Thin Layer Chromatography (TLC) for Polar Lipid


Prepare TLC tank and plates.
- Submerge a 20 cm x 20 cm silica gel coated TLC plate into 0.15 M ammonium sulfate ((NH4)2SO4) solution. Submerge for 30 seconds then dry the plate for at least 2 days in covered container.
- On day of experiment, activate TLC plates by baking in oven at 120°C for 1.5-2.5 hours to remove water from the plate.
- Prepare mobile phase solvent: 91:30:7.5 acetone: toluene: water (winter); 7.0 ml during summer
- Pour mobile phase solvents into TLC tank. Keep lid closed as much as possible.
- Draw a straight line 2 cm from edge of plate across, termed the origin. Do not dig too deeply with mark. Add small tick marks for every 1 cm of origin to help loading.

1. Slowly add 60 µl of total fatty acid on the straight line.
2. Plate TLC plate in the TLC tank with sample ends facing down.
3. The solvent will ascend the plate and the lipids be separated in general about 50-60 minutes for full plate.
4. Remove plate before solvent makes it to the total FA spot.
5. Let plate dry 10-15 min.
6. Stain the plate in the iodine tank.
   - When the lipids are fully shown, take the plate out and take picture fast as iodine evaporates.
   - Trace the spots with the pencil, do this fast before iodine evaporates.
7. Analyze the TLC result: Calculate Rf value and compare the lipid saturation