PAT and beyond
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CHAPTER 2. VIBRATIONAL SPECTROSCOPY

One of the PAT tools is modern process analyzers. A great deal of these analyzers is based on the principles of vibrational spectroscopy. Vibrational spectroscopy techniques are becoming very popular options for a wide range of applications in solid dosage forms manufacturing and has also been the analysis tool in this project.

Vibrational spectroscopy

The most common way of presenting the electromagnetic spectrum is shown in Figure 2. The wavelength is presented in either nanometres or reciprocal centimetres also called wavenumber. Fourier-transform instruments use reciprocal centimetres and filter instruments, array instruments and grating instruments uses nanometres.

Most analytical chemist is familiar with mid-infrared or simply MIR which is the wavelength region from 4000 to 200 cm\(^{-1}\). MIR is primarily used for chemical identification. Different molecular bonds absorbs at specific wave numbers in the MIR region which also is referred to as the “fingerprint region”. When the bonds absorb electromagnetic radiation, the bond will begin to vibrate. There are \(3N-6\) normal modes of vibration (known as fundamentals) of a molecule (\(3N-5\) for linear molecules), where \(N\) is the number of atoms. Two of these modes are called stretching and bending (Figure 3). As an example a three atom molecule is depicted (Figure 3). The centre atom is stationary and the different vibrations are explained by the motions of the two attached atoms. When the bond length between the two atoms and the centre atom simultaneously is increasing or decreasing (in the plane of the paper) it is called symmetric stretch. If one of the bonds is stretching while the other is decreasing in length (still in the plane) the stretch is asymmetric. The bonds can also bend while maintaining the distance to the centre atom. The bending can be symmetric or asymmetric in the plane and also symmetric or asymmetric out of the plane.
The absorption in the MIR region is primarily due to fundamental vibrations. NIR spectral absorption bands exist because the vibration is not a simple harmonic motion and weaker overtones of bond vibrations occur at about two, three, four or five times the wavenumber in MIR. It is actually these overtones that are recognized in the NIR region. The overtones of stretching vibration are usually stronger than bending or rocking modes. In the upper part (5200 – 4000cm⁻¹) of the NIR region in close proximity to the MIR is the combination band region found. These bands appear at frequencies that represent the sum of the two vibrations. The NIR region used to be called the “spectroscopic garbage can” because these were not fundamental absorption bands but weaker overtones. Interpretation was difficult compared to MIR but due to the development of multivariate statistics and increased computer power, the NIR region has become “accessible” to the spectroscopist.

<table>
<thead>
<tr>
<th>nm</th>
<th>20</th>
<th>170</th>
<th>400</th>
<th>800</th>
<th>2500</th>
<th>5000</th>
<th>10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm⁻¹</td>
<td>5x10⁵</td>
<td>60000</td>
<td>25000</td>
<td>12500</td>
<td>4000</td>
<td>200</td>
<td>10</td>
</tr>
</tbody>
</table>

**Figure 2.** The electromagnetic spectrum.
Spectra – structure correlations

Some of the most common groups that are measured with NIR are hydrocarbons, amines and amides, hydroxyls, water, carbonyl compounds and generally just X-H (X=O,N,C) i.e. if a hydrogen bond is present in the molecule it can be detected with NIR. This versatile range of functional groups is one of reasons for the wide range of NIR applications presented in the industry today. Some typical group NIR absorption bands are listed below. If the values are scrutinized it is evident that the peaks are broad and overlap exists. This can in some instances be solved by derivatization of the spectra or the use of chemometrics.

Table 2. List of typical absorption bands (in cm⁻¹) for functional groups in NIR.

<table>
<thead>
<tr>
<th>Group</th>
<th>Combinations</th>
<th>First overtone region</th>
<th>Second overtone region</th>
<th>Third overtone region</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH</td>
<td>4098-4405</td>
<td>5618-5917</td>
<td>8130-8547</td>
<td>10695-11050</td>
</tr>
<tr>
<td>CH₂</td>
<td>4149-4444</td>
<td>5714-5988</td>
<td>8264-8696</td>
<td>10811-11111</td>
</tr>
<tr>
<td>CH₃</td>
<td>4167-4545</td>
<td>5848-6135</td>
<td>8368-8929</td>
<td>10989-11494</td>
</tr>
<tr>
<td>H₂O</td>
<td>5208-5405</td>
<td>6920-7117</td>
<td>10101-10471</td>
<td>13072-13514</td>
</tr>
<tr>
<td>RNH₂</td>
<td>4535-4637</td>
<td>6623-6711</td>
<td>9542-9852</td>
<td>12195-12821</td>
</tr>
<tr>
<td>ArCH</td>
<td>6061-6211</td>
<td>9091-9259</td>
<td>11494-11765</td>
<td></td>
</tr>
<tr>
<td>ROH</td>
<td>4785-4854</td>
<td>6757-7092</td>
<td>10638-10811</td>
<td>13269-13793</td>
</tr>
</tbody>
</table>
Sample presentation in near-infrared

One of the key advantages with NIR, compared to MIR is that no sample preparation is usually needed. Also almost any physical state of a sample can be measured e.g. solid, liquid or gas. Depending on the sample properties it can be measured either in reflectance, transmittance or trans-reflectance mode. In a reflectance measurement (Figure 4.a) the NIR light is radiated on the sample surface and part of the diffuse reflected light is collected by the detector. This measurement type is used for solids e.g. powders. The NIR radiation can also pass through the sample and then be collected by the detector; this is called transmittance (Figure 4.b). Whole tablets can be assessed with transmittance spectroscopy and liquid samples are also measured in transmittance. The trans-reflectance measurement (Figure 4.c) is a combination of the two previous. The NIR radiation is passed through the sample and reflected on a mirror, passing through the sample again and to the detector. When measuring liquids and liquids with suspended solids, trans-reflectance has proven to be useful. It is difficult to give a rule of thumb for what type of sample presentation that provides the best results. This has to be tested on a case-to-case basis.

Figure 4. The three main sample presentations. Reflectance (a), transmittance (b) and trans-reflectance (c). The detector is symbolized as a box with letter D.
Factors effecting near-infrared measurements
NIR spectra are influenced by many physical factors\textsuperscript{11} e.g. particle size, density, temperature. Figure 5 shows reflectance spectra of three different lactose batches. The difference between the chemically identical batches is the mean particle size. This is identified as a clear offset between the spectra.

![Lactose Spectra](image)

**Figure 5. Reflectance spectra of three different Lactose qualities with three different mean particle sizes.**

When quantitative models are made, the non-wanted spectral variation sometimes needs to be minimized in order to get a good predictive model. This can be done by various pre-processing methods\textsuperscript{12-17}. Secondly, if the relevant spectral variation is only taking part in isolated regions of the spectrum model improvements can be made by performing wavelength selection\textsuperscript{18-21}.

The most common way of evaluating the effectiveness of different pre-processing methods and choices of wavelength selection is to compute the prediction uncertainty of an independent test set i.e. the root mean square error of
prediction (RMSEP) or root mean square error of prediction cross-validation (RMSECV) if only a smaller dataset is available. The pre-processing method or selected wavelength region that provides the lowest prediction error is then selected. This requires that a calibration set have been produced and measured with NIR and reference analysis has been performed. If the reference analysis is erroneous or a calibration set is not available, other evaluation criteria have to be used.

Calibration methods based on the calculation of the Net Analyte Signal (NAS) have proven successful. Net analyte signal is defined as the part of a signal that is unique for the analyte of interest. Lorber\textsuperscript{22} demonstrated how figures of merit e.g. multivariate sensitivity, signal-to-noise ratio, selectivity and limit of detection could be computed from the net analyte signal of the analyte. These figures of merit can be used to judge whether a pre-processing method is beneficial for the analytical performance and they can also be used for wavelength selection. Faber\textsuperscript{23} used the inverse multivariate sensitivity of the analyte to judge whether a certain pre-processing method e.g. derivative would improve the predictive ability of the calibration model or not. Xu and Schechter\textsuperscript{24} developed an error indicator for wavelength selection. Boelens et al.\textsuperscript{25} have also demonstrated the usability of NAS for improving detection limit for a spectroscopic process analysis by tuning Savitzky-Golay filters.

We introduced a new error indicator called the signal-to-error indicator (SE)\textsuperscript{26}. A signal-to-error (SE) value is computed for the analyte using various pre-processing methods and wavelength selections applied to the spectra. The highest SE value indicates the optimal pre-processing and wavelength interval. The indicator was used to find the optimal pre-processing method and wavelength regions for two quantitative NIR methods i.e. a method using reflectance spectra of pharmaceutical powder mixtures and a model using transmission spectra of pharmaceutical tablets. The SE indicator was compared