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## Saponification (Base Hydrolysis) of Organic Materials

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### *Introduction*

Many polymeric materials can be decomposed into smaller subunits that are suitable for GC/MS analysis by acidic or basic hydrolysis, and amide bonds are particularly susceptible. The products of ester hydrolysis will be alkanols plus alkanoates (at moderate to high pH) that need to be derivatized before analysis. Saponification (base hydrolysis) is more frequently employed than acid hydrolysis. The procedure is commonly applied to total lipid extracts (TLE) to release ester-bound lipids, but can also be applied to solid materials such as biomass or extracted residues.

### *Materials*

- 0.5N KOH in H<sub>2</sub>O (**note A**)
- 4N HCl
- 5% NaCl in H<sub>2</sub>O
- MTBE (GC grade)
- 40 mL VOA vials
- Teflon capliners
- pH paper
- heating block (70°C)
- TurboVap

### *Special Hazards and Warnings*

- ☒ You are working with a moderately strong base. Be sure to wear gloves and safety glasses. If you don't want small holes to appear in your clothes, a lab coat is recommended.
- ☒ Capped vials can sometimes burst while being heated. Keep the hood sash lowered while samples are heating, and wear safety glasses.

### *Procedure*

1. Transfer sample to a VOA vial using an appropriate solvent (DCM, ether, etc.) and dry in the Turbovap. Add 5mL of 0.5N KOH solution, cap the vial tightly using a Teflon capliner, and heat in the heating block at 70°C for 3 hours (**note B**).
2. Allow to cool, then add 5mL of NaCl solution and shake thoroughly (**note C**).
3. Add 4N HCl dropwise from a pipette to your sample to adjust pH to between 1 and 3. Check the solution pH by transferring a few drops with a clean Pasteur pipette onto wide-range pH paper. This step is necessary to ensure that organic acids are protonated and thus soluble in organic solvents.
4. Add 10mL of MTBE and shake vigorously for 2 minutes. Allow the two phases to separate (**note D**), then pipette off the organic layer (top) and collect it in a second

VOA vial. Repeat 2 more times with fresh 10mL aliquots of MTBE, collecting a total of ~25mL of extract. Dry the extract to ~1 mL under N<sub>2</sub> in the TurboVap.

5. Depending on the next step, you may want to filter the extract through anhydrous sodium sulfate to eliminate any water that might have been collected with the sample.
6. Important! You are extracting a strongly acidic solution with a moderately polar solvent (MTBE). It is likely that your extract will be slightly acidic, especially if you used methanol as the hydrolysis solvent. If the next step is methylation of fatty acids, this is not a concern. However, if you plan on making TMS derivatives and then injecting directly into the GC/MS, this will cause problems with excessive GC column bleed. Neutralize your sample first by shaking with a small amount of 5% NaHCO<sub>3</sub> solution, then dry over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Alternatively, you can extract the aqueous solution with hexane, which will not dissolve an appreciable amount of acid. When in doubt, check the solution pH before injecting into the GC.

### Notes

**A.** Hydrolysis can be conducted in methanol, water, or any other protic solvent. Methanol is somewhat better at solubilizing polymeric organic materials, but there is a chance that some carboxyl groups will be converted to methyl esters. Adding a small amount of water to the reaction will help to limit their formation, as will shaking with acidic water (step 2). If you want to form methyl esters, see the method for alkaline transesterification. You can substitute NaOH for KOH.

**B.** Methods from various labs differ widely in the temperature and time used for saponification. We typically use 3 hours for saponification of total lipid extracts (TLE). However, up to 24 hours is commonly employed for solid materials, including sediments, biomass, and kerogen.

**C.** The NaCl solution is added to partition the KOH into the aqueous phase, away from the organic phase. In a pinch, you can get away with using pure water for this step. However, a NaCl solution is more efficient at this, so we recommend its use when possible.

**D.** Often the hydrolyzed products of fresh biomass produce a thick emulsion that resists separation. Some tricks that may help, in order of increasing effectiveness: *i*) gently stir the emulsion with the tip of a long Pasteur pipette or glass stir rod; *ii*) add more NaCl to the aqueous layer, in an effort to 'salt out' the more polar materials; *iii*) freeze the sample, then allow it to slowly thaw while gently stirring; *iv*) transfer the whole mess to a centrifuge tube and spin at 2000 rpm for 10 minutes. This last option always works, but is slow going.

### Troubleshooting

There is not much that can go wrong with this reaction, aside from the formation of emulsions that are difficult to extract. If the hydrolysis is incomplete, you can increase any of the following: temperature, time, KOH concentration. If the hydrolysis is still incomplete, you can try acid hydrolysis. The most common problem with this procedure is insufficient removal of HCl from your sample (step 6), which leads to other problems in subsequent steps.