

Fatty Acids, Lipids & Membranes

(BE-601)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

Checklist

- 1 bottle PBS Solution (25ml)
- 1 bottle Lipid Extraction Solvent (100ml)
- 6 Glass Slides
- 24 Pestles & Tubes
- 30 Centrifuge Tubes (1.5ml)
- 6 Thin Layer Chromatography (TLC) Plates (6.5x5cm)
- 1 vial Iodine Crystals

SPECIAL HANDLING INSTRUCTIONS

- All reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence*[™] kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
L037	Lipid Extraction Solvent	Flammable
I075	Iodine Crystals	Toxic

ADDITIONAL EQUIPMENT REQUIRED

- Samples for extraction, we suggest nuts as they have a high lipid content
- 250ml glass beaker or similar size glass container for Thin Layer Chromatography
 - Light Microscope

TIME REQUIRED

- **Day 1:** 3-5 hours



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AIMS

- Isolate lipids from a range of samples
- Create and visualize liposomes.
- Separate and view lipids with thin layer chromatography.

BACKGROUND

Lipids are a large and diverse group of naturally occurring organic compounds that are soluble in non-polar organic solvents, including chloroform and acetone, and are generally insoluble in water. Lipids have many key biological functions, such as acting as structural components of cell membranes, serving as energy storage sources and participating in signaling pathways. Lipids can be divided into eight subcategories based on their structural components:

- **Fatty Acyls** (including fatty acids) are the major lipid building block of complex lipids and therefore is one of the most fundamental categories of biological lipids. The carbon chain may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen and sulfur.

Glycerolipids are composed mainly of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol (triacylglycerols), also known as triglycerides. These comprise the bulk of storage fat in animal tissues.

Glycerophospholipids, also referred to as phospholipids, are ubiquitous in nature and are key components of the lipid bilayer of cells, as well as being involved in metabolism and signaling.

Sphingolipids are a complex family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized *de novo* from serine and a long-chain fatty acyl CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids and other species.

Sterol lipids, such as cholesterol and its derivatives are an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins. The steroids, which also contain the same fused four-ring core structure, have different biological roles as hormones and signaling molecules. T

Prenol lipids include carotenoids that function as anti-oxidants and as precursors of vitamin A. Another biologically important class of molecules is exemplified by the quinones and hydroquinones, vitamin E and vitamin K, as well as the ubiquinones, are examples of this class.

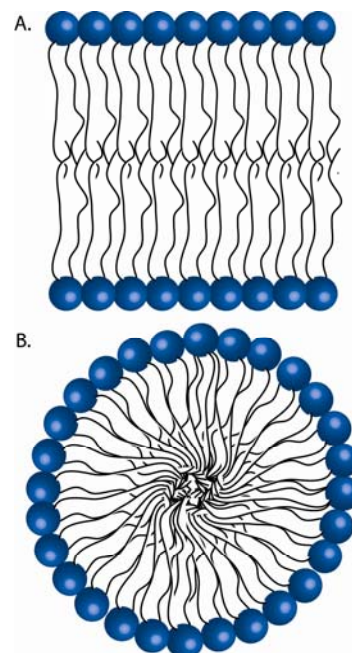
Saccharolipids describe compounds in which fatty acids are linked directly to a sugar backbone, forming structures that are compatible with membrane bilayers.

Polyketides comprise a very large number of secondary metabolites and natural products from animal, plant, bacterial, fungal and marine sources, and have great structural diversity.

Role in Cellular Membranes

The glycerophospholipids are the main structural component of membranes, including the cellular plasma membrane and the intracellular membranes of organelles. The plasma membrane physically separates the intracellular components from the extracellular environment and all eukaryotic cells are compartmentalized into membrane-bound organelles that perform different functions. These glycerophospholipids are amphipathic (both hydrophobic and hydrophilic) molecules that contain a hydrophobic glycerol core linked to two fatty acid-derived "tails" by ester and to one hydrophilic "head" group by a phosphate ester linkage. While glycerophospholipids are the major component of biological membranes, other non-glyceride lipid components such as sphingomyelin and sterols (mainly cholesterol in animal cell membranes) are also found in biological membranes.

A biological membrane is a form of lipid bilayer, as is a liposome. The formation of lipid bilayers is an energetically-preferred process when the glycerophospholipids described above are in an aqueous environment. In an aqueous system, the polar heads of lipids orientate towards the polar, aqueous environment, while the hydrophobic tails minimize their contact with water. The lipophilic tails of lipids tend to cluster together, forming a lipid bilayer (A) or a micelle (B). In this lab activity students will generate liposomes and view them under a microscope.



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TEACHER'S PRE EXPERIMENT SET UP

Samples

1. We recommend supplying students with different nut varieties, such as peanut, walnut and pine nut, for the extraction of lipids. Nuts have a high concentration of lipids. The nuts should NOT be roasted or salted, but in their natural state.
2. Other samples can be used, including animal and plant tissue. We suggest liver or brain tissue, egg yolks and avocado.

Thin Layer Chromatography

1. The Thin Layer Chromatography part of the experiment utilizes the Lipid Extraction Solvent as a solvent for separating the lipids. This solvent is a 2:1 mix of chloroform and methanol. When handling the solvent ensure there are no naked flames around, wear gloves and goggles and perform the chromatography in a fume hood.



CAUTION: The Lipid Extraction Solvent is highly volatile and flammable. Wear gloves and goggles and ensure there are no open flames during this laboratory experiment.

2. For successful chromatography perform in a glass TLC chamber, or if one is not available, use a ≥ 250 ml glass beaker or container. The beaker should be sealed with a glass plate (i.e. A watch glass) or aluminum foil during chromatography.
3. If additional solvent is required, prepare by mixing 2 parts chloroform to 1 part methanol.

Plate Development



CAUTION: Iodine crystals and vapor is toxic. Wear gloves and goggles and perform in a fume hood.

1. After the lipids have migrated through the plates, the plates are air dried.
2. Transfer the TLC plates to dry glass beakers. More than one plate can be placed in a single container as long as there is enough room for all the white surfaces to be exposed.
3. Place the glass containers in a fume hood. Wearing gloves and using forceps or a spatula, divide the iodine crystals between the containers. Place 3-5 crystals into each 250ml container.
4. Lipid spots will appear between 10-120 minutes, depending on the lipid concentration. Once the spots are clearly visible, remove the TLC plates and mark the spots with a pencil circle.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

4 Samples

4 Pestles and Tubes

Lipid Extraction Solvent (shared with class)

PBS Solution (shared with class)

8 Centrifuge Tubes

1 Glass Slide

1 Thin Layer Chromatography (TLC) Plate

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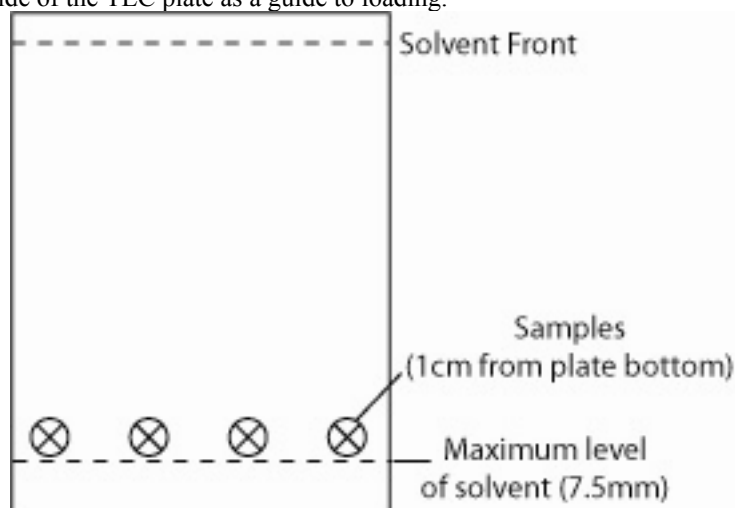
PROCEDURE

1. Each student will extract lipids from a sample supplied by your teacher.
2. Weigh out 50mg tissue and transfer to the grinding tube. Grind the sample with the supplied pestle until a smooth, homogenous paste is achieved.
3. Add 20 volumes of Lipid Extraction Solvent to the ground sample and use the pestle to thoroughly mix the sample and solvent. For each 10mg, add 200 μ l Membrane Extraction Solvent. The Lipid Extraction Solvent contains a mixture of chloroform, an organic solvent, and methanol to extract the hydrophobic lipids.



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4. After a homogenous mixture is achieved, seal the tube and vortex, or vigorously shake the mixture for 1-2 minutes for improved lipid extraction.
5. To recover the liquid phase, the tube is centrifuged for 5 minutes at maximum speed (~14,000 rpm).
6. After centrifugation, transfer the liquid phase to a clean tube and add 40 μ l PBS solution for every 200 μ l Lipid Extraction Solvent used. (For 50mg tissue, use 0.2ml). Vortex, or shake, vigorously to mix.
7. Centrifuge the tube at 2,000rpm for 2 minutes to separate the organic and aqueous phases. Remove the aqueous, upper phase and any cellular debris on top of the lower organic layer and discard. The upper aqueous layer contains the non-lipid contaminants. The lower, organic phase contains the lipids.
8. To create liposomes, transfer 10 μ l organic phase to the bottom of a clean tube. Leave the tube open and air dry for 10-15 minutes or until all liquid has evaporated.
9. Add 10 μ l PBS to the dried lipids and pipette up and down 5-10 times to mix and form liposomes. For visualization of the liposomes, each student transfers 10 μ l liposomes to the same glass slide and view under a light microscope at low power. Do not let the liposomes dry on the glass slide.
10. To visualize the lipids by thin layer chromatography (TLC), using the template below as a guide mark 4 Xs with a pencil on the white side of the TLC plate as a guide to loading.



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11. Slowly, pipette 10 μ l of the extracted lipids (step 7) onto one of the loading guide marks. Each student takes it in turns to load their samples. Allow the samples to dry on the TLC plate before loading next sample.
12. Once all four samples are loaded, transfer the TLC plate to a \geq 250ml glass beaker or container with the samples nearest the bottom of the beaker. Multiple TLC plates can be added to one beaker as long as the white surfaces do not touch.
13. In a fume hood, carefully pipette in Membrane Extraction Solvent, making sure the solvent does not reach the level of the samples.



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14. Cover the beaker with a glass plate (or watch glass) or tightly wrap with aluminum foil. Allow the solvent to migrate to the top of the TLC plate and then transfer the plates to a clean glass beaker. Allow the plates to dry in the beakers.
15. To develop the lipid spots, your supervisor will add iodine crystals that will sublime and the iodine vapor will adhere to the lipids making them visible.



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RESULTS, ANALYSIS & ASSESSMENT

Describe the appearance of the liposomes and explain why they may be used for drug delivery systems?

The liposomes should appear as perfect spheres. They would be good for the delivery of insoluble drugs as the insoluble drugs will be located on the inside of the sphere and the hydrophilic outer lipid coat will allow the liposomes to move through the blood system.

Describe your results.