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An Investigation of Solvent-Mediated Polymorphic Transformation of Progesterone Using in Situ Raman Spectroscopy

Fang Wang,[†] John A. Wachter,*[,‡] Frederick J. Antosz,[§] and Kris A Berglund[†]

Departments of Chemistry and Chemical Engineering, Michigan State University, East Lansing, Michigan 48824, Chemical Process Research and Development, Pharmacia Corporation, 1500-91-2, 7000 Portage Road, Kalamazoo, Michigan 49001-0199, and Analytical Research and Development, Pharmacia Corporation, 1140-230-4, 7000 Portage Road, Kalamazoo, Michigan 49001-0199

Abstract:

Many analytical techniques, such as differential scanning calorimetry (DSC), X-ray diffraction (XRD), infrared spectroscopy (IR) and Raman spectroscopy can be used to differentiate between crystalline polymorphs of the same chemical entity. While all of these techniques are routinely applied to off-line analysis of materials, Raman spectroscopy has the advantage over these other techniques in that Raman technology currently exists for in situ monitoring of the solid-phase behavior within a mixed suspension of liquid and solid. In this work, we present our results from an in situ Raman study, demonstrating the solvent-mediated polymorphic phase transformation of progesterone. In situ Raman analysis has shown that the appearance of Form I progesterone is always preceded by the formation of Form II progesterone. Phase transformation rates were found to increase monotonically as the temperature increases, which indicates that the polymorphic system is monotropic. Form I was found to be thermodynamically more stable than Form II, while Form II was found to be kinetically favored over Form I. The results from this study are consistent with Ostwald's law of stages and lead to an in-depth understanding of the polymorphic transformation process of progesterone. The in situ monitoring capabilities of Raman spectroscopy have allowed us to define the processing parameters required to control the morphology of crystalline progesterone.

Introduction

The pharmaceutical industry is frequently confronted with the presence of multiple crystal polymorphs of the same chemical entity. The presence of multiple polymorphs of the active pharmaceutical ingredient is particularly challenging with solid, oral dosage drug products.[1,2] The characteristics affected by the polymorphism include solubility, dissolution rate, stability, hygroscopicity and solid-state reactivity. The effect of polymorphism on bioavailability is the most important consequence if the bioavailability is mediated via dissolution.[2,3] In situ monitoring of the solvent-mediated polymorphic transformation is therefore of great significance in understanding the thermodynamics of the polymorphic systems and the mechanisms of polymorphic transformation. In situ monitoring offers tremendous potential for efficiently determining the crystallization parameters required to obtain a desired crystal form.

In polymorphic studies, Raman spectroscopy, coupled with an immersible fiber optic probe, has tremendous advantages over the traditional techniques, such as X-ray powder diffraction (XRD), differential scanning calorimetry (DSC), solid-state NMR, and infrared spectroscopy (IR). The fiber optic probe in Raman technique provides the capability of in situ or on-line monitoring of the formation of crystals and the transformation from one polymorph to another. DSC, XRD, and NMR can perform off-line analysis, which can distinguish the polymorph of the end product,[4] but cannot reliably provide insight about transformation processes since a polymorphic transformation may continue to occur during sample filtration, drying, and sampling preparation.

Infrared spectroscopy (IR) can be used to monitor in situ some processes. However aqueous solutions may cause problems in IR measurement.[5] If water is used as either a reagent or a byproduct or a solvent in a process, overwhelming infrared absorption of water will mask most of the useful information from the interested species. In contrast, water is a poor Raman scatterer, which makes Raman preferable to IR when water is present in the processes.[6] This can be explained by the different mechanisms of interaction of electromagnetic radiation with matter in the two spectroscopic techniques. Dipole moment changes during molecular vibrations cause IR absorption, while changes in polarizability associated with molecular vibrations result in Raman scattering. Usually molecules with polar functional groups and asymmetric vibrational modes have stronger absorption in IR and weaker absorption in Raman.[7] In addition to this advantage for aqueous systems, we can use Raman spec-

* To whom correspondence should be addressed. Telephone: (616) 833-3512. Fax: (616) 833−9158. E-mail: john.a.wachter@am.pnu.com.

[†] Michigan State University

[‡] Chemical Process Research and Development, Pharmacia Corporation.

[§] Analytical Research and Development, Pharmacia Corporation.

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troscopy to perform remote sensing since the fiber optic probe can be made as long as 200 m.[8] By comparison, the probe in attenuated total reflectance (ATR)-FTIR can reach no further than 2−3 m.[9] Remote sensing is especially useful in hazardous environments where operators can work safely by staying far from the reactor. This study employed Raman spectroscopy coupled with a fiber optic probe to study the polymorphic transformation of progesterone at a laboratory scale of less than 1 L. The results from this study will be applied to a production-scale process of several thousand liters.

Progesterone has five known polymorphs.[10] For the range of conditions considered in this study, only Form I and Form II will be produced. In this work, we utilized Raman spectroscopy to monitor the interconversion between these two different polymorphs. To verify our intepretation of the Raman spectra, several samples were also characterized by DSC and XRD and compared to known reference standards of these two polymorphs. Raman spectroscopy was also employed to monitor in situ the solvent-mediated phase transformation of progesterone from Form II to Form I.

Experimental Section

The crude progesterone for this study was prepared at Pharmacia Corporation. Recrystallizations of progesterone were carried out in a 1-L automatically controlled LabMax reactor at different temperatures ranging from 5 to 45 °C. The process which we analyzed involved 2 g of progesterone dissolved in 25 mL of organic solvent. This solution was then added to 500 mL of distilled water, which was kept at a constant temperature in the LabMax reactor. Due to the heat released by mixing water with the progesterone solution and crystallization, a jacket supplied with a cooling fluid and close temperature control provided by LabMax were necessary to keep the temperature within 1° of the set-point. Following the completion of the addition, the system was allowed to stir isothermally for several hours. During this post-addition stir period, the polymorphic transformation from Form II (kinetically favored) to Form I (thermodynamically favored) was semi-continuously observed through in situ monitoring with a HoloLab Series 5000 Raman spectrometer coupled to a remote fiber-optic probe equipped with an immersion optic.

Details of the HoloLab 5000 have been described previously.[11,12] Briefly, the 785 nm output from a 250 mW frequency stabilized diode laser is transmitted through a fiber optic cable to the remote probe. The laser light is focused into slurry, using the immersion optic. Backscattered Raman light is collected by the same optic and transmitted back to the spectrometer for analysis. This spectrometer is equipped with a "HoloPlex" holographic transmission grating and a TE-cooled charge coupled device (CCD) for detection. The HoloPlex grating allows simultaneous full spectral coverage (100 to 3450 cm^{-1}) at high spectral resolution (4 cm^{-1}) with no moving parts.

Solid-state Raman analysis of pure Form I and Form II was carried out in aluminum trays. It is important to note that sample preparation for this Raman analysis was minimal. Unlike grinding and compression required for IR and XRD analysis (which may alter the polymorph of the material being analyzed), the sample preparation for Raman analysis consists simply of illuminating the crystalline sample in the vicinity of the focal point of the incident laser from the fiber optic probe. For analysis of prepared mixtures of these two polymorphs, the sample preparation was only slightly more complex. Varying relative amounts of Form I and Form II were stirred in water, and Raman spectra of the aqueous slurry were collected. This slurry technique was used in order to homogeneously distribute the Form I and Form II materials. Because of the very limited solubility of progesterone in water, there was no concern of polymorphic transformation during the 3−5 min required to collect any individual Raman spectrum. Spectra were collected for a total of 11 samples of varying relative compositions of Form I and Form II.

The thermal properties of the polymorphs were characterized on a differential scanning calorimeter (TA instrument model DSC 2920 modulated DSC) and a thermogravimetric analyzer (TA instrument model HiRes TGA 2950).

X-ray powder diffraction patterns of progesterone polymorphs were recorded with a Rigaku Miniflex X-ray diffractometer. The instrument operates using the Cu Kal emission with a nickel filter at 1.50451. For each sample, fine powders were packed in an aluminum sample plate with a 2 mm indent or placed on a zero-background sample holder.

Results and Discussion

Crystallization of progesterone at various temperatures or various time length of mixing may yield different polymorphs. The XRD spectra (Figure 1(A) and (B)) show significant differences between the diffraction patterns of the two polymorphs. At a heating rate of 5 °C/min, the DSC thermodiagrams of Form I and Form II (Figure 2 (A) and (B)) present sharp endotherms with melting points at 129.1 and 121.2 °C, respectively, TGA of both forms indicate no weight loss. The melting points of Form I and Form II are comparable with their literature values.[13]

The Raman spectra of the solid state Form I and Form II are presented in Figure 3. Both XRD and DSC analysis had previously established the solid-phase identity of these samples. There are several spectral regions where peak shifts are present which differentiate between Form I and Form II of progesterone. No attempts were made to assign all of the peaks over the entire spectral region. The region where the most significant difference exists between the Raman spectra of the Form I and the Form II material can be explained by

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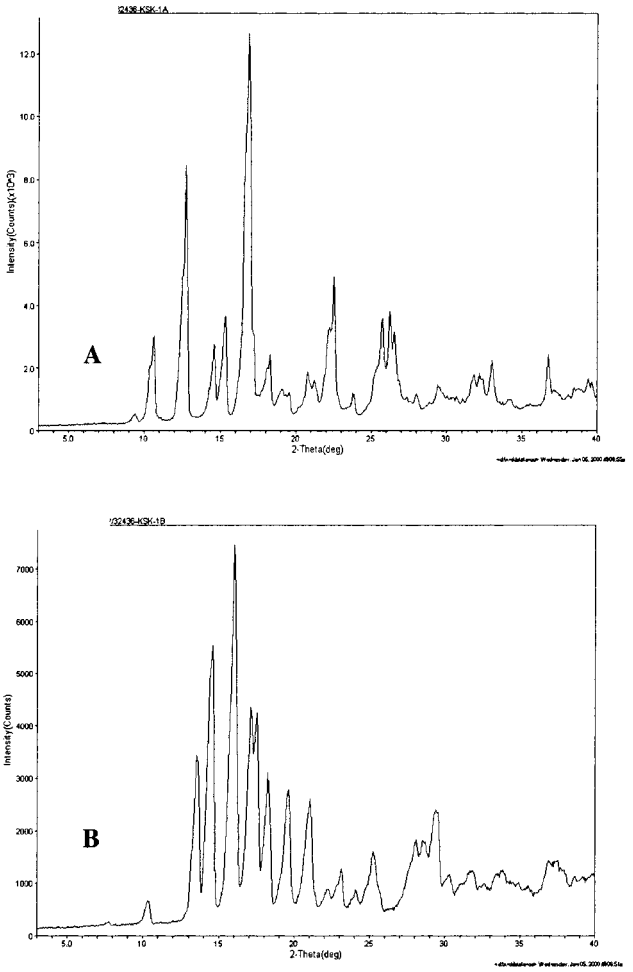

Figure 1. **X-ray diffraction patterns of progesterone polymorphs: (A) Form I and (B) Form II.**

conjugation theory.[14] As shown in the structure of progesterone (Figure 4), the conjugation between the C═O at position 3 and C═C at position 4 results in the delocalization of the π electrons and reduces the double-bond character of both bonds. This causes the absorption at lower wavenumbers than those of the unconjugated systems. As a result of this interpretation, 1617 cm^{-1} is assigned to C═C stretching and 1662 cm^{-1} to C═O stretching for Form I, while 1616 cm^{-1} is from C═C stretching and 1667 cm^{-1} from C═O stretching for Form II. The unconjugated C═O bond at position 20 contributes to the absorption at 1698 cm^{-1} for Form I and 1706 cm^{-1} for Form II. These Raman peak shifts indicate that the two crystalline polymorphs may have different modes of packing. Two ends of the molecule, functional groups around positions 3 and 20, which are less rigid than the rings, are likely affected the most by the different packing environments.

The peak with the largest shift between the two polymorphs (1662 cm^{-1} for Form I and 1667 cm^{-1} for Form II) was used to set up a calibration (Figure 5) to correlate the concentration with the peak position. The spectra of 11 mixtures of varying relative amounts of Form I and Form II show significant peak shift from pure Form I to pure Form

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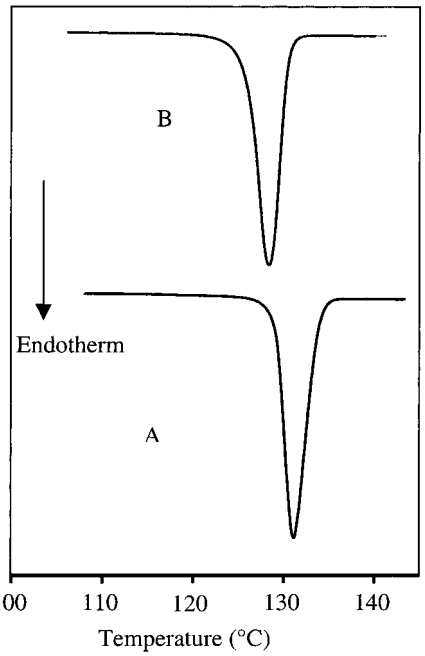

Figure 2. **DSC thermodiagrams of progesterone: (A) Form I and (B) Form II. The heating rate was 5 °C/min.**

II. This calibration curve in Figure 5 clearly indicates that we can adequately predict the relative amount of Form I to Form II simply by the position of the peak in the region from 1662 to 1667 cm^{-1}. While more sophisticated peak deconvolution techniques exist, this straightforward relationship between peak position and solid-phase composition was adequate for the purposes of this study.

Crystallizations were monitored using Raman spectroscopy over a range of temperatures from 5 to 45 °C. Each crystallization was carried out isothermally and allowed to stir for several hours after the onset of the crystallization. During this process, the solid phase of the system was monitored semi-continuously with in situ Raman spectroscopy. The data collected from one of these crystallizations, carried out at 45 °C, are shown in Figures 6 and 7. As shown in Figure 6, the two bold spectra at 1667 and 1662 cm^{-1}, which bracket all of the other spectra, were collected at the beginning and the end of the crystallization, respectively. The initial peak position at 1667 cm^{-1} indicates that Form II formed at the beginning of the crystallization. With additional post crystallization stir time, the peak shifted gradually to 1662 cm^{-1},which indicates that Form II was gradually transforming to Form I. This process of polymorphic transformation is depicted in Figure 7 in which the concentration profiles, calculated from the calibration curve in Figure 5, are plotted against time.

The polymorphic transformation from Form II to Form I was monitored in situ at different temperatures ranging from 5 to 45 °C. The transformation rates were found to increase as the temperature increases, as shown in Figure 8. This trend suggests that this polymorphic system is monotropic and Form I is the thermodynamically stable form, while Form II is the metastable form.[2] This conclusion was confirmed by the thermal analysis result that showed the melting enthalpy of Form I is greater than that of Form II.[11] The melting

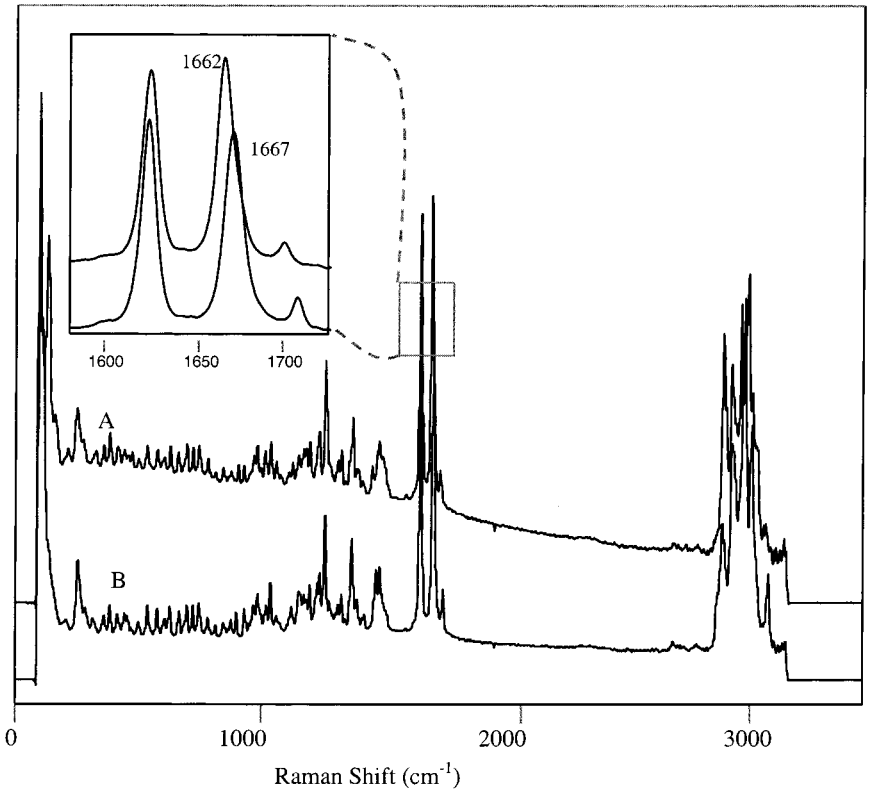

Figure 3. **Raman spectra of solid-state progesterone: (A) Form I and (B) Form II.**

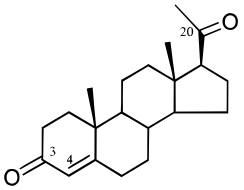

Figure 4. **Molecular structure of progesterone.**

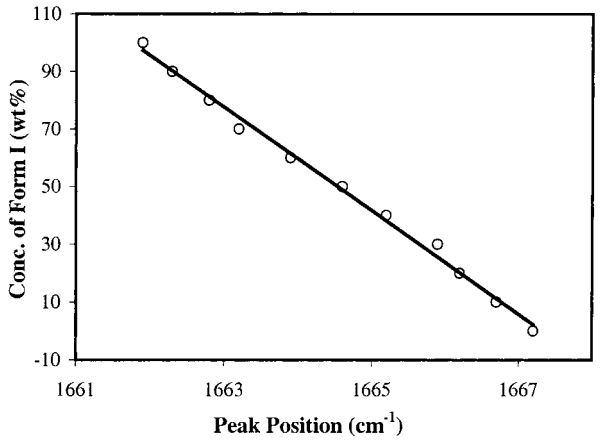

Figure 5. **Calibration curve for the relative concentration of Form I in the mixture of Form I and Form II of progesterone slurried in water.**

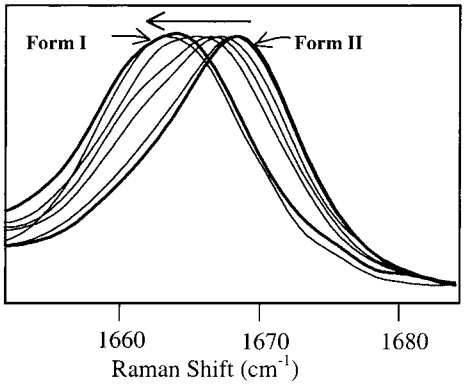

Figure 6. **In situ spectra showing peak shift during the phase transformation process at 45 °C.**

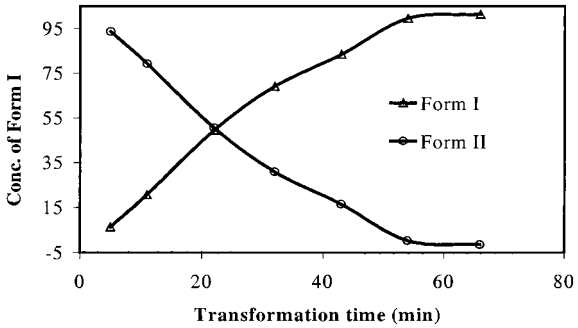

Figure 7. **In situ concentration profiles of Form I and Form II of progesterone throughout the phase transformation process at 45 °C.**

enthalpy rule of Burger states that if the high melting form has the higher melting enthalpy, they are monotrophs. If the high melting form has the lower melting enthalpy, the two forms are enantiotrophs.[16] Therefore, in situ Raman techniques and thermal analysis are consistent in predicting that Form I and Form II are monotrophs. The phase transformation phenomenon observed by Raman spectroscopy is consistent with Ostwald's law of stages, which states that the formation of a metastable phase will precede the

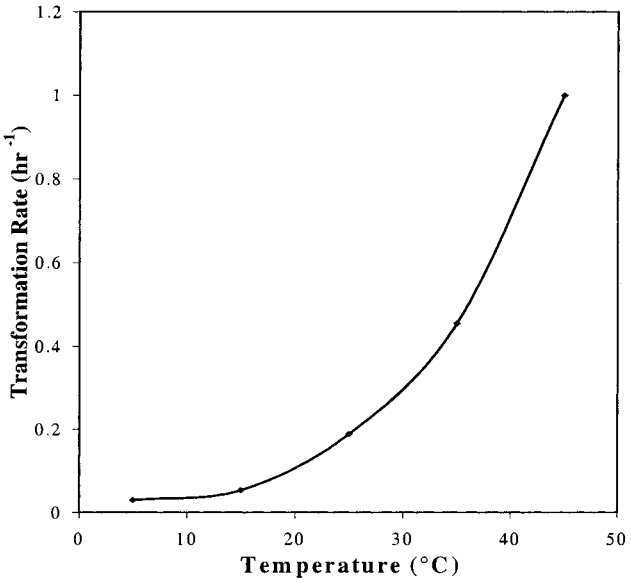

Figure 8. **Temperature dependence of phase transformation rate of progesterone.**

appearance of a thermodynamically stable phase once the supersaturation has spontaneously decreased.[17]

According to Burger,[16] if Form I and Form II are monotrophs and Form I is the stable form, the solubility of Form I will be lower than that of Form II. This is also confirmed by classical nucleation theory, which states that the solid-phase modification that is first formed under given conditions has a higher equilibrium solubility.[18] This is because the nucleation rate is the maximum for those modifications having the lowest surface energy, which is inversely proportional to the equilibrium solubility.[18] The solubility difference between Form I and Form II provides additional insight about the phase transformation process. During the crystallization process, supersaturation is removed with respect to Form II as Form II crystallizes out from the solution as the initial solid phase. At a certain point, the concentration decreases to the solubility of Form II. As the solubility of Form II is higher than that of Form I, the solution is supersaturated with respect to Form I. The solubility difference can be so large that the solubility of Form II lies in the unstable or labile region with respect to Form I, where spontaneous nucleation of Form I occurs. Even if this solubility difference is not so large, it can be reasonably assumed that although Form II is the major solid phase in the mixture, some nuclei of Form I may also be formed.[19] These initial nuclei set the starting point for the transformation process. As the growth of the nuclei consumes the supersaturation of Form I, the solution goes into undersaturated zone with respect to Form II, which makes it possible for Form II to dissolve and produce continuous supersaturation for Form I to grow. The transformation will be complete when all of Form II dissolves and the solution is saturated with respect to Form I.

The advantage of Raman over off-line techniques lies in its ability of monitoring in situ the progress of the phase transformation between polymorphs. By contrast, an off-line analytical technique measures the system several steps removed from the crystallizer. These steps include drying, storage, and sampling that have all been carried out on the material. During these extraneous steps, samples may undergo phase transformation, leading to conclusions from the off-line analysis that might be inaccurate and misleading. On the basis of the powerful insight provided by Raman spectroscopy coupled with an in situ immersion probe, instead of using off-line analytical techniques, we are able to efficiently measure the transformation rate of progesterone from Form II to Form I over a wide range of temperatures in this solvent system. As such, we now fully understand the kinetics of polymorphic transformation of progesterone over a wide range of process temperatures. Consequently, we are now able to specify a process for consistently and reliably producing either of the two known polymorphs.

Conclusions

It is demonstrated that Raman spectroscopy can distinguish between Form I and Form II progesterone crystals. The principal advantage of Raman technique over other methods is the ability to analyze samples in their native state or without the need for sample preparation. More significantly, Raman spectroscopy is proved to be able to monitor in situ the solvent-mediated phase transformation process. Transformation rates were found to increase as the temperature increases, which indicated that Form I and Form II of progesterone are monotrophs. The observations obtained through in situ monitoring with Raman spectroscopy are consistent with Ostwald's rule of stages. Because this system is monotropic, it is expected thermodynamically that Form I has a lower solubility than Form II. The solubility difference and crystallization theory were utilized to explain the mechanism of the phase transformation process. The data collected from in situ monitoring of this system has allowed us to estimate the rate of polymorphic transformation for this monotropic conversion over a wide range of process temperatures.

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