Novel Identification of Pseudopolymorphic Changes of Theophylline During Wet Granulation Using Near Infrared Spectroscopy

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ABSTRACT: The purpose of this study was to demonstrate the efficiency of near infrared (NIR) spectroscopy in studying the pseudopolymorphic changes and the state of water during the wet granulation process. Anhydrous theophylline was granulated in a planetary mixer using water as granulation liquid. NIR spectra and differential scanning calorimetric (DSC) and wide-angle X-ray scattering (WAXS) patterns of theophylline granules, anhydrous theophylline, and theophylline monohydrate were measured. At a low level of granulation liquid (0.3 mol of water per mole of anhydrous theophylline), water absorption maxima in the NIR region occurred first at around 1475 and 1970 nm. These absorption maxima were identical to those of theophylline monohydrate. At higher levels of granulation liquid (1.3–2.7 mol of water per mole of anhydrous theophylline), the increasing absorption maxima occurred at 1410 and 1905 nm due to OH vibrations of free water molecules. X-ray diffraction patterns confirmed the transformation of anhydrous theophylline to theophylline monohydrate during wet granulation. NIR spectroscopy was able to detect different states of water molecules during the wet granulation process faster and in a more flexible manner than conventional methods. © 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 90:389–396, 2001

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INTRODUCTION

In a number of pharmaceutical processes, drugs are exposed to water. Because water molecules may influence the behavior of drugs, it is necessary to understand the state of water. It is generally noticed that water molecules can associate with solids in different ways.[1] Adsorbed water molecules interact only with the surface of the solid. Absorbed water molecules penetrate into the bulk solid structure and they may cause changes in the crystal structure, producing pseudopolymorphs with different chemical and physical properties. Among various pseudopolymorphs, differences occur between packing, kinetic, mechanical, surface, and thermodynamic properties.[2] Detecting the interaction of water and drugs is an effective tool for improving the process control and the final product quality during pharmaceutical processes.

Pseudopolymorphism of theophylline has been studied extensively. It is well known that anhydrous theophylline transforms rapidly into theo-

phylline monohydrate on contact with water molecules. Shefter and Higuchi[3] noticed that the dissolution rate of anhydrous theophylline was much greater than that of theophylline monohydrate. During the pelletization process and storing of tablets under elevated humidity conditions, the transformation of anhydrous theophylline into theophylline monohydrate has been reported.[4–6] Phadnis and Suryanarayanan[7] found thermal dehydration product of theophylline monohydrate (metastable phase of anhydrous theophylline) in tablets. Among these different forms of theophylline and their mixtures, different release rates and solubilities have been reported.[3,4,7–9] Traditionally, pseudopolymorphic changes have been studied using infrared (IR) spectroscopy, thermal methods, optical and electron microscopy, nuclear magnetic resonance (NMR) spectroscopy, and X-ray diffraction.[10–13] These methods are reliable, but relatively slow and limited to off-line and at-line applications. Demand to investigate pseudopolymorphism in-line during pharmaceutical processing has generated a need for faster and more flexible methods of measurement.

Near infrared (NIR) spectroscopy is a rapid, nondestructive method and requires no sample preparation. In the NIR region, absorption bands are mainly caused by overtones and combination vibrations of CH, NH, and OH groups. The most intense absorption bands of pure water in the NIR region are found around 1450 and 1940 nm.[14–16] The band around 1940 nm is caused by a combination of OH stretching and bending vibrations and the band around 1450 nm is issued from the first overtone OH stretching vibrations of water molecules. In addition, absorption bands of water are distributions of vibrations of OH groups with varying energy levels. It is well established that the states of water molecules mainly depend on their hydrogen bonding ability.[17] Using spectral treatment, individual absorption maxima can be distinguished.[15, 18–20]

During pharmaceutical wet granulation processes, the amount of granulation liquid is the most important factor.[21] Intact and rapid NIR spectroscopy offers advantageous possibilities of in-line measurements during processes. The factors affecting the application of NIR spectroscopy to in-line moisture measurement during wet granulation processes have been evaluated.[22,23] However, during a wet granulation process there is an opportunity for a change in the crystal form of a drug. NIR spectroscopy has been applied for studying the polymorphism of different pharmaceutical materials.[24, 25] The purpose of this study was to demonstrate the efficiency of NIR spectroscopy in studying the pseudopolymorphic changes and the state of water during wet granulation of a model compound.

MATERIALS AND METHODS

Materials

Anhydrous theophylline (theophyllinum anhydricum 200M, BASF, Ludwigshafen, Germany) was granulated with a planetary mixer (Kenwood KM400, Kenwood Ltd., UK). The batch size was 300 g of anhydrous theophylline, and five different amounts of purified water were added (Table 1). After the addition of water, the masses were mixed for 5 min. Moisture contents were determined with an IR dryer (Sartorius Thermocontrol YTC01L, Sartorius GmbH, Göttingen, Germany). The IR dryer heated the samples (105°C) until the loss of weight was <0.1% in 50 s. The measurements were performed in triplicate and the results were reported as an average (±standard deviation, SD) of loss on drying (%, wet base).

Theophylline monohydrate was prepared by dissolving anhydrous theophylline in distilled water at 60°C. Saturated solution was slowly cooled, and needle-like theophylline monohydrate was filtered by vacuum. Theophylline monohydrate was dried at room temperature until the moisture content was ~9%. Anhydrous theophylline and theophylline monohydrate were stored in

Table 1. Moisture Contents of Theophylline Granules, Anhydrous Theophylline, and Theophylline Monohydrate

Sample	Computational moisture content, %	Moisture content, % (SD, $n = 3$)	Mole(s) of water per mole of anhydrous theophylline
Anhydrous theophylline	0	0 (0.0)	0.0
Theophylline monohydrate	9	9 (0.0)	1.0
Granule	3	2 (0.0)	0.3
	6	6 (0.2)	0.7
	12	11 (0.1)	1.3
	17	15 (0.1)	2.0
	21	20 (0.1)	2.7

a desiccator at different levels of relative humidity (RH). Anhydrous theophylline was stored with silica gel and the monohydrate with a saturated aqueous solution of NaBr (58% RH).

Near Infrared Spectroscopy

Diffuse reflectance NIR spectra were measured using a Fourier transform (FT)-NIR spectrometer (Bomem MB-160 DX, Bomem Inc., Quebec, Canada). FT-NIR spectra were measured between 4000 and 10,000 cm^{-1} with a resolution of 16 cm^{-1}. Standard reflection was measured using a Teflon background (Labsphere, SRS-99-070, North Sutton, NH, USA). The samples were scanned 32 times, and each spectrum was reported as the average of these scans. Spectral treatment was performed using Grams/32 software (Grams/32, version 4.04, Galactic Industries Corp., Salem, NH, USA).

Differential Scanning Calorimetry

Differential scanning calorimetric (DSC) measurements were performed on a Mettler DSC analyzer (model 821^e, Mettler Toledo Ag, Schwerzenbach, Switzerland) using STAR software (STAR 5.1, Sun Soft Inc., Mountain View, CA, USA). The temperature axis of the equipment was calibrated with zinc and indium. The runs were performed under nitrogen gas flow (50 mL/min) in open aluminum pans, and the weights of the samples were 3–5 mg. The heating rate was 10°C/min over the temperature range 25–300°C.

X-ray Diffractometry

X-ray diffraction patterns were measured using a wide-angle X-ray scattering (WAXS) theta–theta diffractometer (Seifert XRD 3000, Rich. Seifert & Company, Germany). The WAXS experiments of the samples were performed in a symmetrical reflection mode with CuK_{α} radiation (1.54 Å) monocromatized with a graphite monochromator in the scattered beam. The scattered intensities were measured with a NaI(Tl) scintillation counter. The angular range was from 2° to 20° (at θ) with steps of 0.03°, and the measuring time was 15 s/step at all measurements.
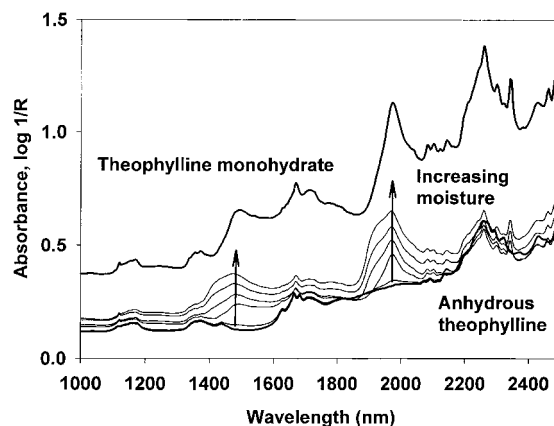
The amounts of anhydrous theophylline and theophylline monohydrate in granules were estimated by fitting a linear combination of the diffraction patterns of anhydrous theophylline and theophylline monohydrate to the diffraction patterns of granules (Matlab v. 5.3, The MathWorks,

Inc., Natick, MA). The crystallinity was estimated by fitting a linear combination of the intensities of the crystalline and amorphous component to the diffraction patterns of the samples. The experimental intensity curve, where the Bragg peaks were subtracted, was used as the amorphous model intensity curve. The crystalline model intensity curve consisted of the diffraction peaks.

RESULTS

Near Infrared Spectroscopy

Anhydrous theophylline had no absorption bands of OH vibrations around 1450 and 1940 nm (Figures 1–3). Theophylline monohydrate had two distinct absorption maxima at around 1475 and 1970 nm. Theophylline granules including 0.3 mol of water per mole of anhydrous theophylline also had two absorption maxima at the same wavelengths, at around 1475 and 1970 nm (Figures 2A and 3A, respectively). Otherwise, the absorption spectrum of granules including 0.3 mol of water per mole of anhydrous theophylline was quite similar to that of anhydrous theophylline. As the moisture content of the granules increased up to 0.7 mol of water per mole of anhydrous theophylline, the intensity of the absorption maxima increased at around 1475 and 1970 nm. Also, some changes in the baseline of spectra occurred due to the increasing particle size.[16, 22, 23, 26–28] When the moisture content of the granules was increased up to 1.3 mol of water per mole of anhydrous theophylline, new absorption maxima occurred at lower wavelengths of

Figure 1. NIR spectra of anhydrous theophylline, theophylline monohydrate, and theophylline granules at 1000–2500 nm.
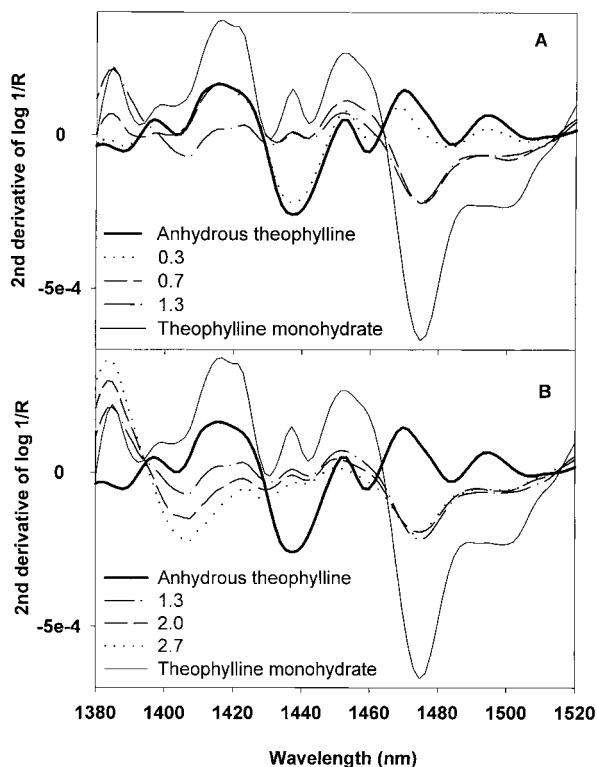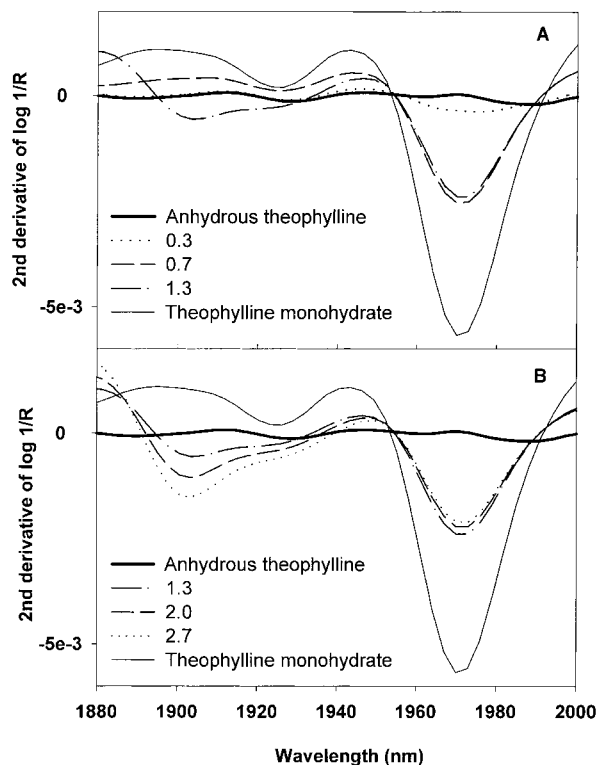
Figure 2. Second derivative of absorbance of anhydrous theophylline, theophylline monohydrate, and theophylline granules at 1380−1520 nm: (A) anhydrous theophylline transformation into theophylline monohydrate at around 1475 nm; (B) effect of free water molecules at around 1410 nm (number indicating mole(s) of water per mole of anhydrous theophylline).


Figure 3. Second derivative of absorbance of anhydrous theophylline, theophylline monohydrate, and theophylline granules at 1880−2000 nm: (A) anhydrous theophylline transformation into theophylline monohydrate at around 1970 nm; (B) effect of free water molecules at around 1905 nm (number indicating mole(s) of water per mole of anhydrous theophylline).

around 1410 and 1905 nm (Figures 2B and 3B, respectively). The intensities of the absorption maxima at around 1475 and 1970 nm were almost the same as for the granules with a moisture content of 0.7 mol of water per mole of anhydrous theophylline. The intensities of these new absorption maxima increased when the moisture content of the granules increased to > 1.3 mol of water per mole of anhydrous theophylline. In practice, the intensity of absorption maxima at around 1475 and 1970 nm remained constant. When the moisture content of theophylline granules increased, the spectral baseline also increased (Figure 1). The baseline was higher for theophylline monohydrate than for the other samples because of the different optical properties of the needle-like crystals of theophylline monohydrate.

Differential Scanning Calorimetry

The DSC dehydration endotherms of the granules started at a lower temperature than the

endotherms of theophylline monohydrate (Figure 4). With increasing water content of the granules, the peak maxima of endotherms occurred at a higher temperature. The endotherm peaks of the granules were observed at lower temperature than the endotherm of theophylline monohydrate, and they were broader.
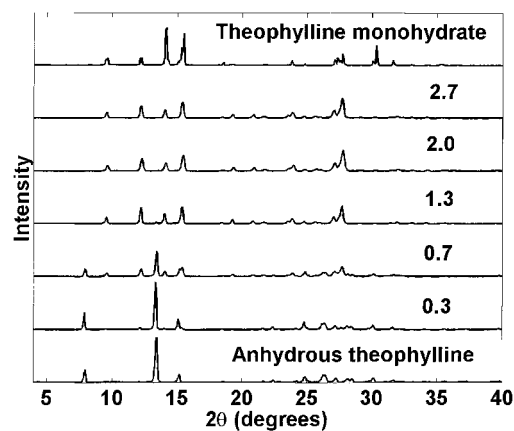
X-ray Diffractometry

The diffraction patterns of anhydrous theophylline resembled closely the intensity curve of orthorhombic anhydrous theophylline ($a = 8.50$ Å, $b = 24.64$ Å, and $c = 3.83$ Å; Figure 5).[29] The diffraction pattern of theophylline monohydrate agreed with the intensity curve of monoclinic theophylline monohydrate ($a = 13.30$ Å, $b = 15.42$ Å, $c = 4.47$ Å, and $\beta = 98.81°$).[30] The diffraction pattern of granules with 0.3 mol of water per mole of anhydrous theophylline was almost identical to anhydrous theophylline. The amount of anhydrous theophylline was 96% (Table 2). The
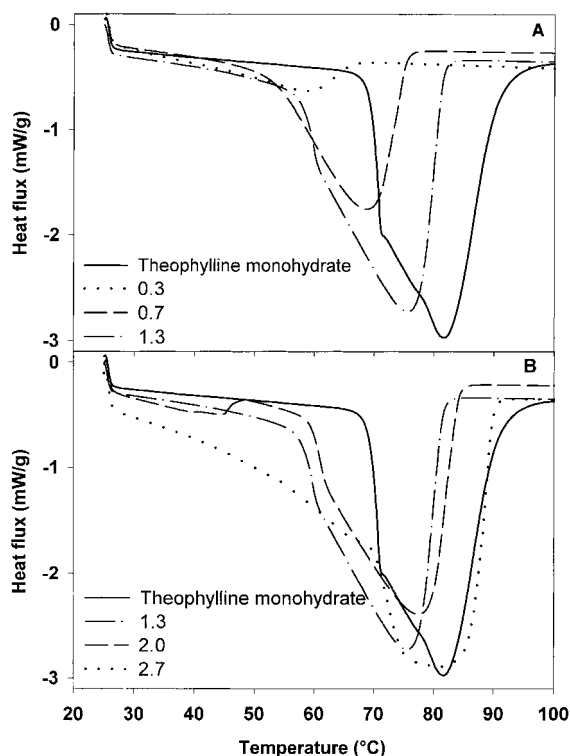
Figure 4. DSC patterns of theophylline monohydrate and theophylline granules: (A) low levels of granulation liquid; (B) high levels of granulation liquid (number indicating mole(s) of water per mole of anhydrous theophylline).

Figure 5. X-ray patterns of anhydrous theophylline, theophylline monohydrate, and theophylline granules (number indicating mole(s) of water per mole of anhydrous theophylline).

diffraction pattern of granules including 0.7 mol of water per mole of anhydrous theophylline had features of both anhydrous theophylline and theophylline monohydrate. The amount of anhydrous theophylline was 78% and the amount of theophylline monohydrate was 22%. The diffraction patterns of granules including 1.3–2.7 mol of water per mole of anhydrous theophylline agreed with the diffraction pattern of theophylline monohydrate. The amount of theophylline mono-

hydrate was between 93 and 94%. The crystallinities of the granules varied from 72 to 75%. They were almost the same as the crystallinities of anhydrous theophylline (71%) and theophylline monohydrate (69%).

DISCUSSION

The different energetic states of water have been studied using the second derivative of NIR absorbance spectra.[19,20,31] The pseudopolymorphic change of anhydrous theophylline during granulation was observed in absorption maxima at around 1475 and 1970 nm (Figures 1, 2A, and 3A). When the moisture content of theophylline granules was low (0.3−0.7 mol of water per mole of anhydrous theophylline), the absorption maxima increased only at around 1475 and 1970 nm. The wavelengths observed with granules were identical to those of theophylline monohydrate. It

Table 2. Polymorph Type and Crystallinity of Anhydrous Theophylline, Theophylline Monohydrate, and Theophylline Granules

Sample	Anhydrous, %	Monohydrate, %	Crystallinity, %[b]
Anhydrous theophylline	100	0	71
Granule (0.3)[a]	96	4	72
Granule (0.7)	78	22	75
Granule (1.3)	7	93	72
Granule (2.0)	6	94	75
Granule (2.7)	6	94	73
Theophylline monohydrate	0	100	69

[a]Moles of water per mole of anhydrous theophylline shown in parentheses.
[b]Accuracy of the method, ±10%.

was concluded that the absorption maxima at around 1475 and 1970 nm resulted from OH vibrations of crystal (monohydrate) water molecules. After the moisture content of granules was 1.3 mol of water per mole of anhydrous theophylline, the intensity of absorption maxima at around 1475 and 1970 nm remained constant and new absorption maxima were observed at around 1410 and 1905 nm (Figures 2B and 3B). These new absorption maxima were due to OH vibrations of free water molecules. After the moisture content of granules had increased to 1 mol of water per 1 mol of anhydrous theophylline, in theory, all anhydrous theophylline was transformed to theophylline monohydrate. After this stage, the rest of the water should remain as free water molecules. Despite this theory, the intensities of absorption maxima at around 1475 and 1970 nm were almost the same with granules up to a moisture content of 0.7 mol of water per mole of anhydrous theophylline. It is suggested that not all anhydrous theophylline was transformed to theophylline monohydrate, and there were some dry regions inside the granules. The radiation at the NIR region is not able to penetrate into granules, and only the surface of the granules was measured. Luner et al.[32] and Seyer et al.[33] have recently determined the accuracy of NIR as a tool for quantifying the different crystalline forms of solids in binary mixtures. They performed calibration using the ratio of two distinct second-derivative peaks related to a specific polymorphic form. The calibration of the in-line (or on-line) NIR signals to quantify the amount of each pseudopolymorphic form during granulation may be performed in the same manner, simply by rationing two distinct second-derivative peaks. However, moving NIR to a harsh process environment will increase the prediction error of calibration models. The issues related to sample representativeness and the process interface should be considered. Further, the effect of formulation should be carefully examined off-line.[23, 31]

The dehydration of theophylline monohydrate is a two-step process.[34−36] The first step is the breaking of hydrogen bonds between theophylline and water molecules, and the second step is the evaporation of the loosened water. However, these steps are overlapping. The DSC curves of the granules already started to slope slowly at low temperatures where the adsorbed water took up heating energy (Figure 4A). Theophylline monohydrate, which lacked adsorbed water, had a more abrupt beginning in the dehydration endotherm. The DSC dehydration curves of the granules at the moisture contents of 1.3 and 2.0 mol of water per mole of anhydrous theophylline resembled that of theophylline monohydrate (Figure 4B). When the moisture content increased further (2.7 mol of water per mole of anhydrous theophylline), the endotherm of the adsorbed water overlapped the hydrate endotherm further. At the highest moisture content, the free water disturbed the DSC measurement to such a degree that the endotherm peak was blunt. Free water disturbs the DSC measurements by its high specific heat. The dehydration endotherms of the granules occurred at a lower temperature probably because the shearing forces of the planetary mixer did not allow regular monohydrate crystals to be formed. When low water contents are used, the granules may be wetted only on the surface. Thus, the water could evaporate more easily from the granules than from the monohydrate crystals produced by crystallizing from water.

Herman et al.[4] studied dry theophylline pellets by X-ray diffractometry. They suggested that anhydrous theophylline with microcrystalline cellulose was transformed into theophylline monohydrate during wet granulation. The WAXS results indicated that with > 1.3 mol of water per mole of anhydrous theophylline, the crystal structure of granules and theophylline monohydrate were almost identical (Figure 5). However, due to the preferred orientation of the crystallites of theophylline monohydrate, the intensities of the reflections of granules and theophylline monohydrate did not match perfectly. As a reason why all anhydrous theophylline did not transform to theophylline monohydrate it is suggested that some dry regions will remain inside the shell of the granules during wet granulation. In practice, the amount of anhydrous theophylline was negligible (Table 2). The X-ray diffraction patterns of anhydrous theophylline, theophylline monohydrate, and theophylline granules indicate that anhydrous theophylline was transformed into theophylline monohydrate during wet granulation.

CONCLUSIONS

Using NIR spectroscopy, anhydrous theophylline transformation into theophylline monohydrate was observed at around 1475 and 1970 nm. Free water molecules were observed at around

1410 and 1905 nm. DSC and X-ray diffraction confirmed this result. Because of the rapidity and the flexibility of the method, NIR measurement is a promising tool in pharmaceutical process and quality control.

ACKNOWLEDGMENTS

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