DIATOM PREPARATION PROCEDURE

SAFETY: See associated COSSH form. Always wear PPE (lab coat, safety glasses and nitrile gloves). Inform safety officer of any spills.

H_2O_2 – mild burns, respiratory irritant
HCl – mild burns, respiratory irritant
Naphrax – highly flammable, irritant

First aid – run with water for ~20 minutes, seek medical help for eye incidents.

CHEMICALS REQUIRED:

- 30% Hydrogen peroxide (H_2O_2)
- 50% Hydrochloric acid (HCl)
- Naphrax diatom mountant
- Distilled water
- Ammonia solution

EQUIPMENT REQUIRED:

- Hotplate / Waterbath
- Fume cupboard
- 100 or 250 ml beaker for each sample
- 19mm diameter round glass cover slips
- 1 ml pipettes for each sample
- 50ml centrifuge tubes
- 50ml tube rack
- Centrifuge
- Glass slides
- Rigid metal tray

PROCEDURE: Hotplate method

Procedure requires close supervision so that beakers do not boil dry. There is also a risk of explosion when very concentrated, hot hydrogen peroxide is rapidly oxidising samples with a high organic content. For this reason it is essential that the fume cupboard window is fully lowered if the heating samples are left unattended, and that the window is lowered to the safe working height and eye protection worn whilst working at the fume cupboard.

1. Place about 0.1 g (dry weight, 1 g wet weight) sediment into a beaker. If diatom concentration is to be assessed, the sediment should be weighed to three decimal places.

2. Add about 20 mls H_2O_2.

3. Heat on a hotplate set at 90°C in a fume cupboard until all organic material has been oxidised (1-3 hours). Coarse plant material in macrophyte samples may be removed after half an hour.

4. Remove the beakers from the heat. Add a few drops of HCl (50%) to remove remaining H_2O_2 plus any carbonates and wash down sides of beaker with distilled water.

5. Allow to cool in the fume cupboard (chlorine is generated from the HCl) and pour into centrifuge tubes, leaving any coarse sand in the beaker. Top up with distilled water.

6. Centrifuge at 1200 rpm for 4 minutes.

7. Decant off supernatant and re-suspend pellet by tapping the base of the tube. Top up with distilled water and centrifuge as before.

8. Repeat washing process at least three times. Clay may be removed during the last wash by adding a few drops of very weak ammonia solution to the sample. The clay is then decanted off with the supernatant. The sample is now ready to make into slides.

PROCEDURE: Water bath method

This method is particularly suitable for large numbers of sediment samples (Renberg, 1990). It can also be safely left without any risk of explosion and there is less risk of samples boiling dry. It may not be suitable for samples which tend to react vigorously with hydrogen peroxide, such as large epilithon and epiphyton samples.

1. Place approximately 0.01 grams of dried sediment (or 0.1 gram of wet sediment) in each tube, weighing to four decimal places if diatom concentrations are to be calculated. Moistening dried sediment with a few drops of H_2O_2 will help its dispersal when the rest of the peroxide is added.

2. Add 5 mls 30% H_2O_2 to each tube and place in a rack in the water bath (in a fume cupboard) at room temperature. If the H_2O_2 does not react violently with the sediment, the temperature of the water bath can be increased to 80°C (or 90°C if using glass tubes). 'Blank' tubes containing only H_2O_2 can be placed at intervals in the rack and analysed to check there is no cross contamination between the tubes during digestion. Evaporation from the water bath is reduced using floating plastic spheres.

3. Heat samples for 2-4 hours, checking the level of H_2O_2 from time to time, until all organic material has been removed. Do not allow the samples to dry. Also keep the water level in the bath topped up with distilled water.

4. Remove the tubes from the bath and add just 1-2 drops of 50% HCl to each tube, which will eliminate any remaining H_2O_2 and any carbonates. The fizzing which occurs also helps to unstick any diatoms which may have become attached to the side of the test-tube.

5. Top up test-tubes with distilled water and leave to settle out overnight at 4°C. This is convenient when handling a large number of samples in glass test-tubes and reduces breakage of fine, filamentous diatoms. The resulting supernatant liquid is then decanted off and the diatoms re-suspended in more distilled water. Alternatively centrifuge

samples in plastic tubes as above.

6. Repeat this washing process four more times, either allowing the diatoms to settle out overnight at 4°C between each wash or by centrifugation. 1-2 drops of weak ammonia (NH_3) solution added to each sample with the final wash will help keep any clays in suspension and will also prevent the diatoms clumping together when making up slides.

PROCEDURE: Addition of microspheres

If diatom concentrations are required, use DVB (divinylbenzene) spheres with a mean diameter of 6.4m (other plastics dissolve in the toluene mountant). These may not survive the digestion process, or if they do the difference in density between the spheres and diatoms could result in some spheres being lost in the washing process following digestion. For these reasons they have to be added to the samples just before the slides are prepared. The DVB spheres are bought as a concentrated suspension. This is sonicated to disperse the spheres before dilution with distilled water to make a concentrated stock suspension which is calibrated using a coulter counter. This is then diluted further to make 1 litre batches of working concentration at around $5x10^6$ spheres per ml. The addition of a small amount of ammonia solution helps to keep the spheres from clumping. To prevent the growth of micro-organisms a very small amount of mercuric chloride is added (<3mg L^{-1}) and the prepared suspension is kept at 4oC.

1. Add the microsphere markers after the last wash, just before the slides are made. Use the suspension ($x10^6$ concentration) stored in the lab fridge. Do not use the stock solution kept in the white polythene bottle in the lab fridge. Because of the toxicity of mercuric chloride it is important to wear gloves when handling the microsphere suspension and to carefully clean up any spills.

2. Initially add 1.5-2 mls of 5×10^6 suspension for each 0.1 grams of dry sediment digested, being careful to shake and sonicate the microsphere suspension before each use to disperse the spheres evenly. A suspension of $5x10^5$ spheres per ml is easier to use for sample sizes of 0.01 gram dry weight when using the waterbath method. In this case it may be advisable to include some replicate samples since the smaller sample size makes this method less suitable for quantitative work.

3. Make a test slide to check the diatom to microsphere ratio (ideally 1:1) and calculate the required amount of microsphere suspension before adding spheres to a whole batch of samples. When making up slides containing DVB microspheres, care must be taken not to heat them above 130°C.

PROCEDURE: Making slides

Diatom slides are usually made up by allowing the diatom suspension to settle out on a cover slip overnight, as described below. This produces an even spread of diatoms over the cover slip but it does take up to two days. It is possible to speed up the procedure (resulting in lower quality slides) by gently heating the coverslips after the diatoms have been allowed to settle for 30 minutes. This may result in some clumping of the diatoms but the slides can usually still be counted.

1. Dilute the cleaned diatom suspension to a suitable concentration. It takes practice to get the concentration right. The suspension should look neither totally clear or milky. Fine particles in suspension should be just visible when the suspension is held up to the light.

2. Place metal settling out trays with cover slips in a position where they will not be disturbed, away from dust sources and air currents.

3. Using the 1 ml pipette, place up to 0.5 ml of well mixed diatom suspension on each cover slip, place a second tray over the first supported on small plastic pots to keep out the dust and leave to dry. This may take up to two days.

4. Heat a hotplate in a fume cupboard to 130°C.

5. Place 1 drop of Naphrax on a glass slide and invert the cover slip with the dried diatoms over the drop.

6. Heat the slide on the hotplate for 15 minutes to drive off the toluene in the Naphrax.

7. Allow the slide to cool and then check that the cover slip does not move when pushed with a fingernail. If it does move then the slide will need to be heated a little longer.

Protocol adapted from UCL DEPARTMENT OF GEOGRAPHY, Physical Geography Laboratory