PHYTOLITH 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
KOH - Highly toxic, severe burns esp. with eye contact, irritant

$(NaPO_3)_6$ – Flammable, irritant
Canada balsam - irritant
First aid – run with water for ~20 minutes, seek medical help for eye incidents.

CHEMICALS REQUIRED:

Distilled water
5% sodium hexametaphosphate $(NaPO_3)_6$
10% potassium hydroxide (KOH)
Sodium polytungstate 2.3-2.35gcm^{-3}
10% hydrochloric acid (HCl)
30% peroxide (H_2O_2)
Canada balsam

EQUIPMENT REQUIRED:

50ml test tubes x40
Tube racks
Caps for test tubes
250ml beakers x20
Large conical cylinder
Large low beakers
15ml glass measuring cylinders
Centrifuge
Retort stand
Stirring rods
Hotplate
Storage bottles for NaPT

PROCEDURE:

1. Subsample

- Weigh and label empty test tubes
- Measure 1 cm^3 of sediment using volume displacement
- Pour into 50 ml tubes
- Centrifuge (3000 rpm, 5 mins) and decant off H_2O
- Put in the drying cabinet overnight to let the sediment dry
- Weigh the test tube plus sediments

2. Carbonate removal

- Add small amount 7% HCl and Whirlymix
- Add 7% HCl to first line test tube rack, washing sediment from sides of tube
- Leave until reaction complete (no more bubbles)
- Add distilled water to the top of the tube, Centrifuge (3000 rpm, 5 mins) and decant off HCl
- Rinse 2 times
- If you suspect the sediments have high $CaCO_3$ content, centrifuge, poor off supernatant and carry the carbonate removal anew. But if the sediment are calcareous, they are not very likely to contain many phytoliths.

3. Deflocculation

- Add small amount of 5% $(NaPO_3)_6$ and whirlymix.
- Add 5 % $(NaPO_3)_6$ to first test tube rack (will need to use a squeezy bottle as pouring from glass cylinder unreliable) (~ 15 ml).
- Place capped tubes in shaker overnight – best to have them slightly on their side as slosh more. You need to set the number of repetitions a minute (use highest) AND set the timer (under mode) to enough minutes to keep going over night (c800 min, max 999). (Alternatively, shake tube regularly throughout a day)
- Centrifuge (3500 rpm, 5 mins) and decant sodium hexametaphosphate off (the supernatant may be quite brown as Na will start to break down humics).
- Rinse until supernatant clear.

4. Organics removal

This stage can be carried out either in the test-tubes in a water bath as detailed below, or alternatively in 250 ml glass beakers on a hot plate.

- Wear goggles
- Add 1 ml of H_2O_2 30%, whirlmix
- Place the samples in the cold water bath and start heating, aiming at 85°C
- Stay nearby and check: that the samples do not dry, that the samples do not start bubbling madly.
- Add little by little more H_2O_2 and whirlmix as appropriate
- You will need to add at least approx 50 ml of H_2O_2 30%,
- Knowing when to stop is tricky. The theory says that supernatant should be clear to yellow with no reddish or brown hue, however, this may take a long time (days).
- Dilute with distilled water, otherwise the hydrogen peroxide will bubble and the pellet will not stick and thus sediment will be lost on pouring.
- Centrifuge (3000 rpm, 5 mins) and decant off H_2O_2
- Rinse 2 times

5. OPTIONAL Humic colloid and excess organics removal

- Add 10 % KOH and heat (no temp specified, 80°C should suffice; on a hotplate in a beaker) for 10 mins

- Centrifuge (3000 rpm, 5 mins) and decant off KOH
- Rinse, centrifuge and decant
- (although this worked well in the sense that it rid my sample of much organic matter, and there were phytolith on my slides, very organic sediments can react virulently with the KOH. It is advised to add it drop by drop, little by little.)

6. Phytolith isolation

- Put the test tubes in the drying cabinet and allow sediments to dry over night
- Grind the dried samples in the test tube using a rod with rounded tip.
- Add 10 ml NaPT at specific gravity 2.3 to 2.35 using gradated pipette
- Whirlimix to ensure good mixing and lifting of phytoliths
- Centrifuge (3000 rpm for 5 mins)
- Decant supernatant into clean weighed labelled tubes (contains the phytoliths)
- Add distilled water to ratio of 2.5:1 to reduce the specific gravity of the solution to <1.5g cm^3 allowing the phytoliths to sink
- Centrifuge at 2500 rpm for 10 mins
- Decant and retain supernatant for NaPT purification
- Reapeat floatation step on remaining sediment another 2-3 times
- Collect all phytolith samples together and rinse again
- Retain soil residue in labelled pot

7. Clay removals

- Add small amount of water, whirlmix, and refill tubes to just above second line of rack (c.6 cm height of water)
- Centrifuge at 3000 rpm for 5 mins
- Repeat centrifuge-decanting step until decanted water is completely clear (i.e. no more clays in the sediment being centrifuged off)
- Check supernatant for presence of phytoliths

8. Dry the pellet in the test tube overnight, weight the test tube plus pellet

9. Microspheres (optional)

- Shake well the bottle of diluted microspheres, and leave in sonic bath for a minute.
- Add between 0.1 and 1 ml of microsphere solution for each $1cm^3$ of sediment started with using gradated pipette
- Centrifuge (3000 rpm, 5 min) and decant supernatant

10. Making slides

- Label slide

- Dilute the phytolith pellet in distilled water so that it is transparent and only slightly miky in aspect.
- Put a big drop of this solution on a cover slip. It should cover the whole cover slip. The phytolith are going to deposit evenly on the cover slip while the water is evaporating (1 day)
- Put 1 drop of Canada Balsam on the labelled slide and put on top of this drop the cover slip, with the side that had the sample solution drop touching the Canada Balsam.
- Heat (mark 4 on our hot plate) the slide so that the Canada balsam bubbles. When it stops bubbling (10 to 15 mins) it's ready. Leave to cool down.

11. Transfer to glass vials

- Label glass vials
- Transfer samples by pipette into small glass vials for storage
- Whirlymix tube's contents and add to vial until tube clean
- Repeat step as many times as possible – have checked what's left in bottom of tube and do loose some phytoliths, so take care
- Wrap vials in tissue to enable retrieval from centrifuge
- Centrifuge (3000 rpm, 5 min) and decant supernatant
- Add 100% ethanol for storage