

## Electrospray Data Processing

In the electrospray spectra of proteins etc, each component produces a range of multiply charged ions in the original m/z spectrum. Therefore additional processing must be performed to produce a molecular mass spectrum.

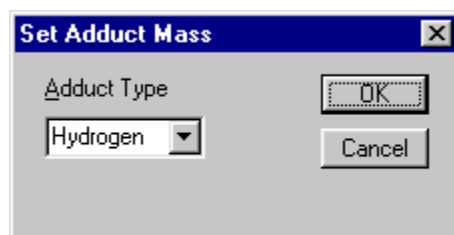
Also, due to the high accuracy required, a special calibration procedure is used.

MassLynx provides two distinct methods for calculating the molecular mass spectrum, **Transform** and **MaxEnt**.

The **Transform** technique requires the prior assignment of charge states to peaks in the electrospray m/z spectrum by the user. This charge state information is then used to transform the electrospray data onto a molecular mass axis.

## Setting Adduct Mass for Transform and MaxEnt

Both the Transform and MaxEnt processes use the value for adduct mass in their calculations. To set the adduct mass value select **Set Adduct Mass** from the Spectrum **Process** menu.



*Figure 4.22 Set Adduct Mass dialog*

The **Adduct Type** can be set to **Hydrogen**, **Potassium** or **Sodium**. Selection of more than one adduct type is not supported.

## Finding Components for Transform

Transform first requires the assignment of charge states, and this is performed on a stick spectrum. Therefore, the first three steps are:

1. Background subtract the data. Suggested parameter values are: **Polynomial order** set to 1 for a flat baseline, or 5 for a curved baseline, **Below curve** set to 40%, and **Tolerance** set to 0.01.
2. Smooth the data with the Moving Mean algorithm. You need to measure the width of a peak in the raw data at half its maximum intensity, and enter this value in the **Peak width** field. Set the **Number of smooths** parameter to 2.
3. Create a stick spectrum with the Center process. Set the **Min peak width at half height** parameter to 4. Select **Top** as the centering method. Ensure the **Create centered spectrum** box is checked, and the **Heights** radio button is also checked. It is most convenient to put the stick spectrum into a new window, so it can be expanded to fill the Spectrum window when multiply-charged series are being identified. Check the **New window** radio button to do this.

Multiply-charged series can now be identified as components. There are two methods of component identification.

The **manual** method requires the user to identify two adjacent peaks in each series. MassLynx then identifies the rest of the series above the threshold and calculates the component's molecular mass and the standard deviation associated with this mass.

The **automatic** method can be used to find each series in the spectrum in turn, or to identify all series in the spectrum. The disadvantage of this method is that a mass range to search over must be known in advance. Using a wide mass range may result in the false identification of spurious series.

For the analysis of a true unknown the **manual** method is preferred, so you can check the reliability of each entry.

### ■ To find components where you do not know the mass range

1. Choose **Component Find Manual** from the Spectrum **Process** menu.

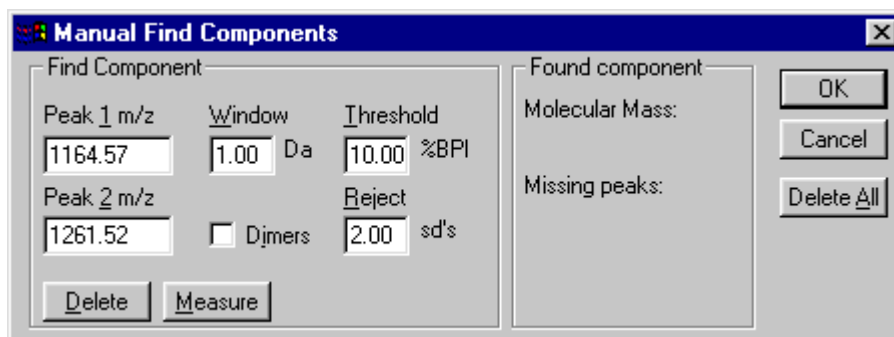


Figure 4.23 Find Components Manual dialog

2. Having visually identified a multiply charged peak series, position the mouse pointer close to one peak in the series. Press the right mouse button. Position the pointer close to an adjacent peak in the series. Press the right button again. The **Peak 1** and **Peak 2** controls will be updated to show the selected masses.
3. Press the **Measure** button. In the Found component section on the right of the dialog box, the mass of the component will be displayed. Also shown are the expected masses of peaks that were not found.
4. If you are satisfied with the identification of the series, proceed to the next one. Otherwise, pressing the **Delete** button will cause the component to be removed from the component table, and you may repeat the process.
5. Press the **OK** button.
6. If you wish to abandon the process and exit the dialog box with no changes to the component table, press the **Cancel** button. If you wish to clear the component table completely, press the **Delete All** button.

You may wish to alter the following parameters:

**Window** Specifies the tolerance on the position of each peak in the series. It may need to be increased from its default value of 0.5Da for statistically poor data. Too low a value will result in the algorithm being unable to identify the whole of the series. Too high a value may result in the algorithm selecting wrong peaks.

**Threshold** Specifies a minimum intensity of peaks for the algorithm to consider. It is specified as a percentage of the intensity of the most intense peak in the spectrum.

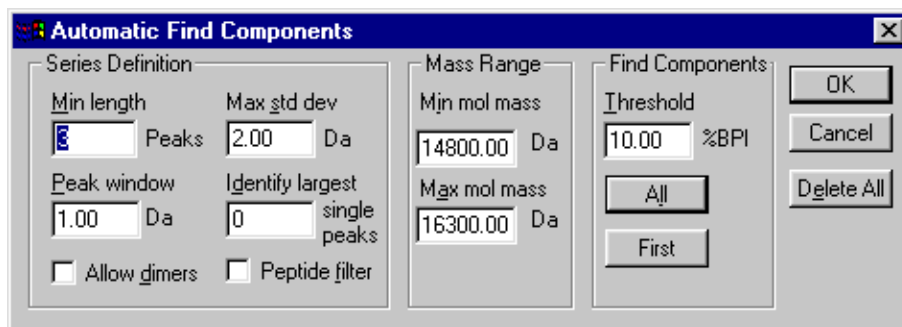
**Dimers** Allows correct charge assignment for the dimeric component in a monomer-dimer mixture. In this case, the monomeric series will obscure alternate peaks in the dimeric series. Therefore to identify the dimer, the algorithm must assume a difference of two charge states rather than one between the two peaks you identify. Checking the **dimers** box causes this assumption to be made.

A molecular mass is calculated for each peak in the series. The mean molecular mass and standard deviation of that mean are then calculated. The **Reject** parameter offers the opportunity to discard any peaks whose molecular mass is too far from the mean value. Such peaks are discarded and the mean is recalculated. This feature prevents outlying peaks from biasing the mean molecular mass measurement. The value specified is a number of standard deviations. The default value of 2.0 means "Reject any peak whose molecular mass lies two or more standard deviations from the mean". Two is a safe value; 95% of the time, masses will be within two standard deviations of the mean.

If you do not wish to use this feature, the **Reject** parameter can be set to some high value (10 is sufficient).

■ **To find components where you have knowledge of the mass range**

1. Choose **Component Find Auto** from the Spectrum **Process** menu.



*Figure 4.24 Find Components Auto dialog*

2. Set up the **Series Definition** parameters. The **Min length** parameter refers to the minimum number of peaks in a series. The **Peak window** parameter is described in the **Component Find Manual** section above. The **Max std dev** parameter allows you to set an upper limit, in Daltons, on the spread of the molecular masses of the peaks in the series. The **Allow dimers** check box is for the analysis of monomer/dimer mixtures as explained in **Component Find Manual** above. Checking **Allow dimers** allows the algorithm to try series with a difference of two charge states between adjacent visible peaks as well as series with contiguous charge states. If you are looking for low mass peptides that are the result of a digest check the **Peptide filter** check box. If you are doing high mass work leave the **Peptide filter** check box unchecked.
3. Set up the **Mass Range** parameters. It is sensible to restrict the range as much as possible; the wider the mass range the algorithm is allowed to search over, the greater the chance of it making a series from peaks in the noise.
4. Set the **Threshold** parameter. A sensible threshold keeps the algorithm out of the noise, and helps to avoid the above problem.
5. Pressing the **First** button causes the algorithm to find the best series containing the most intense unassigned peak. If no such series can be identified, then you need to relax some of the parameters. First, check the **Min length** and **Threshold** parameters. If their values are reasonable, try larger values for **Max std dev** and/or **Peak window**.
6. Pressing the **All** button causes the algorithm to identify all component series present in the spectrum subject to the specified parameters.
7. When all components have been identified, press the **Close** button.
8. If you wish to abandon the process and exit the dialog box with no changes to the component table, press the **Cancel** button. If you wish to clear the component table completely, press the **Delete All** button.

## Editing Components

After you have identified the components present in the sample, you can use the **Edit Components** dialog to:

- Rename a component.
- Delete a component.
- Sort and re-label the components in order of ascending molecular mass.
- Add a component at known molecular mass, for instance singly-charged species.
- Reject a single peak from the peak series. With poor data, this may improve the accuracy of the molecular mass.
- Print a report showing all the peaks in the peak series for one or all components.

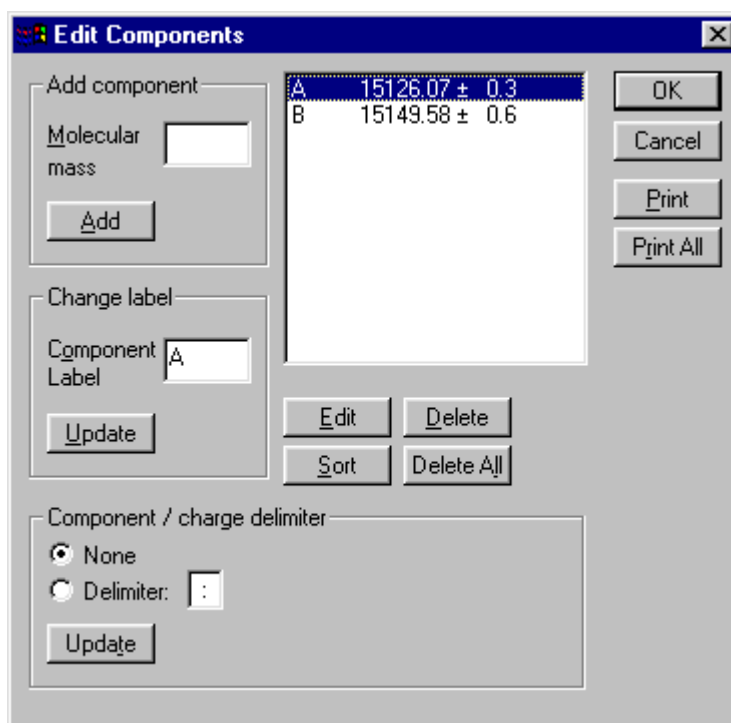


Figure 4.25 Edit Components dialog

### ■ To add a new component at known molecular mass

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Enter the component's mass into the **Molecular mass** box.
3. Press the **Add** button. The component will be inserted into the component table with the next available label.

■ **To change the name of a component**

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Select the component you wish to rename from the list box.
3. Enter the new name for this component (max 3 characters) in the **Component Label** box.
4. Press the **Update** button.

■ **To change which peaks are used in the calculation of a component's molecular mass**

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Select the component whose peak series you wish to alter from the list box.
3. Press the **Edit** button.
4. The **Edit Component** dialog will show you the peak series for that component. The peaks, which are included in the calculation of the molecular mass of that component, are indicated by a check mark [x].

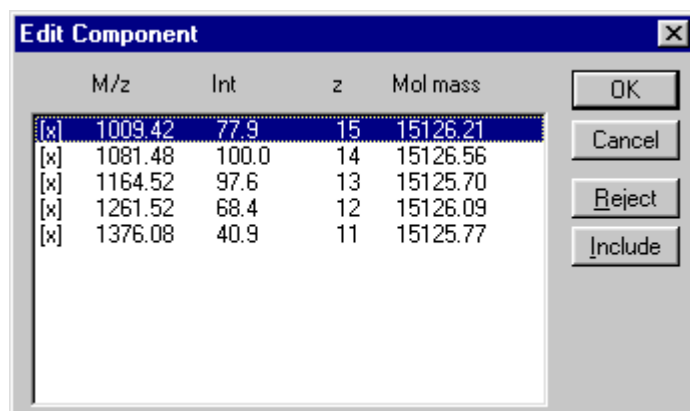


Figure 4.26 Edit Component dialog

■ **To prevent a peak from being used in the calculation of the component's molecular mass**

1. Select the peak you do not wish to be used from the list box.
2. Press the **Reject** button.

■ **To use a peak in the calculation of the component's molecular mass**

1. Select the peak you wish to be used from the list box.
2. Press the **Include** button.

**■ To delete a component**

1. Select **Component Edit** from the Spectrum Process menu.
2. Select the component you wish to delete from the list box.
3. Press the **Delete** button. Press the **Delete All** button to delete all components.

**■ To sort and relabel the components**

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Press the **Sort** button. This will sort the components in order of ascending mass and relabel them, starting at A.

**■ To print the peak series for a single component**

1. Select **Component Edit** from the Spectrum Process menu.
2. Select the component you wish to print from the list box.
3. Press the **Print** button.

**■ To print the peak series for all components**

1. Select **Component Edit** from the Spectrum Process menu.
2. Press the **Print All** button.

**■ To use a component / charge delimiter**

A delimiter can be used to separate the component label from the charge on m/z spectra.

1. Select **Component Edit** from the Spectrum Process menu.
2. To use a delimiter select **Delimiter** otherwise select **None**.
3. Press the **Update** button. The spectrum labels are updated to include the delimiter.

**■ To change the component / charge delimiter**

1. Select **Component Edit** from the Spectrum Process menu.
2. Enter a new character in the **Delimiter** box.
3. Press the **Update** button. The spectrum labels are updated to include the new delimiter.

## Transform

When components have been identified in the spectrum, the data system can assign charge states to each peak. The Transform algorithm uses this information to display the m/z spectrum on a true molecular mass axis.



Figure 4.27 Transform dialog

### ■ To transform an electrospray spectrum onto a molecular mass axis

1. Identify components in the spectrum as described above.
2. Select the **background subtracted continuum** spectrum.
3. Select **Transform** from the **Spectrum Process** menu.
4. Set the mass range you wish to perform the transform over. It is harmless to set a wide range here.
5. If required, you can alter the number of points calculated per Dalton in the Transformed spectrum by altering the **Resolution** parameter. For covering a wide mass range halving the default value, of 0.125 Da between each data point, will halve the run time.
6. **Advanced feature:** The **Cut at** box allows you to specify how the m/z spectrum is to be divided up. With the default setting, **Mid point**, regions of equal charge extend to midway between identified peaks. With **Lowest point** selected, regions of equal charge are divided at the lowest point between identified peaks. Where you have not identified all the components in the spectrum, or the sample contains overlapping series, **Lowest point** may produce a superior transform.

## MaxEnt 1

### ■ Introduction

The **MaxEnt** algorithm uses the method of maximum entropy to produce true molecular mass spectra from multiply-charged electrospray spectra. It has been successfully applied to biopolymers such as proteins and oligonucleotides. The algorithm has several distinct advantages over the **Transform** process.

- MaxEnt automatically finds the molecular weights of the components in a protein mixture without any knowledge other than that they lie within a specified mass range. This can be large e.g. 5-100kDa. To reduce processing time, currently the technique involves a preliminary **survey** run, producing a coarse output to find the approximate masses of the components present.
- The reconstructed MaxEnt spectrum exhibits enhanced resolution and signal-to-noise ratio.
- The reliability of the result can be assessed by probabilistic methods. Thus a probable error range can be calculated for each mass.
- MaxEnt data are as quantitative as any ESMS data. The areas under the peaks in the MaxEnt profile spectrum are representative of the summed intensities of each component's multiply-charged series in the original M/z data.

Transform works from the raw m/z data, combining the peaks from each component into a single peak on the molecular mass scale. Because several peaks in the m/z data are used to produce a single peak in the Transform, the Transformed spectrum exhibits better signal-to-noise than the raw data. However, the Transformed peaks are no better resolved than in the original data.

MaxEnt retains the mass accuracy given by Transform on components that are adequately resolved in the original data. In addition, because of its ability to reveal resolution of peaks which is not apparent in the raw data, MaxEnt allows the mass measurement of components which were previously too poorly resolved for mass measurement in the transformed spectrum.

MaxEnt finds the simplest molecular mass spectrum (spectrum of maximum entropy) that could account for the observed m/z data. The algorithm works iteratively; it takes an initial approximation to the molecular mass spectrum, and then uses programmed knowledge of chemistry and mass spectrometer physics (the **damage model**) to synthesise a corresponding m/z spectrum (the mock spectrum) from this molecular mass spectrum. It then compares the mock data to the observed (real) data, and uses the difference between the two to guide it to an improved molecular mass spectrum. The algorithm terminates when there is sufficiently little difference between mock and real data.

A MaxEnt damage model describes the shape and width of the peaks in the observed m/z data, which is a composite of two effects. One effect is chemical; the distribution of molecular isotopes has a characteristic shape which is a function of molecular mass. The other effect is physical, caused by diffraction effects in the mass spectrometer. You can observe the latter effect alone by running a monoisotopic sample, for instance Caesium Iodide.

The current implementation of MaxEnt provides a single damage model. This is a Gaussian curve of constant width, which is a composite model of both of the above effects. To use this model you need to measure the width of a peak in the observed m/z data at half height.



**Note:** The MaxEnt algorithm needs to accurately measure noise within a data file. For this reason the **Ion Counting Threshold** should be set to zero when acquiring data which will be analysed using MaxEnt. For more information about the Ion Counting Threshold see the **MassLynx Acquisition** manual.

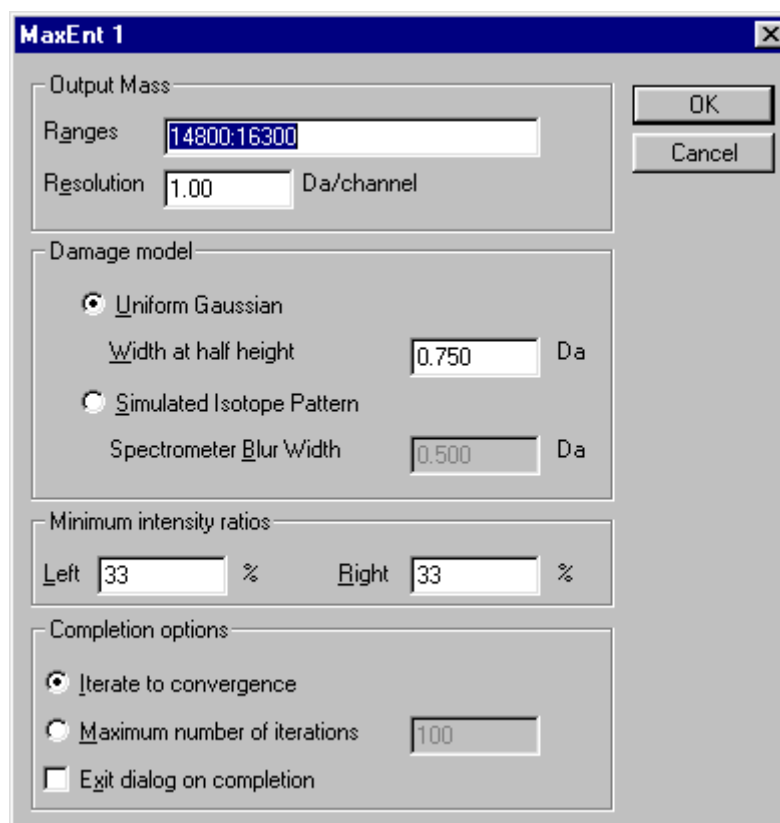
#### ■ To produce a survey spectrum

The sole purpose of producing a survey spectrum is to determine the approximate masses of the components present. It is possible to analyse a complete unknown by selecting a very wide output mass range e.g. 10-100kDa. Usually, the major components are revealed after three or four iterations.

1. **Background subtract** the raw data as described above. Set the parameters to fit an appropriate polynomial with 30-50% of the data below it. 30% usually leaves a low level of noise in the MaxEnt result; you may wish to increase this for noisier spectra.
2. MaxEnt will process the data actually on display. This means that you can rubber-band the display to exclude parts of the spectrum which contain uninterpretable noise. This can improve the MaxEnt result in some cases. Also, if the spectrum has a flat baseline, it is possible to remove this with the mouse also, rubber-banding in the vertical direction.
3. You should leave the **damage model option** set to the **Uniform Gaussian** model since the alternative **Simulated Isotope Pattern** model has not been implemented in the current version. To use the **Uniform Gaussian** model, you need to estimate the average width at half height of a peak in the m/z spectrum.

For a detailed discussion on determining the correct value for the peak width parameter see the next section "**How to Establish the Correct Peak Width Parameter to Use When Processing Multiply Charged Data by MaxEnt**"

4. Select **MaxEnt 1** from the **Spectrum Process** menu.



*Figure 4.28 MaxEnt1 Parameters dialog*

5. Set up the **Output Mass** range. The mass range is given as two numbers separated by a colon. E.g. 10000:100000.
6. Set the **Resolution** parameter to a value in the range 10-25 Da/channel. This parameter controls the "texture" of the result; a value in this range will give a coarse result, not showing fine detail, and without accurate masses, but the spectrum will suffice to locate the major components for a finer run over a smaller mass range.
7. Leave the damage model set to **Uniform Gaussian** and set the **Width at Half Height** as described above.
8. Set the **Left** and **Right Minimum Intensity Ratio** parameters. These parameters place limits on the relative heights of adjacent peaks in the same series. For instance, if the **Left Minimum Intensity Ratio** parameter is set to 30% and the most intense peak in the series is the 15+ peak, then the 16+ peak must be at least 30% as intense as the 15+, the 17+ peak must be at least 30% as intense as the 16+, and so on toward the low mass end of the spectrum. If the **Right Minimum Intensity Ratio** parameter is set to 40%, then the 14+ peak must be at least 40% as intense as the 15+ peak, the 13+ at least 40% as intense as the 14+, and so on. The default values of 33% for each will always work, but for most data sets these values can profitably be increased.

In particular, when doing a survey run, increasing the **Left** and **Right Minimum Intensity Ratios** will give significant reduction in the intensity of the "harmonic artefacts"; the peaks at twice, three times etc. the mass of each component.

9. If you wish the MaxEnt process to continue iterating until it converges select **Iterate to convergence**. Alternatively you can select the **Maximum number of iterations** option and enter a value as an upper limit on the number of iterations which MaxEnt will perform.
10. Select **Exit dialog on completion** if you wish MaxEnt to automatically accept the results, exit the MaxEnt dialog and display the MaxEnt spectrum on completion. If **Exit dialog on completion** is not selected then the MaxEnt dialog will remain displayed on completion, giving you the option to accept the results and save the MaxEnt spectrum or discard the results.
11. Press the **OK** button. The MaxEnt status dialog will appear. The algorithm will initialise itself, then draw molecular mass axes, and the first iteration will start.

■ **How to Establish the Correct Peak Width Parameter to Use When Processing Multiply Charged Data by MaxEnt**

When processing data by MaxEnt, it is crucial that the correct peak width at half height is used. The only sure way to establish this width is to measure it, using peaks that are known to be singlets.

The ideal way is to measure the width of a peak, which is known to be a singlet, in the  $m/z$  spectrum to be processed. For example, in a haemoglobin spectrum, it may be required to separate and measure the components in an unresolved  $\beta$ -globin doublet, when it is known that the  $\alpha$ -globin is a singlet. The measured width of an  $\alpha$ -globin peak near the center of the spectrum may then be used directly in the MaxEnt processing, since the molecular weights of the two globins are similar.

In many situations, however, the peaks in the sample data will not be sufficiently resolved for their widths at half height to be measured. In these cases, it is necessary to measure the peak width from a multiply charged spectrum run under identical conditions as the sample spectrum and known to contain singlets. This can be the spectrum used to calibrate the sample spectrum or another spectrum containing known singlets. In either case, it will generally be necessary to correct the measured peak width in order to find the value to use when processing the data by MaxEnt. This is derived as follows.

Let the measured width at half height of a singlet in the 'calibration' spectrum be  $w_C$  and let the peak have  $n_C$  charges.

Let the molecular weights of the 'calibration' compound and the sample be  $M_C$  and  $M_S$  respectively.

Let the theoretical widths at half height due to the isotopic distribution of the elements in the molecule be  $W_C$  and  $W_S$  for  $M_C$  and  $M_S$  respectively. These may be found from the graph that follows.

It is assumed that the width of a peak in the  $m/z$  spectrum is made up of two components; a component due to the theoretical isotopic distribution and a component due to the instrument itself ( $w_i$ ). These are assumed to be Gaussian, and are added as the root of the sum of the squares.

Hence,

$$w_c^2 = w_i^2 + (W_c/n_c)^2 \quad \text{----- (1)}$$

and

$$w_s^2 = w_i^2 + (W_s/n_s)^2 \quad \text{----- (2)}$$

where  $w_s$  is the width required for processing the sample spectrum by MaxEnt, and  $n_s$  is the number of charges on a peak at a similar part of the  $m/z$  spectrum to that used for measuring  $w_c$ .

Combining (1) and (2) to eliminate  $w_i$ ,

$$w_s^2 = w_c^2 + (W_s/n_s)^2 - (W_c/n_c)^2 \quad \text{----- (3)}$$

### Example

Suppose using myoglobin ( $M_r = 16951.5$ ),  $w_c$  was measured as 1.0 Da for the  $m/z$  1212 peak ( $n_c = 14$ ). From the graph,  $W_c = 8.2$  Da.

Suppose, also, that the sample has a molecular weight of  $\sim 40000$  Da. From the graph,  $W_s = 12.6$  Da. At  $m/z \sim 1212$ ,  $n_s = 33$ .

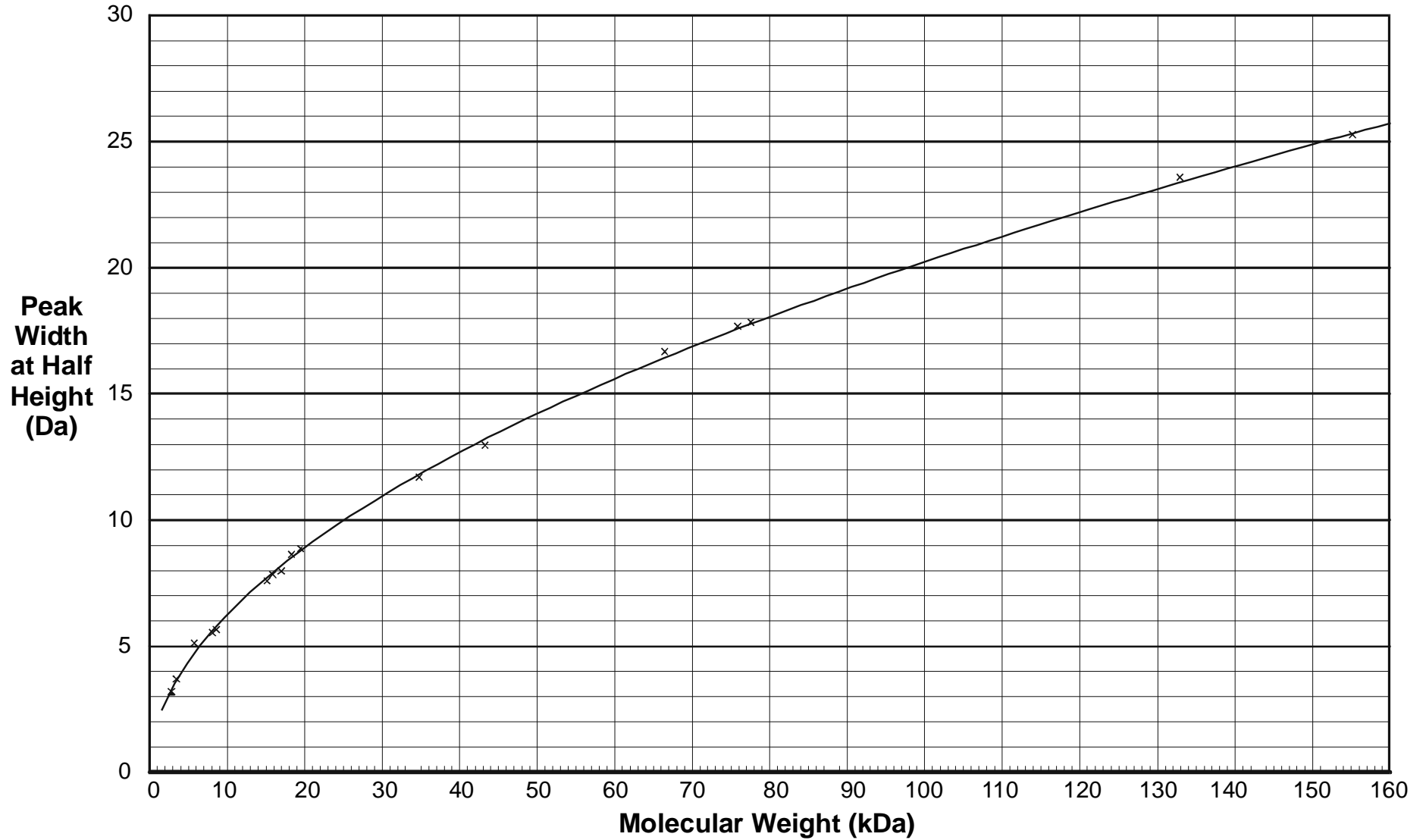
Using equation (3),

$$w_s^2 = 1.0 + (12.6/33)^2 - (8.2/14)^2 = 0.80$$

$$\text{or } w_s = 0.90.$$

If  $w_c = 0.8$  for myoglobin,  $w_s = 0.67$  for the 40 kDa protein.

### Theoretical Peak Width of Proteins due to Isotopic Distribution vs Molecular Weight

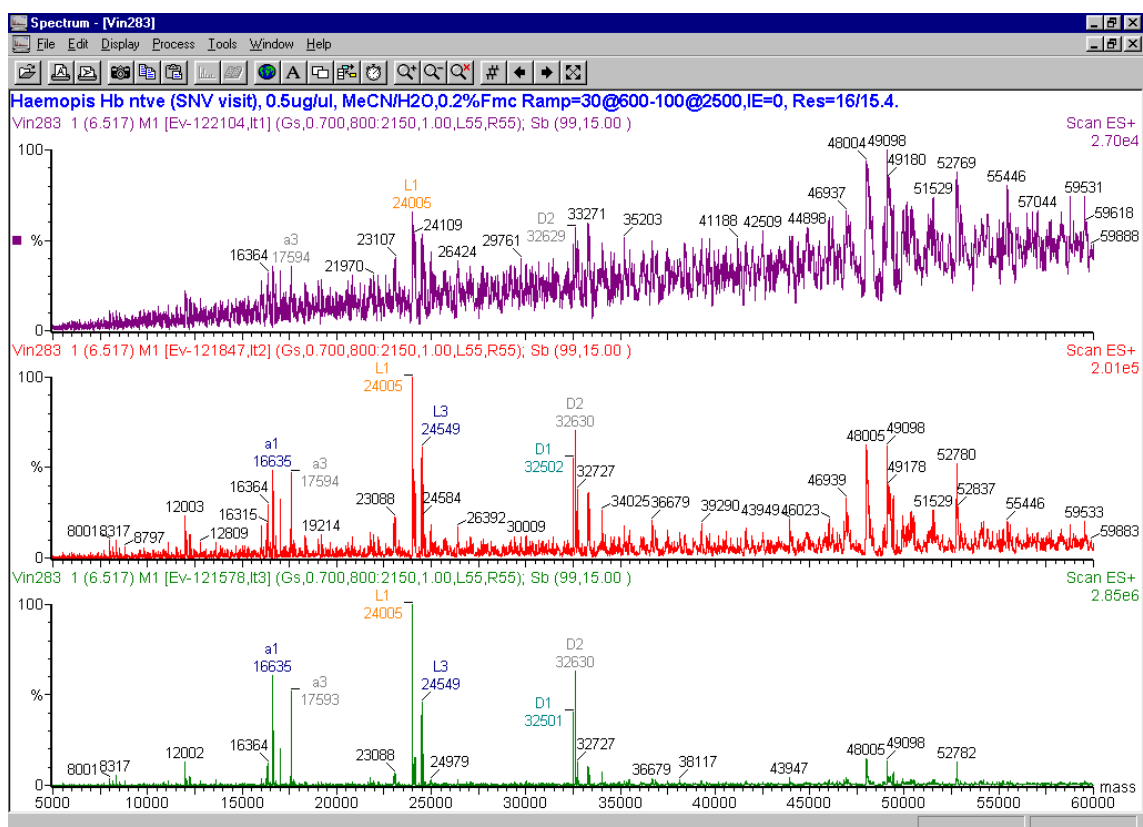


## ■ Interpreting the survey spectrum

**Figure 4.29** shows the first three iterations of a MaxEnt survey run on a data set produced from leech haemoglobin.

After one iteration, the major components are already visible, but the harmonic artefacts at twice the mass of each component are present at significant intensity. Also the background level is high, and rises with increasing mass. After the second iteration, the intensity of artefacts and background level has been greatly reduced. Neither are present with significant intensity after three iterations.

You may also see sub-harmonic artefacts at fractions (half, quarter etc.) of the true molecular mass for the first couple of iterations.



**Figure 4.29** First three iterations of a MaxEnt survey run on leech haemoglobin.

■ **To stop a MaxEnt run before the algorithm converges**

1. Press the **Halt** button
2. You may now accept the result by pressing the **OK** button, or discard it by pressing **Cancel**. You may also restart MaxEnt by pressing the **Restart** button.
3. If you accept the spectrum and at a later time wish to restart MaxEnt, you may do this by selecting **MaxEnt** from the **Spectrum Process** menu again.

■ **To produce the definitive MaxEnt spectrum**

Once the approximate masses of the major components are known, whether from prior knowledge of the sample, or a MaxEnt survey run, the definitive MaxEnt spectrum revealing all the fine structure can be produced.

1. Either select the background subtracted data you used to produce the survey spectrum, or use **Background Subtract** as described above to produce some.
2. Select **MaxEnt 1** from the **Spectrum Process** menu.
3. Set up the **Output Mass** range from your knowledge of the approximate masses of the major components. The run time of MaxEnt is directly proportional to the number of data points in the output, and this number is the product of mass range and reciprocal of resolution. Therefore, do not set the mass range unnecessarily wide.

Note that 2 or more **Output Mass Ranges** separated by commas may be selected e.g. 16500:17500, 24500:26500 (see **Figure 4.28**). Using this facility reduces the processing time. The **Output Mass Ranges** should include all the significant components found in the survey run, in order to make the definitive MaxEnt spectrum a faithful representation of the original data.

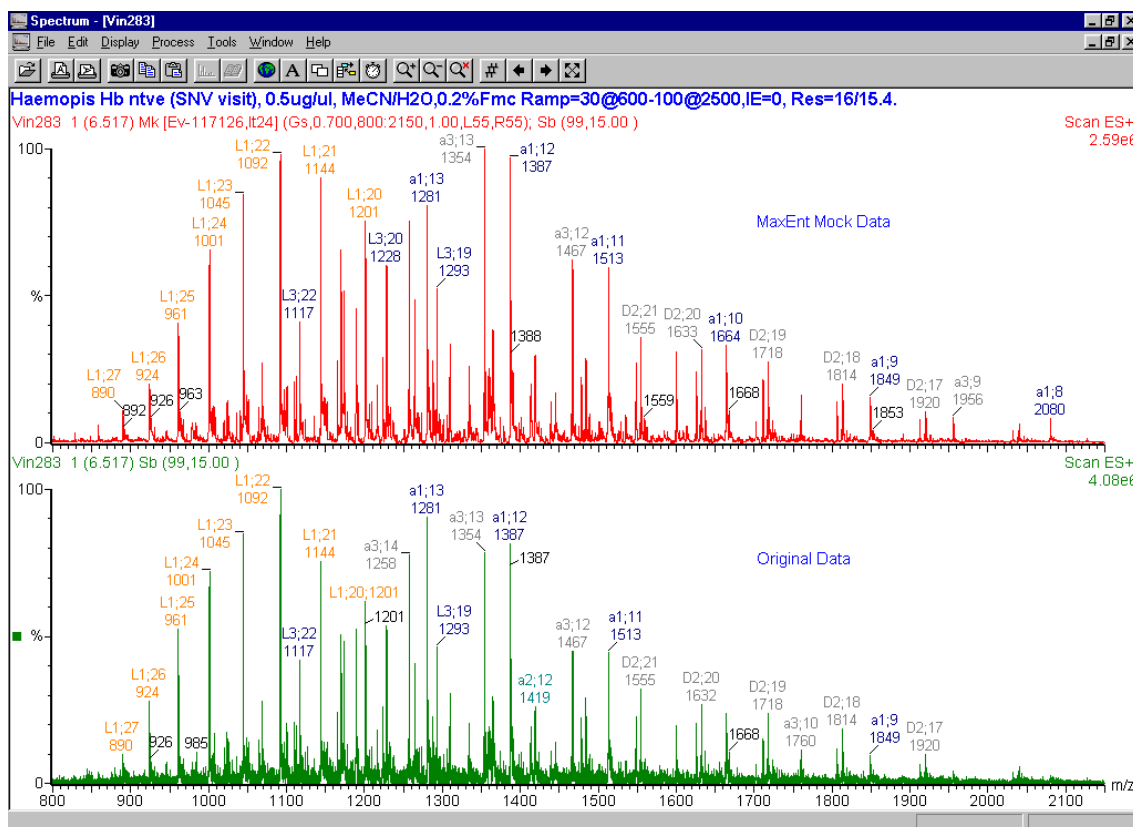
4. Set the **Resolution** parameter to 1.0 Da/channel. Generally, this is sufficiently small to ensure there will be several data points across each peak in the output, and a centroid can be taken to give an accurate mass. Occasionally, a smaller value e.g. 0.5 Da/channel may be necessary. This will, however, increase the processing time.
5. Set the **damage model** and **minimum intensity ratio** parameters as described above.
6. Press the **OK** button.

## ■ Mock data

To get a definitive result, you must allow MaxEnt to run to completion. It will then produce two spectra; one is the MaxEnt result on a molecular mass axis, and the other is the **mock data**, explained above. Examining the mock data can help you decide how good the parameter settings were.

Mock data should fit the observed data within the tolerance of the noise.

**Figure 4.30** shows mock data (upper) and original data (lower).



**Figure 4.30** Original data from leech haemoglobin (lower), and MaxEnt mock data (upper).

## ■ To examine the fit of mock to real data

1. Select **View** from the **Spectrum Display** menu.
2. Check the **Overlay Graphs** box in the **Style** group. This will cause spectra in the same window to be superimposed.
3. Check the **Link Vertical Axes** box in the **Normalize data to** group. This will ensure that both spectra are plotted on the same vertical scale.
4. Press the **OK** button.
5. You now need to display the raw data in the window containing the mock data. Click inside the window containing the mock data, then select **Open** from the **Spectrum File** menu. Ensure that the **Add Data** radio button is checked, then select the raw data from the list box.
6. Press the **OK** button.

The Minimum Intensity Ratio parameters will affect the intensities of the peaks in the mock data, and the appropriate damage model width parameter will affect the widths of the peaks in the mock data.

■ **Mass measurement of MaxEnt spectra**

Special interpretation must be placed on peaks in MaxEnt spectra. The topmost point of the peak is not the most probable estimate of the peak's mass; rather, a centroid must be taken. The height of a MaxEnt peak is an indicator of how good an estimate the algorithm can make of the mass. This means the height is not proportional to the relative concentration of that component in the sample; but the area is.

There are two ways to produce MaxEnt spectra with accurate masses. The first presents the profile spectrum labeled with accurate mass values, as in **Figure 4.31** (upper). The second presents the spectrum as bars, with the height of each bar being proportional to the **area** of the peak in the profile data, as in **Figure 4.31** (lower). Note the apparent ratio of the intensities of the  $\alpha$  and  $\alpha 1$  peaks has altered. The ratios observed in the lower spectrum are definitive, provided **Areas** are used.

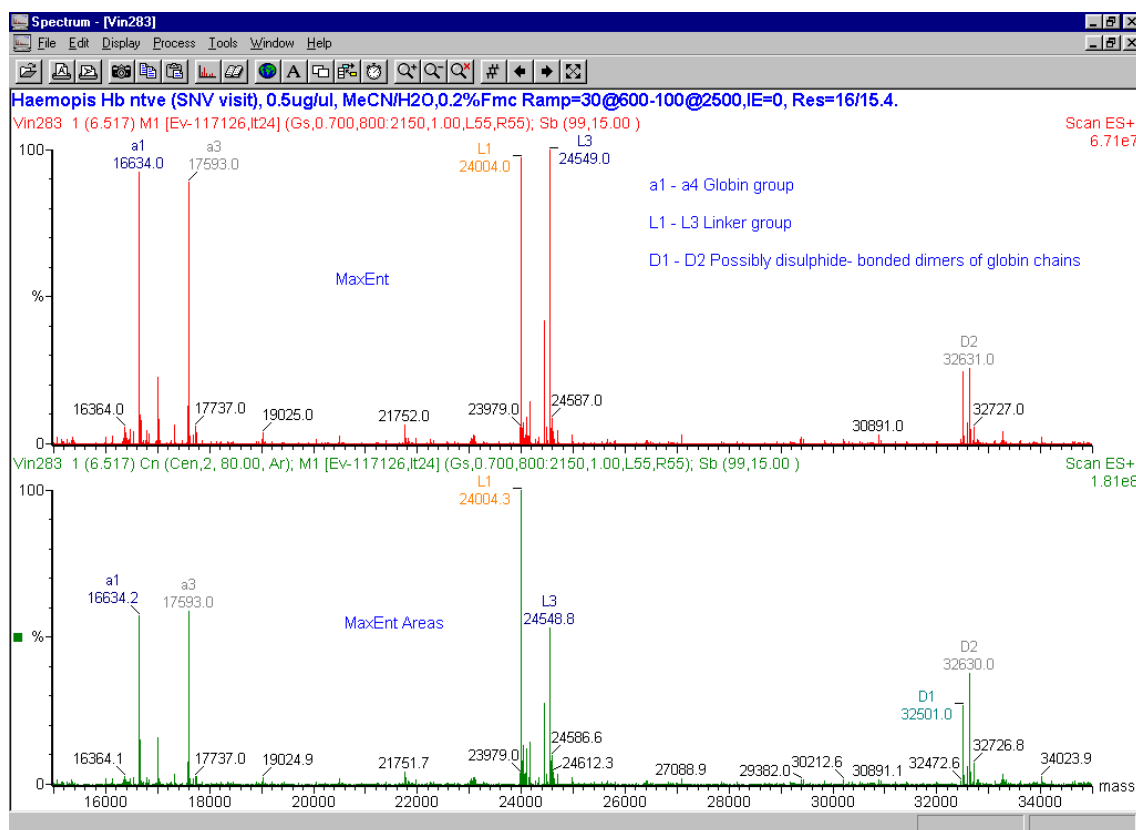


Figure 4.31 MaxEnt results from leech haemoglobin.

■ **To produce a profile spectrum with accurate masses**

1. Click inside the MaxEnt spectrum.
2. Select **Center** from the **Spectrum Process** menu.
3. Set the **Min peak width at half height** parameter to 1. This will interpret the smallest, narrowest feature in the spectrum as a peak. If this does not produce the result you require, you can increase the value of this parameter to group the narrower features together with the wider ones.
4. Select a center method. **Top** is provided mainly for compatibility with the LAB-BASE data system. **Centroid** is the recommended method, since the **Centroid top** parameter can be set to use the well-resolved part of the peak only, keeping clear of baseline effects. Recommended values for **Centroid top** are in the range 70%-90%.
5. Ensure the **Create centered spectrum** box in the **Centered spectrum** group is not checked.
6. Press the **OK** button.

■ **To produce a bar spectrum with heights proportional to component concentration**

1. Click inside the MaxEnt spectrum.
2. Select **Center** from the **Spectrum Process** menu.
3. Set the **Min peak width at half height** parameter as described above.
4. Select a center method as described above, e.g. **Centroid top (%)=90**
5. Check the **Create centered spectrum** box in the **Centered spectrum** group.
6. Select **Areas**.
7. Press the **OK** button.

## ■ MaxEnt Errors

A probable error range can be calculated for the mass of each peak in the MaxEnt spectrum.

This is done by sampling the distribution of possible spectra at about a dozen points near the most probable spectrum. Hence the error analysis requires a further dozen iterations of the MaxEnt kernel, and for this reason, it is a separate process.

Also, in the current release of software, a Gaussian approximation is used to navigate the vector space of possible spectra. The sharper the MaxEnt peak, the further this approximation will take the algorithm from the truth. This leads to pessimistic errors, with sharp peaks having the most pessimistic. An improved algorithm will be available in the future.

## ■ To calculate the MaxEnt errors

1. Form a MaxEnt profile spectrum with accurate masses as described above.
2. Select **MaxEnt errors** from the **Spectrum Process** menu. The status dialog will appear, and the first cloud sample will commence. Twelve samples are performed in all, and after the last one, the spectrum is redisplayed with the errors.
3. Save the errors by selecting **Save spectrum** from the **Spectrum File** menu.

**Note:** You will only see the MaxEnt errors when the **Mass Error** parameter has been selected in the Spectrum **Peak Annotation** dialog.

## ■ MaxEnt Initialisation Errors

Occasionally when you start MaxEnt you may see an error message displayed, **MaxEnt initialisation error -1** or **MaxEnt initialisation error -2**. These errors mean that there is not enough memory available to execute the current MaxEnt operation. In this case you should do the following ;

1. Close down MassLynx and any other Windows programs you are using to free all available memory.
2. Run MassLynx again. Load the spectrum you wish to process and attempt to run MaxEnt again.
3. If the same error occurs then alter the MaxEnt parameters so that less memory is required. Reducing the mass range in the Ranges parameter or increasing the Resolution parameter can do this.