Hydrate Formation during Wet Granulation Studied by Spectroscopic Methods and Multivariate Analysis

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Purpose. The aim was to follow hydrate formation of two structurally related drugs, theophylline and caffeine, during wet granulation using fast and nondestructive spectroscopic methods.
Methods. Anhydrous theophylline and caffeine were granulated with purified water. Charge-coupled device (CCD) Raman spectroscopy was compared with near-infrared spectroscopy (NIR) in following hydrate formation of drugs during wet granulation (off-line). To perform an at-line process analysis, the effect of water addition was monitored by NIR spectroscopy and principal components analysis (PCA). The changes in the crystal arrangements were verified by using X-ray powder diffraction (XRPD).
Results. Hydrate formation of theophylline and caffeine could be followed by CCD Raman spectroscopy. The NIR and Raman spectroscopic results were consistent with each other. NIR revealed the state of water, and Raman spectroscopy gave information related to the drug molecule itself. The XRPD confirmed the spectroscopic results. PCA with three principal components explained 99.9% of the spectral variation in the second derivative NIR spectra.
Conclusions. Both CCD Raman and NIR spectroscopic methods can be applied to monitoring of hydrate formation processes. However, NIR is more suitable for monitoring solid–water interactions.

KEY WORDS: CCD Raman spectroscopy; near-infrared (NIR) spectroscopy; principal components analysis (PCA); pseudopolymorphism, hydrate.

INTRODUCTION

Numerous pharmaceutical compounds can exist as crystalline hydrates (1). As the physicochemical properties of these pseudopolymorphs can differ from those of anhydrous form, their behavior can vary during processing. Of particular importance here are variations in aqueous solubility, which can ultimately result in variations in bioavailability (2).

In various pharmaceutical unit operations, e.g., crystallization, wet granulation, and aqueous film coating, drug substances are exposed to water. Water may influence the behavior of the drugs markedly and can associate with the drug in several ways (3). Exposure to water may cause a pseudopolymorphic change, i.e., uptake of water into the crystal lattice in specific positions in the substances involved in the process (1,3,4). In subsequent drying steps of processing, it is not evident that the substance is transformed into the same anhydrous polymorph as the starting material. Theophylline, for instance, exists as a metastable polymorph after dehydration (5). Low drying temperatures after wet granulation can cause transformation into the metastable polymorph (6). This metastable polymorph has a slower dissolution profile than that of the stable anhydrous theophylline, suggesting that its bioavailability can change. If the water of crystallization does not desolvate during the drying step of manufacturing or desolvates only partially, it can be loosened during storage. The desolvated free water can affect the physical and chemical stability of the product. In addition, other processing steps can cause transformations in the hydration state of drugs. For example, grinding can cause dehydration of pharmaceutical hydrates (7). Processing can also cause polymorphic changes in a drug. Hence, it is important to be able to follow processing-induced transformations, and, by these means, to improve the quality and the degree of safety of pharmaceutical manufacturing (6).

A variety of methods (X-ray diffraction, thermogravimetry, differential scanning calorimetry, hot-stage microscopy, and Karl Fisher titration) are used to study and characterize drug hydrates (8). All of these methods require sampling and sample preparation, and therefore are not easily transferred to process environments. Raman and near-infrared (NIR) spectroscopy can be used in reflectance mode (9,10). Thus, the measurements can be performed directly from powders with no need for either sampling or sample preparation. In both techniques, fiber optics can be employed, enabling on-line measurements. As NIR spectroscopy uses absorption by such bonds as O–H, N–H, C–H, and S–H bonds, water affects the spectra strongly. Raman scattering has different quantum mechanic selection rules, and symmetric vibrations like C–C are strong in Raman spectra. As water is a weak Raman scatterer, Raman spectroscopy reveals changes in vibrations of the drug molecule during hydrate formation. Raman and NIR spectroscopy can also be used to analyze the end products. It is possible to recognize the hydration state of a drug in tablets by Raman spectroscopy (11).

Multivariate data analysis tools have not yet been accepted as standard methods in the field of pharmacy. However, multivariate process monitoring by spectroscopic methods has been reported. Synthesis of drug compounds has been monitored by NMR (12) and Raman (13) spectroscopy. Multivariate process monitoring has also been applied in the manufacture of dosage forms, e.g., powder blending (14) and film coating (15). In these studies principal components analysis (PCA) and partial least squares have been used for data projection.

The aim of this study was to evaluate the applicability of charge-coupled device (CCD) Raman spectroscopy in monitoring pseudopolymorphic transitions during wet granulation and to compare this method with NIR spectroscopy. To perform an at-line process analysis, the effect of water addition was monitored by NIR spectroscopy and principal components analysis. Additionally, the hydration behavior of two structurally related substances, theophylline and caffeine, was investigated.

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MATERIALS

Anhydrous theophylline (theophyllinum anhydricum 200M, Orion Pharma, Espoo, Finland) and anhydrous caffeine (coffeinum anhydricum, Orion Pharma, Espoo, Finland) were used in the granulations. Theophylline monohydrate was prepared by dissolving anhydrous theophylline in heated (60°C) purified water and cooling the supersaturated solution slowly. Caffeine 4/5-hydrate was prepared in the same manner using anhydrous caffeine but the water was heated to 80°C. The crystals formed were filtered by vacuum and dried at room temperature until the moisture content of the hydrate in question was attained. The crystals were stored at 58% relative humidity produced by a saturated salt solution (NaBr).

METHODS

Granulation

Wet masses of theophylline and caffeine were prepared using a planetary mixer (Kenwood KM400, Kenwood Ltd., UK). The granulations were carried out by adding five different amounts (Table I) of purified water at a constant speed into 300 g of anhydrous material with a low agitation speed (60 rpm) and mixing for 5 min with a higher speed (120 rpm) after water addition. Three samples were taken from the masses for each analytical technique and were measured off-line. The samples were measured the same day and were stored in small glass vial filled up to the top to avoid evaporation. The moisture content of each batch was determined with an infrared dryer (Sartorius Thermocontrol YTC01L, Sartorius GmbH, Göttingen, Germany), which heated the samples at 105°C until the weight loss was under 0.1% in 50 s (Table I). The moisture measurements were made in triplicate. The heating effect of mixing was evaluated by measuring the temperature of one theophylline (2.0 mol H_2O/mol anhydrate) and one caffeine (2.1 mol H_2O/mol anhydrate) mass with an infrared noncontact thermometer (KM814, Comark Ltd, Stevenage, England). The temperature of the theophylline and caffeine masses increased with 8 and 5°C, respectively, during the wet massing with the higher speed.

The second part of the study was performed at-line. The effect of water addition was monitored during wet massing of anhydrous theophylline using NIR. The basic idea of this experiment was to add water in small portions and to measure NIR spectra after each water addition. 3 or 6 ml of purified water was added to 300 g anhydrous theophylline while mixing at low speed and then wet massing for 30 s with the higher speed of agitation. Two samples of 5–10 ml were taken to immediate NIR analysis and put back in the mass after analysis. The addition of water was repeated so that a range of 0.2–3.0 moles of water per moles of anhydrate was covered with 0.1–0.2 increments. The temperature of the mass increased with 4°C in the middle of the experiment (0.6–1.4 mol H_2O/mol anhydrous theophylline) and decreased again when more water was introduced (1.5–2.6 mol H_2O/mol anhydrous theophylline).

X-Ray Powder Diffraction

X-ray diffraction (XRD) studies of the caffeine samples were made off-line using an XRD theta-theta diffractometer (Bruker axs D8, Karlsruhe, Germany) with Göbel Mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. All experiments were performed in symmetrical reflection mode with CuK_α radiation (1.54 Å), and the angular range was from 4 to 40° (2θ) with increments of 0.05°. The measuring time was 1 s/increment.

The amount of anhydrate and hydrate in the wet masses was estimated by fitting a linear combination of the diffraction curves of the anhydrate and hydrate to the experimental diffraction curve of the sample. The amount of the anhydrous or hydrate component was calculated as the ratio of the integrated intensities of the component and the studied sample.

NIR Spectroscopy

The diffuse reflectance NIR spectra were measured using a Fourier transform spectrometer (Bomem MB-160 DX, Hartman & Braun, Quebec, Canada) and Bomem Grams software (v. 4.04, Galactic Industries Corp., Salem, NH, USA). The spectra were collected in apparent absorbance (log 1/R) values, and Teflon (99% reflective Spectralon, Labsphere, North Sutton, NH, USA) was used as a reflectance

Table I. Moisture Contents of the Anhydrous Materials, Hydrates, and Wet Masses

Sample	Moles of water per mole of anhydrous material	Computational moisture content, %	Moisture content (wet based), % (mean ± SD, n = 3)
Anhydrous theophylline	0.0	0	0.5 ± 0.0
Theophylline hydrate	1.0	9	9.4 ± 0.2
Wet masses	0.3	3	3.1 ± 0.3
	0.7	6	6.7 ± 0.3
	1.3	12	10.9 ± 0.4
	2.0	17	15.6 ± 0.4
	2.7	21	20.2 ± 0.4
Anhydrous caffeine	0.0	0	0.4 ± 0.1
Caffeine 4/5-hydrate	0.8	7	7.4 ± 0.1
Wet masses	0.3	3	2.3 ± 0.4
	0.6	5	5.9 ± 0.6
	0.9	8	7.5 ± 0.8
	1.5	12	11.4 ± 0.5
	2.1	16	15.8 ± 0.3

reference. The spectra were recorded over a range of 10000–4000 cm^{-1} (1000–2500 nm) with a resolution of 16 cm^{-1} and were averaged over 32 scans. The spectra were measured through the bottom of the glass vial containing the sample. The measurements were carried out in triplicate. Second derivative transformations with 13 point Savitzky-Golay smoothing (16) were performed with Matlab in-house routines (v. 5.3, The MathWorks Inc., Natick, MA, USA).

Raman Spectroscopy

The Raman spectra were measured off-line with a charge-coupled device (CCD) Raman spectrometer prototype (VTT-Electronics, Oulu, Finland) containing an external-cavity diode laser (300 mW) operating at 830 nm as an excitation source and a back-illuminated CCD camera as a detector. The user interface had been programmed with LabVIEW (5.1, Austin, TX, USA), and the spectral information was collected using a fiber-optic probe. The spectra were recorded over a range of 2000–200 cm^{-1} with a resolution of 8 cm^{-1}. A measurement time of 1 s was used and the measurements were performed in triplicate. Spectral intensities were normalized by standard normal variate transformation (17) with Matlab in-house routines (v. 5.3, The MathWorks Inc.).

Principal Components Analysis (PCA)

The spectral data obtained from the at-line process analysis measurements were analyzed with PCA. Principal components were calculated from the second derivative NIR spectra using Simca (8.0, Umetri AB, Umeå, Sweden). The spectral region used was 1100–2050 nm.

RESULTS

X-Ray Powder Diffraction

The measured diffraction patterns of the anhydrous caffeine (18) and caffeine hydrate (19) resembled closely previously obtained intensity curves. The intensities of the reflections of the present and known diffraction patterns did not match perfectly due to preferred orientation of the crystallites. The diffraction curves of the wet masses with the two lowest water contents (0.3 and 0.6 moles of water per mole of anhydrous caffeine) resembled the diffraction curve of anhydrous caffeine (Fig. 1a). The diffraction pattern of the wet mass with 0.9 moles of water per mole of anhydrous caffeine had features of the diffraction patterns of both anhydrous caffeine and caffeine hydrate. The calculated amount of caffeine hydrate in this wet mass was 38% (Table II). The amount of hydrate increased in the wet masses with the two highest amounts of moisture (1.5 and 2.1 moles of water per mole of anhydrous caffeine), but the hydrate formation process was not completed, because the hydrated amount was only 62 and 65%, respectively.

NIR Spectroscopy

The second derivative NIR spectrum of the caffeine hydrate showed a well-resolved water absorbance maximum at around 1960 nm (Figs. 1b and 1c). The anhydrate showed no absorbance in this region. In the caffeine wet masses the increasing water content affected the absorbance maximum at 1960 nm first at the level of 0.9 moles of water per mole of anhydrous caffeine and more clearly at 1.5 moles of water per mole of anhydrous caffeine. With increasing water content of the caffeine wet masses, an increasing absorbance maximum at 1905 nm was observed. Hydrate formation of theophylline detected by NIR spectroscopy has been reported (20).

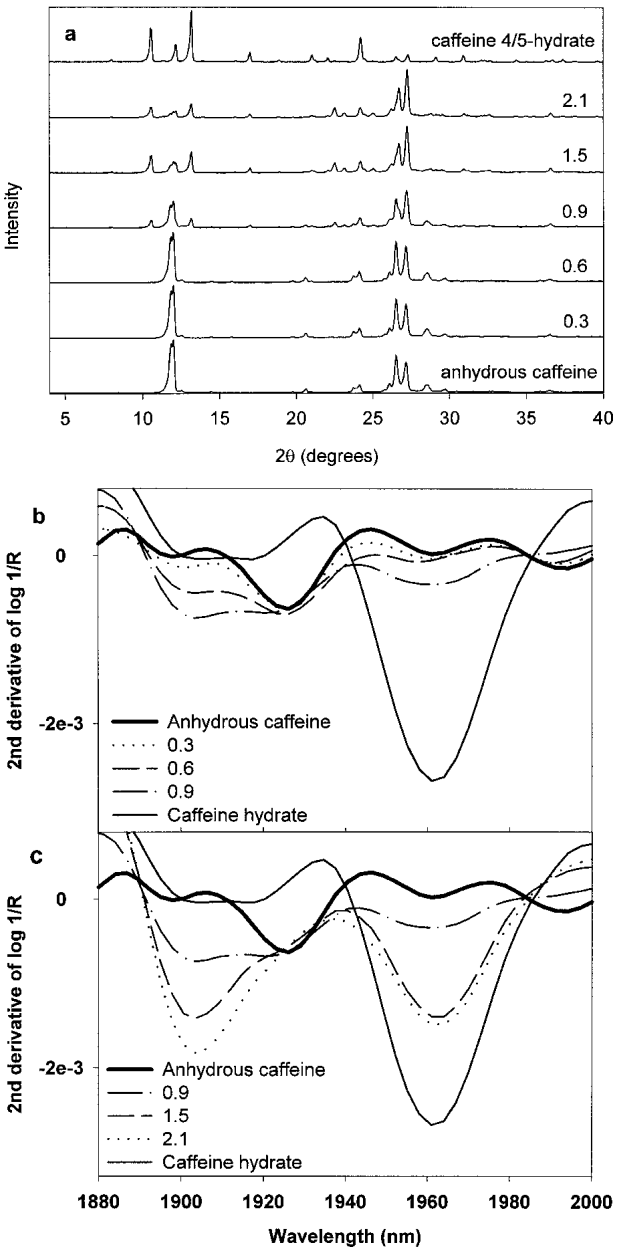

Fig. 1. Characterization of caffeine during wet massing (off-line analysis). (a) X-ray diffraction patterns of anhydrous caffeine, caffeine hydrate, and wet masses. Second derivative NIR spectra of anhydrous caffeine, caffeine hydrate, and caffeine wet masses (b) low levels of moisture, (c) high levels of moisture. (The numbers indicate moles of water per mole of anhydrous material.)

In the second part of the study the spectral data of the water addition to the theophylline wet mass was projected with three principal components (Fig. 2). The three first principal components explained 99.9% of the variation. The loadings (spectral regions) affecting the first principal component

Table II. Phase Composition of the Anhydrous Caffeine, Caffeine Hydrate, and Caffeine Wet Masses

Sample	Anhydrous, %	Hydrate, %
Anhydrous caffeine	100	0
Caffeine hydrate	0	100
Wet mass (0.3)	99	1
Wet mass (0.6)	98	2
Wet mass (0.9)	62	38
Wet mass (1.5)	38	62
Wet mass (2.1)	35	65

Note: Accuracy of the method approx. 10%.

were 1970 and 1650 nm (Fig. 3a). The second principal component was affected mainly by the region between 1600–1700 nm (Fig. 3b). The third principal component was affected by the 1905 nm region (Fig. 3c).

Raman Spectroscopy

In the Raman spectrum of anhydrous theophylline, the bands at 1699 and 1659 cm^{-1} (C=O stretch) disappeared and a band was observed at 1680 cm^{-1} on hydrate formation (Fig. 4a and Table III). The bands at 1606 cm^{-1} (C=C stretch), 1567 cm^{-1} (C=N stretch), 1188 cm^{-1} (C–C vibration), 1423 cm^{-1} (CH_3 deformation), and 926 cm^{-1} (CH_3 rocking) shifted toward lower wavenumbers in the hydrate formation process of theophylline. The bands at 1083–947 cm^{-1} (ring deformation), 1313 and 1285 cm^{-1} (C–N stretching vibrations), 663 and 552 cm^{-1} (O=C–N bend), and 1210 cm^{-1} (H–N=C vibrations) shifted toward higher wavenumbers on hydrate formation. The present assignments are tentative for some bands.

The spectra of the theophylline wet masses with 1.3 and more moles of water per mole of anhydrous theophylline showed no differences from the spectrum of theophylline

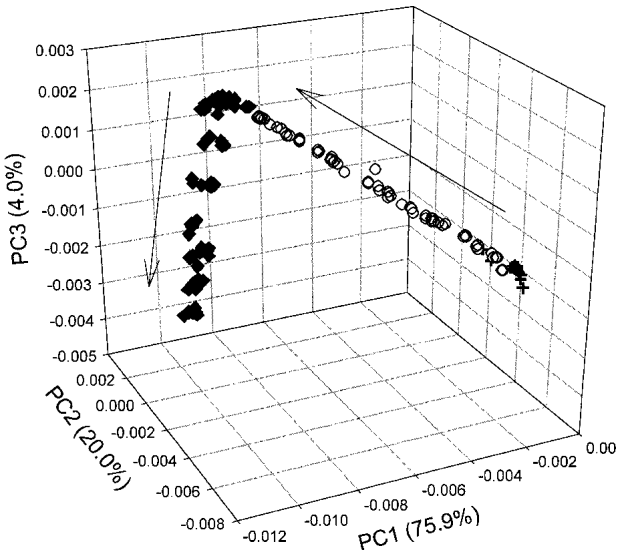

Fig. 2. Principal component scores plot of NIR spectra taken during water addition to a theophylline wet mass (at-line analysis). ✦ Anhydrous (0–0.4 mol H_2O/mol anhydrate); ○ hydrate formation (0.5–1.4 mol H_2O/mol anhydrate); ◆ free water (1.5–3.0 mol H_2O/mol anhydrate. (The arrows indicate increasing water content.)

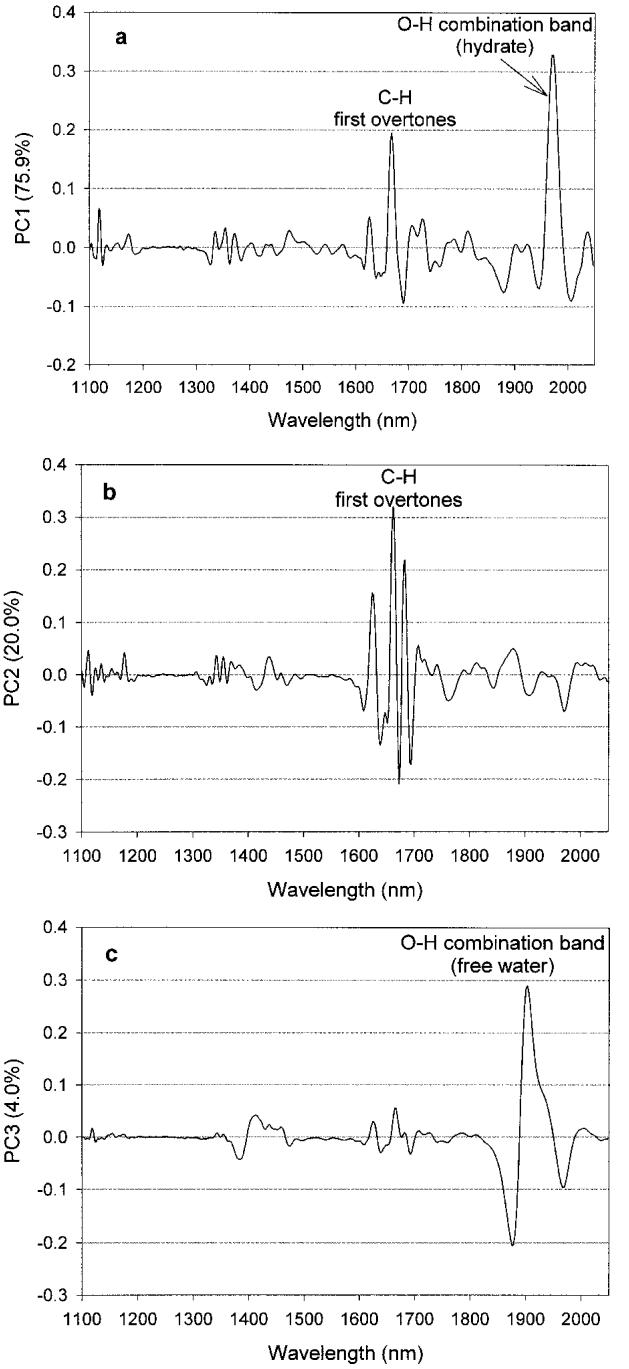

Fig. 3. Principal component loadings of NIR spectra taken during water addition to a theophylline wet mass (at-line analysis). (a) Loadings of the first principal component, (b) loadings of the second principal component, (c) loadings of the third principal component.

monohydrate (Fig. 4a). The spectrum of the wet mass with the lowest water content (0.3 moles of water per mole of anhydrous theophylline) resembled that of anhydrous theophylline. However, small differences were observed throughout the whole spectrum (Fig. 4a), especially for the 1680 cm^{-1} band (C=O stretching), 1170 cm^{-1} band (C–C vibration), and 916 cm^{-1} band (CH_3 rocking). The spectrum of the wet mass with 0.7 moles of water per mole of anhydrous theophylline had features of both anhydrous theophylline and theophylline monohydrate.

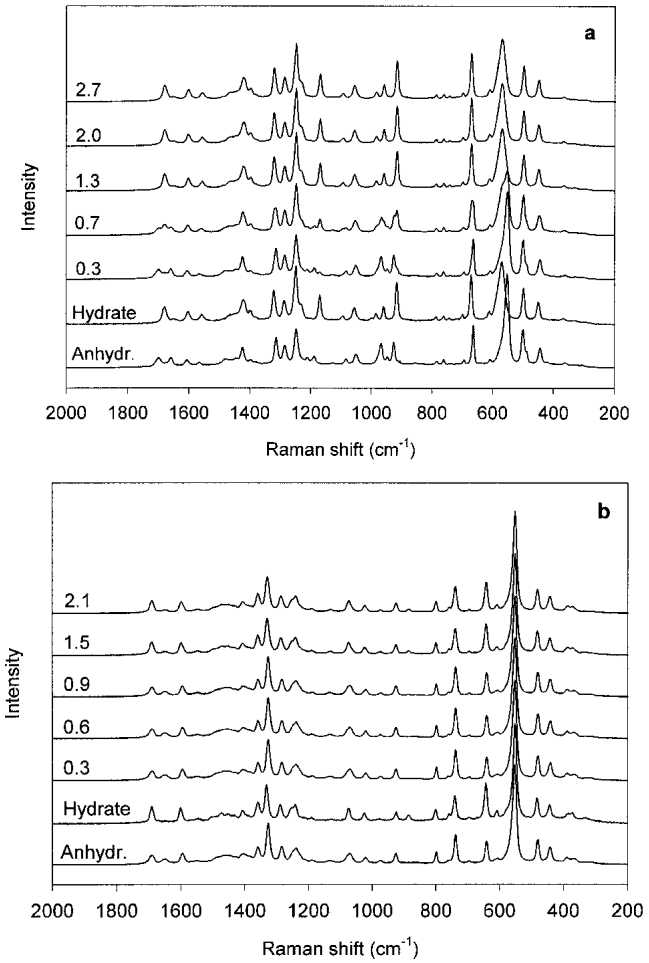

Fig. 4. Standard normal variate corrected Raman spectra of (a) theophylline and (b) caffeine, anhydrous materials, hydrates, and wet masses (off-line analysis). The numbers indicate moles of water per mole of anhydrous material.

Edwards *et al.* (21) previously reported the differences in the Raman spectra of anhydrous caffeine and caffeine hydrate. Our findings agreed with theirs. From the Raman spectra of anhydrous caffeine, a band at 1650 cm^{-1} (C=O) disappeared on hydrate formation (Fig. 4b). Bands at about 1330 (C–N), 1285 (C–N), 1240, 1070 (C–C), 800, 740 (O=C–N), and 640 cm^{-1} shifted toward higher wavenumbers on hydrate formation. Additionally, a new band at around 885 cm^{-1} (H_2O libration) was visible in the hydrate spectra.

The Raman spectra of the caffeine wet masses with 0.3–0.9 moles of water per mole of anhydrous caffeine resembled the spectrum of anhydrous caffeine (Fig. 4b). The spectra of the wet masses with higher water content (1.5 and 2.1 moles of water per mole of anhydrous caffeine) were similar to the spectrum of caffeine hydrate; however, there were some features of the spectrum of anhydrous caffeine. A trace of the 1650 cm^{-1} band (C=O) remained and the up-shifts of the C–N bands at around 1330 and 1285 cm^{-1} were incomplete.

DISCUSSION

Hydrate Formation of Theophylline and Caffeine during Wet Granulation

Hydrate formation of theophylline studied by the off-line measurements started at an earlier stage than that of caffeine.

Table III. Differences in Raman Spectra of Anhydrous and Hydrated Theophylline

Assignment[a]	Hydrate (cm^{-1})	Anhydrous (cm^{-1})
CH_3 str.[b]	3091	3102
	2942	2946
C=O str.	1680	1699
		1659
C=C str.	1603	1606
C=N str.	1557	1567
CH_3 deform.	1421	1423
C–N str.	1321	1313
	1287	1285
H–N=C	1248	1247
	1230	1210
C–C	1170	1188
Deform. ring	1094	1083
	1056	1050
	985	967
	959	947
CH_3 rock	916	926
O=C–N bend.	671	663
	570	552
C=N–C bend. or	499	500
C–C=N bend.		489

[a] Tentative assignment for bands below 1400 cm^{-1}.
[b] Measured with an Andor-Acton device with excitation at 632.8 nm. The experimental details can be found elsewhere (28).

Theophylline was affected already at the moisture level of 0.3 moles of water per mole of anhydrous theophylline (Fig. 4a and Ref. 20). Caffeine showed first signs of hydrate formation at the moisture level of 0.9 moles of water per mole of anhydrous caffeine (Figs. 1 and 4b). The difference is even larger if the fact that caffeine forms a 4/5-hydrate (22) containing 0.8 moles of water is taken into account. This phenomenon, which was apparent in the spectroscopic data, was confirmed by X-ray powder diffraction (Table II, Fig. 1a). Anhydrous caffeine is more stable in these conditions than anhydrous theophylline.

The Raman spectra of caffeine and its hydrate showed smaller differences than between theophylline and its hydrate (Figs. 4a and 4b). This observation suggests that a larger rearrangement occurred in the theophylline crystals on hydrate formation.

Hydrate formation of the caffeine wet masses was not completed with the water amounts used, whereas theophylline transformed almost completely to hydrate. Some features of anhydrous caffeine still remained in the Raman spectrum of the caffeine wet mass with the highest water content (Fig. 4b). This observation was supported by the X-ray diffraction results (Table II, Fig. 1a). Caffeine (23) and theophylline (24) form channel hydrates, caffeine having a larger tunnel cross-sectional area (25). Both hydrate crystals are long and needle-like in habit and, their dehydration is anisotropic, starting at the ends. Caffeine dehydrates more readily than theophylline, having a lower activation energy of dehydration (21,25). The water absorption (1960 nm) in caffeine hydrate NIR spectrum (Figs. 1b, 1c) was at a lower wavelength than that of theophylline hydrate (1970 nm) (20), which indicates a difference in the median energy of the OH bonds.

Small caffeine 4/5-hydrate crystals dehydrate more easily than large crystals (22). Vigorous mixing in the granulation process does not allow formation of long, regular crystals. Therefore, caffeine 4/5-hydrate crystals are likely to dehydrate during mixing although there is theoretically enough water for all the caffeine to be transformed into hydrate.

The mixing time after water addition affected the onset of hydrate formation. In the at-line measurements, when the mass was mixed a shorter period after each water addition, the hydrate formation was observed at a higher moisture content (1.4 moles of water per mole of anhydrous theophylline). Future studies should concentrate on evaluating of the effects of both the process-related and formulation-related variables on hydrate formation.

Comparison of Raman and Near-Infrared Spectroscopy in Studying Hydrates

The same phenomena were observed in both Raman and NIR spectroscopic data (Figs. 1b, 1c, and 4). NIR spectroscopy offers a quick way of determining the state of water, and it can also be used in quantitative water determination (10). However, the strong OH bands dominate other vibrations, and other changes in the molecule are not easily seen when using this technique in the presence of water. Water is a very weak Raman scatterer, and the symmetric vibrations in the drug molecule can be reliably detected and analyzed by Raman spectroscopy. As Raman spectral peaks were much narrower than the bands in the NIR spectra, it was easier to see spectral changes in the Raman spectra (Figs. 1b, 1c, and 4a).

The use of high laser power has caused problems with easily dehydrating material in Fourier transform Raman (11). In the CCD Raman spectroscope the laser power is markedly reduced and the measurement times are significantly shorter, but the dehydrating effect has yet to be investigated.

Both spectroscopic techniques studied here can be applied to on-line process analysis. In NIR spectroscopy, calibrations are often done using multivariate calibration, e.g., partial least squares regression (26). In this case the molecular background of the spectral changes may remain unknown, in which case the multivariate calibration method is used as a black box. Patel *et al.* (27) have successfully developed a univariate NIR calibration for mixtures of two polymorphic forms using spectral bands differing between the polymorphs. These bands relate to the fundamental differences in the crystal packing. In Raman spectra, the bands are sharp and distinguishable from each other. Thus, Raman spectral information can be easily calibrated in a univariate fashion by counting integrated peak ratios. In the case of theophylline, the hydrate formation event can be followed by the ratio of C–C vibrations (Fig. 5a). A peak that does not appear in one of the pseudopolymorphic forms can be used in the case of caffeine, by computing the ratio to the baseline (Fig. 5b). However, a full evaluation of the different calibration approaches goes beyond the scope of this article.

Multivariate Process Monitoring of Different States of Water in Wet Massing

When the NIR spectral data was projected in to three dimensions, the two first principal components explained the main distinctive features in the data (Fig. 2.). The first principal component described mostly the formation of the hydrate by changes in the hydrate water absorbance (1970 nm) (Fig. 3a). The hydrate formation affected also other vibrations in the theophylline molecule. It can be observed from the loadings of the first (Fig. 3a) and second (Fig. 3b) principal components that the C–H bonds (1600–1700 nm) were also involved in the changes of the spectral data during the water addition. In the beginning of the water addition—up to 1.4 moles of water per mole of anhydrous theophylline—movement in the direction of the two first principal components was predominant. After that the data points begun to move in the direction of the third principal component, which was described by changes in the free water region (1905 nm) of the spectra (Fig. 3c). Around this point (1.4–1.5 mol H_2O/mol anhydrous theophylline), granules started to form. From the three-dimensional pattern created by principal component analysis, the hydrate formation and free water could be easily distinguished.

NIR and PCA offer a way to visualize the changes in the state of water. With a PCA projection a three-dimensional path is defined for a granulation process. This path is unique for each formulation and it can be used for process monitor-

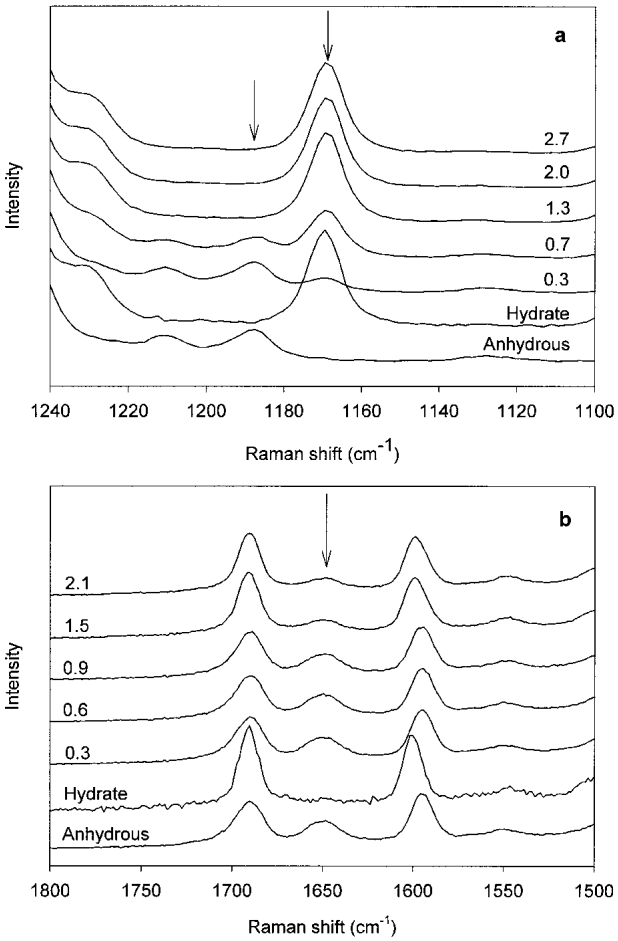

Fig. 5. (a) C–C vibration in the Raman spectra of anhydrous theophylline, theophylline monohydrate, and theophylline wet masses (off-line analysis). (b) C = O vibration in the Raman spectra of anhydrous caffeine, caffeine 4/5-hydrate, and caffeine wet masses (off-line analysis). (The numbers indicate moles of water per mole of anhydrous material.)

ing, even by nonskilled process operators. Those in charge of preformulation work can perform further analysis related to the state of water, e.g., using information in loading values.

CONCLUSIONS

NIR spectroscopy is widely used for quantification of water. Furthermore, it can be used to examine the state of water. Raman and NIR spectroscopy can be used as complementary methods in studying hydrates and hydrate formation. With Raman spectroscopy it is possible to follow the changes on the drug molecule itself during the hydrate formation process. At any rate, NIR spectroscopy seems superior in the study of wet masses and wet granulation due to its capability to differentiate free water and hydrate water. Multivariate process analysis offers a three-dimensional window to the state of water during a wet granulation process.

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