**Author:** Jeff Salacup, Ph.D, UMass – Amherst **Title:** Extraction of Lipid Biomarkers from Geological Archive Sediments – 2. Soxhlet

**Overview:**

The material comprising the living “organic” share of any ecosystem (leaves, fungi, bark, tissue; **Figure 1**) differs fundamentally from the material of the non-living “inorganic” share (rocks and their constituent minerals, oxygen, water, metals). Organic material contains carbon linked to a series of other carbon and hydrogen molecules (**Figure 2**), which distinguishes it from inorganic material. Carbon’s wide valency range (-4 to +4) allows it to form up to four separate covalent bonds with neighboring atoms, usually C, H, O, N, S, and P. It can also share up to three covalent bonds with a single other atom, such as the triple bond in the often poisonous cyanide, or nitrile, group. Over the past 4.6 billion years, this flexibility has led to an amazing array of chemical structures which vary in size, complexity, polarity, shape, and function. The scientific field of organic geochemistry is concerned with the identification and characterization of the whole range of detectable organic compounds, called biomarkers, produced by life on this planet, as well as others, through geologic time.   
 **Principles:**

Soxhlet extraction is likely the oldest form of organic matter extraction. Archeological evidence from Mesopotamia places the use of a soxhlet-like device that utilized hot water at ~3500 BC. Modern soxhlets use sophisticated blown glass condensers and organic solvents in this “continuous” extraction method (**Figure 3**). Solvent is refluxed from a round-bottomed flask upward into a condenser with a recirculating cold water coil. When the gaseous solvent contacts the coil, it condenses into a chamber with a glass fiber thimble holding the sample. This chamber is set with a recirculator, and when a certain volume is reached (generally a volume large enough to submerge the whole sample), the chamber is flushed back into the round-bottomed flask via a built-in siphon, where the lipid extract accumulates while the solvent becomes part of the next cycle. Hence, the term “continuous” extraction. Soxhlet extraction is often used for the extraction of larger (>10 g) samples.

Biomarkers contained in the sample dissolve into the organic phase based on the rules of solubility, which with organic compounds, are controlled primarily by the polarity of both the biomarker and the solvent. This is summarized by the so-called “like dissolves like” rule, whereby relatively apolar biomarkers (those containing exclusively C and H; isoprene) dissolve in apolar solvents (such as hexane, polarity = 0.1) and more polar biomarkers (those containing O, N, S, P; GDGTs) dissolve in more polar solvents (such as methanol or dichloromethane, polarity = 5.1 and 3.1). A mixture of dichloromethane and methanol (9:1) is commonly used.

**Procedure:**

1. Collect the necessary materials:
   1. Extract one sample. Samples (leaves, dirt, fungi, bark, tissue), usually frozen, freeze-dried, crushed, and homogenized prior to extraction, are extracted in groups to maximize efficiency.
   2. Depending on the size of the sample, use vials with volumes ranging from 4-60 mL. For this experiment, use borosilicate glass vials (40 mL) and solvent safe caps. Combust vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of possible organic contaminants.
   3. Dichloromethane and methanol are common in most organic geochemistry laboratories. Use them individually to rinse lab tools and glassware before use. A mixture of dichloromethane (DCM) to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.
   4. Use a soxhlet apparatus. These can be purchased from Fisher Scientific or other science retailer. Wash and combust it at 550 °C for 6 hr prior to use.
   5. Acquire the remaining materials: lab spatula(s), lab scale, hose clamps, Tygon tubing, boiling chips, lab stand, two large glassware clamps, heating mantle with adjustable temperature control, glass fiber thimbles (Whatman makes these. Combust them at 550 °C for 6 hr prior to use), and a solvent approved chemical hood with running water and a drain.
2. Preparation of Sample.
   1. Place a combusted weighing tin on the lab scale and then tare.
   2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.  
      1. The mass of the sample varies depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) may require much less.
   3. Transfer all of the material in the weighing tin into a combusted glass fiber thimble.
3. Extraction.
   1. Transfer ~400 mL of the DCM:MeOH (9:1) mixture into the round-bottomed flask (flask should be more than half full) and put in heating mantle. Add a few (5-10) solvent-rinsed boiling chips.
   2. Place the sample thimble, open end up, into the center piece of the soxhlet apparatus.
   3. Place the center piece on top of the round-bottomed flask and secure with a glassware clamp.
   4. Install the condenser on top of the center piece of soxhlet and secure with a glassware clamp.
   5. Attach one of the cold water lines from the condenser to the cold water line in the hood using a hose clamp. Route the other into the drain.
   6. Turn on the water to ensure proper circulation and drainage.
   7. Turn on the heating mantle and adjust the temperature until the solvent in the round-bottomed flask is lightly boiling.
   8. Monitor the extraction a few times over the next hour.
      1. Check to make sure the temperature is properly set at a low boil, the solvent is condensing in the condenser and dripping into the center piece, the center piece is filling and emptying properly, and the water is properly draining into the hood drain.
   9. Monitor the extraction over the next 36 hours.
      1. Ensure the temperature is properly set at a low boil, the solvent is condensing in the condenser and dripping into the center piece, the center piece is filling and emptying properly, the water is properly draining into the hood drain, and the solvent level in the round-bottomed flask is still about half full.
   10. After 36 hours, stop the extraction by turning off the heating mantle.
   11. Label the flask ‘TLE’.

**Representative Results:**

At the end of extraction, a total lipid extract (TLE) for each sample is evident. Each vial contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can now be analyzed and their chemical constituents identified and quantified.

**Applications:**

Different classes of biomarkers impart information on specific aspects of the Earth system. For example, in its infancy, organic geochemistry was primarily concerned with the formation, migration, and alteration of petroleum, and many of the chemical tools organic geochemists use today are based on those initial investigations. It was through the investigation of a class of compounds called isoprenoids, having a repeating five carbon pattern (**Figure 2**), that scientists discovered petroleum comprised the chemically-altered remains of ancient primary producers, such as plankton in the ocean (converting to oil)(**Figure 4**) or peat bogs on land (coal)(**Figure 5**). Chemists at large oil companies used the ratios of a variety of compounds, each with its own known rate of alteration, to estimate how old petroleum was, where it came from, and if it was worth exploiting. Today, new biomarkers are being discovered, identified, and characterized in modern and ancient samples analyzed in organic geochemistry labs around the world. Many of today’s applications seek to extract environmental information from biomarkers obtained in modern samples (leaves, soil, microbes, water samples, etc.) in order to extend the biomarker’s utility to ancient sediments in an effort to reconstruct the climates, environments, and ecosystems of the past. For example, the distribution of a group of biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs for short), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature. Therefore the distribution of these biomarkers in ancient sediments, or through a series of sediments of known age, can be used to reconstruct air and water temperature back several million years.

**Legend:**

Figure 1: Organic material, such as trees, leaves, and moss, are chemically and visually distinct from inorganic material, such as pavement.

Figure 2: Isoprene comprises five carbon atoms and two double bonds. When added together in biosynthesis reaction, they can form complex molecules diagnostic for the presence of life. For example, 2, 6, 10, 15, 19-pentamethyleicosane, commonly found in cyanobacterial mats.

Figure 3. A soxhlet apparatus.

Figure 4: Illumination of plankton at Maldives.   
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Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.  
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