

JoVE: Science Education
Purification of Complex Organic Mixtures: Urea Adduction
--Manuscript Draft--

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A.



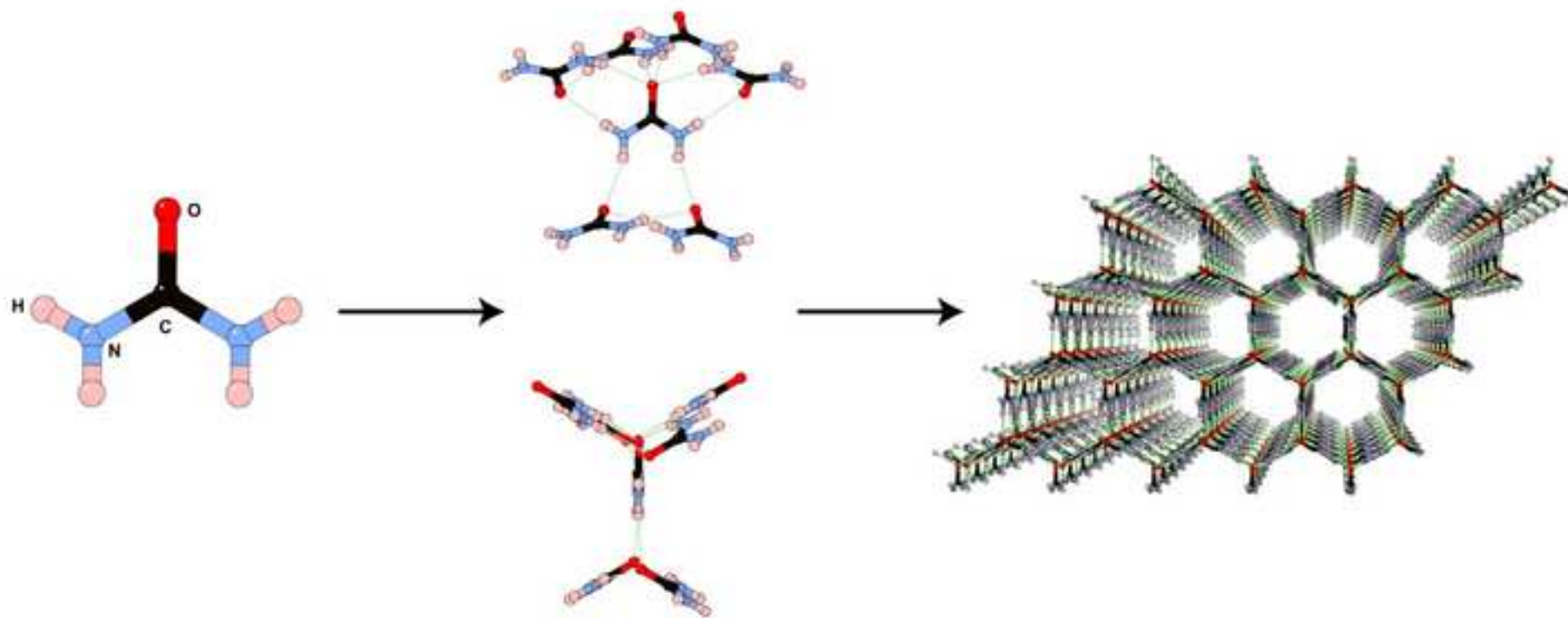
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D.



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Overview:

~~As mentioned in previous videos, 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. ~~In the case of our two organic paleothermometers (Uk'37 and TEX86), we are interested the interest is in only 6 compounds (2 alkenones and 4 isoprenoidal glycerol dialkyl glycerol tetraethers).~~ ~~As discussed in the previous two videos in this series, in order to simplify the sample, and remove unwanted components,~~ purification techniques may be applied ~~in order to pare down the number of compounds in an analyzed sample.~~ These techniques may chemically alter the unwanted components (~~saponification~~), take advantage of the different compound chemistries (~~column chromatography~~), or use the different shapes and sizes of the molecules to include or exclude certain components from the analysis (~~urea adduction~~). The atomic structure of different chemicals leads some organic compounds to form long, narrow, straight chains (~~n-alkanes and alkenones~~), other organic compounds to form complex cyclic structures, ~~and~~ others to form highly-branched structures, ~~and yet others which form both cyclic and branched structures (GDGTs)~~ (**Figure 1**). The different shapes and sizes of the compounds in a sample can be used to separate them from one another, in much the same way as a coin sorter separates coins of different denominations (sizes).

Principles:

Urea adduction is a size exclusion technique that separates straight-chained or rarely-branching structures from highly-branched and cyclic structures. This happens because of the special crystal structure of urea (**Figure 2**). When a urea crystal forms, it creates tiny spaces between the individual crystals. The spaces are long and narrow with an average diameter of five angstroms (5×10^{-10} m). These spaces are large enough to include straight-chain or rarely-branching compounds into the crystal lattice, but too small for highly-branched or cyclic structures. Thus, the latter structures are excluded. The crystals can then be washed, and the ~~highly-branched or cyclic structures can be removed from the sample.~~ Subsequent dissolution of the crystal releases the straight-chained and rarely-branching compounds back into the solution, from which they can be extracted and analyzed.

Procedure:

1. Setup and Preparation of Materials

Commented [JJ1]: Do saponification and column chrom. need to be performed before this step? Does it depend on the sample of interest? Make the relationship between this and the other 2 purifications more obvious.

Commented [JS2R1]: They don't NEED to be accomplished before this step. Very often, a column will be done first. Saponification, rarely, but it does happen in particularly complex samples. I've added some text. Does this help?

Commented [JJ3]: Would you ever want these for any reason?

Commented [JS4R3]: Highly branched and cyclic compounds contain information of their own. Highly branched isoprenoids, produced by sea ice diatoms, can be a proxy for the existence of seasonal sea ice at high latitudes. Cyclic compounds such as PAHs may reflect the presence of fire (wildfires and fossil fuel burning).

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

1.2. Purchase the following materials from any chemical retailer: combusted borosilicate glass pipettes and bulbs; pure water; hexane; Dichloromethane (DCM); methanol; urea.

1.2.1. The reagents should be pure and free from hydrocarbons. Alternatively, pure water can be made in a lab using a water purification system.

1.3. Obtain 4-mL borosilicate glass vials with PTFE-lined caps.

2. Methods

2.1. Make a mixture of 2:1 DCM:Hexane (v:v).

2.1.1. In an Erlenmeyer flask, mix 200 mL of DCM and 100 mL of hexane.

2.2. Make a mixture of urea in methanol (100 mg/mL).

2.2.1. Pour 300 mL of methanol into an Erlenmeyer flask.

2.2.2. Add 300 mg of urea.

2.2.3. Stir on an automatic stir plate until the urea is completely dissolved.

2.3. Start with the dry sample in a 4-mL vial.

2.4. Suspend the sample in 1.5 mL of a 2:1 mixture of DCM:Hexane (v:v).

2.4.1. If the sample does not completely dissolve, sonicate for 5 min.

2.5. To this mixture, add 1.5 mL of urea in methanol.

2.5.1. After this addition, watch for a white precipitate to form. This signals the creation of urea crystals.

2.6. Gently dry the crystals under nitrogen, using gentle heat. Ensure all solvent has evaporated before proceeding to the next step.

Commented [JJ5]: How are you drying these samples between purification techniques?

Commented [JS6R5]: We dry samples between steps using a light stream of pure nitrogen and light heat.

- 2.7. Rinse the crystals ~~3-times~~3x with approximately 1 mL of hexane. Remove the hexane between each rinse ~~by~~ using a glass pipette to transfer it into a vial labeled "non-adduct."
- 2.8. Dissolve the crystals in 2 mL of pure water. Shake to ensure complete dissolution.
- 2.9. Extract the aqueous phase with hexane by adding 1 mL and gently shaking for 5 ~~see~~.
- 2.10. Allow the hexane and water to completely separate before removing approximately 75% of the hexane ~~by~~ using a glass pipette and transferring it into a vial labeled "adduct."
- 2.11. Repeat step 2.10 twice.
- 2.12. Dispose of the water and urea solution in an appropriate waste container.

Representative Results:

This purification technique produces two different vials; one labeled adduct, containing straight-chained and rarely-branching compounds, and another labeled non-adduct, containing highly-branched and cyclic compounds. This procedure has vastly decreased the complexity of any sample to be analyzed on an instrument. This decrease in complexity is often crucial to the accurate analysis of target compounds. For example, in nearshore settings after approximately 1850, alkenones co-elute with troublesome compounds, ostensibly pollutants introduced after the Industrial Revolution, that Revolution, which are not removed using column chromatography or saponification alone. Apparently, the pollutants are cyclic or branched because urea adduction removes them from the sample. The top 160 years of some of these sediment archives can be confidently analyzed for UK'37 only because of the application of urea -adduction.

Applications:

Urea adduction is often used in the purification of n-alkanes, common constituents of leaf wax, in order to remove co-eluting compounds before isotope analysis. The carbon and hydrogen isotope ratios of leaf waxes in plants contain information on the metabolic pathways and environmental conditions the plant used and lived in, respectively. In order to determine the isotope ratios, very large quantities of compound must be loaded onto a GC. Such large quantities often cause compounds that elute close to one another at lower concentrations to co-elute. Often, the compounds co-eluting with alkanes are branched or cyclic moieties of that alkane and can thus be removed using urea adduction.

Commented [JJ7]: As a way to give this video some sense of conclusion, can you analyze both vials? What would be the best way to demonstrate the components of each?

Commented [JS8R7]: We can definitely do this! Easiest way would be to run each fraction on a GC-FID, which we have in our lab. The chromatograms will look quite different.

Commented [JJ9]: It's understandable why this is desirable, but adding an extra step before analysis adds time, cost, possible sources of error, etc. Comment on the fact that this step is worth the downsides.

Commented [JJ10]: This is a good application; what footage/data could you provide to visualize it? What other uses are there for this technique in the geochemistry field?

Commented [JS11R10]: We could look at before and after chromatograms.

Legend:

Figure 1: Comparison of different chemical structures showing decane, a straight-chained alkane (A; from <http://www.bpc.edu/mathscience/chemistry>), cyclohexane, a cyclic alkane (B; from <http://www.bpc.edu/mathscience/chemistry>), a steroid, a poly-cyclic hydrocarbon (C; from www.wikiwand.com), and 2, 2, dimethyl butane, a branched alkane (D; www.wikimedia.com).

Figure 2: Depiction of the chemical and crystal structure of urea (from www.imgkid.com).

Commented [JR12]: If possible, can the images be recreated? It looks like some of the images included are free to use, while others may not be.

Yes, we can recreate molecular models.