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Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 867–873

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Sensitive NIRS measurement of increased moisture in stored hygroscopic freeze dried product

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Received 11 October 2001; accepted 31 October 2001

Abstract

The purpose of this study was to build a best possible NIR prediction model for monitoring of water content in a freeze-dried drug product. The best pre-treatments of the NIR spectra were found to be: transforming from reflection to absorption, baseline correction in the 1845–2165 nm area and a maximum normalisation in the same area. These pre-treatments resulted in a model with the following attributes: SEP of 0.08% (w/w) and one PLS factor, the latter indicating a robust model. The limit of quantification was calculated to 0.24% (w/w). During the stability study an increase in water content in the freeze-dried drug product was revealed, which were found to depend on storage time and temperature. It is believed that the water is derived from the stoppers. The highest increase was found for storage at 40 °C, and was estimated to be 0.04% points a month by weight, from an initial value of about 0.25% (w/w).

Keywords: NIRS; Moisture; Pre-treatment; PLS; Freeze-dried drug product; Hygroscopic; Stability; Stoppers

1. Introduction

The quantitative measurement of small amounts of water content in solids is typically made by the Karl Fischer (KF) method. However, the ambient moisture can influence the KF quantification, and strict precautions have to be taken when handling hygroscopic materials. The KF titration is time consuming and uses toxic reagents. Near infrared reflectance spectroscopy (NIRS) [1,2] is attractive for moisture determination because it is rapid, non-destructive, no sample pre-treatment is needed and water has strong absorption bands in this spectral region that provide the sensitivity needed for accurate determination. The near-infrared spectral signal in the region around 1920 nm is shown appropriate for accurate, precise, robust and sensitive quantification of water content [3]. The method uses the strong O–H vibration overtone generated in this region, but also the vibrations bands around 1450 nm can be used for the water measurements. The

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00668-9

method is less sensitive to the air humidity since the vials remain closed during the analysis. These properties make the method able to perform inspection of all freeze-dried vials, i.e. 100% inspection.

In this study a positive correlation between water content and storage temperature was found probably due to water originating from the stopper. To measure precisely the slight increase in the hygroscopic material with KF was challenging. The results obtained here suggest an improved precision and limit of quantification of NIRS when compared to previous reported results, showing the suitability of NIRS also for small water changes.

The publication is basically divided in two parts. Part one includes a description of the practical work of the validation of the method. Part two consists of the application of the method and learning from the results.

2. Experimental

2.1. Apparatus

All samples were analysed in the laboratory using the Perkin–Elmer IdentiCheck FT-NIRS-system. Titrations were done in a Mettler DL35 KF titrator. The calculations were carried out with UNSCRAMBLER version 6.11 (Camo, Norway).

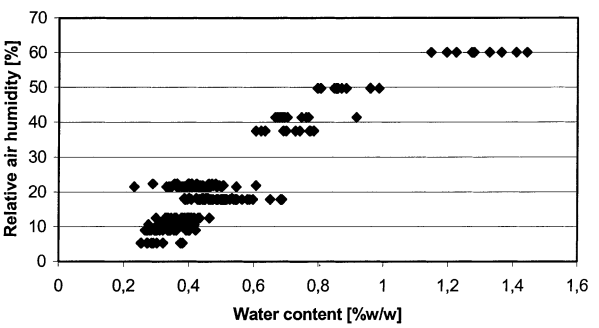

Fig. 1. Karl Fischer measurements at different humidity.

2.2. Samples and methods

Unopened vials containing freeze-dried drug were placed on the FT-NIRS IdentiCheck Reflectance Accessory and spectra were acquired. Two or three spectra of each sample were collected in the 3.500–10.000 cm^{-1} region, by rotating each sample vial in different positions.

Afterwards the product was analysed for water content by KF titration. The drug exposure to air was timed and registered, and the air humidity was measured with an electronic hygrometer. A new weighing boat was used for each new sample. Some vials were exposed to humid air in effort to acquire higher water content, by loosening the rubber stopper on the vials. After a fixed time the stoppers were fastened. Some hours later spectra were recorded and the moisture content analysed by KF. Vials that were expected to contain low levels of water (below 1%) were stabilised for at least 4 h, before the spectra were recorded and analysed.

The samples used in the calibration model were exposed to air at 22% relative humidity (RH) for 1, 4, 8, 16 and 24 h. The samples in the calibration model used for the lowest level of water content were analysed immediately after opening the vials and at 5.3 and 9.0% RH to minimise errors. (These low values for RH are possible in the lab when the weather conditions outdoor are optimal; below -10 °C and dry air. When the outdoor temperature is 20 °C, a relative air humidity of 50% and above is the normal.)

Karl Fischer measured a total of 80 samples after the scanning by NIRS two or three times each, summing up to 180 scans.

3. Results and discussion

3.1. Building the model-calibration and validation

Samples were analysed at various RH (5.3–60.1%). The analysis time, i.e. exposure to air, varied from 55 to 105 s influencing the results slightly. A clear relationship was found between the room humidity and measured water content by KF, see Fig. 1. Based on these results the need for a more robust method was desirable.

3.1.1. Pre-treatments

A study was performed to establish a NIRS method for determining the moisture level in a hygroscopic freeze-dried drug substance. A calibration model was developed from the sample set described in Section 2. The calibration set contains both release samples analysed under optimal conditions and samples exposed to air humidity.

A sensible pre-treatment of the spectra is crucial to obtain good results. Appropriate pre-processing reduces noise and emphasises the important attributes. It also reduces the need of many principal components in the model and hence the chances of incorporating noise in the model. Often derivatives, and especially second derivatives are used when pre-treating NIRS spectra [1,2]. It is not always ideal that all the time every derivative increases the noise/signal ratio in the spectra. Derivatives should therefore be avoided if possible. The most appropriate pre-treatment of NIRS spectra were here found to be:

- Transforming the spectra from reflection to absorption.
- A baseline correction in the 1845–2165 nm area.
- A maximum normalisation in the same area.

These pre-treatments will emphasise the relevant attributes in the spectra. Fig. 2 shows all the steps in the pre-treatments of the spectra.

3.1.2. Validation of the model

To validate the model, the sample set was divided in a calibration set and a test set.

The calibration set included samples exposed to humid air for 1(f), 4(e), 8(d) and 24(a) h and samples measured at an atmospheric humidity of 5.3% (i). The test set included samples exposed to humid air for 16(c) h and samples measured at 9.0% air humidity.

Fig. 3 shows the calibration model and Fig. 4 shows the predictions from the test set, and indicate that the model performs well.

In the final model, intended for use, all the samples from the calibration set and the validation set (test set) were used to build a new calibration model.

After the right pre-treatments are done, the final model for use has the following important attributes: an SEP of 0.08% (w/w) and one PLS factor. More than one PLS factor did not improve the model worth mentioning. The one factor that covers the principal property that is interesting is the variation in water content.

One could most certainly have used one wavelength instead of one PLS factor in this application, but the use of a PLS factor instead of a wavelength will stabilise the model better.

Moreover, the use of a PLS factor will 'classify' the spectrum. If one uses one wavelength-model from a different sample, one may predict a plausible answer, a value that seems right. But with the use of a PLS factor, the prediction error will unveil the sample in this example to be large.

A complete GMP validation was also performed to ensure the quality of the method.

The numbers derived in this validation illustrates the expected performance of the model.

Accuracy was decided for release samples and a sample where moisture was added (Table 1).

Accuracy as recovery (%) = [measured amount (NIRS)/reference amount (KF)] × 100%.

The reason for difference in accuracy for the two release samples is the difference in relative air humidity when measured with the reference method (60.1 vs. 5.3%).

The *repeatability* was tested at the 100% level, which is the expected level for future freeze-dried samples. Six spectra were collected from a vial by randomly rotating the vial in six different positions. The average value was 0.26% (w/w) (0.259, 0.258, 0.264, 0.264, 0.263 and 0.254) with SD = 0.004 and RSD = 1.5%. The 95% confidence interval is here (0.252–0.268).

The *intermediate precision* was tested on ten vials. Two spectra were acquired from each vial a day over a 3 day period. Table 2 shows predicted NIRS values.

The average values from the 3 days are: day 1, 0.26% (w/w); day 2, 0.28% (w/w); and day 3, 0.27% (w/w). The grand average is 0.272% (w/w), with SD = 0.024 and RSD = 8.8%.

The 95% confidence interval is (0.224–0.320).

The *specificity* is ensured by the strong water absorptions around 1920 nm.

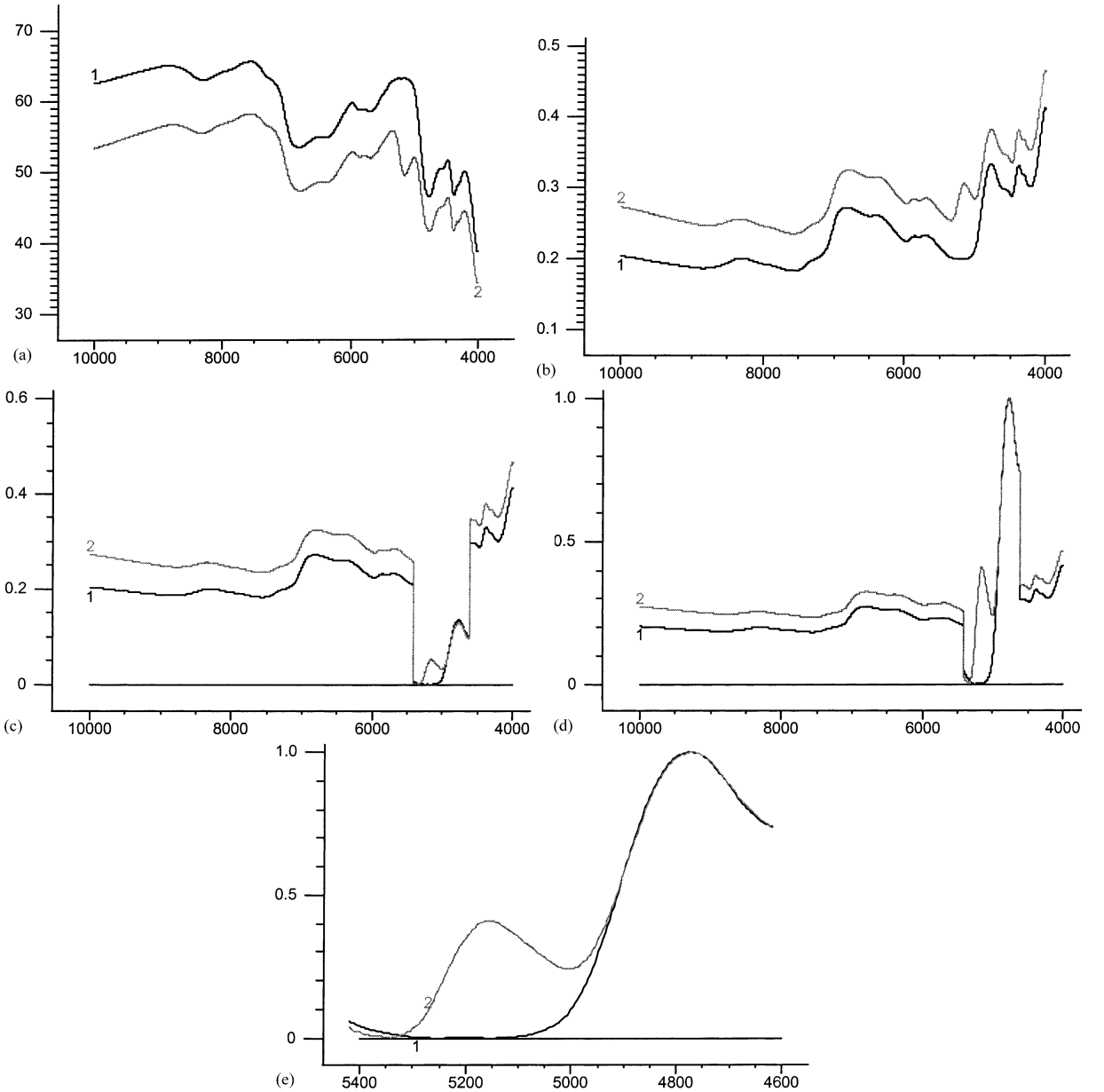

Fig. 2. Pre-treatments of the NIR spectra (a) Start reflection spectra, (b) Step 1, to absorption spectra, (c) Step 2, baseline correction, (d) Step 3, maximum normalization, (e) Used for modelling, maximum normalization zoomed.

The detection limit (DL) and the quantitation limit (QL) can be expressed as: $DL = (3.3S_B)/b$ and $QL = (10S_B)/b$ [4], where S_B is the standard deviation for the intermediate precision (Table 2) and b (value, 0.9939) is the slope of the final calibration model.

Selecting the worst case for S_B in Table 2 gives the following values for DL and QL: DL = 0.08% (w/w) and QL = 0.24% (w/w).

All the later results in this publication are given on the basis of prediction from NIR spectra.

A 3 year old batch was analysed. The individual vials were first analysed by NIR and then by KF. Three different storage conditions were tested to confirm the model. The results are shown in Table 3 and confirm the model and the anticipations done. The reason why the release values measured by NIRS in Table 4 are lower than the values measured by KF under optimal conditions [Figs. 1–3 (used in the calibration model)], is that these samples are not release samples, but shelf life samples. The samples are therefore expected to be slightly higher in water content than the release values indicated by NIRS measurements.

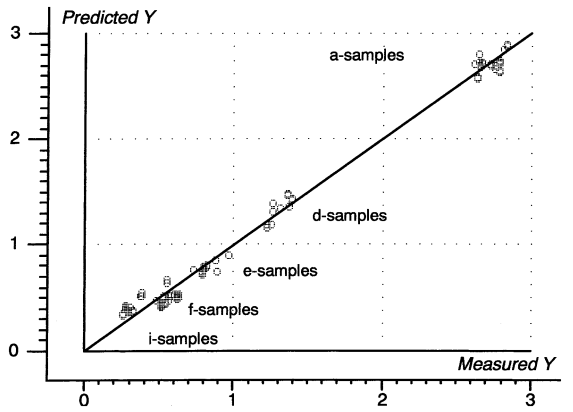

Fig. 3. Calibration samples for prediction model.

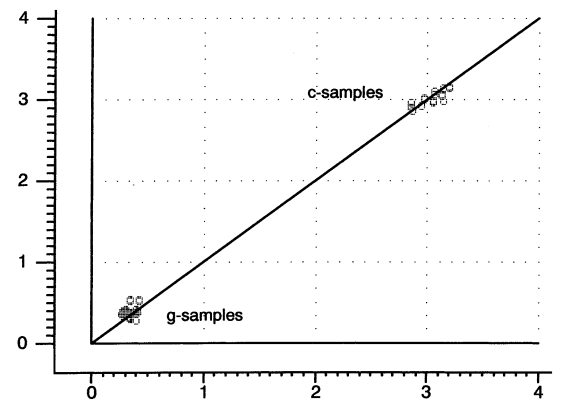

Fig. 4. Prediction of test set samples versus KF measurements.

Table 1
Accuracy of the NIRS method [1]

NRS [%w/w]	KF [%w/w]	Accuracy (%)
0.25	1.44	17
0.37	0.33	112
3.01	3.05	99

3.1.3. Application of the model

The validated model was used for the determination of water content in the freeze-dried product during stability studies.

The freeze-dried product are stored under four different conditions: 4 °C and ambient humidity, 25 °C and 60% RH, 30 °C and 60% RH, and 40 °C and 75% RH.

The samples are produced in batches that are analysed at release. The batches are produced in the period between January 1999 and March 2001.

From each batch ten vials are randomly selected and analysed by NIRS. Table 3 below shows the average of ten randomly selected vials from seven different batches with SD and RSD values.

The results from Table 4, when compared with the results in Table 1 indicate that NIRS is a much more robust technique than KF. The NIRS technique is clearly less dependent on weather conditions than the KF technique. Results also show that the freeze-drier process is controlled excellently.

Two of the batches were analysed over a 2 year period, and scanned every third month for the four different conditions. Ten vials were analysed each time. Where no numbers are added in the table it means analyses were not performed, e.g. after 3 months.

As seen from Table 5 there is clear relationship between moisture content and temperature and moisture content and storage time. Moisture content in the freeze-dried product increases with storage time and storage temperature. Fig. 5 shows how the water content in the matrix increases over 2 years at 40 °C and 75% RH for batch 906025 and batch 907027.

It is clear that there is an increase in moisture content in the vials, but is there an external or internal source to the moisture?

Other chemical analyses performed on the product and mass balances of possible degradation-paths show that the water increase is unlikely to be caused by the degradation of other compo-

Table 2
Intermediate precision, water content [%(w/w)] [1]

Day	Vial									
	1	2	3	4	5	6	7	8	9	10
Day 1	0.255	0.303	0.237	0.287	0.261	0.262	0.265	0.230	0.268	0.251
Day 1	0.225	0.291	0.251	0.289	0.267	0.300	0.255	0.233	0.259	0.244
Day 2	0.267	0.316	0.261	0.300	0.273	0.334	0.280	0.243	0.292	0.259
Day 2	0.259	0.298	0.268	0.293	0.273	0.302	0.284	0.248	0.286	0.258
Day 3	0.245	0.303	0.255	0.276	0.256	0.310	0.269	0.246	0.282	0.265
Day 3	0.259	0.314	0.254	0.299	0.281	0.312	0.266	0.246	0.278	0.252
Average	0.252	0.304	0.254	0.291	2.269	0.303	0.270	0.241	0.278	0.255
SD	0.015	0.009	0.010	0.009	0.009	0.024	0.011	0.008	0.012	0.007
RSD	6.0	3.0	3.9	3.1	3.4	7.9	4.1	3.3	4.3	2.7

nents, because the increase is too great. Moreover, the only conceivable degradation of component B to component C, is a step that consumes water. A degradation of component A, which is the major component in the matrix production of water, is inconceivable.

Therefore, it is safe to conclude that the water in question has an 'external' source. The only possible sources can then be leaky vials or moisture derived from the stoppers.

The storage temperatures of 25 and 30 °C has the same humidity, but the level of moisture is in general higher in the 30 °C samples. It is therefore reasonable to believe that the water is released from the stoppers. Also the extremely hygroscopic nature of the matrix indicates that leakage is not a possibility.

A well-known problem in freeze-drying process is drying of the stoppers. Rubber stoppers extract water when they are washed [4] and have to be dried before use. Experience show that to obtain stoppers with no water is very difficult.

The amount of water in the stoppers is decided by the drying cycle and the rubber composition of the stoppers, and hence also the quality of the stoppers.

Freeze-drying is done to preserve the matrix, so that the desired properties of the drug product are maintained over time. The level of water in the matrix is of course crucial. Too much water will make the freeze-dried cake collapse. It is difficult to predict the exact level of this undesirable phenomenon.

With the non-invasive NIRS measurements one can obtain precise empirical data for the level of water which causes collapse in a hygroscopic matrix.

It is reasonable to believe that the factors that can change the composition in a chemical matrix are time, light, oxygen and water. Even though the increase in water content is not caused by the degradation of other components, water may cause degradation of other components. Therefore, the content of water can indicate the level of quality of the product. Water content under a given level may ensure that other parameters are satisfactory.

NIRS can therefore be an excellent tool to monitor quality.

Table 3
Comparison of NIR and KF results [1]

Sample	4 °C	25 °C	30 °C
KF/NIR 1	0.459/0.311	0.583/0.472	0.716/722
KF/NIR 1	0.503/0.245	0.574/0.528	0.806/0.651
KF/NIR 1	0.500/0.331	0.549/0.447	0.860/0.689
KF/NIR 1	0.525/0.340	0.563/0.469	0.780/0.573
KF/NIR 1	0.511/0.319	0.602/0.508	0.786/0.651
KF/NIR 1	0.545/0.317	0.622/0.457	0.798/0.691
Average KF	0.51	0.58	0.79
Average NIR	0.31	0.48	0.66
Δ(KF-NIR)	0.20	0.10	0.13

Table 4
Water content %(w/w) measured by NIRS [1]

Batch	901002	902009	906025	907027	908029	10129724	10137183
Average	0.25	0.24	0.24	0.27	0.25	0.25	0.24
SD	0.01	0.02	0.01	0.03	0.04	0.01	0.02
RSD	4.9	7.1	4.1	11.7	15.8	3.5	7.7

Table 5
Water content in freeze-dried product [% (w/w)] [1]

Batch number		906025				907027			
Stability temperature		4 °C	25 °C	30 °C	40 °C	4 °C	25 °C	30 °C	40 °C
Time	Release	–	0.24	–	–	–	0.27	–	–
	3 months	–	–	–	–	–	–	–	–
	6 months	–	–	–	0.55	–	–	–	0.44
	9 months	–	0.41	0.45	0.66	–	0.31	0.38	0.52
	12 months	0.38	0.42	0.50	0.76	0.32	0.37	0.44	0.62
	15 months	0.39	0.50	0.58	0.98	0.38	0.40	0.48	0.78
	18 months	0.36	0.44	0.56	0.96	0.31	0.37	0.49	0.87
	21 months	0.46	0.46	0.64	1.10	0.41	0.41	0.49	1.01

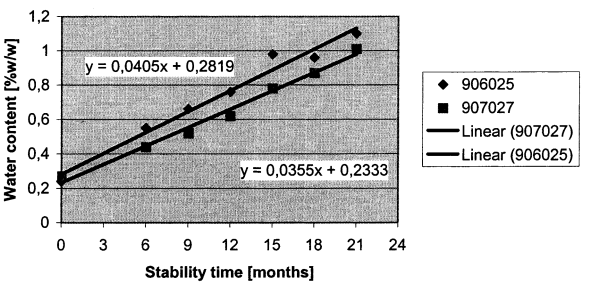

Fig. 5. Moisture development for 40 degrees and 75% RH samples.

4. Conclusions

This study has stressed the importance of proper data pre-processing and the awareness of how air humidity influences hygrosopic freeze-dried samples. This knowledge was used to build an NIR prediction model with an SEP of 0.08%, one PLS factor and a limit of quantification of 0.24% (w/w).

The prediction model was used to monitor the freeze-dried drug product during a stability study, and revealed that the rate of water which is released from stoppers, and hence the amount of water in the hygroscopic drug product, is dependent on the storage temperature and time.

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