In-Situ Near-Infrared Spectroscopy Monitoring of the Lyophilization Process

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Purpose. The purpose of this work was to demonstrate the feasibility of using near-infrared spectroscopy (NIRS) to monitor the freeze-drying process *in-situ*.

Methods. The experiment was performed in a pilot-scale freeze-dryer, in which the NIRS probe was interfaced using a lead-through to the lyophilizer. Special equipment for the sample presentation was developed. NIRS measurements were made using a FT (Fourier transform)-NIR spectrometer fitted with a single fiber reflectance probe.

Results. The physical changes, that is, freezing, sublimation, and desorption, generated significant spectral changes. There was good agreement between NIRS monitoring and product temperature monitoring about the freezing process and the transition from frozen solution to ice-free material. The NIRS monitoring also provided new information about the process that was not possible to detect with product temperature monitoring, such as the rate of the desorption process and the steady-state where the drying was complete. The NIRS monitoring yields significantly more information about the actual process and essentially explains the observed changes of the product temperature during the lyophilization process.

Conclusions. NIRS monitoring is a viable tool for *in-situ* monitoring, both qualitatively and quantitatively. It can facilitate investigations of the drying process within a sample. The small volume monitored makes sample presentation very important.

KEY WORDS: lyophilization; freeze-drying; process; NIR; monitoring; *in-situ*.

INTRODUCTION

Freeze-drying, or lyophilization, has developed into a widely used method for the stabilization of otherwise easily degraded substances. Its use in the preservation of microorganisms, food items, biologic products, and pharmaceuticals is well documented in the literature (1–4). It is also a fact that the regulatory authorities accept lyophilization as a suitable unit operation in the manufacture of therapeutic products (5). Although the most common application of pharmaceutical lyophilization is in the production of parenterals, the process is also used in the production of diagnostics and, occasionally, for oral or ophthalmic solid dosage forms where a very fast dissolution rate is desired (6,7).

A conventional lyophilization apparatus comprises a vacuum chamber in which the material to be lyophilized is placed. During the lyophilization process, the temperature of the material is monitored by thermocouples. These are arranged in contact with the material, which is distributed in samples within the vacuum chamber. It is also common to monitor the moisture content in the vacuum chamber during the freeze-drying process by means of a comparative pressure measurement (8), a pressure rise measurement (9), an electronic hygrometer (10,11), or residual gas analysis (12). These techniques are indirect and as such are capable of identifying the global end point of the lyophilization process, but the moisture content of the material itself cannot be readily assessed during the process. Furthermore, the relationship between measurement response and actual moisture content of the material has to be established empirically for each type of material and lyophilization apparatus, which is a laborious task. Other analytical techniques, such as low temperature X-ray powder diffractometry (13), low-resolution pulse nuclear magnetic resonance (14), IR spectroscopy (15), and visual microscopic observation (16) have been used for *in-situ* characterization of samples being lyophilized in a special lyophilization stage in direct contact with the instrument but it will be very difficult to use these techniques in a conventional lyophilizer.

In recent years near-infrared spectroscopy (NIRS) has gained increasing attention for the monitoring and controlling of pharmaceutical manufacturing processes, such as blending (17,18), granulation (19,20), and film coating (21–25), both at-line and in-line/on-line. NIRS has been used for several years off-line for noninvasive, nondestructive, and rapid residual moisture determinations of the final lyophilized product (26,27), but to the best of our knowledge it has not been used for *in-situ* monitoring of the lyophilization process. The application of *in-situ* NIRS for process monitoring of the lyophilization process provides completely new possibilities to study both the product and the process in real time in a conventional lyophilizer. Existing monitoring techniques as mentioned above, deal with indirect measurement of the dryness of the product, with the exception for product temperature monitoring. The advantage of using NIRS as an *in-situ* monitoring method is that it makes it possible to directly study the product characteristics that are important for the final quality of the lyophilized product. NIRS is a technique ideally suited for analysis of the lyophilization process because it displays spectroscopic features that can be assigned to the variables that are most important for lyophilization, such as moisture and structure characteristics. The measurement can be conducted in the lyophilizer wherever necessary via the use of fiberoptic probes. This means that individual product packages can be studied. The positioning of the probes is flexible because relatively small single-fiber probes can be used, and still obtain a satisfactory spectroscopic signal. A multichannel NIR instrument equipped with several probes can be used to monitor different packages simultaneously at different positions in the batch. Interpretation of the NIRS signal is preferably made with multivariate analysis. The aim of this study was to demonstrate the feasibility of using NIRS to monitor the lyophilization process *in-situ*.

MATERIALS AND METHODS

Materials

The experiment was conducted with 10-mL injection vials made of colorless borosilicate glass tubing of Ph. Eur. and

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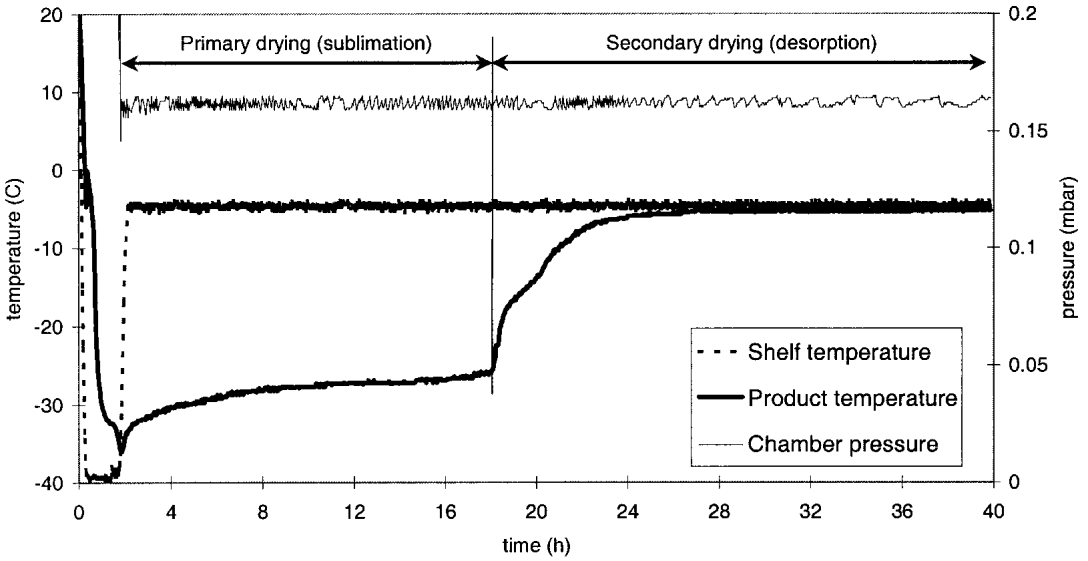

Fig. 1. Diagram showing the changes over time of shelf temperature, product temperature, and chamber pressure during the freeze-drying process, as measured by conventional thermocouples and a pressure gauge.

USP type 1. The dimensions of the vials were according to size 10 R in ISO 8362-1 (Münnerstädter Glaswarenfabrik, Münnerstadt, Germany). The model compound used was polyvinylpyrrolidone (PVP), i.e., Polyvidon K25. The solution was made with Polyvidon K25 (BASF, Minden, Germany) and distilled water.

Lyophilizer

The lyophilization process vessel was manufactured by Edward Kniese & Co. Hochvakuum GmbH, Marburg, Germany. The fully automated, programmable, and steam sterilizable dryer had a condenser capacity of 11 kg and temperature-controlled shelves, each with a shelf area of 0.14 m^2. The distance between the shelves was 110 mm. The condenser was situated in a separate chamber, which was connected to the cabinet by a valve. The lyophilizer was automatically controlled by a programmable logic controller (Siemens AG, Fürth, Germany) that could be programmed from a PC. The condenser was cooled to about –80°C whereas the temperature of the polished stainless-steel shelves was varied between –45°C and –5°C. The temperature of the circulating silicone oil that controlled the temperature of the shelves was measured both at the inlet and at the outlet of the shelves. A platinum probe at the outlet was used for the feedback loop control of the shelf temperature. The operating pressure was regulated with a nitrogen injection through a microvalve and with the valve to the vacuum pump. The chamber pressure was measured with two pirani manometers (Edwards High Vacuum International, West Sussex, England and Leybold Vacuum, Cologne, Germany) and a capacitance manometer (MKS Instruments, Inc. Andover, MA). The capacitance manometer was used for the regulation and its operating range was 0.01–100 Pa with an accuracy of ±0.5%. Temperatures in the chamber were measured with fine wire thermocouples type K of 0.13 mm diameter. Experimental data were logged

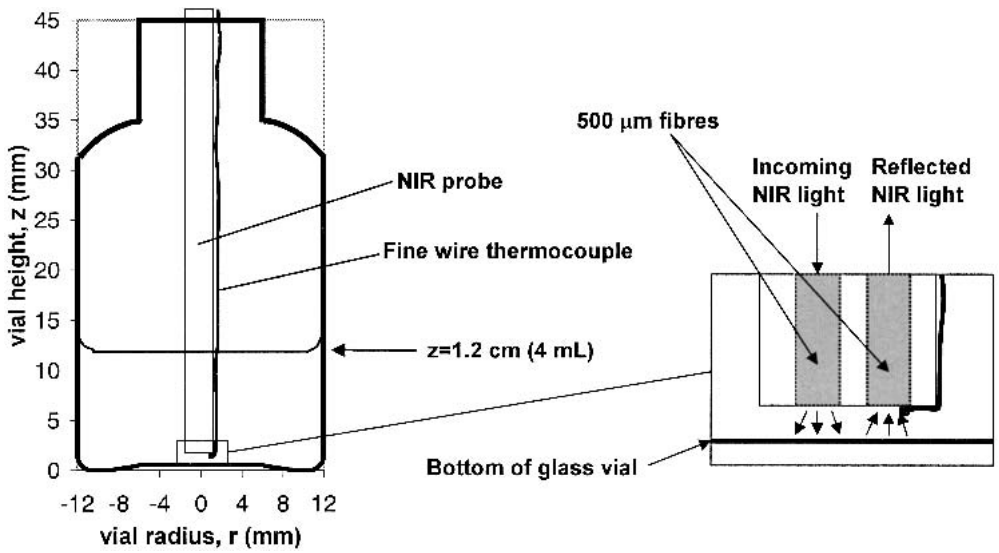

Fig. 2. A cross section of a 10-mL vial showing the position of the near-infrared spectroscopy probe and the thermocouple.

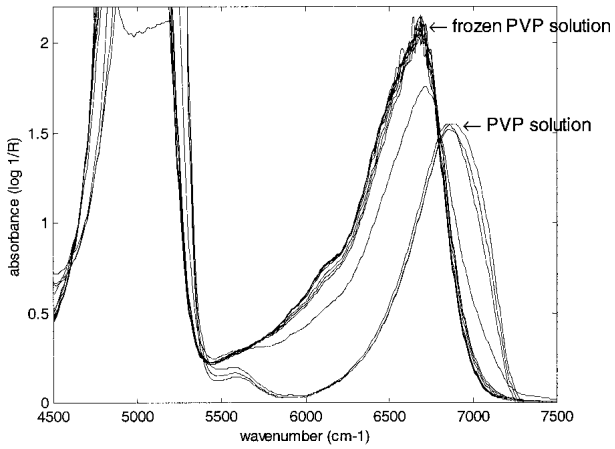

Fig. 3. Near-infrared spectra during the transition from polyvinylpyrrolidone solution to frozen polyvinylpyrrolidone solution. The plot shows 13 spectra during a period of 1.5 h.

on the connected PC. A special interface for the NIRS probe was developed and installed.

Lyophilization Cycle

The lyophilization cycle is illustrated in Fig. 1. The plot shows the product temperature, the shelf temperature, and the chamber pressure during the process. The shelf temperature was changed from 20°C to –40°C when the lyophilization cycle was activated. The shelf temperature was then kept at –40°C for 1 h to let the PVP solution freeze completely. Sublimation was initiated by evacuating the chamber to a pressure of 16 Pa and changing the shelf temperature from –40°C to –5°C. The chamber pressure and shelf temperature were kept constant at these levels for approximately 38 h. The ice in the vial sublimated during the first phase of the drying process. This phase is called "primary drying" and lasts until all of the ice in the product has been removed. The last stage of the drying process, which is called "secondary drying", involved the removal of water adsorbed to or trapped by the solid matrix.

NIRS Instrument

NIRS measurements were made using a Fourier transform-NIR spectrometer (ABB Bomem, Inc., Quebec, Canada) fitted with a 500-μm single fiber reflectance probe and an InGaAs detector. Spectra were recorded over the range 3996 to 12004 cm^{-1} (830 to 2500 nm) with a resolution of 8 cm^{-1}, and each spectrum was the average of 128 scans. This resulted in an acquisition time of approximately 1 min. One spectrum was recorded approximately every 7 min.

Sample Preparation and Presentation

A total number of 215 vials were filled with 4 mL of a 40 mg/mL PVP solution. Rubber stoppers were mounted in the vials except in the one in which the NIRS probe and the thermocouple were placed. One fine wire thermocouple was placed alongside the NIRS probe and the soldered end of the thermocouple, where the temperature was measured, was placed in front of the NIRS probe. The measuring point for the probe and the thermocouple were therefore as close together as possible. The probe was placed in the center of the vial 1 mm above the bottom (see Fig. 2). All vials were placed directly on the middle shelf in a hexagonal packing array. A specially designed shelf border in stainless steel surrounded the vials. The probe was attached to the border to enable adjustment and fixation of its position. The monitored vial was placed in the center of the shelf and neighboring vials therefore surrounded it.

Data Analysis

Principal component analysis was performed on the spectral data (28). There was no pretreatment of the spectral data except that only data in the range 4405 to 9003 cm^{-1} (1110 to 2270 nm) was used in the analysis due to the signal to noise characteristics of the detector element. The result from the principal component analysis, i.e., scores from the first principal component, was compared with temperature and pressure data from the lyophilizer. It was mainly the first compo-

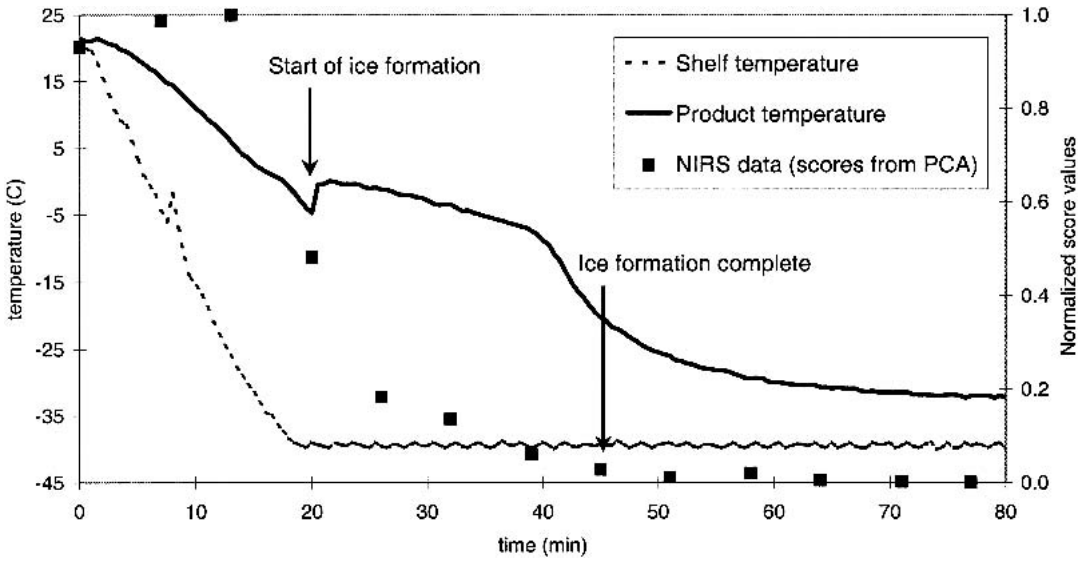

Fig. 4. Near-infrared spectroscopy data compared with product temperature and shelf temperature during the freezing phase.

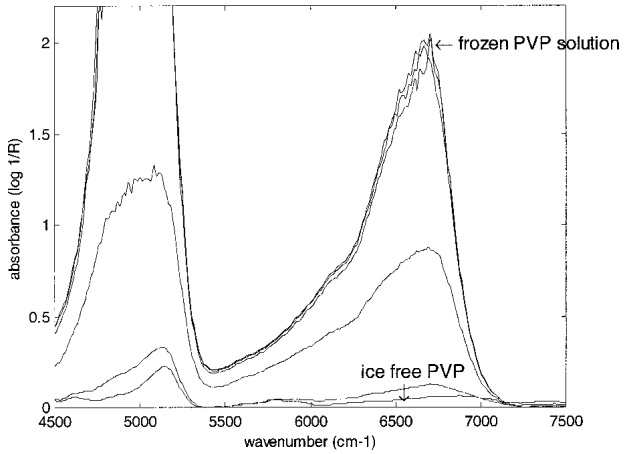

Fig. 5. Near-infrared spectra during the transition from frozen polyvinylpyrrolidone solution to polyvinylpyrrolidone material locally free from ice. The plot shows 5 spectra during a period of 35 min.

nent that was significant for these data and it could explain 92 to 98% of the variation of the NIRS data.

RESULTS AND DISCUSSION

Freezing Phase

It can be seen in Fig. 3 that the water peak at approximately 6900 cm^{-1} (1450 nm, first overtone) in the spectrum for the PVP solution shifted distinctly to approximately 6700 cm^{-1} (1490 nm) in the spectrum of the frozen PVP solution. It is also illustrated that there was another strong absorption, which is also caused by water, between 4800 to 5200 cm^{-1} (1920 to 2080 nm, combination band). It can be seen in Fig. 4 that there is good agreement between NIRS data and product temperature data about the starting point of the ice formation. It can also be seen that the product temperature was almost constant during the ice formation process, which lasted approximately 20 min, and then suddenly dropped exponential-like at the same time as the NIRS data indicates that the ice-formation was complete. NIRS monitoring gives additional information about the freezing process, which can explain the observed behavior of the product temperature.

Primary Drying Phase (Sublimation)

It is illustrated in Fig. 5 that the spectrum of the frozen PVP solution and the spectrum of the PVP material locally free from ice are very different and that the NIRS signal therefore changed significantly during the transition. It can be seen in Fig. 6 that there is good agreement between NIRS monitoring and product temperature monitoring concerning the transition from frozen PVP solution to ice-free material. The product temperature shows a very steep increase at the same time as the NIRS data indicate that the ice has been removed. The NIRS monitoring gives additional information about the sublimation process, which can explain the observed behavior of the product temperature.

The probe was situated at the bottom of the sample where the material dried last. During the first 16 h of the sublimation process, i.e., before the transition point, only spectra of the frozen PVP solution could be observed, and there was no trend but only a natural variation of these spectra. It can therefore be concluded that it was only a small amount of material in front of the probe that was monitored, and no information about the drying of the material above the probe can be deduced. NIRS monitoring consequently gave only local information about the material in the vial.

It can be seen that the product temperature changed during the sublimation process before the transition point. The decrease of the product temperature at the start of the sublimation was the result of the fact that the frozen solution adopted the equilibrium temperature that corresponded to the prevailing water vapor pressure above the ice surface. The continuous increase in product temperature that started after approximately 30 min of sublimation was caused by a mass transfer resistance in the dry product layer that was formed on the surface of the frozen solution and which grew as the drying process proceeded. These two changes of the product temperature cannot be detected in the NIRS data and it can therefore be concluded that these processes did not change

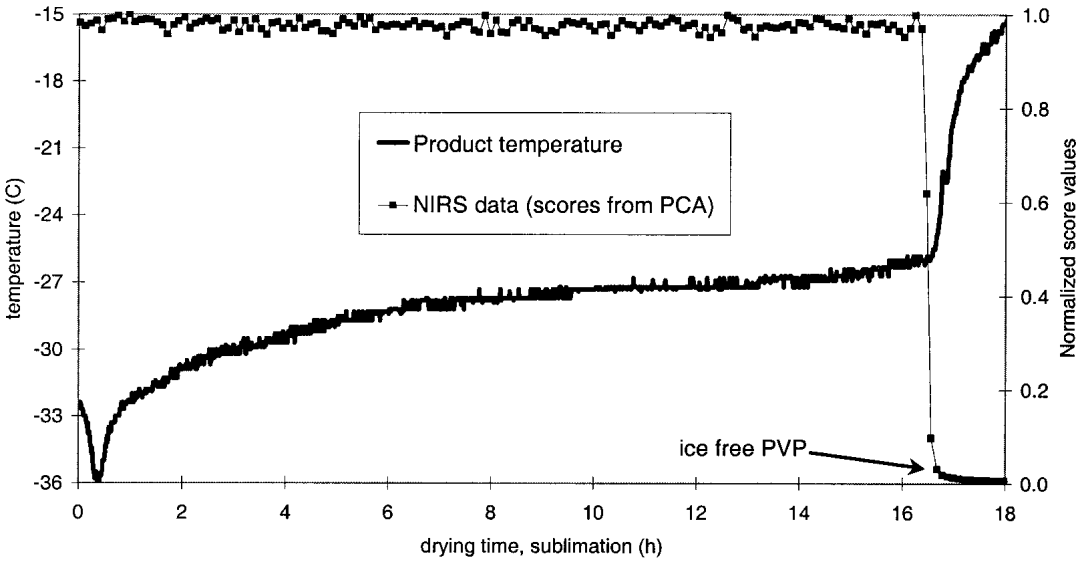

Fig. 6. Near-infrared spectroscopy data compared with product temperature and shelf temperature during the sublimation phase.

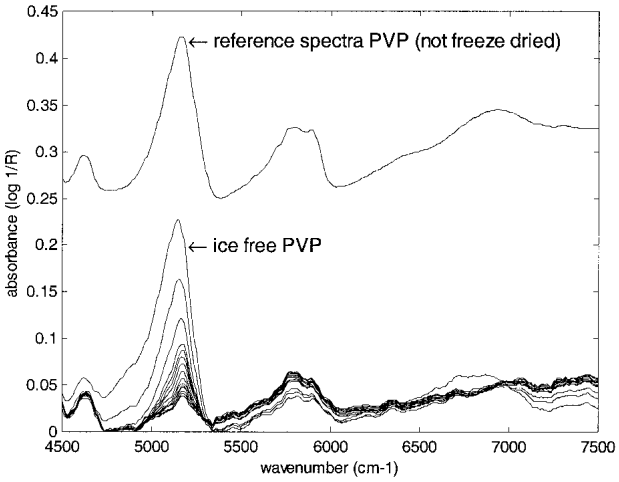

Fig. 7. Near-infrared spectra during the desorption phase. The plot shows 15 spectra during a period of 15 h, i.e., 1 spectrum/h, and above these a reference spectra of the polyvinylpyrrolidone raw material that has not been lyophilized.

the frozen solution and/or the sensitivity of the NIRS measurement was not high enough to detect these changes.

Secondary Drying Phase (Desorption)

It is illustrated in Fig. 7 that the dominating change in the spectra during the desorption phase was the significant decrease in the water peak at 5100 to 5200 cm^{-1} (1920 to 1960 nm). A spectrum of PVP raw material, with a water content of approximately 8%, that had not been lyophilized is also shown above the process spectra as a reference. It can be seen in Fig. 8 from the NIRS data that the desorption progressed through two different drying phases with an intermediate short transition period. It is apparent that the area of the water peak diminished more rapidly during the first phase and that the drying rate was much faster in that phase. The drying rate seemed to be more or less constant in each phase and an attempt to illustrate this has been made by adding two lines in the figure. This information could not be obtained from the product temperature monitoring. The temperature data shows the same pattern as the NIRS data during the initial stage of the desorption process up to about 3 h, but it is not possible to see the two different drying rate phases in the product temperature data. The product temperature reached a steady-state after approximately 8 h, whereas drying continued for another 10 h until the water content reached steady-state. NIR monitoring therefore provides new information about the desorption process and it is clear that the drying rate and the steady-state at which the drying was complete could be precisely detected. However, it was only local information originating from a relatively small amount of the sample directly in front of the probe and conclusions about the whole sample could not be made. This feature makes the position of the probe extremely important. This feature can however, also facilitate investigations of the lyophilization process within one sample, such as a study of the desorption in an ice-free part of the sample before the sublimation has been completed in other regions.

CONCLUSIONS

The freezing point, the completeness of the ice formation process, and the transition from frozen solution to ice-free material could be precisely detected with NIRS monitoring and the detected transition points correlated well with the results obtained from product temperature monitoring. The NIRS data yields significantly more information about the actual process and essentially explains the observed temperature changes during the process. It was clear that the rate of the desorption process and the steady-state at which the drying was complete could also be precisely monitored with NIRS. NIRS monitoring therefore provided new information about the drying process and the changes in the material during the process that was not possible to detect by product temperature monitoring. NIRS has thus been demonstrated to be a viable tool for in-line monitoring of the lyophilization process, both qualitatively and quantitatively. The tested NIRS monitoring can facilitate investigations of the drying process within a sample and it will be possible to generate

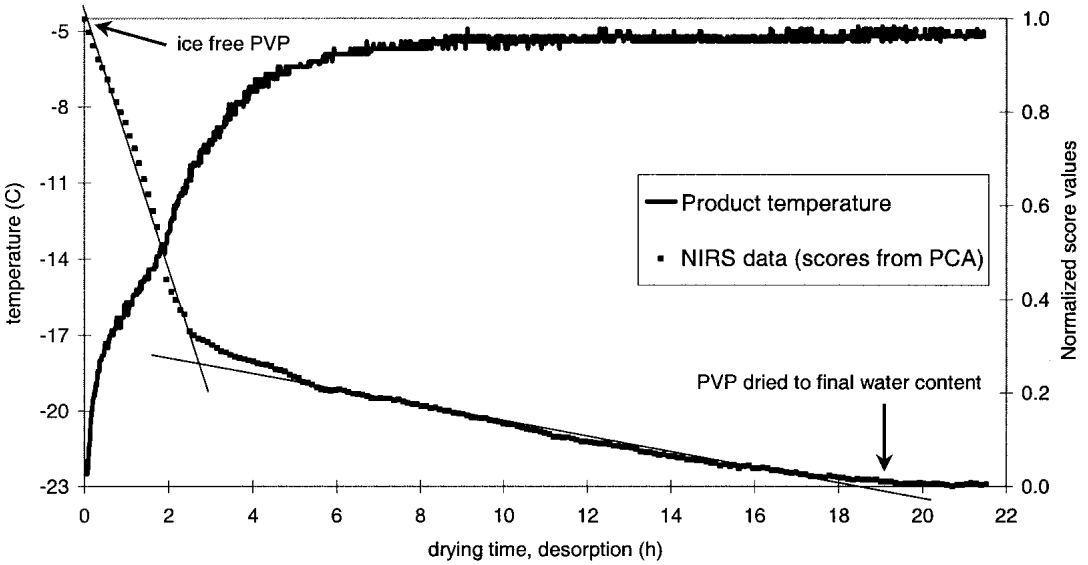

Fig. 8. Near-infrared spectroscopy data compared with product temperature during the desorption phase.

new information that can increase our understanding of the lyophilization process. The small volume that is monitored will make sample selection and sample presentation very important.

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