Measurement of starch in paper by high-pressure liquid chromatography

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ABSTRACT: A quick and easy method using high-pressure liquid chromatography (HPLC) is proposed for determining starch in paper. The starch is converted into dextrose by hydrolysis of the starch in paper and the use of enzymes. Adding a known amount of starch to pulp and analyzing for it yielded a 97% recovery. A 97% recovery was also obtained when starches of different dgrees of substitution were analyzed. It is shown that the HPLC method is more reproducible than the spectrophotometric method.

KEYWORDS: Amylase, enzymes, glucose, high pressure, liquid chromatography, measurement, paper, starch.

The quantitative determination of the amount of starch in paper is based on a high-pressure liquid chromatography (HPLC) assay of the converted starch into a monosaccharide. This procedure involved the hydrolysis of starch in paper with the use of enzymes. Starch is converted to dextrose with alpha-amylase and glucoamylase. An HPLC is used to determine the amount of starch in the paper which is equivalent to 90% of the amount of dextrose because every time dextrose is formed, a molecule of water is added with a molecular weight of 18.

$$\text{Starch} + n\text{HOH} \rightarrow (n-1)\ \text{dextrose}$$

$$\text{Starch} + \text{HOH} \rightarrow \underset{\text{OH}}{\text{dextrose}} + \underset{\text{H}}{\text{dextrose}}$$

$$162/180 = 0.90$$

Experimental

Apparatus

Volumetric flasks, 250 mL
HPLC instrument with integrator
HPLC column: Biorad, Amineh carbohydrate HPH-42 A,
Cat 125-00597
Syringe for sample injection (10–50 µL)
Filtering flask, 500 mL
Buchner funnel, 500 mL
Filter paper No. 40, ashless, Whatman
Water bath with thermostat

Reagents

Hydrochloric acid, concentrated
Alpha-amylase, Canalpha, Biocon, 600,000 units/mL
Glucoamylase, Enzyme Development, 220 mg/mL

One to two grams of paper were cut up into 1-cm squares and placed into a 4-oz. Osterizer miniblend container. Then 90–100 mL of distilled water was added into the container. The mixture was ground in an Osterizer blender until the pulp was shredded to fine particles. In the meantime, a water bath was heated to 70–80°C (temperature for optimum activity of alpha-amylase, Canalpha, Biocon). pH was maintained at 6–7. After the mixture temperature reached 70–80°C, 10 drops (USP) of alpha-amylase were added. The mixture was maintained at these conditions with occasional stirring for 30 min. Dilute HCl was added to the mixture to reach pH 4.1–4.3. The temperature of the bath was raised to 85°C, and 10 drops (USP) of glucoamylase from Enzyme Development was added and stirred. These conditions were maintained with occasional stirring for 1–1.5 h. Dilute acid was added to pH 3, and the mixture was allowed to remain in the water bath for at least 10 min. This mixture was filtered hot through a Buchner funnel with No. 40 Whatman filter paper. Two portions of 10 mL each of hot distilled water were washed through the pulp on the filter paper. The solution was transferred into a 250-mL volumetric flask that contained 0.5 mL of concentrated HCl. The solution was swirled and set on a steam bath for 10–15 min to ensure the deactivation of the enzymes. It was cooled to room temperature and distilled water was added to volume. One mL of this solution was filtered through a 0.2µ Millipore syringe filter. This solution was injected into an HPLC, 10 µL were delivered automatically, and the area of the dextrose portion of the chromatogram was measured. The exact time of the dextrose peak was determined beforehand. A blank con-

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taining 150 mL distilled water and the same amount of each enzyme was used. This mixture underwent the same conditions as the samples.

Calculation:

% Dextrose = % starch (0.90)

% Starch = area (slope) 250 mL/g paper sample

If the blank (as described in the previous procedure) showed an area for dextrose, this was subtracted from the area of the equation above.

A calibration curve was made by making varying amounts of dextrose concentrations of 0.01–0.08% from a 1% stock solution of 0.5 g of dried analytical grade dextrose dissolved in 500 mL distilled water. The area of each concentration was plotted against concentration of dextrose.

The slope was taken after a linear regression was drawn from this curve.

Discussion

Starch is vital in the production of paper because it enhances appearance, strength, and printability. This article suggests a simple, quick and easy method for measuring starch in paper. A spectrophotometric method has been used *(1, 2)*. The reaction of partially hydrolyzed starch with a dilute solution of phenol, and a small amount of concentrated sulfuric acid results in an amber-colored solution that absorbs at 490 nm. This method is good for clear, colorless solutions. If the paper is colored, absorbance measurements are difficult, so carbon black is added to the solution before filtration. The naked eye cannot determine if all the color has been removed and/or all the carbon black has been removed. The addition of a dilute solution of phenol and sulfuric acid might not always produce the same intensity of color for any given sample and the presence of lignin in colored paper will interfere in the analysis. Concentrated sulfuric acid presents a possible hazard of handling and environmental concern, i.e., disposal of very acidic solutions. Therefore, a new method was studied that gave reproducible results.

A TAPPI method *(3)* in the procedures manual utilizes absorbance measurements of a colored solution resulting from the reaction of starch and iodine, and another method uses reflectance measurements *(4)*.

I. Comparison of HPLC and spectrophotometric methods

	Spectrophotometric	*HPLC*
Paper 1 N=5	5.20% Starch SD 0.81	6.86% Starch SD 0.183
Paper 2 N=5	3.67% Starch SD 0.094	4.14% Starch SD 0.080

II. % Recovery study (HPLC method) Addition of starch to paper

Starch added	*N*	*% Recovery*
Pearl (unsubstituted)	4	97.61
Cationic (Apollo 600)	4	97.50
Hydroxyethylated (PG 280)	4	95.68

Examples of starch analysis by HPLC:

Paper 1 LM 68-91:

This paper was an alkaline reply card with Penford Gum added.

Paper 2 A 4721:

This was newsprint paper that was coated with 4.69% Penford Gum 295. The two sets of data in **Table I** show that the HPLC method of starch analysis is more reproducible than the previous method. Adding a known amount of starch to pulp and analyzing the total amount of starch showed that recovery was about 97%. A study was also made on the recovery of starch with different degrees of substitution added to pulp, e.g., pearl starch that does not have any substitution, a cationic starch, and a hydroxyethylated starch produced at Penford Products Co. These also showed about 97% recovery. The paper used for this study was Whatman No. 541, a filter paper that does not have starch.
(**Table II**).

HPLC should be performed the same day that the enzyme hydrolysis is done because the dextrose solution is not stable even if refrigerated.

Literature cited

1. Boettcher, N. B., Penford Products Co., Jan. 7, 1988 [adapted from Ref. *(2)*].
2. Dubois, M., Gilles, K. A., Hamilton, *et al.*, Anal. Chem. 28(3): 102(1956).
3. TAPPI Test Method T 638 cm-85 "Analysis of starch and starch products."
4. Boczkowski, R. J., Tappi J. 65(12): 102(1982).

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