14 chapter

Total Carbohydrate by Phenol-Sulfuric Acid Method

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14.1 INTRODUCTION

14.1.1 Background

The phenol-sulfuric acid method is a simple and rapid colorimetric method to determine total carbohydrates in a sample. The method detects virtually all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides. Although the method detects almost all carbohydrates, the absorptivity of the different carbohydrates varies. Thus, unless a sample is known to contain only one carbohydrate, the results must be expressed arbitrarily in terms of one carbohydrate.

In this method, the concentrated sulfuric acid breaks down any polysaccharides, oligosaccharides, and disaccharides to monosaccharides. Pentoses (5-carbon compounds) then are dehydrated to furfural, and hexoses (6-carbon compounds) to hydroxymethyl furfural. These compounds then react with phenol to produce a yellow-gold color. For products that are very high in xylose (a pentose), such as wheat bran or corn bran, xylose should be used to construct the standard curve for the assay and measure the absorption at 480 nm. For products that are high in hexose sugars, glucose is commonly used to create the standard curve, and the absorption is measured at 490 nm. The color for this reaction is stable for several hours, and the accuracy of the method is within ±2% under proper conditions.

Carbohydrates are the major source of calories in soft drinks, beer, and fruit juices, supplying 4 Cal/gram carbohydrate. In this experiment, you will create a standard curve with a glucose standard solution, use it to determine the carbohydrate concentration of soft drinks and beer, then calculate the caloric content of those beverages.

14.1.2 Reading Assignment

BeMiller, J.N. 2017. Carbohydrate analysis. Ch. 19, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

14.1.3 Objective

Determine the total carbohydrate content of soft drinks and beers.

14.1.4 Principle of Method

Carbohydrates (simple sugars, oligosaccharides, polysaccharides, and their derivatives) react in the presence of strong acid and heat to generate furan derivatives that condense with phenol to form stable yellow-gold compounds that can be measured spectrophotometrically.

14.1.5 Chemicals

	CAS No.	*Hazards*
D-Glucose ($C_6H_{12}O_6$)	50-99-7	
Phenol (C_6H_6O)	108-95-2	Toxic
Sulfuric acid (H_2SO_4)	7664-93-9	Corrosive

14.1.6 Reagents

(** It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Glucose std. solution, 100 mg/L **
- Phenol, 80% wt/wt in H_2O, 1 mL **
  Prepare by adding 20-g deionized distilled (dd) water to 80 g of redistilled reagent grade phenol (crystals).
- Sulfuric acid, concentrated

14.1.7 Hazards, Cautions, and Waste Disposal

Use concentrated H_2SO_4 and the 80% phenol solution with caution. Wear gloves and safety glasses at all times, and use good lab technique. The concentrated H_2SO_4 is very corrosive (e.g., to clothes, shoes, skin). The phenol is toxic and must be discarded as hazardous waste. Other waste not containing phenol likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

14.1.8 Supplies

(Used by students)

- Beer (lite and regular, of same brand)
- Bottle to collect waste
- Cuvettes (tubes) for spectrophotometer
- Erlenmeyer flask, 100 mL, for dd water
- 2 Erlenmeyer flasks, 500 mL, for beverages
- Gloves
- Mechanical, adjustable volume pipettors, 1000 μL and 100 μL (or 200 μL), with plastic tips
- Pasteur pipettes and bulb
- Parafilm®
- Pipette bulb or pump
- Repipettor (for fast delivery of 5-mL conc. H_2SO_4)
- Soft drinks (clear-colored, diet and regular, of same brand)
- 20 test tubes, 16–20-mm internal diameter
- Test tube rack
- 4 Volumetric flasks, 100 mL *or* 2 Volumetric flasks, 1000 mL
- Volumetric pipette, 5 mL
- 2 Volumetric pipettes, 10 mL

14.1.9 Equipment

- Spectrophotometer
- Vortex mixer
- Water bath, maintained at 25 °C

14.2 PROCEDURE

(Instructions are given for analysis in duplicate.)

1. Standard curve tubes: Using the glucose standard solution (100 mg glucose/L) and dd water as indicated in the table below, pipette aliquots of the glucose standard into clean test tubes (duplicates for each concentration) such that the tubes contain 0–100 µL of glucose (use 1000-µl mechanical pipettor to pipette samples), in a total volume of 2 mL. These tubes will be used to create a standard curve, with values of 0–100-µg glucose/2 mL. The 0-µg glucose/2 mL sample will be used to prepare the reagent blank.

	µg Glucose/2 mL					
	0	*20*	*40*	*60*	*80*	*100*
mL glucose stock solution	0	0.2	0.4	0.6	0.8	1.0
mL dd water	2.0	1.8	1.6	1.4	1.2	1.0

2. Record caloric content from label: You will analyze for total carbohydrate content: (1) a regular and diet soft drink of the same brand, and/or (2) a regular and lite beer of the same brand. Before you proceed with the sample preparation and analysis, record the caloric content on the nutrition label of the samples you will analyze.
3. Decarbonate the beverages: With the beverages at room temperature, pour approximately 100 mL into a 500-mL Erlenmeyer flask. Shake gently at first (try not to foam the sample if it is beer) and continue gentle shaking until no observable carbon dioxide bubbles appear. If there is any noticeable suspended material in the beverage, filter the sample before analysis.
4. Sample tubes: So the sample tested will contain 20–100-µg glucose/2 mL, the dilution procedure and volumes to be assayed are given below.

	Dilution	*Volume assayed (mL)*
Soft drink		
Regular	1:2000	1
Diet	0	1
Beer		
Regular	1:2000	1
Lite	1:1000	1

Recommended dilution scheme for 1:2000 dilution:

(a) Pipette 5 mL of beverage into a 100-mL volumetric flask, and dilute to volume with dd water. Seal flask with Parafilm® and mix well (this is a 1:20 dilution). Then, pipette 1.0 mL of this 1:20 diluted beverage into another 100-mL volumetric flask. Dilute to volume with dd water. Seal flask with Parafilm® and mix well.

OR

(b) Pipette 1.0 mL of beverage into a 1000-mL volumetric flask, and dilute to volume with dd water. Seal flask with Parafilm® and mix well. Then, in a test tube, combine 1 mL of the 1:1000 diluted beverage and 1 mL dd water. Mix well.

Recommended dilution scheme for 1:1000 dilution:

(a) Pipette 10 mL of beverage into a 100-mL volumetric flask, and dilute to volume with dd water. Seal flask with Parafilm® and mix well (this is a 1:10 dilution). Then, pipette 1.0 mL of this 1:10 diluted beverage into another 100-mL volumetric flask. Dilute to volume with dd water. Seal flask with Parafilm® and mix well.

OR

(b) Pipette 1.0 mL of beverage into a 1000-mL volumetric flask, and dilute to volume with dd water. Seal flask with Parafilm® and mix well.

5. After dilution as indicated, pipette 1.0 mL of sample into a test tube and add 1.0 mL of dd water. Analyze each diluted sample in duplicate.
6. Phenol addition: To each tube from Parts 1 and 4 containing a total volume of 2 mL, add 0.05-mL 80% phenol (use 100 or 200-µl mechanical pipettor). Mix on a Vortex test tube mixer.
7. H_2SO_4 addition: To each tube from Part 6, add 5.0-mL H_2SO_4. The sulfuric acid reagent should be added rapidly to the test tube. Direct the stream of acid against the liquid surface rather than against the side of the test tube in order to obtain good mixing. (These reactions are driven by the heat produced upon the addition of H_2SO_4 to an aqueous sample. Thus, the rate of addition of sulfuric acid must be standardized.) Mix on a Vortex test tube mixer. Let tubes stand for 10 min and then place in a 25 °C bath for 10 min (i.e., to cool them to room temperature). Vortex the test tubes again before reading the absorbance.
8. Reading absorbance: Wear gloves to pour samples from test tubes into cuvettes. Do not

rinse cuvettes with water between samples. Zero the spectrophotometer with the standard curve sample that contains 0-µg glucose/2 mL (i.e., blank). Retain this blank sample in one cuvette for later use. Read absorbances of all other samples at 490 nm. Read your standard curve tubes from low to high concentration (i.e., 20 µg/2 mL up to 100 µg/2 mL), and then read your beverage samples. To be sure that the outside of the cuvettes are free of moisture and smudges, wipe the outside of the cuvette with a clean paper wipe prior to inserting it into the spectrophotometer for a reading.

9. Absorbance spectra: Use one of the duplicate tubes from a standard curve sample with an absorbance reading of 0.5-0.8. Determine the absorbance spectra from 450–550 nm by reading the tube at 10-nm intervals. Zero the spectrophotometer with the blank at each 10-nm interval.

14.3 DATA AND CALCULATIONS

1. Summarize your procedures and results for all standards and samples in the tables immediately below. Use the data for the standard curve samples in the first table to calculate the equation for the line, which is used to calculate the concentrations in the original samples reported in the second table.

Standard Curve:

Sample identity	*A_{490} msmt 1*	*A_{490} msmt 2*	*Avg.*
Blank			
Std. 20 µg			
Std. 40 µg			
Std. 60 µg			
Std. 80 µg			
Std. 100 µg			

Samples:

				Glucose equivalent	
Sample identity	*A_{490}*	*ug glucose/ 2 mL*	*Dilution scheme*	*µg/mL, original sample*	*g/L, original sample*
Soft drink, reg.					
Soft drink, reg.					
Soft drink, diet					
Soft drink, diet					
Beer, reg.					
Beer, reg.					
Beer, lite					
Beer, lite					

Sample calculation for soft drink, regular:

$$\text{Equation of the line: } y = 0.011x + 0.1027$$

$$y = 0.648$$

$$x = 49.57\,\mu\text{g} / 2\,\text{mL}$$

$$C_i = C_f\left(V_2 / V_1\right)\left(V_4 / V_3\right)$$

(See Chap. 3 in this laboratory manual, C_i = initial concentration; C_f = final concentration)

$$C_i = \left(49.57\,\mu\text{g glucose} / 2\,\text{mL}\right) \times \left(2000\,\text{mL} / 1\,\text{mL}\right) \times \left(2\,\text{mL} / 1\,\text{mL}\right) = 99\,140\,\mu\text{g} / \text{mL}$$
$$= 99.14\,\text{mg} / \text{mL}$$
$$= 99.14\,\text{g} / \text{L}$$

2. Construct a standard curve for your total carbohydrate determinations, expressed in terms of glucose (A_{490} versus µg glucose/2 mL). Determine the equation of the line for the standard curve.
3. Calculate the concentration of glucose in your soft drink samples and beer samples, in terms of (a) grams/liter and (b) g/12 fl. oz. (*Note:* 29.56 mL/fl. oz.)
4. Calculate the caloric content (based only on carbohydrate content) of your soft drink samples and beer samples in term of Cal/12 fl. oz.

Sample	*g Glucose/ 12 fl. oz.*	*Measured Cal/12 fl. oz.*	*Nutrition label Cal/12 fl. oz.*
Soft drink			
Regular			
Diet			
Beer			
Regular			
Lite			

5. Plot the absorbance spectra obtained by measuring the absorbance between 450 and 550 nm.

wave-length	450	460	470	480	490	500	510	520	530	540	550
Abs.											

14.4 QUESTIONS

1. What are the advantages, disadvantages, and sources of error for this method to determine total carbohydrates?
2. Your lab technician performed the phenol-H_2SO_4 analysis on food samples for total carbohydrates but the results showed low precision, and the values seemed a little high. The technician had used new test tubes (they had never been used, and were taken right from the cardboard box). What most likely caused these results? Why? Describe what happened.
3. If you started with a glucose standard solution of 10-g glucose/liter, what dilution of this solution would be necessary such that you could pipette 0.20, 0.40, 0.60, 0.80, and 1.0 mL of the diluted glucose standard solution into test tubes and add water to 2 mL for the standard curve tubes (20–100 μg/2 mL)? Show all calculations.
4. If you had not been told to do a 2000-fold dilution of a soft drink sample, and if you know the approximate carbohydrate content of regular soft drinks (US Department of Agriculture Nutrient Database for Standard Reference indicates ca. 3 g carbohydrate/fl. oz.), how could you have calculated the 2000-fold dilution was appropriate if you wanted to use 1 mL of diluted soft drink in the assay. Show all calculations.
5. How does your calculated value compare to the caloric content on the food label? Do the rounding rules for Calories explain any differences? (See Metzger and Nielsen, 2017, Table 3.3). Does the alcohol content (assume 4–5% alcohol at 7 Cal/g) of beer explain any differences?
6. Was it best to have read the absorbance for the standard curve and other samples at 490 nm? Explain why a wavelength in this region is appropriate for this reaction.

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RESOURCE MATERIALS

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