

# Moisture determination in hygroscopic drug substances by near infrared spectroscopy

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Received 14 March 1997

## Abstract

The moisture level in a hygroscopic drug substance was successfully determined by near infrared spectroscopy using coulometric Karl Fischer titration as the reference method. The importance of sample handling and proper application of the reference technique are stressed for this difficult sample type. Samples were prepared with moisture levels from 0.5 to 11.4% (w/w) and reflectance spectra were collected over the spectral range 1100–2500 nm. Calibration models were built using partial least squares (PLS) regression analysis. Optimum models were found by choosing proper spectral ranges and number of PLS factors. The best calibration models were built using first derivative spectra, a spectral range of 1850–1936 nm and 5 PLS factors. The corresponding standard error of prediction was 0.11% (w/w) water. © 1998 Elsevier Science B.V. All rights reserved.

*Keywords:* Near infrared; NIR; NIRS; Drug substance; Moisture; PLS; Karl Fischer

## 1. Introduction

Traditional methods for moisture determination in pharmaceutical drug products are based on weight loss on drying and Karl Fischer titration [1,2]. Gas chromatography is another method which could provide comparable results [3]. However, each has pros and cons in terms of accuracy, speed and ease of operation. Weight loss on drying is limited if the chemicals are unstable at elevated temperatures or if other volatile species are present. Gas chromatography is more expensive and standardization can be difficult. Some

volatile drug substances might interfere with the measurement. Karl Fischer titration is time consuming and uses toxic reagents. When determining low moisture levels and for hygroscopic samples, all three methods are influenced by the ambient moisture if strict precautions are not taken.

Near infrared reflectance spectroscopy (NIRS) is attractive for moisture determination because it is rapid, non-destructive, no sample pretreatment is needed and water has strong absorption bands in this spectral region that provide the sensitivity needed for accurate determination [4–7]. For example, water of freeze-dried drug in a vial has been measured with a calibration equation based on the absorbancies at one or two wavelengths [4].

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Similarly, moisture in antibiotic powders has been measured with multiple linear regression (MLR) based on the information at four wavelengths [5]. However, the limits of accuracy have not been fully explored. It is necessary to establish optimum methodology for the reference measurement, for the NIRS measurement, for sample handling during the entire process and for the calibration model, in order to build an NIRS method which is accurate, precise and robust.

A study was performed to establish a near infrared reflectance (NIR) method for determining the moisture level in a hygroscopic bulk drug substance. This hygroscopic test material was chosen because it is a challenging sample type for building an optimum NIR calibration model. Experimental techniques and data handling methods are presented which result in the most accurate NIR spectroscopic method. These techniques include a reliable reference method based on coulometric Karl Fischer titration which minimized the interference from ambient moisture and an optimized PLS regression model.

## 2. Experimental

### 2.1. Apparatus and materials

Diffuse reflectance spectra were obtained with an NIRSystems 6500 spectrophotometer (Perstorp Analytical, Silver Spring, MD) equipped with a Rapid Content Analyzer (RCA). Karl Fischer titration was performed with a 737 KF Coulometer (Metrohm, Switzerland). Samples were introduced using 3 and 10 ml plastic syringes (Becton Dickinson, Franklin Lakes, NJ) with special 10 cm long needles (22 gauge) (Metrohm, Switzerland). The KF reagent was HYDRANAL<sup>®</sup>-Coulomat AG (Crescent Chemical, Hauppauge, NY).

The drug substance used in this study is very hygroscopic. The water content can vary from 0.5 to 14% (w/w) at a rate of up to 0.02% min<sup>-1</sup> as ambient humidity changes. In order to protect the material from humidity changes, the powder was capped in 20 ml clear glass bottles with flat bottoms (Kimble Glass, Vineland, NJ). A moisture-tight closure was obtained with Teflon coated

low-moisture stoppers (Diakyo D777-3 Gold Butyl stoppers, The West Company, Lionville, PA) and an aluminum crimp seal. Each bottle contained approximately 150 mg of powder which was loosely packed. Prior to capping, moisture levels of these powders were adjusted from as dry as possible to about 11% (w/w) using a humidity controlled glove box. First, all powder samples were dried to the same level by applying a vacuum at 80°C for approximately 2 h. Approximate target levels were then obtained by exposing various bottles to humidity between ambient and 80% R.H., and stoppering vials at appropriate times. After capping and prior to the Karl Fischer titration, NIR spectra were obtained for each bottle. Just before spectrum collection, each bottle was gently shaken and then tapped gently in a way that was shown to achieve reproducible packing.

### 2.2. Coulometric Karl Fischer titration and calculation

The coulometric Karl Fischer titration method used for this study was an in-house procedure for the determination of moisture levels in freeze-dried pharmaceutical products. A plastic syringe was used to draw and dispense KF reagent from the titration vessel until the drift value remained below 12 µg min<sup>-1</sup>. The reagent in the dried syringe was then injected through the stopper into weighed bottles, completely dissolving the powder. A syringe was also dried by the same 'draw and dispense' process before drawing sample from a bottle. The sample was injected into the titration vessel and the amount of sample added was obtained from the weight difference of the syringe before and after the injection. All the operations were optimized to minimize interference from ambient moisture. All vials used in this study were assayed in triplicate by this procedure. Moisture values were in the range 0.5–11.4% (w/w) with a standard deviation of 0.09% (w/w). The Karl Fischer titration results are listed in Table 1.

The percent (w/w) moisture level ( $C_{w\%}$ ) of the sample powder was calculated by the following equation:

Table 1  
Total number of samples and the corresponding moisture levels, as measured by the coulometric Karl Fischer method

Moisture level	Moisture % (w/w)
1	0.53, 0.46, 0.54, 0.53
2	1.72, 1.74, 1.74, 1.70
3	2.22, 2.16, 2.13, 2.16
4	4.09, 4.06, 3.95, 3.80
5	4.82, 4.93
6	5.72, 5.66, 5.77, 5.54, 5.60, 5.77, 5.70, 5.76, 5.81, 5.65
7	6.02, 6.08, 5.83
8	6.11, 6.04
9	6.53, 6.39, 6.40
10	7.82, 7.74, 7.79
11	11.47, 11.08, 11.03, 11.32

$$C_{w\%} = \left( W_w - \frac{W_d}{W_c + W_d} \cdot W_s \cdot C_b \right) \cdot \frac{W_d + W_c}{W_s} \cdot \frac{100\%}{W_c \cdot 10^6}$$

where  $W_c$  is the weight in grams of the powder in the bottle;  $W_d$  is the weight of diluent (g) added to the bottle for reconstitution prior to titration;  $W_s$  is the weight of liquid sample (g) injected into the titrator;  $W_w$  is the titrated weight of water ( $\mu\text{g}$ ); and  $C_b$  is the amount of water in the blank diluent in  $\mu\text{g}$  of water  $\text{g}^{-1}$  of diluent. By testing the addition and removal of dried solvent to empty, dry bottles, we have determined that the typical blank level is  $10 \mu\text{g g}^{-1}$  and use this value in all of our calculations.

### 2.3. Spectrum collection and processing

Each spectrum was the average of 16 scans over the range 1100–2500 nm. The ratio of each reflectance spectrum against the corresponding reference spectrum of a white ceramic was taken. Absorbance spectra were generated as the negative logarithm of the ratios of the spectra. After approximately 30 min of measurement, a new reference spectrum was collected for subsequent sample spectra. Three spectra were collected sequentially for each sample. Spectra collected over 3 weeks were included in this study. A total of 129 spectra were obtained for the 43 samples. The

software package accompanying this instrument (NSAS version 3.50) was used to collect the spectra. Partial least squares (PLS) regression was performed with PLSplus/IQ in GRAMS/32 (Galactic Industries, Salem, NH).

## 3. Results and discussion

### 3.1. Spectral regions

Overlap of spectral bands is a typical characteristic in the NIR region. It is difficult to find an absorption band unique to a specific molecule in a mixture of compounds. Fortunately, water has large absorption bands with peak maxima around 1420 and 1920 nm in the near infrared region. These absorption bands are not only strong but typically well resolved from the absorption bands of other chemicals, especially the first combination bands of O–H around 1920 nm [9]. The exact position and width of these bands might vary slightly depending on the chemical environment [10].

Significant changes in spectral features of the sample studied have been observed when the moisture level varies. Figs. 1 and 2 show the effect of water on the raw reflectance spectra of these samples, along with the corresponding first and second derivative spectra. In Fig. 1, the absorbance around 1900 nm increases when the water level increases from 0.53 to 11.08% (w/w). These variations in the raw absorbance spectra are retained in the corresponding first and second derivative spectra. By visual inspection of these representative spectra, it is clear that a quantitative relationship exists between the magnitude of absorption and the moisture level.

### 3.2. PLS regression

Simple linear or multiple linear regression are used for most applications of NIRS for moisture analysis. This is because the water band is strong and usually isolated from other significant spectral interference. In this work, we chose to use PLS regression for construction of calibration models. The key element of this work was to

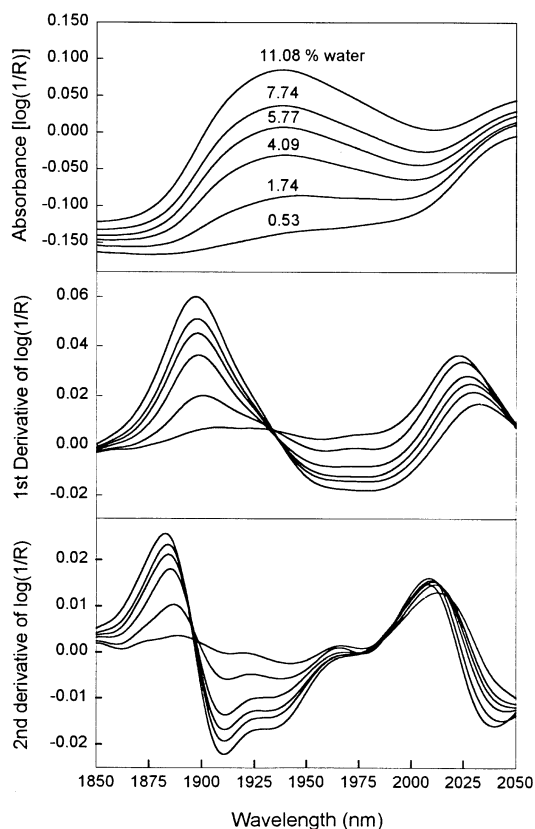


Fig. 1. Reflectance, first, and second derivative spectra of the bulk material with different moisture levels (expressed as percent w/w) in the spectral range 1850–2050 nm.

develop a reference technique and method of handling samples that reaches the limit of precision and accuracy. If experimental conditions are chosen properly, PLS is less likely to result in a model that is limited by minor sources of variance and should be the best choice for illustrating the quality of our overall process. Also, for this chemical system, there are potential sources of nonlinearity related to a spectral feature around 1925–1950 nm. Even with these considerations, the best MLR model with this data set results in only slightly larger errors in moisture prediction.

PLS is a factor-based analysis [8]. During this process the variables of the original data matrix are reconstructed with new variables (factors) which are linear combinations of the original vari-

ables in the absorbance matrix and concentration matrix. The factors are believed to better describe the analytical variations in the data matrix with fewer dimensions. One major advantage of such factor-based methods is that the factors are orthogonal to each other and thus eliminate collinearity without deletion of spectral information. Noise is usually reduced because it spreads throughout all the factors while the variations of interest (such as concentrations) are modeled into the first few factors. PLS is believed to be the best multivariate regression tool to remove spectral noise and other irrelevant information [8]. In addition, it can model nonlinearity [11].

The samples were divided into two data sets, one for calibration and one for prediction. There were 90 spectra from 30 samples in the calibration

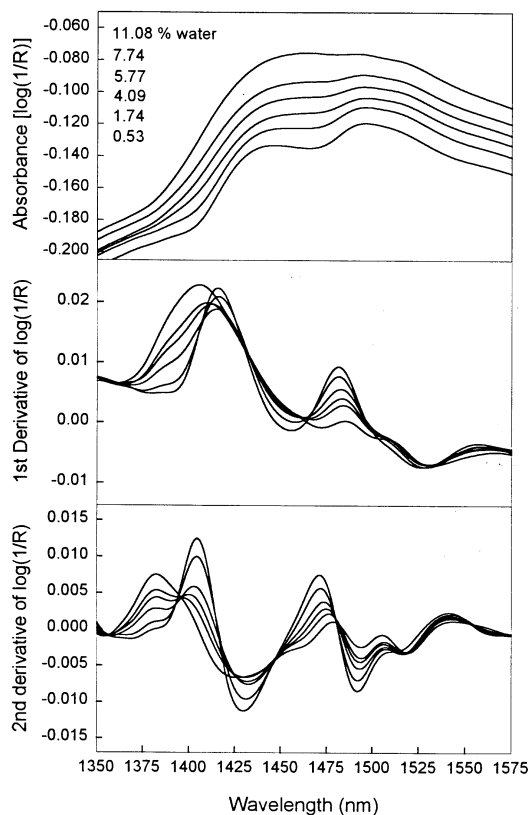


Fig. 2. Reflectance, first, and second derivative spectra of the bulk material with different moisture levels (expressed as percent w/w) in the spectral range 1350–1575 nm.

set and 39 spectra from 13 samples in the prediction set. The former is used to build the calibration models while the latter is used to evaluate the performance of these calibration models.

The spectral range and the number of PLS factors are the two most crucial parameters in the PLS regression process [8]. Based on the absorption features in Figs. 1 and 2, we investigated several sub-ranges within the 1350–1500 and 1850–1990 nm ranges. The first region focuses on the 1420 nm absorption bands. The second one covers the 1920 nm absorption bands. Calibration models were built by using various combinations of spectral range and data pretreatment methods. Each pretreatment method performs differently and the corresponding standard errors of prediction (SEP) for the prediction data set are listed in Table 2.

The optimum number of PLS factors was determined for each set of parameters. For each combination of parameters in Table 2, the number of

factors listed corresponds to the factors recommended by the PLS-1 program [13] based on the *F*-test of the prediction residual error sum of squares (PRESS). The number of PLS factors suggested by this method is expected to coincide with the optimum number of PLS factors. However, caution is advisable, due to the fact that the number of PLS factors recommended by this method sometimes does not equal the number of factors which provide the lowest SEP to the prediction data set. Therefore, the number of factors was corrected to correspond to the lowest SEP under the same conditions. In the spectral range 1350–1500 nm, for example, the optimum calibration model built with the second derivative spectra uses 6 PLS factors and provides an SEP of 0.21% (w/w).

### 3.3. Spectral pretreatment

Obviously, PLS regression is a powerful tool that can extract the spectral variation related to varying moisture level even without any data pretreatment (see Table 2). In other literature, spectral pretreatment has been shown to improve the quality of the spectra and thus improve the performance of the calibration models [12]. In this application, spectral pretreatment by multiplicative scatter correction (MSC) did not improve the performance of the calibration models. Spectral pretreatment with derivative calculation does slightly improve the performance of the calibration models in some cases. Most calibration models built with derivative spectra perform similarly, regardless of whether the first or second derivative is used. More PLS factors are needed to account for the variations in the data set using a larger spectral range. On the other hand, fewer PLS factors are usually required after spectral pretreatment by derivatives. This effect is minor here, because strong absorption bands of water are involved in this study. In addition, in all cases (see Figs. 1 and 2) baseline shift is minimized after derivative pretreatment.

The best calibration model was obtained with first derivative spectra over the 1850–1936 nm spectral range with 5 PLS factors. This model has an SEP of 0.11% (w/w). The concentration corre-

Table 2  
Results from calibration models built by PLS regression with different data pretreatment methods in different spectral regions

Spectral range (nm)	Data pretreatment	PLS factors	SEP %(w/w)
1350–1500	None	8	0.21
1350–1500	MSC	6	0.27
1350–1500	Mean center	7	0.23
1350–1500	First derivative	7	0.25
1350–1500	Second derivative	6	0.21
1850–1936	None	5	0.16
1850–1936	MSC	7	0.20
1850–1936	Mean center	5	0.15
1850–1936	First derivative	5	0.11
1850–1936	Second derivative	5	0.22
1850–1990	None	8	0.13
1850–1990	MSC	6	0.23
1850–1990	Mean center	6	0.13
1850–1990	First derivative	6	0.13
1850–1990	Second derivative	6	0.15

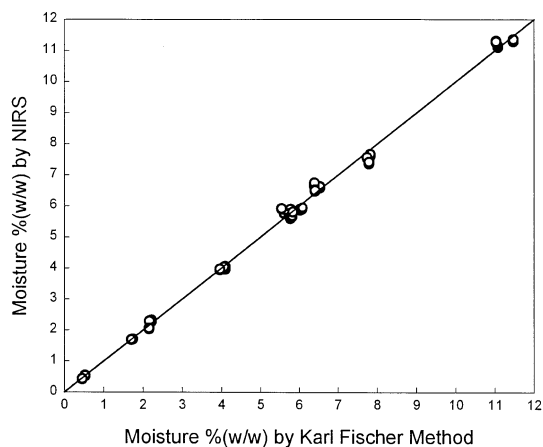


Fig. 3. Concentration correlation plot for the calibration set. The calibration model is built with the first derivative spectra and five PLS factors in the spectral range 1850–1936 nm. The solid line is a unity line.

lation plot of the calibration data set for this best calibration model is presented in Fig. 3. Regression analysis ( $R^2 = 0.999$ ) indicates a slope of 1.0132 as well as a  $y$ -intercept of  $-0.1026\%$  (w/w). For this type of correlation plot, a slope of 1.0 and  $y$ -intercept of 0.0 is ideal. The corresponding concentration correlation plot of the prediction data set is illustrated in Fig. 4, where the slope and the  $y$ -intercept of regression analy-

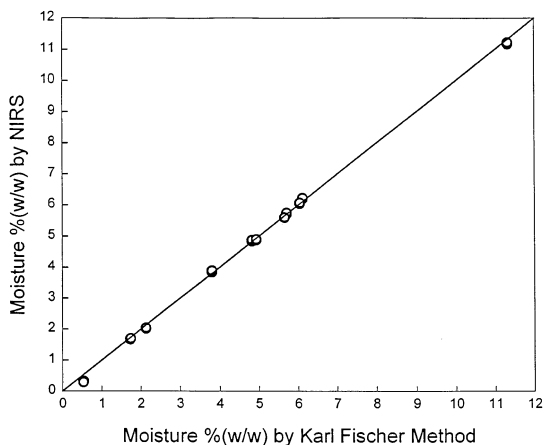


Fig. 4. Concentration correlation plot for the prediction set. The calibration model is built with the first derivative spectra and five PLS factors in the spectral range 1850–1936 nm. The solid line is a unity line.

sis ( $R^2 = 0.997$ ) are 0.9978 and 0.0107% (w/w), respectively.

#### 4. Conclusion

Moisture levels in drug substances can be measured quite accurately by NIR spectroscopy, even for a difficult to handle hygroscopic material, with a standard error of prediction of 0.11% (w/w) in the range 0.5–11.4% (w/w). This result is only possible because of the accuracy and precision of our reference method, which provides a standard error of deviation of 0.09% (w/w) for three replicates at moisture levels of 0.5–11.4%. The handling and transfer of samples in sealed bottles further ensures the accuracy of the reference measurement, by eliminating interference from ambient moisture. The other key element of this accurate and precise method is the optimization of the PLS regression. Valid calibration models can be constructed by PLS regression in the spectral regions of the first overtones and first combination bands of water. Spectral pretreatment by derivative techniques improves the quality of the spectra in some cases. In this work, the first derivative calculation in the spectral range 1850–1936 nm provides the best calibration model. The results of this study explore the limits of accuracy and precision for water determination by NIRS.

#### Acknowledgements

The authors would like to thank Dr Timothy Wozniak, Dr Lian Yu and Mr Michael Skibic for their helpful discussion.

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