

CD - Cuvette Cleaning

Note:

Never Use sulfochromic acid or strong bases, such as potassium or sodium hydroxide, they can damage the cuvettes.

Low Molecular Weight, Water Soluble Samples

Simply rinse with purified water followed by acetone or ethanol and blow dry with clean, dry, compressed air or nitrogen. Make sure that you do not build up pressure in the cuvette. Don't use house air without having a filter installed. This is often sufficient to clean the interior of the cuvette. Clean the exterior of the cuvette by wiping gently with acetone or ethanol on soft lint free tissue.

Biomacromolecules

Particularly denatured proteins can be quite sticky and their removal from quartz surfaces often needs more aggressive cleaning methods.

- 1) Starna and Hellma supply their own brands of cuvette cleaning agents, usually solutions of anionic and non-ionic detergents in the pH range 9.5 to 12. Their websites also give guidance on cuvette cleaning and their recommended protocols are ok. However, they are not always enough to remove protein baked onto the cuvette surfaces at high temperatures.
- 2) "Nano Strip" (from Cyantek) or Piranha solution works well (be careful it is named so for a reason!!! Wear lab coat, goggles, gloves, closed-toed shoes and long pants. Work in a fume hood!!!!). For use follow the Nitric Acid protocol from below using Nano Strip or Piranha instead. *Be Careful!!!!!! Plastic transfer pipette or flat gel loading tips do not hold up to Nano Strip or Piranha solutions.*
- 3) **My favorite method is using Nitric Acid**

(Be careful- wear lab coat, goggles, gloves, closed-toed shoes and long pants. Work in a fume hood)

- I. After use, take the cuvette from the holder and remove as much of the sample as possible using a fine-tipped disposable transfer pipette or a flat gel loading tip for 0.5 mm cuvettes. Rinse four or five times with either buffer or purified water (a buffer rinse is used for samples such as some proteins that precipitate in purified water). For each rinse, introduce and remove the rinsing agent with the pipette.
- II. If a buffer rinse is used, it should be followed by further rinsing with purified water.
- III. Rinse several times with acetone or ethanol, then blow dry thoroughly with clean dry compressed air or nitrogen. *It is particularly important that the cuvette is completely dry before the next step.* If it is not, heat from mixing acid with water or ethanol can damage the cuvette! Make sure that you do not build up pressure in the cuvette. Don't use house air without having a filter installed.
- IV. Immerse in concentrated nitric acid, making sure that the air in the cuvette is fully displaced with the acid.

Caution:

Make sure that the vessel containing the acid is clearly marked with its contents and is stored in a safe place. Make sure that the cuvette does not get damaged while moving the vessel around.

- V. Allow to soak for 10 minutes to 1 hour (or overnight with 2 M nitric acid) if the cuvette has been used at high temperatures (with Piranha/Nano-Strip 30 min should be enough).
- VI. Over a drip tray, carefully remove the cuvette from the acid using stainless steel tweezers, gripping the cuvette lightly by the neck, not by the optical surfaces.
- VII. Using a fine-tipped disposable transfer pipette or flat gel loading tips, carefully remove as much of the acid as possible from the cuvette. (Again Piranha or Nano-strip is not compatible with plastic pipettes)
- VIII. Carefully rinse the cuvette with purified water several times. Introduce and remove the water with a fine-tipped pipette for each rinse.
- IX. After at least 7 rinses, measure a CD spectrum (180 nm to 260 nm for 1 and 0.5 mm cuvettes, 200 nm to 260 nm for longer path lengths, go to longer wavelengths if your signal was at longer wavelengths) of the water filled cuvette

to prove that it is clean. Save the spectrum as "water_after" to document that the cuvettes were left clean. If you used multiple cuvettes save a water_after spectrum for each!

- X. Rinse the clean cuvette with acetone or ethanol, than blow dry thoroughly with compressed air or nitrogen. Make sure that you don't build up pressure in the cuvette. Do not vacuum dry as this may introduce stresses resulting in birefringence in the cuvette.
- XI. Gently clean the outside of the cuvette with acetone or ethanol using a soft lint free tissue. Check that the cuvette is clean by holding it up to the light. There should be no visible contamination or smears.
- XII. If it is not needed immediately, store the cuvette in the box they were in (if not available, store in a box lined with a soft material) a clean and dry environment.