

STAINING INULIN CRYSTALS IN PLANT TISSUES¹

GLENN W. BLAYDES
The Ohio State University

Inulin, a levulosan, is found widely distributed among numerous angiosperm families. This carbohydrate occurs in solution in the cell sap of some species, in others it accumulates as an amorphous material, and in some plants inulin crystals are found deposited in living tissues. Inulin stains brown in iodine and will not reduce Fehling's solution. It may be precipitated as sphaero-crystals within the cells of some tissues when immersed in 70 per cent ethyl alcohol. The enzyme inulase in plant tissues hydrolyzes inulin to fructose. A microchemical test for inulin consists of treating freehand sections of plant tissues with a drop of 15 per cent thymol (15 grams of thymol dissolved in 85 cc. of 95 per cent ethyl alcohol). This treatment is followed by a drop of concentrated sulphuric acid. If inulin is present a bright red color is evident at once and soon disappears. If the inulin is in the form of crystals the red color becomes evident as the crystals dissolve.

For class study, freehand sections are usually prepared and examined as temporary mounts, or they are mounted in glycerine jelly. These preparations are often unsatisfactory since beginning students have trouble finding the transparent crystals. Inulin crystals in glycerine jelly preparations often dissolve within a few months after mounting.

The following method has been devised for the preparation of permanent microscopic mounts with the inulin differentiated by its reddish pink color when stained with phloxine. Inulin crystals in tissues prepared by this method 10 years ago are as satisfactorily differentiated and brilliantly stained now as when made. The aniline blue counterstain has faded in these balsam mounts. It is probable that this fading of the counterstain can be counteracted to some extent by mounting in xylene clarite. Further tests with clarite as the mounting medium are in progress. Bismarck brown Y (certification No. CN-3) may be used as a counterstain instead of the aniline blue. It is much more permanent, but the color contrast is not as satisfactory as with the aniline blue. A saturated solution of Bismarck brown Y in 70 per cent ethyl alcohol may be used. Staining for 3 minutes gave the most satisfactory results.

Dandelion (*Leontodon taraxacum* L.) root is excellent as a source of tissue containing inulin. These roots should be collected during the summer or autumn. Roots collected during the winter period (January and February) have not proven satisfactory, apparently because of the low inulin content. Several fixatives have been tried, including formalin-acetic acid-alcohol and Carnoy's fluids. Dissolution of the inulin crystals occurred in all these fixatives. At length 70 per cent ethyl alcohol was resorted to as a killing agent, and at the same time causing crystallization of the inulin. Some plasmolysis of cortical cells occurred. However, this is of little importance since the preparation is intended as a food storage demonstration. Pieces of dandelion roots were cut into appropriate lengths and dropped into the alcohol. A faucet vacuum pump may be used to advantage in removing air from the tissues. Wittlake's (2) vacuum apparatus is ideal for this purpose.

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The following schedule outlines the procedure in detail:

1. Use dandelion roots which are approximately $\frac{3}{16}$ " in diameter and cut into blocks of about the same length.
2. Place the pieces of root in 70 per cent ethyl alcohol (70 parts of 95 per cent ethyl alcohol to 25 parts distilled water).
3. Remove air from the tissues by a faucet vacuum pump. Allow the alcoholic treatment to continue about 72 hours after pumping, or longer if more convenient.
4. Complete the dehydration in 80, 95 and 100 per cent alcohols at intervals of 3 hours.
5. Transfer root pieces to ether-absolute ethyl alcohol (half and half mixture) for 3 hours.
6. Pass through a 2, 4, 6, 8, 10, 12, 14 and 16 per cent collodion² series at intervals of 24 hours, using the hot method of Wetmore (1).
7. Imbed in collodion on hardwood blocks and harden in chloroform for 12 hours.
8. Store in glycerine-alcohol (half glycerine to half 95 per cent ethyl alcohol) for 3 days or until needed.
9. Section on a sliding microtome. Good sections for general study may be made from 24 to 30 microns in thickness. The knife should be sharpened after cutting about 5 sections since the inulin crystals rapidly dull the edge. Keep the knife and tissue block flooded with the glycerine-alcohol mixture.
10. Transfer the sections to 95 per cent ethyl alcohol, rinsing 5 or 6 times to remove the glycerine.
11. Transfer to a half and half mixture of ether and absolute ethyl alcohol to remove the collodion.
12. Rinse in 95 per cent alcohol.
13. Stain in phloxine³ (1 gram to 100 cc. of 90 per cent ethyl alcohol) for about 10 minutes.
14. Rinse sections in 95 per cent alcohol to remove the excess phloxine.
15. Counterstain in aniline blue (1 gram to 100 cc. of 95 per cent ethyl alcohol) for 15 to 30 seconds. Care must be taken to prevent overstaining in the aniline blue. The inulin should remain a pinkish red color. If the inulin crystals absorb too much of the blue stain, restain by transferring to the phloxine again.
16. Rinse in 95 per cent ethyl alcohol.
17. Transfer to absolute ethyl alcohol for 3 minutes.
18. Clear in xylene for 5 minutes. Wintergreen oil (methyl salicylate) may be used to advantage before the xylene treatment. This must be short, however, since it appears to intensify the aniline blue and to cause the inulin crystals to appear purplish.
19. Mount in neutral balsam, or in xylene clarite.

REFERENCES

- (1) **Wetmore, R. H.** The use of collodion in botanical technic. *Stain Tech.*, 7: 37-62. 1932.
- (2) **Wittlake, Eugene B.** An efficient vacuum apparatus for microtechnic. *Ohio Jour. Sci.*, 42: 65-69. 1942.

²Collodion Cotton or Pyroxylin, prepared and sold by the J. T. Baker Chemical Co., Phillipsburg, N. J., is excellent and inexpensive for this purpose.

³The phloxine originally used was manufactured by Coleman and Bell, Norwood, Ohio. More recent tests have been made with phloxine from The National Aniline & Chemical Co., New York, N. Y., certification No. 9, total dye content 82 per cent.