The Basic Assumption of Pollen Analysis

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Pollen analysis is a technique for reconstructing former vegetation by means of the pollen grains and spores it produced. Fossil pollen grains form a continuous record in the sediments accumulating in lakes and peat bogs. These records provide one of the richest sources of information about the terrestrial environment of the past.

Pollen analysis is based upon three basic assumptions as follows:

(1) Pollen and spores has the variation in the size and general shape of the entire grain, and sculpture of their outer (the exine). Using light microscopy, the majority of pollen grains can be identified only to genus or family, but sometimes can be identified to species.

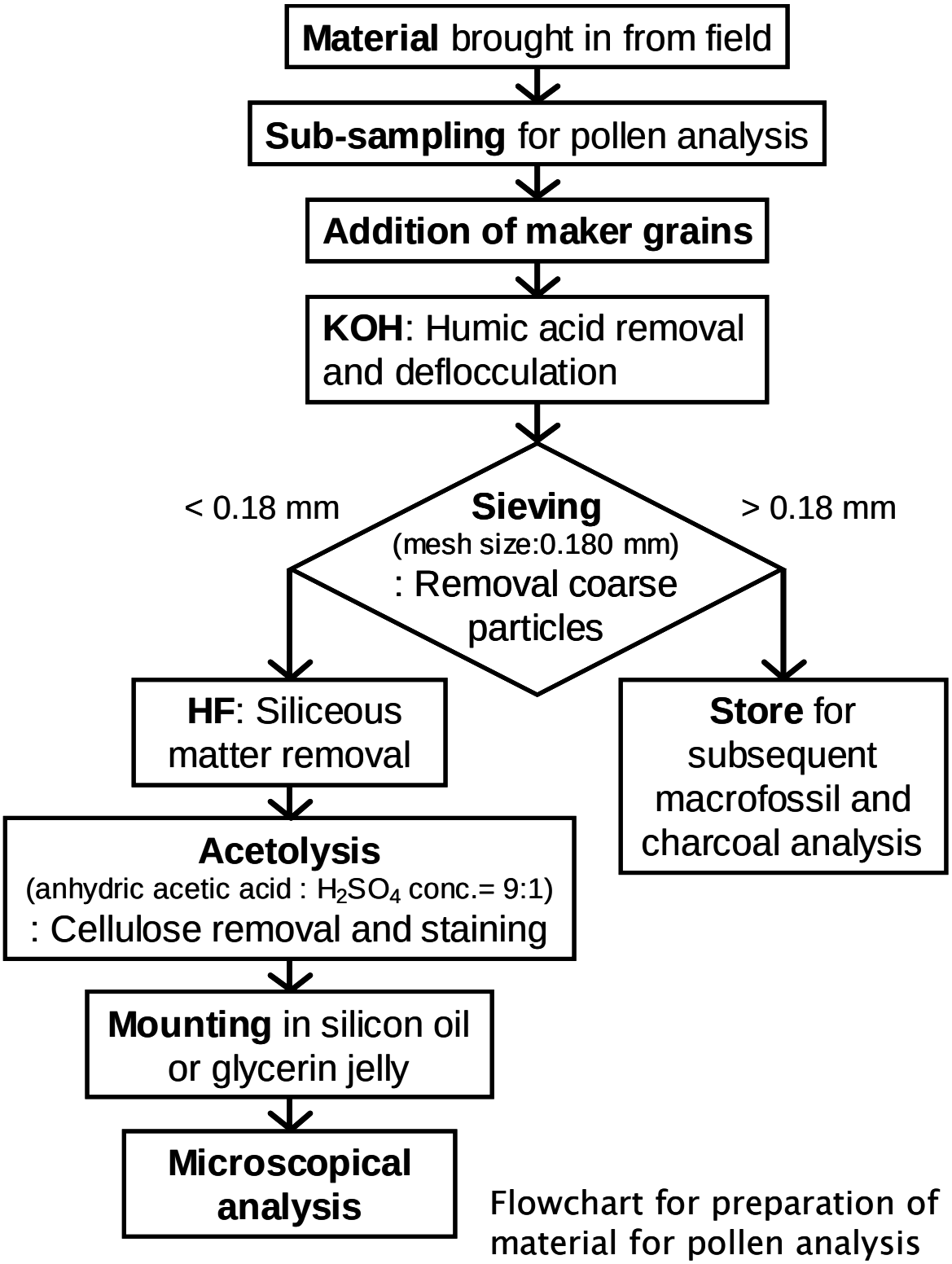
(2) The outer (the exine) of pollen grains and spores are preserved in sediments for a long time. The chemistry of the exine renders them resistant to decay and wherever microbial activity is depressed, whether due to wetness and low oxygen availability, there is a chance of pollen preserved well.

(3) Pollen grains and spores are produced in large numbers and widely and uniformly spread. Especially, wind-pollinated species produce extremely large numbers of pollen.

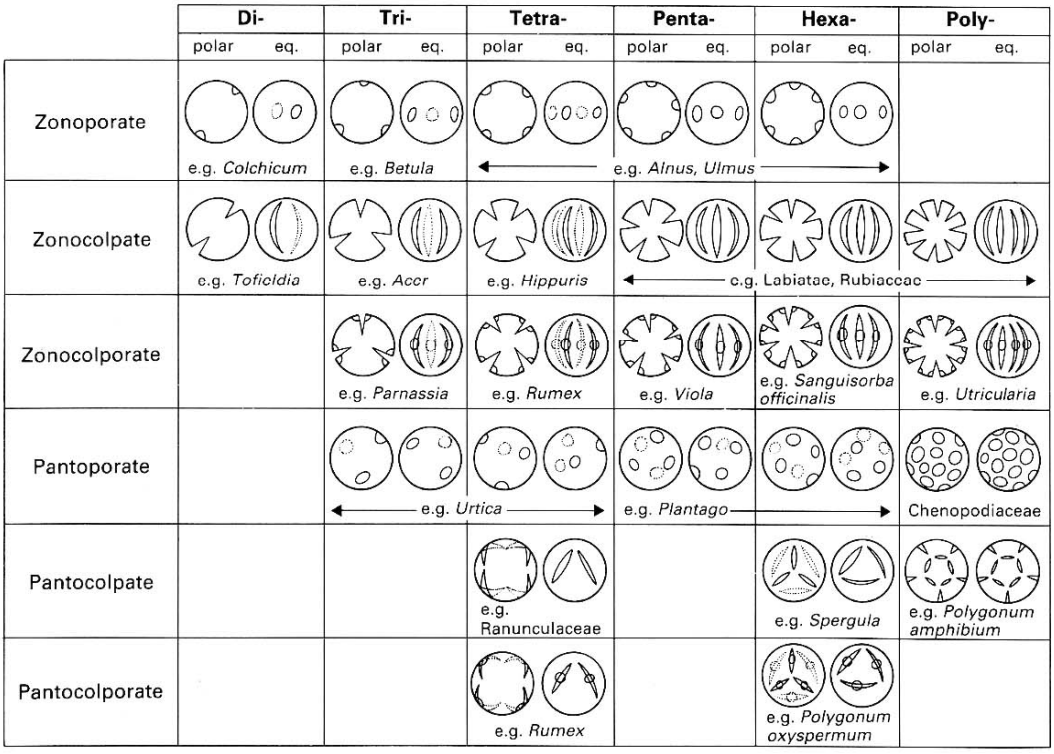
Further Readings

Faegri, K. and Iversen, J. 1989. Textbook of pollen analysis. 4th edition. John Wiley & Sons Ltd.

Moore, P. D., Webb, J. A. and Collinson, M. E. 1991. Pollen analysis. 2nd edition. Blackwell Scientific Publications.



Flowchart for preparation of material for pollen analysis



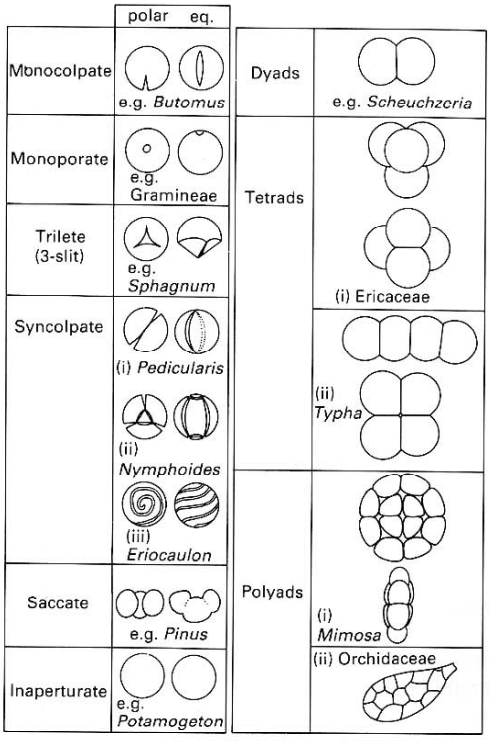


FIG. 5.4. Diagram showing the range of aperture number, position and character. Some of the possible combinations have no example within the British flora. Classification of pollen types based upon the number and arrangement of apertures. Examples are shown in polar and equatorial views. Dotted lines indicate a different focal plane. Empty positions denote the lack of a North West European example.

From Moore *et al.*, 1991

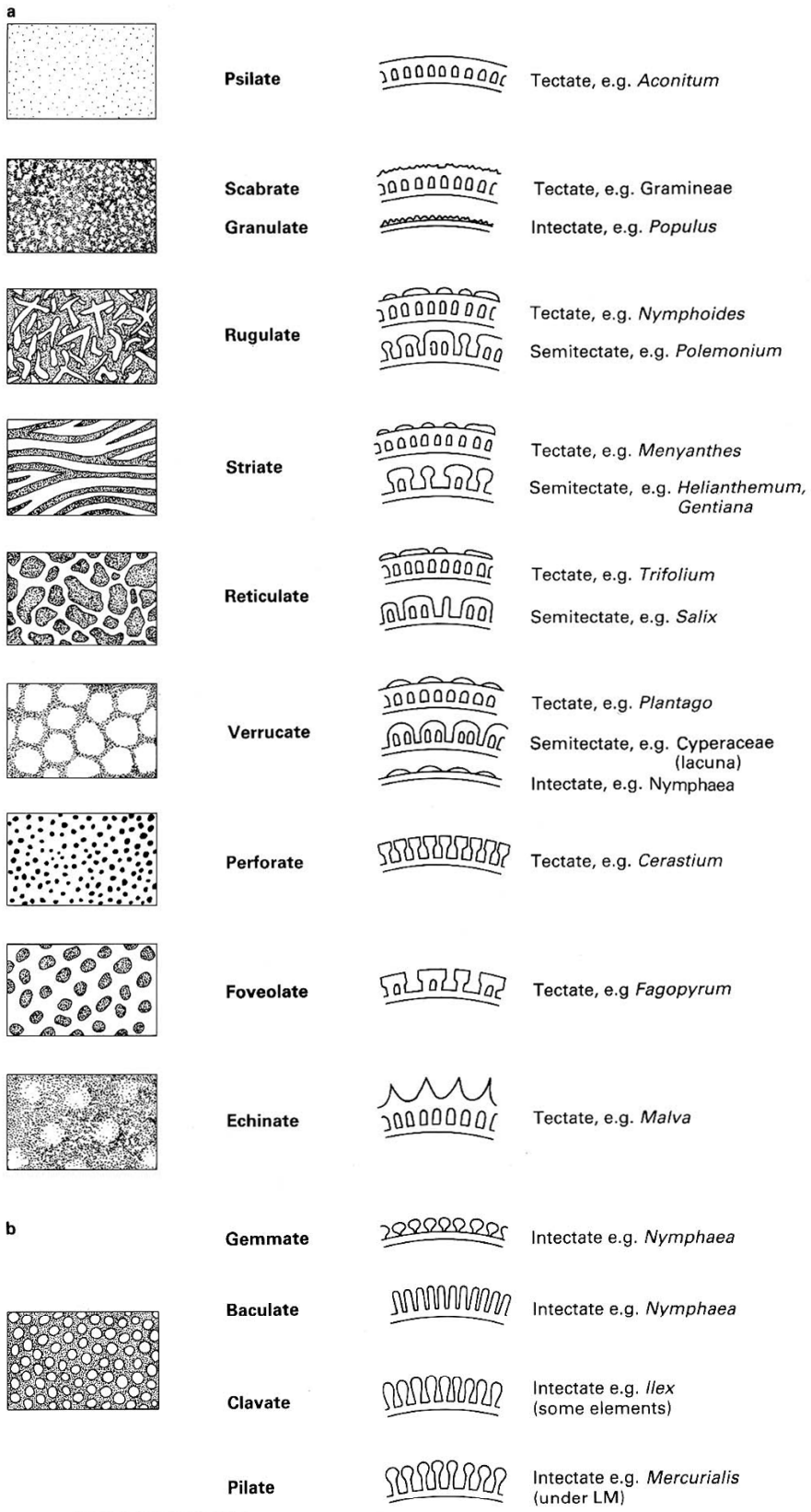


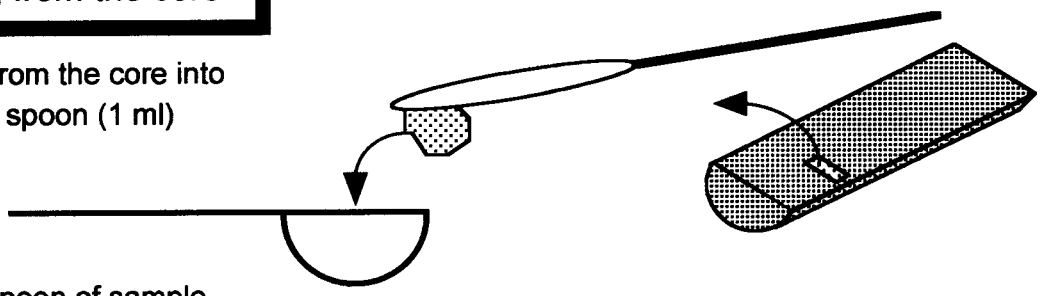
FIG. 5.8. (a) Diagrams of sculpturing types visible in surface view and optical section showing possible underlying exine types. In the sculpturing types all raised areas are shown light, all lower areas or holes are shown dark. It is possible that one sculpturing type, e.g. verrucate, may be produced by three different exine structures. Other sculpturing types, e.g. perforate, can be produced by only one exine structure. (b) Here the same surface pattern is produced by four different sculpturing types. Gemmate, baculate, clavate and pilate all refer to the shape of the projecting processes (see Table 5.1). Theoretically all these types of process could occur on top of the tectum of a tectate grain.

From Moore *et al.*, 1991

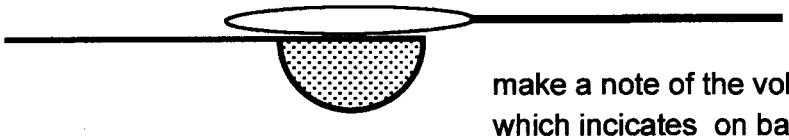
Technique for Pollen Analysis

1. Sampling from the core

Put sample from the core into the measure spoon (1 ml)



make a level spoon of sample.



make a note of the volume which incicates on back of spoon gripe

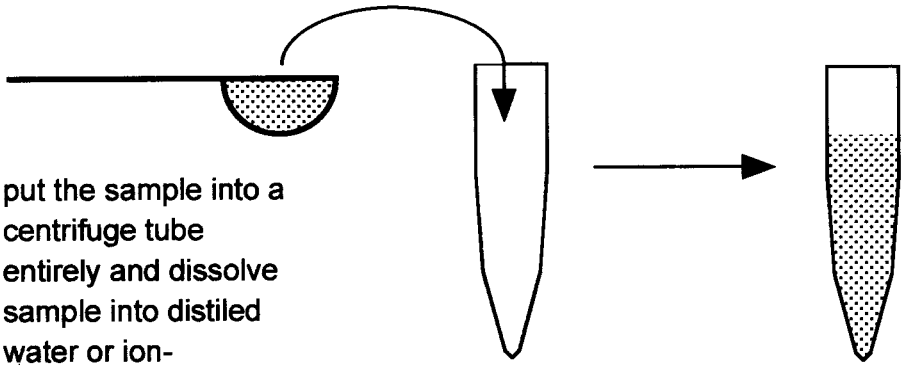
Make a note of each items for each samples on your lab note as the following example.
Also, it is important to write down the date of your work and the date of the Marker suspension prepared.

Number of the centrifuge tube

date: the date of Marker suspension prepared :

sample No	depth	volume	marker added	No	description of sample	note
KPU-20	20-22 cm	1.16 ml	1 ml	1	peaty with small plant remains	

In the column for the description of samle, you should note a detailed description for the sample as follows. plant remains, clay, gravel, charcoal and so on. After sieving, more detailed description will be done.

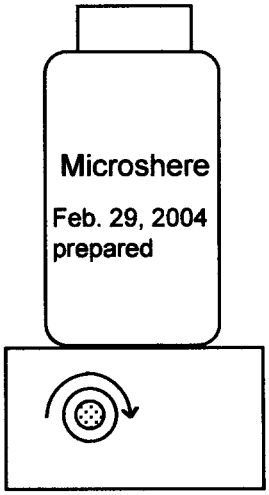


put the sample into a centrifuge tube entirely and dissolve sample into distiled water or ion-exchanged water.

2. admixture of the marker to measure the absolute pollen grain

After putting all samples into centrifuge tubes, add one ml of the marker suspension (Plastic grains:Microshere) into each centrifuge tubes.

For dispersion, stir the mixture on the magnetic stirrer for 30 minutes before using

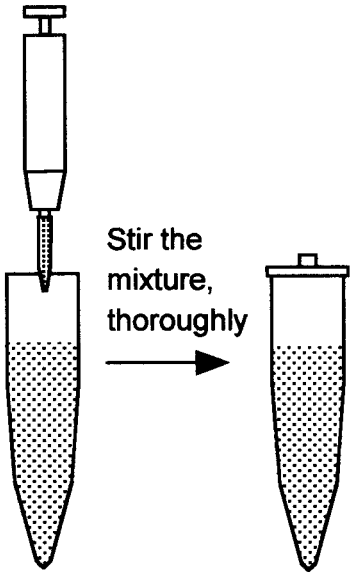


Marker suspension

Never shaking and accelerating the magnetic stirrer, not to bubble up!

Add 1.0 ml of the marker suspension into the centrifuge tube, precisely by using of FIN pipette for 1 ml. Be careful not to make double addition or no addition.

Pop the cap back on the centrifuge tube, after the addition, in order to avoid such a mistake.

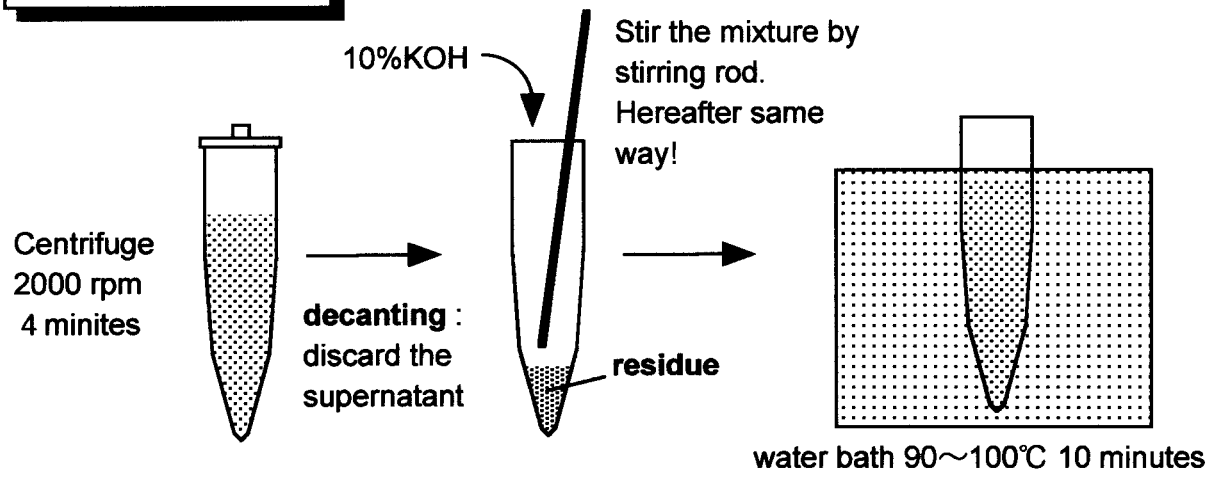


Stir the mixture, thoroughly

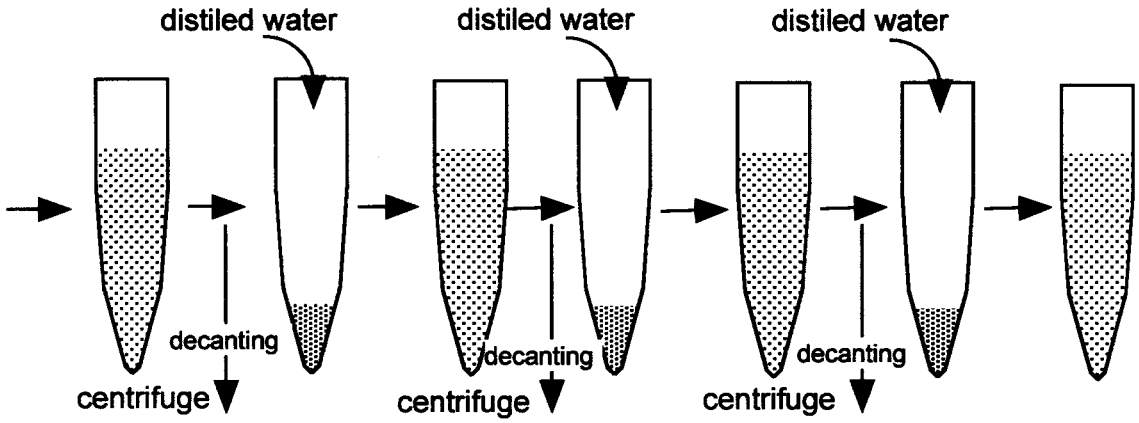
Warning: Even tapwater can contain pollen, so be sure you always use distilled water in the course of pretaration procedures.

3. Potassium hydrate (KOH) treatment

To decompose the humic acid and make deflocculation of sample, simultaneously.

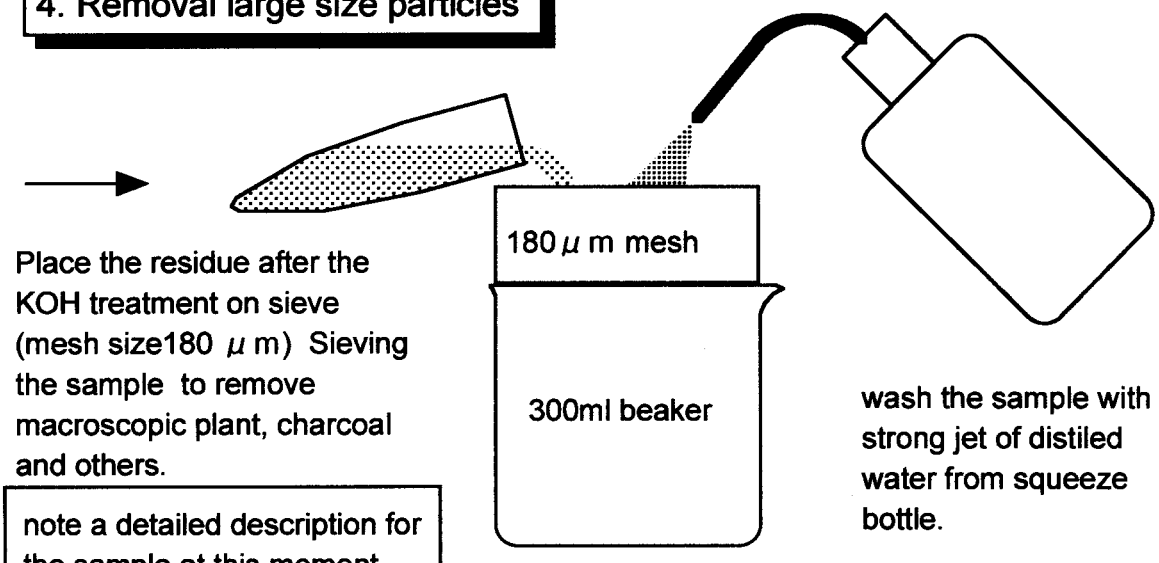


Warning ! : Do not use the KOH procedure without wearing eye glasses or safety grasses. KOH solution affects your eyes (cornea), severely.



Discard the waste fluid of KOH into the bottle for KOH waste. Aftrer neutralization, you can dispose it

4. Removal large size particles



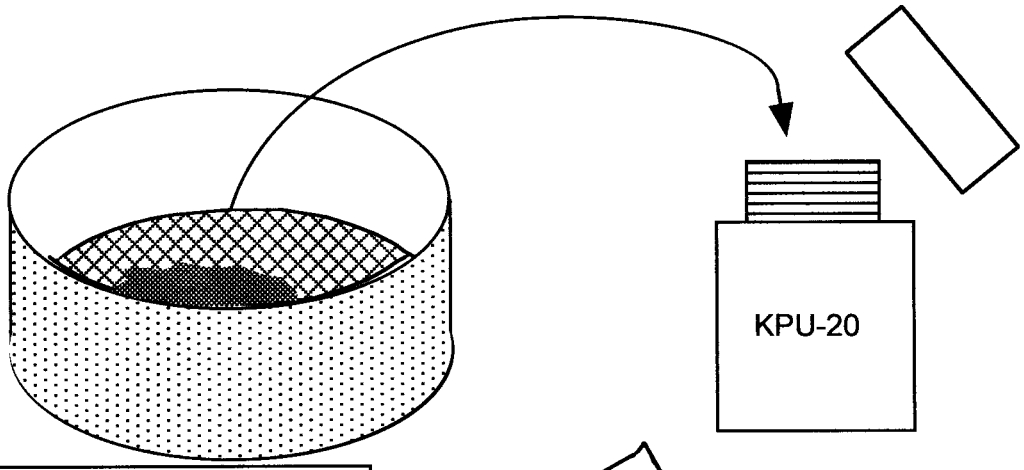
Place the residue after the KOH treatment on sieve (mesh size180 μ m) Sieving the sample to remove macroscopic plant, charcoal and others.

note a detailed description for the sample at this moment

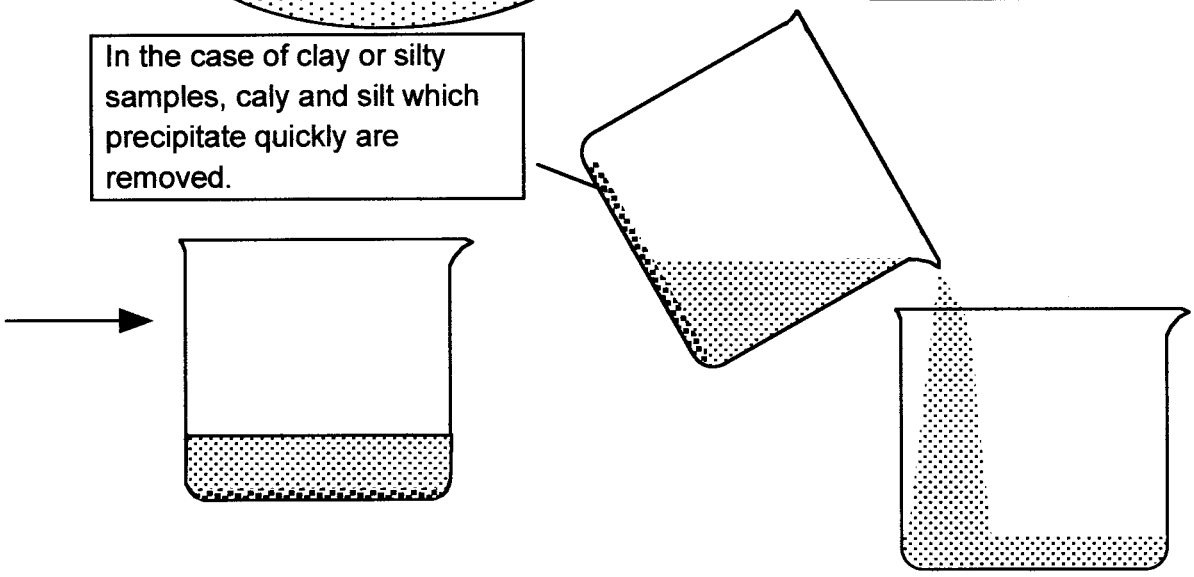
wash the sample with strong jet of distiled water from squeeze bottle.

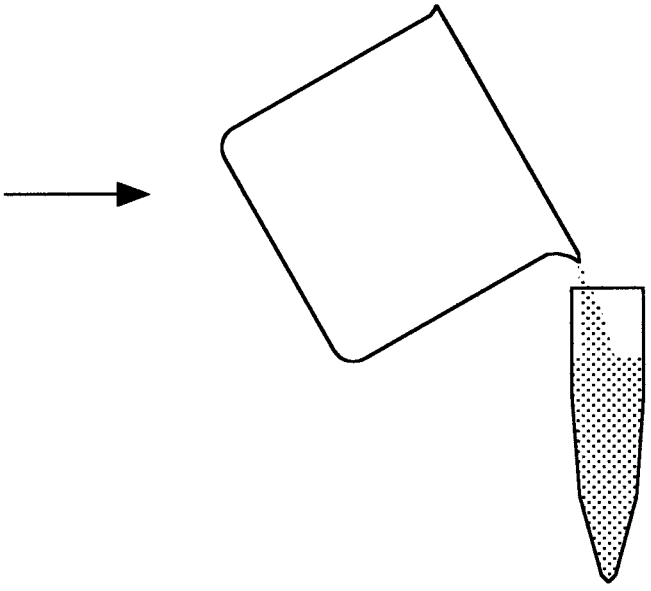
Put large things on the mesh into a labeled sample bottle and preserve in refrigerator.

If you find any seed or needle, put them into other sample bottle and preserve in refrigerator.



In the case of clay or silty samples, caly and silt which precipitate quickly are removed.





In the case of much more suspension, you can use several centrifuge tubes to recover the suspension.

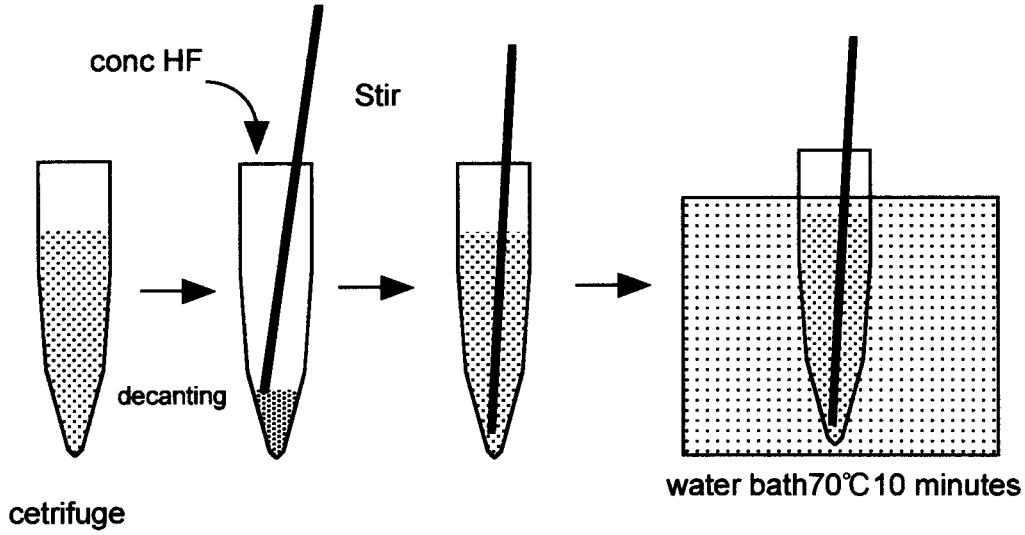
5. Hydrofluoric acid (HF) treatment

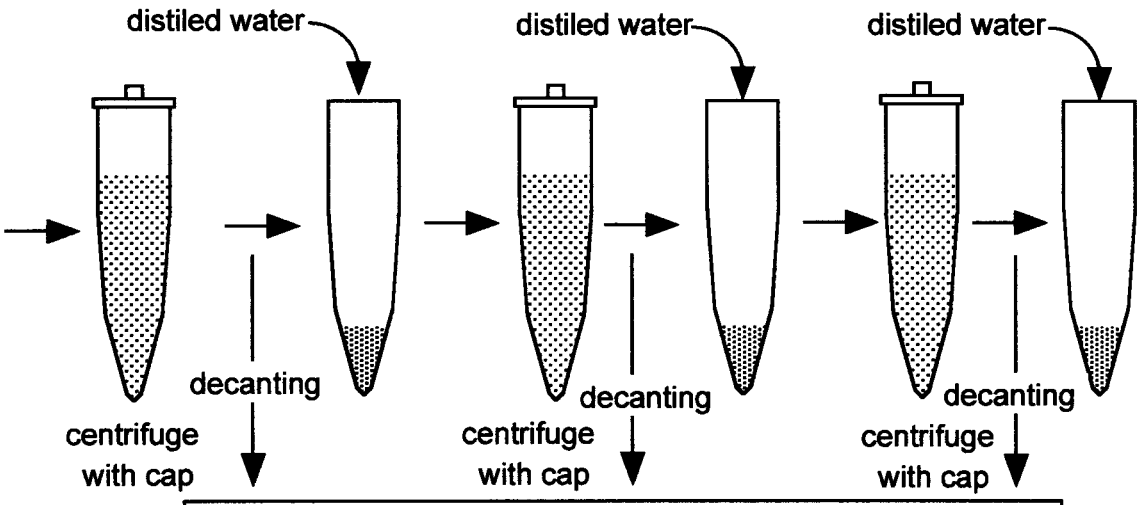
To remove siliceous material.

Warning ! !!
No one use the Hydrofluoric acid treatment without wearing safety glasses and gloves. Any contact of Hydrofluoric acid with the skin and nail should be avoided and the after-effects are persistent. Also, the vapour affect your lungs severely.Never inhale the vapour !!!

This treatment should be done in the draft chamber.

Gas of HF corrodes glass





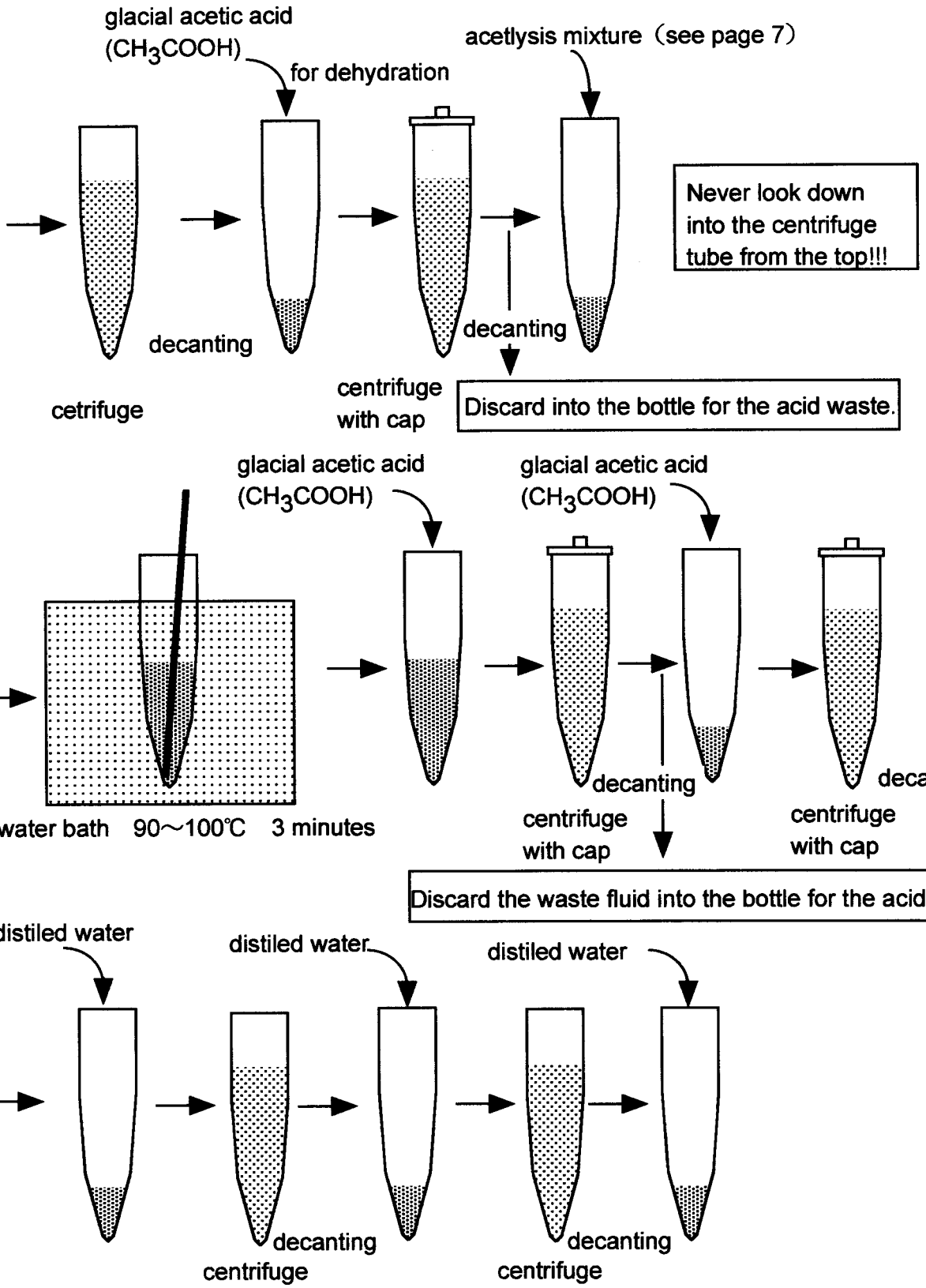
Discard the waste fluid of HF into the bottle for the HF waste.

6. Acetlysis treatment

To hydrolyze cellulose in plant remains.

Warnig : Keep away from water to avoide the explosive reaction of acetlysis mixture with water.

This treatment should be done in the draft chamber.



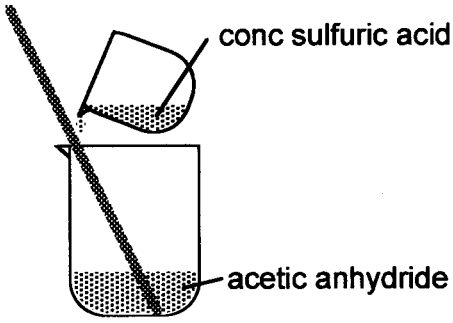
Preparation of acetolysis mixture

This treatment shold be done in the draft chamber.

Make a mixture of conc sulfuric acid (H_2SO_4) and acetic anhydride ($(CH_3CO)_2O$).
The proportion of H_2SO_4 to ($(CH_3CO)_2O$) in the volume is one to nine.

conc sulfuric acid (H_2SO_4) : acetic anhydride ($(CH_3CO)_2O$Åj = 1 : 9

stir by dropping of conc sulfric acid.



Take care about the heat!!!

Warnig!!
★Use glass wares as beakers and stirring rods without water.
★Drop by drop conc sulfuric acid to acetic anhydride by stirring. Take care about the heat
★Never looking down into the tube from the top!
★The mixture cause severe burns
★The mixture must be mixed up freshly each time you use.

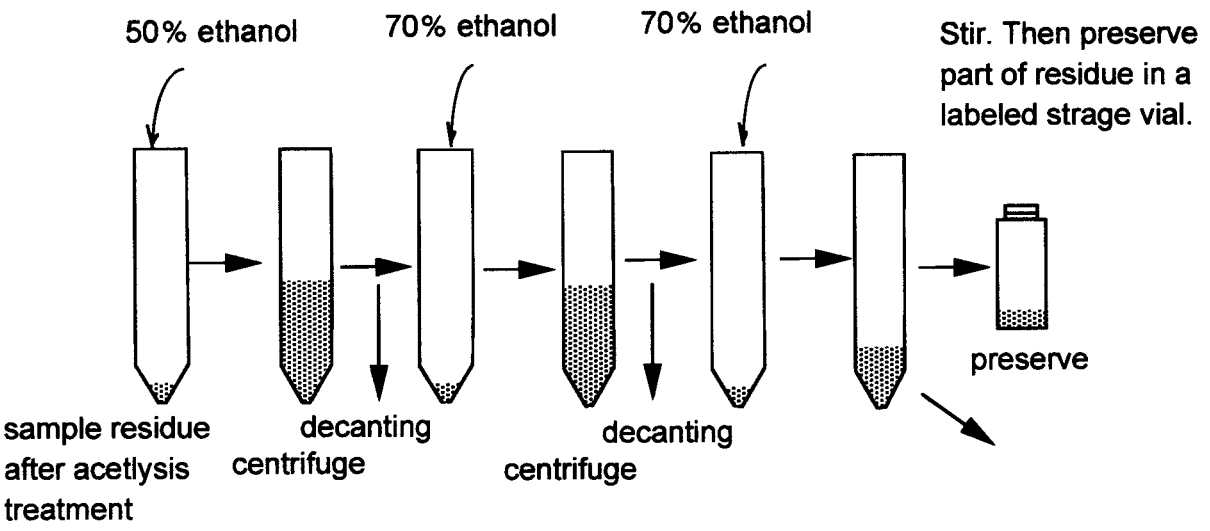
Preparation of glycerine jelly

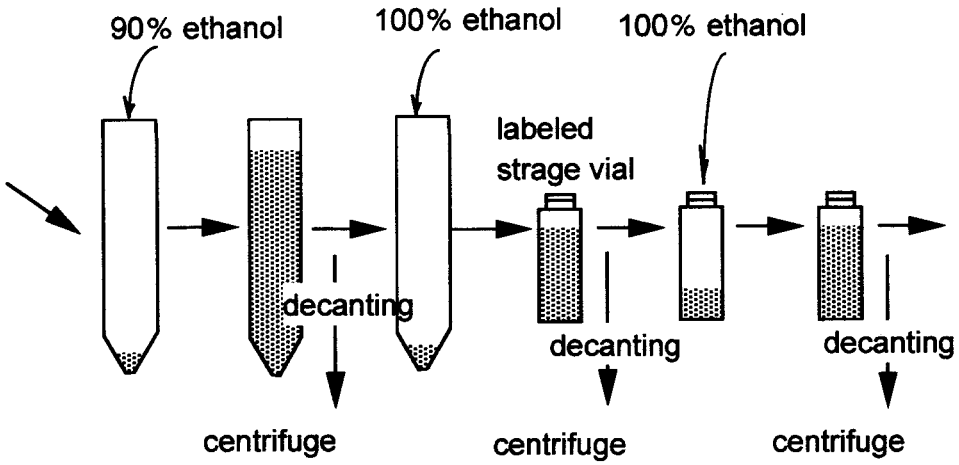
1. Swelling gelatine in beaker with distiled water half a day.
2. Add phenol into melted gelatin in water bath. Then stir gentlle to prevent form
   Any contact of phenol with the skin should be avoided. Phenol will cause burns.
3. If need, small clumps can be removed by sieving with gauze.
4. keep it in a bottle with cap in refrigerator

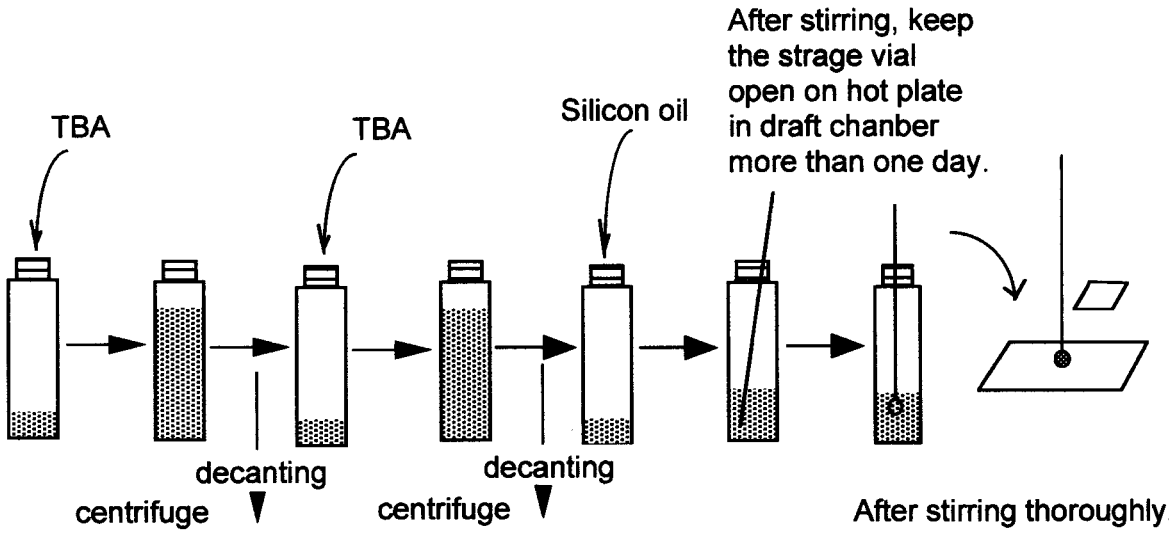
Warning : Never dispose the material with phenol.
If need, discard it in the waste bottel for phenol.

material :
distiled water 44g,
gelatine13g
phenol 0.5g, glycerin 38g

Mounting in silicon oil





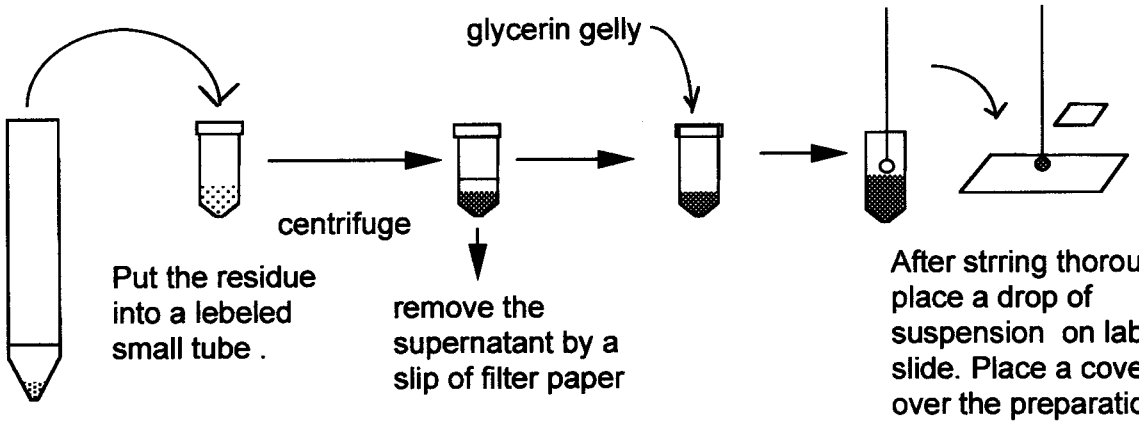


Discard the waste TBA into the bottle for TBA waste

After stirring thoroughly, place a drop of suspension on labeled slide. Place a coverglass over the preparation.

TBA : tertiary butyl alcohol

Mounting in glycerin gelly



Melt glycerin gelly in water bath before use.

warning : Never dispose glycerin gelly into sink, because it contains phenol.