material is preferred).
5. Starch Indicator Solution, 1%

PROCEDURE

Weigh 10.0 g of sample, transfer to a 200-mL Kohlrausch flask, dilute to volume with water, shake for 30 minutes and gravity filter through Whatman No. 1 filter paper; collect the filtrate in a clean and dry flask. Alternatively, centrifuge suspension to clear supernate.

Pipet 10.0 mL of Fehling's A solution and then 10.0 mL of Fehling's B solution into 1 250-mL Erlenmeyer flask. Add 20.0 mL of sample filtrate, or supernate solution, and 10 mL of purified water to bring total volume of reaction mixture to 50 mL. Mix contents of flask by gentle swirling.

Add 2 small glass beads to prevent bumping while boiling and cover the mouth of the flask with a small glass funnel or glass bulb. Place the flask on a hot plate adjusted to bring the solution to a boil in 3 minutes (Note 3), and continue boiling for exactly 2 minutes (total heating time of 5 minutes). Cool quickly to room temperature in an ice bath or a cold water bath.

Add 10 mL of 30% potassium iodide solution and 10 mL of 28% sulfuric acid solution; titrate immediately with standard 0.1 *N* sodium thiosulfate solution. Near the end, point add 1 mL of starch indicator solution, and continue titrating

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carefully while agitating the solution continuously until the blue starch-iodine color disappears.

Conduct two blank determinations in identical fashion substituting purified water for the sample filtrate.

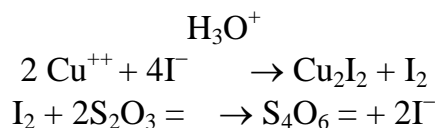
CALCULATION

Subtract sample titer from the average blank titer. Find reducing sugar content (expressed as dextrose) equivalent to the titer difference by reference to the accompanying table (Note 4).

$$\text{Reducing Sugars, \% (as Dextrose)} = \frac{\text{mg Dextrose (From Table)} \times 200 \text{ mL} \times 100}{\text{Sample Wt. (g)} \times 20 \text{ ml} \times 1000 \text{ mg/g}}$$

NOTES AND PRECAUTIONS

- 1 The Fehling's solution recommended here is equivalent to that used in Dextrose Equivalent Methods E-26 and F-22 according to Lane and Eynon at constant volume, where careful standardization of the reagent is essential. However, copper concentration in the Schoorl method is not highly critical and adjustment may be unnecessary. Satisfactory results can be obtained with reagents giving blank titers ranging from 27.5 to 29.5 mL of 0.1 *N* sodium thiosulfate solution, and adjustment of the copper sulfate solution (Fehling's A solution) concentration to provide blank titers in this range is recommended.
2. Copper oxidation of carbohydrates is not a simple reaction; end products are mixtures of acids, so equations are not usually written. Consequently, quantitative analysis of reducing sugars by copper oxidation is strictly empirical, that is, its success depends on maintaining the conditions of the test. Iodometric determination of excess copper (II) is summarized:



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3. Alternatively, the flask may be placed on a wire gauze and heated over a Bunsen Burner, but control of the heating cycle is more difficult by this technique. Heating on a hot plate with a stepless control is recommended for best precision.
4. Use of the accompanying table for conversion of titer difference to mg of reducing sugar (expressed as dextrose) presumes ability of the analyst to duplicate exactly the conditions under which the table was developed. This approach is quite accurate in most cases but it does entail a risk of error. The risk of error can be avoided by careful duplicate standardization using known quantities of pure dextrose. The standardization plot is slightly curvilinear, passing through the origin.

REFERENCE

Food Chemicals Codex, Fourth Edition (1996), First Supplement, National Academic Press, p. 18.

REDUCING SUGARS — continued**Conversion of Net Titer to Reducing Sugars**

<u>Net Titer*</u>	<u>0.0</u>	<u>0.1</u>	<u>0.2</u>	<u>0.3</u>	<u>0.4</u>	<u>0.5</u>	<u>0.6</u>	<u>0.7</u>	<u>0.8</u>	<u>0.9</u>
Reducing Sugars (As Dextrose), mg										
0.0 mL	0.0	0.3	0.7	1.0	1.3	1.6	1.9	2.2	2.5	2.8
1.0	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9
2.0	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.5	8.8	9.1
3.0	9.4	9.8	10.1	10.4	10.7	11.0	11.4	11.7	12.0	12.3
4.0	12.6	13.0	13.3	13.6	14.0	14.3	14.6	15.0	15.3	15.6
5.0	15.6	16.3	16.6	16.9	17.2	17.6	17.9	18.2	18.5	18.9
6.0	19.2	19.5	19.8	20.0	20.5	20.8	21.1	21.4	21.8	22.1
7.0	22.4	22.7	23.0	23.3	23.7	24.0	24.3	24.6	24.9	25.2
8.0	25.6	25.9	26.2	26.6	26.9	27.3	27.6	28.0	28.3	28.6
9.0	28.9	29.3	29.6	30.0	30.3	30.6	31.0	31.3	31.6	31.9
10.0	32.3	32.7	33.0	33.3	33.7	34.0	34.3	34.6	35.0	35.3
11.0	35.7	36.0	36.3	36.7	37.0	37.3	37.6	38.0	38.3	38.7
12.0	39.0	39.3	39.6	40.0	40.3	40.6	41.0	41.3	41.7	42.0
13.0	42.4	42.8	43.0	43.4	43.7	44.1	44.4	44.8	45.2	45.5
14.0	45.8	46.2	46.5	46.9	47.2	47.6	47.9	48.3	48.6	48.9
15.0	49.3	49.6	49.9	50.3	50.7	51.1	51.4	51.7	52.1	52.4
16.0	52.8	53.2	53.5	53.9	54.2	54.5	54.9	55.3	55.6	56.0
17.0	56.3	56.7	57.0	57.3	57.7	58.1	58.4	58.8	59.1	59.5
18.0	59.8	60.1	60.5	60.9	61.2	61.5	61.9	62.3	62.6	63.0
19.0	63.3	63.6	64.0	64.3	64.7	65.0	65.4	65.8	66.1	66.5
20.0	66.9	67.2	67.6	68.0	68.4	68.8	69.1	69.5	69.9	70.3
21.0	70.7	71.1	71.5	71.9	72.2	72.6	73.0	73.4	73.7	74.1
22.0	74.5	74.9	75.3	75.7	76.1	76.5	76.9	77.3	77.7	78.1
23.0	78.5	78.9	79.3	79.7	80.1	80.5	80.9	81.3	81.7	82.1
24.0	82.6	83.0	83.4	83.8	84.2	84.6	85.0	85.4	85.8	86.2
25.0	86.6	87.0	87.4	87.8	88.2	88.6	89.0	89.4	89.8	90.2
26.0	90.7	91.1	91.5	91.9	92.3	92.7	93.1	93.5	93.9	94.3
27.0	94.8									

*mL of 0.1000 N sodium thiosulfate solution.