Chapter III Extraction of Membrane Lipids

Anjni Koul and Rajendra Prasad

1 Background

Lipids form a group of organic compounds which is widely distributed in living systems. The term lipid cannot be defined concisely, but it refers to a large number of compounds which have similar solubility properties (for more details on type of membrane lipids, the reader is referred to Chap. I, this Vol.). Membrane lipids are amphipathic molecules which are water-insoluble and can be generally extracted from different sources by treatment with the combination of polar and non-polar organic solvents. The aim of the present chapter is to consider practical aspects of isolation of lipids. The chapter is intended to provide the reader with necessary information for the extraction of membrane lipids with confidence. The extraction protocols described herein should provide the reader with sufficient background to adopt the procedure to fit to the experimental requirements.

For the purpose of extraction, membrane lipids can be classified into three distinct groups:

Non-Polar Lipids

This group includes carotenoids, hydrocarbons, sterol esters, wax esters and glycerides. These lipids are often extractable

School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

R. Prasad (ed.), *Manual on Membrane Lipids*

with ether or chloroform, since they are weakly bound to each other and to the hydrophobic regions of the membrane by Van der Waals forces.

Polar Lipids

This group includes phosphoglycerides, glycolipids and sterols which are not only held by Van der Waals forces but are also bound by hydrogen bonds and, in some cases, by electrostatic interactions. These bonds are less readily disrupted and, therefore, lower alcohols or acetone must be used to free these lipids from membranes. Chloroform:methanol (2:1, v/v) is commonly used as an extraction mixture which removes polar lipids from membranes. However, acidic phospholipids and polyphosphoinositides are not generally extractable by this combination of polar and non-polar solvents, one must consider adding 0.25% HCl (conc.) to the solvent mixture for complete extraction of these lipids. Since plasmalogens would be hydrolysed by the acid, the use of acid should be avoided if plasmalogens are under investigation (see Chap. VII, this Vol. for more details).

Anchored Lipids

This group includes fatty acids and hydroxy fatty acids bound covalently through ester, amide or glycosidic bonds, predominantly to polysaccharide structures. These fatty acids are extractable only after acidic or basic hydrolysis of the covalent bonds.

Before the extraction of membrane lipids, it is imperative to know that one is dealing with desired pure membrane fraction (discussed in Chap. II, this Vol.). Membrane lipids from animal, plant and microorganism sources should ideally be extracted as soon as possible after their preparation to avoid

changes in lipid components. When this is not possible, the membrane fraction should be frozen rapidly on dry ice and stored in a sealed glass container at –70 °C in an atmosphere of nitrogen. Under these conditions, the sample can be kept for several months.

2 Extraction Protocols of Membrane Lipids from Different Sources

General Principles of Extraction. Folch and Stanley (1957) procedure of lipid extraction is the most common method used. However, modifications to this method have also been adopted. Twenty volumes of chloroform:methanol (2:1, v/v) mixture per volume of tissue or membrane pellet or suspension is recommended to yield a single homogeneous suspension. If the ratio is small (12–15 volumes) two phases will be formed, more solvent would be required to obtain a single phase. The single phase of solvent provides better interaction of polar and non-polar solvents with membrane lipids. High ratio is probably harmless to some extent, but too high a ratio of solvent will lower the water content, making polar lipid extraction incomplete.

There are several protocols of lipid extraction which differ slightly from each other. Membrane lipid extraction protocols commonly used for membranes derived from different sources are described below. These protocols can also be used for the extraction of total lipids from tissues of different origin.

Reagents and solvents

- Benzene
- Chloroform
- Methanol
- Propan-2-ol
- $CaCl_2$ anhydrous

- 0.9% NaCl
- Nitrogen gas

Equipment

- Thermal block or rotary evaporator

Extraction from red blood cells

1. Resuspend the RBC membrane fraction pellet directly in glass tubes (with stoppers) in at least 20 volumes of chloroform:methanol (2:1, v/v) or more commonly used alcohol:ether (3:1, v/v). Shake the suspension vigorously for 2–3 min. (When using tissue for lipid extraction, it is necessary to homogenise the tissue extract in a glass homogeniser for uniform suspension.)

! **Note.** Normally 20 volumes of solvent mixture is sufficient to make a single homogeneous phase. If the suspension does not form a single phase, more solvent mixture can be added.

2. Flush the suspension with N_2 gas and tightly close with stoppers. The tubes can be shaken gently in a shaker at room temperature for 15–30 min.

3. Separate the denatured protein either by filtration or centrifugation. We recommend centrifugation where denatured proteins can be removed by discarding the pellet.

4. Transfer the supernatant to another clean and dry graduated centrifuge tube and mix with 0.2 volumes of 0.9% NaCl (to remove non-lipid contaminants). The resulting turbid suspension could separate into two phases within ca. 2 h. However, a quick centrifugation in any bench top centrifuge will separate the two phases within a few minutes.

5. Remove the upper layer containing non-lipid contaminants carefully with a Pasteur pipette or aspirate off using a suitable device.

6. Recover the lower clear phase containing most of the lipids in another tube and concentrate as described below (for complete extraction of membrane lipids, e.g. for the extraction of polyphosphoinositides, lysophospholipids and gangliosides special steps are necessary; see Chap. VIII, this Vol.). For quantitative extraction, insoluble residue of step 3 can be re-extracted with fresh solvent.

Extraction from plant tissues

The method of lipid extraction is essentially similar to animal tissues. However, in order to inactivate hydrolytic enzymes, e.g. lipases, which are activated in the presence of chloroform and diethyl ether, it is necesssary to boil the plant tissue or its membrane fraction in propan-2-ol before chloroform: methanol extraction (Nichols 1963). In addition, for quantitative extraction of polar lipids, it might be necessary to use high salt buffer to partition the solvent phase. A typical protocol for the extraction of plant lipids is described below:

1. Boil the plant tissue or protoplast or membrane fraction prepared from it in five volumes of propan-2-ol (v/v) for 5 min.
2. Macerise the boiled fraction in a mortar in the presence of washed inactive sand and propan-2-ol.
3. Transfer the extract to a glass stoppered tube and add 20 volumes of chloroform:methanol. Flush the tube with N_2 and shake the mixture for 5 min.
4. Filter the total extract through Whatman # 1 filter disc. For quantitative extraction, the residue on the filter paper can be re-extracted with fresh solvent mixture.
5. Wash the filtered extract with 0.2 volumes of NaCl (0.9%) to remove non-lipid contaminants and transfer the extract to a separating funnel to separate aqueous and non-aqueous phases. After flushing with N_2, leave the funnel overnight for the separation of two phases.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.