LABORATORY MANUAL AND METHODS

University of Exeter
Department of Geography
Physical Laboratories

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C:\Recovered Data\manuals\labmanual.doc
## Laboratory Manual Table of Contents

*Soil Preparation and Particle Sizing*

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SOIL PREPARATION

The soil, on receipt in the laboratory, is logged and assigned a LABREF number. The sample is then air-dried. (See below)

If the original field volume of the soil is known, then the sample is now weighed, so that any results could be recalculated in terms of field volume.

1) Air Drying of Sample

Samples from the field must first be air-dried. This is usually done by putting the samples onto porous plates and leaving them on the drying racks in the Field Equipment Store until they feel dry. This can take considerable time and so alternative methods are;

   a) Putting the porous plates into an oven at 40°C
   or b) by freeze-drying the samples.

2) 2mm Sieving

When the samples have been air-dried the next stage is to separate out the stone fraction.

The samples are first ground using a mortar and pestle. A rubber pestle is preferable, as this tends not to break the primary particles. The stones can then be sieved out using a 2mm sieve. The grinding and sieving is repeated until only the stones are left (it may even be necessary to brush the fines fraction off the stones).

The weights of both the stone fraction and the fines fraction are then taken and recorded.

3) Stone Analysis

A nest of sieves greater than 2mm is constructed such that the largest sieve is just bigger than the largest stone. The stones are then added to this nest and this is then placed on the sieve shaker.

After shaking the fractions are all weighed and recorded. The best technique is to weigh the fraction in the collecting pan, and then invert each sieve in turn over this pan and gently brush the stones onto it.

4) Silt / Clay Analysis

Using a splitter a fraction of about 10g of the fines is placed in a 60 ml bottle and to this is added about 30ml of 0.4% Sodium Hexametaphosphate solution.

(6.67 g Sodium Hexametaphosphate + 1.3g Sodium Carbonate to 2l distilled water).

The suspension is then shaken and then dispersed using the Ultrasonic Disintegrator probe at a setting of 16 for 5 minutes. The dispersed sample is then screened through a 63 µm sieve collecting the liquid, which is then stored ready for analysis on the Mastersizer. On storage there could be a tendency to flocculate and so this time should be kept to a minimum.
5) Sands Analysis

About 40-50 gm of the fines are accurately weighed into a clean dry beaker of known weight and then dried overnight in an oven at 105°C.

The beaker + soil is then cooled in a dessicator before being reweighed to give the oven dry weight.

To this is now added about 50 ml of 0.4% Sodium Hexametaphosphate (see above) and the dispersion completed by the Ultrasonic Disintegrator (again see above). The dispersion is then screened through the 63 µm sieve allowing the liquid to run to waste and the sand fraction is washed with tap water until the liquid is clear. It is then washed with distilled water. It is essential to give the sands a thorough wash to remove the Sodium Hexametaphosphate.

The sands are then washed back into the beaker of known weight and dried in the oven overnight at 105°C.

The beaker + sand allowed to cool in a dessicator (for about 30 minutes) and then weighed to give a weight of total sands.

A nest of sieves is now constructed from 2mm down to 63µm and the sands can then be sieved, weighed and recorded.
Pre-treatment of Samples Prior to Particle Size Analysis

Reagents

- 100vol hydrogen peroxide
- 0.4% sodium hexametaphosphate
  - 6.7 gm sodium hexametaphosphate
  - 1.3 gm sodium carbonate
  - to 2 litre with distilled water

Method

This method is a general one where removal of organic material is required prior to particle size analysis by instruments such as the Saturn Digisizer (screening at 1000µm) or Mastersizer (screening at 63µm).

1. The soil is first air-dried and then ground and sieved through a 2 mm sieve, weighing both fractions.

2. 2 - 10 gm (depending on estimated sand content) of the < 2 mm fraction is then accurately weighed into a clean dry beaker. The soil is then dried overnight at 90°C and then re-weighed to give the oven-dry weight.

3. 10 ml of water is then added to each sample + 5 ml of the hydrogen peroxide. It is often necessary to wait and observe the reaction and to control the effervescence by addition of a few drops of IMS.

4. After 2-3 hours, if the frothing has ceased a further 5 ml of hydrogen peroxide is added and the sample allowed to stand overnight. (More peroxide is added if a higher organic content is suspected)

5. The beakers are now warmed on a hotplate (starting at 80°C and gradually increasing the temperature to 100°C) until the reaction is complete and there is a clear supernatant above the sample.

6. The whole of the beaker contents are now carefully transferred to a weighed centrifuge tube, using a policeman on a rod to clean the sides of the beaker. The tubes are centrifuged at 2500 rpm for 1 hour.

7. The centrifuge tube is then dried overnight at 90°C before being reweighed.

8. Approximately 30 ml of the 0.4% sodium hexametaphosphate solution are added to each tube and initially disaggregated with a spatula.

9. The soil suspension is then screened through a sieve (63µm if using the Mastersizer, 1000µm if using the Saturn digisizer) collecting the screened fraction in a 125 ml bottle. The centrifuge tube is washed with a small amount of the sodium hexametaphosphate and this too is passed through the sieve.

10. The residue (>63 µm or >1000µm) is washed with water, and the centrifuge tube is now completely washed with distilled water, passing the washings through the sieve. The residue on that sieve is now completely washed until no more fines are washed through and all the sodium hexametaphosphate has been washed away. The residue is now washed into a weighed dry 60 ml bottle with distilled water and dried overnight. This is then reweighed (and stored for sand fractionation if >63µm).
11. The screened suspension is then placed in an ultrasonic bath where it is treated for 5 minutes before being run on the MasterSizer (<63µm) or on Digisizer (<1000µm).

12. (If the sand fractionation is done, this uses a series of 100 mm sieves, usually 1000 µm, 500 µm, 250 µm, 125 µm, and 63 µm. Each fraction is accurately weighed on a 4-place analytical balance.)

13. **Calculations - Digisizer:** An excel template can be used to calculate the size distribution (1000µm Digisizer Template.xls found in Arts Directory on the Digisizer computer).

14. **Calculations – Malvern Mastersizer:** If Robin’s ‘Cosed’ program is to be used then the files from the Mastersizer are converted to ASCII format (using the Convert utility). The data is then taken on disc to a computer with ethernet capability to be transferred to the mainframe computer (using FTP or EWAN - use Wordpad to select the data onto the Clipboard and then use shift + Insert when in Vi), the soil weights and sand weights etc. need also to be entered into a separate file, before being merged with Art’s mc program (See the Aid file on Art's parsiz directory for instructions on how to enter the data and run the programs).
Pre-treatment of Samples (less than 1000 µm) prior to Particle Size Analysis

Reagents

100vol hydrogen peroxide
0.4% sodium hexametaphosphate

6.7 gm sodium hexametaphosphate
1.3 gm sodium carbonate

to 2 litre with distilled water

Method

This method is a simplified method of the previous method above where removal of organic material is required prior to particle size analysis by instruments such as the Saturn Digisizer (screening at 1000µm) and the samples are known to be less than 1000µm

1. The soil is first air-dried and then ground.

2. 1-2 gm (depending on estimated sand content) is then put into a clean dry beaker. About 10 ml of water is then added to each sample + 5 ml of the hydrogen peroxide. It is often necessary to wait and observe the reaction and to control the effervescence by addition of a few drops of IMS.

3. After 2-3 hours, if the frothing has ceased a further 5 ml of hydrogen peroxide is added and the sample allowed to stand overnight. (More peroxide is added if a higher organic content is suspected)

4. The beakers are now warmed on a hotplate (starting at 80 °C and gradually increasing the temperature to 100 °C) until the reaction is complete and there is a clear supernatant above the sample.

5. The whole of the beaker contents are now carefully transferred to a centrifuge tube, using a policeman on a rod to clean the sides of the beaker. The tubes are centrifuged at 2500 rpm for 1 hour.

6. Approximately 30 ml of the 0.4% sodium hexametaphosphate solution are added to each tube and initially disaggregated with a spatula.

7. The suspension is then run on the Digisizer (this runs samples in the range 0.1 - 1000µm)

8. Calculations - Digisizer: An excel template can be used to calculate the size distribution (less than 1000µm Template.xls found in Arts Directory on the Digisizer computer)
Mastersizer Running Instructions

Start-up

Please contact the technician if you are unsure of any stage of this procedure or if any part is out of specification. **DO NOT attempt to change lenses or clean cells without prior consultation with the technician, as this could result in costly damage.**

Switch on at the mains

a) The Imspin magnetic stirrer
b) The Ultrasonic bath (if using Ultrasound)
c) The Mastersizer MS20
d) The Olivetti computer
e) The 3 place magnetic stirrer (if more than 1 sample)
f) The Malvern printer (if to be used)

Place bottle containing sample with magnetic follower in the ultrasonic bath and turn on the stirrer (and Ultrasound if needed). Place other samples with followers on the 3place stirrer to disperse whilst awaiting analysis.

Press **Clean Fill** on the Mastersizer MS20 Sample Presentation Unit. The 1 litre bath will now fill with clean water. When this is full press **Clean Fill** again to give the system a good flush.

The computer meanwhile will have gone through its checks and the main Menu will appear. Press F1 to run the Mastersizer software. Several files will then be loaded and then a graphic of the system will appear. Press space bar at this point.

If the clean fill process is operational at this time the software may force an error message. Pressing return will clear the message and the prompt **Commands:-** will show. Sometimes the Sample Presentation Unit will have flipped from manual to computer mode. Typing in **man** at the **Commands:-** prompt will revert it back to manual. If an error has been forced then the usual software can be restarted by typing in **run 16** at the **Commands:-** prompt.

The computer will go through some text help screens. The first screen is an introduction. The second screen reminds you (if you have not already done so) to give the system a good flush by pressing **Clean Fill** on the MS20. The data and result files should then be set up by the commands **dat filename** and **res filename**.

As the software overwrites files it is a good idea to check that your chosen filename has not been used before or else you could wipe out valuable data. The commands are **rec res** and **rec dat** and should show all records in the files to be empty.

When the files have been set-up it is also a good idea to check that the system is set up for what you want by entering **dis par**. If all is OK then another worthwhile check at this stage is **m b** which measures the background. The graphics of the laser intensity should be noted (which should be in the Good zone and preferably just below the Good letters) and the values for background should be less than 25 and preferably less than 20.

If you are happy with all the settings and states then press F3 (return) to give the next text help screen. This will give you an option of using the loop sequence (F5 return) or single samples (F6 return).
Running in the loop

This is a program set up so that the samples can be run one at a time with the results and data being automatically saved in the respective files. **Note that the series always starts at one so never restart a loop, as it will overwrite previous records.**

- The first operation is an automatic alignment. Do not touch the computer.

- Next is the editor (sam edi). Editing is done by using the cursor keys to move the cursor and overwrite (insert mode can be obtained by INSERT then key).
  The first line is usually the Labref number and owner (the correct format for Robin’s program must be as this example i.e. 1001/AA) and is what will identify the record in the file when a record is performed - see below.
  The next line usually is the Site, description etc. with the third line also description or sample no as you wish. (Note Robins program requires something there so if you wish the line to be ignored type in ***)
  Below the line are the MS15 parameters and it is quite useful for future reference to enter these.
  When the sample details have been satisfactorily entered press END on the keyboard to leave the editor.

- A text help screen now reminds you to set up the pump speeds stirrer Ultrasonics etc. - press any key when these are set to carry on.
  The computer now does a m b (measure background) Do not touch the keyboard until the values are displayed for the 32 rings. Check these values and if any are greater than 25 inform the technician who will then clean the cell. If all is OK press any key to continue

- Ins li (Inspect live) is next and it is at this point that you add the dispersed sample (a syringe is a useful means) to the tank. Add sample until the obscuration (bottom left on screen) is in the range .150 - .200. If the obscuration is appreciatively higher than this hold down the dispersant button on the Sample Presentation Unit.
  When a stable reading is obtained in the correct range press the space bar. The computer now measures the sample and when this is finished a text help screen reminds you to now clean the cell by pressing CLEAN FILL on the Sample Presentation Unit.

- Whilst this is being done (which takes a fair time) the computer calculates the results from the ring data, displays the results and then saves them to the files before displaying the current state of the file. Pressing SPACE BAR at this point then brings in a graphic of the distribution of frequency and oversize. Pressing any key after this returns you to the editor and the sequence restarts from there (i.e. everything other than the original alignment).

- To break out of the loop press F1 which aborts the programme after it has finished the operation it is currently doing. It is preferable to do this when the graphic screen is being or has been plotted.
Individual Samples

If you have pressed F6 this will run samples one at a time with the following sequence (see running in a loop for more details of individual stages)

a) **a a**: The automatic alignment (This can be avoided by entering run 23 rather than F6)
b) **sam edi**: The sample Editor
c) **m b**: Measures background
d) **ins liv**: Add sample until obscuration is in range 0.150 - 0.200
e) **m s**: Measures sample
f) **c**: Calculates results (Press CLEAN FILL at this point)
g) **dis res**: Displays results

At this point the data and results can be saved by entering

\[
\text{sa dat},n \quad \text{where } n \text{ is the record number} \\
\text{sa res},n
\]

However take care not to give the same number for different samples as the original will be overwritten.

Further samples can be processed by f6 (with a a ) or by entering run 23 (rtn)

Shutdown

Before exiting from the Mastersizer software, it is desirable to change the active data files (to avoid accidental overwriting of data) and this is achieved by dat rub and res rub. The Mastersizer software can then be exited by typing end. This then saves all the parameters in a configuration file and returns you to the main menu. You can then use CONVERT to convert files to ASCII format saving the result file in C:RES and then transferring this to a floppy in the A drive. This disc can then be taken to a machine which is connected with an ethernet card in order that the transfer can take place.

If you want to finish completely press ESC to return to MS-DOS and then enter DPARK (return). This will park the hard disk and so minimise any danger of damage or possible loss of data.
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Malvern Mastersizer (Care and Maintenance)

These operations should only be carried out by one of the Technical staff

Cleaning of Cell Windows

Care must be taken not to scratch the windows

1) The Cell is removed from the Cell Holder.

2) Each Window in turn is removed from the cell block using the special tool.

3) The internal side of the window (i.e. next to suspension) is washed gently under the tap and then carefully dried using Lens Cleaning tissues.

4) The external side (next to air) is cleaned by gently blowing on it and wiping dry with a lens tissue.

5) The cleanliness can be checked by holding the window up to the light

6) The cell block should also be wiped clean with a tissue before replacing the windows and securing them with the special tool

7) Check the background with the command m b

Changing of Lenses

Care must be taken not to damage the detectors by attempting to change the lenses whilst the focus is in the 45mm position and to ensure no damage should be performed in the 100 mm position

1) Ensure that the detectors are well away from the cell holder/lens by typing dis par whilst in Mastersizer mode. The focus should be 100 or 300. If the focus is 45 then enter foc 100 or foc 300 as necessary.

2) Release the cell holder using lever and remove from the optical bench

3) Changing from 45 mm lens:
   a) Unscrew the 45 mm lens from the front of the cell holder
   b) Screw the correct lens onto the optical bridge behind the cell holder

   Changing from 100 or 300 mm lens:
   a) Unscrew the lens from behind the cell holder
   b) Replace with correct lens (NB the 45mm lens screws onto the front of the cell holder)

4) Replace the cell holder and secure with lever

5) In Master Mode select ali aut to realign the laser

6) Check the background with m b
**Operating Instructions for Coulter LS130**

*Check that the correct module is installed. If in any doubt seek advise from the technical staff.*

1. Switch on computer and screen.
2. Wait until Program Manager page of Windows has appeared.
3. Double click on the LS Control icon.
4. Press OK on the next screen. The Title Bar should now read LS130.
5. Click on *Control* and select *Pump on*. Listen to the Variable speed fluid module (VFM) to make sure pump is running.
6. Click on *Run* and select *Cycle*. The secondary title bar should now read Fluid module.
7. Click on *New sample*. The various operations will now be highlighted by a X in the relevant box.
8. Check that the PIDS box and also the Autorinse box both have a X in them.
9. Set pump speed to 30-35 for most samples (Less than this may cause large particles to settle - too high a setting may introduce air bubbles).
10. Click on *Start*.
11. The software will now go through the following cycle;
   
   a) Measurement of Offsets: Electronic zeroing of detectors with laser off (This is omitted if performed in previous two hours).
   b) Alignment of laser: (Again this is omitted if it has been done in previous 2 hours).
   c) Background: This measures background of cell and is usually a maximum of about $800 \times 10^3$ but a value up to $1500 \times 10^3$ is acceptable.
   d) Loading: This is the point when sample is added to the fluid module tank. The Obscuration and PIDS are measured and shown on the Title Bar and is OK in the range 45-55%. Select *Done* when this is OK and stable.
   e) Sample Info: Warning! Pressing Enter at any time leaves this page. Therefore use the mouse or tab to go between fields and use the OK box when finished. Enter a Labref number and initials into the group ID if you want to use RAC's program. This also becomes the filename. Enter relevant data into the other fields. Click on *OK* when finished.
   f) Run Info: This sets parameters for the measurement and report. Ensure that the Compute and Save boxes are marked with a X and that the Fraunhofer model is selected. Click on OK when finished.
   g) The sample is now measured and the size distribution calculated. The message *Using model Fraunhofer Cancel?* will appear during the calculation. Be patient and do not cancel but wait for the Auto rinse.
   h) Auto rinse: This rinses the fluid module tank. During this phase the results can be exported to the A drive (see below).
12. The next sample can then be run by closing the secondary window and then clicking on *Run* and selecting *Start Cycle* and proceeding from 7 above.
**Closing down of machine after run**

1. Ensure that the cell has been well rinsed and is left clean.
2. Click on *Control* and select *Pump Off*.
3. Listen to the fluid module to ensure the pump is off.
4. Close the LS program window.
5. Switch off the computer.
6. Do not switch off the Optical bench.

**Exporting of result files**

1. Click on *Runfile* and select *Export*.
2. Ensure that the boxes *Sample Info* and *Listing* are selected with a X.
3. Select *Comma delimited* (filled circle).
4. Ensure that the directory is A drive by:
   a) Clicking on *Directory*
   b) Selecting A drive
   c) Clicking on *Change*
   d) Clicking on *OK*.
5. Enter filename (The same as entered in Group ID above).
6. Enter the extension (lis works OK).
7. When all is prepared click on *Export*. 
Installation of Micro Volume Module in Coulter LS130

This operation should only be carried out by the Technicians!

DANGER The Optical Module must be off during the changeover.

1. Switch on the computer and wait for the Program Manager Page Of Windows.
2. Double click on the LS Control icon.
3. Press OK on the LS page. The Title bar should now show LS 130.
4. Click on Run and select Shutdown Optical Module. The Title Bar should now show Coulter LS.
5. Switch the power off on the Bench extinguishing the green light.
6. Remove the following connectors;
   a) The plastic 25 way D computer plug
   b) The metal high voltage power plug (unscrew)
   c) The black lead with the BNC plug
7. Open the door of the optical bench.
8. Release the 4 latches at the back of the cell assembly.
9. Withdraw the whole cell assembly.
10. Insert the micro-volume-cell assembly using the locating pin on the back plate of the bench. Secure by pushing the 4 latches down (NB the two bottom latches secure at a slight angle unlike the two top latches which are flush).
11. Connect the metal power cable and screw home.
12. Fill the cell with diluent (water?) and locate the cell onto the assembly.
13. Switch on power on bench and check that stirrer is functioning and that there are no air bubbles in cell.
14. Close door and secure firmly.
15. Click on Run and then select Use Optical Module. The Title Bar should now show LS130 again.
16. Click on Run and select Start Cycle.
17. The secondary title bar should now show Micro Volume Module.
18. The cell is now ready for analyses.
Saturn Digisizer

Modus Operandi
The Saturn digisizer is used for particle sizing up to 1000µm. Each sample has its own file and the autosampler can be used to batch up to 18 samples at a time (even more can be added whilst the autosampler is in operation). An ‘empty’ file, containing just parameters, is created for each sample, and then once the sample has been run the data is automatically added and saved. Customer designed reports can then extract results which can then be cut and pasted into other packages. Files (new ones) can be created and samples added to the autosampler schedule even when the machine is running.

Connecting
1) Switch on at mains the digisizer 5200, Sonics, Mastertech, Aquaprep before switching on the computer
2) Password on the computer is null (just hit return)
3) Double click on Saturn digisizer icon and then wait as the software downloads data from the Digisizer – this takes about 1 minute

Ensure that the deionised water tubs are full (deair if necessary beforehand) and waste tub is empty

Rinsing and Background
1) Click on Unit 1 – Rinse – Digisizer – suggest do 2 rinses
2) Click on unit1 – Background – next – next
3) Background takes about 8 minutes (may be longer if starting from cold). When background is finished, click on next and then look at trace. It should reach zero on the x-axis before the end of the run and also there should be a double drop around about the 6 level

Setting up empty files
1) These must be set up before running the autosampler – however new files can also be set up as the autosampler is processing other samples
2) Click on File – Open – Sample Information (or press F2)
3) The file will just be a number.SMP which is automatically incremented. Press OK. Press Save
4) Now click on Replace All – this enables parameters to be set from a previous file to be carried into this file. Pick up a suitable data file from before (usually the last one created) – click on OK – change sample details (and anything else as necessary) and then click on save and then close.

Setting up a schedule for the autosampler
1) Click on Unit1 – MasterTech schedule
2) Click on insert
3) Check (or change) beaker number
4) Click on Browse and pick up the relevant empty sample file
5) Click on OK (Header – Sample Information File)
6) Click on OK (Header – Mastertech Schedule Insert)
7) Samples in the schedule can be edited or deleted by first highlighting them and then pressing the Edit or Delete button
8) Repeat for all the samples in the autosampler and then click on Start
9) Further samples can be added so long as their Sample Information Files have been created
Reports
1) Click on Reports – start report
2) Pick up relevant file
3) Click on OK
4) Choose from lists of ‘Reports to generate’ by double clicking option
5) Click on OK
6) A good idea is to check ‘Goodness of Fit’ – the blue trace and the red line should coincide and the fit should be as low a number as possible – preferably less than 5. If curve turns down at top, then repeat but add more sample!
7) Various parts can then be extracted to the clipboard by right clicking by that data

Exporting data to Excel
1. Click on Reports - start report - pick up relevant file – OK - OK
2. Click on Frequency Graph page of the report
3. Right click on graph. If more than 1 graph than the best graph can be chosen by ‘hiding’ the other graphs. Whilst on right click choose ‘Copy as Text’. This puts the results onto the clipboard and they can then be easily pasted into an Excel spreadsheet. The actual graphic can be exported similarly by using ‘Copy as metafile’

Stopping an autosampler run
1. When the MasterTech is ‘Waiting to redisperse beaker’, click on Suspend. (then Yes to confirm)
2. Wait until the Digisizer has completed the sample and is now showing Idle
3. Click on Stop and then Cancel. Click in box to confirm ‘Cancel the MasterTech operation’
4. Click on Close – (the MasterTech schedule is now frozen but will be available again even after closing the computer down by clicking on MasterTech schedule)

Finishing Off
1) Click on Unit1 – Rinse – MasterTech then Digisizer.
2) Place an empty beaker on the autosampler and choose that beaker position to rinse to
3) When rinse is completed, add a couple of ml of Sodium Hexametaphosphate to tank. Allow this to circulate a few minutes.
4) Close down software and computer
5) Switch everything off at the mains
6) Empty the waste tub!

Overlay
1) Click on File – Open – Sample Information
2) Pick up first file of sequence
3) Click on Report Options - From the box titled Reports to Generate, highlight the type of graph that is to be overlayed (e.g. cumulative) – click on Edit (just to the right of box)
4) Go to Overlay (right hand side of next window) and choose Samples from the drop down menu - Click on OK (Repeat steps 4 and 5 for any other suitable graphs)
5) You now are returned to Report Options - Click on Overlays (bottom left of screen)
6) Pick up files you wish to overlay by clicking on the Browse button for each overlay
7) Click on OK and then Save and Close. The overlays are now added to that first file.
8) Click on Reports and then Start Report. Pick up the file to which the overlays have just been added. The graphs can be exported by first right clicking in the graph and saving as a metafile – this just puts the graphics on a clipboard. Open another package such as Word and paste the metafile directly in (Ctrl + V)
**Problems and Maintenance**

**Cleaning of Cell**

The manual is very good on this, (Section 9, page4) but as there is risk of contamination getting onto the rear lens, this should be performed by a Technician. The technique is as follows

1. Select Unit - Drain on the computer. This drains the cell and moves the rotation arm clear
2. Open the front cover (‘ring pull’) and undo the three thumb screws to the sample cell cover – remove that cover
3. To avoid any fingermarks on the cell, disposable gloves are to be worn. Loosen the two knurled nuts on the top of the cell assembly and push up the top so that the cell can be removed by slight rotation. Avoid touching the cell windows. Note the orientation of the cell (one side is angled)
4. Put a drop of the lens cleaner (Tech Spec) on the Flexitip swab and clean the inside of the cell (holding cell at edge)
5. Rinse inside well with deionised water.
6. Check outside of cell window and clean with lens tissue if required.
7. Replace cell in assembly in correct position and rotate to fit. Clamp with the knurled nuts (tight but not too tight).
8. Click on Unit - Rinse to fill the cell and check that it is not leaking. (Especially important that nothing gets on the lens behind the cell)
9. Replace cover and tighten the three thumbscrews
10. Shuttle main cover
11. Recheck background

**AutoSampler**

**Table Disturbed**

There is a black plastic arm under the beaker in the front active position that moves up and down as the autosampler goes around. This can stick in the down position, which the autosampler sees as a problem and flags Table Disturbed on the Instrument Schematic. This can even stop the run. This can be easily remedied by just tapping this arm back to the up position e.g. with a spatula.

**Initialise Mastertech**

The autosampler can forget where it was either by the Table Disturbed fault above, or if you remove the sample cup ring and cannot remember which position it was originally in. This is overcome by placing the ring with position 18 as the active position at the front and then clicking on Unit - Initialize MasterTech.

The initialisation can be checked by doing a MasterTech Rinse (Unit - Rinse - MasterTech) and making sure that it goes to the beaker that you have chosen.
MICROMERITICS GEMINI SURFACE AREA APPARATUS

Note: Care must be taken with the handling of liquid nitrogen. Gloves and eye protection must be worn and care taken not to spill the liquid nitrogen (it cracks the floor, but more importantly any significant spillage will produce a great deal of nitrogen and cause a serious depletion of oxygen which can be fatal)

Sample Preparation
1) Samples should be prepared the day before analysis so that they can be degassed overnight. Up to 6 samples may be done at one time.

2) Obtain the special sample tubes and rubber bungs, normally kept in the Instrument lab. Make sure they are clean and dry and not cracked. There needs to be $1\text{m}^2$ of surface in the sample tube for analysis. If you suspect that the surface area of your sample will be $< 2 - 3\text{m}^2/\text{g}$ you need to use the bulb sample tubes with filler rods — follow the instructions in the manual A (Operators), Appendix C.

3) Identify which sample is to be used with a particular tube and bung (these all have a letter written on them so they can be distinguished from one another).

4) Weigh the empty tube plus bung in each case, using an analytical balance 4-figure (160g).

5) For each sample, use the funnel from the Gemini drawer (small funnel + plastic tube) to put an appropriate quantity in the tube. The quantity required will vary according to the type of sample — a typical amount might be around 0.3g. It is not necessary to know the exact sample weight at this stage — it will be weighed the next day after degassing. Try not to get any sample around the top of the tube where it might get on the bung.

Degassing (References are to Manual C- FlowPrep)
1) Take the samples into the instrument room where the surface area equipment and degasser are located.

2) Turn on the nitrogen flow at the tap behind the instrument.

3) Turn on the degasser. The temperature should be pre-set for 100°C. If not, it can be set using the controls on the machine. It will heat up to the required temperature in a few minutes. (p1.15)

4) For each sample, take one of the degassing tubes. Open the nitrogen flow for that tube by flipping the switch above it to the open position. Check the flow rate by attaching the bubble manometer from the C/N Analyser (adapter in drawer) – this should be up to 10ml min-1. Only after the gas is flowing should the degassing tube be placed into the sample tube (p1.28 et seq)

5) Take the bung out of the sample tube and put the nozzle of the degassing tube into the sample tube such that it goes down to the bottom of the sample. Put the bung back loosely.

6) Place the sample tube into one of the heating ports of the degasser. These are the back-row ports, outlined in red. Ensure that the tube is fully inserted.

7) Leave the samples overnight. Put a notice next to the degasser explaining that it should be left on and including your name and the date.
Performing the Analysis (References are Manual A – Operator and Manual B - Stardriver)

1) Check how long it is since the saturation pressure was measured. If it is more than a few months, re-measure it before proceeding with the analysis –see below for procedure.

2) When it is time to perform the analysis, the following items need to be turned on:
   - N₂ gas (tap)
   - He gas (tap)
   - vacuum pump
   - Gemini surface area unit
   - computer

3) At the computer, start the surface area software by double-clicking the STARDRVR icon.

4) Take one of the sample tubes out of the heating port in the degasser. Carefully remove the nozzle of the degassing tube, if necessary tapping it on the inside of the sample tube to dislodge any particles of the sample, which are clinging to it. Put the bung fully into the top of the sample tube. Only after removing the degassing tube should the N₂ flow for that tube be turned off.

5) Leave the sample tube to cool off for about 10 minutes in one of the degasser's cooling ports (the blue-marked ones at the front. Clean the nozzle of the degassing tube with a tissue. (Manual C p1.30)

6) While the sample is cooling, remove the Dewar flask from the Gemini and fill it with liquid nitrogen. After it has stopped boiling, top up the flask to within about 1cm from the top. (Manual A –p4.2)

7) Check that the tube in the balance port is the same type as your sample tube, and contains no filler rod.

8) Take the sample tube back to the balance and determine the total weight of tube plus bung plus sample. By subtracting the weight of the empty tube plus bung measured the previous day, the exact weight of the degassed sample can be obtained.

9) Bring the sample tube to the Gemini. Remove the bung and, ensuring that the small rubber O-ring is correctly positioned inside the aperture, very carefully (they are extremely fragile) insert the tube upwards (it may help to twist slightly) until it is level with the reference tube. Tighten the nut to hold the sample tube in place. Slide the semi-circular polystyrene cover onto the sample tube so it is level with the one on the reference tube. (This should be at the height of the dewar when in the raised position) (Manual A-p4.3)

10) Check that there is an adequate quantity of liquid nitrogen in the Dewar flask, and place it on the platform below the sample and reference tubes. Close the doors of the machine.

Software: The software creates an empty file (.MGD) containing parameters that is then filled with the analysis data. (Manual B –p4.1) To set this up

11) Select File...New. Select the standard setup file, C:\STARDRVR\FIONA.MGS — a technician in reading (Fiona) suggested setup (or C\Stardrvr\Data\setup.mgs)— and click on "OK". (Manual B p4.5)

12) Select Sample...Sample Information and enter a description of your sample in the "Sample ID" field. Click on "OK" , then use File...Save_As to save this file. Select the C:\STARDRVR\DATA directory and enter your filename with the .MGD extension provided. Click on "OK" to confirm this. (Manual B –p4.10 and 4.32)

13) Select Analyze...Start_Analysis. Select "Unit 1" (2360) and click "OK". Enter the weight of your degassed sample in the sample weight field. Check that your sample is ready in position, and click "OK" to commence the analysis. (Manual B p4.36 et seq.)

14) When the run has finished, open the Gemini's doors, remove the Dewar, and remove the sample tube.

15) Analysis of the next sample can now be done by cooling the tube, etc. (i.e. from step 4). Initiate a new analysis on the computer by selecting File...New, and proceed as before. Remember for each sample to top up the liquid nitrogen in the Dewar.

16) When all samples have been analysed, move your results from the C:\STARDRVR\DATA directory to your own floppy disc. This can be accomplished using the Windows File Manager.
**Switching off**

Shutting down the apparatus is essentially the reverse of switching it on.

Close any applications running on the computer.

Switch off the Gemini, the vacuum pump and the degasser (if not already off).

Shut off the N₂ and He supplies.

**Measuring the Saturation Pressure**

This is performed by

1. Attaching an empty tube to the analysis port, the same size as the tube in the balance port.

2. Add liquid nitrogen to the dewar (allow the liquid nitrogen temperature to stabilise and then refill).

   Place dewar onto elevator.

3. From software goto Po | Start Measurement. *(Manual B p4.35)*

**Data Reports**

1. Goto File | Open and pick up the relevant .mgd file
2. Click on Report …. Regular ……….. *(Manual B – p4.13)*
3. Click on next to select page – surface area reports seem to be on page 3
4. To export any data suggest setting up Acrobat Distiller as the default printer – print the report to the Distiller and then cut and paste from the .pdf file
Pollen Preparation

Pollen preparations need great care. Cleanliness and good technique are essential for accurate preparations with no contamination. Even more importantly hazardous chemicals are used and so advise must be sought on handling and protective clothing gloves and goggles / facemasks must be worn.

There are many variations on the technique depending on the nature of the sample and so advice must again be sought before the preparation.

AA) Treatment of exotic standard.
This only applies to those samples where an absolute value for the pollen is to be estimated
1. Decide on the number of exotics to be added.
2. Using forceps add these exotic tablets to the 50 ml boiling tube.
3. Add ca. 30 ml of distilled water and 1 ml of conc. Hydrochloric acid to each boiling tube.
4. After the effervescence has died down, centrifuge the tubes at 2500 rpm for 20 minutes.
5. Decant the supernatant, ensuring no loss of pollens and wash with 40 ml of distilled water
6. Centrifuge at 2500 rpm for 20 minutes
7. Repeat stages 5 and 6.

A) Initial Preparation – Wet Samples
1. Decide how much sample to take - usually 0.4 ml.
2. Add a small amount of distilled water to a 5 ml measuring cylinder. To this add sample with a micro-spatula until 0.4 ml (or designated volume) is displaced.
3. Add this mixture to the boiling tube and wash cylinder with the Sodium Hydroxide, adding washings to the boiling tube. Repeat until all the sample has been transferred.
4. Boil the centrifuge tubes in a water-bath at 100 °C for 20 minutes, stirring regularly.

A) Initial Preparation – Dry samples
1. Decide how much sample to take - usually 0.2 – 0.5g. Weigh the sample into the boiling tube, being careful to not cross contaminate between samples. Note the exact weight (4 decimal places)
2. Add 40 ml 10% Sodium Hydroxide, to the boiling tube.
3. Boil the centrifuge tubes in a water-bath at 100 °C for 20 minutes, stirring regularly.

A) Initial Preparation (Calcareous samples)
1. To each boiling tube weigh out 2gm of sample (weight may vary according to expected pollen)
2. Add a small amount of IMS (to stop frothing) and 1ml of 10%HCl – wait for effervescence to subside. Repeat until no more effervescence on adding the acid
3. Centrifuge at 2500 rpm for 10 minutes – discard the excess acid. Wash with distilled water and centrifuge at 2500 rpm for 10 minutes – discard the supernatant.
4. Add 40ml of 10% Sodium Hydroxide to each boiling tube
5. Boil the centrifuge tubes in a water-bath at 100 °C for 20 minutes, stirring regularly.

Note: Alternatively, the addition of the hydrochloric acid can be performed after the distilled water wash following the first screening (see below), but extra care must be taken with the addition of the acid because of the smaller volume of the test tube.
B) Screening

1. Onto a funnel a 10µm sieve (nylon cloth) is placed. Above this is a 'cut away' funnel and then a 106 µm brass sieve.
2. Pour the contents of the beaker through this, collecting the liquor as waste in a bottle. Wash the 106 µm sieve with distilled water, collecting the <106 µm fraction on the 10µm sieve.
3. When the 106-µm sieve has been thoroughly washed, then stir the liquor on the 10µm sieve and wash this with distilled water until the waste is clear.
4. Wash the residue towards the lip of the sieve, and then using a wash bottle fitted with a jet, wash this entire residue into the 10 ml centrifuge tube.
5. Centrifuge the tubes at 2500 rpm for 10 minutes. Discard the supernatant.
6. Retain the 10 µm sieve as this is used again after the hydrofluoric acid stage.

The macro on the 106 µm sieve is washed into a 100 ml bottle and retained for possible analysis.

The brass sieves are then placed in an ultrasonic bath for thorough cleaning before being soaked overnight in peroxide.

C) Hydrofluoric Acid Digestion

This removes silicas and so may not be necessary for organic samples.

**This is a VERY HAZARDOUS substance and so must only be used under supervision and in a fume cupboard with full personal protection (gloves, facemasks, etc.)**

1. Add about 6 - 8 ml of the Hydrofluoric acid to the residue in each of the tubes.
2. Stir with a plastic rod (not glass!)
3. Place in a waterbath at 100 °C for 30 minutes.
4. Centrifuge at 2500 rpm for 10 minutes.
5. Discard supernatant into a tub of water.
6. If high amounts of silica anticipated then repeat stages 1 - 5.
7. Wash residue with distilled water.
8. Sieve through the relevant 10 µm sieve, and wash residue back into the 10ml centrifuge tube.
9. Centrifuge at 2500 rpm for 10 minutes.
10. Discard supernatant, taking care as, when in water, the pellet is often not very firm.

The waste Hydrofluoric acid from stage 5 is neutralised with Sodium Hydrogen Carbonate in the fume cupboard sink before disposal.
D) Acetolysis

The acetolysis mixture reacts VIOLENTLY with water and so great care must be exercised, again using a fume cupboard and full personal protection.

The Acetolysis Mixture is 9 parts Acetic Anhydride + 1 part conc. Sulphuric Acid
This should be prepared by a Technician using a fume cupboard

1. About 2 ml of Glacial Acetic Acid is added to the residue and stirred. The tube is then centrifuged at 2500 rpm for 10 minutes and the supernatant is discarded into a tub of water.
2. Stage 1 is then repeated to ensure that all water is removed
3. About 6 - 8 ml of the Acetolysis Mixture is carefully added to the residue
4. After stirring the tubes are heated at 100 °C for 5 - 10 minutes in a waterbath.
5. The tubes are then centrifuged at 2500 rpm for 10 minutes
6. The supernatant is then cautiously discarded into a DRY beaker and disposed of by the Technician.
7. If the organic content of the sample is high then repeat stages 3 - 6
8. Ca. 2 ml of glacial acetic acid are added to the residue and stirred. The tubes are centrifuged at 2500 rpm for 10 minutes and the supernatant discarded.
9. The residue is now washed with distilled water, centrifuged and then the supernatant discarded.
10. Stage 9 is then repeated.

E) Finishing off (Silicon Oil)

1. To the residue is added ca. 2 ml of 95 % alcohol (IMS). After stirring, the tubes are centrifuged at 2500 rpm for 10 minutes. The supernatant is discarded
2. Stages 1 is now repeated to ensure total removal of water
3. Ca. 2 ml of 100 % alcohol (iso - propanol) is added to the residue. Again this is centrifuged and the supernatant discarded.
4. Ca. 1 ml of tertiary butyl alcohol (TBA) is added to the residue. Again this is stirred using the vortex stirrer, centrifuged and the supernatant discarded.
5. 1 ml of tertiary butyl alcohol is again added to the residue and stirred
6. A small amount of silica oil is added to a labelled vial and the contents of the centrifuge tube are then transferred to this vial using the TBA.
7. The vial is then placed in a heating block at 40 °C until the TBA has evaporated (ca 2 days)

E) Finishing off (Glycerol)

1. Wash the residue into a labelled vial with distilled water and centrifuge gently at 1500 rpm for 10 minutes
2. Decant off the water, (the last drop can be dealt with by a piece of tissue, but ensure that there is no loss of solid)
3. Add a few drops of the glycerol and stopper the vial for storage

An essential part of the preparation is the use of clean equipment. Therefore after use, all tubes, stirrers, funnels, sieves etc. must be thoroughly washed and then soaked overnight in Hydrogen Peroxide before rinsing with distilled water and stored separately from general laboratory equipment.
1. Slowly dissolve 200 gm of calcite in cold concentrated Hydrochloric acid, controlling the effervescence by the addition of alcohol (IMS). Centrifuge \(^1\) and decant. If the calcite is very pure, and forms little solid residue, add 10 - 15 gm of sand before the first centrifuging, in order to produce a pellet, thus not risking loss during the decanting.

2. Wash with distilled water, centrifuge and decant

3. Add 10 - 15 drops of 10% Hydriodic acid, mix, adding distilled water, centrifuge and decant\(^2\)

4. Dilute the pellet in about 75 ml of Thoulet solution\(^3\), transfer into a Waring Blender (MC-3) and mix for 10 minutes, transferring back to the centrifuge tube

5. Centrifuge for 20 minutes and decant the supernatant liquid through a filter, Schleicher & Schüll no 604

6. Repeat operations 4 & 5, then rinse the blades and lid of the mixer with 50 ml of Thoulet solution, centrifuge, and filter

7. Put the filter in a nickel crucible and boil in 40% Hydroflouric acid for 10 minutes, cool for 30 minutes, centrifuge & decant

8. Treat with cold 10% Hydrochloric acid, centrifuge & decant

9. Wash with distilled water, centrifuge & decant.

10. Remove the water with acetic acid for 10 minutes, centrifuge & decant

11. Heat in a water bath at 100\(^\circ\) C with acetylosis mixture (9 parts acetic anhydride + 1 part concentrated sulphuric acid). Leave to cool for 30 minutes, centrifuge & decant

12. Wash with glacial acetic acid, centrifuge & decant

13. Wash with distilled water, centrifuge & decant

14. Dilute the pellet in the solution for final mounting

With two centrifuges of capacity 4 x 100 ml, the whole of the operations 2 to 14 need about 6 working hours for 8 samples. The dissolving of the calcite and removal of the Hydrochloric acid by centrifuging takes about 2 hours of preparation spread out over a period of two days. For the method above it is therefore possible to prepare 8 samples of stalagmite (or stalagmite slices) in 8 hours of preparation

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\(^1\) Unless indicated elsewhere, the centrifuging should be 4000 rpm for 5 minutes - in 100 ml tubes for operations 1 to 6, in 50 ml. tubes for operations 7 to 11, and 15 ml. tubes for operations 12 & 13

\(^2\) This operation is withdrawn if one uses recovered Thoulet solution saturated with iodides

\(^3\) A litre of Thoulets solution of specific gravity 2 is obtained by dissolving in 604 ml of distilled water, 664 gm of Potassium iodide & 732 gm of Cadmium Iodide. This Thoulet solution can be recovered and reused, after passing through a filter, Schleicher & Schüll no 589 and readjust to the specific gravity of 2 by simmering
Procedure for floatation method - Art - 12 June 1998

1. 1 lycopodium tablet washed with hcl
2. 1.5 ml sample added to 2ml. Of water
3. Washed into boiling tube with sodium hexametaphosphate
4. Centrifuged at 2500 for 20 mins
5. Supernatant discarded - residue washed with distilled water - centrifuged at 2500 for 20 mins
6. Above repeated
7. 30 ml of 10 % sodium hydroxide - warm @ 90ºc for 10 mins
8. Centrifuge at 2500 for 6 mins
9. Wash with distilled water - centrifuge at 2500 for 6 mins
10. Repeat above
11. Wash with 5 ml. Conc hydrochloric acid - centrifuge at 2500 for 6 mins
12. Add 20 ml dense media (230 g ZnCl to 100ml water adjusted to s.g. of 1.88)
13. Mix well - centrifuge at 1800 rpm for 20 mins
14. To supernatant
   a) Dilute to s.g. < 1.3 (20 ml.. Added to 80 ml water in beaker)
   b) Transfer to test tube & centrifuge at 2500 for 6mins - discard supernatant
   c) Repeat b until all of the liquid has been centrifuged
15. To pellet
   a) Screen through 106 @ 10 µm sieve
   b) Boil with HF for 1 hour
   c) Centrifuge - decant
   d) Repeat a & b
   e) Screen through 10 µm sieve
16. Dehydrate with glacial acetic acid (twice)
17. Warm with acetolysis mixture (as normal) twice
18. Dehydrate with glacial acetic acid
19. Dry with alcohols as normal (see standard pollen preparation)
20. Mount in silicon oil
Preparation for Diatoms

Reagents

A) Potassium Dichromate           10 g to 100 ml distilled water
B) Conc. Sulphuric Acid
C) Hydrogen Peroxide (100 vol.)

Method

1. A trace of sediment is placed in a test tube using a clean spatula
2. To this is added 1 ml of the Potassium Dichromate solution (A)
3. 5 ml of conc. Sulphuric acid are now carefully added
4. The reaction is now left for 10 minutes (with occasional stirring)
5. 2 drops of Hydrogen Peroxide are now added
6. The reaction is again left for 10 minutes
7. The tubes are now centrifuged at 2500 rpm for 10 minutes
8. After decanting the supernatant the residue is washed with distilled water
9. The tubes are again centrifuged for 10 minutes at 2500 rpm
10. The supernatant is again discarded
11. The residue is transferred to a vial with a small amount of water
EPMA SLIDE AND SAMPLE PREPARATION;
Nick Woolley 22/3/94.

The general aim of this procedure is to extract tephra shards from their host material (e.g. peat) and mount them in an araldite resin to allow the production of a polished grain mount slide suitable for Electron Microprobe WDS and EDS analysis.

ACID DIGESTION:
The sample size is obviously dependent on the amount of material available together with the adopted sampling strategy (e.g. 1cm intervals). However the bigger the sample size available for acid digestion the better, particularly with material like peat where the actual tephra concentrations are relatively small.

1) Place the sample in a conical flask and add 50ml> of 98% sulphuric acid. The amount of acid added will not significantly affect the tephra, so larger amounts than 50ml can be used in order to digest the organic material.

2) Add 10-20ml of nitric acid to the flask. This should be done in stages to avoid too violent a reaction, and the whole process can be carried out cold if desired.

3) As soon as all the nitric acid has been added place the flask on a hotplate, (in a fume cupboard). When the acid digestion mixture starts to boil reduce the temperature and allow to simmer until the mixture becomes relatively clear.

4) When the mixture has cooled add DH2O and centrifuge at 2500 for 15 minutes. Pour ALL the supernatant off and wash and centrifuge with more DH2O, repeating this process a few times until the sample has become neutralised, (pH5-6 is best).

5) Transfer the water-and hopefully tephra etc.- to a labelled glass vial and allow to settle.

SLIDE PREPARATION;
Slides should measure 48mm long by 1 inch wide, and should be frosted using either 600um or 120um grit. The idea of using 120um frosting is to provide a surface onto which the araldite resin can firmly adhere, alternatively some use 600um as a precaution against tephra grains resting between scratches.

1) The slide should be cleaned with methylated spirit and kept free of all dirt and grease during the preparation.

2) The working area should be marked off with a pencil. The top and bottom edge must be kept clear, (raised ends will interfere with the position of the slide and subsequently focusing in the microprobe), as must the sides, (to allow efficient insulation later).

3) Sample areas together with appropriate samples must then be marked on. Usual numbers of samples on an individual slide can range from 2-6, (depending on the relative concentration of the samples being examined).

4) The slide thickness must be measured, (in a number of places as thickness can vary across a single slide), using a gauge micrometer.
ADDING SAMPLE TO SLIDE;

1) Place slide and the araldite on a hotplate at 70C.

2) Using a pipette suck off the bottom sediment from the sample vial. Allow the suspended material to settle in the pipette and then drop a small amount onto the appropriate sample area on the slide. When the DH\textsubscript{2}O has evaporated repeat this procedure until a residue has built up on the sample area.

3) When residues have been built up for all the samples on one slide add nine drops of the warmed araldite to a watchglass together with one drop of hardener, (N.B remember to keep pipettes for the araldite and hardener separate). Mix the two together on the watchglass.

4) Take a blob of the araldite resin, (using a cocktail stick etc.), and place it onto the sample residue. Mix the residue into the resin, (the idea is to get the sample fully suspended in the araldite resin).

5) When all the sample residues have been treated in this way use the remaining resin to cover the titles etc.. on the slide, (this will protect them from grinding and polishing later).

6) Leave the slide on the hotplate for an hour in order to let the resin cure and harden.

GRINDING;

A series of silicon-carbide paper discs are used for the grinding- starting on 180 (6/1000 inch), 400 (4/1000 inch) and finally 600 (3/1000 inch). The discs are mounted onto glass sheets and DH\textsubscript{2}O is used as the grinding lubricant. It is important to apply an even pressure with your fingers over the whole slide while you are grinding, (N.B if 180 grinding goes too far for example then the scratch marks will not be removed by the 600 disc).

The idea is to achieve a resin thickness of plus 6/1000 inch relative to the slide thickness, so constant measuring with a micrometer is necessary.
Macrofossil preparation

Reagents

A) 10% potassium hydroxide (100 g KOH to 1000 ml distilled water)

Method

It is custom to express numbers of seeds etc. per unit weight or unit volume of soil. Therefore weigh a sample and place in a beaker. Add enough KOH solution to cover the sample, and carefully heat on a hot-plate. Boil for several minutes. The actual boiling time depends on the material. Well-preserved peats may only take a few minutes to disintegrate, strongly humified peats and detritic material will take up to 15 minutes or more. Wash down any material on the beaker with distilled water.

The next step is sieving. The choice of sieve depends on the material. If the samples are to be used together with the coarse fraction left from pollen preparation, a 106 µm sieve should be used. Other standard mesh sizes are 150 µm and 200 µm.

Transfer the sample into the sieve and gently wash with distilled water. If you are not planning to use the sample for anything else (for instance radiocarbon dating) you can also use tap water. Transfer the residue to a bottle with some water.

If after standing for a while the water colours dark brown, not all the KOH has been washed out. Wash carefully over a sieve to remove the KOH. Repeat until the liquid remains clear.
Extraction of Carbonaceous particles (SCP)


Reagents
Nitric Acid (concentrated)
Hydrofluoric Acid (40%)
Hydrochloric Acid (6M) – 500 ml HCl to 1 l with distilled water
Distilled water
Napthrax

Safety Notes
The method involves Hydrofluoric acid (HF). This is a VERY HAZARDOUS substance and so must only be used under supervision and in a fume cupboard in the Clean Laboratory, with full personal protection (gloves, facemasks, etc.) and having the antidote gel available.

*Nitric acid / Hydrochloric acid also pose normal acid hazards and so gloves / eye protection must be worn and all operations carried out in the fume cupboard*

Method
1. Weigh 0.1 – 0.15 g of dry sediment accurately into a 12ml centrifuge tube
2. Add 1.5ml conc. nitric acid – leave overnight in fume cupboard
3. Add further 1.5 ml nitric acid and heat in water bath at 80 – 90°C for 2 hours
4. Fill tubes with distilled water and centrifuge at 1500 rpm for 5 minutes – discard supernatant
5. Add 3ml of HF and heat at 80 – 90°C for 3 hours
6. Centrifuge at 1500 rpm for 5 minutes – discard supernatant
7. Add 3ml of 6M HCl – heat at 80 – 90°C for 2 hours
8. Centrifuge at 1500 rpm for 5 minutes – discard supernatant
9. Add distilled water - Centrifuge at 1500 rpm for 5 minutes – discard supernatant
10. Repeat stage 9
11. Wash residue into labelled vial with distilled water – this can then be stored
12. Pipette out known amount of sample onto a slide and evaporate to dry – mount with Napthrax
Diatom preparation

Reagents

A) Hydrogen peroxide (H₂O₂, 30% or weaker)
B) Hydrochloric acid (HCl, 10%)
C) Ammonia (NH₃, 1-2%)
D) NAPHRAX diatom mountant

Precautions

Heating H₂O₂ with strongly organic sediment can lead to violent reactions. Both HCl and NH₃ give off strong fumes, and these chemicals must be used in a fume cupboard. NAPHRAX diatom mountant contains toluene, and must be used in a fume cupboard as well.

Method

The method described here follows that of Battarbee (1986). Refer to this paper for a full discussion of diatom analysis and preparation. Since publication of the paper, the method (see figure 26.1) has changed on two points. HCl treatment is now usually done after H₂O₂ digestion, and the flotation agent ZnBr₂ has been replaced with the slightly less environment unfriendly NaWO₃.

Place approximately 0.1 g (dry weight) or 1.0 g (wet weight) of sediment in a beaker. Add a small quantity of H₂O₂, around 20 ml usually is sufficient but more may be necessary for samples with a high organic content. Carefully heat on a hot-plate until all organic material has been removed. Alternatively, the samples can be left standing overnight (or longer) until all organic material has been removed.

Add a few drops of HCl to remove carbonates and any remaining H₂O₂ from the sample (in fume cupboard). After cooling, pour the sample into a clean centrifuge tube. Any coarse sand may be left in the beaker and thrown away.

Centrifuge the samples for 4 minutes at 1200 rpm. Higher centrifuge speeds will cause increased breakage in the diatoms. Decant the samples discarding the supernatant, re-suspend the sediment by carefully tapping the tube and fill the tubes with distilled water and centrifuge again for 4 minutes at 1200 rpm. Repeat the washing and centrifuging at least 3 times to remove all chemicals. During the last washing, clay can be removed by adding a few drops of very dilute NH₃. However this may not remove all clay, especially in sediment rich samples. Careful sieving over a very fine sieve (10 µm or less) may help here.

Slide preparation

Dilute the diatom suspension to the right concentration. This is something the obviously depends on the material, and it takes practice to get it right. Place a round coverslip on a flat, even surface and with a pipette put some of the diatom suspension on the coverslip. With some care, a nice meniscus can be created. When uncertain about the concentration of the suspension, a second coverslip with a more dilute suspension can be made. Leave the coverslips to dry overnight, making sure that they will not be disturbed by drafts, dust or cleaners. The drying can take up to two days.

When the coverslips have dried they can be mounted on microscopic slides. Heat a hot-plate in the fume cupboard to 120 °C. Place a drop of NAPHRAX diatom mountant on the slide and place the coverslip with the sediment down on the drop. Carefully heat the slide on the hot-plate to remove the toluene from the NAPHRAX. Allow the slide to cool, and test if the coverslip moves. If so, it will need to be heated a little longer. Two coverslips (of the same sample) can be mounted on one slide.

Literature

Battarbee, R.W. (1986) Diatom Analysis; in: Berglund, B.E. (ed.) Handbook of Holocene palaeoecology and palaeohydrology, p. 527-570 Further information and advice on diatom analysis can be found on the UCL-ECRC website http:\geog.ucl.ac.uk\..... or by contacting one of their technical staff (see website for addresses)
Degree of Humification Methodology


Samples are extracted contiguously over 2cm intervals, of size 5-9g wet weight, and dried at 70 c overnight, taking care not to burn the sample.

Once dry the samples are ground and a 0.2g subsample extracted and placed in a 200ml flask, with 100ml of freshly mixed 5% NaOH from a measuring cylinder. At this point the time is recorded, because from the addition of the NaOH to the recording of the % transmission, the remainder of the experiment should be completed within 4 hours, any time in excess of this then a correction should be applied to the transmission value.

The mixture is now brought to the boil on a hot plate, and is then simmered for one hour.

The samples are allowed to cool, topped up to 200ml mark with distilled H₂O, and thoroughly shaken.

The samples are then filtered through Whatman (Qualitative 1) Paper. 50ml of the filter solution is poured into a 100ml flask (preferably during filtration - saves washing up) and diluted with 50 ml of distilled H₂O.

This diluted filtrate is then thoroughly shaken before measuring the % transmission on the UV Vis Spectrophotometer at 540nm wavelength. The spectrophotometer is set to Transmission, at 540nm, and zeroed at 100% using distilled water. The samples should all have less than 100% transmissivity.

The range of values encountered can vary from 70% down to 5%. Higher values are indicative of poorly humified peats, and lower percentages are indicative of well humified peats.
PARAFFIN FLOTATION FOR INSECTS

This technique can be used to extract fossil insect macrofossils, especially Coleoptera (beetles), from unconsolidated sediments. It can also be used to extract modern specimens, or recently deceased individuals from contemporary sediments (e.g. river and lake sediments) and other materials (e.g. dung, leaf litter, hay).

Materials & reagents

<table>
<thead>
<tr>
<th>Paraffin (kerosene)</th>
<th>Optional:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Vacuum pump &amp; flask</td>
</tr>
<tr>
<td>2 x 300 micron sieve</td>
<td>Calgon</td>
</tr>
<tr>
<td>2 x spouted bucket</td>
<td>potassium hydroxide</td>
</tr>
<tr>
<td>rubber gloves</td>
<td>acetic acid</td>
</tr>
</tbody>
</table>

Optional:

- Vacuum pump & flask
- Calgon
- Potassium hydroxide
- Acetic acid

Stage 1: Disaggregation

First stage is to break down (disaggregate) the sediment matrix to free up the fossil contents.

1. Place sample in large plastic bucket and soak in warm water. It may be necessary to gently tease apart lumps, but try to avoid any unnecessary mechanical disaggregation (e.g. crumbling between fingers) as this will further fragment the fossils. If the sample disaggregate easily in to a slurry proceed to step 6. If the sample does not disaggregate readily proceed to step 2.

2. If the sample is clayey proceed to step 3. If the sample is calcareous proceed to step 4. If the sample comprises compacted plant debris, felted peats, or other such dense fibrous sediments proceed to step 5.

3. Clayey samples can be treated using by soaking in 'calgon' and warm water. If the matrix still doesn't crumble, try gently working it between your fingers to help it fall apart, using a jet of warm water to wash away sediment. Avoid excessive force as this will crush fossils. Go to step 6.

4. Calcareous sediments can be soaked in dilute acetic acid. If the matrix still doesn't break down, try placing sediment samples in an ultrasonic bath, and then re-soak in fresh acetic acid. Go to step 6.

5. Dense peaty samples can be treated by soaking in cold KOH. If the matrix still won't break down, boil the sample in KOH (use fume cupboard). Go to step 6.

6. Wash material through a 300 micron sieve to wash out fines (clay, silt) using warm water. Avoid slopping material over the sides of the sieve. Retain the contents of the sieve.

7. Allow the sieve contents to drain, but do not dry out. Excess water can be removed by passing the sieve over a vacuum flask.

Stage 2 Paraffin flotatation

This stage separates and concentrates the fossil insect remains from the sediment matrix.

8. Place the contents of the sieve in a clean spouted bucket.

9. Add paraffin (about 0.25 litres is usually plenty) and gently mix in with sediment using your fingers (wear rubber gloves). Anything with a waxy surface (e.g. insect fossils, and some plant remains) should adhere to the paraffin. Obviously the more you mix, the more likely you are to coat the fossil, but also at same time you may damage them. Be gentle!
10. Pour in cold water to fill bucket close to brim, stirring up the contents of the bucket.

11. Leave to stand for 15-25 mins. The paraffin should float to form an oily scum on the surface, containing insect fossils and some other organic debris. Allow the water and paraffin flot to separate out, and non-insect matter to settle to bottom. The paraffin 'flot' should now contain concentrated insect remains, so it is important not to lose any of this material.

12. Pour off paraffin flot into a 300micron sieve by tilting the bucket. Keep pouring until the paraffin and its insect content has been decanted into the sieve, and/or you start to stir up the sediment at bottom of bucket. A constant, steady pouring is necessary. If you stop and start it will re-suspend the bottom sediment. This will mean that you have more work in stage 3!

13. Repeat floatation process several times until paraffin flot has nothing in it. i.e. add cold water, leave to stand, pour off into the sieve. The number of re-floats required depends on the material, but is usually 3 to 4 times. If little floats, you may consider repeating from step 7 (i.e. re-paraffining) to confirm that no insects are present.

14. You should now have a 300micron sieve containing an oily mixture of insect remains and plant debris (proportions will vary). Wash off paraffin using detergent and warm water, until clean of paraffin and detergent. Allow the contents of the sieve to drain, drive off water using ethanol or if excessively wet (e.g. high moss content) use vacuum pump (see step 7).

15. Using ethanol, sluice the sieve contents into a beaker or water-tight bottle. Be careful not to spill any of the flot.

**Stage 3: sorting**

This involves separating out the insects from the other debris in the flot, which usually comprises fragments of plant tissue, seeds and some sand grains.

1. Place small amounts of the flot in a petri dish and add ethanol to a depth of 5mm below the rim. Under a low-power binocular microscope at about X 8 or 10 magnification, pick out fossil insect remains and store them separately in ethanol (i.e. in a glass tube).

2. When you have sorted through the flot, place the debris in a 300 micron sieve and drive of the remaining ethanol (use vacuum flask) and archive (i.e. double bag and label).

3. The insect remains can now be sorted and identified.

NB labelling samples: write your name and sample details in pencil on a piece of card and insert this into the storage jar. If you label the outside of the jar with a sticker, or permanent marker pen there is a risk that the information will rub off, especially if it comes into contact with ethanol.
**Phosphate Analysis (Sodium Hydroxide / Hydrochloric Acid Extraction)**


<table>
<thead>
<tr>
<th>Reagents</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ppm P solution</td>
<td>4.3943 gm KH$_2$PO$_4$ to 1 litre with water</td>
</tr>
<tr>
<td>5 ppm P solution</td>
<td>5 ml of 1000ppm P to 1 litre with water</td>
</tr>
<tr>
<td>1.0 N Hydrochloric acid</td>
<td>86 ml conc. HCl to 1 litre with water</td>
</tr>
<tr>
<td>1.0 N Sodium Hydroxide</td>
<td>80 gm NaOH to 2 litres with water</td>
</tr>
<tr>
<td>4.0 N Sulphuric acid</td>
<td>56 ml conc. H$_2$SO$_4$ to 500 ml with water</td>
</tr>
<tr>
<td>Ammonium Molybdate solution</td>
<td>6.4 gm Ammonium molybdate to 200 ml water</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>20 gm per litre of colourimetric solution</td>
</tr>
<tr>
<td>Potassium Antimony Tartrate</td>
<td>1.08 gm APT in 100 ml water</td>
</tr>
<tr>
<td>Colorimetric Reagent</td>
<td>500 ml of 4.0 N H$_2$SO$_4$</td>
</tr>
<tr>
<td></td>
<td>150 ml of Ammonium Molybdate soln</td>
</tr>
<tr>
<td></td>
<td>50 ml of APT solution</td>
</tr>
<tr>
<td></td>
<td>20 gm ascorbic acid</td>
</tr>
<tr>
<td></td>
<td>+ distilled water to 1 litre</td>
</tr>
</tbody>
</table>
Extraction

N.B. All glassware must be scrupulously clean and acid washed before use.

Approximately 0.5 gm of the soil or sediment is accurately weighed into a 50 ml polypropylene centrifuge tube. (Also prepare a blank by following all the next steps, but without any soil/sediment)

To this is added 40 ml of 1.0N Hydrochloric Acid.

The tube is then stoppered and shaken for 16 hours (i.e. overnight).

After this shaking the tube is centrifuged at 2500 rpm for 15 minutes and the supernatant transferred to a labelled 250 ml conical flask.

40 ml of 1.0 N Sodium Hydroxide is then added to the residue in the tube, which is then stoppered and shaken for 4 hours.

The tubes are then centrifuged again at 2500 rpm for 15 minutes and the supernatant again added to the relevant flask.

A further 40 ml of 1.0 N Sodium Hydroxide is added to the residue, the tube is stoppered and shaken and before being placed in an oven at 90 °C for 16 hours.

Again the tube is centrifuged at 2500 rpm for 15 minutes and the supernatant added to the relevant flask.

6 ml of conc. Hydrochloric Acid is then added to the flask and the contents transferred to a 200 ml volumetric flask. This is then made up to the 200 ml mark with distilled water and then returned to the conical flask.

Calibration standards

For both the total and inorganic phosphorous a series of standards are prepared to calibrate the UV/VIS spectrophotometer. The series is as follows

0,2,4,6,8,10 ml of 5ppm P

To this is added 5 or 10ml of the blank reagents, depending on how much aliquot is taken for the samples (see below). Then the colorations for both total and inorganic P are obtained as in the following methods for the samples, from the point in the methods when the aliquots have been taken.

A calibration equation can then be obtained using a regression package such as Minitab, or by using advanced regression in Qpro or Excel if that spreadsheet is used
A) Determination of total phosphorous

The 200ml extract from above is thoroughly mixed and a 5ml or 10ml aliquot is taken and transferred to a 125ml conical flask. (10ml if low values are suspected, otherwise 5ml)

A few anti-bumping granules are added and the solution is then boiled on a hotplate in the fume cupboard until almost dryness.

The flask is then removed from the hotplate and when cool, 1ml of perchloric acid is added. The flask is then returned to the hotplate where it is heated with care until almost dry. White fumes will then come from the flask and a few drops of water are added. The flask is then repeatedly heated and water added until there are no more perchloric fumes.

The flask is then removed from the hotplate and allowed to cool before the addition of the colorimetric reagent (see below).

B) Determination of inorganic phosphorous

The 200ml extract is thoroughly mixed and then about 40ml is transferred to a 50ml centrifuge tube where it is centrifuged at 2500 rpm for 15 minutes.

Again choose 5 or 10 ml of the supernatant, which is then transferred to a 100 ml beaker

Coloring

The colorimetric solution (see recipe above) is prepared fresh daily.

For the Total Phosphorous determinations, 10ml of the colorimetric solution are pipetted into a 50 ml volumetric flask which is then made up to the mark with water.

The entire contents of the 50ml flask are then added to the dry residues in the 125ml conical flasks from A above.

For the Inorganic Phosphorous determinations, 10ml of the colorimetric solution are pipetted into the 100ml beakers from B above and the entire contents are then transferred to a 50ml volumetric flask. This is then made up to the mark with distilled water before being returned to the 100ml beaker

Both sets of determinations are then allowed to develop for between 30 and 120 minutes.

The absorption at 890 nm is then measured on the SP6 UV/VIS Spectrophotometer.

Calculation

\[
\text{Factor } f = \text{ units on UV/VIS per ml of 5 ppm P} \\
\text{Reading } r = \text{ units on UV/VIS of sample} \\
\text{Volume } v = \text{ ml. of extract used (normally 5 or 10)} \\
\text{Weight } w = \text{ wt. of soil used in g}
\]

\[
\text{ppm P} = \frac{1000 \times r}{f \times v \times w}
\]
Phosphorous Absorption Isotherms

Reagents

0.02M KCl  
1.291gm KCl to 1l distilled water

5000 ppm P  
10.9844 gm KH$_2$PO$_4$ to 500 ml water

20 ppm P  
2 ml 5000 ppm P to 500ml with 0.02M KCl

Standard P solutions  
see table 1 below

Table 1

<table>
<thead>
<tr>
<th>ppm required</th>
<th>ml of 5000ppm P to 1000 ml with 0.02M KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>................................... 5</td>
</tr>
<tr>
<td>50</td>
<td>................................... 10</td>
</tr>
<tr>
<td>100</td>
<td>................................... 20</td>
</tr>
<tr>
<td>300</td>
<td>................................... 60</td>
</tr>
<tr>
<td>500</td>
<td>................................... 100</td>
</tr>
</tbody>
</table>

Ammonium Molybdate  
3.0 gm of Ammonium Molybdate  
+0.08 gm Antimony Potassium Tartrate  
+ 37 ml conc. Sulphuric acid  
to 2 Litres with distilled water

Ascorbic Acid  
3.0 gm Ascorbic Acid to 200ml water (make fresh daily!!)

Calibration standards  
see table 2 below

Table 2

<table>
<thead>
<tr>
<th>ml of 20ppm P to 50ml</th>
<th>ppm P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
</tr>
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<td>5.0</td>
<td>2</td>
</tr>
<tr>
<td>7.5</td>
<td>3</td>
</tr>
<tr>
<td>10.0</td>
<td>4</td>
</tr>
<tr>
<td>12.5</td>
<td>5</td>
</tr>
<tr>
<td>15.0</td>
<td>6</td>
</tr>
</tbody>
</table>
Method

All glassware must be acid-washed to ensure that it is phosphate-free.

If fresh soil is used, then a separate moisture determination is required.

1) Portions of approximately 5 gm (oven-dry-equivalent) of fresh soil are weighed into stoppered 200 ml flasks

2) 100 ml of the respective standard P solutions are added to each flask.

3) The flasks are now stoppered and shaken for 24 hours.

4) The contents are then filtered through a Whatman 542 filter, collecting the filtrate.

5) Dilutions are now made from the filtrates using the following table:-

<table>
<thead>
<tr>
<th>ppm P</th>
<th>ml taken</th>
<th>made up to</th>
<th>dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>5.0</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>2.5</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>300</td>
<td>1.0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>500</td>
<td>1.0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

6) 5ml aliquots of the calibration standards are now taken, as are 5ml aliquots of the diluted filtrates and the diluted standard P solutions.

7) To these are added :-

   20ml of the Ammonium Molybdate solution
   5ml of the ascorbic acid solution

8) The solutions are now left for 30-120 minutes for the colour to develop before being measured on the UV/Vis at 890 nm.

9) A calibration is obtained from the calibration standards, and the P content of the filtrates calculated. The P concentration of the original P standard solutions are also estimated.

10) The amount of phosphate absorbed by the soil can now be calculated and a graph plotted of :-

    g P absorbed/g of sample vs. Original P solution (ppm)
Phosphorous (Olsen's Method)

**Extraction**

*Reagents*

42g Sodium Hydrogen Carbonate to 1l and adjust to pH=8.5 with 50% sodium hydroxide.

*Method*

5g of soil is accurately weighed into a 250 ml centrifuge bottle and 100ml of the sodium bicarbonate solution is accurately added. This is then stoppered and shaken for 30 minutes in the constant temperature room at 20 C. The suspension is then filtered through a fluted Whatman no 2 filter collecting the filtrate for analysis.

**Coloration**

*Reagents*

A) Standard P (1000 ppm) 0.879g KH₂PO₄ to 200 ml

B) Standard P (20 ppm) 10ml A to 500ml with the sodium bicarbonate solution

C) Ammonium Molybdate (Stock) 30g Ammonium Molybdate

+ 0.75g Antimony Potassium Tartrate
+ 370 ml Sulphuric acid
+ water to 5litres

D) Ammonium Molybdate (working) 250ml C to 1litre

E) Ascorbic acid 1.5g to 100ml water (fresh daily)

F) Sulphuric acid 80ml conc. H₂SO₄ to 1litre (caution eye protection!!)

G) Standards (0 to 7ppm)

Aliquots of 0.5, 1.0, 15, 2.0, 25, 30 and 35 ml of 20ppm P (solution B) are accurately pipetted into 100ml standard flasks and made up to the mark with the sodium bicarbonate solution.
**Method**

A colour solution is made fresh daily consisting of:

- 20 parts D (Ammonium Molybdate working solution)
- 5 parts E (Ascorbic acid)

This should be a straw yellow colour and if this turns blue then contamination has occurred and the solutions remade.

To a 100ml flask is added accurately

- 5 ml of filtrate (or 5ml of standard)
- 1 ml of the sulphuric acid (solution E above)
- 25 ml of the colour solution

and the flask is then allowed to stand for between 30 and 120 minutes for the blue colour to develop.

If the soil extract is coloured then it may be advisable to make a blank of 5ml filtrate +1ml E + 25ml water

After the allotted time the absorbance of each solution is read on the UV/Vis Spectrophotometer at 880nm. If blanks are needed then these are also read at this wavelength and their values subtracted from the absorbances obtained from those which had the colour solution added.

**Calculation**

A graph of absorbance against ppm is plotted for the standards (alternatively Minitab can be invoked and the regression coefficients found, or a regression tool from spreadsheets such as Qpro or Excel)

It will then be possible to read off the values for the ppm P of the filtrates.

The original concentration of P in the soil can then be calculated by

\[ \text{ppm P} = 100 \times \frac{y}{w} \]

where \( y \) = ppm P in filtrate
\( w \) = wt of soil in gm
Estimation of Carbon and Nitrogen

The NA2500 Elemental Analyser

Principle
The Elemental Analyser is based on a series of sequential steps. The sample is burnt and then the gases produced are flushed with pure helium through various stages to a detector. Output from the detector is interfaced to a computer to give the final determinations.

Sample preparation
The sample is accurately weighed into a small tin boat, which is then closed.

The Burn
There is a controlled combustion of the sample. The capsule containing the sample is introduced into a tube at 1000 °C. Here it meets the helium stream, and also a measured amount of oxygen. There is a strongly exothermic oxidation reaction, which causes a ‘Dynamic Flash Combustion’ and locally raises the temperature to 1800 °C, optimising the sample combustion

Catalysis
The combustion gases are driven through an oxidative catalyst layer (chromic oxide and silvered cobaltous cobaltic oxide) where oxidation is completed. The gases are now present in the form of carbon dioxide, various oxides of nitrogen, and water.

Reduction
The gases then flow over a tube containing pure copper, heated at 780 °C. This reduces nitrogen oxides to elemental nitrogen and also scrubs out any excess oxygen.

Water Removal
The gases now pass through a scrubber tube containing anhydrous magnesium perchlorate to remove any water produced by the initial combustion, thus leaving just nitrogen and carbon dioxide.

Separation and Estimation
Separation is achieved by passing the gases through a gas chromatography column (containing PoroPack PQS) maintained at 50oC. The nitrogen is eluted first and the carbon dioxide is usually about 15 –20 seconds afterwards. The gases flow over a thermo conductivity detector (The TCD is basically a heated wire whose conductivity is measured by a Wheatstone bridge arrangement – this conductivity changes as various gases pass over it).

Computer Interface and Final Calculations
The electrical signal from the TCD is interfaced with the computer, and a trace appears on the screen.

Software allows an integration window to be set, and the area of the peak to be calculated. This is then reconciled by the area of the blank sample and then compared with a known standard (in our case EDTA) in order to arrive at the final estimate of the carbon and nitrogen content of the sample. The software allows for peaks to be checked to ensure full compliance and to be reprocessed should there be any errors in the peak identification. Final results are then exported in a format suitable for spreadsheet packages.
### Layout Configuration:

<table>
<thead>
<tr>
<th>Element</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHN</td>
<td>1</td>
</tr>
<tr>
<td>CHNS</td>
<td>3</td>
</tr>
<tr>
<td>NC</td>
<td>5</td>
</tr>
<tr>
<td>Sulphur</td>
<td>7</td>
</tr>
<tr>
<td>Oxygen</td>
<td>2</td>
</tr>
<tr>
<td>NCS</td>
<td>4</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>8</td>
</tr>
<tr>
<td>N/protein</td>
<td>9</td>
</tr>
</tbody>
</table>

### NC Layout Using N.A. 1500

![Diagram](image)

- **Autosampler**
- **Quartz Wool**
- **Chromium Oxide**
- **Ag/Cobaltous Co Oxide**
- **Copper Oxide**
- **Copper Grain**
- **Anhydride**
- **Detector**
- **GC Column**
Carlo Erba C/N Analyser

Start-up - The NA2500 C/N Analyser

1. Ensure that the oxygen and helium are turned on at the regulators and set to 1.5 bar and 3 bar respectively.
2. On the panel press SPC FUN (Special Functions) and then the right arrow - this will show the STD BY flashing.
3. Press ENTER on the panel. The panel should now show ST-BY YES, O2 OFF, He OFF.
4. Press ENTER again so that it now reads ST-BY NO, O2 ON, He ON.
5. Press SPC FUN again to return to the temperatures.

The C/N Analyser will now warm up the reaction and reduction furnaces, a process which takes approximately 30 minutes. The lights on the panel graphic showing the furnace heaters will remain steady until the set temperatures are reached, when they will flash on or off.

Do a leak check by blanking the vent, waiting 2 minutes and then winding out the helium thumbscrew. The pressure should not drop. Remove blank and set the helium back to its setting of 150.

Start-up - Computer

1. Check that the computer has the correct dongle on the parallel port, and the correct 9 way connector in the COMS 1 serial port. (The computer is also used for the UV/VIS so check or ask.)
2. Switch on the computer.
3. Type Win to enter the Windows environment.
5. Wait a little, even when the hour glass symbol changes back to the normal arrow. This initialises the Eager card.
6. A Grey Window eventually appears showing the serial number of the Eager Card. Click on OK and wait again for 30 seconds or so until the main Eager Window appears.

Cleaning of Tin Boats

It is essential that the tin boats are cleaned and dried before being used for analysis.

1. The waterbath is heated to at least 60°C in the fume cupboard.
2. The waterbath is then switched off so as to minimise risk of fire from the chloroform fumes.
3. The boats are put in a beaker, and Chloroform added to just cover the boats. The beaker is then placed in the warm waterbath for about 5 minutes with regular swirling.
4. The Chloroform is then carefully decanted into the Waste Chlorinated Solvent bottle (IMPORTANT that the correct waste bottle is used)
5. Acetone is now added to the boats and again warmed for 5 minutes with occasional swirling.
6. The acetone is then carefully decanted into the Waste Acetone Bottle (again important that correct waste bottle is used).
7. The beaker and boats is then placed in an oven at 105° C for ca. 1 hour to dry, before being placed in a dessicator to cool.
**Weighing of Samples**

1. The samples must first be ground to $<425 \mu m$.
2. The balance used is the Sartorius Supermicro in room 360A. This is a very expensive and delicate balance and so initial instruction must be made by a technician. If the pan becomes dirty then consult the technician for cleaning - *it can be very easily broken!*
3. The required sequence is 1 blank, followed by a range of 3 calibration standards, usually EDTA, followed by samples, with the odd check standard.
4. The balance is first set up as follows:
   a) Turn the RH knob to read W - this may need pulling out before turning if it has been left in it’s correct TA position.
   b) Turn the LH knobs from the TA position to read 0 000 when level with the steel button. One knob is turned anticlockwise, the other clockwise.
   c) Position the rocker switch at the RH back to on. The display should now go through a self check and then zero itself.
   d) Ensure that the door to room 360A is closed so as to minimise draughts.
   e) Press the MOTOR key on the keypad. This opens the internal door and swings out the weighing pan. Open the RH door on the balance. Press MOTOR again to return to the weighing pan. Repeat this twice to fully acclimatise the weighing chamber to the room temperature. You are now ready to weigh.
5. Press MOTOR so as to bring the weighing pan out of the weighing chamber.
6. A tin boat is taken using the tweezers provided and carefully placed on the weighing pan.
7. Press MOTOR to return the pan to the weighing chamber and close the external RH balance door.
8. Wait for the reading to stabilise and then press ZERO.
9. Press MOTOR so as to bring the weighing pan out of the weighing chamber.
10. Remove the boat carefully from the weighing pan using the tweezers.
11. Using the micro scoop, add either 20 - 30 mg of sample (about 3 scoops) or up to 15 mg of standard as required.
12. Whilst holding the boat with the tweezers, tap the tweezers so that the sample settles in the bottom of the boat. Put boat in the number 3 hole of the forming block. Using the ‘sugar tongs’ compress the top of the boat to form ‘bomb fins’. (to prevent errors due to slight moisture changes).
13. Return the boat + sample to the weighing pan, close the external door, and press MOTOR to return the pan to the weighing chamber.
14. Wait for the reading to stabilise and note the weight.
15. Press MOTOR to remove pan from weighing chamber, open external door and remove boat using the tweezers.
16. Carefully fold over the ‘fins’ with tweezers and a micro spatula. Taking care not to puncture the boat, form a cube using the tweezers and micro spatula, ensuring that there are no loose edges that could be caught in the injection mechanism of the C/N analyser.
17. Place the boat in the correct number of the transport block. (With practice the forming and folding can be done whilst the next boat is being zeroed.)
18. After all samples have been weighed, the ShutDown of the balance is the reverse procedure - Turn off the rocker switch, put both the LH & RH dials to TA and replace cover.
Eager Programs

Sample Table
This is used to input the details of the samples and also their weights. It can also used to change which sample is the next one to be analysed. There are features such as ‘Fill Table’ which can be used to expedite entries. The filename is usually unique and a different file is produced for each chromatogram. Note that if the file already exists (directory is C:\EAW\DATA\CHNS) then the new filename will automatically be changed by it's first character being updated e.g. art1.dat will go to brt1.dat

1. The first sample in the table is usually a 'bypass'. This is a sample of anything which is used to condition the system and to ascertain just where the Nitrogen and Carbon peaks are being eluted. (see component table below)

2. The next sample is a blank, followed by three standards, and then the unknowns with the odd check standard.

3. The entry of Blank, Bypass, Standard or Unknown is done through the check box that appears. If Standard is chosen then a choice of standards will appear. These all have the percentages of C and N already calculated. At present we are using EDTA but new standards can be chosen, but these would need the Standard Table edited.

4. Finally the weight of each standard / sample is entered

5. Before quitting Sample Table, the sample to be acquired is set to the first sample, by simply clicking in the top Left Hand cell of the sample table and checking the sample to be acquired box that then appears. - this facility can be used to alter which sample is being acquired

6. The sample table can only be saved by saving the complete method -see below

Component Table
This is used to identify and set the times of the expected component.

1. The bypass sample is run (see running of samples below)

2. From the Main Menu, Component Table is clicked and the peak times and names can then be entered.

3. The first peak is Nitrogen - usually ca. 75 sec., with a second Carbon peak at ca. 120 sec.

4. These times can be more precisely using the displayed chromatogram, along with a window setting so that the peak will fall within that window.

If, despite this, some peaks are missed during the actual run, this operation can be still be carried out after the run, and the peaks can then be integrated.

Setting up of Run Times etc.
Care must be taken with adjustments of Run Times as the time set on the instrument must concur with that set in the software.

1. On the Main Menu, click on Integration Parameters. This then brings up a box for analysis time, minimum areas etc., and can be changed by clicking in the relevant box.

2. To change the run time on the instrument itself, press SetUp followed by SetCyc followed by Enter. The run time can then be altered by using the Up/Down arrows.

3. Press SetUp (not Enter) when correct time has been selected.

4. Always have same time in software and machine.

Calculation Parameters - These are set by the default method, the important parameter here is that the calibration is set to K-factor
Running of the Samples

1. The first, most important step is to check which sample is the sample to be acquired, by looking in Sample Table. This can be set as described above by clicking in the Act. Column.
2. Sample Table is then closed.
3. From the main menu, the option View is clicked, followed by Sample being acquired.
4. In the next window, click on Run followed by Start single analysis when doing the initial bypass, or Run followed by Start analysis for all the samples.
5. As the samples are being run, the chromatogram is shown. Scaling of this can be altered via View.

Calculating and Displaying Results

1. It is well worth looking at the baseline and the start / end of peaks. This is performed by following the methods below for recalculation
2. Once the baselines are satisfactory then from the Main Menu, click on Recalculation, followed by Summarise Results. The calculation takes a while, but then displays the results, which can then be exported (via File) to an Excel.XLS file.
3. If peaks are missing, then parameters can be changed in the Components Table, or Integration Parameters (see above) before recalculating by Recalculation - Recalculation - Summarise results. (see below)

Checking of Calibration

From the Main Menu - click on View followed by View Calibration Curve

Saving

1. The parameters and sample table are maintained by saving the method from the main menu. This file (filename.MTH) can then be zipped with the data and text files - see below - it might be worth zipping the .SUM file into this record file as well

ShutDown - The NA2500 C/N Analyser

1. Press SpcFun and then right arrow to get St-By.
2. Press Enter and then Enter again so that it reads Yes.
3. Press SpcFun again to return to temperatures.
4. Note that the standby mode still keeps a trickle of gases going through and so it may be safer, especially for a long shutdown to close the cylinder regulators of both the helium and oxygen.

ShutDown - Computer

1. Close the main Erba window.
2. Tidy up the disc by zipping the .dat files in c:\eaw\data\chns and copying the .zip file to zipfiles (and floppy). Delete the .dat & .txt files.
3. Close down Windows.
4. Switch off computer.
**Recalculation**

Care must be taken with the reintegration option as this will revert the chromatogram to original default settings, overwriting any changes previously made manually.

1. If the zipfile including the .MTH file is transferred to the Pentium Opus (directory C:\eaw\data\chns) and unzipped then recalculation can be performed quite quickly.

2. From the main menu choose *Recalculation - Recalculation*.

3. Click on the boxes marked **Reintegrate**, **Identify Peaks**, **Review Integration**, **Save After reintegration**.

4. Enter the sequence and then click on OK.

5. As the peaks appear, choose the relevant peak by clicking below it, outside of the white section and just in the grey.

6. Set the scale so that the baseline is magnified (from View)- Alt +V,C will set the magnification to fit the current peak, or Alt +V, S will allow settings to be made manually.

7. Set the baseline at the beginning of the peak by Ctrl + F2, positioning the baseline using the mouse and then clicking. The end of the peak is set similarly using Ctrl + F3.

8. Repeat with other peaks until all seems OK.

9. Close the window by clicking on the X in the top RH corner - an error message will then appear asking you to save the file - Click on OK.

10. On the Save Chromatogram Window - click on OK and then Yes for the replacement.

11. **NB** Once amended do not reintegrate that file again or it will revert to the default settings.

12. If all the files are recalculated then a complete summary file will be produced.

13. If only some of the files are recalculated, then a complete summary file is produced by choosing *Recalculation - Recalculation* and then ensure that none of the Integration options boxes are marked with a X. Set up the sample sequence as before from the first to last samples and then click on OK.

14. The summary file can be reviewed by choosing *Recalculation - Summarise results* from the main menu. This is possible on the Opus Pentium computer.
**Problems**

All maintenance should be done in the Std-By mode when the gases will be turned off.

**Leaks**

Perform a leak check as described above in the Start Up section. There is a pressure sensor in the machine, and so it is essential that leak checks are allowed to stabilise for 2 minutes or else there will be natural fall back. To identify the source of any leak, the pneumatic circuit is blanked off, section by section, and checks made, thus identifying the offending section.

**Poor burns / Peak spreading**

The usual cause of this is too much ash in the combustion furnace. When removing this ash take care as the tools do get **Very Hot**.

1. With the machine on Standby, unscrew the sampler and push it back a little.
2. Using our special tool (bung on brass tube), and the vacuum cleaner, vacuum out the loose ash.
3. The more compact ash can be removed by first loosening it with the ‘grauncher’, and then vacuuming out. Take care not to vacuum out the filling of Chromic Oxide (green).
4. Replace the sampler and do a leak check.

**High Carbon Peak**

The usual cause here is a failure of the water trap, with the water peak appearing as a long tail.

1. The remedy is to put the machine on Std-By to shut down the gases
2. Remove the water trap. Refill with magnesium perchlorate. Replace
3. A leak check should then be performed

**High Nitrogen Peak**

The usual cause is depletion of the copper in the reduction furnace. Take care with removal of the tube as it is **Very Hot**, so wear protective glasses and heat resistant gloves.

1. Take off the cover of the sampler (or you will not be able to raise the tube)
2. Unscrew the connecting tube on top of the reduction furnace
3. Take off the furnace compartment cover and remove the support of the reduction furnace.
4. Unscrew the bottom connector of the reduction furnace and remove this and the O-ring
5. The reduction tube can then be lifted clear.
6. Allow to cool on asbestos pads, also covering the tube with pads.
7. When unpacking the tube, try to retain the copper oxide which is at either end. The spent copper can be tapped out of the tube, with the more compact being ‘graunched’ out.
8. Refer to the following diagram from the manual for re-packing the column, with the copper oxide at both ends. This also shows the packing for the combustion furnace.
Easily oxidisable Carbon  (Walkley-Black)

**Chemicals**

- Potassium dichromate 1N (M/6) 98g to 2l
- Ferrous sulphate N/2 (M/2) 139g to 1l + 10ml H₂SO₄
- Barium diphenylamine p-sulphonate 0.1g +16g BaCl₂ to 200ml (Warm to dissolve)

**Method**

The soil is first ground to 0.425mm using a pestle and mortar.

A quantity of soil is then accurately weighed into a 250ml conical flask. The amount of soil to be weighed depends on the estimated carbon content. For highly organic soils the maximum amount is about 40-50mg but this can be raised to near 500mg for more mineral soils.

To each flask is now added accurately 10 ml of the 1N Potassium Dichromate. Blanks containing no soil also have to be done (3 blanks are suggested as this result is critical to the accuracy of the other determinations.)

To each flask is then added 20ml conc. sulphuric acid from a dispenser. This should be done in a fume cupboard wearing eye and hand protection as the reaction can be quite fierce. The flask is gently swirled so that all the soil is in contact with the acid-dichromate mixture and then allowed to gently mix by placing on the orbital shaker for exactly 30 minutes.

After the 30 minutes have elapsed 10ml of orthophosphoric acid are added to each flask (again using a dispenser) and also about 150ml of water.

The blanks are treated in exactly the same manner.

A magnetic follower is now placed in the flask and also a few drops of the indicator. The flask is then placed on a magnetic stirrer (the lmspins are preferred) under a 50ml burette containing the Ferrous sulphate solution. Ferrous sulphate is then added and the colour should go from a muddy brown through a blue stage to a green. As the green stage is approached the ferrous sulphate is best added more slowly. At this point a further 0.5 ml of Potassium dichromate is accurately added and the titration completed until the solution just goes green. The reading is then taken.

**Calculation**

a) Calculation of Ferrous Sulphate strength.

\[ N(Fe) = \frac{10.5}{B} \]  where B is the titre for the blank

b) Calculation of Carbon content

1) Easily oxidisable carbon

\[ \%EOC = \frac{300 \cdot N(Fe) \cdot (B-T)}{W} \]  \[ T = \text{Titre for sample} \]

\[ W = \text{Weight taken in mg} \]

2) Total oxidisable carbon

\[ \%TOC = \%EOC \cdot 1.3 \]
Loss on Ignition

1. Dry crucibles at 105 °C for at least 1 hour
2. Cool in dessicator for 30 minutes.
3. Weigh crucible \((W_C)\)
4. Add sample, say about half full
5. Dry crucible + sample in oven (preferably 105 °C overnight)
6. Cool crucible in dessicator & reweigh \((W_S)\)
7. Place crucible onto the furnace tray and put tray (remember to have the hole nearest you in order to use the extraction tool later on) into furnace at 500 °C - leave for 4 hours
8. Using the special tool, extract furnace tray, and place tray onto heat resistant sheet for at least 5 minutes to initially cool, before placing crucibles in a dessicator to fully cool
9. Reweigh crucible + ash \((W_A)\)
10. If carbonate estimate is to be done, return samples to furnace at 850 °C for 1 hour, cool as above and reweigh \((W_{CA})\)

Calculation: Loss on ignition = \[
\frac{(W_S) - (W_A)}{(W_S) - (W_C)} \times 100\%
\]

Calculation: Carbonate = \[
\frac{(W_A) - (W_{CA})}{(W_S) - (W_C)} \times 100\%
\]
Luminescence Spectrophotometer LS-50B - Solid Sample Analysis

1. Attach cuvette holder on to the Luminescence Spectrophotometer LS-50B and place quartz cuvette filled with fresh deionised water into the holder.
2. Turn on LS-50B on wall.
3. Turn on computer.
4. Wait 15-20 minutes for machine to warm up. In the meantime you can set up the computer…
5. Open up the icon "Fl Winlab"
6. Open up "utilities", then “configuration”. Change the location where the data will be stored to the place that you want. The computer will create any new folders etc., so you can be as specific as necessary (e.g c:\flwinlab\data\andy\1998\feb\30th).
7. Open up "application". Click on "Validate LS50-B, but don't do any more! Once 15 minutes have elapsed…

8. Answer yes to all questions. The computer will check that the machine is correctly calibrated. At the end of this procedure (c. 5 minutes), a data table is presented. The values should all be within the accepted range. If any column says "check", do not proceed and get advice. If all data is okay, enter data into the machine notebook and continue by clicking on “exit”
9. Go back to "application". This time open "scan".
10. Click on "file", click on "open method", click on either:
   a) "solst" - this opens a method designed to analyse solid stalagmite samples.
   b) "bog" - this opens a method designed to analyse peat samples

From now on it is essential that care is taken, as UV light is used in this method. The UV light source in the machine is low power, and thus not too dangerous, but staring at the source and exposure to skin should be avoided.

Methods "solst" and "bog" varies the excitation (input) wavelength of light in the UV range (300-380 nm). The emitted luminescence is measured over the range 390-500 nm (blue light). The program works by starting at the lowest excitation wavelength, and scans over the whole emission ranges. Then it increments the excitation wavelength a little, and scans again, and so on, for the total number of scans specified.

If the LS-50B is to be opened (e.g. to attach the fibre-optic) or the fibre-optic is visible to the naked-eye (e.g when moving the sample), then for health and safety reasons it is essential that the excitation wavelength is set to a value between 410 and 600 nm (i.e. within the visible light range.)

11. Change the value of "excitation" to a value between 410 and 600 nm. Click on the "traffic light". The program will now run.
12. As soon as data appears on screen, click again on the "traffic light". The program will stop.
13. It is now safe to open the front of the machine.
14. Remove the cuvette holder. Replace this with the fibre-optic. A blue or green light should be visible coming out of the end of the fibres - if not, you have done something wrong and UV light could be coming out. Beware!
15. Set up the sample to be analysed under the fibre-optic. Then either
   a) Ensure all lights are turned off and blinds drawn, that the door is locked and that
      you are wearing eye protection or preferably
   b) Black out the sample with the photographic blanket.

16. On the computer, click on "parameters" tab.
   a) Change the value of "excitation" back to the original value.
   b) Double-click on the "filename", and change this to an appropriate name. Note that
      the software only allows for 5 digit filenames and they must not end with a number.

17. Click on the traffic light. Data collection will commence and take 5-10 minutes. During this
    time, you can interpret the previous data collected (see 23 below) or word-process, etc..

18. The traffic light will change colour (to green) once data collection has finished.

19. Click on "parameters" tab, and change the value of "excitation" to a safe value (within the
    range 410-600 nm). Change the filename (or else it will overwrite your new data!)

20. Click on the traffic-light, and wait until some data appears on screen. Click on the traffic
    light again to stop data collection.

21. Go to 15.

22. Repeat until bored!

23. To look at the data analysed,
   a) Open up the "FlWinlab" folder again.
   b) Click on the "application" "3-D View".
   c) "Open" the data that you have just collected. A 3-D graph will be drawn.
   d) Initially, the scale is likely to be incorrect, so click on "view", then "format", and
      change to "contour with false colour", and change the scale from a minimum of 0, a
      maximum of 50, and 10 contours. Hopefully, the graph will now show a peak
      somewhere in the middle.
   e) You can place a cross-hair on the peak (see tools at the top), and thus find the
      location of maximum luminescence.
   f) Write down the values of the excitation and emission wavelengths of this maximum
      luminescence. These values will normally increase with decreasing humification
      (wetter climate).
Luminescence Spectrophotometer LS-50B - Water Sample Analysis

1. Attach cuvette holder on to the Luminescence Spectrophotometer LS-50B and place quartz cuvette filled with fresh deionised water into the holder.

2. Turn on LS-50B on wall

3. Turn on computer

4. Wait 15-20 minutes for machine to warm up. In the meantime you can set up the computer…

5. Open up the icon "Fl Winlab"

6. Open up "utilities". Change the location where the data will be stored to the location that you want. The computer will create any new folders etc., so you can be as specific as necessary (e.g c:\andy\1998\feb\30th).

7. Open up "application". Click on "Validate LS50-B", but don't do any more!

Once 15 minutes have elapsed...

8. Answer yes to all questions. The computer will check that the machine is correctly calibrated. At the end of this procedure (c. 5 minutes), a data table is presented. The values should all be within the accepted range. If any column says "check", do not proceed and get advice. If all data is okay, enter data into the machine notebook and continue….

9. Go back to "applications". This time open "scan".

10. Click on "file", click on "open method", click on "water". This opens a method designed to analyse water samples.

From now on it is essential that care is taken, as UV light is used in this method. The UV light source in the machine is low power, and thus not too dangerous, but staring at the source (i.e. looking directly into the machine when loading / unloading water samples) should be avoided.

Method "water" varies the excitation (input) wavelength of light in the UV range (310-350). The emitted luminescence is measured over the range 390-500 nm (blue light). The program works but starting at the lowest excitation wavelength, and scans over the whole emission ranges. Then it increments the excitation wavelength a little, and scans again, and so on, for the total number of scans specified.

11. Open the machine and remove the cuvette.

12. Empty the distilled water, and fill the cuvette with the water sample to be analysed (shake bottle well before pipetting).

13. Replace the cuvette into the machine. ****Use the same orientation each time. To maintain conformity with previous work, have the cuvette face with writing on facing the operators chair (e.g facing SE)****

14. On the computer, double-click on the "filename", and change this to an appropriate name. Note that the software only allows for 5 digit filenames and they must not end with a number.

15. Click on the traffic light. Data collection will commence and take 5-10 minutes. During this time, you can interpret the previous data collected (see below) or word-process, etc..

16. The traffic light will change colour once data collection has finished.

17. Click on "set-up".

18. Go-to 11. Remember to triple rinse the cuvette and pipette with distilled water between each sample. Run blank samples which contain distilled water every 10-15 samples. Repeat until bored!
To look at the data analysed,

1. Open up the "FlWinlab" folder again.
2. Click on the "application" "3-D View".
3. "Open" the data that you have just collected. A 3-D graph will be drawn.
4. Initially, the scale is likely to be incorrect, so click on "view", then "format", and change to "contour with false colour",
5. Change the scale from a minimum of 0, a maximum of 50, and 10 contours.
6. Hopefully, the graph will now show a peak somewhere in the middle.
7. You can place a crosshair on the peak (see tools at the top), and thus find the location and intensity of maximum luminescence.

Write down the values of the excitation and emission wavelengths of this maximum luminescence.
Short instructions for the Unicam 939 Atomic Absorption Spectrophotometer
- Flame Mode

*These notes are just basic instructions. Contact the technicians if you are new to, or unsure of, anything. Although there are several safety features such as gas interlocks, this is still a dangerous machine (and VERY expensive), so err on the side of caution.*

**Starting Up**

1. Switch on the extraction fan
2. Switch on the gases at the cylinders outside and at the valves behind the AAS, and turn on the compressed air tap
3. Switch on the Atomic Absorption Spectrophotometer
4. Switch on the computer - wait for virus checks
5. Type `win` at C:\ prompt
6. Double click on the SOLAAR icon - wait for this to load and wait further until the word ONLINE appears in the bottom right-hand corner of the screen.
7. The different sub windows are then cascaded. A useful way to rearrange them is to use one of the pre-set positions (Pull down the Window menu followed by Load Positions and choose a file such as `art.fmt`).

**Adding Lamps**

1. The Unicam manual is very good for this. See the Technicians, though, for instructions on which lamps to use, what their lamp currents are to be set to (sheet behind AAS) and how to perform the alignments. The lamp should be kept in their cradles as this is very close to the alignment.
2. It should be noted that a few of the lamps are datacoded and these will automatically register on installation. They still have to be properly aligned.
3. The lamp elements, currents and positions etc. are set by clicking on the (red) lamp icon

**Setting up the Run Parameters (Element, Sequence, Calibration etc) – the Easy way**

The easiest way to set these is to use data from a previous user. This can then be modified for your purpose as detailed in sections below

1. Click on Load – Results and then choose a file from a previous analysis for your element. When this has loaded click on Finished. This removes the results.
2. Perform a Set up Optics (second icon on icon bar)
3. Check the Sequence is what you require and alter as necessary
4. Check the Flame conditions are what is needed
5. Light flame and check a few samples against standards so that you can estimate the best standards (alter sensitivity as required)
6. Check Calibration parameters and alter as required
7. Click on the three green arrows and off you go!
Setting up the Sample Sequence (Single Element only)

1. The Sequence Window allows you to set up a task list for calibrations and samples
2. To call up the Sequence, double click on the Sequence icon (Right Hand side halfway up screen)
3. If this has details from a previous run this can be cleared by dragging the mouse over any unwanted detail and then deleting by pulling down the Sequence menu and clicking on Delete (Alt+N, D)
4. To insert calibrations, samples etc., pull down the sequence menu, followed by Insert (Alt+N,I) Click on down arrow by the combo box which says Blank. Choose what you wish to insert e.g. Calibration, Sample(s) etc.. If you choose samples, drag mouse over the 1 in the number box and enter your number of samples. Then click on Insert
5. Minimise the window, as it is not really needed anymore.

Setting up the Calibration

1. Click on the Calibration Window
2. Pull down the Calibration menu (Alt + C)
3. Click on Parameters. It is advisable to change the Method to Quadratic Least Squares Fit
4. Enter the number of standards, and the values for those standards
5. Click on OK

Choosing the Element

1. Click on the Spectrophotometer Window.
2. Pull down the Spectrophotometer menu (Alt + P )
3. Click on Element and select the element from the Periodic Table.
4. Click on Default Parameters
5. Pull down the Spectrophotometer menu again and this time click on Parameters
6. Check the parameters, especially to see if the background correction is on.
7. Click on OK
8. Again pull down the Spectrophotometer menu and click on Lamps.
9. See which turret position your lamp is in and click on the correct turret position.
10. Wait for turret to get into correct position - this takes a few seconds.
11. Click on OK

Setting up of Flame

1. Click on the Flame Parameters icon on the toolbar (see Help | 939/959Help | Bookmark | Toolbar for a picture of this icon )
2. If you need to change the flame type this can be done by clicking on the down arrow at the side of the combo box
3. Check the fuel flow value
4. When all is set up, click on OK

Setting up of Optics

1. Pull down the Actions menu followed by Set Up Optics ( Alt + A , O ) - or use the second icon on the tool bar
2. Wait until the ONLINE again shows in the bottom right hand corner.
**Lighting of Flame**

1. Press the white flashing Ignite button and hold until the flame ignites.
2. If this does not happen within 15 seconds, or the flame goes out, GET A TECHNICIAN.
3. Wait for a few minutes for the burner temperature to stabilise.

**The Analysis - Running the Sequence**

1. Click on the icon with the 3 green arrows.
2. Wait whilst optimisation of the optics etc. takes place.
3. Follow the prompts for the standard / sample aspirations.
4. You can stop at any time by clicking on Stop on the menu bar.
5. Otherwise continue until the final prompt says that the Analysis has been completed.

**Post Analysis**

1. At the Prompt - Analysis is completed - Click on OK.
2. The Stop on the Menu Bar now becomes ‘Finished’. Do NOT click on this Finished command.
3. Save the results by pulling down the File menu, followed by Save and then Results With Signals (Alt + F, S, W).
4. Type in the results filename (keep the data directory tidy using FileManager afterwards).
5. To put results into suitable formats for Qpro or Excel etc. pull down the File menu, followed by Export Results (Alt + F, X, C), saving as a CSV (comma separated variable) file. This saves the file with quotes and commas as the separators.
6. Now you can click on Finished and go on to your next operation.

**Shut Down**

1. Extinguish flame by pressing the red button on the AAS (both for air / acetylene and also for the nitrous oxide flame).
2. Shut off gases at the cylinders and at the compressed air tap.
3. Switch off the lamps (Spectrophotometer menu | Lamps | click off each lamp in turn).
4. Close down the SOLAAR software (File | Exit (Alt + F, E)).
5. Shutdown Windows (Alt + F4).
6. Switch off computer.
7. Switch off AAS.
**Elements for STAT**

<table>
<thead>
<tr>
<th>I b</th>
<th>II b</th>
<th>III b</th>
<th>IV b</th>
<th>V b</th>
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<td>(Ga)</td>
<td>(Ge)</td>
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<tr>
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<td>Cd</td>
<td>(In)</td>
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<td>Hg</td>
<td>Tl</td>
<td>Pb</td>
<td>Bi</td>
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</table>

**Setting up the STAT**

1. Remove burner head and screw the STAT attachment onto the burner head.
2. Rotate the STAT until it clips down on top of the burner.
3. Rotate the tube until the longer of the two slots is directly above the burner slot (the smaller slot is now on the top).
4. Adjust the burner height down so that the burner head can be refitted.
5. Click on the GFTV and adjust the burner height (and possibly the lateral knob) so that the light is passing through the tube.
6. Click off the GFTV.
7. Rotate the STAT so that it is unclipped and the flame can be lit.
8. Once the flame is lit rotate STAT back to clipped position.
## Lamp Parameters AAS –normal settings

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</table>
Short instructions for the AAS Furnace

1. **Physical Set-up**
   a) Switch on
      i) Furnace Power Supply
      ii) AAS
      iii) Computer
   b) Turn on
      i) Water at tap (green line)
      ii) Argon at cylinder - turn on a full turn

2. **Initial Checks and Alignments**
   a) Check that the autosampler is clean - if not wash it to prevent sticky operation.
      With the furnace removed align the lamps to be used (See Instructions for Flame)
   b) Place furnace into position (Technician job) and align to minimum absorbance reading
   c) Add autosampler and align capillary tip (Actions | Align Capillary Tip) by adjusting knobs on Autosampler, and recheck! Use the GFTV option to set the depth of the probe - this will vary as to the volume injected.

3. **Software Preparation**
   a) **Spectrophotometer Menu**
      **Parameters**
      a) Enter number of resamples (2 should be enough)
      b) Alter Signal to Height
      c) Put background correction on where applicable
   b) **Furnace Menu**
      i) **Parameters**
         Set Cuvette Type - At present this is Normal but check with Technicians
      ii) **Program** -(hopefully this can be short circuited by using programs from our library- Load in appropriate file from File | Load | Analysis Parameters)
         If unavailable then bring up the Furnace|Program, highlight stage and use Insert and/or Edit button to modify the default program
         a) Add 2-stage drying
            (1) 90 °C, 10 seconds, 30 °C/sec ramp
            (2) 110 °C, 10 seconds, 1 °C/sec ramp
         b) And a clean phase at the end. (ca 100-150 °C higher than atomise, 3 seconds, Temperature Control ON, gas flow 1)
         c) Change other temperatures as necessary
            (see Technician, ‘Rubber Book’, Unicam Methods Book)
      iii) **GFTV** This can be used to switch on the GFTV to Analysis
c) **Autosampler Menu**

i) **Parameters**
   a) Set working volume (usually 10 µl)
   b) Set Standard Preparation to Fixed Volume
   c) Set Standard Preparation to Intelligent Dilution
      (This automatically dilutes and re-runs high results)
   d) Go to More and then enter details of Matrix modifier (if used)
      Enter name, volume to be added and Set Method to Wet

ii) **Volumes**
    Displays volumes to be used (useful for checking that the standards are correct)

iii) **Reagent Details**
    This is just a description of the reagents - used when loading the Autosampler (see Sequence)

d) **Calibration Menus**

Parameters
   a) Set the Method (Segmented curve or Quadratic)
   b) Enter the number of Standards
   c) Enter the value for those standards

e) **Sequence Menu**

i) Enter the Sample details - see Instructions for Flame
ii) Check the loading of the Autosampler using ASLG... and load accordingly

4. **Initial Analytical Checks**

Single Solution Checks of Blank, Standards & Samples
   i) In sample details select Calibration
   ii) Select Actions from the top toolbar and then Single solution (This will give an analysis of the blank as a check)
   iii) Repeat this, but this time select Standard
   iv) Watch the signal - especially in the atomise and clean zones to ensure that all the sample is atomised and cleaned out
   v) Again select sequence and this time select a sample
   vi) Repeat the single solution test

5. **Running of samples**

a) Just click on the three green arrows. The AAS will then do an optical alignment before asking you to load ‘Carousel 1’. Click on OK

b) Observation of important criteria can be achieved by loading the positions fur.fmt. (Windows | Load Positions). Right clicking in the signal window will bring up the display parameters. Changing to the atomise and clean phases will be the most useful.

c) When the Analysis is complete, save the results in the normal manner before clicking on Finished

6. **Shut Down**

a) Close all Windows and switch off Computer
b) Switch off AAS
c) Switch off Furnace Supply
d) Turn off Argon at Cylinder
e) Turn off Water at tap
Atomic Absorption Spectrophotometer – M Series (using local menu)

Please see next page for running using the computer

Startup
1. Turn on the acetylene, air and also the extraction fan
2. Turn on the AAS (mains + possibly rocker switch RH rear of instrument) and the printer
3. When AAS has started, press Go (bottom of the 5 RH soft keys)
4. Light flame by holding in the white button on the LH side of the AAS until flame is lit. Allow to stabilise for 10 minutes before doing any analyses. (The button should be flashing when unlit and steady when lit – call technician if not.)

Switching on Lamps
1. Press System Setup and then Setup Lamps
2. In turn select each lamp (highlighted in black) and press Turn Lamp On (Top RH button)
3. Turn Deuterium lamp on (if required) – Bottom LH Key
4. When all necessary lamps are on – Click on done and Done again to return to the Home Menu

Choosing the element and setting up Spectrophotometer and Flame
1. This is automatically done through Run Analysis, but it is useful to do it before and it is necessary in order to get the flame set at the right angle.
2. With the flame lit, aspirate the blank
3. Click on Element Sets, choose the correct element, then Setup (top RH key)
4. Click on Setup Spectrophotometer and then Setup Optics
5. When setup, aspirate the top standard and rotate the burner head until the absorbance reads .850 to .950.
6. Click on Done and then Done again to return to the Home Screen

Running the Analysis
1. Click on Run Analysis
2. Check adequate number of samples (more than your run) click on next
3. If the active element and method is the one you want, then go to step 5
4. To change active element, first click on Remove to leave a blank window. Then Click on Insert and choose from the list of pre-prepared sets – press OK.
5. Click on OK
6. Wait until ‘Base Sample name’ appears (change if required, but it takes ages)
7. Click on OK
8. Wait for instrument to set itself up, then when prompted do blank and standards followed by the samples.
9. When samples are completed, press stop
10. To autozero if drift (look at absorbance in top RH of screen), press Pause, then Actions, Autozero then Continue.
11. To see results click on Done then View Results (they can be printed from this page). This is the way to overcome printer problems as the results stay in memory at this time.

Switching Off
1. Press the red button (LH side of AAS) to extinguish the flame
2. Switch off acetylene and air at taps
3. Hold the red button for about 15 seconds until you hear a faint click – then release and you should hear the lines being purged
4. Switch off the AAS and the printer
5. Switch off the air extraction
Atomic Absorption Spectrophotometer – M Series (using SOLAAR AAS PREDEFINED METHOD Software)

1. Switch on the computer and the AAS.
2. Click on the SOLAAR icon and input the password (if you do not have one set up for you, then username ADMINISTRATOR and password SOLAAR will work).
3. When connected the AAS will show ON LINE at the bottom right of the screen.
4. Turn on the extractor fan and the gasses then ignite the flame by pushing the white button on the front of the AAS.
5. When using a predefined method the wizard on screen may be closed down.
6. Turn the required lamps on using the icon on the toolbar (6th from right), then close.
7. Go to FILE and select new results, enter filename and save. All data is now saved to this file until it is closed and a new filename entered.
8. Go to the methods folder on the toolbar (5th from right). Select the required element from those listed and load. At this stage it is possible to change any of the method parameters such as standards, wavelength etc. When ready select O.K.
9. Select the run icon (12th from right) this will then tune the lamp and optics.
10. Enter analysis name and any details then click analysis and aspirate with water as prompted. At the point where the blank is asked for DO NOT press O.K. but instead aspirate the top standard and angle the burner to give 0.6-0.7 absorbance units. When this has been done aspirate the blank (water) and click O.K.
11. Aspirate standards and samples as prompted (here you may give individual sample ID’s as required).
12. When finished, click on stop (at this point the data is automatically saved).
13. To change element, select the method folder icon or to run more of the same element use the run icon and repeated as above.
14. At the end of the session select file and then close the results.

DATA REMOVAL

1. Go to file select open results (for the required filename).
2. Open the Wizard (4th icon from the right).
3. Select view results and follow the prompts to add the desired results to the results window, close the wizard. Check all the required results are in the results table window then go to file and export the results as a CSV file.
4. Select any additional details required (none for just results, ID and signals).
5. Allocate a filename and save the data.
Cation Exchange

Reagents

M/2 Ammonium Acetate pH = 7
120 ml of 0.88 ammonia
+ 1 l distilled water
+ Acetic acid to pH = 7 (ca 115-120 ml)
+ distilled water to 2 l

Standards for AAS
A mixed matrix standard is made from 1000 ppm standard diluted with Ammonium Acetate to give

K = 0 - 30 ppm
Mg = 0 - 10 ppm
Ca = 0 - 60 ppm
Na = 0 - 10 ppm

Method

About 10 gm (dry equivalent) of fresh soil is weighed accurately into a 250 ml beaker and 100 ml of the Ammonium acetate solution is added and left to stand overnight. The solution is then filtered through a 542 filter, collecting the filtrate in a 200 ml standard flask. The residue is then leached with successive washings of the Ammonium acetate until 200 ml has been collected. This is then retained for analysis on the Atomic Absorption Spectrophotometer. Using the mixed matrix standard, the AAS is set up for Na at 589 nm, Ca at 422.7 nm, Mg at 285.2 nm and K at 766.5 nm respectively, using the air/acetylene flame throughout. Interference can be minimised by use of lanthanum chloride, or by emission mode for K and Na.

Calculation

\[ N = 1 + \frac{\text{Moisture}}{100} \]

\[ \text{cation (meq/100g)} = \frac{N \times a \times y}{10 \times E \times w} \]

where

- \( a \) = ppm of cation
- \( y \) = ml. of extract
- \( E \) = Equivalent Weight of cation
- \( w \) = gm of wet soil

\[ E(K) = 39.1 \quad E(Na) = 23.0 \]
\[ E(Mg) = 12.15 \quad E(Ca) = 20.04 \]
Iron Aluminium and Manganese using Pyrophosphate / Dithionite extraction

**Reagents**

**Pyrophosphate**

33 gm potassium pyrophosphate to 1 l with distilled water.

**Acetate buffer**

33 gm anhydrous sodium acetate
+ 160 ml glacial acetic acid
+ distilled water to 1 litre

**Dithionite/acetate**

4 gm of sodium dithionite is added to each 100 ml of acetate buffer. (Make fresh just before extraction).

**Fe/Al AAS standards**

Using standard 1000 ppm solution, dilute with the extracting media to give the ranges:

- Fe 0 - 100 ppm
- Al 0 - 20 ppm
- Mn 0 - 20 ppm

**Method**

Approximately 1 gm (or dry equivalent) of soil is accurately weighed into a 250 ml centrifuge bottle.

To each bottle is accurately dispensed 100 ml of the pyrophosphate reagent and shaken - usually overnight - for 16 hours.

The bottles are then centrifuged at 2500 rpm for 20 minutes, and the supernatant is decanted and stored in a clean glass bottle for analysis.

The residue is then washed with 50 ml of distilled water and then centrifuged at 2500 rpm for 20 minutes. This supernatant is discarded.

100 ml of the dithionite/acetate buffer solution is then accurately dispensed to the residue and again shaken overnight.

The bottles are then again centrifuged at 2500 rpm for 20 minutes and the supernatant decanted and stored for analysis.

The analysis is performed on the Atomic Absorption Spectrophotometer with each extract - using the standards specific to that extract. Fe is determined at 248 nm using an air/acetylene flame, whilst Al requires a nitrous oxide/acetylene at 309 nm. (Care is needed for this flame - see manual).

**Calculations**

\[
\text{ppm in soil} = \frac{\text{ppm Fe or Al in extract} \times \text{extraction volume}}{\text{Wt. of soil}}
\]

\[
\text{Fe (total)} = \text{Fe (pyro.) + Fe (dith.)}
\]

\[
\text{Activity Ratio} = \frac{\text{Fe (pyro.)}}{\text{Fe (total)}}
\]
Estimation of Iron and Manganese by Acid Ammonium Oxalate Extraction

N.B. This extraction must be done under DARK conditions. Also precautions must be observed as to the poisonous nature of the extractant.

Reagents

Acid Ammonium Oxalate 126 gm Oxalic acid + 4.5 l of distilled water
Add Ammonia until pH = 3.
Make up to 5 l with distilled water.

Fe standards for AAS : Using 1000 ppm Fe standard, dilute to give range between 0 - 100 ppm with the Acid Ammonium Oxalate

Mn standards for AAS : Using 1000 ppm Mn standard, dilute to give range between 0 - 10 ppm with the Acid Ammonium Oxalate

Method

Approximately 1 gm of soil (or dry equivalent) is accurately weighed into a 250 ml. centrifuge bottle.

100 ml. of the Acid Ammonium Oxalate solution is then accurately dispensed into each centrifuge bottle, which is then sealed and shaken for 4 hours in the dark.

The bottles are then centrifuged at 2500 rpm for 20 minutes, and the supernatant is decanted into a clean glass bottle before being stored, in the dark, prior to analysis.

The Iron (Fe) is measured on the Atomic Absorption Spectrophotometer at 248 nm using an air/acetylene flame. Care must be taken to flush the nebuliser regularly between samples to prevent the oxalate crystallising.

Manganese (Mn) is measured on the AAS at 279 nm, again using an air/acetylene flame

Calculation

\[
\text{ppm Fe (oxalate)} = \frac{\text{ppm Fe in extract} \times 100}{\text{Wt. of soil}}
\]
Heavy Metals by Atomic Absorption Spectroscopy

Reagents
1. Conc. Nitric Acid
2. Conc. Hydrochloric Acid
3. Mixed matrix standard 
   Zn, Pb, Cr, Cu 
   This is made from 1000 ppm standard diluted with a 
   solution containing 
   12 ml conc. nitric acid 
   + 2 ml conc. hydrochloric acid 
   to 100 ml with distilled water 

It depends upon estimated results Ours is :-

<table>
<thead>
<tr>
<th>Element</th>
<th>ppm Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0 - 10</td>
</tr>
<tr>
<td>Pb</td>
<td>0 - 4</td>
</tr>
<tr>
<td>Cu</td>
<td>0 - 4</td>
</tr>
<tr>
<td>Cr</td>
<td>0 - 4</td>
</tr>
</tbody>
</table>

Method

All glassware must be scrupulously clean, and preferably acid washed.

Between 0.5 - 1.0 gm of sediment is accurately weighed into a 100 ml beaker.

3ml of conc. nitric acid is then added and the beaker warmed to dryness on a hotplate. The beaker is then removed from the hotplate and 3ml of conc. nitric acid is again added, together with 0.5 ml of conc. hydrochloric acid. This is now warmed until the appearance of the nitrogen dioxide brown fumes. The beaker is then removed from the hotplate to cool.

The contents of the beaker are then transferred with as little distilled water as possible to a 50ml centrifuge tube and centrifuged at 2500 rpm for 20 minutes. The supernatant is decanted into a 25 or 50 ml standard flask, and the residue washed with a small amount of distilled water. This is again centrifuged at 2500 rpm for 20 minutes, with the supernatant again being added to the standard flask. The flask is then made up to volume with distilled water, and the solution retained for analysis on the Atomic Absorption Spectrophotometer.

An air/acetylene flame is used for all the elements, except for Chromium, where we use an acetylene/nitrous oxide flame. (The graphite furnace does increase the sensitivity and detection limits greatly - see separate instructions on usage)

The wavelengths used are :-

Zn  213.9  Pb 217.0  Cu 324.8  Cr. 357.9

Calculation

\[
\text{ppm in sediment} = \frac{\text{volume} \times \text{ppm of solution}}{\text{weight of sediment in gm}}
\]
MARS Microwave digestor

The microwave digestor is used to heat samples with acid in a closed vessel at high pressures resulting in better extractions of metals etc. Care must be taken that this is done properly as improper use would be highly dangerous (both with the microwaves and also the pressurised containers holding hazardous chemicals) and also could lead to very expensive damage.

Therefore no-one is to use this without permission and training.

Weighing of samples

There are a maximum of 12 vessels that can be used at any one time. One of these vessels MUST be the ‘control’ vessel which is slightly larger than the others and has ability to be connected to the pressure monitor and temperature probe.

When doing a run, every vessel must have similar contents i.e. approximately same amount of soil and acid(s). This is so that the measurements in the control vessel are meaningful for the other vessels.

By the same token, blanks must not be done at the same time as samples but instead separately in a different run.

The first sample is weighed into the dry liner of the ‘control’ vessel as follows.

1. Tare out the weight of this Teflon liner.
2. As there is sometimes static electricity associated with the Teflon and it is imperative that the sample is at the bottom and not on the edge, create a paper liner by rolling up some paper and push this right into the Teflon liner before adding the sample. Then withdraw the paper before weighing to give the sample weight.
3. Suggest 1 – 2 gm for our soils.
4. Repeat with the other Teflon liners as required, but always having approximately the same weight of soil.

Addition of acid

In fume cupboard pipette out the acid as required. Suggest 9ml Nitric acid and 1ml Hydrochloric acid. Same amount must be put into each liner.

Microwave turntable

The turntable is removable, but on replacement, make sure that the spacer is fitted in the back left of the chamber.
**Construction of 'Control' vessel**

1. Slip liner into Kevlar sleeve – Take care not to get this sleeve wet.
2. Add safety membrane to grey cap. These membranes should not be used more than 3 or 4 times. Screw grey cap onto side of side arm (quite tightly) on the cap.
3. Check tightness of top knurled nut and inner knurled nut in cap – should be hand tight. Place cap into liner
4. Put white spacer on top knurled nut with the side of the spacer that is recessed uppermost.
5. Put beige spacer into this recess with the side of the beige spacer that is beige uppermost
6. Put liner into wide part of frame and twist clockwise (looking from top) until side arm touches edge of frame
7. With one hand positioning liner so that it fits neatly on the bottom teflon disc of the frame, hand tighten the hexagon nut on top of the frame.
8. If everything is correctly in place, tighten the hexagon with the torque spanner until that clicks.
9. Attach the pressure hose to the top connector on the side arm of the cap (light hand tight). Place fibre optic temperature probe through hole in hexagonal nut and into the liner. CARE – these probes are expensive and easily damaged – do not bend or force it
10. Open the microwave (grey button on top of microwave) and place controller into position 1 (lug at back of frame fits into its slot in carousel)
11. Holding the white section of the temperature fibre optic, push this into the fitting in the roof of the microwave chamber.
12. Push the end of the pressure hose into the connection top right of the microwave chamber and push and twist until you hear a click
13. Secure the pressure hose by use of the little white clip in the roof of the chamber.
14. Check the movement of the carousel by pressing the button with circular arrow (pressing this button toggles carousel between stop / start)

**Construction of standard vessel**

1. Slip liner into Kevlar sleeve – Take care not to get this sleeve wet.
2. Add safety membrane to grey cap. These membranes should not be used more than 3 or 4 times. Screw grey cap onto side arm (quite tightly) on the cap.
3. Place cap into liner
4. Put beige spacer into recess of cap with the side of the beige spacer that is beige uppermost
5. Put liner into wide part of frame and twist clockwise (looking from top) until side arm touches edge of frame
6. With one hand positioning liner so that it fits neatly on the bottom teflon disc of the frame, hand tighten the hexagon nut on top of the frame.
7. If everything is correctly in place, tighten the hexagon with the torque spanner until that clicks.
8. Open the microwave and place frame into relevant position (lug at back of frame fits into its slot in carousel) – if less than 12 vessels then have them spaced out
9. Movement of the carousel is achieved by pressing the button with circular arrow (pressing this button toggles carousel between stop / start)

**Digestion of Samples**

1. Switch on Microwave at mains and allow it to go through the initialisation screens until the CEM METHOD MENU is shown. The current menu is at the bottom of the screen.
2. Choose the method most relevant to your number of samples. To load a different method, highlight the ‘Load Method’ by pressing the RH grey arrow soft key and then the soft key marked SELECT. Select ‘User Directory’ again with the RH Key and Select. Scroll down to the chosen method and press SELECT
3. Press start
4. Periodically check that control is showing correct pressure and temperature
5. After digestion allow vessels to cool as long as is needed. *(Take extreme care as they are still under pressure)*
Removal of Sample – control vessel  (Take extreme care as they are still under pressure)

1. Holding the white portion, pull down the temperature optic fibre from its slot in the roof of the microwave chamber
2. Unclip the pressure tube and pull tube out of the slot on the right of the chamber
3. Remove vessel from microwave. Remove temperature optic fibre and place in its box – disconnect the pressure tube
4. Take vessel to fume microwave and relieve pressure by slightly slackening the membrane cap on the side arm
5. Slacken hexagon nut with torque wrench remove vessel from frame and dismantle taking care not to get the Kevlar collar wet.
6. Pour contents of Teflon liner into the centrifuge tube – rinse and add to tube

Removal of Sample – other vessels  (Take extreme care as they are still under pressure)

1. Remove vessel from microwave.
2. Take vessel to fume cupboard and relieve pressure by slightly slackening the membrane cap on the side arm
3. Slacken hexagon nut with torque wrench remove vessel from frame and dismantle taking care not to get the Kevlar collar wet.
4. Pour contents of Teflon liner into the centrifuge tube – rinse and add to tube

Cleaning of Teflon liners

The liners can be washed as per normal with detergent. Nitric stains can be removed by periodically (say 20 runs) heating the tubes overnight at 140 °C. New liners should be baked before use

Methods (Optimum specification)

1 –3 vessels 300w
4 – 8 vessels 600w
6 – 12 vessels 1200w
(possibly choose 600w for 4+ and longer times)
% power 100%, Ramp could be 5 minutes, but for better instrument life, 12 –15 minutes
PSI = 300 for soils
°C 200 – hold 20 minutes

Use blue membranes which blow at 500
Sequential Extraction of Heavy Metals

*Note: Hazardous chemicals are used in these extractions. The digestions must be carried out in a fume cupboard and full personal protection worn. Consult the technician before starting for full COSSH assessments especially relating to the use of Hydrofluoric acid*

**Chemicals**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Chloride</td>
<td>1M</td>
<td>203.3 gm/dm³</td>
</tr>
<tr>
<td>Sodium Acetate</td>
<td>1M</td>
<td>82.03 gm/dm³ adjusted to pH=5 with acetic acid</td>
</tr>
<tr>
<td>Hydroxylamine hydrochloride</td>
<td>0.04M</td>
<td>2.596 gm/dm³ in 25% (v/v) acetic acid</td>
</tr>
<tr>
<td>Ammonium Acetate</td>
<td>1.2M</td>
<td>92.946 gm/dm³ in 20% (v/v) nitric acid</td>
</tr>
<tr>
<td>Hydroflouric Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric Acid</td>
<td>0.02M</td>
<td>1.3 ml/dm³ in 1 litre</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Specialised equipment** - Teflon Beakers, 25 ml. plastic volumetric flasks

**Method**

1. **Magnesium Chloride extract**
   a) Weigh 1 gm of dry sample into a plastic centrifuge tube
   b) Add 8ml 1M Magnesium Chloride
   c) Shake for 1 hour
   d) Centrifuge at 2500 rpm for 20 minutes
   e) Store supernatant

2. **Sodium Acetate extract**
   a) To residue add 8 ml. 1M Sodium Acetate
   b) Shake for 5 hours
   c) Centrifuge at 2500 rpm for 20 minutes
   d) Store supernatant

3. **Hydroxylamine hydrochloride extract**
   a) To residue add 20 ml. 0.04M hydroxylamine hydrochloride
   b) Heat in waterbath at 96°C ± 3°C for 6 hours
   c) Centrifuge at 2500 rpm for 20 minutes
   d) Store supernatant

4. **Ammonium Acetate extract**
   a) To residue add 3ml of 0.02M Nitric Acid + 5ml of 30% Hydrogen peroxide adjusted to pH=2 with Nitric acid
   b) Warm in a waterbath at 85°C ± 5°C for 2 hours
   c) Add 3ml 30% Hydrogen peroxide (adjusted to pH=2 with Nitric acid)
   d) Warm in a waterbath at 85°C ± 5°C for 3 hours
   e) Cool and add 5ml 1.2M ammonium acetate in 20% (v/v) Nitric acid
   f) Dilute to 20 ml and shake for 30 minutes
   g) Centrifuge at 2500 rpm for 20 minutes
   h) Store supernatant
**Hydroflouric acid extract**

i) Transfer residue to Teflon beaker with minimum amount of distilled water

j) Add 2ml. of conc. Hydrochloric acid , 6ml. of conc. Nitric acid & 3ml. of 30% hydrogen peroxide

k) Digest on a hotplate in a fume cupboard until almost dry

l) Add 3ml. conc. Nitric acid , 10 ml. Hydroflouric acid, & 3ml. 30% Hydrogen peroxide

m) Digest on a hotplate in a fume cupboard until almost dry

n) Add 1ml. conc. Nitric acid , 3 ml. Hydroflouric acid, & 1ml. 30% Hydrogen peroxide

o) Add 20ml. distilled water

p) Digest on a hotplate in a fume cupboard until about 15ml. volume

q) Dilute to 25ml with distilled water

r) Transfer to centrifuge tube

s) Centrifuge at 2500 rpm for 20 minutes

t) Store supernatant

**Analysis**

All analyses are performed on the Atomic Absorption Spectrophotometer. (see separate method sheet)

**References**


Dawson & Macklin (unpub.)
**Estimation of Suspended Sediment**

*Equipment*
- Glass fibre filters (Whatman GF/C 125mm diameter)
- Hartley pattern filtration system
- Measuring cylinder
- Vacuum Pump
- Balance (0.1 mg sensitivity)
- Oven (105°C)

*Method*

1. **For each filter**
   a) Assemble Hartley pattern filtration system with clean filter  
   b) Remove loose fibres in filter by washing with 100ml of distilled water under vacuum  
   c) Place filter in oven at 105°C for at least 1 hour  
   d) Remove filters in oven and cool in dessicator for about 10 minutes  
   e) Mark filters with a pencil and weigh them accurately (W₁)

2. Measure 500ml of river water containing sediment. This volume can be varied depending on the expected sediment concentration - too much sediment can block filters - too little will not be accurate, but note volume taken (V)

3. Assemble Hartley pattern filtration system with marked weighed filter

4. Slowly add the river water with as little vacuum as possible to avoid blocking. Increase vacuum as necessary

5. When all the water has passed through the filter, turn off vacuum, remove filter and dry in oven at 105°C (time depends on quantity of sediment)

6. Cool filters in dessicator and reweigh (W₂)

   \[ \text{Sediment concentration (ppm)} = 10^6 \times \frac{(W₂ - W₁)}{V}. \]
KCl Extraction for Nitrate / Nitrite / Ammonium from Soils

Reagents

2M Potassium Chloride 149.1g to 1 litre

Method

• Weigh 10 gm of soil in a 250 ml centrifuge bottle.
• Add exactly 100 ml 2M Potassium Chloride
• Shake for 1 hour
• Filter through Whatman 42 (or 542) discarding first 5 ml
• Collect enough liquid to perform analyses on the AutoAnalyser
BRAN+LUEBBE CONTINUOUS FLOW AUTOANALYSER

1. Switch the system on starting with the XY sampler, pumps, heating baths but not the colorimeters
2. Place all tubing in deionised water and run for about 5 minutes
3. After this time place the tubes marked Brij and SDS (aresol 22) into the wetting agents
4. Make up the chemicals as required for each of the analysis that are being run and place each of the marked tubes into the corresponding reagent. Allow the system 30 minutes too come to equilibrium.
5. Turn on the computer followed by the colorimeters, at this point the pumps will stop.

USING THE AACEV 5.40 SOFTWARE

1. Double click the AACE desktop icon to open the programme. The last active analysis will be shown in a window on the bottom left corner.
2. Select Charting and if this is the required analysis select O.K. If not, select Cancel, which will open the select analysis option. Double-click the required analysis folder and select O.K (for setting tray protocol etc see below).
3. In either case the system will then connect to the AA3 (after a 30 second delay).
4. When prompted for a Download, select NO. After a further delay the system will prompt for a further download again select NO. The system will now enter charting mode.
5. Wait for the baseline to appear on the charts, then select each in turn by placing the mouse arrow over the chart and use the right hand click to select SET BASELINE (in the case of Alkalinity select INVERSE CHEMISTRY first).
6. Next run the Primer (position 91). On the X Y sampler select manual, press 9 1 Enter then 120(sec.) Enter and Run.
7. It will take about 10 mins for the primer to appear on the charts, at which point each window should be selected in turn using a right click on the mouse and selecting SET GAIN (the primer must be drawing a flat peak when this is done) to set full scale deflection.
8. Once the charts return to baseline (NOT BEFORE) the analysis may be started.
9. For ALKPHOSTONCL quick start Maybe selected (the running man icon). For other methods see **below
10. The quick start window will open at this point it is necessary to set the end sample number (between 1 and 36) and the site ID (under comment).
11. It is also possible to deselect any of the channels by selecting 1, 2, 3 or 4 in the lower portion of the window.
12. Once all the choices are made click OK and the system will start.
13. In the case of any different analysis run start will have to be selected and a previously created run including the tray protocol recalled.

** If a different analysis (e.g. Sulphate) is required it will be necessary to change the active analysis. To do this, select charting and when prompted cancel the active analysis and choose the new analysis from the directory. A new run must now be created (quick start only works for ALKPHOSTONCL) and a unique tray protocol created from the template. This will involves changing the number of samples used, this is done in TRAY PROTOCOL using the delete option.
Determination of Nitrate on the Old Autoanalyser

Standards

1000 ppm stock solution  1.6308g Potassium Nitrate to 1 litre distilled water

<table>
<thead>
<tr>
<th></th>
<th>High Range</th>
<th>Low Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ppm * 2</td>
<td>5 ppm * 2</td>
<td></td>
</tr>
<tr>
<td>30 ppm * 1</td>
<td>3 ppm * 1</td>
<td></td>
</tr>
<tr>
<td>20 ppm * 1</td>
<td>2 ppm * 1</td>
<td></td>
</tr>
<tr>
<td>10 ppm * 1</td>
<td>1 ppm * 1</td>
<td></td>
</tr>
<tr>
<td>5 ppm * 1</td>
<td>0.5 ppm * 1</td>
<td></td>
</tr>
</tbody>
</table>

Reagents

Copper Sulphate stock solution  3.9 gm Copper Sulphate to 1 litre Distilled water

A) 0.2N Sodium Hydroxide  4.4 gm to 500ml dist. water + 2 ml Brij

B) Copper Hydrazine  1.5 gm Hydrazinium Sulphate
+ 3 ml Copper Sulphate soln.
to 1 litre with distilled water

C) Sulphanilamide  10 gm Sulphanilamide
100 ml Orthophosphoric Acid (low in nitrite)
0.5 gm NEDD
to 1 litre with distilled water

* NEDD is N-1-NaphthylEthlyeneDiamineDihydrochloride

Procedure

1. Switch on Transformers and Colorimeter.
2. Place relevant tubing into the solutions A B & C.
3. Start pump.
4. Switch on Chart recorder - Wait for stable baseline.
5. Load tray with standards and samples.
7. Press Power On on Sampler.
8. The first peak appears after ca. 18 samples.
9. Transfer the standards to the end of the sample batch to bracket the calibration.
10. Place the autostop in line with last standard.
11. At end of runs place all tubing in water to wash lines for about 10 minutes before switching off.

Settings

Use a 520 nm filter.

<table>
<thead>
<tr>
<th></th>
<th>High Range</th>
<th>Low Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td>Jim's machine</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Schematic Diagram for Low Nitrate

A  Sampler  Orange/orange
B  Sodium Hydroxide  Orange/orange
C  Water  White/white
D  Copper Hydrazine  Black/black
E  Sulphanilamide  Yellow/orange
X  Dialyser
Y  Waterbath
Z  Colorimeter
Ammonia in Waters and KCl by Old Autoanalyser

**Standards**

1000 ppm NH$_4$-N

| Ammonium Chloride | 3.8189 gm |
| Distilled Water   | 1000 ml   |

Use 4.3.2.1.0.5 and 0.25 ppm NH$_4$Cl-N

**Reagents**

A) Hypochlorite

- Teepol Bleach (4% available chlorine) 15 ml
- Distilled water 135 ml
- Wetting agent (Aerosol 22) 1 drop

B) Nitroprusside

- tri-Sodium Citrate 12.0 gm
- Sodium Salicylate 5.26 gm
- Sodium Nitroprusside 0.15 gm
- ** tri-Sodium Orthophosphate 6.0 gm (Waters)
  ** 1.5 gm (CaCl/KCl extracts)
  ** 12.0 gm (acid digests)
- Distilled Water 300 ml
- Wetting agent (Aerosol 22) 1 drop

C) Wash Water

- Wetting Agent (Aerosol 22) 15 drops / litre

**Procedure**

1. Switch on Transformers and Colorimeter
2. Place relevant tubing into solutions A B & C.
3. Start pump. Switch on chart recorder and wait for stable baseline.
4. Load tray with standards and samples.
5. Line 1st standard with marker on autosampler.
6. Press Power on Sampler.
7. The first peak appears after about 7 samples.
8. Transfer the standards to the end of the sample batch to bracket the calibration.
9. Place the autostop in line with the last standard.
10. At end of run place all tubing in water to wash lines for about 10 minutes before switching off.

**Settings**

- Use a 660 nm filter
- Gain between 1 and 2 (actually 7.4 on present pot)
- Use the 6 place adjustable cam.
# Analysis of Waters for Phosphorous using Ammonium Molybdate / Ascorbic Acid

## Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 ppm P solution</td>
<td>4.3943 gm KH$_2$PO$_4$ to 1 litre with water</td>
</tr>
<tr>
<td>5 ppm P solution</td>
<td>5 ml of 1000ppm P to 1 litre with water</td>
</tr>
<tr>
<td>4.0 N Sulphuric acid</td>
<td>56 ml conc. H$_2$SO$_4$ to 500 ml with water</td>
</tr>
<tr>
<td>Ammonium Molybdate solution</td>
<td>6.4 gm Ammonium molybdate to 200 ml water</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>20 gm ascorbic acid in 200 ml water (Make fresh daily)</td>
</tr>
<tr>
<td>Potassium Antimony Tartrate</td>
<td>1.08 gm APT in 100 ml water</td>
</tr>
<tr>
<td>Colorimetric Reagent</td>
<td>375 ml of 4.0 N H$_2$SO$_4$</td>
</tr>
<tr>
<td></td>
<td>110 ml of Ammonium Molybdate soln</td>
</tr>
<tr>
<td></td>
<td>36 ml of APT solution</td>
</tr>
<tr>
<td></td>
<td>150 ml of ascorbic acid soln</td>
</tr>
<tr>
<td></td>
<td>+ distilled water to 750 ml</td>
</tr>
</tbody>
</table>

## Method

20 ml. of the water to be analysed is pipetted into a 100 ml. flask. To this is added 5 ml. of the colorimetric solution and allowed to develop for 60 min. - 2 hours.

A series of standard solutions of 1, 2, 3, 4, & 5 ml of 5 ppm P are pipetted into 25 ml volumetric flasks. To these are added 5 ml. of the colorimetric solution and made up to the mark with distilled water. Again these are allowed to develop for 60 minutes - 2 hours.

The absorbance of each solution is then read at 890 nm on the UV - Vis spectrophotometer.

The ppm P can then be calculated using the absorbance of the standards as calibration.
The following procedures allow the calculation of various fractions of sediment bound phosphorus. This enables the characterisation of the forms of phosphorus forms and will aid studies on phosphorus dynamics within the sediment. The methods described are all relatively simple and do not require the use of sophisticated equipment.

The procedures included in this handbook include the determination of soluble phosphorus concentration and fractionation of sediment bound phosphorus into:

- Total phosphorus
- Algal available phosphorus
- Non-apatite / apatite phosphorus
- Inorganic / organic phosphorus
Phosphorus Analysis Of Water Samples

High Phosphorus Concentrations (>0.05 Mgl⁻¹ P₀₄-P)

SWQRG METHOD 1

REAGENTS
1. Ammonium molybdate solution : 6.4g Ammonium molybdate to 200 ml water. Store solution in glass bottle in a refrigerator. The solution is stable for several months.
2. 4.0N Sulphuric acid : 56 ml conc. H₂SO₄ to 500 ml with water. Store in refrigerator for up to 2 months.
3. Potassium Antimony Tartrate : 1.08g APT to 100 ml water. Refrigerate.
4. Ascorbic acid : 20g ascorbic acid to 200 ml water. Make fresh each day.

STANDARDS
1000 mgl⁻¹ P solution : 4.3943g KH₂PO₄ (oven dried to 110°C) to 1 litre of water.

PROCEDURE
1. Prepare colorimetric solution;

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>250 ml</th>
<th>500 ml</th>
<th>750 ml</th>
<th>1 litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0N H₂SO₄</td>
<td>125 ml</td>
<td>250 ml</td>
<td>375 ml</td>
<td>500 ml</td>
</tr>
<tr>
<td>Molybdate</td>
<td>37 ml</td>
<td>74 ml</td>
<td>111 ml</td>
<td>148 ml</td>
</tr>
<tr>
<td>APT</td>
<td>12 ml</td>
<td>24 ml</td>
<td>36 ml</td>
<td>48 ml</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>50 ml</td>
<td>100 ml</td>
<td>150 ml</td>
<td>200 ml</td>
</tr>
<tr>
<td>Water</td>
<td>27 ml</td>
<td>54 ml</td>
<td>81 ml</td>
<td>108 ml</td>
</tr>
</tbody>
</table>

This solution must be made fresh each day.

2. Add to a 50 ml centrifuge tube
   i. 5 ml colorimetric solution
   ii. 5 ml sample
   iii. 5 ml water

3. Use standards of range 0.0, 0.05, 0.1, 0.5, 1.0, 5.0 mgl⁻¹ P₀₄-P.

4. Allow colour development between 30 to 120 minutes.

5. Measure absorption at 890 nm on SP6 uv/vis spectrophotometer.

SOURCE

SWQRG/MAR/96
The Determination Of The Sediment Bound Algal-Available Phosphorus

SWQRG METHOD 2

INTRODUCTION
The amount of phosphorus available to algae in aquatic ecosystems is the main determinant of eutrophication (Schlindler, 1977). This available fraction exists as inorganic dissolved phosphorus and as a proportion of phosphorus sorbed on sediment particles. In most natural fluvial environments, the majority of phosphorus entering the system is sediment bound. Therefore, a measure of the proportion of sediment associated phosphorus must be determined to produce more realistic estimates of the amount of the algal available phosphorus (Paa).

Traditionally, the Paa fraction is estimated by bioassays (Dorich et al., 1980, Williams et al., 1980b) but this method is both time consuming (around 14 days, though Hegemann et al., 1983, suggests 100 days to produce an accurate result) and requires specialist expertise. Williams et al. (1980b) report that apatite phosphorus and organic phosphorus could not support algal growth, but a fraction of the non-apatite phosphorus (phosphorus sorbed onto iron and aluminium hydrous oxides) extracted by 0.1M NaOH could support algal growth. Dorich et al. (1985) compared several methods that have previously shown to have relationships with Paa with 2 day and 14 day bioassays; sequential NaOH and HCl (Sagher et al., 1975), sequential NH₄F, NaOH, HCl (Dorich et al., 1980), NTA (Goltermann, 1976) and anion exchange resin (Huettl et al., 1979). They determined that 0.1M NaOH was the best estimator of Paa.

Laboratory derived values will represent the maximum Paa in ideal conditions. Many factors control the desorption of Paa from the sediment such as the soluble phosphorus concentration in the water, the algal species and abundance, light, temperature, pH and whether the sediment has settled (Sonzogni et al., 1983). Also the duration of sediment-algae contact time is important. Dorich et al. (1980) found that after 14 days incubation, there was no soluble reactive phosphate (SRP) in solution. This implies that in short river systems, the Paa will be much lower than in large river systems where the water residence time in the network is much longer.

A measure of Paa;
1. will provide a more accurate estimate of the algal available phosphorus fluxes in the river systems than simply dissolved phosphorus concentrations.
2. will emphasise the role of sediment in contributing to eutrophication problems.
3. may help in the development of controls on phosphorus pollution by indicating hydrologic and geomorphic controls.

REAGENTS
1. 0.1M Sodium hydroxide solution : 4g NaOH to 1 litre of water

PROCEDURE
1. Weigh accurately approximately 1.5g of fresh sediment to a 250 ml centrifuge bottle. (NB: The soil moisture content of the sediment must be known)
2. Add 200 ml of reagent 1 and shake for 17 hours on an mechanical shaker.
3. Centrifuge at 2500 rpm for 20 mins and analyse the supernatant for phosphorus as described in method 1

SOURCE

Inorganic Phosphorus Fractionation
INTRODUCTION

The fractionation of the various phosphorus forms within sediments can be a very important tool in investigating the biogeochemical processes controlling phosphorus dynamics in the fluvial environment. In general phosphorus fractionation schemes quantify the size of the iron, aluminium and calcium inorganic phosphate fractions. Changes in these inorganic phosphate fractions may be related to biogeochemical or hydrological changes within the river environment such as reduction processes in the sediment or storm events respectively. Phosphorus fractionations may also reveal information on the source of the sediment, i.e. sediments rich in calcium phosphates (apatite) may have derived from areas of calcareous bedrock.

Phosphorus fractionation schemes are based on the principle that apatite is soluble in acid solutions and that iron and aluminium hydrous oxides (non-apatite) are soluble in alkaline solutions. An additional procedure consisting of a citrate-bicarbonate buffered dithionite solution for removing the occluded fractions of iron and aluminium is commonly applied. The discovery that aluminium can be selectively extracted by ammonium fluoride lead to the development of the Chang and Jackson (1957) fractionation scheme. However, it has been demonstrated that this strict fractionation scheme has severe limitations when applied to soils and sediments rather than pure chemical systems.

The Exeter Laboratory tested a number of phosphorus fractionation procedures such as Dorich et al. (1984), Hieljes and Lijkema (1980) and Olsen and Sommers (1982) and Williams et al. (1976, 1980). It was determined that the Williams et al. (1976) procedure described below best separated the non-apatite and apatite fractions.

REAGENTS

1. 0.3M citrate solution : 88g sodium citrate dihydrate (Na₃C₆H₅O₇·2H₂O) to 1 litre of water
2. 1.0M Sodium bicarbonate solution : 84g NaHCO₃ to 1 litre of water
3. 0.1M Sodium hydroxide solution : 4g NaOH to 1 litre of water
4. 1.0M Hydrochloric acid solution : 86 ml conc. HCl to 1 litre of water
5. 5.0M Sodium hydroxide solution : 200g NaOH to 1 litre of water

PROCEDURE

1. Weigh accurately approximately 1g (<2 mm) dry sample into 50 ml centrifuge tube.
2. Add 35 ml reagent 1.

3. Add 5 ml reagent 2.

4. Place test tubes upright in a water bath at 85°C.

5. When heated, add approximately 1g Sodium dithionite (Na₂S₂O₄·2H₂O) and stir rapidly.

6. Continue heating at 85°C for 15 minutes.

7. Centrifuge at 2500 rpm for 20 minutes.

8. Dilute supernatant to 200 ml using a volumetric flask and store (supernatant A).


10. Shake on mechanical shaker for 17 hours.

11. Repeat stage 7 and dilute to 50 ml using a volumetric flask (supernatant B).


13. Shake for 1 hour on a mechanical shaker.

14. Repeat stage 7 and dilute to 50 ml using a volumetric flask (supernatant C).

15. To test supernatant A for phosphorus, all the added sodium dithionite must be first destroyed:

   i) Add 5 ml supernatant A to a 125 ml conical flask
   ii) Add a few anti-bumping granules and boil to dryness.
   iii) Add 1 ml 60% perchloric acid and continue boiling in a fume cupboard. White fumes will evolve.
   iv) Add water sparingly until no more white fumes are evolved.
   v) Boil to dryness
   vi) Cool and test for phosphorus (Method 1)

16. Analyse supernatants B and C by the Murphy and Riley procedure (Method 1). However, supernatant C must be first neutralised by adding 0.5 ml reagent 5. Remember to treat the standards similarly.

**SOURCE**
Adapted from;


SWQRG/MAR/96
The Determination Of Total Sediment Bound Phosphorus

SWQRG METHOD 4

PROCEDURE

1. Weigh accurately approximately 0.5g (<2 mm) dry sediment into 250 ml conical flask

2. Add 7.5 ml 60% perchloric acid and heat to a few degrees below boiling point on a hot plate.

3. Do not allow particles to stick to the sides of the flask. If necessary, add an additional 1 or 2 ml of perchloric acid to move these particles.

4. Heat on hotplate until white fumes cease, but do not allow the sediment to burn.

5. Allow to cool.

6. Add distilled water to obtain a volume of 250 ml and mix the contents.

7. Allow the particles to settle and then test for phosphorus in the supernatant (Method 1).

SOURCE


SWQRG/MAR/96
# Fractionation Calculations

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>CALCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TOTAL PHOSPHORUS CONTENT</td>
<td>METHOD 4</td>
</tr>
<tr>
<td>2. INORGANIC CONTENT</td>
<td>( \Sigma ) (METHOD 3)</td>
</tr>
<tr>
<td>3. NON-APATITE CONTENT</td>
<td>( \Sigma ) (METHOD 3 {SUPERNATANT A+B})</td>
</tr>
<tr>
<td>4. APATITE CONTENT</td>
<td>METHOD 3 {SUPERNATANT C}</td>
</tr>
<tr>
<td>5. ALGAL AVAILABLE CONTENT</td>
<td>METHOD 2</td>
</tr>
<tr>
<td>6. ORGANIC CONTENT</td>
<td>METHOD 4 - ( \Sigma ) (METHOD 3)</td>
</tr>
</tbody>
</table>

SWQRG/MAR/96
Calcium Fractionation

INTRODUCTION

This method sequentially extracts exchangeable and mineral forms (calcium carbonate) of calcium in soil and sediment samples. The procedure also allows the calculation of the Cation Exchange Capacity of the sample.

REAGENTS

1. 0.5N BaCl₂ : Add 61.07g BaCl₂ to 1 litre of water.
2. 0.001M or 0.01M NaOH : Add 0.08g or 0.8g NaOH to 2 litres of water.
3. 1.0M HCl : Add 43 ml of conc. HCl to 500 ml of water.

PROCEDURE

1. Weigh accurately approximately 2.0g of sediment to a 50 ml centrifuge tube.
2. Add 30 ml BaCl₂ and shake for 2 hours.
3. Pour contents into a buchner funnel in side-arm conical flask (Whatman Paper No 541).
4. Wash tube with 4 successive 25 ml aliquots of BaCl₂. Pour washings into buchner flask. Ensure all the sediment is washed out. Dilute to 200 ml using a volumetric flask.
5. Leave to filter for 20 mins and finish filtering under vacuum. (Supernatant A)
6. Titrate 50 ml of the filtrate with 0.01M or 0.001M NaOH depending on the calcium content of the samples, to pH 7.0 using a pH meter.
7. Transfer filter paper and sediment to a 250 ml centrifuge bottle.
8. Add 200 ml 1.0M HCl and shake for 1 hour.
9. Centrifuge at 2500 rpm for 20 minutes. (Supernatant B)
10. Determine by AAS; Al, Ca, Mg, Na and K in supernatant A (exchangeable forms) and Ca in supernatant B (calcium carbonate).

SWQRG/MAR/96
Preparation Of Water Samples For Total N + P Analysis

This method simultaneously oxidises nitrogen and phosphate compounds by the action of a persulphate solution. All the phosphate compounds are converted to soluble reactive phosphorus and nitrogen compounds are converted to nitrate. Common practice is to use an alkaline persulphate solution (Method 1), but this digestion may cause the precipitation of CaSO₄ with subsequent phosphate precipitation. Therefore, when analysing water samples with high calcium concentrations it is advisable to use an acid persulphate digestion to give maximum recovery of phosphate (Method 2).

REAGENTS

METHOD 1: ALKALINE PERSULPHATE

1. 0.375M Sodium hydroxide : 15g NaOH to 1 litre of water
2. Oxidising reagent : Add 5g potassium persulphate and 3g orthoboric acid to 100 ml of reagent 1. Store in a tightly stoppered polyethylene bottle wrapped with aluminium foil at room temperature. The reagent is stable for at least a week.

METHOD 2: ACID PERSULPHATE

1. Sulphuric acid solution : Add 50 ml conc. H₂SO₄ to 200 ml of water.
2. Oxidising reagent : Dilute 10 ml of reagent 1 to 200 ml of water. Add 10g potassium persulphate. This reagent must be made up immediately before use.

PROCEDURE

1. Add
   i) 5 ml of the oxidising reagent to 50 ml of sample in a screw topped flask (Method 1) or
   ii) 4 ml of the oxidising reagent to 50 ml of sample in a screw topped flask (Method 2)

2. Ensure water covers the bottom platform of the autoclave.

3. Place flasks in wire baskets in autoclave.

4. Close autoclave lid ensuring the lid is sealed.

5. Open vent valve (pointing upwards).

6. When the temperature reaches 100°C, close vent valve.

7. Turn autoclave off after 30 minutes at 15 lbf/in² (internal scale).

8. Allow to cool.

SOURCE

Dissolved Inorganic Silicate Analysis

REAGENTS
1. Sulphuric acid : Dilute 12.5 ml conc. H₂SO₄ to 50 ml.
2. Acid molybdate solution : Add 6.3 g ammonium molybdate to 50 ml distilled water in a PLASTIC beaker. Add 50 ml reagent 1. This solution is stable for a month.
3. Oxalic acid solution : Add 10 g oxalic acid to 100 ml distilled water in a PLASTIC beaker. This solution is stable indefinitely.
4. Ascorbic acid solution : Add 2.8 g in 100 ml distilled water. Make fresh each day.

STANDARDS
Using the 1000 mg l⁻¹ silicon standard, prepare standards of range 1, 2, 5, 10 mg l⁻¹ Si.

PROCEDURE
1. To a 50 ml centrifuge tube add
   i. 10 ml filtered sample
   ii. 10 ml distilled water
   iii. 1 ml acid molybdate solution
2. Mix well and leave for 10 minutes.
3. Add 1 ml oxalic acid solution and 0.5 ml ascorbic acid solution to the tubes.
4. Allow 30 to 60 minutes for colour development.
5. Measure absorption at 810 nm on SP6 uv/vis spectrophotometer.

SOURCE
**Determination Of Chlorophyll-A In Water Samples (Acetone)**

This procedure is a quick and simple measure for chlorophyll-a concentrations in river water samples. It is desirable to conduct this procedure as soon as possible after sampling. The filtering and addition of acetone can be easily conducted in the field. In rivers with low phytoplankton populations, large water samples are needed for analysis of the order of 2 litres. Volumes of approximately 500 ml are necessary in rivers with large phytoplankton populations.

**PROCEDURE**

1. Filter a known volume of river water through a Whatman GF/C filter paper.
2. Tear the filter paper into 5 or 6 pieces and insert into a 50 ml centrifuge tube.
3. Add a known volume of acetone (30 ml) to cover the filter paper.
4. Leave the tube in the fridge overnight.
5. Stir the resulting solution and filter paper vigorously and centrifuge at 2500 rpm for 10 minutes.
6. Using a GLASS 1 cm cuvette, measure the extinction at 665 nm.

**CALCULATION**

\[
\text{Chl-a (ug/l)} = \frac{A \times E \times V}{V_f \times L}
\]

Where:

- \(A\) = absorbance coefficient of chlorophyll-a in acetone (11.9).
- \(E\) = absorbance value from spectrophotometer.
- \(V\) = volume of acetone used for extinction (ml).
- \(V_f\) = litres of water filtered.
- \(L\) = path length of cuvette.

**SOURCE**


Dionex Operating Instructions

If any doubt on the operation please check with the Technicians. Errors can cause great expense

1. Ensure that the correct columns are installed by checking inside the BASIC CHROMATOGRAPHY MODULE.
2. Check that the correct detector is on and connected to the eluant from the column.
3. Prepare the correct eluants.
4. Filter and degas the eluant.
5. Put the eluant into the correct container (check line number)
6. Turn Nitrogen on at cylinder (set at 90 psi).
7. Switch on the Dionex by pressing POWER ON.
8. Select the correct eluant line (Choose from 1 to 4 on ANALYTICAL PUMP).
9. Press START on ANALYTICAL PUMP.
10. Prime pump by:
    a) attach syringe to luer fitting to left of pumps
    b) turn lever to face syringe
    c) extract a syringe full of eluant
    d) remove syringe and push out any air
    e) replace syringe on luer fitting
    f) loosen thumbwheel in centre of pumps
    g) place beaker under tube between pumps
    h) push contents of syringe through pumps
    i) turn lever back to 90 degrees from syringe
11. Let eluant run for sufficient time for stabilisation
12. Switch on computer, screen and printer
13. Click on method.exe and choose appropriate method.
14. Click on run.exe
15. Load method
16. Load autosampler with one standard
17. Put Autosampler to RUN
18. Put Detector to REMOTE
19. Put Pump to REMOTE
20. Click on Run and then Start followed by OK
21. Allow standard to run
22. Check that all the peaks are found on printout: If not then recall method and change retention times etc.
23. Set up Schedule using schedule.exe
24. Load Autosampler (with regular standards)
25. Recall run.exe window and load schedule followed by run and then Start
26. At end of run switch off Dionex and turn off Nitrogen at the cylinder.
Changing from Transition Metals to Anions

1. Stop everything
2. Turn off lamp
3. Disconnect tubing marked EXTERNAL DETECTOR (check PAR does not siphon)
4. Disconnect tubing from valve to guard
5. Disconnect separator column (from back of external detector)
6. Take brown connector off anion column set and use this to join the transition metal columns and put them in drawer
7. Take blank screw out of ELUANT OUT on membrane
8. Connect tube from conductivity cell into ELUANT OUT
9. Take blank screw out of ELUANT IN on membrane
10. Connect separator column to ELUANT IN
11. Suck out eluant at Luer fitting
12. Push this through pump
13. Set flow to 9ml/min
14. Put a tissue at valve outlet (where guard column connects)
15. Pump anion eluant (Na$_2$CO$_3$/NaHCO$_3$) until liquid at valve outlet is alkaline
16. Switch between Inject and Load to clear the sample loop
17. Set flow to 2ml/min
18. Stop pump
19. Connect guard to valve
20. Change electrical connections on integrator
21. Start pump
22. Switch on Conductivity Cell
Use of Dionex for Anions

Reagents

Note: Use only the deionised water from the Permutit Plus system

Eluant.............. 1.8mM Na₂CO₃ / 1.7mM NaHCO₃ 0.143g Na₂CO₃ + 0.191 NaHCO₃ to 1 litre with water (Filter through a GF/F filter and degas using vacuum dessicator)

Regen ............. 25mM H₂SO₄ 2.8 ml conc. H₂SO₄ to 4 litres with water

Standards (1000 ppm)

<table>
<thead>
<tr>
<th>Anion</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>K₂SO₄ ...... 5.4350 g/l</td>
</tr>
<tr>
<td>NO₃</td>
<td>KNO₃ ........ 1.6308 g/l</td>
</tr>
<tr>
<td>Cl</td>
<td>NaCl ........ 1.6500 g/l</td>
</tr>
<tr>
<td>SO₄</td>
<td>K₂SO₄ ........ 1.8141 g/l</td>
</tr>
</tbody>
</table>

Modus Operandum

Guard AG4A
Column AS4A
Flow Rate 2.0 ml/min
Detector Conductivity
Use of Dionex for Nitrate / Nitrite in KCl

Reagents

Note: Use only the deionised water from the Permutit Plus system

Eluant........... 35mM NaCl 2.0 g NaCl to 1 litre
(Filter through a GF/F filter and degas using vacuum dessicator)

Standards (1000 ppm)

Nitrate - N ....................... 7.2221 g Potassium Nitrate to 1 litre
Nitrite - N ....................... 4.9486 g Sodium Nitrite to 1 litre
Nitrate - NO₃ ................... 1.6308 g Potassium Nitrate to 1 litre
Nitrite - NO₂ .................... 1.5000 g Sodium Nitrite to 1 litre

(Dilute Standards to required level with the KCl extract)

Modus Operandum

Guard CG5
Column CS5
Flow Rate 1.0 ml/min
Detector UV/Vis (Deuterium lamp)
Wavelength 215 nm
Use of Dionex for Transition Metals and Ferrous / Ferric

Reagents

Note: Use only the deionised water from the Permutit Plus system

Eluant
6mM PDCA 1.003 g/l (pyridine-2,6-dicarboxylic acid)
50mM Acetic Acid 3 ml to 1l
50mM Sodium Acetate 4.1 g/l

(Filter through a GF/F filter and degas using vacuum dessicator)

Post Column Reagent
0.3mM PAR 0.0712 g/l (4-(2-pyridylazo)resorcinol)
1M Acetic Acid 60 ml to 1l
3M Ammonium Hydroxide 146 ml to 1l

Other Reagent ........ 0.1M Sodium Sulphite 25.22 g/l

Standards (Stock solutions)

Fe\(^{3+}\), Cu, Zn, Mn use 1000 ppm standard stock solutions
Fe\(^{2+}\) (100 ppm) 0.175gm Ammonium Ferrous Sulphate in 250 ml water
+ 1 ml Sulphuric acid

Modus Operandum

Guard CG5
Column CS5
Flow Rate 1.0 ml/min (eluant)
0.6 ml/min (post column)
Detector UV/Vis (tungsten 520nm)

NB Reduce lines by running 0.1M sodium sulphite for at least 1 hour prior to use.
Short Instructions for Sample Changing on Gamma Spectrophotometers

Series 35

1. Set switch to 1/1. Push COLLECT to stop counting.
2. Set switch to 1/4. Push HOME and then go through peaks using Index
3. Take readings of Time T, Area A and Integral I write in book
5. Change sample writing relevant information in book including type of pot
6. CLEAR DATA with switch in position 1/1 to clear all or 1/4 2/4 etc.
7. When all is ready push COLLECT

Ace (Room 363)

To toggle between detectors use ctrl+f1,f2 or f3. This corresponds to detectors 5,6 & 7.

1. Press shift+6 to move to ROI. ( 6 is on numeric keypad as arrow)
2. Press alt+5 to move to buffer
3. Press f4 to show contents of buffer.
4. Press alt+6 to calculate
5. Press alt+3 to give areas
6. Read Time (Live Time), Area (gross area) and I (net area)
7. Go to next peak with shift+6 followed by alt+3 to give areas
8. Change sample writing relevant information in book including type of pot.
9. Press f4 to go back to MCB
10. Press alt+0 to go to main menu
11. Press alt+2 to stop collection
12. Press alt+3 to clear
13. Press alt+1 to start

Adcam (Room 364) - also room 355

To toggle between detectors use ctrl+f1,f2 etc. as marked on the books

1. Press shift+6 or shift+arrow to move to ROI (home is an option)
2. Press alt+5,
3. Press alt+6 (note 2 & 3 only once when multiple peaks are read)
4. Press alt+c
5. Press alt+a - read Time(LvTm) , Net Area(A) & Gross Area(I)
6. Go through next peaks with shift+arrow, and use alt+c followed by alt+a to read areas
7. Save spectra by alt+f, alt+s
8. Change sample writing relevant information in book including type of pot.
9. Press f4
10. Press alt+2 to stop
11. Press alt+3 to clear
12. Press alt+1 to restart
**Sodium Iodide Detector**

1. Press `ctrl + f4` as the detector is in Channel 4
2. Measure a sample from deep in the core to get a background reading (`alt + 1` to collect, `T = set at 3000 secs`)
3. Save background sample by transferring to the buffer (`alt + 5`, `alt + 6`)
4. Then `alt + F`, `alt + S` and name as `filename.BAC`
5. Press `f4` to get out of the buffer
6. Clear data with `alt + 3` and collect first sample using `alt + 1`
7. Transfer to buffer with `alt + 5`, `alt + 6`
8. Strip the background (`alt + C`, `alt + P`) - pushing `END` will get last saved file `filename.BAC` then ENTER. On prompt for ratio enter 1
9. Obtain Area in normal way (`alt + C`, `alt + A`)
10. Transfer out of buffer using `f4` and restart from 5 above

**Resetting of Automatic Cut-out (Power Cuts)**

*This is best left to the Technicians as expensive damage could occur*

The detector HV supplies are on a special circuit which does not reset after power cuts. This is a safety device as a surge of high voltage could cause expensive damage.

Therefore if power cuts have occurred and the HV supplies are off,

1. Note the settings of the individual HV bias voltages (settings on the pots)
2. Reduce these settings to 0 (zero) by turning the pots anticlockwise
3. Reset the main circuit (button on wall in room 363 marked MANUAL RESET and found just above the computer and between the bottom shelves) - note some of the detectors in room 364 have separate in-line cut-outs/resets
4. Wind up each HV supply very slowly to its noted value
5. It may be necessary to recalibrate the detectors especially if the computers have also suffered from the power cut

**Resetting of Automatic Low Nitrogen Cut-out**

*This at present only applies to the well detector (Detector 10)*

The alarm will sound (beep) when the liquid Nitrogen is low. If this happens,

1. Switch HV off on HV supply 659 (the one with LED’s and a bias voltage of 2300)
2. Reduce bias voltage to 0 (zero) on pot
3. Make sure liquid Nitrogen is now full
4. Switch HV back on (remember bias must be 0 (zero))
5. Press reset button to remove shutdown light
6. Very slowly dial up bias voltage until 2300 is reached
Nomad Spectroscopy System

Setting up

1. Fill the dewar with liquid nitrogen at least 2 hours before use (or the night before). It will need filling in the morning and at the end of the day if it is to be use again the next day (holding time approx. 8hrs.)

2. Make sure both NOMAD and laptop are charged fully.

3. The cables are connected as follows:
   a) Short D connector from NOMAD to laptop
   b) One BNC connectors from the shutdown on the detector to SD on the NOMAD
   c) The other BNC connectors from output 1 on the detector to AMP IN on the NOMAD
   d) SHV cable from HV on the detector to the HV on the NOMAD
   e) Pre-amp power, connect 9 pin D plug to NOMAD

4. Turn the power to the NOMAD on, then run the required program to start the system. Note the power to the HV and all the amplifier functions are controlled via the M1MAIN / MAESTRO 2 emulator programs.

Program usage

All programs can be run from the desktop by using the shortcuts provided.

M1INCALP – runs the initial instrument calibration. This program is only needed if the detector is changed or a new detector added to the system. It must also be used if an additional point source is used in calibration of the system (i.e. a different radio nuclide).

M1DCALP – runs the program which is used to calibrate the detector each time before it is used in the field. Before running this program it is necessary to run M1MAIN.

M1GO – runs the field analysis.

Running In The Field

M1DCALP
1. Switch the power to the NOMAD on.
2. Run M1MAIN, select F10 (MCA control).
3. Enter MAESTRO 2 emulator (F2).
4. Use SERVICES to switch on the HV via 92X control.
5. Select DET BIAS then ON.
6. Use ESC to return to the main screen then select PRESETS, live time and set to 300 sec.
7. Now quit the program.
8. Run M1DCALP from the desktop. A spectra will now collect for 300 sec. After which time the system will calibrate itself. The system is now ready for use.

M1GO
1. Check that the HV power is on as above (this must be done every time the nomad power is switched off).
2. Run M1GO from the desktop, enter spectra id. etc. and the time for which you wish to collect.
3. Enter the data by processing the screen (F1). The rest is automatic, with a read out at the end of each run.
Lichen Analysis

AAS Analysis of Metal accumulation in Lichens

Reagents

Nitric Acid 50%
Nitric Acid 1N 63 ml conc. Nitric acid to 1 litre with water
Perchloric Acid
AAS Standards as required

Method

1. Collect 0.5 - 1.0 g of lichen

2. Dry Samples at 50 °C over three days

3. Weigh 0.5 - 1.0 gm of lichen accurately using a 4-place balance into a clean dry beaker

4 Digest samples by adding 20 ml 50% Nitric Acid and boil (reflux) for 8 hours covering beaker with a watchglass
   Evaporate the solution to dryness.
   Add 1 ml conc. perchloric acid. Evaporate to dryness.
   Dilute to 50 ml with ~ 1N nitric acid

5 Analyse for Pb, Ni, Zn, Fe, etc. ....using standard AAS techniques (see method for heavy metals.

6 Calculate metal concentration per gm of dry weight of lichen

Measuring SO₂ damage to lichens using electrolyte leakage

1 Place lichen samples on moist filter paper in a closed container for two hours

2 Rinse samples for three seconds.

3 Take 10 - 60 mg of lichen and place in a beaker containing 20 ml of distilled water.
   Allow to stand for 5 minutes to allow electrolyte to leak out.

4 Remove lichen and measure conductivity

5 Dry retrieved lichen at 80°C for 24 hours and weigh.
   Calculate electrolyte leakage as conductivity per gram of dry weight of lichen per ml of solution.
Determination Of Chlorophyll-A In Water Samples (Methanol)

PROCEDURE

1. Filter a known volume (250 ml) of water through a Whatman GF/C filter paper. This may be stored frozen (-20°C) until analysis is carried out.

2. Tear the filter paper into 5 or 6 pieces and insert into a 50 ml centrifuge tube.

3. Add a known volume of methanol (20 ml) to cover the filter paper, shake and vortex until the filter paper has broken up.

4. Stopper with a closure and leave the tube in the fridge overnight.

5. Centrifuge at 3200 rpm for 10 minutes. Pour off the supernatant into a 1cm cuvette.

6. Measure the extinction at both 665 nm. and 750nm (zero with methanol).

CALCULATION

\[
\text{Chl-a (ug/l)} = \frac{(\text{Abs}[665\text{nm}]-\text{Abs}[750\text{nm}]) \times A \times Vm}{Vf \times L}
\]

Where:

- \(A\) = absorbance coefficient of chlorophyll-a in methanol (12.63).
- \(Vm\) = volume of methanol used for extinction (ml).
- \(Vf\) = litres of water filtered.
- \(L\) = path length of cuvette.

SOURCE


Calcium Titrations using EDTA

Reagents

Na$_2$EDTA 0.02M  
Dry Na$_2$EDTA at 80°C  
Dissolve 3.722g Na$_2$EDTA in 500 ml water  
Make up to 1 litre

Sodium Hydroxide  0.1M  
4 g NaOH to 1 litre of water

Murexide Powder

Method

1  Measure 25 - 50 ml of water into a conical flask.
2  Add 5 ml of 0.1M Sodium Hydroxide and ca. 20 grains of murexide powder
3  Titrate against EDTA solution from burette  
   (Endpoint is when pink colour turns blue)

Calculation

\[
Ca = \frac{C \cdot V}{S}
\]

where
\[C = \text{conc. of EDTA in mmol l}^{-1} \quad (20 \text{ if EDTA made accurately})
\[V = \text{titre of EDTA}
\[S = \text{volume taken of sample} \]
Useful Reagent Formulae

\[ pH = 4 \text{ Buffer} \]
\[ 10.211 \text{ g Potassium hydrogen phthalate to 1 litre water} \]

\[ \text{Redox Standard} \]
\[ 1 \text{ litre pH 4 Buffer saturated (ca 3 g) with Quinhydrone} \]
\[ \text{Reading should give + 218 mv} \]

\[ \text{Calibration Standard} \]
\[ (5000 \text{ ppm formazine}) \]
\[ 181.8 \text{ g Hexamine} \]
\[ 18.18 \text{ g Hydrazinium Sulphate} \]
\[ 4 \text{ litres distilled Water} \]

\[ 1M \text{ Hydrochloric Acid} \]
\[ 86 \text{ ml conc. Hydrochloric acid to 1 litre with water} \]

\[ 1M \text{ Nitric Acid} \]
\[ 63 \text{ ml conc. Nitric acid to 1 litre with water} \]

\[ 1M \text{ Sulphuric Acid} \]
\[ 56 \text{ ml conc. Sulphuric acid to 1 litre with water} \]
\[ \text{(Add acid carefully to water)} \]

\[ 1M \text{ Sodium Hydroxide} \]
\[ 40 \text{ gm Sodium Hydroxide to 1 litre with water} \]
### Cylinder sizes of gases used in the department

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<thead>
<tr>
<th>Gas</th>
<th>Type</th>
<th>Size</th>
<th>Spares</th>
<th>Depot</th>
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<td>Worsley</td>
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<tr>
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<td>CP</td>
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</table>

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