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Note

A *p*-hydroxybenzoylhydrazine method for the analysis of cellulose ethers and water-soluble cellulose derivatives

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Many spectrophotometric methods have been reported for the determination of reducing carbohydrates and polysaccharides. Our studies required sensitivity in the range of 0–10 p.p.m. of cellulose methyl ethers in solution. The phenol–sulfuric acid[1] or anthrone[2–4] methods for polysaccharides did not appear to provide the required sensitivity without considerable prior concentration of the sample. Recently, a new method was developed for determination of D-glucose based on the use of *p*-hydroxybenzoylhydrazine[5]. This method formed the basis for the spectrophotometric assay described herein.

The initial process needed for a method based on the foregoing chemistry was a suitable procedure for hydrolyzing the cellulose methyl ethers to the monomeric

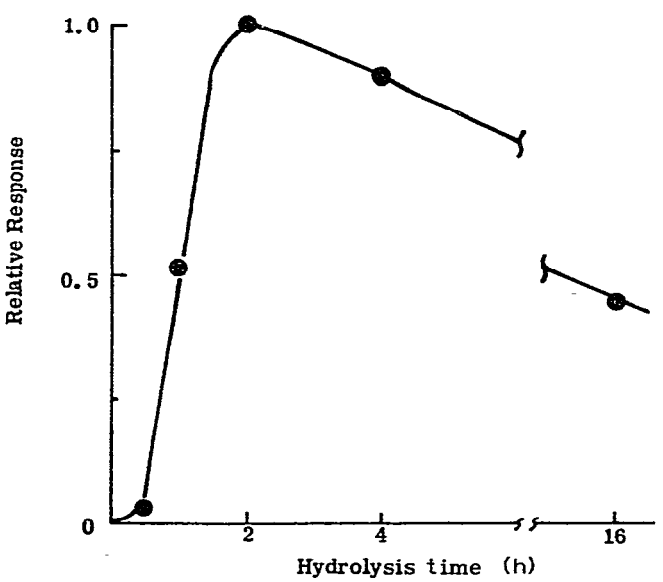
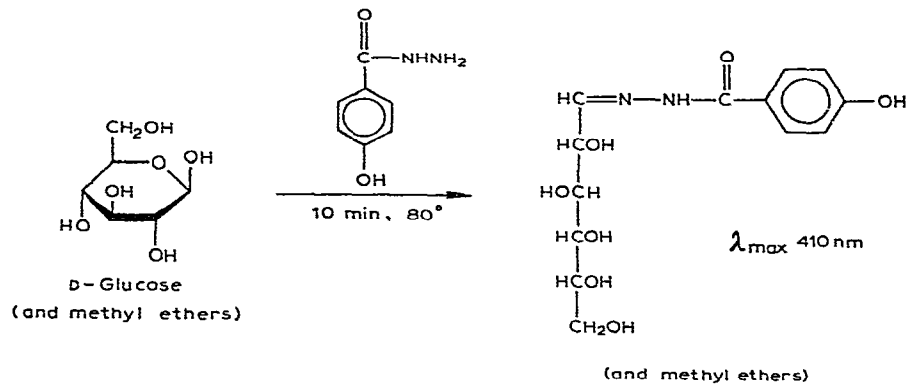


Fig. 1. The effect of hydrolysis time (h) in the *p*-hydroxybenzoylhydrazine method.

methylated D-glucose derivatives. A common procedure used with polysaccharides involves hydrolysis overnight in cold, concentrated sulfuric acid followed by a second hydrolysis in dilute acid under more-vigorous conditions. Sulfuric acid is then removed by precipitation with barium hydroxide. A more-rapid method was required for our purposes.

Trifluoroacetic acid has been employed previously for the hydrolysis of plant cell-wall polysaccharides[6]. Yields of monosaccharide by this hydrolytic method have been found comparable to those obtained by hydrolysis with mineral acid and, furthermore, trifluoroacetic acid may be readily removed by evaporation. Trifluoroacetic acid was found useful for hydrolysis of cellulose ethers. Subsequent to further development of the method, it was shown that hydrolysis for 2 h in vials (sealed with Teflon-lined caps) kept in an oven at 120° provided the optimum response in the hydrazone formation step, as shown in Fig. 1. The substituted D-glucose derivatives were then recovered by evaporating off the trifluoroacetic acid at 50° under a stream of filtered nitrogen.



The complete reaction-sequence for the method is indicated in the accompanying chart. The hydrazone chromophore is prepared by treatment of the hydrolyzate with *p*-hydroxybenzoylhydrazine under alkaline conditions. Although formation of the monoarylhydrazone is indicated, osazone formation may also occur[5,9]. For analysis of D-glucose, a reaction time of 5 min in a water bath at 100° was determined to be optimal[5]. With the mixture of D-glucose and its methyl ethers obtained from the hydrolysis of cellulose ethers, a longer reaction-time was needed, as shown in Fig. 2. However, we have shown that color development may be reproducibly obtained after a reaction time of 10 min in a bath at 80°. The absorption spectrum of the *p*-hydroxybenzoylhydrazones of substituted D-glucose derivatives is shown in Fig. 3, where curve A represents the hydrolyzed cellulose ether heated with the hydrazine and scanned against the reagent blank, and curve B is the reagent blank scanned against water. The absorption maximum was found to be 390 nm. The ratio of absorbance

of derivative to absorbance of reagent blank is maximal at about 410 nm, and this wavelength was utilized in subsequent investigations. A typical calibration-curve based on 10-ml aliquots containing 0–10 p.p.m. of cellulose methyl ether in solution is shown in Fig. 4.

The method described is dependent to a certain extent on the degree of substitution (d.s.) by methyl of the cellulose ether. Materials of lower d.s. produce a larger proportion of osazone relative to the simple hydrazone, and these thereby affect the observed molar absorptivity[7,8]. Therefore, calibration curves need to be developed for each material investigated. We have also utilized the method for *O*-2-hydroxyethyl, *O*-3-hydroxypropyl, *O*-carboxymethyl, and other water-soluble cellulose derivatives.

We have found the sensitivity (μg level) provided by this spectrophotometric procedure to be adequate for our needs. However, a fluorometric method could be developed to increase the sensitivity to the ng level by utilizing the fluorescence of the lanthanum chelate of the D-glucose *p*-hydroxybenzoylhydrazone[9]. An automated method has also been described[10].

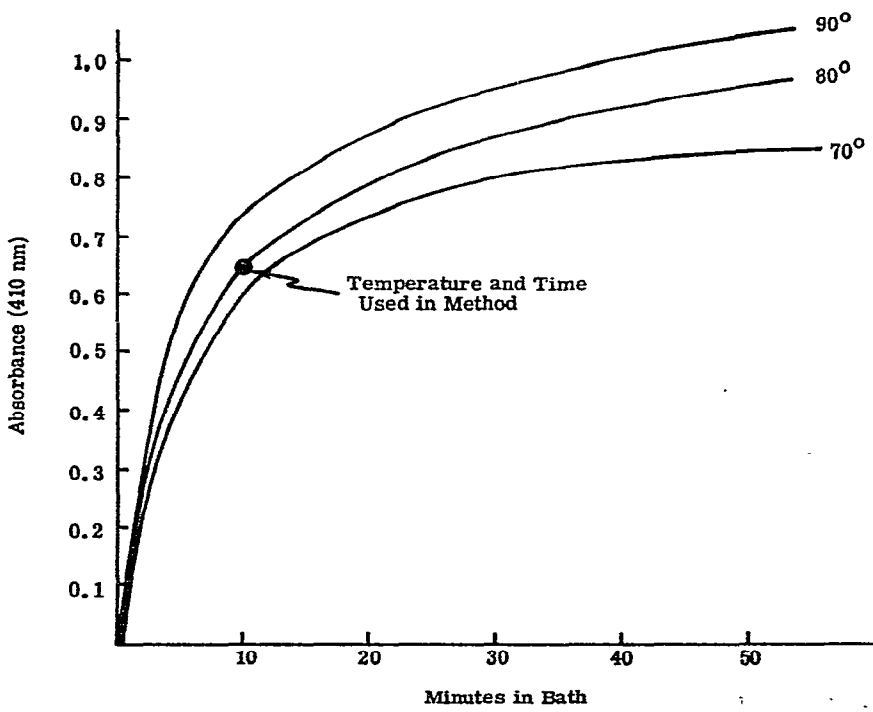


Fig. 2. Kinetics of hydrazone formation as a function of time and temperature.

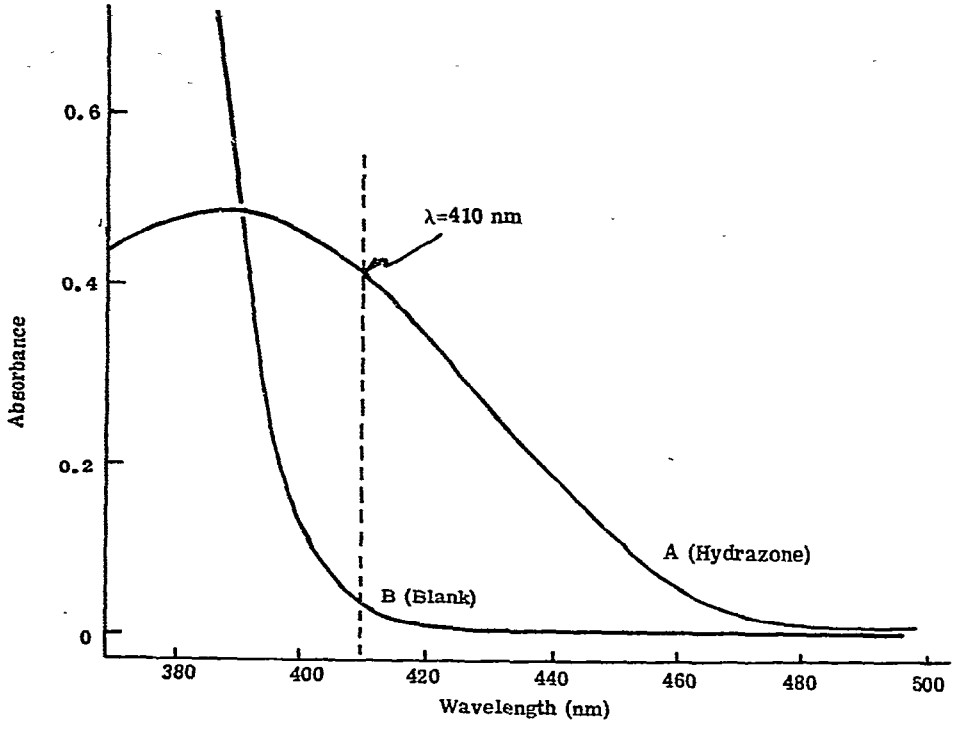


Fig. 3. Absorption spectrum of the *p*-hydroxybenzoylhydrazone of hydrolyzed cellulose ether.

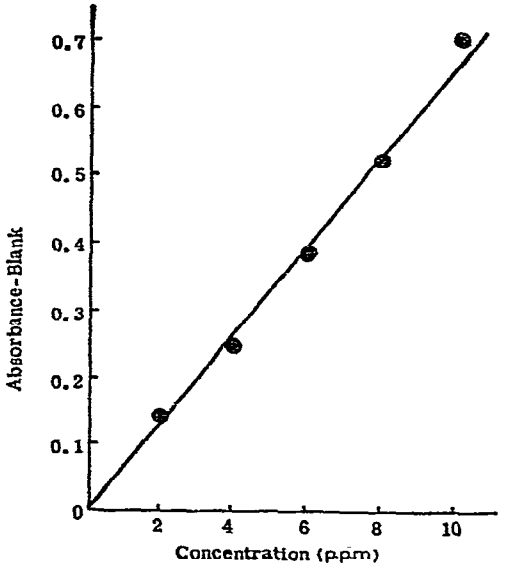


Fig. 4. Typical calibration curve for a cellulose ether.

EXPERIMENTAL

Materials. — The cellulose derivatives used were commercial Dow materials (Methocel series) or laboratory-prepared materials. *p*-Hydroxybenzoylhydrazine was obtained from Aldrich, and trifluoroacetic acid from Eastman. Laboratory distilled water was utilized.

Method. — A series of standard solutions of cellulose ethers is prepared. A good working-range is 0–10 p.p.m., or aliquots that contain 20–100 μg of the cellulose ether. For the 0–10 p.p.m. range, an aliquot (10 ml) is placed in an appropriately sized vial. Evaporation to dryness at this point in a water bath at 100° under a stream of nitrogen improves the precision of the method. After evaporation, trifluoroacetic acid (2–3M, 2 ml) is added. [If the samples are not evaporated initially, trifluoroacetic acid (1.5 ml) may be added directly.] The vials are sealed with Teflon-lined caps, and the contents hydrolyzed in an oven at 120°. The cooled vials are opened, and the contents evaporated to dryness as described. A stock solution of *p*-hydroxybenzoylhydrazine is prepared by weighing 5 g into a volumetric flask (100 ml) and dissolving it with hydrochloric acid (0.5M). An aliquot of this solution (50 ml) is pipetted into a volumetric flask (250 ml), which is made up to volume with 0.5M sodium hydroxide. The alkaline solution of hydrazine (4 ml) is pipetted into the vials. Formation of the hydrazone is accomplished by heating in a water bath for 10 min at 80°. Care should be taken to prevent the solutions from coming into contact with the cap liners. The solutions are allowed to cool for 15 min, and the absorbance is recorded at 410 nm *vs.* distilled water, using 1-cm cuvettes.

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