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Extraction of Lipid Biomarkers from Geological Archive Sediments - 2. Soxhlet

--Manuscript Draft--

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Corresponding Author:	Jeff Salacup UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
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First Author:	Jeff Salacup
First Author Secondary Information:	
Order of Authors:	Jeff Salacup
Order of Authors Secondary Information:	

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.

Comment [DM1]: This is the same as the Overview for the Sonication MS.

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:

    1.1. 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.

    1.2. 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.

    1.3. 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.

1.4. 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.

1.5. 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.

2.1. Place a combusted weighing tin on the lab scale and then tare.

2.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

2.2.1. 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.

2.3. Transfer all of the material in the weighing tin into a combusted glass fiber thimble.

3. Extraction.

3.1. 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.

3.2. Place the sample thimble, open end up, into the center piece of the soxhlet apparatus.

3.3. Place the center piece on top of the round-bottomed flask and secure with a glassware clamp.

3.4. Install the condenser on top of the center piece of soxhlet and secure with a glassware clamp.

3.5. 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.

3.6. Turn on the water to ensure proper circulation and drainage.

3.7. Turn on the heating mantle and adjust the temperature until the solvent in the round-bottomed flask is lightly boiling.

3.8. Monitor the extraction a few times over the next hour.

3.8.1. 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.

3.9. Monitor the extraction over the next 36 hours.

3.9.1. 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.

3.10. After 36 hours, stop the extraction by turning off the heating mantle.

3.11. 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:

Comment [DM2]: This is also identical to the Applications section for the Sonication manuscript.

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.

Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.

Photo or Graphic File
Click here to download Photo or Graphic File: Salacup_Sonication_Figure 4.jpg

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.~~

Every lab needs standards that ~~with which to~~ track the performance, accuracy, and precision of its instruments over time to ensure a measurement made today is the same as a measurement made a year from now (**Figure 1**). Because standards must test the performance of instruments over a long period of time, large volumes of the standards are often required. Many chemical standards can be purchased from retail scientific companies, like Sigma-Aldrich and Fisher. However, some compounds that occur in nature and that are relevant to paleoclimatic studies have not yet been isolated and purified for purchase. Therefore, ~~we need to extract~~ these compounds need to be extracted from natural samples, and ~~and~~ because of the large volumes of standards required, ~~we often need to extract~~ large volumes of sediment need to be extracted. ~~.~~ The Accelerated Solvent Extraction (Dionex) and sonication extractions are not appropriate for the extraction of such large sediment volumes. In these circumstances, ~~we instead use~~ a Soxhlet extraction is used.

Comment [A1]: We can't have the same Overview section in 3 (maybe 4) videos. If you want to demonstrate multiple ways to perform extractions, there needs to be 1) different components being extracted, 2) comparison between the techniques, and when you'd choose one over another.

Comment [DM2]: This is the same as the Overview for the Sonication MS.

Principles:

Soxhlet extraction is likely the oldest form of organic matter extraction. Archeological evidence from Mesopotamia places the use of a Ssoxhlet-like device that utilized hot water at ~3,500 BC (Levey, 1959; Jensen, 2007). Modern Ssoxhlets use sophisticated blown glass condensers and organic solvents in this "continuous" extraction method (**Figure 2~~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. Setup and Preparation of Materials.

~~1.1.~~ Collect ~~athe necessary materials:~~

~~1.2.~~

Comment [A3]: This is interesting, we'd like to briefly mention it. Do we have any idea what they looked like, or has it been lost to time?

Comment [JS4]: I don't know what it looked like but there may be something in the Levey reference. My library doesn't have it but I have requested a loan from another library.

Comment [A5]: But WHY would you use a Soxhlet? (e.g. Used for non-soluble analytes.)

Comment [JS6]: See last sentence in paragraph.

Comment [A7]: This is that same as in the other 2. These videos need to be differentiated.

Comment [A8]: Collapse this section down, maybe into a "Setup" section. Each of these steps should be a filmable component of performing the procedure. Caveat: Any notes you need to add (cautions, things to look out for, etc.) can be implemented as text overlays in the video.

Comment [JS9]: I'm confused here. This is the format that was used in the sonication MS. Can you give me an example of what you're looking for?

Comment [JR10]: Hi Andrew, I took a shot at changing this into the format you suggested. Let me know if it needs adjustment.

~~1.3.~~1.1. ~~Extract one s~~S sample of~~. Samples (leaves, dirt, fungi, bark, tissue), usually We'll use~~ frozen, freeze-dried, crushed, and homogenized ~~prior to extraction, are extracted in groups to maximize efficiency.~~ marine sediment. A sample like this ~~as it should~~ contains many of the compounds ~~we'll~~ needed for~~as~~ standards.

~~1.4.~~1.2. Depending on the size of the sample, use vials with volumes ranging from 4-60 ml~~L~~. For this experiment, use borosilicate glass vials (40 ml~~L~~) and solvent safe caps. Combust the vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of possible organic contaminants.

~~1.5.~~1.3. Obtain ~~D~~dichloromethane and methanol ~~are~~ (both are common in most organic geochemistry laboratories), then~~.~~ ~~U~~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.

~~1.6.~~1.4. Acquire ~~Use~~ a soxhlet apparatus to use in this experiment~~.~~ (~~T~~these can be purchased from Fisher Scientific or other science retailer), then~~.~~ ~~W~~ wash~~-~~ and combust it at 550 °C for 6 hr prior to use.

~~1.7.~~1.5. Obtain ~~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 (can be purchased from Whatman ~~makes these~~) and~~.~~ ~~C~~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.

2.1. Place a combusted weighing tin on the lab scale and then tare.

Comment [A11]: How do you collect/pre-treat your samples? Different sample types (mentioned in 2.2.1) might be a way to differentiate the videos.

Comment [JS12]: Samples are collected differently based on what they are. Sediments may come from the sediment water interface of a basin of water, or from a sedimentary core spanning thru time. Biological tissues will be cultured or sampled from living natural organisms (trees). Pre-treatment includes freezing and freeze drying.

2.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

2.2.1. 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.

2.3. Transfer all of the material in the weighing tin into a combusted glass fiber thimble.

3. Extraction.

3.1. 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 several~~a few~~ (5-10) solvent-rinsed boiling chips.

3.2. Place the sample thimble, open-end up, into the centerpiece of the S~~s~~oxhlet apparatus.

3.3. Place the centerpiece on top of the round-bottomed flask and secure with a glassware clamp.

3.4. Install the condenser on top of the centerpiece of the S~~s~~oxhlet and secure with a glassware clamp.

3.5. 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.

3.6. Turn on the water to ensure proper circulation and drainage.

3.7. Turn on the heating mantle and adjust the temperature until the solvent in the round-bottomed flask is lightly boiling.

3.8. Monitor the extraction a few times over the next hour.

3.8.1. 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.

3.9. Monitor the extraction over the next 36 h~~ou~~r~~s~~.

3.9.1. 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.

3.10. After 36 h~~ou~~r~~s~~, stop the extraction by turning off the heating mantle.

3.11. Label the flask "TLE".

Representative Results:

At the end of extraction, a total lipid extract (TLE) for ~~each~~ the sample is ~~evident~~produced. ~~Each vial~~The round-bottomed flask contains the extractable organic matter from ~~a sediment, soil, or plant tissue~~the sediment sample. This~~ese~~ TLE~~s~~ can now be analyzed, and ~~their~~ its 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~~

Comment [A13]: What makes these videos different are the extraction methods, not the extractant. This should be an Applications section unique to Soxhlet.

Comment [DM14]: This is also identical to the Applications section for the Sonication manuscript.

~~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.~~

The extract from the marine mud contains compounds called alkenones, which ~~that~~ are used in paleoceanography. Alkenones are long--chained alkyl-ketones produced by certain classes of haptophyte algae that live in the sunlit surface ocean (**Figure 3**). The two most common alkenones are 37 carbon atoms long and have two or three double bonds in them.

The haptophytes adjust the ratio of these two alkenones in their cells according to the temperature of the water they live in. Alkenones are preserved in sediments dating as far back as the Early Eocene (~56 million years ago). Knowing the distribution of alkenones in a sediment core throughu time relates information on the evolution of sea surface temperature at that location. However, it's~~we~~ necessary to first ~~need to~~ make sure the~~our~~ instrument accurately and precisely measures the ratio of the two alkenones, and that is why ~~we need~~ standards are needed.

Legend:

Figure 1: Schematic depicting how chemical standard tracks the performance of an instrument through time. The dash line represents a 1:1 relationship between the accepted and measured (on the instrument) value of a variable. Each star is a weekly measurement of the chemical standard. Green stars represent standards that are accurate. Red stars reflect those that are not accurate indicating that the instrument requires corrective maintenance.

~~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 2: A soxhlet apparatus.

Figure 3~~.~~: Alkenones with 2 (C37:2) and 3 (C37:3) double bonds (left) are produced by certain haptophyte algae that live in the sunlit surface ocean (right). (Photo courtesy of Tim I. Eglinton, Woods Hole Oceanographic Institution)

~~Figure 4: Illumination of plankton at Maldives.~~

~~Copyright PawelG Photo.~~

~~Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.~~

~~Copyright Dr. Morley Read~~

References:

The Origin of the Soxhlet Extractor William B. Jensen Vol. 84 No. 12 December 2007 • Journal of Chemical Education 1913

M. Levey, Chemistry and Technology in Ancient Mesopotamia, Elsevier: Amsterdam, 1959, pp. 33-34.

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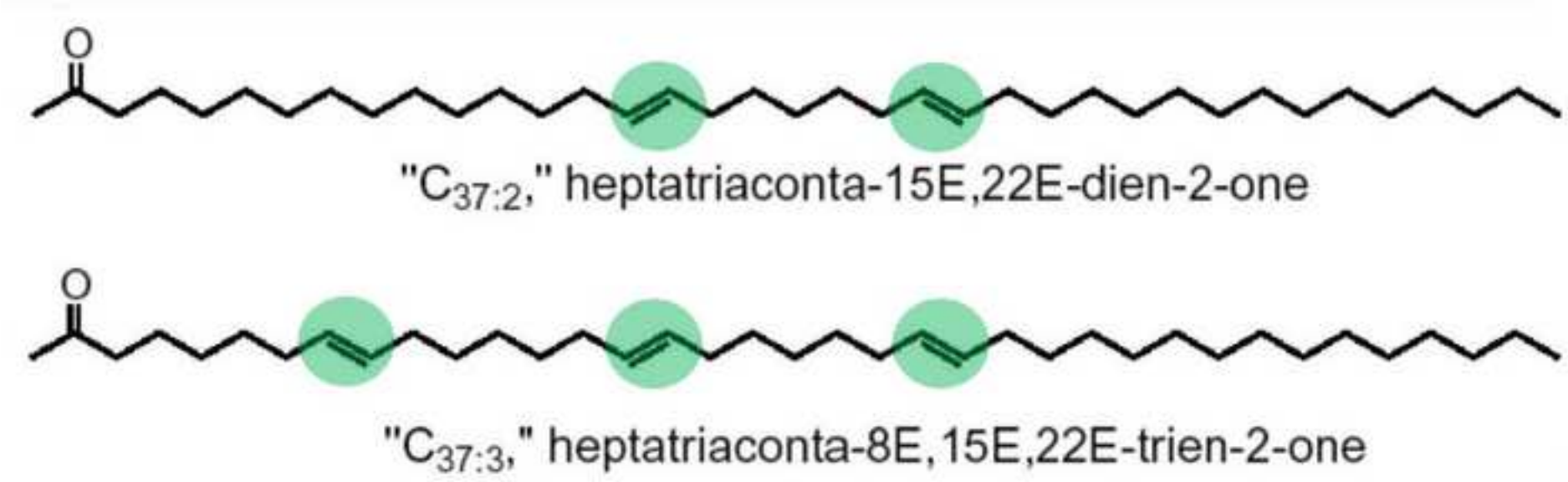

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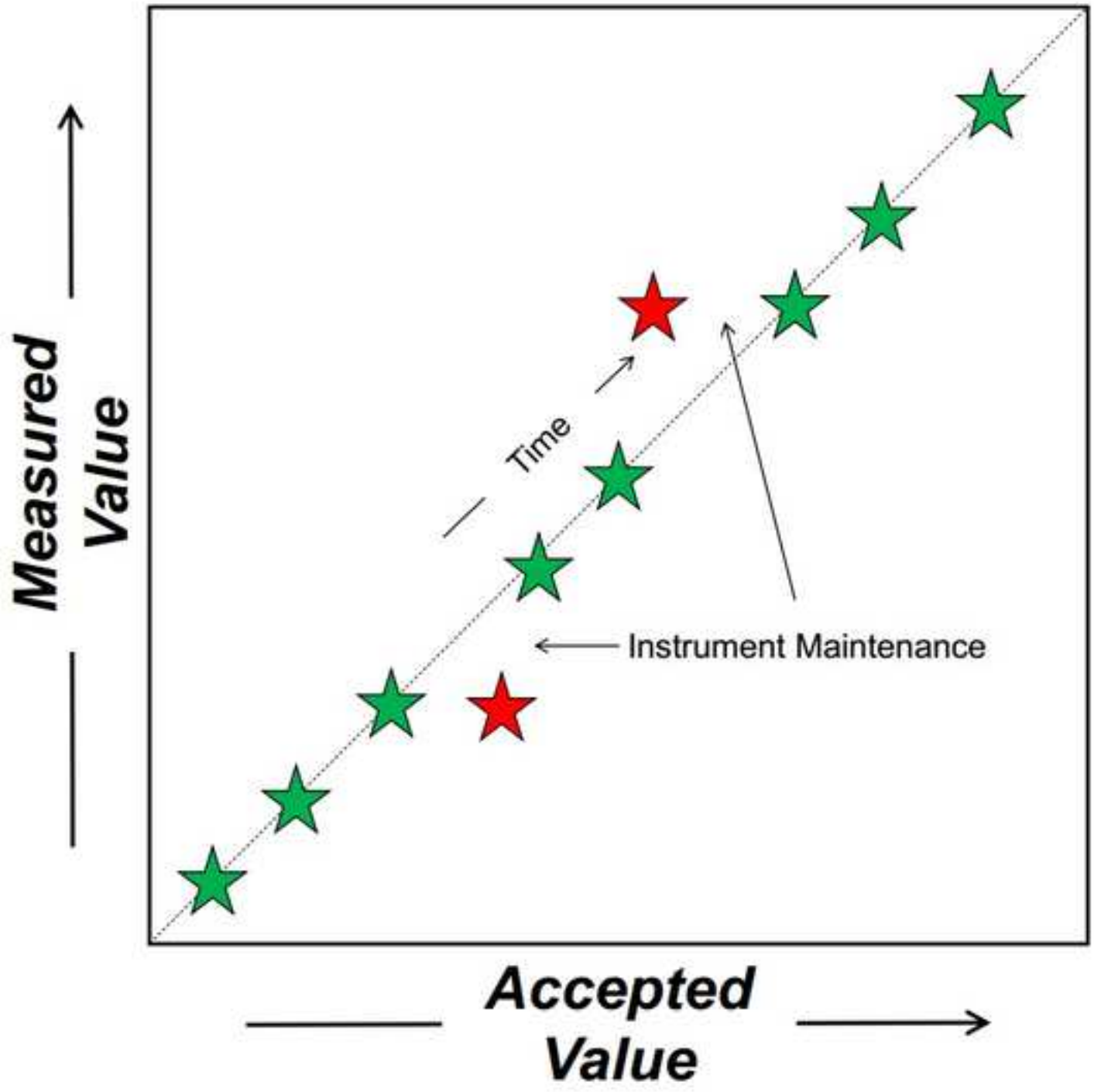

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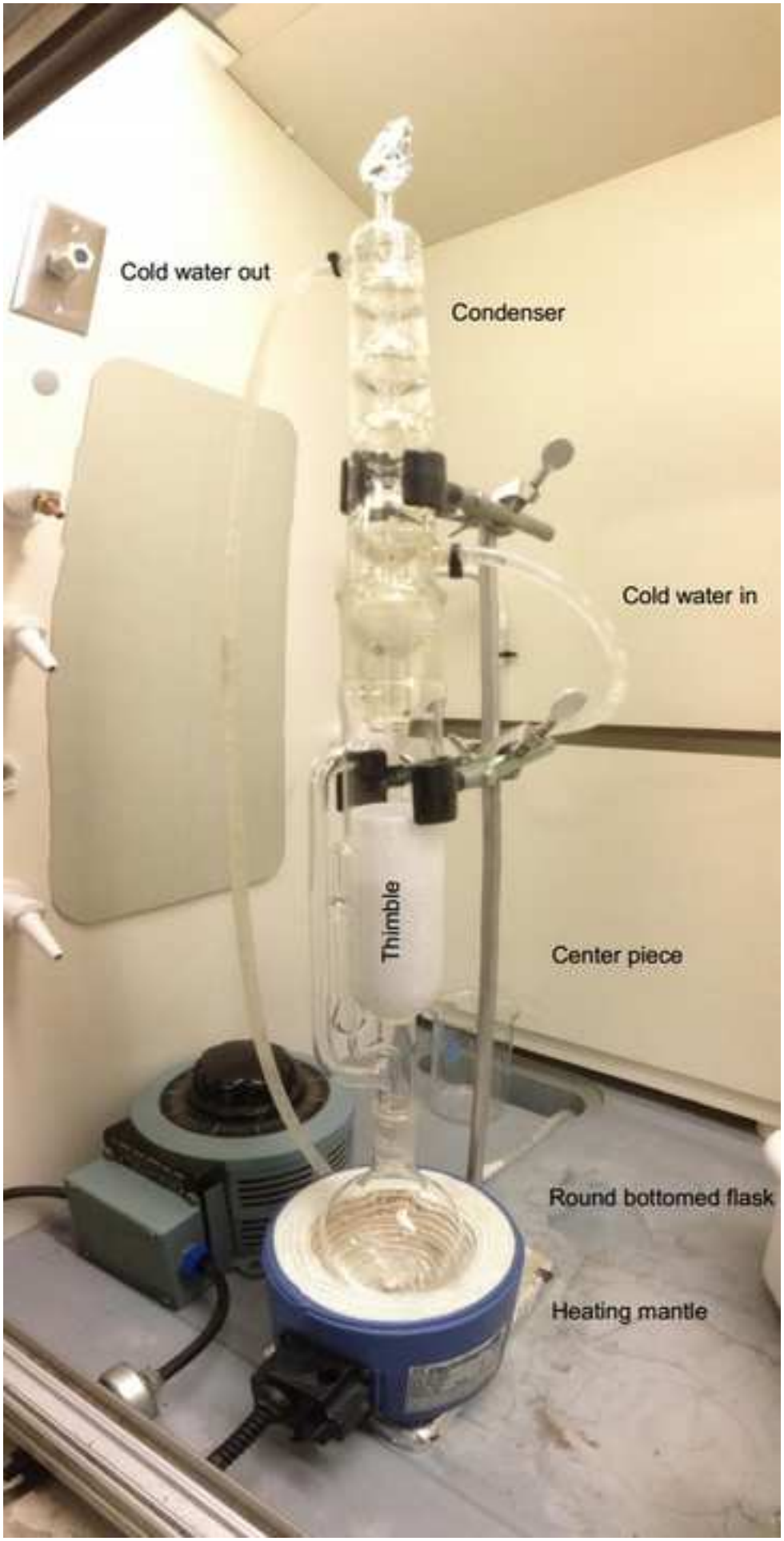

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Principles:

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 ~3,500 BC (Levey, 1959; Jensen, 2007). Modern Soxhlets use sophisticated blown glass condensers and organic solvents in this “continuous” extraction method (**Figure 2**). 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.

Procedure:

1. Setup and Preparation of Materials

1.1. Collect a sample of frozen, freeze-dried, crushed, and homogenized marine sediment. A sample like this contains many of the compounds needed for standards.

1.1.1. Standards are often made from sediments that are left over after a coring expedition or analysis. For example, ~~for the purpose of this video~~in this experiment, ~~we'll extract~~ sediment that was obtained from the 'Mud Patch' located just south of Cape Cod is extracted. This sediment was taken as part of a coring expedition but will not be used to answer scientific questions. We can therefore use it to make a standard.

1.1.2. Place a ~100 g chunk of the sediment into the freezer overnight so that it freezes thr~~u~~ough.

1.1.3. Once the sediment is completely frozen, turn on the freeze dryer (available from many scientific equipment retailers like Fisher) and wait until the condenser reaches it setpoint (often ~-30C).

1.1.4. Load the sediment sample into the freeze dryer and close the purge to be~~ing~~gin pulling a vacuum on the sample.

1.1.5. Depending on the amount of water in the sediment, and the size of the sample, it may take several days for the sample to dry.

1.1.6. Once the sample is dry, turn off the freeze dryer, vent it, and remove the sample.

~~1.1.1.~~1.1.7. Place the sample in a solvent rinsed mortar and grind to a powder using a pestle. Do this to the entire sample and store in a glass jar in the freezer until ready to extract.

1.2. 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 the vials, borosilicate glass pipettes, and weighing tins at 550 °C

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Comment [AW1]: Please provide more information on this procedure (similar to sections 2 and 3). We want to make sure we can accurately script these steps (correct order, apparatus used, time and temperature parameters, etc.)

for 6 hr prior to ensure removal of possible organic contaminants.

1.3. Obtain dichloromethane and methanol (both are common in most organic geochemistry laboratories), then 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.

1.4. Acquire a soxhlet apparatus to use in this experiment (these can be purchased from Fisher Scientific or other science retailer), then wash and combust it at 550 °C for 6 hr prior to use.

1.5. Obtain glass fiber thimbles (can be purchased from Whatman) and combust them at 550 °C for 6 hr prior to use.

2. Preparation of Sample

Comment [AW2]: What sample will you be using in this demonstration? If your lab does the collection, include the process, even if we can't film it on the shoot date.

Comment [JS3]: We'll use sediment obtained from a nearby location described in better detail now above. It was collected by another lab.

2.1. Place a combusted weighing tin on the lab scale and then tare.

2.2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.

2.2.1. 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.

2.3. Transfer all of the material in the weighing tin into a combusted glass fiber thimble.

3. Extraction

3.1. 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 several (5-10) solvent-rinsed boiling chips.

3.2. Place the sample thimble, open-end up, into the centerpiece of the Soxhlet apparatus.

3.3. Place the centerpiece on top of the round-bottomed flask and secure with a glassware clamp.

3.4. Install the condenser on top of the centerpiece of the Soxhlet and secure with a glassware clamp.

3.5. 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.

3.6. Turn on the water to ensure proper circulation and drainage.

3.7. Turn on the heating mantle and adjust the temperature until the solvent in the round-bottomed flask is lightly boiling.

3.8. Monitor the extraction a few times over the next hour.

3.8.1. 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.

3.9. Monitor the extraction over the next 36 hr.

3.9.1. 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.

3.10. After 36 hr, stop the extraction by turning off the heating mantle.

3.11. Label the flask “TLE”.

Representative Results:

At the end of extraction, a total lipid extract (TLE) for the sample is produced. The round-bottomed flask contains the extractable organic matter from the sediment sample. This TLE can now be analyzed, and its chemical constituents identified and quantified.

Applications:

The extract from the marine mud contains compounds called alkenones, which are used in paleoceanography. Alkenones are long-chained alkyl-ketones produced by certain classes of haptophyte algae that live in the sunlit surface ocean [*Conte et al.*, 1994] (**Figure 3**). The two most common alkenones are 37 carbon atoms long and have two or three double bonds in them. The haptophytes adjust the ratio of these two alkenones in their cells according to the temperature of the water they live in. The ratio of the two alkenones defines the Uk’37 ratio:

Eq.1) $U^{k'}_{37} = (C_{37:2}) / (C_{37:2} + C_{37:3})$ [*Brassell et al.*, 1986; *Herbert*, 2003]

Culture [*Prahl and Wakeham*, 1987; *Prahl et al.*, 1988] and core-top sediment [*Müller et al.*, 1998] calibration studies led to the development of the $U^{k'}_{37}$ Index as a quantitative SST proxy. In this work we use:

Eq. 2) $U^{k'}_{37} = 0.034(SST) + 0.039$; ±1.4°C from 0 to 28°C

[culture-based; *Prahl et al.*, 1988]

Comment [AW4]: This is a very interesting application, but I don’t want to use it here, if it would fit better in a different, analysis-focused video. If you think that is not the case, we can use it here. We do need more details: What is the correlation between ratio and temperature? How accurate/sensitive is the relationship? Etc.

Comment [AW5]: We would ideally like 3 applications. Now that the video’s focused has shifted to the extraction of standards, the other Applications could briefly demonstrate the extraction of different types of standards; especially if the procedure deviates from the current Procedure in some way that we can demonstrate. Sediment vs. leaf tissue, for example.

Comment [JS6]: You want three applications per extraction script? I’m not sure this will work well. There are only a few reasons why we extract anything in our lab. Creation of standards is why we use a soxhlet.

Alkenones are preserved in sediments dating as far back as the Early Eocene (~56 million years ago) [*Marlowe et al.*, 1990]. Knowing the distribution of alkenones in a sediment core through time relates information on the evolution of sea surface temperature at that location. However, it's necessary to first make sure the instrument accurately and precisely measures the ratio of the two alkenones, and that is why standards are needed.

Legend:

Figure 1: Schematic depicting how chemical standard tracks the performance of an instrument through time. The dash line represents a 1:1 relationship between the accepted and measured (on the instrument) value of a variable. Each star is a weekly measurement of the chemical standard. Green stars represent standards that are accurate. Red stars reflect those that are not accurate indicating that the instrument requires corrective maintenance.

Figure 2: A soxhlet apparatus.

Figure 3: Alkenones with 2 (C37:2) and 3 (C37:3) double bonds (left) are produced by certain haptophyte algae that live in the sunlit surface ocean (right). (Photo courtesy of Tim I. Eglinton, Woods Hole Oceanographic Institution)

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