Putting Near-Infrared Spectroscopy (NIR) in the spotlight

Outline

- What is NIR good for?
- A bit of history and basic theory
- Applications in Pharmaceutical industry
- Development Quantitative prediction models
What is NIR good for?

**Medical devices**
- HemoNIR™
- HemoNIR™ Med
- CardioNIR™
- GlucoNIR™
- LiproNIR™

<table>
<thead>
<tr>
<th>Analytes</th>
<th>HemoNIR™</th>
<th>HemoNIR™ Med</th>
<th>CardioNIR™</th>
<th>GlucoNIR™</th>
<th>LiproNIR™</th>
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<tbody>
<tr>
<td>Total-Hb, Carboxy-Hb, Met-Hb</td>
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<tr>
<td>Total-Hb</td>
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<td>Total cholesterol</td>
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<td>Glucose</td>
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<td>Total cholesterol, Chol-LDL, Chol-HDL, Triglycerides</td>
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**Agriculture / Food**

**Pharmaceutical industry**

**Paper industry**

**Oil / Petrochemicals**
Attributes of NIR Spectroscopy

- Fast
  - Results (quantitative, qualitative) immediately available
- No sample preparation required
- Non-destructive
- Measurements possible direct through glass
- Fibre optic cables can be used
- Good signal to noise ratio is achieved
History / Theory
The Herschel experiment - 1800

Sir Frederick William Herschel with his prism (Science Museum London)

Temperature highest “above” the visible red light
The Electromagnetic Spectrum

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<th>Frequency</th>
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</tbody>
</table>

- Radio waves
- Microwaves
- Far IR
- Mid IR
- Vacuum UV
- X-Rays
- Gamma Rays

Near-IR: 2500-780nm
Visible: 780-380nm
UV: 380-200nm
NIR sources and detectors!

Emission of a tungsten light bulb
Basics of Vibrational Spectroscopy

- Continuous bending / stretching
- Frequency depends on the
  - mass of molecules
  - position in molecule
  - strength of the bond
- Organic molecules show absorption in NIR
When a photon with a certain energy hits a bond, it is absorbed, and the size (amplitude) of the motion increases.
Anharmonic Oscillator
Relative Intensities of Infrared Bands

- NIR intensity weaker than IR
- No “dilution” required
NIR absorptions

- The bond must have charge separation (dipole moment)
- The intensity of absorption depends on the degree of anharmonicity
- Hydrogen is the lightest atom and will vibrate with the largest amplitude in stretching
- Strong absorption where hydrogen is bonded to another atom:
  - C-H
  - N-H
  - O-H

![Absorption Spectrum](image-url)
When Light Interacts with Solid Matter

- Absorption
- Specular Reflectance
- Transmittance
- Diffuse Reflectance
Example of NIR spectrometer
NIR Methods of Tablet Analysis

- **Reflectance**
  - Surface Measurement
  - Small Portion of Sample Analysed
  - Sensitive to physical properties
  - E.g. Powders, tablet coating

- **Transmission**
  - large portion of sample analysed
  - Good for tablet content analysis
Effect of Particle Size

Long pathlength

Short pathlength

Absorbance vs. Wavelength

Coarse particle size

Fine particle size
Standard Normal Variate (SNV)

- Contains physical and chemical information
  - Particle size and density difference will be seen as baseline offsets.
  - The effects of physical properties can be removed

Effect of normalisation on physical differences
Case studies

- Tablet content determination
- Dissolution prediction
- Blend monitoring
- Mapping / Imaging
Tablet Content determination

- Tablets with 75 - 125 % label claim manufactured
- 5 batches of 150 mg tablets used
- Tablets measured in transmission over 10 days
- All tablets analysed by HPLC for Content Uniformity
PLS result: comparison of HPLC vs. NIR

Prediction vs True [mg] / Cross Validation

Rank: 5  $R^2 = 99.19$  RMSECV = 2.22
Dissolution testing by NIR

- Release profile of formulation dependant on coat thickness
- Samples with different coat thickness scanned by NIR and then analysed by conventional dissolution test.

(Note that 1 dissolution run takes 18 hours)
On-line blend monitoring

- Blending process
- NIR attached
- 1 spectra per revolution

Typical rapid improvements in blend homogeneity

Blending generally complete at about the same time but well before the end of the nominal blend time!
Chemical Imaging: NIR spectroscopy

NIR map of tablet
2 x 2 mm²

- API
- Excipient 1
- Excipient 2
Conclusions

- NIR is
  - a very versatile analytical tool
  - fast, non-destructive
  - suitable for on-line control applications
  - Potential for real-time release
Acknowledgements

- Simon Maris
- Rachel Brody
- Slobodan Sasic
- Ian Clegg
- Elleanor Wood
Backup slides
How to develop a quantitative NIR calibration method

- NIR calibration model different from other analytical techniques
  - Not a primary measurement system
  - Requires training with results from primary measurement method (e.g. HPLC, KF)
  - Requires chemometrics to extract information

- Prework required (for a feasibility study)
  - Scan ingredients of matrix if available
  - Scan small number of samples of product (e.g. 10)
  - Spike samples if possible to check linearity
  - Determine analytical wavelengths
  - Optimise sample presentation
Choice of Samples

- Good choice of samples is essential for a robust calibration model.
- Badly chosen sample sets will produce non-robust calibrations.
- Samples in the calibration set should represent all the normal and expected variations in the product.
- The range of constituents must be uniformly distributed.
The number of calibration samples should be based on the complexity of the matrix to be analysed.

For complex matrices a minimum of 50 samples is recommended.

These variations should be included in the calibration set (these are very important otherwise the method will not be robust):
- Range and distribution of constituent values
- Origin/composition of the samples
- Sample preparation/texture
- Presentation of the sample to the instrument
Reference Analysis

- Based on good laboratory practice and a proven methodology

- NIR calibrations will inherit the error of the reference method

- The NIR method cannot have a smaller standard error than the reference method
Partial Least Squares is a multivariate correlation technique

The reference value is assigned to the NIR spectra

Spectra containing higher constituent concentrations are weighted more heavily than those with low concentrations

Because the entire spectrum (or spectral blocks) are used, a large number of wavelengths are averaged into the calibrations. Thus PLS models matrix effects as well as changes in concentration of the constituents of interest.
Partial Least Squares

In all cases, choice of wavelength regions should be justified based upon scientific knowledge of the sample matrix and examination of the sample spectra

- Included regions must be specific to the analyte
- The primary reasons for exclusion are typically noise or regions sensitive to moisture changes. The general ‘rule’ is to use a minimum number of “Factors”
Chemometrics: Principal Component Analysis

- Spectrum consists of e.g. 1500 wavelengths (variables)

- Chemometrics such as Principal component analysis required to “reduce” the important information to a few “scores” and “loadings”

- One spectra becomes one data point in a multi dimensional space
Principal component analysis of NIR data

**Grouping of material with similar properties**

RESULT2, X-expl: 98%, 2%