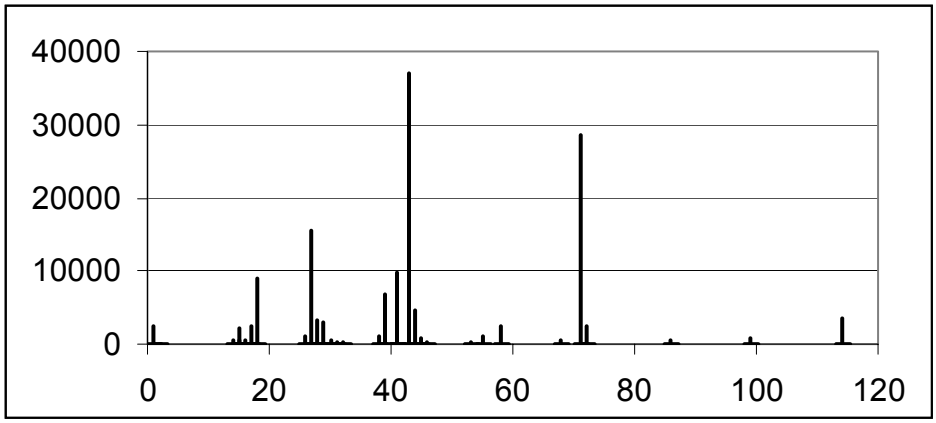
7.13 MASS SPECTROMETRY (6 points)



Outline

This experiment illustrates the basic principles and applications of mass spectrometry, including mass determination, interpretation of cracking patterns and the use of mass spectra as molecular fingerprints. The technique is of central importance in chemistry, and is widely used to identify compounds and to determine their molecular structure in fields such as fundamental research, the chemical industry, medicine and forensic science. For further examples, consult the ASMS web site whose URL is given below.

Background Information

This experiment requires no specific background knowledge, although the lecture courses on quantum chemistry (Michaelmas, 2nd year), valence (Hilary, 2nd year) and photochemistry (Michaelmas, 3rd year) contain some relevant material.

Web-based Resources

http://ptcl.chem.ox.ac.uk/~hmc/tlab/experiments/713.html is the PTCL web site address for this experiment, providing links to safety and other information.

http://webbook.nist.gov/chemistry/ is a link into the NIST web site which contains numerous mass spectra. You may wish to use this site to help in your interpretation of the spectra you will record.

http://www.asms.org/whatisms/index.html offers a comprehensible introduction to MS and its uses.

Safety

(i) Chemicals
Wear safety glasses during this experiment because the glass inlet tubes are evacuated and the samples are eye irritants. Handle the samples in a fume cupboard except when you need to bring them to the apparatus. You can find safety data for the chemicals used in this experiment at the URL given above. You will use very small amounts of these chemicals, and although most are highly flammable, they should not present a significant risk to health.

(ii) Liquid nitrogen
Take care when handling liquid nitrogen; wear safety glasses (Dewars can implode) and avoid spills onto clothing. If a spill onto clothing in contact with the skin occurs, remove the garment immediately to avoid potentially unpleasant burns.

(iii) High voltages
The flight tube uses very high voltages: its connectors must not be disturbed in any way. For your own safety and to protect the apparatus please do not change the settings of any controls apart from those specifically indicated.

Preparation

Start by reading through these instructions in their entirety. If you are not familiar with mass spectrometry, you will find it helpful to spend some time reading about the principles of the technique and about cracking patterns, in a general physical or analytical chemistry textbook. Some texts more focused on the subject are listed in the next section. In addition, the ASMS web site, whose URL is given earlier, contains must useful information about the technique.

General References

1. *Interpretation of mass spectra* F.W. McLafferty [Copies in RSL Stack 1857 d. 252, Queen's College N.e. 189]
2. *Mass spectrometry for chemists and biochemists* R.A.W.Johnstone and M.E.Rose, 2nd edn. 1996, Cambridge University Press. [Copies in RSL Stack 96.B04318.]
3. *Introduction to mass spectrometry* H.C. Hill [Copies in RSL Stack 193975 d. 46, St Anne's 543.7 31, St Peter's Ch 2/HIL.]
4. *Mass spectrometry* J. Barker, 2nd edn., 1998, Wiley. [Copies in RSL Stack 99.E00265, St Peter's Ch 4/BAR.]

Theory

The mass spectrometer in the Teaching Laboratory is a purpose-built instrument made in the PTCL workshops; it uses the ion "time-of-flight" method to separate masses. A liquid sample (or a gas) is introduced into the apparatus and allowed to evaporate. A pulse of electrons ionises the gas-phase molecules and immediately afterwards a pulsed electric field accelerates all ions of the same charge to the same energy, after which they travel rapidly to a distant detector. The kinetic energy given to the ions is equal to the product of their charge q and the applied voltage V.

$$\tfrac{1}{2}mv^2 = qV$$

Although the ions have identical energies, their velocities will differ since they have different masses. For a flight distance D, the flight time t is

$$t = D/v$$

It transpires that the flight time is determined by the mass divided by the charge; spectra are always shown on a scale for ions of a single charge, so doubly (or triply) charged ions, of which a small number may be generated, appear at half (one third) of their real masses.

One of the most useful capabilities of mass spectrometers is to determine molecular masses. In high resolution instruments masses are determined with such precision that molecular formulae can be determined directly from a measurement of the mass, because the fractional part of the exact mass is different for each atomic (isotopic) composition at a single nominal mass number. The same thing can be done in favourable cases even with a low resolution instrument; you will do it in this experiment.

Cracking patterns

The ionising electrons have an energy of 70 eV which is a universal standard in mass spectrometry. The energy actually transfered to molecules on ionisation is usually

much less than this, because the departing electrons also carry away energy. The energy given to the molecules ranges from the minimum needed for single ionisation up to the energy needed for double or even triple ionisation.

The positive ions formed by impact with an electron may be in their ground electronic state, but a high proportion will be in an electronically excited state. The excited states in large molecular ions rapidly undergo *non-radiative transitions (internal conversions*), reaching the ground electronic state with much vibrational energy, causing dissociation. The excited molecular ions break into a mix of smaller ions and neutral fragments, mostly within picoseconds of ionisation; the mass spectrum shows the ions remaining in the ionisation region after a few nanoseconds. The pressure is so low in this spectrometer that there are no bimolecular reactions between ions and neutral molecules; all the ions seen in the spectrum are either parent ions or breakdown products.

The intensity distribution over the different ion masses constitutes the cracking pattern; it is characteristic of the individual molecule and relates systematically to the molecular structure. In part of the practical you will measure the cracking patterns of a series of monofunctional molecules, to investigate their breakdown behaviour and the rules which govern it.

Procedure

1. Check the vacuum

Before starting, look at the diagram of the apparatus and identify the main parts and controls.

First, check the vacuum. The spectrometer operates under high vacuum, maintained by two turbopumps which share a common rotary backing pump. The vacuum controls are at the lower front of the apparatus, behind a perspex panel.

Check that:

- Both rotary pumps are on (orange and green lights lit)
- The backing vacuum (left digital pressure gauge) is below 2×10^{-2} mbar (reading 2.0 -2 or less),
- Both turbopumps are on and "normal" ("power" and "normal" lights lit on the units at left and right).

If all is OK, turn on the ionisation (vacuum pressure) gauge controller by depressing the power switch to the right of the gauge, setting the range switch to 10^{-4} and briefly pressing the switch below the range knob to "ON"; the glass gauge head on top of the apparatus will light up like a light bulb and a reading will show on the meter.

The reading will rise at first as the gauge head degasses itself, then it should quickly (within a minute or so) show 10^{-4} mbar or less. Wait about 10 minutes for the reading to stabilise; change the range switch to the 10^{-5} range; the pressure should be below 2 $\times$ 10^{-6} mbar. If not, check with a demonstrator.

Having completed the checks, turn the ionisation gauge to the 10^{-4} range. **Close the perspex panel** to avoid accidental contact with the vacuum controls.

2. Turn on the spectrometer electronics

Turn on the electronics rack at the single main switch on the right hand side and the NIM bin rocker switch at the bottom right of the lowest stack.

Check that:

- The filament current is about 2 amps (left hand meter in RH unit of top electronics rack).
- The MCP supply voltage is about 2.7 kV (LH unit in the top electronics rack).
- The accelerator voltage is 1.5 kV (central unit in top electronics of rack).

The spectrometer is now on, and even without any added sample a signal of about a hundred counts per second should show on the ratemeter at the right hand end of the lowest unit in the rack; this arises from residual air and other material in the flight tube. Note that the ratemeter is set on its logarithmic scale.

If there is no signal, consult a demonstrator.

3. Turn on the computer and gather a background spectrum

If the computer is not already on, turn it on by pressing the Power button. (You might wonder why we use an old computer to control a new instrument. This computer is in fact particularly suitable, since we run it under DOS to allow the Mass Spectrometer to write data directly into the computer's memory - something that is not possible under Windows.)

At the DOS prompt (`C:>`) type `CD MASS` and when the `C:\MASS>` prompt appears type `MASPEC` and press `ENTER`. (Note that if the computer is already running the MASPEC program when you start, you must quit the program by selecting choice 5 on the main menu, then restart by typing `MASPEC` and pressing `ENTER`.)

When the program starts the main menu will appear. Select choice 1 "gather spectrum" by pressing `1 ENTER`.

Even though you have not put in any sample, a spectrum will start to build up. It shows the background gases that are always present in vacuum systems (water, residual air,

traces of previous samples.) As the spectrum accumulates, compare it with the standard background spectrum provided. If your spectrum is similar to the standard, then all is well. If you notice big differences, consult a demonstrator. Small extra peaks are not important, but if the peaks are much broader than in the standard, or if the approximate mass calibration is wildly out (e.g. no peak at mass 18 for H_2O^+), tuning may be necessary before you go any further.

While the spectrum is being gathered practise controlling the sensitivity range of the displays (Page up/down and up/down arrows) and move the blown-up region (lower panel) around the main spectrum (R/L arrows and CNTRL-arrows). The instructions for using these keypress commands are given on screen. To end data gathering and return to the main menu press Q. Pressing Q (or sometimes pressing the spacebar) will return you to the menu from all parts of the program. You can clear the current spectrum and restart gathering by pressing C.

Stop gathering when the peaks are well defined, e.g. when the strongest peak has reached 800 counts or more when blown up (lower panel). Now explore the spectrum using choice 2 on the main menu; the key instructions for using this part of the program are indicated on-screen, and explained in more detail in a sheet on the bench.

The mass calibration can be reset, if necessary, using 1 for H^+ and 18 for the H_2O^+ peak, or 18 for H_2O^+ and 28 for N_2^+. These will give only a crude calibration, however. For a good calibration you need two masses as far apart as possible, such as 1 for H^+ and 100 from a hexanone. When you are satisfied with the mass calibration and familiar with this part of the program, press Q to return to the main menu.

Next create a stick diagram spectrum using choice 3. You will be asked to choose a mass range and a sensitivity. A maximum mass of 100 is appropriate for the background spectrum and 2 is a good first choice for sensitivity. This means that, for peaks to be shown in the spectrum, they must have areas greater than 2% of the strongest peak ("base peak").

At this point you may wish to print out the stick diagram spectrum. To do so, consult "Printing spectra" at the end of these instructions. When you press the spacebar to leave the stick diagram, you are asked if you want to save it; if you do, press Y for yes. You can create different stick diagrams from the same main spectrum as often as you like; try varying the scale and sensitivity until you are happy with the result.

You can give each saved spectrum a name, which will be printed on it, but you should also record its identity in your experimental notebook. The computer will tell you what number is used for identification before the spectrum is saved.

4. Measurement of an air sample and verification of the mass scale

Before adding a sample, study the inlet vacuum system. Samples will be contained in tubes connected to the down-pointing steel cone; tap A leads from the cone to a manifold, tap B to the vacuum pump and tap C to the sample reservoir and to the spectrometer via an electromagnetic (EM) valve controlled by a pushbutton.

The pressure in the reservoir is measured by a thermal conductivity gauge and is displayed digitally behind the perspex panel below the inlet system. Take a moment to check the digital display; e.g. 3.2 -2 means 3.2×10^{-2} mbar. If the pressure in the reservoir is more than 1.1 mbar, or if tap C is open, the EM valve leading to the spectrometer cannot be opened and the pushbutton marked READY will be dark; it lights up when the pressure is below 1 mbar. One press opens the EM valve (warning light on), a second press shuts it.

The inlet should have been left with tap A and the EM valve closed, B and C open to evacuate the reservoir; if it is not so, set it so now. When the reservoir pressure is below 5×10^{-2} mbar, close tap C.

A clean glass sample tube should already be on the inlet, and should have been evacuated. If there is no sample tube on the inlet:

- Clean any grease off the steel cone using tissue paper, inside as well as outside.
- Apply a minimum quantity of grease ("glisseal" or Apiezon N or L) to the cone.
- Select a clean sample tube and fit it onto the cone. (If all the sample tubes have been left dirty (test by smell), clean one first as described below.)
- When a sample tube is in place, rotate it to ensure an even film of grease, then open tap A to evacuate the inlet tube for about a minute.

Close tap A. <u>Carefully support the steel line connected to the cone</u>, and pull the sample tube off with a gently twisting motion and replace it immediately (this traps a sample of air).

Close tap B, open tap A. (This expands the sample, reducing its pressure). Close tap A again (for safety). Check that the EM valve is closed (red light off).

Gently open tap C and watch the reservoir pressure as you do so. It may overshoot the desired 1 mbar when the tap opens. If it does, close C for the moment, open B to evacuate the manifold, then gently open tap C to reduce the sample pressure in the reservoir to somewhere between 0.5 and 1 mbar; the READY light should come on.

Finally close tap C and press the READY button to admit the sample to the spectrometer; the Valve Open light should come on.

Gather the spectrum as before, until the peaks including Ar^+ at mass 40 are well defined. Press the READY button to shut off the flow of sample; press Q on the keyboard to stop data gathering.

Open taps B and C to pump the air sample away.

5. Analysis; theory of the TOF mass spectrometer

If you plan to do the write-up at home using your own computer, save the whole spectrum now using choice 4 on the main menu (not a stick-diagram version). To analyse the data:

1. Review the equations relating kinetic energy, charge, mass velocity, flight distance and flight time. Work out how flight time should be related to the mass and charge of the ions, given that ions are rapidly accelerated to 1.5 keV energy, and their flight distance is about 0.50 m.

Examine the spectrum of air, using choice 2 on the main menu, to get data to check your theory. The position of a broad cursor is shown on the lower figure as a yellow highlighting of several contiguous channels. This cursor can be moved slowly using the left and right arrow keys, or more rapidly by using CTRL / $\leftarrow$ or CTRL / $\rightarrow$. As you move the cursor onto each peak the flight time in ns is shown at the lower left of the screen, calculated as the centroid of the points covered by the cursor. The next two figures are the lower and upper limits of the cursor, whose width you can set with the < and > keys. The approximate mass is given at the far right. Use the positions of the peaks with the exact masses listed below to complete two tasks:

(i) Verify that the relationship that you have derived between mass, charge and flight time has at least roughly the right form and that the constant in it is approximately correct. (Don't forget the Ar^{2+} ion.)

(ii) Show that the functional form of the mass dependence is exactly right and is followed with great precision. Note that because of imperfections in the electronics, there may be a constant (small) time offset,

$$t = \text{constant} \times f(m,q) + t_0$$

You should think carefully how to demonstrate exact agreement, because a simple linear graph of t against f(m,q) can not be drawn precisely enough to be a good test. Try to think of a way to eliminate the main part of the variation of t with m so as to bring out any residual deviations.

2. The resolution of a spectrometer of this type is a number equal to m/dm where dm is the mass width. Verify from your equation that $m/dm = \frac{1}{2} t/dt$. Measure the

width of a peak (mass 28 or 32 is suitable) by adjusting the cursor to fit the top half of the peak and noting the upper and lower limits; hence determine the resolution of the spectrometer. One could get a different answer by taking the width at the base of the peak, but here we use the conventional definition based on "Full width at half maximum" (FWHM).

Ion	Nominal mass	Exact mass
H^+	1	1.00782
H_2O^+	18	18.01055
N_2^+	28	28.00615
O_2^+	32	31.98983
Ar^+	40	39.96239

6. Mass spectra of the ketones

You will measure the mass spectra of a series of liquid ketones, to establish some systematics of their molecular ion breakdown. Use the minimum quantity of sample each time, to protect the environment, to protect the apparatus and to make the measurement process quick and efficient.

You will need:

(i) a Dewar ("Thermos") of liquid nitrogen, which the technician will supply,
(ii) a small Dewar,
(iii) at least two sample tubes,
(iv) a thin glass rod,
(v) vacuum grease,
(vi) tissue paper.

To work through the samples, you will repeat a series of operations. Start with the spectrometer as it should have been left after the air sample, i.e.:

Tap A shut; taps B and C open; EM valve shut. Sample reservoir pressure below 5×10^{-2} mbar.

Gather a background spectrum to check that any previous sample has been removed. If water is the main peak, all is well. If not, pump some more. Remember that you can start a new spectrum at any time while gathering data by pressing C (clear).

Put a clean empty sample tube on to the inlet cone. (There is no need to regrease the cone every time, but you should do so if the layer of grease between cone and socket is visibly incomplete, or if you suspect that the grease is contaminated. In this case clean all old grease off before putting new grease on.)

Close tap C and then open taps A and B to pump on the empty sample tube. This is the best way to ensure that all traces of volatiles (previous samples, cleaning solvent) have been removed. After 30 s close tap A, remove the sample tube. You have to pull it off against the vacuum, so twist the tube as you pull, and support the steel tube.

Take the sample tube to the fume cupboard and select the next sample. Dip a **clean** glass rod into the liquid, then touch it to the bottom of the sample tube (preferably without touching the sides). This will transfer a tiny sample to the tube. Put the sample tube back on the entrance cone of the spectrometer.

Freeze the sample in liquid nitrogen, using the small Dewar to cool only the bottom of the tube. Usually you will see white crystals appear when the sample freezes. Now open tap A to pump all the air away; this will take about 15 s; you will *hear* when it is done as the sound of the vacuum pump changes. Close tap B, open tap C and remove the liquid nitrogen to allow the sample to warm up. Watch the sample reservoir pressure gauge as it does so; as soon as the pressure reaches 1 mbar or becomes steady at a lower pressure, close tap C. If you overshoot, refreeze the sample and open taps A and C to remove some vapour from the reservoir, then close the taps again.

You can now run the spectrum. Press the READY button to let sample flow in, and then set the computer to gathering (choice 1 from main menu or press C if gathering is already going on).

As soon as the spectrum is strong enough press the READY button to stop sample flow, and terminate gathering by pressing Q.

Examine the spectrum to check that it looks OK (not just background!). If it looks OK do not pause long to study it or print it, but immediately refreeze the sample in liquid N_2, then open taps A and C to remove remaining sample from the reservoir. You can watch the reservoir pressure fall as the sample condenses. When the pressure is below 0.1 torr, or steady, close tap A, open tap B to pump out any residue, and carefully remove the cold sample tube to the fume cupboard to thaw there. (The point of this is that the longer a sample stays in the machine, the more it is absorbed on surfaces and in rubber gaskets, and the more difficult it is to pump away.)

While the pumps clear the spectrometer for the next sample, you can examine the spectrum in more detail. Check the mass calibration (all these ketones show a molecular parent ion) and reset it if necessary. Then make a stick diagram spectrum and save it, noting the details in your laboratory notebook.

When the sample has thawed in the fume cupboard, swill the tube out three times with small quantities of acetone into the appropriate residues bottle. Then dry the tube in a flow of air from the compressed air line (red pipe) at the tap near the large optical

spectrograph. It is now ready for reuse. Clean the glass rod with tissue paper and acetone.

Choice of samples and interpretation of the spectra

Run the spectra of:

2-butanone, 2-pentanone, 3-pentanone, 2-hexanone, 3-heptanone

To interpret the spectra, consider the following:

- Are the intense fragment peaks at even or odd mass number ?
- What are the neutral leaving groups (Parent - fragment ion) ?

When you have thought about the spectra a bit, consult a book, the Internet or a Demonstrator to check out the principal fragmentation mechanisms followed by ketone ions. Note your conclusions in your practical notebook.

7. Accurate mass determination

In the spectrum of 3-pentanone you will have found a strong peak at mass 29. This ion could be either $C_2H_5^+$ or CHO^+, both of which are stable ions found in the spectra of these sorts of compounds. We can check which it is by determining its exact mass using nitrogen and oxygen in air as local calibrants.

Measurement

Feed an air sample of 0.5 mbar into the reservoir. Then carefully add 3-pentanone to make the total pressure up to 1 mbar. Leave the gases a few minutes to mix, then gather the spectrum until the peaks at mass 28, 29 and 32 all look nicely smooth. Then pump all the sample away (the pentanone would not diffuse fast enough through the air to make freezing out practical).

Calculation

Examine the spectrum using choice 2 from the menu. Because the peaks are not perfectly separated at this resolution, use the same cursor width on all three, wide enough to take in the whole of each peak, and note the peak positions (centroids) as determined by the program.

Now use the exact masses of N_2^+ and O_2^+ given in the Table above, together with the functional dependence of flight time on mass as checked in Part 2, to determine the exact mass of the 29 peak. Compare with the possible values, 29.0027 for CHO^+ and 29.0391 for $C_2H_5^+$. Estimate the uncertainty in your answer from the uncertainty in

the times of the peaks (you can use the uncertainty in time of mass 29 alone as an approximation). From the estimated uncertainty, decide whether or not you have proved that the ion is one or the other species. Does your result agree with your interpretation of the spectrum ?

8. Unknowns

Using the same procedure as for the liquid ketones, measure the spectra of unknown A (which is an aliphatic ketone) and all the other unknowns B through E.

You may be able to deduce the identity of A from your newly acquired knowledge of the spectra of ketones. For the other unknowns you can try two different strategies. To give you a good chance of successful identification, the parent ions appear in all the spectra. You could use one or more of the following approaches:

1. Use general observations (e.g. carefully smell each sample) and some simple principles of interpretation (Appendix) to guess the identity. Identification can be checked by getting the known mass spectrum of the guessed compound over the internet (NIST website), or from a library compilation (EPA/NIH Mass spectral database NSRDS-NBS 63, 8-peak index of mass spectra etc.)

2. Compare the spectrum as a fingerprint, without interpretation, against a set of spectra in a database. A limited database and a clever search program are provided on the computer, and instructions for using them are on a separate sheet.

3. Work through one of the texts and learn the art of assigning spectra, including the identities of common fragment ions, leaving groups and rearrangement mechanisms, then apply the knowledge to the spectra. Unfortunately, this might take rather too long. One of the very first "expert" computer programs (DENDRAL) was designed to analyse mass spectra in this way.

Shutting down

Please leave the spectrometer as you would wish to have found it, i.e.

A clean sample tube on the cone, already evacuated
Tap A closed
Taps B and C open
EM valve closed (Warning light off)

All pumps on.

Main spectrometer switched off (Check zero filament current)

NIM bin rocker switch off

Computer showing the DOS prompt, monitor turned off

Appendix

Some principles for interpreting mass spectra

The atomic composition of an ion can sometimes be deduced from its isotope abundance pattern, e.g. if Cl or Br is present.

If the parent molecular ion mass number is odd, it must contain an odd number of N atoms.

For a compound $C_xH_yN_zO_n$ the total number of rings and double bonds is $x - \frac{1}{2}y + \frac{1}{2}z + 1$.

Simple bond cleavage of an even-mass molecular ion produces fragment ions at odd mass numbers, with even numbers of electrons. Fragments at even mass numbers come from rearrangements or two bond breakages. Intense odd-electron ions are usually structurally significant. If the parent ion is odd mass, then odd mass fragments are the unusual ones.

If the parent ion or parent group of peaks is more intense than the fragment ions, an aromatic ring structure likely.

Only a limited number of stable neutral fragments are commonly lost in ion fragmentation. Look at the mass losses from the parent ion to provisionally identify groups present, e.g 15 = $-CH_3$, 29 = $-CHO$ or $-C_2H_5$, 31 = $-OCH_3$ etc. *(Table on bench)*

Printing spectra

The computer running DOS will not communicate satisfactorily with a laser or inkjet printer, so you need to copy onto a floppy disk the spectra that you want to print and then print them elsewhere. The most flexible way of doing this is to feed the spectra into Excel.

Save your spectra, then close down the maspec program and insert a floppy disk. Copy the spectra that you wish to print using the copy command, for example `COPY STK?.msp a:` in which ? is replaced by the number of the spectrum you wish to print. MS files contain the full spectrum while STK files contain the stick diagram.

Go to a PC that has Excel available and insert the floppy disk. Start Excel, choose the `Open...` command within the `File` menu and select a STK file from the floppy (you will need to choose to display all files to be able to see the files).

Although the file you are reading is not in Excel format, the program will be able to parse (i.e. interpret it). Choose `Delimited` data on the first window that appears, click on `Next` and select `Space delimiters` on the next window. Click on `Finish`

The data should appear in columns 1-3. The first entry is the number of sticks, the final one is the name you gave your spectrum. Remaining entries are the stick heights and positions.

Once you have read in the data, click and drag with the mouse to select the x/y values for your spectrum. When you have highlighted the relevant values, choose `Insert` and add a chart. Select the `X/Y (Scatter)` option and click `Finish`.

This procedure will generate your mass spectrum, but in an odd form, since each peak will be represented as a single point at the relevant intensity. To create a conventional stick diagram you need to

(i) join each point to the baseline with a line, and
(ii) remove the marker for the point.

To accomplish this, double-click on one of the points; this should bring up the points properties dialogue. In the marker section click on `None` for the type of marker. Now select the Y Error Bars tab. Choose to draw an error bar downwards (the `Minus` option), click the `Percentage` radio button and set the length to be 100%. Click on OK; the spectrum will appear in the correct format.

Taking the data to analyse in College or at home

Copy onto your floppy disk all the full mass spectra (MS?.dat) and stick spectra (STK?.msp) that you have created. Also copy the program "Maspec3.exe". This is a version of the program without the interface to the spectrometer, which would cause computers without the hardware to crash.