HYDROXYETHYL SUBSTITUTION LEVEL

PRINCIPLE

The hydroxyethyl group is cleaved from the starch by hydrogen iodide in the presence of adipic acid and an organic solvent containing a known concentration of toluene as an internal standard. All hydroxyethyl groups are quantitatively converted to iodoethane, which is detected by gas chromatography and measured relative to the area of the toluene peak. Comparison with a standard curve gives the concentration of iodoethane which is then used to calculate the weight percent of hydroxyethyl groups in the starch (Note 1).

SCOPE

This method is applicable to hydroxyethyl starches. It has been validated for hydroxyethyl starches made from ordinary corn starch. It is applicable, with modifications, to other hydroxyethyl starches and hydroxyethyl celluloses.

SAFETY NOTE

Since the procedure involves the use of hazardous chemicals, the heating of sealed vials under pressure and flame ionization detection, all appropriate safety precautions and waste disposal requirements should be reviewed before carrying it out. The analyst should wear appropriate eye and hand protection and handle reagents in a fume hood. In addition, the analyst should heat and handle the sealed reaction vials with heavy rubber gloves behind a safety shield in the hood.

SPECIAL APPARATUS

1. A gas chromatograph equipped with flame ionization detection and a computing integrator.

2. Reacti-Therm Heating Module (available from Pierce Chemical Company, 3747 N. Meridian Rd., P.O. Box 117, Rockford, IL 61105), Product #18870 (single block) or Product #18835 (triple block), or equivalent (Note 2).

3. Aluminum heating block, Product #18802, available from Pierce Chemical Company, or equivalent.

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4. Reacti-Vials, 5.0 mL capacity, Product #13223 with open top screw caps and Tuf-Bond Discs (Product #12718), available from Pierce Chemical Company, or equivalent (Note 3).

5. Pipets, 2 mL TD.

REAGENTS

1. Mixed Xylenes, ACS reagent grade, free of toluene, Aldrich cat #44,337-9, or equivalent. Check for the absence of toluene by running a blank by gas chromatography.

2. Iodoethane (ethyl iodide), 99%.

3. Toluene, ACS reagent grade.

4. Internal Standard Solution: 5 mg/mL toluene in xylene. Weigh 5.00 ± 0.01 g toluene and dilute to 1 L with xylene.

5. Standard Stock Solution: Tare a 25 mL volumetric flask with glass stopper. Add 1 mL of iodoethane to the flask. Reweigh to determine the amount of iodoethane in the flask. Dilute to volume with the internal standard solution.

6. Hydriodic acid, 57%: A high purity constant boiling acid (HI) of specific gravity 1.7 must be used. Available from G. Frederick Smith Co., 867 Mckinley Avenue, Columbus, OH 43223.

7. Adipic acid, mp 151-153 °C .

8. Gas Liquid Chromatographic Column: Stainless steel, 10 ft x 1/8 inch, packed with 10% SP2100 on 80/100 mesh Chromosorb W-HP is suggested (available from Supelco, Supelco Park, Bellefonte, PA 16823-0048). Other non-polar methyl silicones can be used but the chromatographic parameters given here are for SP2100 (Notes 2 & 3).

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CHROMATOGRAPH PARAMETERS

1. Column Temperature: 100 °C
2. Injector Port Temperature: 180 °C
3. Detector Temperature: 250 °C
4. Gas Flow Rates: Nitrogen 20 mL/min.

PROCEDURE

Standardization: Pipet 0.6 mL of the standard stock solution into a 25 mL glass vial. Add 7.4 mL of the internal standard solution and mix. Pipet 1.0 mL of the solution into a 5 mL Reacti-Vial, add 2 mL of hydriodic acid and 150 mg of adipic acid. Mix and inject 1.0µL of the upper (xylene) layer into the gas chromatograph.

Determine the area of the toluene peak and the iodoethane peak. Calculate the amount of iodoethane in the standard using the following calculation:

$$\frac{\text{iodoethane, } mg}{\text{mL}} = \frac{(\text{wt iodoethane, } mg)(0.6)}{(25)(8)}$$

Determine the amount of ethylene oxide using the following formula:

$$\frac{\text{mg ethylene oxide}}{\text{mL}} = \frac{(\text{mg iodoethane})(44.05)}{(\text{mL})(155.97)}$$

This solution will also contain 5.0 mg of toluene per mL.
Determine the response factor (RF) using the following formula:

$$\text{RF} = \frac{(\text{iodoethane, } mg)(\text{area of internal standard})}{(\text{area of iodoethane})(\text{internal standard, } mg)}$$

where the weight of the internal standard is 5.0 mg and the weight of iodoethane was calculated above (equation 1).

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Wash and dry a representative sample of starch (Note 4 & 5). Accurately weigh about 80 mg (± 3) d.b. of starch and transfer to a 5 mL Reacti-Vial. Add 150 mg of adipic acid. Add 2 mL (using a 2 mL TD pipet) of mixed xylenes which contains 5.0 mg/mL of toluene, followed by 2.0 mL of hydriodic acid. Wrap the threads of the vial with teflon tape and cap the vial. Weigh the vial. Heat the vial at 150 °C for 3.5 hrs., inverting it every 10 min. during the first hr. and every 40 mins. thereafter. Cool to room temperature and reweigh the vial. If the vial has lost less than 50 mg, centrifuge it at about 800 rpm for 10 mins. Vials with a weight loss of more than 50 mg should be rejected and the analysis repeated. Inject 1.0 µL of the xylene (upper) layer into the gas chromatograph. Determine the areas of the internal standard and iodoethane.

CALCULATION

$$\text{mg iodoethane} = \frac{(\text{area iodoethane})(\text{wt internal standard})\,\text{R.F.}}{(\text{area internal standard})}$$

$$\%\ \text{ethylene oxide} = \frac{(44.05)(\text{mg iodoethane})(100)}{(155.97)(\text{sample weight d.b.(mg)})}$$

NOTES AND PRECAUTIONS

1. This method is derived from the classical Zeisel procedure (CRA method C-30). As originally developed, the distillation method is discussed in reference 1. A more complete discussion is found in reference 4.

2. The single block heating module will heat form ambient temperature to 150 °C (temperature control: ±0.5 °C). The triple block heating module can be operated to 200 °C. Both are also available with magnetic stirring capability, respectively, as products #18970 and #18935.

3. The Tuf-Bond™ disc is designed to provide for good seal/reseal and adequate resistance to pressure. However, published information in References 2 and 4 below was obtained after combining open caps with Mininert7 Valves (Pierce catalogue #10127). Use of closed caps is less convenient: vials need to be open for sampling and injection into GC. Also, holding open vial for multiple injections could cause loss of alkyl iodide.

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4. The gas chromatographic conditions for different liquid phases such as OV-101 (a dimethyl silicone) and UCW 98 (a methyl silicone) should be determined by experimentation.

5. Hydriodic acid degrades some of the starch and materials from the reaction are deposited on the column. The column should be cleaned by heating it to 180 °C for at least three hrs.

6. Ethylene glycol may be present as a by-product of manufacturing the starch derivative and could form iodoethane, therefore should be removed by washing with water.

7. The starch could be dried essentially to zero moisture, but it may be used as is after determining moisture by an approved method.

REFERENCES

1. Lortz, H. J., *Anal. Chem.*, 28, 892-895 (1956).

2. Hodges, K. L., Kester, W. E., Wiederrich, D. L. and Grover, J. A., *Anal. Chem.*, 51,2172- 2176 (1979).

3. West, I. R., Hedges, A. R., Owen, R. L. and Yahl, K. R., American Maize Products Company, unpublished report (1982).

4. Lee, Y. Baaske, D. M. and Carter, J. E., *Anal. Chem.*, 55, 334-338 (1983).

METHOD HISTORY

Corn Starch (Modified), Hydroxyethyl Substitution Level (C-31), Date of Acceptance 6-30-1998.