4

Processing

4.1 Introduction

Acquiring your data is just the first step in producing a useful spectrum. Fortunately, systems are normally set up so that they perform the processing steps automatically. Most of the time they do an excellent job and your data is fine. Sometimes you may have special requirements and other processing will be required. This chapter looks at some of the things that can be altered to improve the appearance of the data for you. Note that most of the examples are for 1-D proton spectra but all of the sections are valid for certain types of 2-D experiment.

4.2 Zero Filling and Linear Prediction

Because we are always in a hurry (so many samples, so little spectrometer time) we always try to acquire that little bit faster than we should. This is particularly true with 2-D acquisitions which can be very time-consuming. As discussed previously, we try to minimise the number of increments to save time. This gives rise to highly truncated data sets and poor resolution. This can be made to look a little prettier by adding a load of zeros to the experiment before Fourier transforming it. We call this (somewhat obviously) 'zero filling'. Note that this doesn't add any information but it does make the result look nicer.

Linear prediction works in a different way by predicting what the missing (future) values would be, based on the existing (past) values. This approach is more powerful than mere zero filling but it also brings with it some risks (artefacts). You can't linear predict infinitely and so we tend to advise that one degree of linear prediction is about all the data can reliably take without going into the realms of fantasy. If we take the example of our COSY spectrum, we would probably linear predict out once (to double its size to 512) and then zero fill once or twice to take the final size to 1024 or 2048 points (in the indirect dimension). It is also possible to 'backward linear predict'. This allows us to reconstruct the first part of the FID which we can't collect because we have to wait a finite time for the transmit pulse signal to die away. This effect is known as 'ring down' and causes baseline distortion. Backward linear prediction allows us to throw these points away and replace them with what might have been there.

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4.3 Apodization

Sometimes the FID doesn't behave as we would like. If we have a truncated FID, Fourier transformation (see Section 4.4) will give rise to some artefacts in the spectrum. This is because the truncation will appear to have some square wave character to it and the Fourier transform of this gives rise to a Sinc function (as described previously). This exhibits itself as nasty oscillations around the peaks. We can tweak the data to make these go away by multiplying the FID with an exponential function (Figure 4.1).

This has the effect of smoothing the FID away to zero, thus yielding lovely peaks. We call this 'exponential multiplication' for obvious reasons!

It is also possible to play other mathematical tricks with the FID. For example, we may want to make our signals appear sharper so we can see small couplings. In this case, we want our FID to continue for longer (an infinite FID has infinitely thin lines when Fourier transformed). To do this we use 'Gaussian multiplication'. This works exactly the same way as exponential multiplication but uses a different mathematical function (Figure 4.2).

It should be noted that Gaussian multiplication can severely distort peaks and also reduce signal-to-noise of the spectrum so it is not a good idea to do this if you have a very weak spectrum to start with. Spectrum 4.1 shows a real case where Gaussian multiplication has been able to resolve a triplet. Note that it is possible to just make out the triplet nature of the peak in the unmultiplied spectrum – Gaussian multiplication helps verify this and also allows us to measure the splitting pattern.

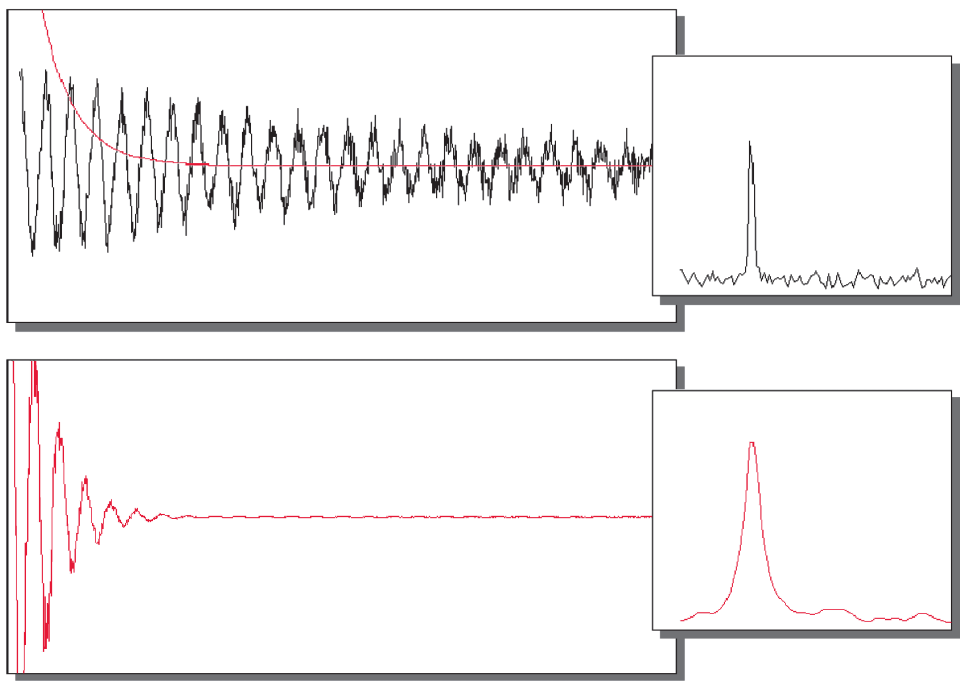


Figure 4.1 Exponential multiplication.

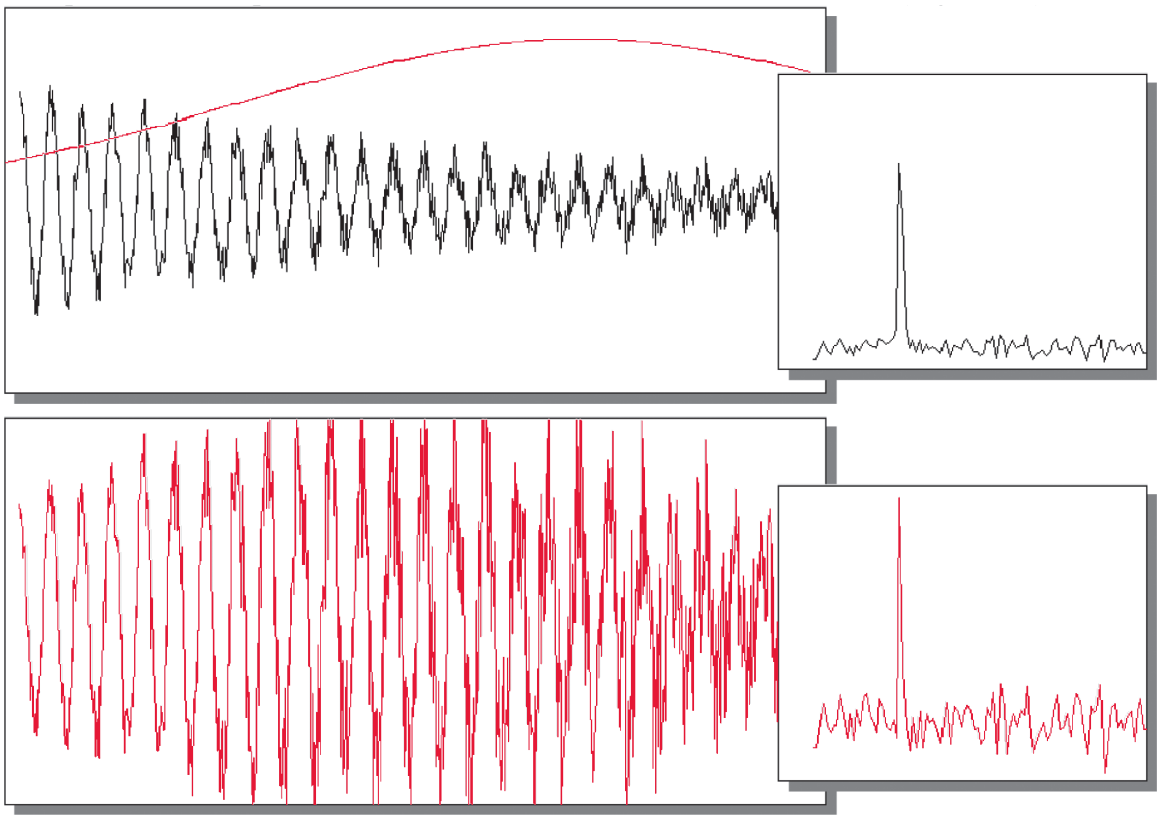
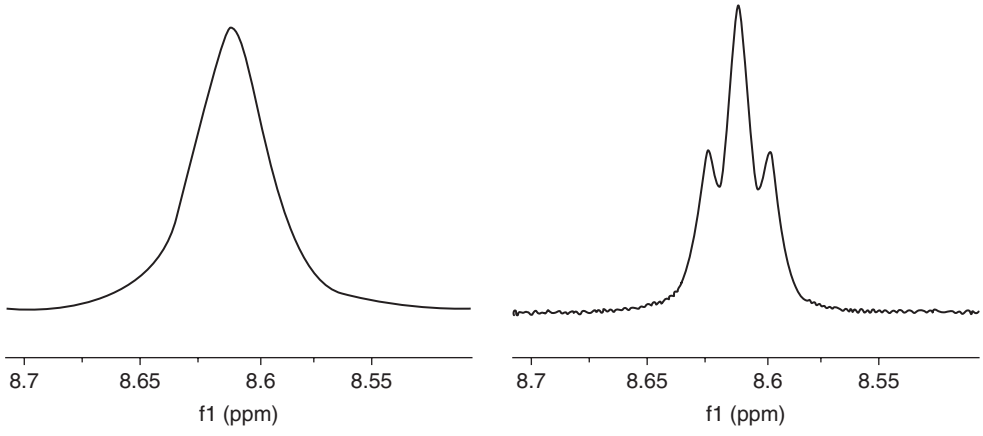


Figure 4.2 Gaussian multiplication.



Spectrum 4.1 Gaussian multiplication in action.

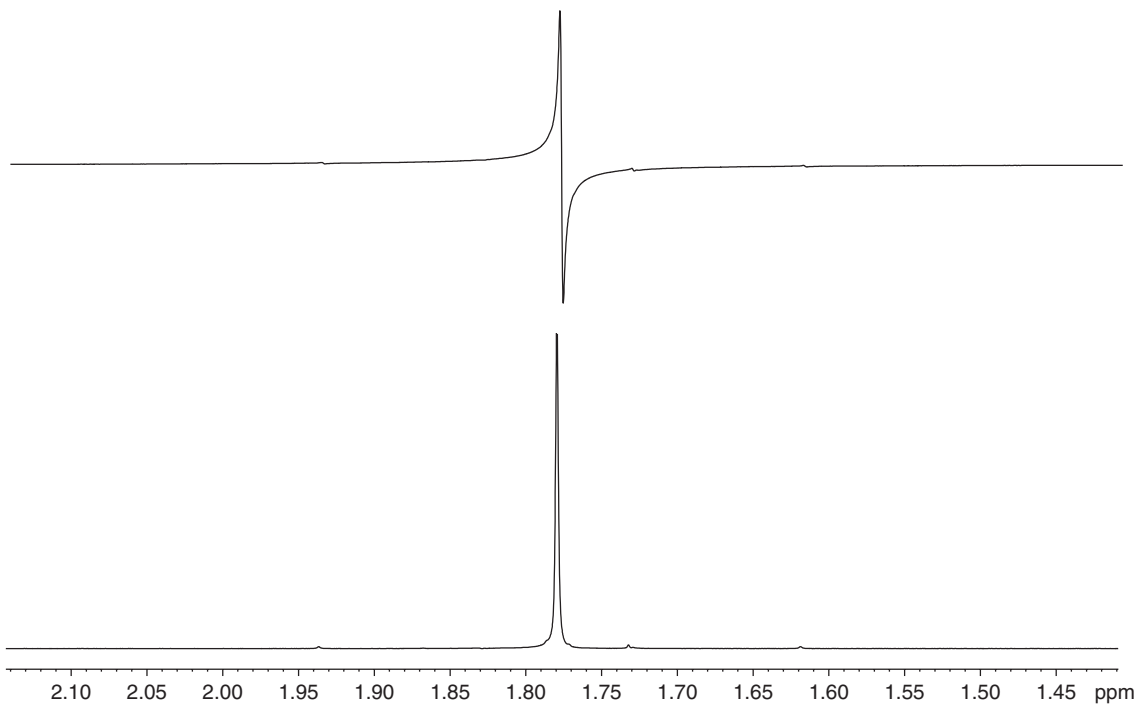
There are many other apodization functions which are used for specific types of NMR data. In fact you can make up your own if you want to but for most data sets, the 'canned' ones that are shipped with the instrument are more than adequate.

4.4 Fourier Transformation

As mentioned earlier, we acquire data in the 'time domain' but to make sense of it, we need to view it in the 'frequency domain.' This is where the Fourier transformation comes in. There is not too much to do here – there are no parameters to change. It is a necessary step but the automatic routines will perform this for you with no input.

4.5 Phase Correction

For several technical reasons, it is not possible to acquire NMR data with perfect phase. One reason is the inability to detect XY magnetisation correctly; another is the fact that we are unable to collect the data as soon as the spins are excited. These limitations mean that we have to phase correct our spectrum so that we end up with a pure absorption spectrum. What we *don't* want is a dispersion signal (see Spectrum 4.2).



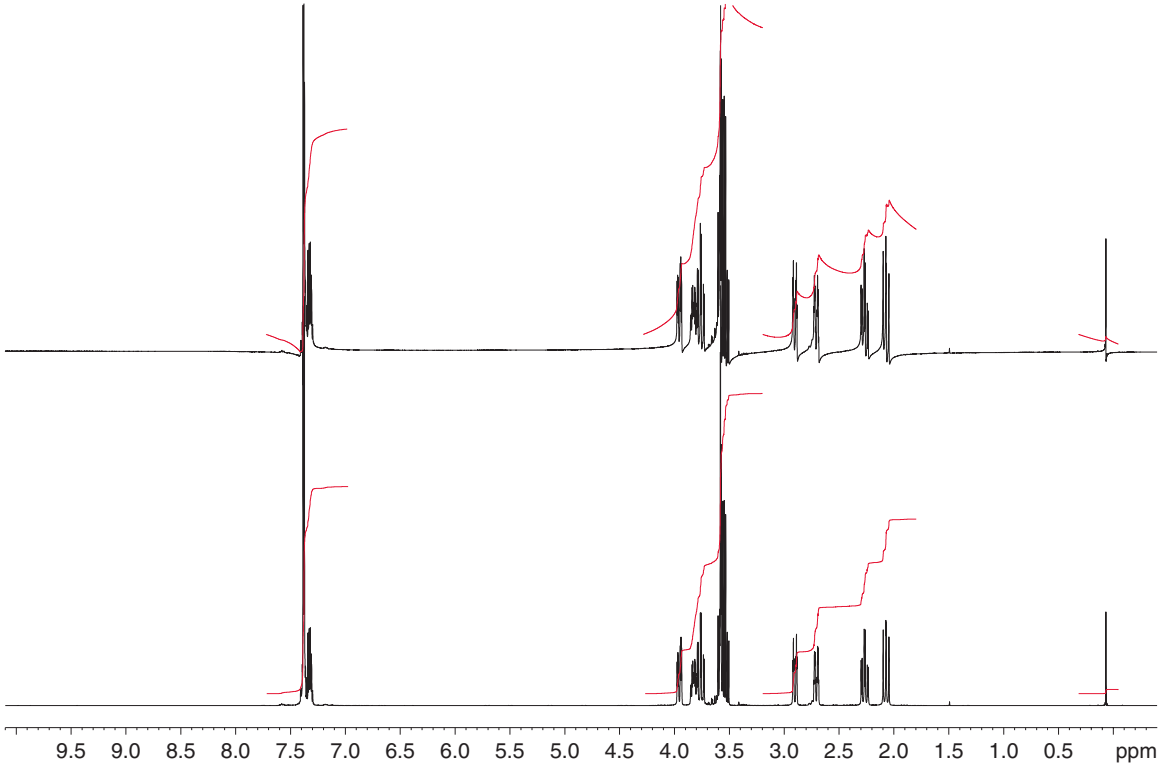
Spectrum 4.2 An absorption signal (below) and dispersion signal (above).

The XY problem gives rise to a constant phase error across the spectrum, the delay problem gives a linear phase error. To correct for this, we have two phase adjustment parameters at our disposal: zero and first order.

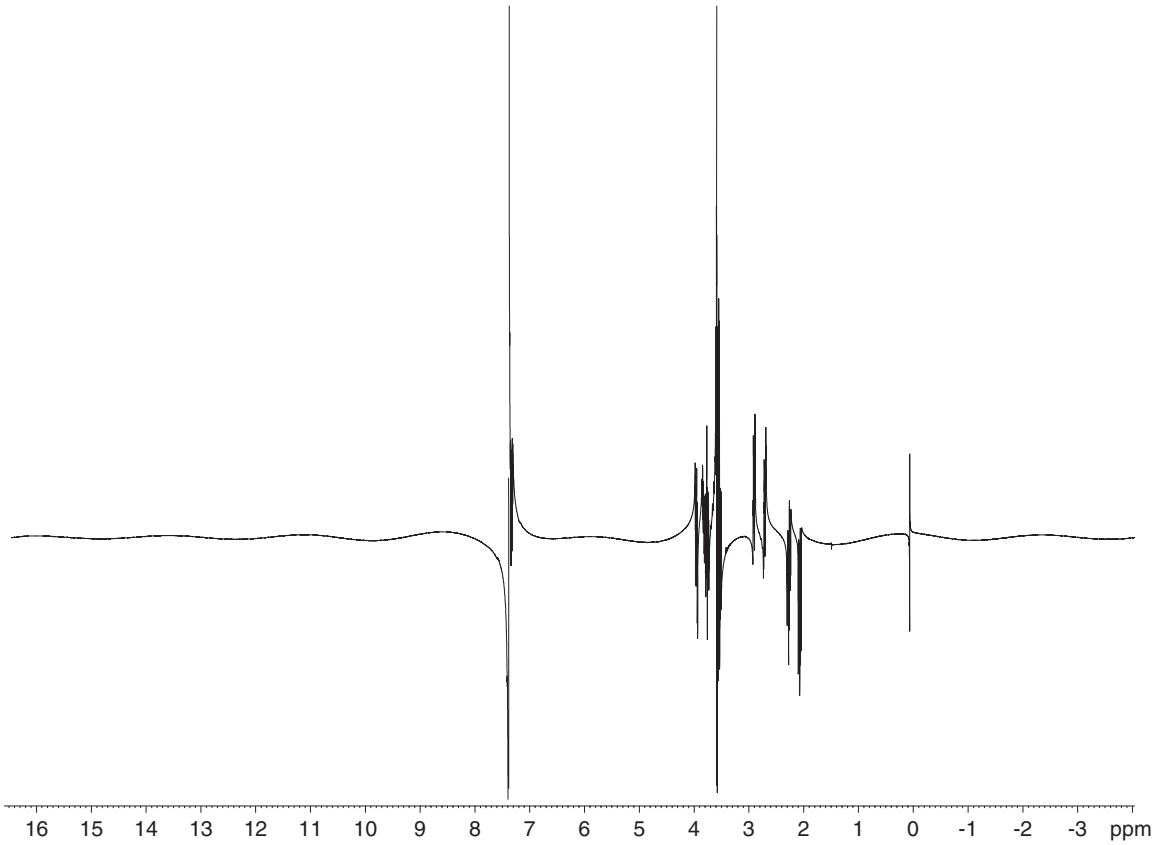
Modern NMR software comes with very good automatic phase routines so most of the time you should end up with a beautifully phased spectrum. Sometimes, however, the software doesn't quite perform and you may need to tweak the phase manually. It can take a bit of familiarity to get this right but it is just a matter of practise. If you remember that the zero order adjustment works constantly across the spectrum and that the first order doesn't, it is quite easy to see what is going on. Normally the software gives you an option of setting the 'pivot point' of the first order adjustment (i.e., the frequency in the spectrum where there is no effect from the first order adjustment). This pivot point is normally set to the largest peak.

Spectrum 4.3 shows how the phase can be improved with a manual tweak. Note that in a poorly phased spectrum, the integrals will be distorted such that they are pretty much unusable.

So far, we have talked about phasing 1-D spectra but this is also valid for some 2-D experiments. Phase-sensitive 2-D experiments also require phasing in one or both dimensions. Similar approaches are used as described here. Note that this is not necessarily the case for all 2-D experiments as some of them are collected in 'magnitude mode' where we look at only the intensity of the signals, not their sign.



Spectrum 4.3 A well phased spectrum with reliable integrals (below) and a badly phased spectrum with unusable integrals (above).



Spectrum 4.4 Too much first order phase!

One last cautionary note: the first order phase can be increased beyond +/– 360° – but shouldn't be! If this happens, you will end up with a distorted, 'wavy' baseline. A sine wave is in effect superimposed on the spectrum, so if you see a wavy baseline, check that you haven't wrapped the phase too far. Spectrum 4.4 shows what happens when you go a bit mad with first order phase! If you end up in this position, do not attempt any kind of baseline correction as this will add to your problems. Just set both your phase parameters back to zero and start again. . .

4.6 Baseline Correction

There are many reasons why your baseline may not be as flat as you would like. Many of them are hardware-related; some are brought about by having a distortion in the early points in the FID. They can also be caused by background in the probe (this is often the case for Fluorine spectra due to PTFE in the probe). Whatever their cause, bad baselines not only make the spectrum look poor, they also give rise to poor integrals. Whilst there has been a lot of work at improving the hardware, there is still a need to massage the baseline to make it look good. There are numerous algorithms to help with your

baseline and these will generally be applied automatically by the software that was used to acquire your data. These poor baselines are particularly noticeable when you have a very weak sample (for example, carbon spectra). It is also possible to manually correct your baseline if the automatic algorithms fail. In this case, you tell the software where the baseline should be and it then performs a spline-fit to level it.

4.7 Integration

As you are no doubt aware, integrals are one of the key parameters in the interpretation of proton spectra and are pivotal in quantification. They measure the area under a peak and this is directly proportional to the number of protons (in the case of proton NMR) in that environment. Most software will automatically try to identify the peaks in your spectrum and integrate them for you. If you need to do it yourself, then it is a fairly trivial matter of defining the start and end point of the integrals of interest. The only complication is that you may need to tweak the slope and bias of the integral. This should be unnecessary if you have got the phase and baseline of your spectrum correct. If you find that you need to adjust slope and bias, we suggest that you go back and try to sort out baseline and phase a bit better.

Integrals may appear low on signals that have a long relaxation time (see Chapter 3). If this is the case, then you should acquire your data with a longer relaxation delay. This is likely to be most noticeable on singlets and isolated protons as these tend to have quite long relaxation times. If you have poor signal to noise, this will also affect the accuracy of your integrals.

4.8 Referencing

As mentioned in Chapter 3, we standardise our reporting of chemical shifts with reference to TMS or the residual solvent peak. Your spectrometer software should do this for you automatically. If it gets it wrong (which is possible if you have a mixed solvent or a spurious peak near TMS), then you can set it manually using your software.

4.9 Peak Picking

If you want accurate chemical shifts or splittings, peak picking can help. However, it is worth issuing a health warning here! The accuracy of your chemical shifts and your splittings is limited by the digital resolution of your spectrum. This means that whilst the computer is happy to spout figures to four decimal places, in reality you may not be able to measure to better than +/– 0.5 Hz. Always check your digital resolution before trying to quote things too accurately. Don't forget, your chemical shifts will be influenced by concentration, temperature and pH so it is probably pointless quoting chemical shifts to a greater accuracy than 0.05 ppm except in special circumstances. Also, be warned that measured splitting is influenced by line width, so very broad peaks (or very close peaks) may show a smaller value than the real value.