Determination of Moisture in a Protein Sample Using a Portable NIR Instrument

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INTRODUCTION

Lysophotometry is a well-established procedure for the preservation and storage of proteins and other biochemical materials in both the food and the pharmaceutical industries. Monitoring protein moisture content is an important process as moisture affects both the physical and the chemical state of the material. In particular, the detection of proteins in pharmaceutical processes requires monitoring of these samples as any excess moisture, or moisture lower than optimum, will impact the usability and the shelf-life of the samples.

RESULTS AND DISCUSSION

Data preparation and generation

Spectral data from the microPHAZIR RX was combined with the reference moisture values from the LOD balance for all samples. The moisture values ranged from approximately 9 to 13 wt/wt%.

The collected spectra of the moisture in casein samples are shown in Figure 1. Color coding is by sample number with each sample containing different moisture levels.

Figure 1. The collected spectra of the moisture in casein samples

Spectra preprocessing was done to optimize the robustness of subsequent models. In this case preprocessing consisted of Standard Normal Variates (SNV) to compensate for packing or density differences, and Savitly-Goaty (1st derivative-5 point smooth-2nd order polynomial). Preprocessing was kept to a minimum as the sample set was used as viable product improving operational efficiency. It is possible to perform 100% inspection of samples to comply with regulatory requirements and ensure that anomalous samples will be identified.

The method is destructive so samples cannot be used again causing unnecessary loss of product. The process is expensive and time-consuming due to the materials involved and the specialized training required to perform the relevant protocols.

In contrast the use of NIR technology is fast (seconds), easy to use, and allows for accurate determination of the moisture level in a closed sample vial. Scanning of the sample can be performed through the glass vial without compromising the sample integrity. The vial can be used for multiple scans as viable product improving operational efficiency. It is possible to perform 100% inspection of samples to comply with regulatory requirements and ensure that anomalous samples will be identified.

EXPERIMENTAL DESIGN

Replicate casein samples were made using casein from bovine milk (Sigma Aldrich). Twenty standard samples were placed in glass vials in a saturated NaCl solution within a humidity chamber set to produce 75% humidity. Samples were sealed in the humidity chamber for varying periods of time to produce a range of moisture levels within a protein sample. The results generated with the portable NIR instrument are also evaluated and compared with a laboratory based NIR instrument in order to demonstrate the comparable performance and improved utility of the portable instrument.

CONCLUSION

The similarity between the Antaris and the microPHAZIR RX is further corroborated in the RMSE results. RMSE, which incorporates standard deviation of prediction error, is a consistent indicator of model performance and robustness, and as Table 2 shows, both instruments are expected to have excellent accuracy predicting moisture in the casein protein samples. Accuracy with respect to the RMSE for calibration and cross validation is similar for the Antaris and the microPHAZIR RX. Note the low and consistent standard deviations indicating both precision in the obtained results and consistency between devices observed.

Figure 4. Correlation plots between calibration and validation values for microPHAZIR RX and Antaris, respectively

The results are indicated in terms of coefficient of determination (R²), which is a measure of the strength of the linear relationship between the observed and predicted values. An R² value of 1 indicates a perfect fit, while an R² value of 0 indicates no correlation. Table 2 shows the RMSE results for the Antaris and the microPHAZIR RX, along with the R² values for cross validation and calibration.

Table 2. RMSE comparison of Antaris and MicroPHAZIR RX methodologies

<table>
<thead>
<tr>
<th>Moisture Content</th>
<th>Antaris</th>
<th>microPHAZIR RX</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSEC</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>RMSEV</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>RMSECV</td>
<td>0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td>1.00</td>
</tr>
<tr>
<td>StDev, Residual</td>
<td>0.11</td>
<td>0.11</td>
</tr>
</tbody>
</table>

The resulting PLS model was built to utilize these 3 factors. The subsequent correlation plot of reference moisture values and the predicted moisture values is shown in Figure 4. The plot shows the calibration (blue) and the validation data results (red). As clearly seen, both calibration and validation results are linear, indicating good agreement between predicted and actual results. The results from the Antaris data set are also shown for comparison. Again, a high degree of agreement is seen with the predicted and reference values. Both plots also show a similar response to expected model predictive behavior as they correspond to a very similar linear pattern.