

3.5.11

AOAC Official Method 948.02

Starch in Plants

Titrimetric Method

First Action 1948

Final Action 1962

A. Reagents

(a) *Iodine-potassium iodide solution*.—Grind 7.5 g I₂ and 7.5 g KI with 150 mL H₂O, dilute to 250 mL, and filter.

(b) *Alcoholic sodium chloride solution*.—Mix 350 mL alcohol, 80 mL H₂O, and 50 mL 20% NaCl solution, and dilute to 500 mL with H₂O.

(c) *Alcoholic sodium hydroxide solution*.—0.25M. Mix 350 mL alcohol, 100 mL H₂O, and 25 mL 5M NaOH, and dilute to 500 mL with H₂O.

(d) *Dilute hydrochloric acid*.—0.7M. Dilute 60 mL HCl to 1 L with H₂O.

(e) *Somogyi phosphate sugar reagent*.—Dissolve 56 g anhydrous Na₂HPO₄ and 80 g Rochelle salt in ca 1 L H₂O, and add 200 mL 1.00M NaOH. Slowly add, with stirring, 160 mL 10% CuSO₄·5H₂O solution. Dissolve 360 g anhydrous Na₂SO₄ in this solution, transfer to 2 L volumetric flask, and add exactly 200 mL 0.0167M KIO₃ solution (3.5667 g/L). Dilute to volume, mix well, let stand several days, and filter through dry paper into dry flask, discarding first 50 mL filtrate. Store reagent at 20–25°C. It is 0.00167M with respect to KIO₃; 5.00 mL is equivalent to 10 mL 0.005M Na₂S₂O₃.

Determine glucose factor of reagent as follows: Accurately weigh 150 mg NIST glucose SRM into 1 L volumetric flask, dissolve in H₂O, dilute to volume, and mix well. Transfer 5 mL aliquot to 25 × 200 mm Pyrex test tube, add exactly 5 mL Somogyi reagent, stopper with size 00 crucible, and heat (together with several blanks containing 5 mL H₂O and 5 mL reagent) exactly 15 min in boiling water bath. Titrate as in determination. From difference between blank and standard titrations, calculate mg glucose equivalent to 1 mL exactly 0.005M Na₂S₂O₃. Effective range for determination is 0.05–1.0 mg glucose in 5 mL aliquot.

(f) *Sodium thiosulfate standard solution*.—0.0055M. Dissolve 2.73 g Na₂S₂O₃·5H₂O in H₂O and dilute to 2 L. Standardize daily as follows: Add 1 mL KI solution, (g), and 3 mL 0.75M H₂SO₄ to 5 mL Somogyi sugar reagent. Let stand 5 min, and titrate with Na₂S₂O₃ solution, adding starch indicator, (h), just before end point.

(g) *Potassium iodide solution*.—2.5%. Stabilize with little Na₂CO₃.

(h) *Starch indicator*.—Make 1.5 g soluble starch into paste with few mL H₂O, and add slowly, with stirring, to 300 mL boiling H₂O.

B. Determination

Select test sample as in 922.01 (see 3.1.01), remove all foreign matter, dry, and grind to pass No. 80 sieve. Accurately weigh

0.1–1.0 g powdered test portion containing ca 20 mg starch into 25 × 150 mm Pyrex test tube. Add ca 200 mg fine sand and 5 mL H₂O, and mix well with stirring rod to wet sample. Heat tube in boiling water bath 15 min to gelatinize starch. Cool to room temperature, and place in 22–25°C bath. Add 5 mL 60% HClO₄ rapidly with constant agitation. Grind tissue against lower wall of tube with stirring rod for approximately 1 min at time. Repeat grinding frequently during 30 min; then without delay, transfer quantitatively to 100 mL volumetric flask with H₂O. Add 3 mL 5% uranyl acetate solution to precipitate protein, dilute to volume with H₂O, mix well, and centrifuge portion of mixture. Pipet 10 mL clear supernate into 25 × 150 mm test tube. Add ca 100 mg Celite, 5 mL 20% NaCl solution, and 2 mL I₂–KI reagent, A(a), and mix well. Let stand overnight, centrifuge, and decant.

Wash starch–I₂ precipitate by suspending it in 5 mL alcoholic NaCl solution, centrifuge, and decant. Add 2 mL alcoholic NaOH solution to packed precipitate. Gently shake and tap tube until precipitate is no longer blue. (Do not use stirring rod; allow ample time for complex to decompose.) Wash walls of tube with 5 mL alcoholic NaCl solution, A(b), centrifuge liberated starch, and wash with 5 mL alcoholic NaCl solution as before. Add 2 mL 0.7M HCl to precipitate. Stopper tube loosely with size 00 crucible, and heat 2.5 h in boiling water bath. (Bath should have cover with holes to accommodate tubes; holes not occupied by tubes must be covered.)

Cool, and transfer quantitatively to 25 mL volumetric flask. Add drop phenol red, 941.17A (see A.1.05), and neutralize with 1M NaOH. Discharge color with 0.05M oxalic acid, dilute to volume, and mix well. Transfer 5 mL aliquot to 25 × 200 mm Pyrex test tube, add exactly 5 mL Somogyi reagent, and stopper tube with size 00 crucible. Heat together with several blanks containing 5 mL H₂O and 5 mL Somogyi reagent in vigorously boiling water bath exactly 15 min. Remove tube from bath and cool to 25–30°C. Add 1 mL 2.5% KI solution, A(g), down wall of tube without agitation and then add 3 mL 0.75M H₂SO₄ rapidly with agitation. After all Cu₂O dissolves, titrate solution with 0.0055M Na₂S₂O₃, adding starch indicator, A(h), just before end point is reached. Treat blank solutions similarly.

$$\text{Starch, \%} = \frac{[50(\text{mL blank} - \text{mL test solution}) - 0.90/\text{mg test portion}]}{(M/0.005) \quad G \quad 100}$$

where 50 = dilution factor, 0.90 = factor glucose to starch, M = actual molarity Na₂S₂O₃ solution, and G = mg glucose equivalent to 1 mL 0.005M Na₂S₂O₃.

References: *Anal. Chem.* **20**, 850(1948).

JAOAC **39**, 423(1956).