

Appendix 2 Analysis of workflows and experimental methodology

1) Infra-red Spectroscopy (IR)

INSTRUMENT USED – NICOLET 380 FT-IR SMART ORBIT

INFORMATION STORED ON JCAMP-DX FILE:

- TITLE
- JCAMP-DX
- DATATYPE
- ORIGIN
- OWNER
- LONGDATE
- TIME
- DATA PROCESSING
- XUNITS
- YUNITS
- FIRSTX
- LASTX
- MAXX
- MINX
- MAXY
- MINY
- XFACTOR
- YFACTOR
- NPOINTS
- DELTA
- XYDATA

NO INFORMATION ON SCAN RATE.

NO INFO ON INSTRUMENT SERIAL NUMBER

NO INFO REQUIRED TO ENTER INTO COMPUTER BEFORE RUNNING SPECTRA.

Things to consider when doing IR:

What information will the instrument offer?

- Structural determination
- Indications of polymorphism
- Assess purity

What information e.g. decomposition temperature, melting point, etc, is required before running analysis with the instrument?

- N/A

How should this information be determined?

- N/A

How should the instrument be set up to obtain specific information?

Instructions for use

Turn on computer and IR at mains.

Choose OMNIC operating system for using IR on computer.

Can then choose smart orbit – most common setting for running spectra.

Or go to collect, choose experiment set up to change parameters according to what information is wanted to be collected, e.g. when looking to identify polymorphs a minimum range is required as information on polymorphs is found in the lower frequency range.

Parameters include:

- No. of scans
- Resolution
- Final format
- Correction
- Background handling
- Min/max range limit
- Gain
- Velocity
- Aperture
- Quality on spectrum, parameter, background, interferogram.

Go to collect, click on background, press confirm – shows water, carbon dioxide and diamond frequencies. This information automatically saves and normalises results.

To run sample place a small amount on the window and screw down contact till finger tight.

Go to collect – collect sample.

Gives 32 scans and shows 4, these parameters can be altered according to accuracy, time and necessity.

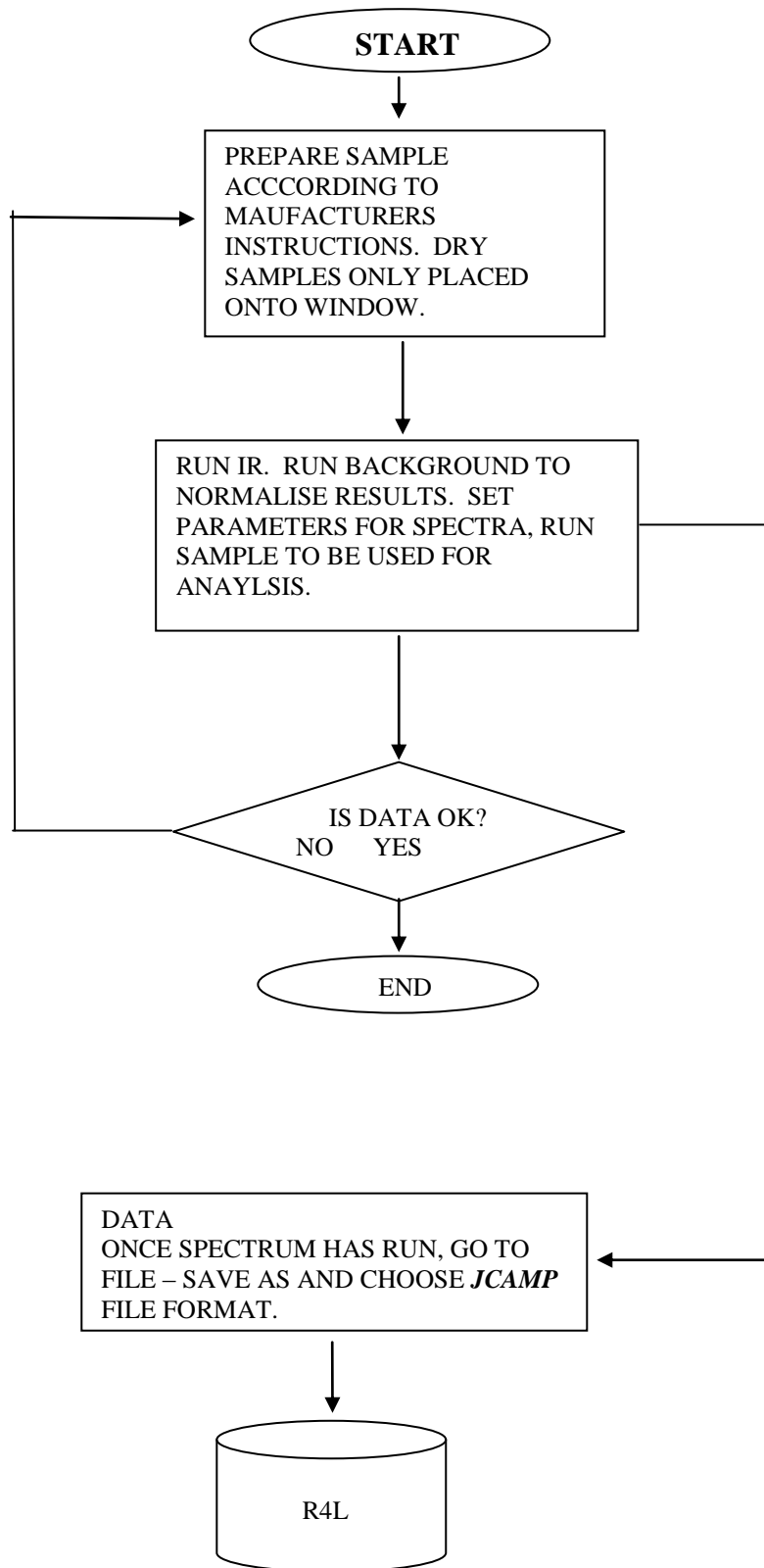
Once spectra has been run screen pops up a sample file to enter.

Once file is named go to file – save as – choose **JCAMP** file format/extension

Computer is networked so should be able to access data from else where.

Clean contacts with acetone once finished.

Workflow



2) Differential Scanning Calorimetry (DSC)

INSTRUMENT USED: PERKIN ELMER DIAMOND DSC WITH AUTOSAMPLER – PART NO. N5360024, REVISION 0.01 SERIAL NO. 536N3052801, FILE NO. LR23329C.

SOFTWARE VERSION: 6.0.0.0033
FIRMWARE VERSION

USED IN CONJUNCTION WITH PERKIN ELMER CRYOFILL LIQUID NITROGEN COOLER AND PERKIN ELMER ELIMINATOR II DESICCANT DRYER DE350-P04

INFORMATION STORED ON TEXT FILE:

- FILE NAME
- OPERATOR ID
- SAMPLE ID
- COMMENTS
- SERIAL NUMBER
- DATE AND TIME DATE COLLECTED
- SAMPLE WEIGHT
- VALIDATED BY
- DATE
- CALIBRATION INFORMATION
- FILE NAME
- DATE/TIME
- TEMPERATURE
- Y INITIAL (HEAT FLOW)
- SAMPLE RATE – doesn't indicate actual rate just gives statement e.g. fast this information is given at end of scan
- BASELINE FILE NAME
- END CONDITION
- TOTAL NUMBER OF POINTS RECORDED
- METHOD STEPS
- SCAN DATA – time, unsubtracted heat flow, baseline heat flow, program temperature, sample temperature, approximate gas flow, heat calibration
- DSC AUTOTUNE CALIBRATION VALUES
- DSC SAMPLE TEMPERATURE CALIBRATION VALUES
- DSC FURNACE TEMPERATURE CALIBRATION VALUES
- FURNACE CALIBRATION COMPUTED RESULTS
- DSC HEAT FLOW CALIBRATION VALUES
- HEAT FLOW CALIBRATION COMPUTED RESULTS
- PROFILE VALUES FOR THIS DATA – software version, part number, revision, load temperature, go to temperature rate, maximum allowed temperature, type purge gas, liquid nitrogen used, data taken using,

filter factor, lag compensation, cooling device (Cryofill), filter algorithm (adaptive boxer)

Considerations for using DSC:

What information will DSC give for analysis?

- Melting point
- Re-crystallisation temperatures
- Heat capacity
- Glass transition temperature (for polymers)
- Sample purity

What information is required before running a DSC?

- Decomposition temperature

How should this information be determined?

- Using manual melting point apparatus

However it is a little hard to determine the decomposition temperature, so I advise that once the melting point of the compound has been roughly determined, heat for a further 10°C to check that the compound doesn't decompose or evaporate and then set this temperature as the limit for the DSC.

How should the instrument be set up for:

- Melting point?
- Re-crystallisation temperature?
- Heat capacity?
- Glass transition temperature?

INSTRUCTIONS FOR PERKING ELMER PYRIS DIAMOND DSC AUTOSAMPLER:

- **Preparing machine:**

Vent large nitrogen cooling cylinder to prevent spewing nitrogen from out when fitting the Cryofill liquid nitrogen cooling system. Once this is fitted to the top of the cylinder turn off the vent. The cooling system cools nitrogen gas to liquid. Attach nitrogen gas to the back of the cooling system, use ECD grade nitrogen as this is pure. Also make sure nitrogen gas is attached to the machine arm and the helium purging gas is attached to diamond DSC, turn on all the attached gas cylinders and switch on cooler.

In the machine check that there is blue silica crystals in there to take moisture out of the environment and reduce the risk of freezing.

At the computer go into start menu and click on Pyris manager. An icon then appears on the screen, click to connect online, **ensure cooler is switched on or will not work**. When screen appears click on 3 icons Cryofil, cover heater and purge gas. Also click on open

cover after about 15 minutes to ensure it doesn't freeze on. Once the system is purged the continual hissing of nitrogen gas will stop and you will hear it sporadically, this means the machine is ready to use.

- **Preparing sample:**

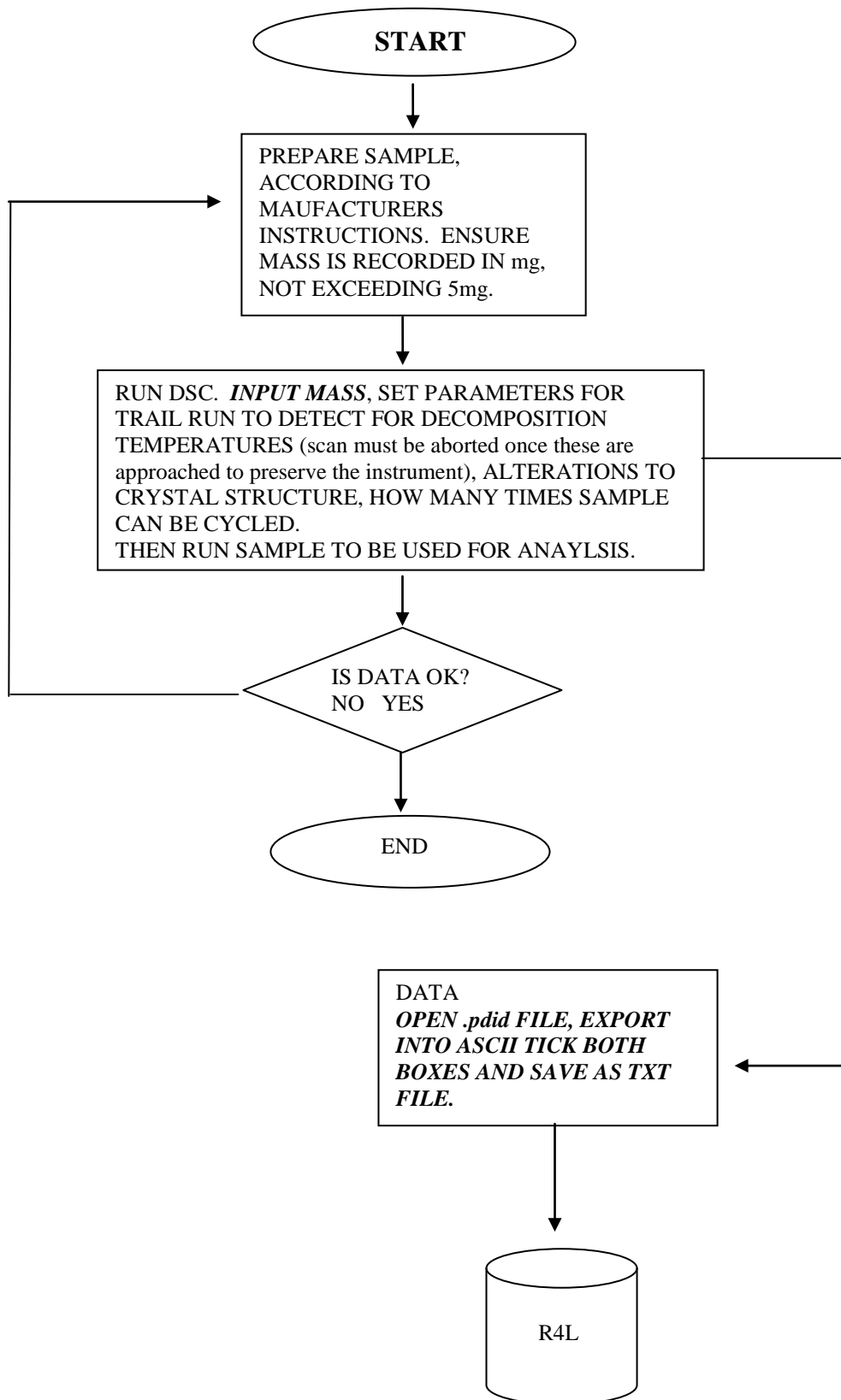
All samples are required to be under 5mg, however it is advisable that the sample mass be 0-2mg to prevent overspill and machine damage. It is important not to touch the pans during preparation due to residues on the skin contaminating the sample. Record the mass of the sample.

- **Running the DSC:**

Check that the temperature on the LHS of the screen is above 0°C. It is necessary to run a calibration of an indium sample to begin with. In auto sampler control place number in the box that indium sample occupies and click on load sample. Load reference sample (an empty pan). The furnace lid loads automatically. If either sample is set wonky open the cover and correct quickly. Click on OK and enter data, where the data should be saved, mass of the sample and ensure the Zero box is displaying 0. Ensure program temperature and sample temperatures are the same. Click on data sample option, make sure seconds between the points is displayed, and select desired value (how frequently points are recorded on the graph). Click on go to temperature to ensure both program temperature and sample temperatures are the same. Machine automatically sets this temperature at 30°C. Click on start icon. Files will automatically be saved in the appointed place and can be opened using a Perkin-Elmer program. Run indium calibration 3 times at the beginning and at the end of running the samples. It should be noted that if the tracer behaves erratically the sample has reached its decomposition temperature and should be stopped immediately. Bring up the auto sampler control screen and unload indium from furnace, load sample for analysis, and continue as before.

- **Reading results:**

Bring plot onto screen, right click on curve – remove curve. Click on curve tab – heat flow, choose heating/cooling. Click on temperature/time icon, RHS of lower icons. Click on calc – peak area – baseline sigmodal onset – calculate – choose p -calculate. Record onset and delta enthalpy.



3) Mass Spectrometry (Mass Spec)

INSTRUMENTS USED: THERMO ELECTRON IONISER TRACE GC,
WATERS ZMD ELECTROSPRAY AND WATERS PLATFORM
ELECTROSPRAY.

THERMO ELECTRO-IONISER SAVES INFORMATION AS **.raw** FILE,
INFORMATION READABLE ON FILE:

Initial Temperature (C):	40
Initial Time (min):	4.00
Number of Ramps:	1
Rate #1 (deg/min):	10.0
Final Temperature #1 (C):	320
Hold Time #1 (min):	6.00
Post Run Temperature:	Off
Enable Cryogenics:	Off
Maximum Temperature (C):	350
Prep Run Timeout (min):	10.00
Equilibration Time (min):	0.50
Right SSL Method	
Base Temperature:	On
Base Temperature (C):	220
Mode:	Splitless
Split Flow:	On
Split Flow Flow (ml/min):	200
Splitless Time (min):	1.00
Surge Pressure:	Off
Surge Pressure (kPa):	3.00
Surge Duration (min):	0.00
Constant Purge:	On
Stop Purge At: (min):	0.00
Right Carrier Method	
Mode:	Constant Flow
Initial Value:	On
Initial Value (ml/min):	1.00
Initial Time:	1.00
Gas Saver:	On
Gas Saver Flow (ml/min):	20
Gas Saver Time:	2.00
Vacuum Compensation:	On
No Left Inlet	
No Right Detector	
No Left Detector	
No Aux Detector	
Aux Zones	
Aux Temperature 0:	Off
Aux Temperature 0 (C):	30
Aux Temperature 1:	Off
Aux Temperature 1 (C):	30

Aux Pressure 0: Off
Aux Pressure 0 (kPa): 0.00
Aux Pressure 1: Off
Aux Pressure 1 (kPa): 0.00
Aux Pressure 2: Off
Aux Pressure 2 (kPa): 0.00

Run Table

External Event #1 Prep-Run Default: Off
External Event #2 Prep-Run Default: Off
External Event #3 Prep-Run Default: Off
External Event #4 Prep-Run Default: Off
External Event #5 Prep-Run Default: Off
External Event #6 Prep-Run Default: Off
External Event #7 Prep-Run Default: Off
External Event #8 Prep-Run Default: Off

Sample Volume: 1.0 ul
Air Volume: 1.0 ul
Injection Delay: 2 sec
Pullout Delay: 1 sec
Injection Speed: 10 ul/sec

Sample Cleans: 2
Pull Ups Pumps: 3
Pull Ups Volume: 3.0 ul
Pull Ups Delay: 1.0 sec

Pre Injection Washes: 2
Pre Injection Volume: 5.0 ul
Pre Injection Solvent: A
Pre Wash every time

Post Injection Washes: 2
Post Injection Volume: 5.0 ul
Post Injection Solvent: A
Post Wash every time

No Internal Standard
General Details

=====
Method Type: TRACE MS
Method Version: 3

Ionization Mode Settings
=====
Ionization Mode: Electron Impact (EI+)

Analysis Settings
=====
Data

Detector Voltage (V): 350.0

Acquire with reference gas on: No

Wait until setpoints achieved: Yes

Tune File:

C:\Xcalibur\methods\03Jan06.tun

Analog Settings

+++++

(Unused)

Acquisition Settings

=====

Acquisition Type: Full Scan

Scans per Second: 2.0

Scan Range Start (amu): 20

Scan Range End (amu): 540

Event Control

+++++

Start acquiring after (min): 3.0

Stop acquiring when GC run finishes.

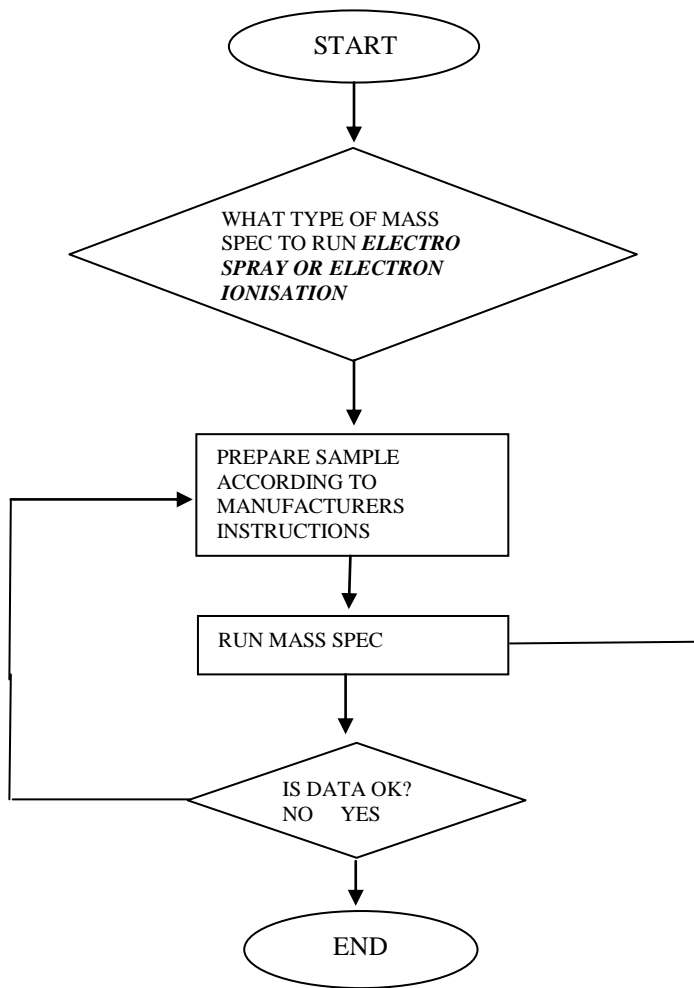
No spectral information is readable from this file.

**WATERS ZND AND PLATFORM ELECTROSPRAY SAVE INFORMATION
AS A FOLDER THAT INCLUDES THE FILES:**

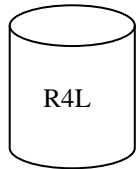
- _EXPMENT.INF
- _FUNC001.DAT
- _FUNC001.IDX
- _FUNC002.DAT
- _FUNC002.IDX
- _FUNC003.DAT
- _FUNC003.IDX
- _FUNCTNS.INF
- _HEADER.TXT
- _HISTORY.INF
- _INLET.INF
- _PROC001.DAT
- _PROC001.IDX
- _PROC001.SIG
- _PROC002.DAT
- _PORC002.IDX
- _PROC002.SIG

THE ONLY FILE READABLE IS THE HEADER FILE. LIST OF INFORMATION WITHIN THIS FILE:

- VERSION
- AQUIRED NAME
- JOB CODE
- TASK CODE
- USER NAME
- INSTRUMENT
- CONDITIONS
- LABORTORY NAME
- SAMPLE DESCRIPTION
- SOLVENT DELAY
- SUBMITTER
- BOTTLE NUMBER
- PLATE DESC
- CAL MS1 STATIC PARAMS
- CAL MS1 DYNAMIC PARAMS
- CAL TIME
- CAL DATE
- CAL FUNCTION 1
- CAL FUNCTION 2
- CAL MS1 STATIC
- AQUIRED DATE
- AQUIRED TIME



DATA COMES OUT DIFFERENTLY ACCORDING TO INSTRUMENT USED. A COMPREHANSIVE LIST OF FILES GIVEN ARE BELOW. ALL INSTRUMENTS GIVE A *.raw* FILE THAT IS CONVERTED TO A *.swx* AND LOADED ONTO AN ONLINE SERVICE WHERE YOU CAN VIEW **ONLY** YOUR OWN SPECTRA. NEED TO DECODE THE *.swx* .



4) Nuclear Magnetic Resonance Spectroscopy (NMR)

INSTRUMENT USED: BRUKER 400MHz

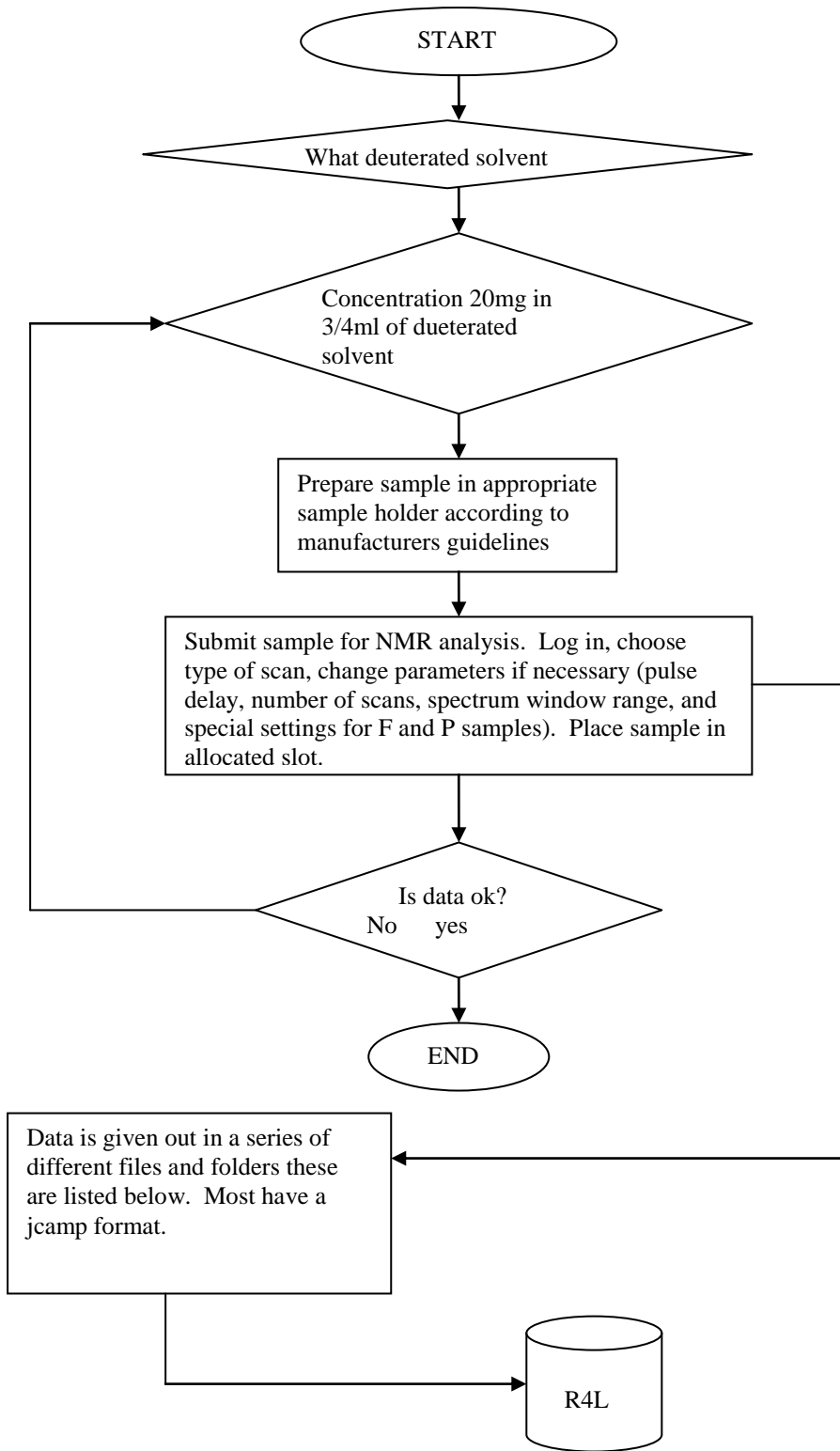
Files are given in 2 folders.

1st folder contains 2nd folder pdata and:

- Acqu jcamp
- Acqus jcamp
- Audita jcamp
- Fid unreadable
- Format
- Prosol_History
- Pulseprogram
- Scon jcamp
- Uxnmr jcamp

In pdata folder

- 1i unreadable
- 1r unreadable
- Auditp jcamp
- Intrng
- Last_plot
- Meta
- Outd jcamp
- Parm what appears on print out
- Peak numerical representation of what appears on print out
- Peaks unreadable
- Proc jcamp
- Procs jcamp
- Title personal identification of compound

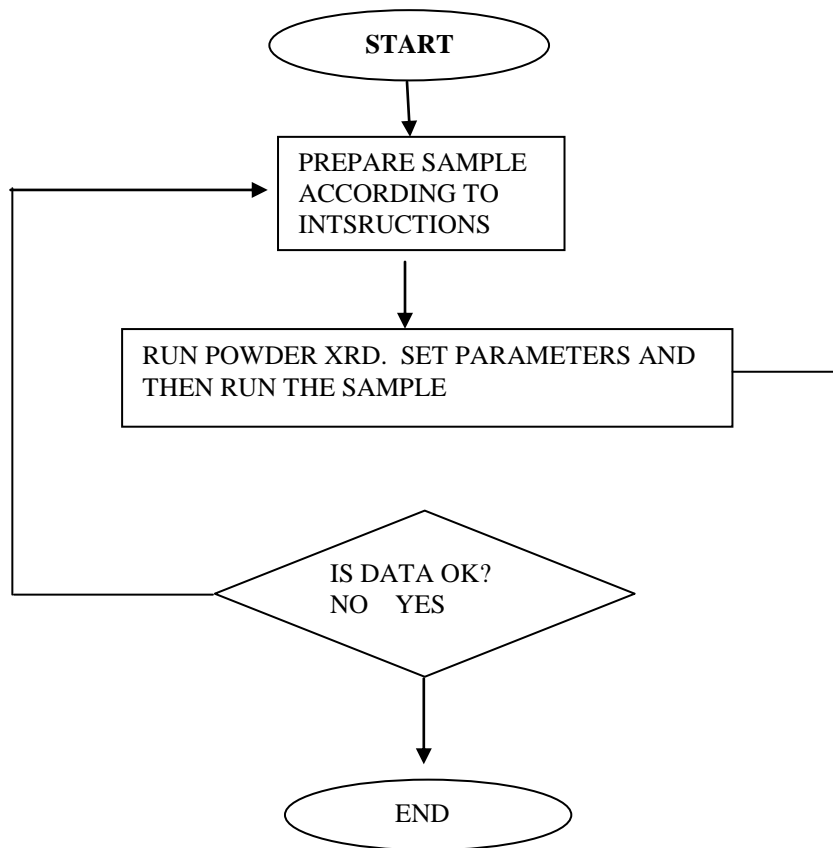


5) Powder X-ray Diffraction

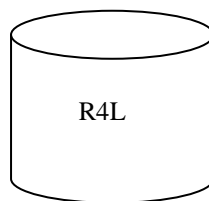
INSTRUMENT USED: SIEMENS DIFFRAKTOMETER WITH SIEMENS KRISTALLOFLEX.

INFORMATION STORED ON TEXT FILE:

- X DATA POINTS
- Y DATA POINTS



**DATA
SAVE AS .raw FILE, CONVERT
TO .dat FILE USING ConvX,
OPEN USING EXCEL, SAVE AS
.xls FILE, DATA COMES OUT IN
TWO COLOMN FOR x AND y
POINTS.**



6) UV-Visible Spectroscopy (UV-Vis)

INSTRUMENT USED: PERKIN ELMER LAMBDA 25 4444 UV

INFORMATION STORED IN EXCEL/TEXT FROM JCAMP FILE:

- TIIILE=
- JCAMP-DX=
- DATA TYPE=
- ORIGIN=
- OWNER=
- DATE=
- TIME=
- SPECTOMETER/DATA SYSTEM=
- RESOLUTION
- DELTAX
- XUNITS=
- YUNITS=
- XFACTOR=
- YFACTOR=
- FIRSTX=
- LASTX=
- NPOINTS=
- FIRSTY=
- MAXY=
- MINY=
- XYDATA=

DOES NOT GIVE DETAILED ACCOUNT OF INSTRUMENT e.g. SERIAL NUMBER ETC.

DOES NOT GIVE SCAN RATE.

NO PLACE TO INPUT SOLVENT OR CONCENTRATION, DATA NOT GIVEN.

DOES NOT GIVE BACKGROUND SCAN INFORMATION.

ASCII FORMATS DO NOT REPRODUCE DATA TO THE STANDARD THAT JCAMP DOES.

Considerations for using UV-vis:

What information will UV-vis give for analysis?

- Extinction coefficients, concentration of a sample using Beer Lambert
- Electron transfer from HOMO→LUMO, thus identifying conjugated structures.

What information is required before running the UV-vis?

- N/A

How should this information be determined?

- N/A

INSTRUCTIONS FOR UV/ VIS

Turn on instrument and computer.

Left click on the RHS of autozero to set parameters:

- Start/end wavelength
- Data interval
- Number of cycles
- Ordinate min/max
- Display
- Scan speed
- Ordinate mode
- Lamp UV
- Lamp vis
- Slit
- Lamp change

Place cuvettes with solvent in both sample holders of instrument.

Click on autozero to zero the instrument.

Remove front cuvette and replace with sample.

Click on start and then ok.

Scan will show on screen.

Computer is not networked so have to transfer files using memory stick.

