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| Standard Operating Procedure for:  **Use of the FTIR Spectrometer** | PPE required: |
| **Introduction**  A spectrometer measures the intensity of absorption or transmission of light through a sample as a function of wavelength/-number. In the case of Fourier-Transform -Infrared spectroscopy, the light is infrared radiation. Absorbance/transmittance of the sample depends on its composition as different chemical bonds react differently to the incoming light, resulting in spectra which are as individual as fingerprints. Hence FTIR can be used to   * identify unknown materials * determine the quality or consistency of a sample * determine the amount of components in a mixture. |
| **Sample preparation**  **Risk and COSHH assessments must be complete for the sample you are measuring.** This instrument can measure solid (powdered) and liquid samples (high concentrations for aqueous solutions). The amount of sample you need is tiny (about 1 mm3). If you measure wet solid samples or aqueous samples, the spectrum will show your sample peaks as well as the water peaks.  **Starting up the instrument**   1. Make sure the instrument is connected to a power plug and a PC/Laptop using a USB cable. 2. Switch on the instrument using the button located at the bottom left (will change from red to green within 10 s; red indicates that instrument could not connect to PC/laptop) 3. Leave for 2 – 3 hours to warm up and drive water out of the instrument (necessary to obtain spectra with low background) 4. Open MicroLab PC on the Desktop and log on (Username: Admin, PW: admin)   **Cleaning procedure**  If the sample area is not clean, your spectra will have a very noisy background and potentially negative peaks. It should be cleaned before you start, between samples, **and when you have finished**.   1. Wipe with a dry tissue, 2. Wipe with a tissue slightly wet with deionized water, 3. Wipe with a tissue with some isopropanol or ethanol, 4. Finally dry with a dust free tissue (e.g. Kimwipe).   **Closing down procedure**   1. When you are done measuring, click on “Home”. This will take you to the initial screen. 2. To close the software, click on “Logoff” and then click on “Exit”. 3. Switch off the instrument by pressing the green button (will go back to red). | **Hazard symbols**:  See risks from individual experiments. |
| **Significant hazards:**  See risks from individual experiments. |
| Hazard phrases (R): |
| Can it be done out of hours?  **See risks from individual experiments.** |
| **This SOP is not relevant in the following circumstances:**   1. SOP does not cover specific experimental risk these must be covered by user’s assessments 2. Any other situation where the procedure may result in harm to yourself or others. | |

**Running Samples**

After starting up the instrument and cleaning the sample area you can start to run your sample. Ensure that and risks of the samples to yourself or those around you have been identified.

1. Click on “Method” and select the method which you want to use. Usually, method “1024 scans” is used. This method consists of the addition of 1024 scans to obtain an FTIR spectrum and includes a background measurement (and subtraction) every 30 minutes. Click on “Activate”.
2. Click start and follow the instructions step by step.
3. During roughly 5 minutes, the instrument will collect background scans. There is nothing to do but wait.

IMPORTANT: do not touch the instrument and avoid any vibration when the instrument is running. That would be translated into noise in the FTIR spectrum.

1. If your sample is solid, put a very small amount (1 mm3) on the crystal area and then press it down using the metallic pressing device. After that, click on “Next”.

If your sample is liquid, just place one single drop of the liquid on the crystal area. Do not close the pressing device. Once you have done this, click on “Next”.

1. Once you click next, you will go to a new screen. This screen is important: it shows you how the spectrum looks like in real time (1 scan per second). You need to check that the FTIR spectrum is clearly visible before continuing. If the FTIR spectrum is not visible at all (i.e.: you have a flat line) or if the intensities are very small (absorbance <0.2), you can open the metallic pressing device and add more sample or place it better and see if the intensity of the peaks in the FTIR spectrum gets higher (no action with the computer needs to be taken). Once you feel comfortable with the FTIR spectrum, click on “Next”. Note: some substances may have a flat FTIR spectrum between 650 and 4000 cm-1 (e.g., NaCl).
2. Now, the sample will be measured. This will take approximately 10 minutes. The background will be automatically subtracted.
3. Once the sample is measured, the program will display the final FTIR spectrum (addition of 1024 spectra). The only thing you need to do is to export the data. In order to export the data you need to click on “Data Handling”. (DO NOT click on “Done”; if you do it, all the data will be lost).
4. Once you click on “Data Handling”, you will go to a new screen. There are two things you need to do:

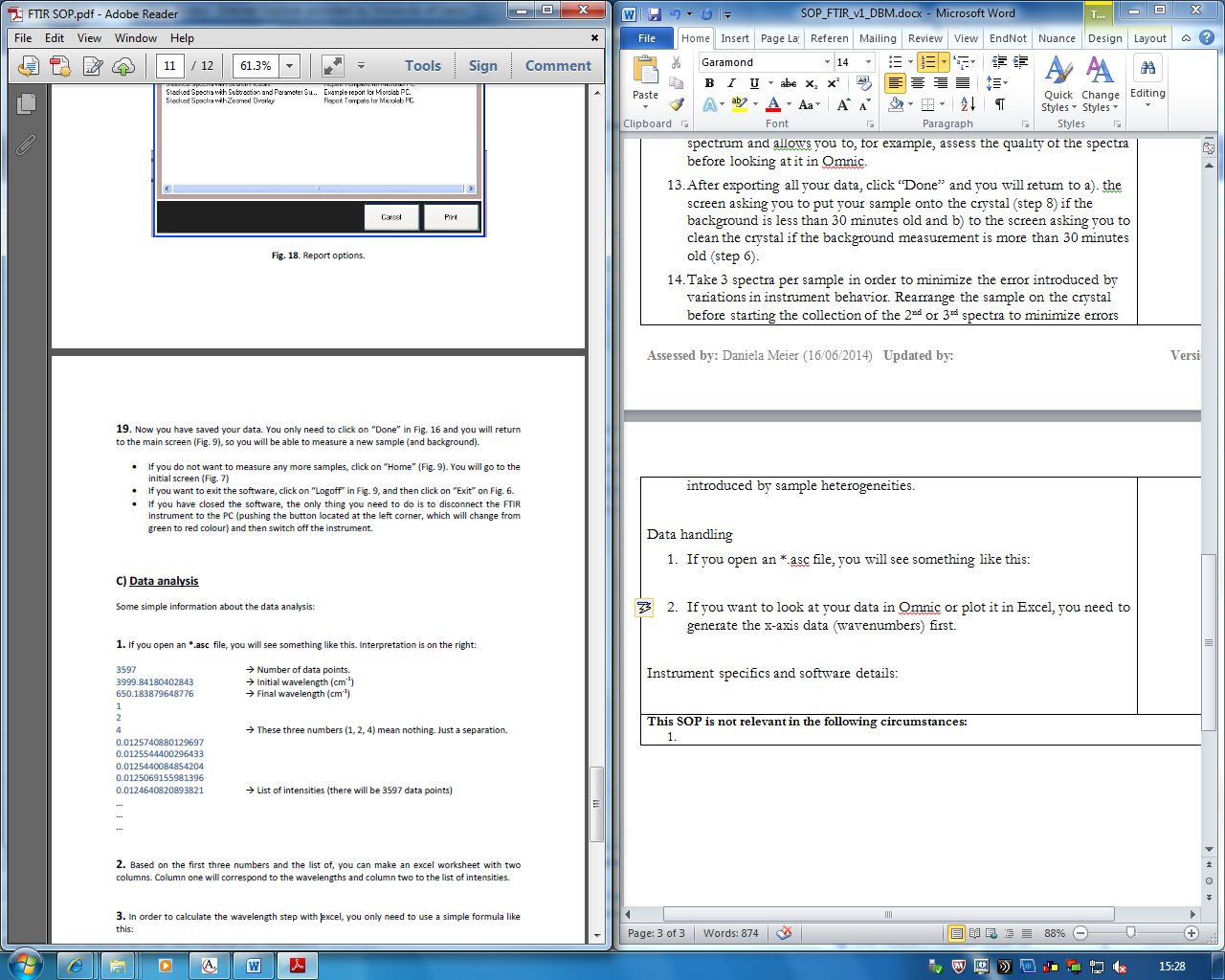
• Click on “Export” and select “Thermo Grams ASCII”. This way, you will export the data as an .asp file. This is the most important thing you need to do in order to keep your data safe and eventually be able to study it in Excel and/or Omnic.

• Click on “Create a report” and choose the first option. A report is quite useful because it consists of a PDF file with a graph of your FTIR spectrum and allows you to, for example, assess the quality of the spectra before looking at it in Omnic.

1. After exporting all your data, click “Done” and you will return to a). the screen asking you to put your sample onto the crystal (step 8) if the background is less than 30 minutes old and b) to the screen asking you to clean the crystal if the background measurement is more than 30 minutes old (step 6).
2. Take 3 spectra per sample in order to minimize the error introduced by variations in instrument behavior. Rearrange the sample on the crystal before starting the collection of the 2nd or 3rd spectra to minimize errors introduced by sample heterogeneities.

**Data handling**

1. If you open an \*.asp file, you will see something like this (interpretation on the right):



1. If you want to look at your data in Omnic or plot it in Excel, you need to generate the x-axis data (wavenumbers) first. All the information needed to do this is hidden in the first three numbers of the .asp file.

Wavelength Step = (Initial wavelength - Final wavelength) / (Number of data points - 1)

WS = (3999.84 - 650.18) / (3597 - 1) = 0.93

You can then start by subtracting the calculated wavelength step from the maximum value. From this newly calculated step you subtract the wavelength step again etc. until you have arrived at the final wavelength.

If you do this in excel, it will be a very quick job.

1. Then copy the data from the .asp file (starting below the cells with 1 2 4) into your newly created excel containing the wavenumbers. This file has to be exported as .csv file for Omnic to be able to read it.

Windows X: File -> Save & send -> Change file type -> Other file types -> CSV (comma delimited) (\*.csv)

Windows 10: File -> Export -> Change file type -> Other file types -> CSV (comma delimited) (\*.csv)