Determination of moisture in hard gelatin capsules using near-infrared spectroscopy: applications to at-line process control of pharmaceutics

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Abstract

A method is proposed in which diffuse reflectance near-infrared spectroscopy (NIR) is applied in an at-line process analytical interface to determine moisture content in bulk hard gelatin capsules. Capsule samples were equilibrated at various relative humidities and the moisture contents were determined using loss on drying (LOD). Different multivariate calibration methods using multiple linear regression (MLR) and partial least squares regression (PLSR) and various spectral pretreatments were compared. No sample pretreatment was required and the analysis time was 1–2 min. The investigated range for the moisture determination was 5.6–18.0% w/w and the root mean square error of prediction (RMSEP) value was 0.1% w/w © 1997 Elsevier Science B.V.

1. Introduction

This paper presents a rapid at-line process analytical method for determination of moisture in intact bulk hard gelatin capsules using near-infrared spectroscopy (NIRS).

Gelatin capsules are a common dosage form. The physical characteristics of gelatin capsules are strongly affected by moisture. After manufacture, the moisture content is of the order of 15% and it is desirable to maintain this level if the capsule shells are to behave satisfactorily in high speed capsule filling machines. Below about 10% moisture the gelatin shells become brittle and can fracture, releasing the contents. Above about 18% moisture the shells soften and become sticky. The stability and microbiological activity of gelatin capsule products are also known to depend on moisture [1]. The moisture content of each batch must be checked prior to capsule filling and packaging. Thus, there is a need for a rapid process analytical method for moisture determination.

The most important methods for the determination of moisture content in the pharmaceutical

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industry are the loss on drying (LOD) method and the Karl Fischer titrimetric method. Measurement of water activity and NIR spectrometry are other methods for determination of moisture. LOD is a thermogravimetric method commonly used to determine moisture. The method is not selective to water but determines the total of all compounds volatile under the conditions used. The reference method used for gelatin capsules at Astra Production Tablets AB is loss on drying at 105°C for 16 h. This method requires manual sample preparation and weighing. Filled gelatin capsules must be opened and emptied manually before analysis.

Near infrared reflectance spectroscopy is easily adaptable to in situ analysis of samples containing moisture, and a large number of NIR papers and commercial applications deal with moisture determination [2].

Pharmaceutical applications of process NIRS offers rapid, noninvasive analysis but are not as frequently reported as applications in the food and polymer industries [3] for example. NIR determination of moisture still has the potential to become an important tool in pharmaceutical production and quality control. Applications of NIR reported from the pharmaceutical industry includes for example identification of raw materials and final products, determination of moisture and active, blending validation and quality control [4–6]. The near infrared absorption spectra of dried gelatin and gelatin saturated with water vapor were compared, without quantitative applications, in a work by Ellis and Bath (1938) [7]. Recently, the convenience of near-infrared determination of water uptake in individual hard gelatin capsules has been demonstrated [8].

2. Experimental

2.1. Materials

Hard gelatin capsules manufactured by Capsugel (a Division of Warner-Lambert, Greenwood, SC) were obtained from Astra Production Tablets AB.

2.2. Instrumentation

Spectral data where collected by a NIRSystems 6500 (NIRSystems, Silver Spring, MD) spectrophotometer equipped with a Sample Transport Module NR-6511 and an elongated coarse sample cell. This sample transport module was designed for coarse irregular samples such as gelatin capsules and provides sample averaging over the 60 square centimetres sampling area. The sample cell is moved up and down through the sample beam while scans are co-added to provide a representative spectrum. Diffuse reflectance data where obtained from capsules every 2 nm from 1100 to 2500 nm. Data analysis was performed on a personal computer using MATLAB for Windows version 4.2c.1 (The MathWorks, Natick, MA).

2.3. Procedure

A calibration set of gelatin capsules with different moisture content was prepared, according to Table 1, by conditioning of samples at various constant relative humidities using salt solutions in glass desiccators. Seven saturated salt solutions where prepared using Merck pro analysi quality salts, according to Table 1. Small portions of the salt solutions, with excess salt crystals, were added to seven glass desiccators to obtain the relative humidities listed in Table 1 [9,10]. In each desiccator 140 empty capsule shells were equilibrated at room temperature for 20 days. Another 140 capsules were dried over desiccant for 5 days. Before equilibration, the moisture content of the capsules were 14.4%. In order to obtain an approximate equilibrium condition in only 20 days, capsules equilibrated over lithium chloride and potassium acetate were initially dried over desiccant for 48 h to a moisture content of 8%. Capsules equilibrated over magnesium chloride and potassium carbonate were initially dried over desiccant for 24 h to a moisture content of 10%.

After equilibration each set of 140 capsules were scanned in the NIR spectrophotometer, and then 40 capsules were placed in two preweighed glass containers. Each spectrum was obtained as an average of 32 spectral scans. Two spectra were collected from each sample with the capsules re-

Table 1
Salt solutions for conditioning of capsule samples and corresponding LOD moisture contents used for calibration

Salt solution	Relative humidity at 20°C (%)	Moisture content of capsules (%)
Lithium chloride	11.3	8.02
Potassium acetate	22.5	10.34
Magnesium chloride	33.1	12.31
Potassium carbonate	44	13.69
Magnesium nitrate	54.5	14.67
Sodium nitrite	66	15.76
Sodium chloride	75.5	18.05
Capsules before conditioning		14.44
Capsules dried over desiccant for 5 days		5.56

moved and refilled into the sample cell in order to check the repeatability. The capsules were then analysed using the LOD reference method, drying at 105°C for 16 h. The wet and dry weights were obtained on an electronic balance with a resolution of 0.1 mg. The LOD moisture is expressed as: calculated mass of moisture per mass of wet capsules.

Calibration models were obtained using inverse multiple linear regression (MLR) and partial least squares regression (PLSR) of the NIR spectra on the LOD data [11,12]. The calibration data set consisted of 18 NIR spectra (objects) and 700 wavelengths (variables).

3. Results

In general, water causes prominent absorption peaks at about 1440 and 1930 nm [7] which is possible to use for measurement. The diffuse reflectance spectra are influenced, for example, by additive and multiplicative shifts, as a result of scattering effects and specular reflectance etc. (see the spectra in Fig. 1). Consequently, a multivariate data analysis approach is often required using information from several wavelengths.

An MLR approach was initially tested. Collinear data are not suitable for MLR calibrations. By an iterative procedure the best combinations of two and three wavelengths were selected. Wavelengths were selected with the criterion of being at least 10 nm apart and to minimize the

root mean square error of prediction (RMSEP) in a full cross validation of the capsule spectra [11]. By using MLR satisfactory results were obtained (Table 2).

RMSEP was calculated as:

$$\text{RMSEP} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2} \tag{1}$$

where n = the number of samples (18); y_i = the LOD reference value of moisture content; $\hat{y}_i$ = the predicted value of moisture content for sample i.

Since collinearity is not a problem in PLSR all 700 wavelengths were used in the PLSR calibration models. Another advantage of using all wavelengths is the ability to perform data pretreatment and transformation. In this study, pretreatment using multiplicative signal correction (MSC) [11] and the Savitzky-Golay convolution
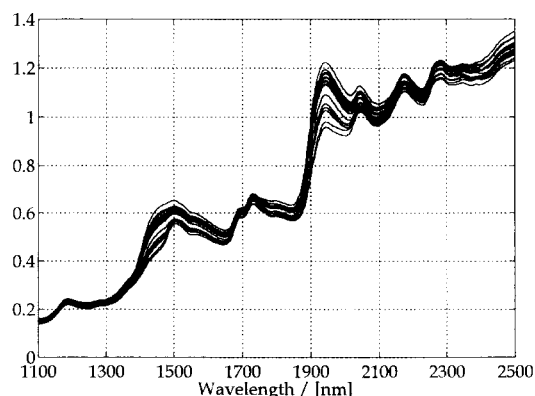

Fig. 1. NIR raw spectra of gelatin capsules.

Table 2
Summary of results from various MLR and PLSR calibration models

Calibration model	RMSEP (%)	Correlation coefficient[a]	Slope/intercept[a]
MLR using 2 wavelengths $y = 43.7242 - 472.26*\lambda_{1396} + 449.31*\lambda_{1406}$	0.2050	0.9985	0.9993/0.0061
MLR using 3 wavelengths $y = 9.730 + 235.8*\lambda_{1592} - 1521.6*\lambda_{1692} + 1296.1*\lambda_{1708}$	0.0907	0.9997	0.9996/0.0017
PLSR model without pretreatment, 3 factors	0.4068	0.9940	0.9951/0.0490
Optimum of 5 factors	0.1986	0.9986	1.0005/0.0063
PLSR model using MSC[b], 3 factors	0.2850	0.9971	0.9972/0.0392
Optimum of 6 factors	0.1389	0.9993	0.9990/0.0382
PLSR model using 2nd derivative[c], 3 factors	0.1875	0.9987	0.9970/0.0364
Optimum of 7 factors	0.1203	0.9995	0.9945/0.0703

[a] Linear regression analysis of LOD moisture content vs. predicted data from the cross validation.
[b] MSC base wavelengths: 1100–1298, 1600–1798, 2200–2398 nm.
[c] Savitzky-Golay convolution derivation using 4th order polynomials and 13 data points.

2nd derivation [13,14] were tested (Table 2). The RMSEP was evaluated as a function of the number of latent variables, factors, kept in the PLSR model. The optimum number of factors was that yielding the smallest value of RMSEP in the cross validation. No outliers were removed in the PLSR calibrations.

The repeatability of the LOD reference method measured as the standard deviation of the mean of two replicate determinations is 0.05% w/w.

4. Discussion

The best calibration model was obtained using MLR based on three of the NIR wavelengths. This implies that a relatively inexpensive filter photometer system could be used with good results. However, the three selected wavelengths are not among the most water-absorbing wavelengths. Fig. 2 shows the apparent difference spectrum of gelatin-bound water, that is, a spectrum of dry gelatin (6% w/w) subtracted from a spectrum of wet gelatin (18% w/w).

When comparing MLR with PLSR the latter offers the useful advantages of being more robust and able to detect outliers. When comparing dif-

ferent spectral pretreatments it can be concluded that both the MSC and the 2nd order derivative pretreatment significantly improves the PLSR calibration model. In the MSC method each spectrum is 'corrected' in offset and in slope by comparison with the mean spectrum of the total data set [11]. The MSC method adjusts for light scattering effects common in diffuse reflectance data. The Savitzky-Golay convolution derivation decreases the spectral noise since the transformed points have been fitted to a polynomial curve.
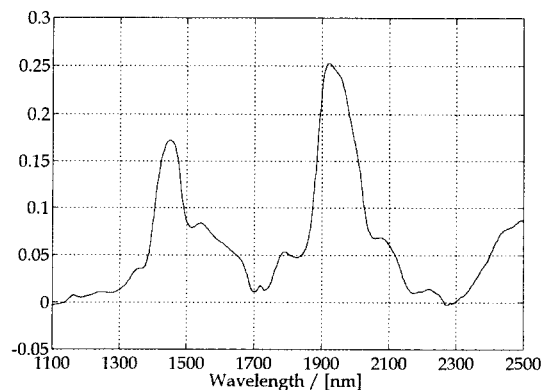

Fig. 2. Gelatin-bound water NIR difference spectrum: a spectrum of dry gelatin (6% w/w) subtracted from a spectrum of wet gelatin (18% w/w).

Table 3
Raw data comparing the moisture contents by LOD and NIR as means and S.D. in % w/w for the humidities described in Table 1, and the overall means

LOD		NIR, MLR using 3λ		NIR, PLSR using 2nd derivative and 7 factors	
Mean	S.D.	Mean	S.D.	Mean	S.D.
5.56	0.002	5.55	0.08	5.45	0.15
8.02	0.12	8.05	0.06	8.14	0.10
10.34	0.07	10.34	0.02	10.48	0.16
12.31	0.03	12.30	0.11	12.31	0.14
13.69	0.05	13.68	0.04	13.73	0.14
14.44	0.05	14.37	0.02	14.32	0.07
14.67	0.02	14.71	0.03	14.74	0.01
15.76	0.11	15.79	0.07	15.67	0.09
18.05	0.16	18.04	0.16	18.00	0.04
12.538	0.067	12.538	0.064	12.539	0.100

Derivative pretreatment is known to be capable of handling nonlinearities and multiplicative light scatter variations [11].

The PLSR loading vectors are strongly weighted towards the water absorbing NIR wavelengths indicating that the PLSR calibration models mainly use the information at these wavelengths. The first loading vector is almost identical to the difference spectrum in Fig. 2 and the following loading vectors all have prominent peaks in the water absorbing NIR regions.

The cross validation of the PLSR calibration indicates that 3 factors give good predictions and that 5–7 factors give the best predictions. A larger data set is required to fully evaluate the prediction ability of the PLSR and the MLR calibrations. The moisture contents by LOD and NIR are compared in Table 3.

It has recently been reported from measurements of diffuse reflectance on silica gel layers that there was an effect of moisture content at all NIR wavelengths, even where water absorbed minimally [15,16]. The adsorption of water onto the silica gel plate apparently increased the scattering of incident radiation across a wide range of wavelengths. The change in scattering was registered as a wavelength independent baseline offset in the NIR spectra [16]. In the work reported here, no such wavelength independent baseline offset was noticeable. There were no correlations between the moisture content and the MSC offset and slope variables.

The wider implication of these results is that an on-line method for determination of moisture in hard gelatin capsules can be envisioned. This could be applied to the quality control of pharmaceutics and to optimization of pharmaceutical production processes. The present LOD method requires manual sample preparation and weighing in a production laboratory. Filled capsules must be opened and emptied manually before analysis. If the LOD method could be replaced by a NIR method, a considerable decrease in analysis time could be achieved. Further, the simple handling would make it easier to introduce process operators to the method.

The work reported here has been performed on empty gelatin capsule shells but the NIR method can also be applied to filled capsules. However, a separate calibration is required for each type of capsule filling.

The main advantage of this NIR method is that the analysis time can be reduced from 16 h to 1–2 min with a corresponding improvement in production speed. Furthermore, no manual sample pretreatment is needed and the analysis can be performed by production operators. The at-line configuration is appropriate for an introduction of NIR spectroscopy to the production unit and for gradually building up knowledge and experi-

ence. The final goal should be to develop an in-line process control of moisture, e.g. in capsule drying and capsule filling processes.

5. Conclusions

The developed at-line method has been shown to work well for a set of empty hard gelatin capsules. It could also have a large potential for replacing the traditional LOD method in the quality control of pharmaceutics and pharmaceutical processes involving gelatin capsules. Both identity and functionality could be recorded. The investigated range for the moisture determination was 5.6–18.0% w/w. The RMSEP value was 0.1% w/w, which is comparable to the S.D. of the reference method.

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