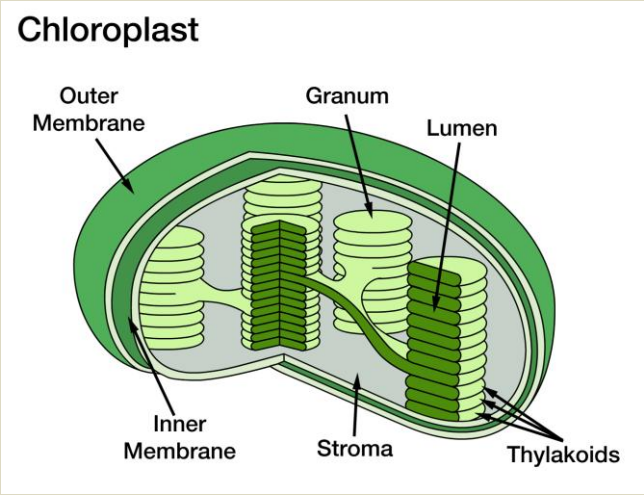
ISOLATION OF CHLOROPLASTS

chloroplast

The *chloroplast* is the place in a plant cell and green algae where photosynthesis happens. Photosynthesis the process by which light energy is converted to chemical energy, resulting in the production of oxygen and energy-rich organic compounds.

Chloroplast is the combination of two biological terms, *plastid* (an organelle in a plant cell), and *chloros,* which means green



Materials (per grope)

- Fresh spinach
- clean sharp sand
- 50 mL 0.5 M sucrose
- cheese cloth, 12 x 12 inches
- Ice
- 25 mL graduated cylinder
- mortar and pestle (or blender)
- Centrifuge
- glass filter funnel
- two 16x150 mm test tubes in rack
- three 13x100 mm test tubes in rack
- plastic capped 15 mL centrifuge tube
- glass stirring rods

Procedures

1-**Prepare, weigh and homogenize:**

Grind 8 g deveined spinach with ½ tsp clean sharp sand in mortar and pestle to a paste.

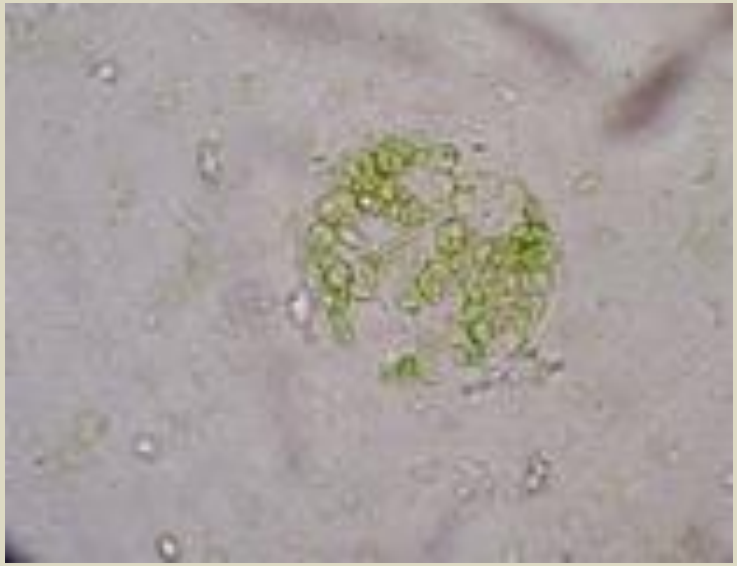
2-**Suspend in 0.5 M sucrose:**

Measure out 16 mL ice-cold 0.5 M sucrose solution in a 25 mL graduated cylinder. Add in 3-4 mL increments, grind to smooth pulp with each addition.

3-**Filter**

homogenate through about eight layers of clean cheese cloth in a glass funnel into an iced 16x150 mm test tube

4-Pour filtrate back into 25 mL cylinder and **record volume**. Save ~0.5 mL of the filtrate and examine it at 400x under microscope.



5-**Centrifuge at low speed:**

prepare a balance tube against the filtrate in a 16x150 tube and spin at 50x g for 10 minutes

6- Decant the top 10 mL into a clean cold centrifuge tube, discard sediment. Record volume. Save ~0.5 mL supernatant to examine under microscope .

7- Centrifuge the supernatant at 1000x g for 10 minutes to precipitate chloroplasts. Carefully decant *all* of the supernatant into 16x150 mm tube but save the pellet. Discard supernatant *if* you have a significant pellet

8-Resuspend pellet from the last step the filtrate in ice-cold 0.5 M sucrose with a clean, ice cold stirring rod. Record final volume. Keep on ice at all times. Examine suspended organelles under microscope .