

◀RAMBLE'S SOUP CO.▶

MEMO

TO: Product Testing Lab

FROM: Vice President, New Products

PROBLEM: The product development group is proposing to use a carbohydrate polymer as a thickening agent in a new product line. The plant gums (polysaccharides) they are considering (arabic or ghatti) are reported to contain some portion of the following monosaccharide units: galactose, mannose, xylose, arabinose, rhamnose and glucuronic acid. We are concerned about the breakdown of the product by acid hydrolysis when the soup is heated which will adversely affect the consistency. Some soups in the *Lumpy Soup* product line contain about two tablespoons of vinegar per serving. We would like to know if significant hydrolysis occurs during normal heating times. Investigate hydrolysis of the gums under acidic conditions and at boiling. Also determine the relative amounts of hexose and pentose sugars in the gums (o-toluidine colorimetric method). Determine which of the monosaccharide components listed are contained in each of the gums by thin layer chromatography following hydrolysis.

REPORT: For your sample, report the hydrolysis results and conclusions, the component residues and the percentages of pentose and hexose components. Your report is needed in two weeks.

Test for Polysaccharide Hydrolysis

Benedict's Test - This is a test for reducing sugars. These are any carbohydrate with a potential aldehyde group (hemiacetal) or potential ketone group (hemiketal). Carbohydrates yielding positive tests are monosaccharides or small oligosaccharides without linked anomeric carbons. Large polysaccharides, where the majority of anomeric carbons are involved in linkages, will test negative. Therefore, unhydrolyzed polysaccharide will test negative while hydrolyzed will test positive.

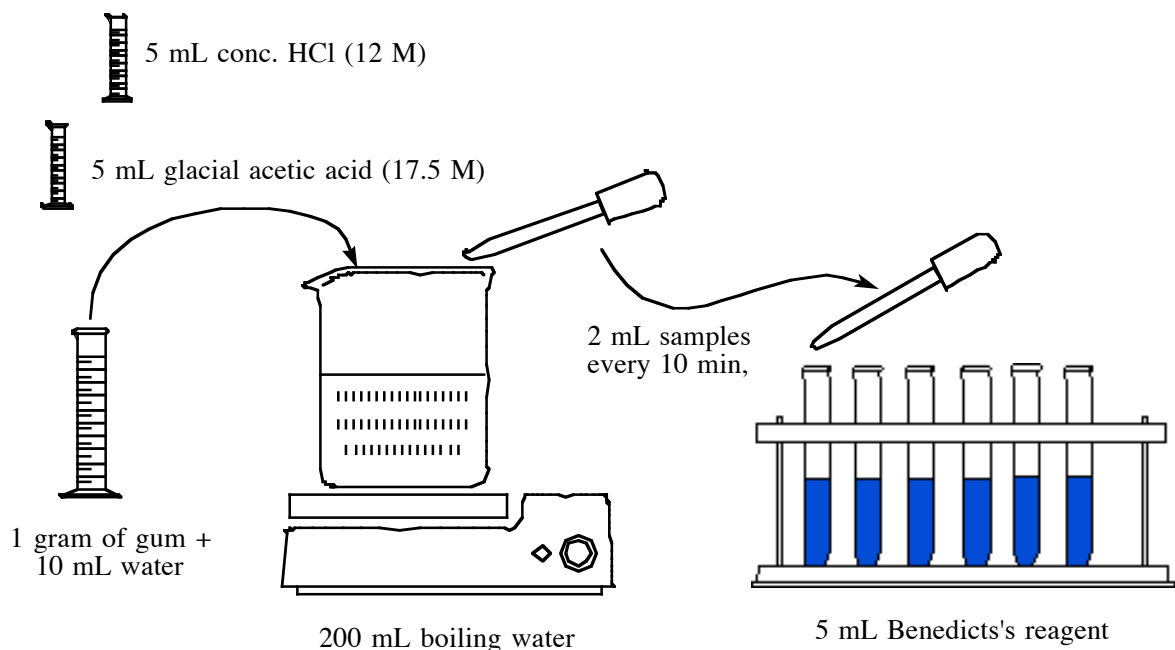
Benedict's qualitative reagent is a solution of sodium carbonate, sodium citrate and copper sulfate. When it is heated (5 minutes boiling water) with a reducing sugar, the Cu^{2+} ion is reduced to Cu^{1+} which then precipitates as a brick-red oxide, Cu_2O . Red precipitate indicates a positive test. Compare results with a 1% galactose and a 1% sucrose solution.

Solutions:

- 1) Glacial acetic acid
- 2) Concentrated hydrochloric acid
- 3) 10% sodium hydroxide
- 4) Benedict's reagent

Laboratory:

1. Add about one gram of the polysaccharide gum to 10 mL of cold water. Make note of the solubility.
2. Heat 200 mL of water to boiling and add the gum suspension to the hot water. Do an initial Benedict's test by removing about 2 mL from the solution and transferring to a test tube containing 5 mL of Benedict's reagent. (Heat tube in a boiling water bath for 5 minutes).
3. Next, add 5 ml glacial acetic acid to the boiling "soup". After 10 minutes, remove about 2 mL from the solution and transfer to a test tube, cool and neutralize with 10% NaOH and do another Benedict's test.
4. Repeat this procedure again after 20 minutes.
5. After removing the 20 minute sample, add 5 mL of concentrated HCl to the hot solution and continue heating. After 10 minutes with HCl, remove 2 mL, cool and neutralize with 10% NaOH, and do another Benedict's test.



o-Toluidine Colorimetric Method for Hexoses and Pentoses

Reference: Goodwin, J.F. *Anal. Biochem.*, 1972, 48, pp 120-128.

Aldohexoses react with o-toluidine in hot acetic acid to produce a mixture of glycosylamines and the corresponding Schiff base which have an absorbance maximum around 630 nm. Aldopentoses react and the products show an absorbance maximum around 480 nm. Simultaneous quantification of both components is possible by measuring the molar absorptivities of the individual components at both wavelengths, and then measuring the absorbances of samples at the same wavelengths.

Solutions:

- 1) Acetic acid reagent - 1.5 g thiourea and 3.5 g boric acid in one liter of glacial acetic acid.
- 2) Amine reagent - add 5.0 mL of o-toluidine to 95.0 mL of the acetic acid reagent.
- 3) Standard monosaccharide solutions - accurately weigh 0.1 g of hexose or pentose into 100 mL volumetric flask and dilute to mark with distilled water. Calculate molarity.
- 4) Polysaccharide sample - accurately weigh 0.1 g of polysaccharide into a test tube. Add 10 mL water and 1 mL concentrated HCl. Heat in boiling water bath for 30 minutes. Pour solution into 100 mL volumetric flask and dilute to the mark. Calculate concentration in grams/Liter.

Laboratory:

- 1) Prepare a blank by mixing 5.00 mL of amine reagent and 0.1 mL water.
- 2) Prepare a standard solution for each of the hexose and pentose solutions. Mix 5.00 mL of amine reagent with 0.050 mL of standard sugar and 0.050 mL of distilled water to total 0.100 mL.
- 3) Prepare two standard mixture samples by combining 5.00 mL of amine reagent, 0.050 mL of the standard hexose and 0.050 mL of the standard pentose.
- 4) Prepare two unknown solutions by mixing 5.00 mL of amine reagent, 0.05 mL of hydrolyzed and diluted polysaccharide sample, and 0.05 mL distilled water.
- 5) Heat all solutions in boiling water for 10 minutes - cool. Read the absorption spectra of the individual pentose and hexose solutions from 450 nm to 650 nm. Read the absorbances of mixtures and unknowns at 480 nm and 630 nm relative to the blank.

Cramer's Rule for Simultaneous Equations

$$A_{480} = a_{P,480}p + a_{H,480}h$$

$$A_{630} = a_{P,630}p + a_{H,630}h$$

A = Absorbance at wavelength shown

a = molar absorptivity of pentose or hexose at wavelength shown

p = molarity of pentose

h = molarity of hexose

$$p = \frac{\begin{vmatrix} A_{480} & a_{H,480} \\ A_{630} & a_{H,630} \end{vmatrix}}{\begin{vmatrix} a_{P,480} & a_{H,480} \\ a_{P,630} & a_{H,630} \end{vmatrix}}$$
$$h = \frac{\begin{vmatrix} a_{P,480} & A_{480} \\ a_{P,630} & A_{630} \end{vmatrix}}{\begin{vmatrix} a_{P,480} & a_{H,480} \\ a_{P,630} & a_{H,630} \end{vmatrix}}$$

Thin Layer Chromatography of Monosaccharides

Reference: White, B.J.; Robyt, J.F. *J. Chem. Ed.*, **1988**, 65(2), pp 164-166.

Solutions:

- 1) Mobile phase - acetonitrile:water, 85:15 (v,v)
- 2) 1% (w,v) solutions of galactose, mannose, arabinose, xylose, rhamnose and glucuronic acid.
- 3) Polysaccharide Acid Hydrolysate - Add 0.2 g of polysaccharide to 10 mL of water in a test tube, and add 1 mL trifluoroacetic acid. Tightly stopper and heat in boiling water bath for 30 minutes.
- 4) Developing Spray - methanol:sulfuric acid, 4:1 (v,v)

Laboratory:

- 1) Prepare plate (Whatman K5 Silica Gel) by using a dull pencil to draw a horizontal line 2.5 cm from the bottom of the plate. Place four equally spaced dots on the line for four ascent lanes.
- 2) With a capillary tube, spot galactose and arabinose in lane 1; mannose and xylose in lane 2; rhamnose and glucuronic acid in lane 3; and the acid hydrolysate in lane 4. Allow to dry thoroughly.
- 3) Prepare chromatography chamber and allow to equilibrate. Run two mobile phase ascents with thorough drying in between.
- 4) Allow to dry completely after second ascent. Lightly spray plate with developing solution and place in oven at 110°C for 10 min.
- 5) Identify spots and measure migration distances.

Report:

Include as part of the report:

Experimental

- polysaccharide (g/L?), acetic acid (M?), hydrochloric acid (M?) concentrations in hydrolysis test mixture; procedure followed
- Standard solution M of pentose and hexose; polysaccharide (g/L) used for o-toluidine method

Results

- Sample calculations: Calculation of molar absorptivity from absorption spectra; sample calculation using Cramer's Rule
- Table summarizing hydrolysis (Benedict's test) results
- Figures for each of the two standard solutions showing the absorption spectrum from 450 nm to 650 nm.
- Spreadsheet Table showing Cramer's Rule calculation of the percent hexose and percent pentose in the 2 known mixtures (compare these to the known concentrations) and the 2 unknown solutions. Use the 4 molar absorptivities calculated from the absorption spectra, along with the absorbances of the solutions.
- Table showing structures of all monosaccharides used for TLC
- Figure showing the results of the TLC experiment; all spots should be identified and labeled