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Purification of Complex Organic Mixtures: Column Chromatography

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

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formula[1]	group/compound name
R—OH	hydroxyl: alcohol (R = alkyl group) phenol (R = phenyl group)
—C(=O)—R	carbonyl: aldehyde (R = H) ketone (R = alkyl or phenyl)
—C(=O)—OR	carboxyl: carboxylic acid (R = H) ester (R = alkyl group)
—O—	ether
—NH_2	amine
—C(=O)—NH_2	amide
—SH	thiol (or mercaptan)
—S—	sulphide
>C=C<	alkene

[1]R is used to represent aliphatic chains (alkyl groups) or aromatic rings. The latter can also be called **aryl** groups, and are sometimes represented by Ar.

Photo or Graphic File
Click here to download Photo or Graphic File: Jeff Salacup_Column Chromatography_Figure 2.jpg

Author: Jeff Salacup

Earth Science Education Title: Purification of Complex Organic Mixtures: Column Chromatography

Overview:

The product of an organic solvent extraction, a total lipid extract (TLE), is often a complex mixture of hundreds, if not thousands, of different compounds. The researcher is often only interested in a handful of compounds. The compounds of interest may belong to one of several classes of compounds, such as alkanes, ketones, alcohols, or acids (**Figure 1**), and it may be useful to remove the compound classes to which it does not belong in order to get a clearer view of the compounds you are interested in. ~~For example~~For example, a TLE may contain 1,000 compounds, ~~250 of which belong to each of these four compound classes~~, but the Uk'37 sea surface temperature proxy is based on only two compounds (alkenones) and the TEX86 sea surface temperature proxy is ~~only~~ based on only four (glycerol dialkyl glycerol tetraethers). ~~In this example, i~~It would behoove the researcher to remove ~~the 750 compounds from the classes~~as many of the compounds they are not interested in. This makes the instrumental analysis of the compounds of interest (alkenones or GDGTs) less likely to be complicated by other extraneous compounds.

In other cases, an upstream purification technique may have produced compounds you wish to now remove from the sample, such as the production of carboxylic acids during saponification in our previous video. In both of the above cases~~this case,~~ the purification technique called column chromatography is very useful.

Principles:

Silica gel column chromatography is a purification technique that utilizes the differing associations of discrete compound classes for a silica solid phase, a fine powder called a gel. A small pipette is loaded approximately half full with the gel (**Figure 2**). This column is then saturated with an apolar solvent, often hexane. A sample is then loaded onto the top of the gel in the column, and a series of solvents of increasing polarity is sequentially passed through the sample in order to separate it into separate compound classes. The separation is based on the affinity of the different compound classes for either the solid phase or the solvent phase. Polar compounds bond more strongly to the silica and therefore take more polar solvents to be washed from the column. Thus, using one column and one sample, that sample can be separated into several fractions; for example, apolars (hydrocarbons), mid-polars (ketones and alcohols), and polars (acids and other functionalized compounds).

Procedure:

1. Setup and Preparation of Materials

Commented [JJ1]: In the theoretical sections, make it more obvious this process is downstream of the saponification, and how they relate to one another.

Commented [JS2R1]: So column chromatography is actually more often used without saponification. Almost every lab performs a column on every sample they analyze, but only certain labs and certain samples use saponification. Saponification almost always requires a column but not the other way around. It would be a narrow view to take to explain columns only within the context of saponification.

Commented [JJ3]: This manuscript should either be 1) a more general write-up on column chromatography and its use in geochemistry, or 2) a part of the larger whole that is the fabrication of the paleothermometer.

Commented [JS4R3]: I have updated the text to gear this towards the paleothermometer. Sorry, I missed this comment during my prior review.

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Commented [JJ5]: In the same way the Saponification manuscript covered the specific class of molecules that needed to be dealt with, go into detail about the carboxylic acids: Why they need to be removed, their elution vs. the alkenones, etc.

Commented [JS6R5]: As mentioned above, saponification is a much more specific technique. Columns are used for a HUGE variety of reasons?

Commented [JJ7]: Is this term more common than "non-polar" in the geochemistry field?

Commented [JS8R7]: Yes, we more often use apolar.

1.1. Obtain a total lipid extract (TLE) using a solvent extraction method (Sonication, Soxhlet, or Accelerated Solvent Extraction (ASE)).

1.2. Gather the following: combusted borosilicate glass pipettes and bulbs, silica gel, hexane, dichloromethane (DCM), and methanol.

1.2.1. These materials can be purchased from any chemical retailer. The reagents should be pure and free from hydrocarbons.

1.3. Also obtain combusted glass wool and 4-mL borosilicate glass vials.

1.4. Make sure to have a means of supporting the column and the collection vials during the procedure. For example, a stand with clamps and a vile rack. Many labs have engineered custom-made racks that hold several columns and collection vials (**Figure 2**). This allows many columns to be run at the same time.

2. Methods

2.1. Start with the dry sample in a 4-mL vial. If ~~your~~ the sample weighs more than ~ 10 mg when dry, ~~you~~ it may need to be split ~~it~~ before performing the next steps as the silica gel can only react with a finite mass of organic matter.

2.2. Suspend the sample in a small amount of hexane. This is the first and the least polar of the three solvents used in this experiment.

~~2.2.~~2.3. If there is sample stuck to the inside of the vial, sonicate the samples for 5 min.

~~2.3.~~2.4. Load a small amount of glass wool into the top of a pipette using a set of clean tweezers. Gently push the glass wool to the bottom of the pipette, using another pipette, to form a plug.

~~2.4.~~2.5. Carefully transfer silica gel into the pipette until it is approximately half full.

~~2.5.~~2.6. Place a 4-mL waste collection vial under the column.

~~2.6.~~2.7. Soak the silica gel in the pipette with three volumes of hexane. This conditions the column, removes air bubbles, and rinses any impurities off the silica gel.

~~2.7.~~2.8. Once the final wash is done, remove the waste collection vial and replace it with a vial to collect the apolar fraction.

~~2.8.~~2.9. Carefully transfer the entire sample in hexane onto the column using a pipette. Rinse the sample vial two more times with small volumes of hexane, and transfer to the column. Allow the hexane the sample was transferred in to completely soak into the silica gel. At no time during the procedure should the silica gel dry out.

~~2.9.~~2.10. Continue adding hexane to the top of the sample until the collection vial below the column is nearly full (~4 mL).

~~2.9.1.~~2.10.1. Allow all of the hexane to enter the silica gel before starting with the next solvent.

~~2.10.~~2.11. Place the mid-polarity collection vial under the column.

~~2.11.~~2.12. Add DCM to the top of the column until the collection vial is nearly full. Again, allow all of the DCM to enter the collection vial before starting the next solvent.

~~2.12.~~2.13. Place the polar collection vial under the column.

~~2.13.~~2.14. Add methanol to the top of the column until the collection vile is nearly full.

Representative Results:

This purification technique produces three different vials, each containing a different compound class or group of compound classes. The complexity of any sample to be analyzed on an instrument has been vastly decreased. This process also removes compounds, such as acids produced during a saponification, that can actually stick to parts of the instruments, because of their low volatility, which would decrease their accuracy, precision, and lifetime.

Applications:

Alkenones and isoprenoidal GDGTs are both very common constituents of marine sediments and can be found across the world's oceans. Alkenones are being increasingly detected in lake sediments, although the organisms responsible for their production are different than in the ocean, and thus the relationship between the Uk'37 ratio and water temperature (calibration) is different from the ocean and even between separate lakes. Isoprenoidal GDGTs are found in some large lakes and just like alkenones, often need a local calibration.

The alkenones and GDGTs we are interested in come out in the ketone and polar fractions, respectively. In marine sediments we often analyze both SST proxies from one

Commented [JJ9]: If you place the DCM vial under the column at this point, wouldn't you collect a column's worth of hexane?

Commented [JS10R9]: Yes, but 1) there is only a small amount of hexane actually in the pore space of the gel and 2) nearly all of the hexane soluble biomarkers have been washed from the gel by the time 4 mL of solvent has moved thru it.

Commented [JJ11]: As a way to give this video some sense of conclusion, provide a chromatogram pre-chromatography, and one of the fraction of interest. Why are acids bad for the instrument?

Commented [JS12R11]: Do you want these now or later, for the video?

sample. This allows the construction of two independent SST records which show the evolution of water temperature at the core site thr~~u~~ough time. This comparison, called a multi-proxy approach, often highlights times when the two proxies agree and times when they don't. This agreement or discrepancy itself contains information. If the two proxies agree, maybe the producing organisms occupied the same depth habitat, or maybe they lived at separate depths but a ~~well mixed~~well-mixed water column led to the vertical homogenization of temperature (water usually cools with depth). If the two proxies disagree, it could be that the two populations lived at separate depths; one living in warm, shallow waters and one in cooler, deeper water. Or it could be that the compounds were produced during different times of the -year and so reflect the temperatures of different seasons. These questions are created by the analysis of two different SST proxies at the same site and they highlight the care organic geochemists and paleo-climatologists need to take when interpreting their data.

Because of the high relative stability of apolar hydrocarbons, the apolar fraction contains many interesting organic compounds. Alkanes are important constituents of a leaf's outer waxy layer and they are used in sediment records for many reasons. Their average chain length (number of carbon atoms) contains information on the dominance of aquatic vs. terrestrial plants, temperature, and precipitation. The isotopic ratio of carbon in alkanes is related to the C3 vs. C4 plant-type of the plant that produced it and the hydrogen isotopic ratio is related to local to global temperature and precipitation. Steranes and hopanes are also found in the apolar fraction. These biomarkers are the geostable versions of bioactive compounds like hopanoids and steroids, which serve important biochemical roles in prokaryotes and eukaryotes, respectively.

The mid-polarity fraction contains ~~ketones~~our alkenones. Alkenones are ketones which are important recorders of ancient surface temperatures via the Uk'37 sea surface temperature proxy. Some ketones also come from the same leaf waxes the alkanes do, although there are generally far less.

The polar fraction contains carboxylic acids, another important constituent in leaf wax, that is slightly less specific and harder to work with than alkanes (low volatility) but can nonetheless relate some of the same information. Glycerol dialkyl glycerol tetraethers (GDGTs) are in the polar fraction and are another important recorder of ancient water and air temperatures.

Legend:

Figure 1: Geochemically important functional groups. From (KILLOPS and KILLOPS, 2005).

Figure 2: Image of a custom-made rack that allows the purification of up to 12 samples at a time.

References:

Killops, S. and Killops, V., 2005. *Introduction to Organic Geochemistry*. Blackwell Publishing, Malden, MA.