Application Note: 109. Italian Barley Calibration: Cropscan 1000B

Introduction:

The calibration for protein in Barley is an on going problem. We have not been able to identify why the SEC for protein in barley is in excess of 0.5%. This report outlines steps taken to improve the SEC.

Description:

43 samples of barley were scanned on a Cropscan 1000B. 10 spectra were collected for each sample. Figure 1 shows these spectra.

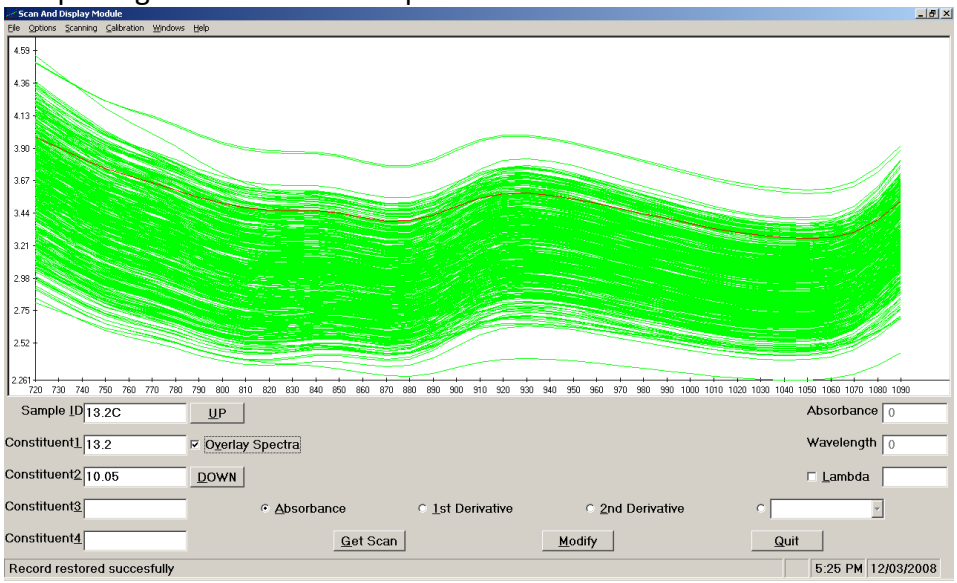

Figure 1. NIT spectra of barley.

A PLS calibration was performed on these spectra and the protein values for each sample. Figure 2. shows the plot of the NIT Protein vs the Reference Protein.

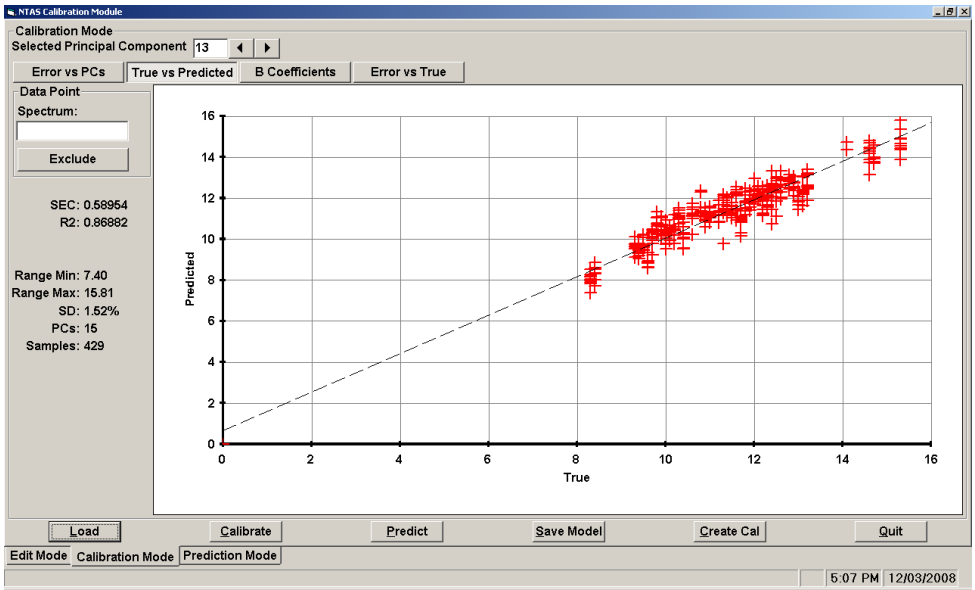

Figure 2. Calibration plot of protein in barley.

There were several observations made concerning these spectra.

1) There are two types of barley spectra. Figure 3. shows Type A and Type B spectra.

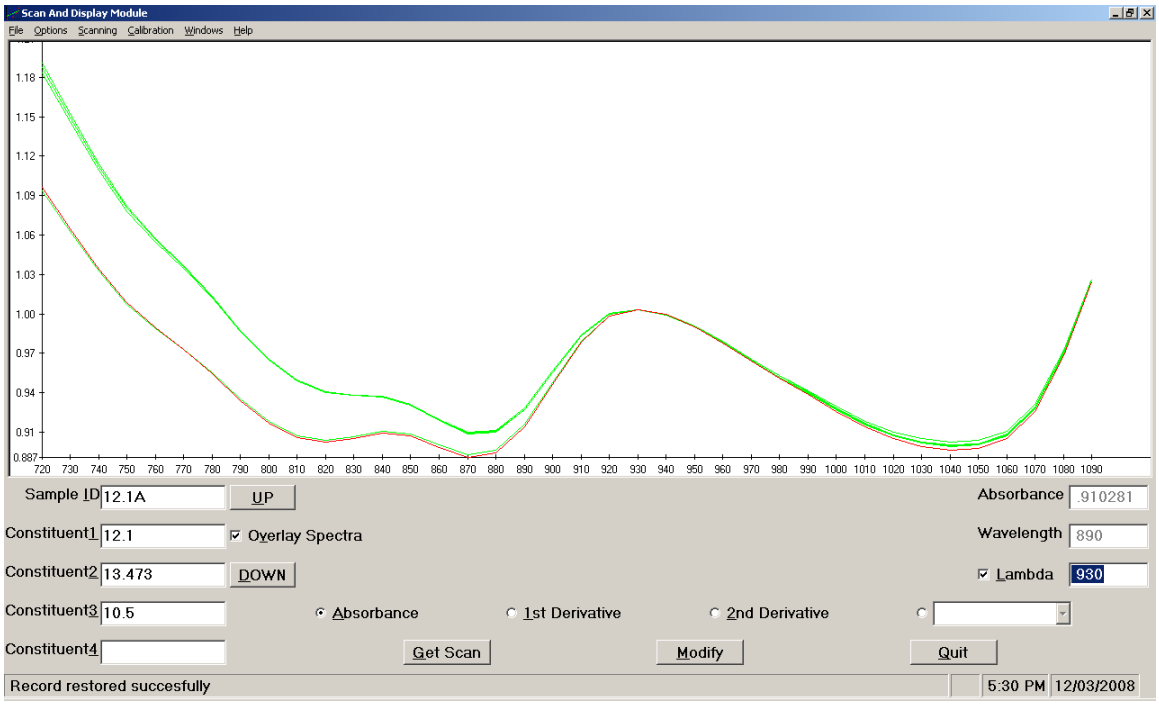

Figure 3. Type A and Type B spectra.

Type A spectra have a more definite band at 840nm(starch), where as Type B spectra have a sloping curve which covers up the 840nm band.

2) Of the 10 scans collected for each sample, it is observed that often the first scan is considerably different to the rest. Figure 4. shows the 10 spectra of sample 14B.

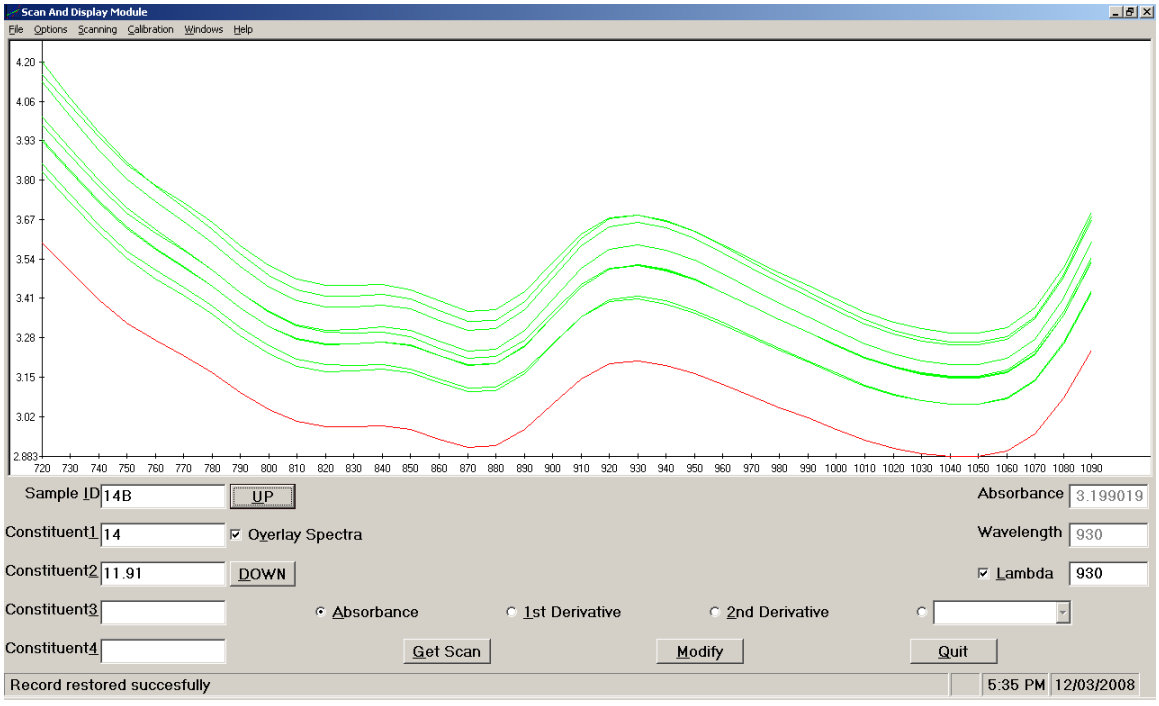

Figure 4. 10 scans of sample 14B.

The highlighted scan, ie, red line, is the first spectrum collected. It is believed that this low absorbance scan is caused by the fact that when the sample is poured into the funnel, the packing of the sample is loose and therefore more light passes through the sample. When the brush rotates and the sample drops, the barley packs tighter and therefore less light passes through the sample. This first scan often gives a lower protein reading than the other scans and therefore biases the results.

It is also noticed that the last scan can often show the same effect. It is believed that when there is not enough grain to fill the cell, then the packing is less ad too much light passes through the cell.

Sorting By Spectral Type:

The spectra were sorted into Type A and Type B spectra. There were approximately half of each type. The spectra were averaged into sets of the first 5 averaged spectra, all 10 averaged spectra and the average of scans 2 to 8. Figures 5 and 6. show the two sets.

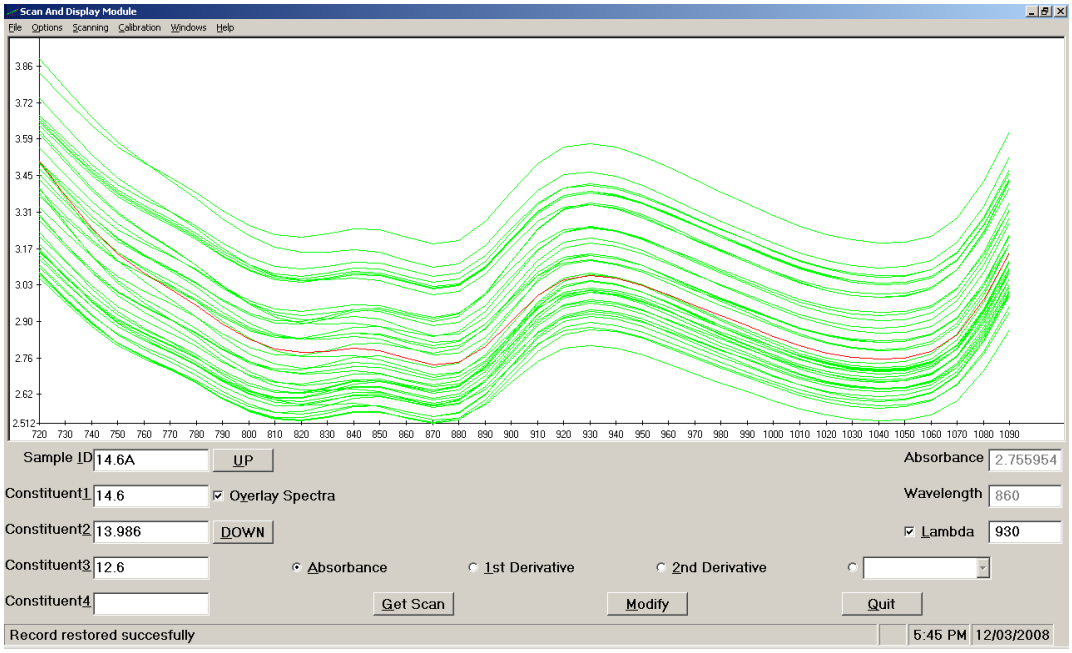

Figure 5. Type A Spectra Averaged

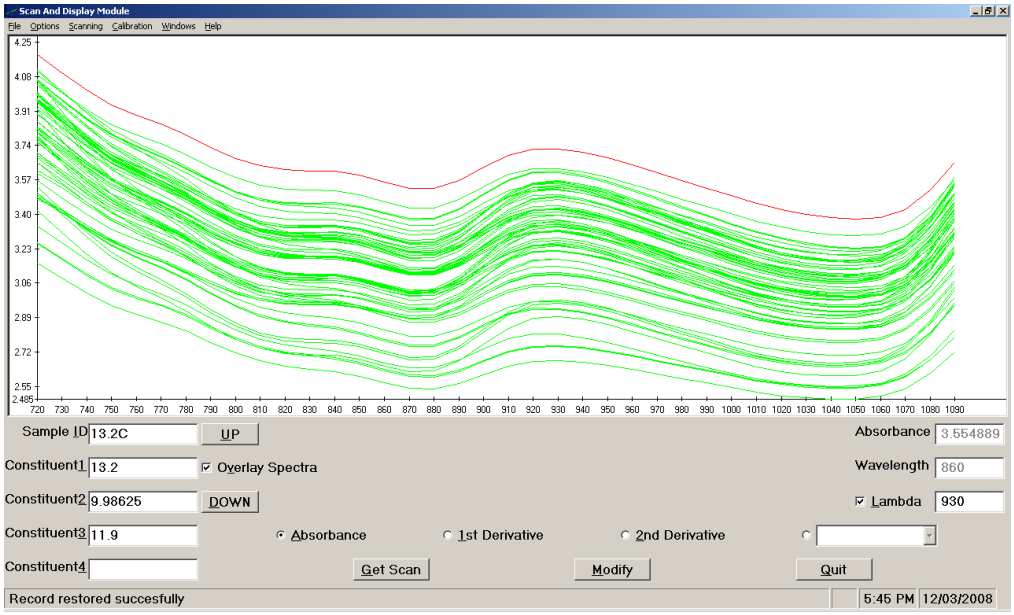

Figure 6. Type B Spectra Averaged.

Calibration:

A PLS calibration was run on each set of spectra. Figure 7a and b. show the calibration plot for Type A spectra.

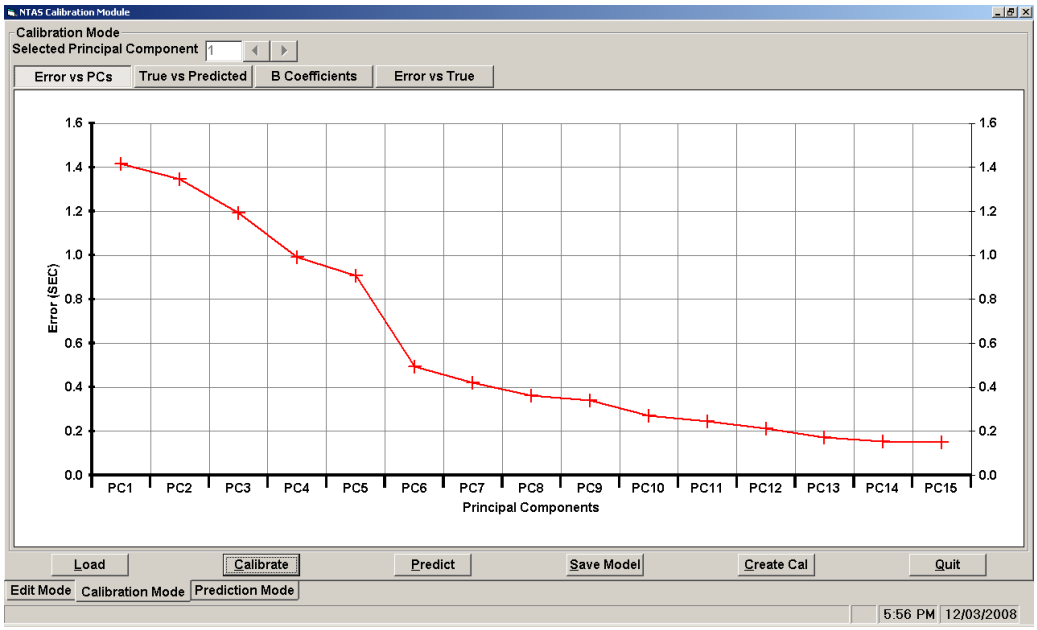

Figure 7 a. Plot of SEC vs PC for Type A

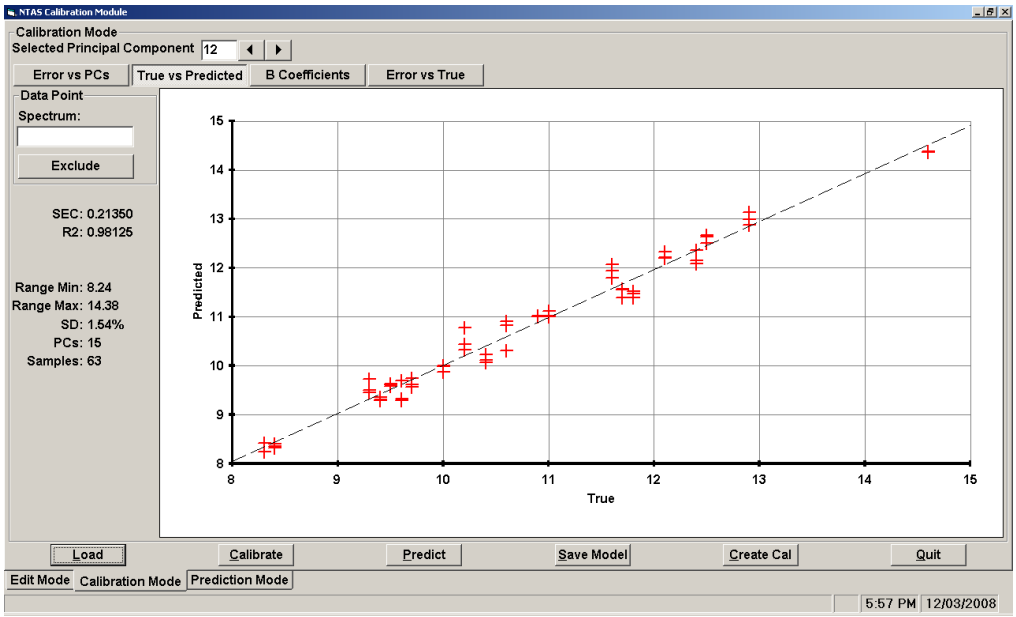

Figure 7b. Plot of NIT Protein vs Ref Protein for Type A spectra.

Figure 8 a and b show the calibration plots for Type B spectra.

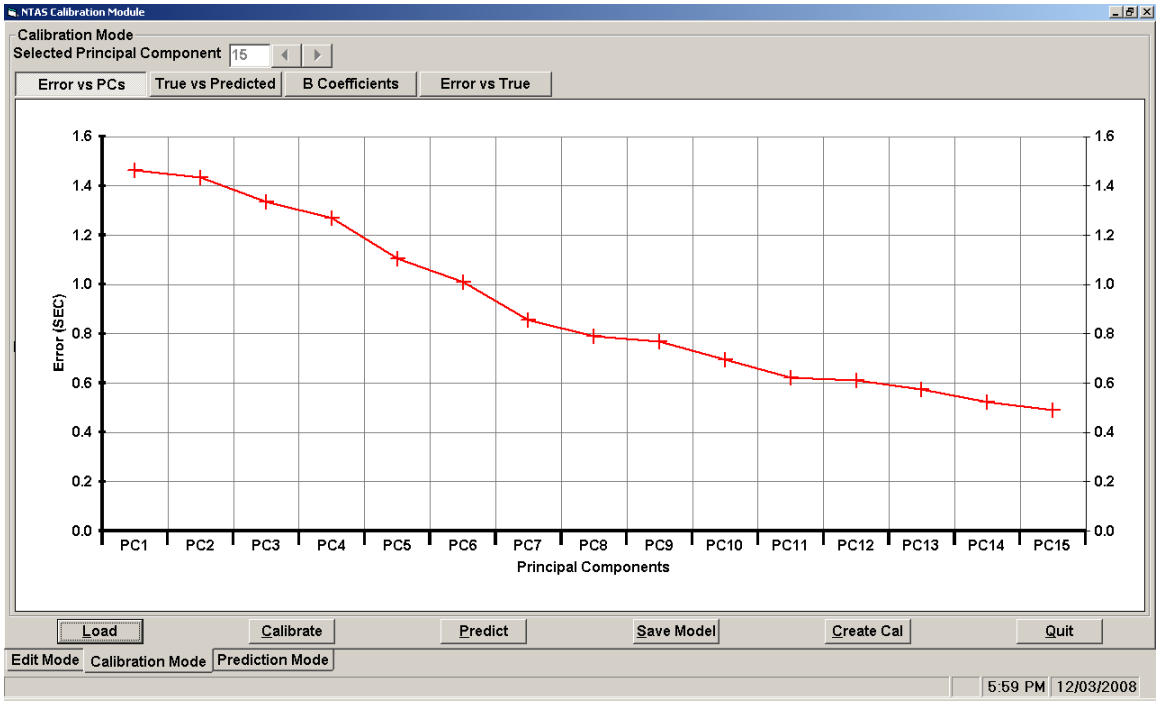

Figure 8a. Plot of Sec vs PC Type B Spectra.

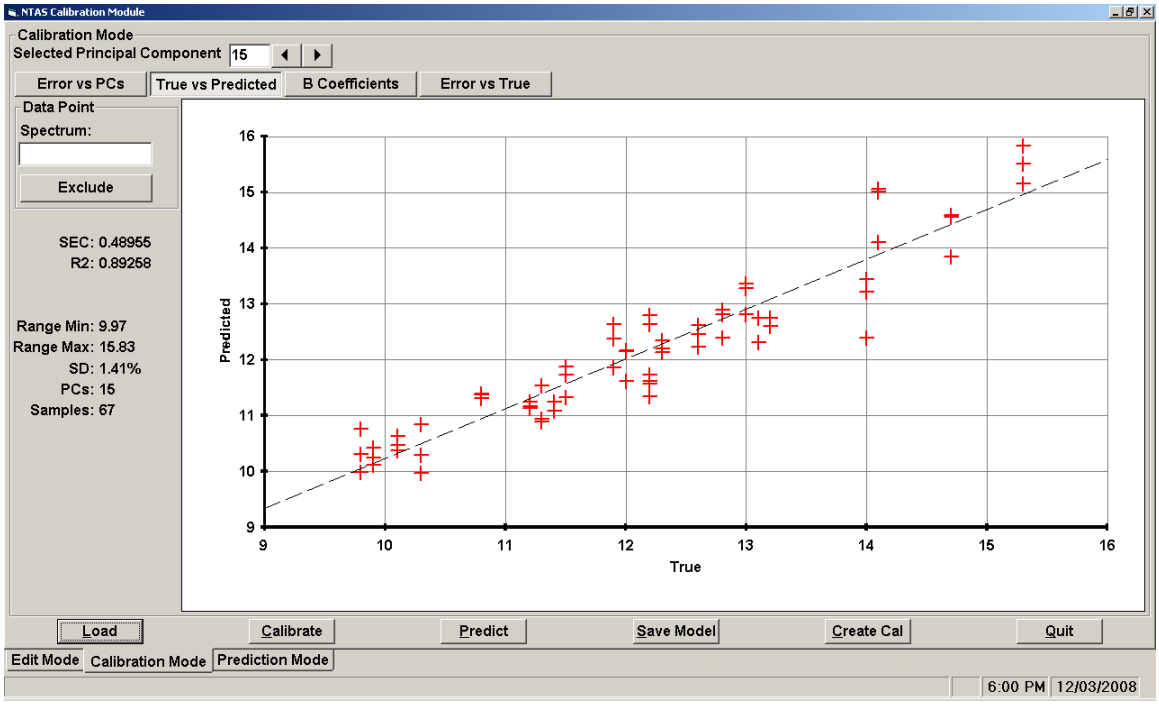

Figure 8b. Plot of NIT Protein vs Ref Protein for Type B Spectra.

By plotting the Error vs True Proteins, it can be seen that in several samples there is big difference between the 3 sets of averages for a single sample. Figure 9 shows the Type B spectra plot.

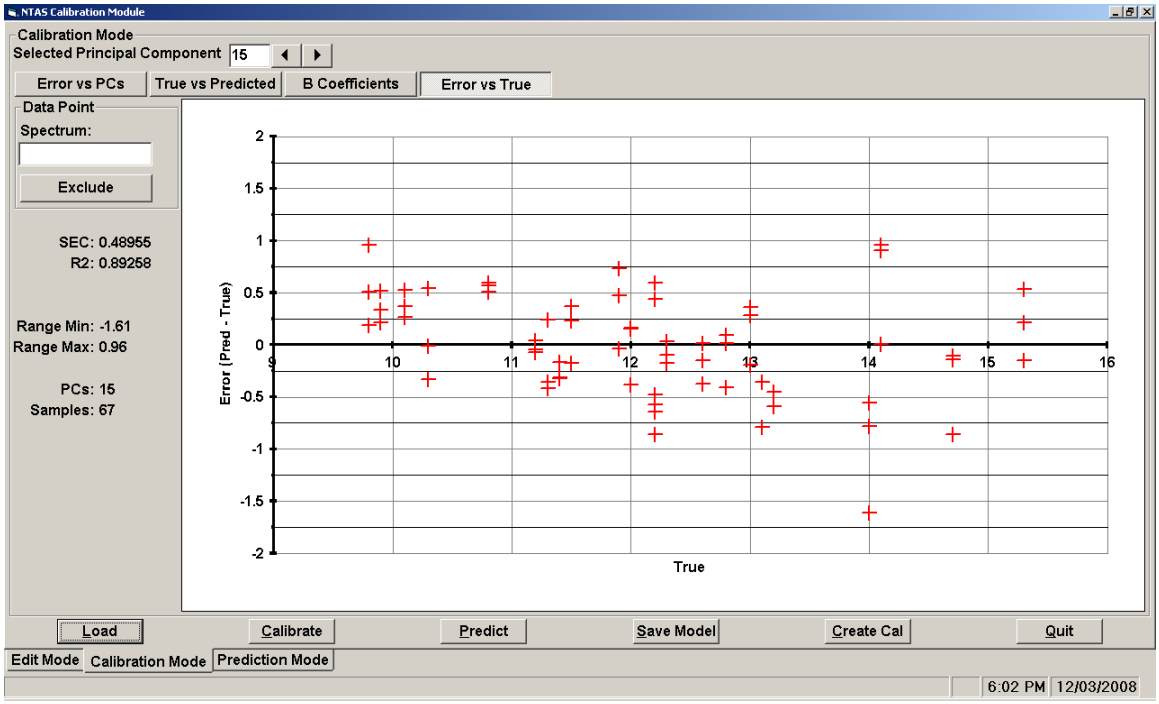

Figure 9. Error vs True Plot for Type B spectra.

Note that the sample with a reference value of 9.8, 14, 14.1 and 14.7 have big variations between the three averaged spectra. It was observed that the average of scans 2 to 8 always gave the best agreement with the reference data and that for these sets of scans, the first and or last spectra were effected by packing.

As such, these spectra were removed from the calibration set of Type B. Figure 10 a and b show the calibration plots with these spectra removed.

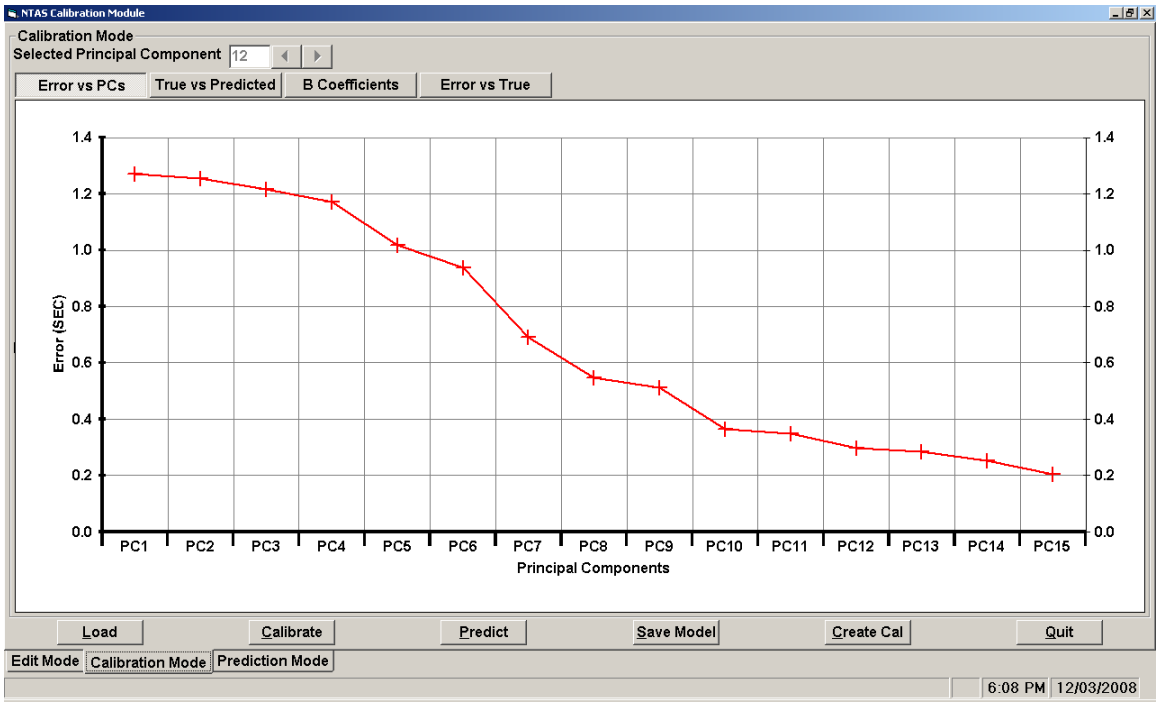

Figure 10a. Plot of SEC vs PC for Type B spectra with outliers removed.

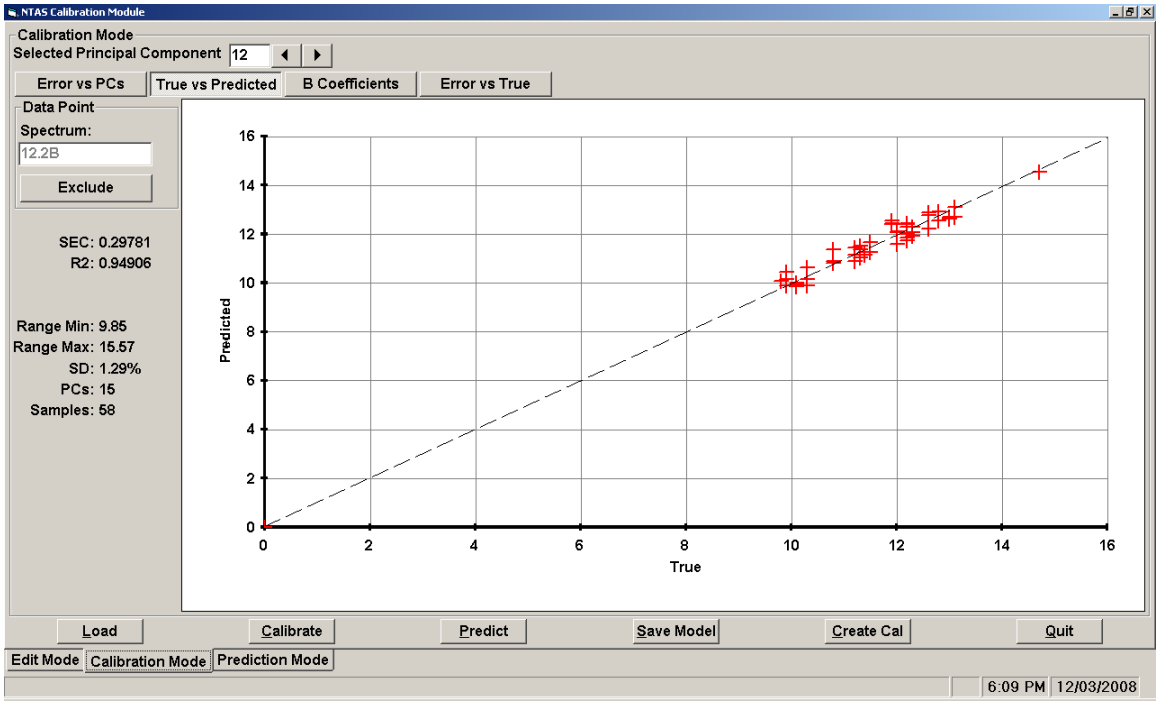

Figure 10b. Plot of NIT Protein vs Ref Protein for Type B spectra with outliers removed.

Combined Average Spectra Calibration:

By combining the two types of barley spectra, there is a considerable increase in SEC. Figure 11 shows the plot of the combined calibration data with the same spectra removed.

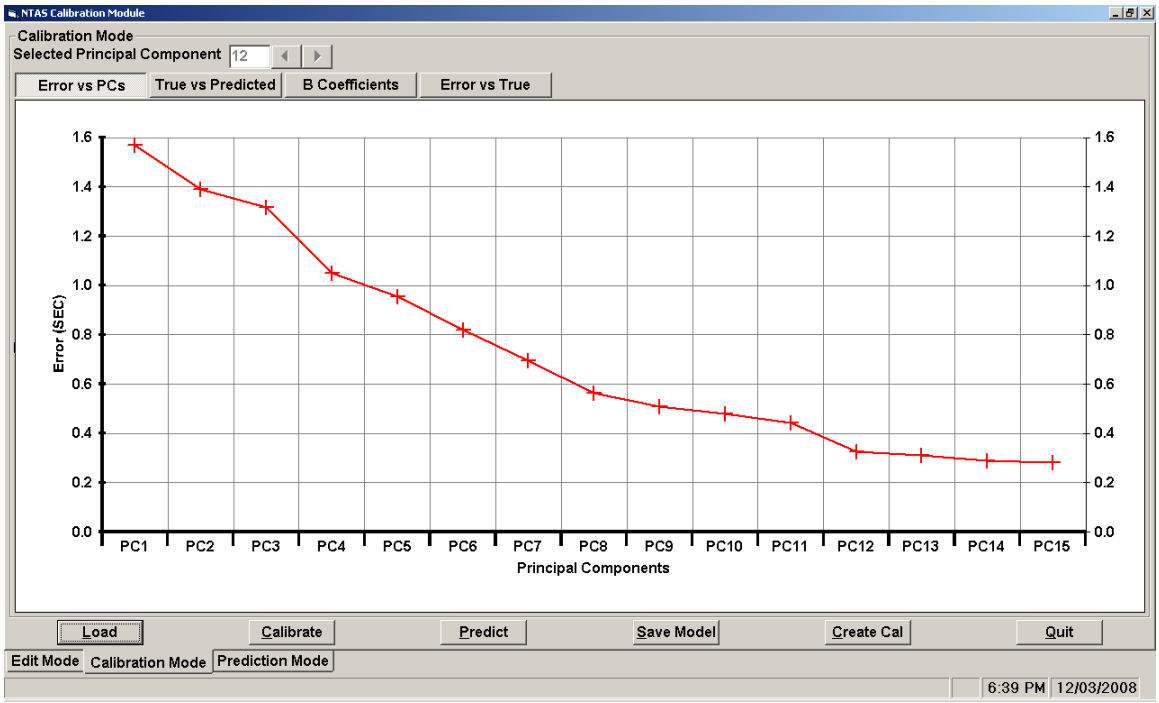

Figure 11a. Plot of SEC vs PCs for Types A and B spectra.

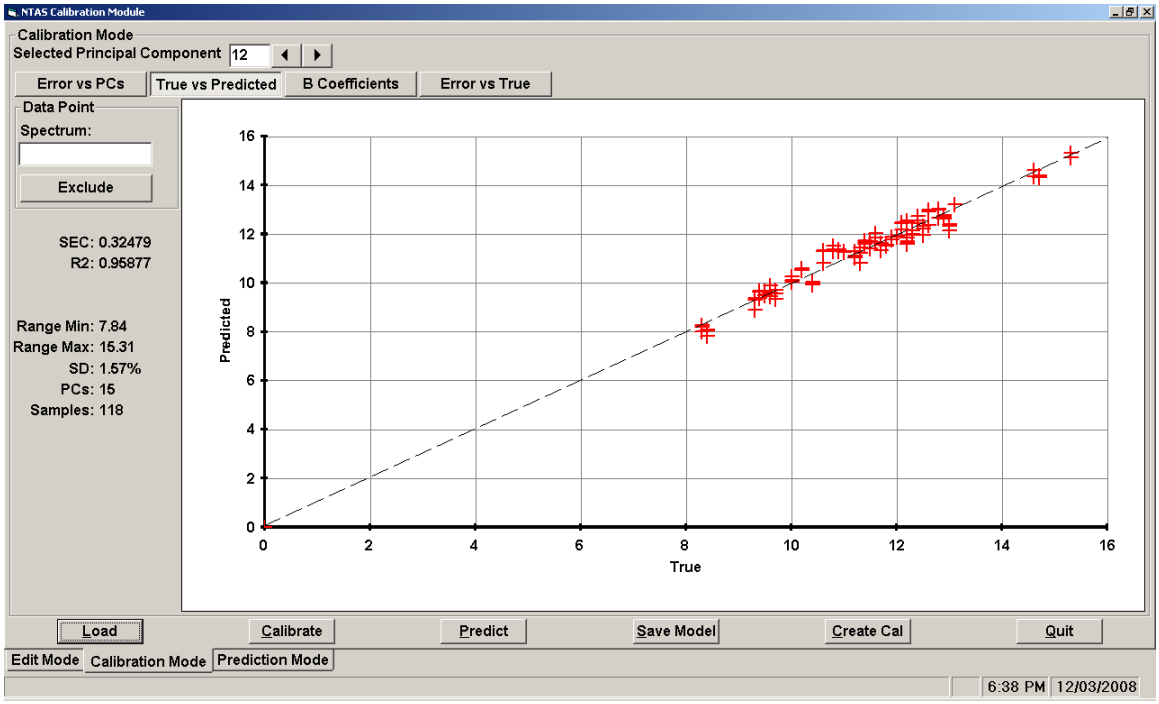

Figure 11b. Plot of NIT Protein vs Ref Protein for Types A and B Spectra.

Note that all PLS calibrations were performed with Sp 1-6 and 34-38 eliminated.

Conclusion:

This study strongly suggests that the problem with calibrating for barley samples is two fold, ie, different types of barley and packing density.

The issue of different barley types may be addressed by using the new software, ie, Multiple Calibration Selection. The difficulty is to discriminate between the two sets of spectra. If this is possible then the analyser can decide which calibration to use based on the spectra type.

The issue of packing density can be fixed in two ways. Firstly by using the outlier detection software, ie, Z Score or Min Max, then outliers will be removed from the calculation when predicting samples. Secondly, the latest version of software, WGA6.14 rotates the brush wheel before the first scan is collected. This is done to ensure that the sample packing is consistent. Also by ensuring that a full 500ml, ie, a cup full of sample is used, will help to maintain the packing density.

The last point to make is that when developing calibrations, it is critical to use the average spectra rather than the unaveraged spectra. As can be seen from this study, the original unaveraged spectra gave an SEC of 0.59%, where as the averages spectra gave a SEC of 0.32%.

The data presented in this study is limited to calibration data only. It is important that more samples be scanned and used as prediction data and then added back to the calibration data.

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