

## WAXY AND NONWAXY

### PRINCIPLE

The starch sample is stained with iodine in dilute aqueous slurry. Waxy starch stains red and nonwaxy starch stains blue (Note 1). The stained sample is examined with a microscope, and the concentration of waxy and nonwaxy starch is determined from the relative quantities of red and blue granules in the field of observation.

### SCOPE

The method applies generally to waxy and nonwaxy starches from corn.

### SPECIAL APPARATUS

1. Microscope: Use a microscope capable of 250 power-magnification, equipped with polarizing filters, and a light source for substage illumination.
2. Counting Chamber: The Levy improved Neubauer (bright line) double ruling haemocytometer counting chamber, with cover glasses, is recommended.
3. Hand Tally Counter (optional)

### REAGENTS

Iodine Solution, 0.02 *N*: Dilute 0.1 *N* iodine solution with purified water. Prepare fresh daily.

### PROCEDURE

Clean the haemocytometer counting chamber and the cover glass with lens tissue, using purified water if necessary.

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Weigh 0.30 g of sample (Note 2) into a 250 mL beaker, add 15 mL of purified water and stir to disperse the starch (Note 3). Mix 3 mL of iodine solution with 85 mL of purified water, and add to the starch dispersion while stirring vigorously with a fire-polished glass rod. Withdraw the rod immediately and transfer the adhering drop to the counting face of the haemocytometer. Drop the cover glass into place and position the haemocytometer on the microscope stage.

With substage illumination and 250 power-magnification, align the counting chamber so that the finely-ruled center section of 25 squares appears in the microscope field (Note 4). Count separately the red and blue granules in the 25 square section, counting in squares in regular progression from left to right and from top to bottom (Notes 5 and 6).

**CALCULATION (Note 7)**

$$\% \text{ Nonwaxy Starch} = \frac{\text{Total Blue Granules} \times 100}{\text{Total Blue Granules} + \text{Total Red Granules}}$$

**NOTES AND PRECAUTIONS**

1. Waxy starch is composed entirely of branched polymers which stain red with iodine. Nonwaxy starch contains amylose (linear fraction) which stains blue with iodine.
2. The sample size is chosen to give a total count of 350-600 granules in the center section. Differences from this total count are largely a matter of technique; starch granules settle rapidly in the dispersion thereby affecting the number in the drop clinging to the stirring rod. If the total count varies significantly from the stated range, stir the dispersion vigorously, refill the counting chamber and repeat the count.
3. In alkaline dispersions, starch granules stain nonuniformly or not at all with iodine. If the sample dispersion is alkaline, acidify with dilute hydrochloric or acetic acid prior to addition of iodine solution.
4. The clear (or mirrored) counting face of the chamber contains a ruled square 3 mm wide which usually can be located by visual inspection with

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reflected light. This square is subdivided into 25 squares, each of which is finely ruled into 16 squares.

5. Clumped granules are indicative of inadequate dispersion. In such cases accurate counts are difficult to obtain and a more vigorous dispersing technique should be used.
6. Many of the "blue-staining" granules will appear almost black under the microscope. Slight changes in focus will serve to show the blue color at the edges of the granule. Particles which stain colors other than blue or red represent nonstarch constituents which should not be counted.
7. Duplicate analyses are recommended and results should agree within 2% absolute. Report the average of duplicate results, retaining 2 significant figures.