

# HPLC Pigments – Sample Filtration

Updated 9/18/08 WKoz

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## HPLC Filtration Set-up

### Equipment needed:

- 6 place filtration manifold (wide spacing)
- HPLC filtration rack
- 5 gallon glass carboy in square gray crate
- 500 ml Erlenmeyer flask with sidearm (used as trap)
- 3 pieces of vacuum tubing
- Gast vacuum pump (set to no higher than 12 psi)
- 6-2L narrow mouth amber Nalgene polypropylene bottles
- 6-200 ml Gelman plastic funnel cups
- 6 plastic frits and funnel bases in stoppers
- GF/F filters
- 6-1.8 ml Nunc cryovials (preferably not Nalgene brand; label these with PENCIL)
- aluminum cryocanes
- plastic cryosleeve for cryocanes
- forceps
- 0.2 $\mu$  filtered sea water in wash bottle

### Secure the vacuum tubing as follows:

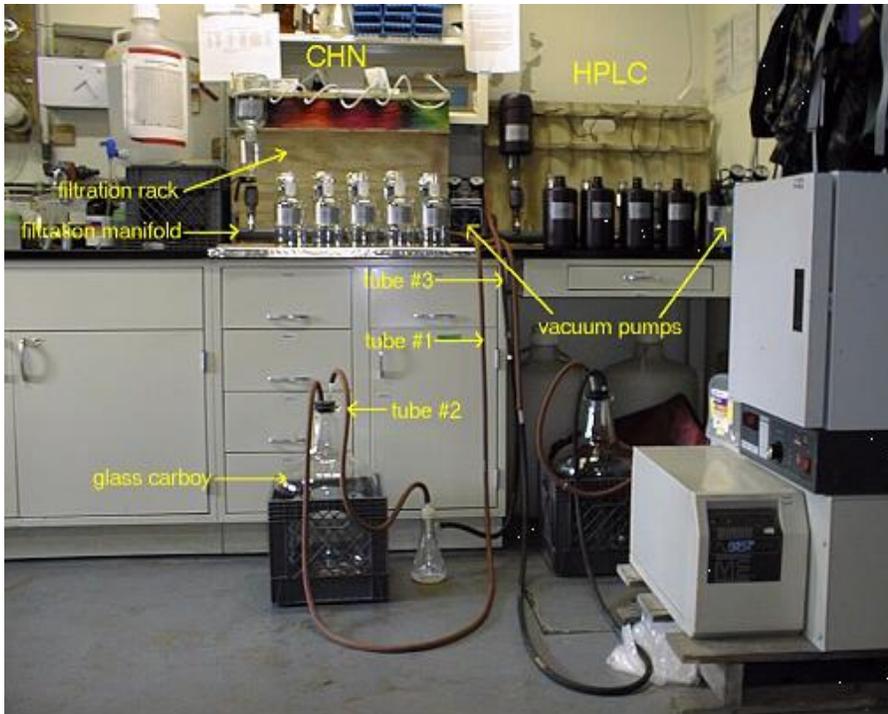
- tube #1 - end of manifold > stopper in carboy
- tube #2 - stopper in carboy > stopper in flask
- tube #3 - sidearm of flask > vacuum pump

Place a 6 inch piece of plastic tubing in the ends of the vacuum tubes that will be secured to stoppers.

Then push the plastic tubing through the stoppers.

In the carboy, the end of the plastic tube from the manifold should be deeper in the container than the other tube.

*\*Tip: To get the plastic tubing through the stoppers, use a piece of sturdy tubing (Nalgene tubing works well). Put this tubing through the stopper, being careful to ensure it goes below the point at which the side-arm branches off. This reduces the amount of water sucked into the vacuum pump. Once the tubing is through the stopper it can be fit into the larger plastic tubing coming from the end of the manifold.*



## HPLC Filtering

### Sample Water

0.5 to 3.0 liters of sample water at each of the light levels: 100, 50, 25, 10, 5, 0.5 station

### Important Tidbits

- This filtering produces samples that will later be extracted for total pigments using the HPLC (high performance liquid chromatography).
- The goal for filtering is to end with filters that have “**good color**”, much more than is needed for CHN.
- "Good color" means that the filters should have a distinctly visible circle of colored material on the filter.
- The appropriate amount of water to filter is going to vary, but usually **one liter** is a good starting amount.
- After a good amount of colored material is achieved, filters are put in labeled 1.8 mL **cryovials**.
- All vials from each station (6 total) are put in a single cryocane with a plastic cryosleeve and stored in **liquid nitrogen**.
- Pigments are extremely affected by light, thus all filtration should be done **in the dark**.
- Temperature also impacts pigments, so the goal is to spend the least amount of time possible filtering while still getting an appropriate amount on the filter.
- If some water samples (certain depths) finish filtering before others, those filters should be put in cryovials and kept in a bench top cooler (frozen in the -20°C freezer) for a

short period of time or stored in the  $-20^{\circ}\text{C}$  freezer if it will be longer, until all 6 depths are done and they can be stored in liquid nitrogen.

- Label the end of the cryocane appropriately (with a sharpie).
- In between different stations wash bottles and funnels in deionized water.