# Determination of very low unsaturation levels in oleochemicals by NIR

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#### Introduction

Many applications in the oleochemical industry, such as hydrogenation, require a quick and reliable determination of very low unsaturation levels (Iodine Value [IV] below 1 g I / 100 g sample). The well-known wet chemistry method using iodine monochloride (Wijs) solution to halogenate the double bonds is, in its original (widely applied) version,<sup>1</sup> long and laborious, involves toxic solvents and generates large quantities of chemical wastes. Introduction of alternative reagents has improved analysis speed<sup>2</sup> and reduced toxicity issues.<sup>3</sup> However, high accuracy and precision of very low IV by wet chemistry remain difficult to achieve in a production environment.

Numerous alternative instrumental methods for the measurement of unsaturation levels have been published. Most of them rely on FT-IR spectroscopy with a transmission cell of narrow pathlength (< 100  $\mu$ m)<sup>4-5</sup> or an ATR crystal.<sup>6,7</sup> Although IV can be measured in less than two minutes, transfer of this technology to direct in-line analyses remain limited, as spectrophotometers are not welcome in the production area, and the use of fibre optics in the IR range is restricted to a few metres. Based on a study of available literature,<sup>8,9</sup> the application of Raman spectroscopy to measurement of low IV seems very challenging (due to either fluorescence or sensitivity issues) and the cost of its implementation is in any case prohibitive. By contrast, NIR technology is now widely accepted for process control of IV down to 5 g I / 100 g sample.<sup>10,11</sup> However, it has focused on the measurement of major components, as NIR spectra do not exhibit vibrational absorption wavelengths specific to C–C double bonds and quantitative analysis relies on complex multivariate algorithms. In addition, its application to the determination of low IV levels is often limited by the poor accuracy and precision of the wet chemistry reference method.

This paper investigates the effect of pathlength on the determination of very low levels of unsaturation in oleochemicals, with a view to improving sensitivity of the NIR technique. Analyses are carried out using two probes of fixed pathlength (5 and 20 mm), and under conditions of precisely controlled sample temperature. In order to overcome poor precision of the referencemethod, the calibration set is built by mixing a small number of process fatty acid samples with well-defined IV. Linearity of the detector response when working with the 20 mm pathlength probe is checked. NIR models for IV determination are made for each pathlength, and their predictive performances are compared. In addition, detection limits of the two methods are discussed. The effect of small variations of uncontrolled parameters during the measurement is also investigated.

#### Material and method

#### Instrumentation

NIR spectra have been recorded using a process type analyser (Prospec IV, Guided Wave) equipped with a grating and an InGaAs detector. Scanning covers the region 1000 to 2100 nm, with a resolution of 1 nm. The instrument is connected through optical cables (2 times 20 m single-strand fibres, Guided Wave) to a single side transmission (SST) probe (Guided Wave). Two different

pathlengths (5 mm and 20 mm) have been used. Temperature regulation ( $\pm$  0.5 °C) is ensured by a semi-automatic sampling / measuring unit,<sup>12</sup> developed for at-line analyses.

#### Samples

Two stearic acid samples produced from the same raw material but with different residual unsaturation levels have been collected. Their iodine values (IV = 0.13 and 13.5 g I / 100 g) have been determined by the modified Wijs method.<sup>3</sup>

The calibration set has been generated by mixing these two stearic samples in various proportions. Eight additional samples have hence been prepared and their IV have been calculated. The sample with an IV  $\sim 2$  g I / 100 g has been prepared in triplicate and the sample with an IV = 0.13 g I / 100 g has been measured 3 times, leading all together to 14 spectra.

#### Method

Scanning parameters are based on AOCS standard procedure for IV determination by NIR<sup>13</sup>:

- Sample temperature: 75°C
- Scanning time: 2 min (29 scans)
- Reference: empty sampling unit

NIR spectra have been exported to Unscrambler® software (7.8, Camo AS) and corrected for baseline drift (one-point correction at 1100 nm) before investigation.

Modelling also roughly follows the AOCS guidelines. NIR models for IV prediction have been built on part of the CH second overtone range (1100 - 1212 nm) after normalisation (range normalisation over 1098 - 1323 nm). The Partial Least Squares (PLS1) approach has been selected to ensure all relevant structure of the data matrix to be used, despite the small IV range spanned by the calibration set. Because of the limited number of samples, all models have been evaluated by full cross validation (10 segments, including each all spectra related to the same sample).

#### Results

Effect of pathlength increase on second CH overtone.

The prediction by NIR of IV requires a correlation between changes in unsaturation levels and changes in absorption intensity of =CH band. Figure 1 gives a typical NIR spectrum profile recorded for one of the stearic acid samples (IV = 4.6 g I / 100 g) using the standard 5 mm pathlength probe. Already with this accessory, the absorption intensity in the first CH overtone falls well outside of the linearity range of the detector (above 2 A.U.). We have therefore concentrated our study on the second CH overtone (1100 - 1210 nm). As already mentioned, this is also the range recommended by the AOCS method for IV determination by NIR<sup>13</sup>.



Figure 1. Profile of raw spectrum of stearic acid (IV = 4.6 g I / 100 g & pathlength = 5 mm)

To further check this relationship, Figure 2(b) overlays the spectrum profile characterizing the 5 mm pathlength probe, with  $\frac{1}{4}$  of the spectral absorbance recorded for the 20 mm pathlength

accessory. The picture clearly shows a very close match, with the exception of the wavelengths around to peak maximum (~ 1205–1215 nm). Therefore, even with the 20 mm pathlength, the detector signal remains linear. Note that the small deviation around 1210 nm might be explained by the absorbance intensities somewhat above 1.5 A.U. Furthermore, increasing the sample volume tested does not lead to any significant light scattering effects, as no baseline drift or / and slope change are visible when overlaying raw spectra recorded with the two different probes. As a result, extra spectral treatment is not required, when working with a 20 mm pathlength

Figure 2(a) shows the change in spectral absorbance when going from 5 mm to 20 mm pathlength. As expected from the Lambert–Beer law, the absorbance intensity drastically increases from about 0.4 to about 1.6 A.U., thus by the same coefficient "4".



Figure 2. Effect of pathlength on CH  $2^{nd}$  overtone region (after baseline correction) – (a: right). Increase of spectral absorbance from pathlength = 5 mm to pathlength = 20 mm – (b: left). Overlay of spectrum profile recorded for pathlength = 5 mm with  $\frac{1}{4}$  of spectral absorbance recorded for pathlength = 20 mm

Effect of pathlength increase on signal to noise ratio and detection limit

In order to compare the signal to noise ratio characterising the two probes in the CH second overtone range, spectra of two different samples ( $\Delta$  IV = 4.8 g I / 100 g) have been recorded, and after normalisation, their absorbance intensities have been subtracted. Figure 3 shows the difference spectrum profiles obtained respectively with the 5 mm and the 20 mm pathlength probes.



Figure 3. Effect of pathlength on signal-to-noise—difference between spectrum absorbance recorded for 2 samples (normalised spectra,  $\Delta$  IV = 4.8 g I / 100 g)—(a) with pathlength = 5 mm—(b) with pathlength = 20 mm.

• For the signal, the absorbance intensity corresponding to a  $\Delta$  IV of 4.8 g I / 100 g has been measured at the wavelength characterising the =CH band maximum (1162 nm);

• For the noise, the difference between maximum and minimum absorption in the baseline region around 1300 nm has been determined.

Due to normalisation, the =CH band maxima have about the same intensity; however, the noise level is decreasing by about a factor "4" when increasing the pathlength from 5 to 20 mm.

Based on these spectral data, the detection limit can then be estimated, using the following formula:<sup>14</sup>

$$LOD = 3 \times noise \times \frac{\Delta IV}{signal}$$
(1)

The results are summarised in Table 1 below. Increasing the pathlength from 5 to 20 mm, improves the detection limit by a factor of "4" and should bring the quantification limit (factor of "10" instead of "3" in Equation 1 above) below 0.1 g I / 100 g.

#### Effect of pathlength increase on NIR model performances

Based on the same small calibration sample set, two NIR models for IV determination have been made following closely the AOCS standard procedure;<sup>13</sup> the first one uses spectra recorded with the 5 mm pathlength probe, while in the second one includes spectral data collected with the 20 mm pathlength accessory. Figures 4(a) and 4(b) below show the loading plots obtained for these two PLS models.

The first PLS component exhibits a very similar profile in both cases; however, the explained variance (based on full crossed validation) decreases from 98 to 92%, when increasing the pathlength by four. In other words, it seems that the spectral data set generated by the 20 mm probe contains additional small systematic variations to be explained and correlated to the unsaturation level. In addition, the second PLS component is less noisy with the 20 mm pathlength than with the 5 mm one. Therefore, longer pathlength should lead to more accurate and precise predictions.



Figure 4. Effect of pathlength on loadings plots of PLS models for IV determination – (a: right). With pathlength = 5 mm – (b: left). With pathlength = 20 mm.

Table 1 summarised the root mean square error of prediction (*RMSEP*) calculated by full cross validation for the two models for an optimised number of PLS components. As expected from the loading plots, increasing the pathlength results in a significant decrease of the *RMSEP*; this

conclusion remains true, when focussing on the low IV samples. However, based on these results, the use of longer pathlength seems somewhat less beneficial than expected from the detection limit calculation. A possible explanation could be that the PLS algorithm enables to extract spectral information despite the noise level. On the other hand, the quantification limit for the model based on NIR spectra recorded with the 20 mm probe seems to lie around 0.3 g I / 100 g. This is higher than the first estimate made directly from the NIR spectra, as the RMSEP reflects both modelling error and reference method error. Note that the performance of both NIR models should be somewhat improved by using a sample set of larger size.

|             | Spectral data |        |           | PLS model (full cross validation) |            |             |               |
|-------------|---------------|--------|-----------|-----------------------------------|------------|-------------|---------------|
|             | Signal        | Noise  | Detection | Number PLS                        | RMSEP      | RMSEP       | RMSEP         |
|             | IIIA.U.       | IIIA.U | (gI/100g) | components                        | An samples | < 1 gi/100g | < 0.5 gi/100g |
| 5 mm probe  | 5.7           | 0.050  | 0.084     | 3                                 | 0.24       | 0.17        | 0.20          |
| 20 mm probe | 6.8           | 0.017  | 0.024     | 4                                 | 0.17       | 0.14        | 0.14          |

Table 1. Comparison of detection limit and RMSEP for pathlength = 5 & 20 mm

#### Effect of small variations of uncontrolled parameters on low IV determination

Even when working with a longer pathlength, determination of low unsaturation levels by NIR depends on small intensity changes of the second CH overtone. Accuracy and precision are likely to be affected by variations of uncontrolled variables. For example, as measurements are carried out using an open cell maintained at 75°C, traces of water could evaporate from the stearic acid matrix. A typical sample (IV = 13.5 g I / 100 g) has been continuously scanned, while being heated. Although the sample temperature remains pretty stable during the experiment [random variations of  $\pm 0.02^{\circ}$ C, Figure 5(a)], the absorption intensity characterising =CH<sub>2</sub> maximum decreases in the time (by more than 0.001 A.U.), while the absorption intensity recorded for the CH<sub>2</sub> maximum keeps increasing [by about 0.003 A.U., Figure 5(b)]. Simultaneously, the absorption intensity of the water band maximum at 1443 nm decreases significantly (by more than 0.05 A.U.).



Figure 5. Effect of small variations of uncontrolled parameters on low IV determination—(a: left). Variation in time of sample temperature and water absorption intensity (1443 nm)—(b: right). Variation in time of absorption intensities of =CH maximum (1162 nm) and CH, maximum (1214 nm)

These spectral changes seems to be related to loss of water traces, as  $CH_2$  concentration is then expected to increase slightly and the OH band at 1141 nm<sup>15</sup> could lead to the decrease of the absorption intensity of =CH maximum. This proposed explanation was confirmed by spiking the

sample with water. Note that the observed variation in absorption intensity of =CH maximum corresponds roughly to a change in IV prediction of 0.4 g I / 100 g.

# Conclusions

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This paper investigates the effect of pathlength increase on the determination of very low levels of unsaturation in oleochemicals, with a view to improving sensitivity of the NIR technique. Based on the above discussion, the following conclusions can be drawn:

- It is possible to use a 20 mm pathlength for NIR determination of IV in fatty acid samples.
- Based on the limited data set tested, the longer pathlength achieves a the quantification limit between 0.3 and 0.1 g I / 100 g. Increasing the pathlength from 5 to 20 mm improves the predictive abilities of NIR models in the low IV range.

• Determination of low IV requires careful control of analytical conditions (for example, use of bypass).

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