Practical Botany – The Maltese Cross

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Abstract

One of the simplest tests to identify particles as starch is to view their appearance in polarizing light. Polarizing filters for student microscopes were made from inexpensive, long-lasting polarizing film. With their high degree of internal organization and relatively round shape, starch grains are almost crystalline in structure and exhibit birefringence under cross-polarized light. The starch grains appear bright against the dark field and each starch grain shows a Maltese cross when viewed with the light microscope. The presence of the telltale Maltese cross can be used to determine if starch is present and has many practical applications. In this laboratory exercise, students examine the properties of polarizing light and look for the presence of the distinctive Maltese cross in a variety of known and unknown substances to determine if starch is present.

Introduction

Normally, light is unpolarized. Light waves emanating from the sun, a lamp, or a candle are examples of unpolarized light in which the light waves coming off the source vibrate in all directions. In contrast, polarized light waves vibrate in just a single plane. A polarizing filter can convert unpolarized light into polarized light. As light passes thorough the filter, only light waves oriented in a single plane are transmitted because the filter blocks all other vibrational planes. If another polarizing filter is placed in the path of polarized light, the amount of light transmitted will depend upon the placement of the second filter. If the second filter is parallel to the first – in other words, the second filter has the same plane of polarization as the first – light will pass through the second filter. If two polarizing filters are crossed or oriented at right angles to one another, no light will pass through the second filter.

Certain biological materials may act like polarizing filters. The biological material must have a highly ordered internal structure, approaching a crystalline pattern of organization, to partially polarize light. If such materials are placed between crossed filters, some of the light waves are polarized in a new direction and will pass through the second filter. This property is known as *birefringence* because the material bends or refracts light into two planes. The biological material will be bright against a dark background since the crossed polarizing filters still prevent light from passing through everywhere else. A few examples of biological material that are birefringent are: skeletal muscle fibers, the radula of a snail, wood, and starch.

Starch is one of the most abundant carbohydrates in the plant world, second only to cellulose. Plants store more starch than any other food material and it is commonly accumulates in seed endosperm and tubers. Starch is a polymer composed of many smaller units or monomers. In the case of starch, the monomer is the sugar glucose, which forms a ring upon itself. The way in which the glucose units are joined together determines whether the type of starch is amylose or amylopectin. Amylose is linear, composed of unbranched chains of glucose. Picture glucose as a paper clip and then join the paper clips one to another in a straight chain -- that's amylose. Add side branches to the chain at approximately at every 20-30th paper clip and you've created amylopectin. Typically, amylose makes up about 20-30% of starch, and amylopectin, 70-80% but the exact composition varies with the plant source.

As plants photosynthesize, the accumulated glucose is converted into starch and stored temporarily in the chloroplast. Eventually, the starch formed in the chloroplast is broken down and its components transported out and the starch re-formed as starch grains in a specialized plastid, the amyloplast. Each amyloplast may contain one to several starch grains. In some cases, the starch grains may fuse to form compound grains. Starch grains are composed of both amylose and amylopectin layered around a nucleation center called the hilum. Often, the layers in many starch grains are visible when viewed with the light microscope. The structure, shape, and size of starch grains are often distinctive for particular plant species and can be a valuable diagnostic tool in identifying starch from different plants.

One of the easiest tests to identify particles as starch grains is their appearance in polarizing light. With their high degree of internal organization and relatively round shape, starch grains are almost crystalline in structure and exhibit birefringence under cross-polarized light. The starch grains appear bright against the dark field and each starch grain shows a Maltese cross (Esau, 1977). The crossing point of the Maltese cross is located at the hilum.

The presence of the telltale Maltese cross can be used to determine if starch is present and has many practical applications. Starch grain identification can be a means of verifying if a particular product is what the seller claims it to be, such as finding out if cereal grains have been added to ground meat. The indication of starch grains in stomach contents can be useful in determining the time of death in forensic science. Starch residues on ancient tools and pottery have been useful in determining the diet of ancestral peoples.

In this laboratory exercise, you will use polarizing filters to transmit polarized light and view the birefringent properties of starch grains under the conditions of cross–polarization. You will look for the presence of the distinctive Maltese cross in a variety of known and unknown substance to determine if starch is present.

Learning Objectives

1. Understand the principles of polarization and birefringence.
2. Recognize the diagnostic Maltese cross found in starch grains in cross–polarized light.
3. Use the Maltese cross to identify the presence of starch in known and unknown substances.

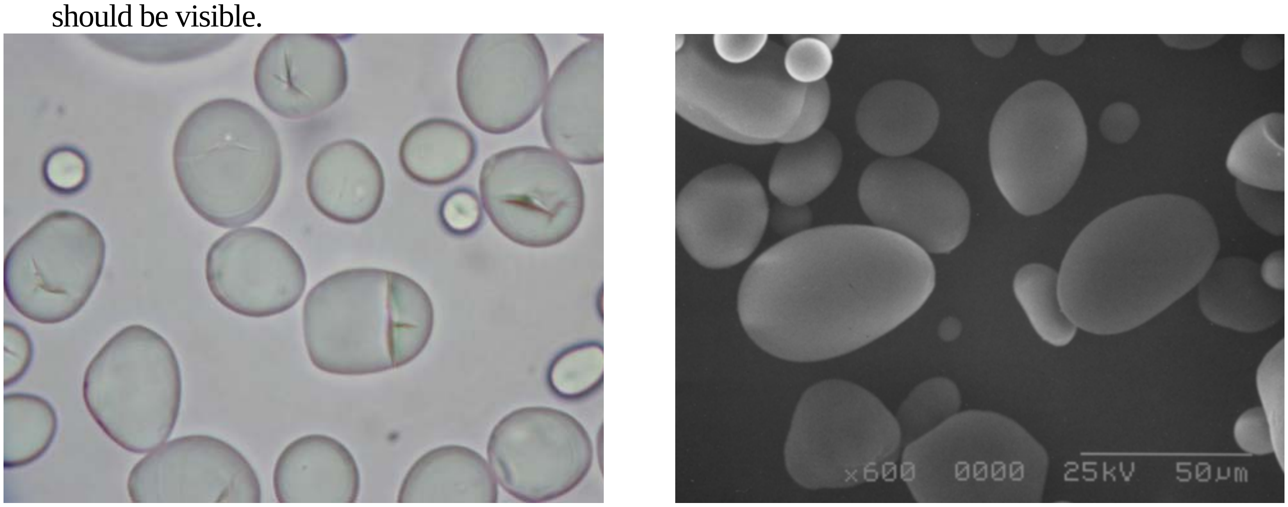
Procedure

Materials Needed

- Compound light microscope, (monocular)
- Coverslips
- Dissecting needles
- Dropper bottle of distilled water
- Glass slides
- Flours/powders, variety
- Polarizing filters

Protocol

1. Obtain two squares of polarizing filters (film). Take one filter and look at an object through the filter. Note that the filter does not distort the shape or dimensions of any object; but it does produce a dimmer image. Why?
2. Now view an object looking through both filters together. Slowly rotate the second filter. What is the orientation of the polarizing filters when the object is visible? What is the orientation of the filters when all light from an object is blocked and the object is no longer visible?
3. Place a drop of distilled water on the center of a glass slide. Using a dissecting needle, obtain a small sample of potato (*Solanum tuberosum*) starch and put this sample in the drop of water. Cover with a coverslip. Focus first under scanning (4X objective) or low (10 X objective) to view the starch grains. Bring up to high power (40 X objective) to see more clearly. Potato starch grains (Fig. 1) are some of the largest starch grains of all of the commercially used flours. Concentric layers within the oval grains should be visible.



A. **B.**

Figure 1. The starch grains of white potato have an ellipsoidal shape as seen in light (A) and scanning micrographs (B).

4. For best results set the substage lamp so it is at maximum brightness. Place one polarizing filter over the built-in lamp under the stage. Hold the second filter so that it covers the eyepiece lens. While looking into the microscope, rotate this second filter, taking care to keep the filter closely pressed against the eyepiece. Stop the rotation when the background is darkest; at this point the filters are crossed. Each starch grain will be bright against the dark background even though the polarizing filters are crossed. Why?
5. Observe the diagnostic Maltese cross (Fig. 2) that appears in each starch grain of potato in cross-polarized light.

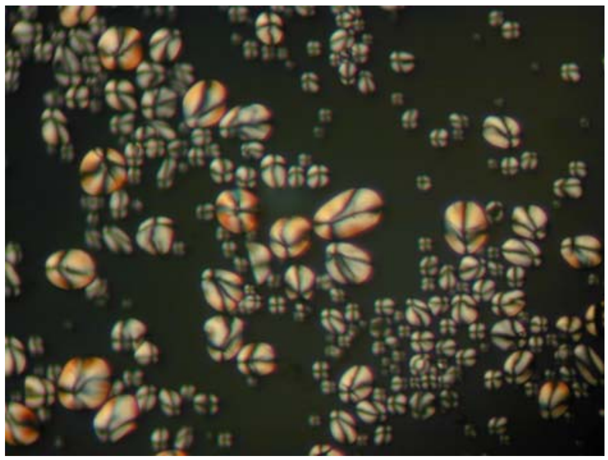


Figure. 2. Each potato starch grain shows a Maltese cross when polarizing filters are crossed.

6. Make slides of the corn (*Zea mays*) starch and arrowroot (*Maranta arumdinacea*) flour provided following steps 3-4 above. The starch grains of corn have a polygonal shape (Fig. 3). Observe that the starch grains of arrowroot are tiny which makes the flour easy to digest.

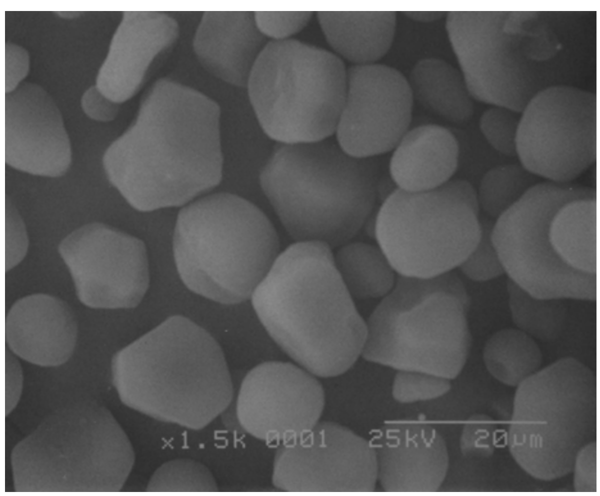


Figure 3. The starch grains of corn have a polygonal shape (SEM).

7. Make a slide of the non-dairy creamer. What can you conclude about its composition?
8. Make a slide of wheat (*Triticum aestivum*) flour. As you scan the field note that wheat starch grains are of two size classes: large and small. This bimodal size distribution makes wheat starch less valuable for industrial applications that require starch grains of a uniform size.

9. Amaranth (*Amaranthus spp.*) is a high-protein, noncereal grain valued for centuries by the Aztecs. It is currently popular as a wheat flour substitute for those who suffer from intolerance to gluten, a protein found in wheat flour. Make a slide of amaranth flour to verify that it is indeed wheat-free.
10. Make a slide of the nutritional powder. Note the uniform oval particles that make-up the powder. Are they starch grains?
11. Soy (*Glycine max*) flour is a high protein product made from the soybean. It can be used as a nutritional and relatively inexpensive (at $1.69/lb) ground meat extender. Cheaper, less nutritious cereal flours (at $ 0.89/lb) can be used for this purpose as well. Substance A was used at a food processing plant as a meat extender. Is it a soy product or a cereal starch?

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Notes to the Instructor

1. Polarizing filters can be made from sheets of linear polarizing laminated film. Edmund Optics sells several brands (Tech Spec and 3M) and different sizes of sheets. I have used the commercial quality large sheets and cut them to the sizes I need. Each microscope will need two filter squares approximately 10 x 10 cm square. These filters can be used over and over again. The ones I use in my laboratory classes are approximately 15 years old and still work perfectly.
   Linear Polarizing Laminated Film, TECH SPEC, 3M
   Edmund Industrial Optics: <*www.edmundoptics.com*>
2. I have not been able to use this exercise with a binocular compound light microscope. Although I have tried several different methods, I am not able to completely block light from the unused eyepiece and consequently am unable to make the light completely cross-polarized. Without cross-polarized light, the background does not became completely black and the Maltese cross does not appear in each starch grain.
3. The common flours/powders can be obtained from a supermarket. Some of the more unusual varieties can be found from specialty shops, such as health food stores. You can also purchase purified starches from any chemical or biological supply house (Sigma, Fisher, Carolina, etc.)
4. You can also take a small sample of fresh tissue (such as the flesh of white potato or banana) and spread thinly in a drop of water to see starch grains.
5. Starch grains have different degrees of polarization varying on a scale from 0 (very low intensity) to 4 (very high). Some examples of starch grain intensity are:

Arrowroot 3	Oats 2
Barley flour 2	Pea 1-3 (depending on variety)
Banana 2	Rice 2
Buckwheat 3	
Corn 3	Rye 2-3
Fava beans 4	White potato 4
Lima bean 3	Wheat 1
Manioc (tapioca)	

   I have found the following starch grains to polarize especially well: arrowroot, buckwheat, corn, and potato. Barley, wheat, and rice starch grains also polarize.

6. The following are the answers to the questions posed in the laboratory exercise:
   - Q. 7. Non-dairy creamer (Coffee-mate ® brand) contains corn starch grains that will show the distinctive Maltese cross in cross-polarizing light.
   - Q. 9. Amaranth contains no starch grains and, of course, will not show the distinctive Maltese cross.
   - Q. 10. The nutritional powder I used was Brewer's yeast bought from a national health food chain. Although the yeast shape is similar to starch grains it will not show the distinctive Maltese cross in cross-polarizing light
   - Q. 11. Soy flour was added to extend the meat and has no starch grains. Other flours/powders that you can use as unknowns that do not contain starch grains and thus do not show the distinctive Maltese cross are gelatin and nonfat dry milk.