Preparation and Characterization of Solvent Induced Dihydrated, Anhydrous, and Amorphous Paclitaxel

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The control of solid drug properties is one of the current issues in the pharmaceutical industry.[1,2,3] Many drugs have different applications according to characteristics of their different solid states.[4,5,6,7] Sometimes, the solubility of a drug in water or other solvents is largely dependent on its solid properties, such as the degree of hydration.[6,7,8] Moreover, the stability of the drug may have a close relationship with the interconversion of the drug among morphologically different states. Therefore the control of solid states and their characterization of a drug have practical as well as academic importance.[2,3] Paclitaxel, which is one of the most potent anticancer drugs, is relatively complex organic drug having several solid structures. In relation to this, Burt and *et al.* reported the solid-state characterization of the different paclitaxel in their recent studies.[1] According to the studies, there are four different states of solid paclitaxel and these can be characterized using solid-state analytical tools, such as X-ray powder diffractometer (XRPD) and differential scanning calorimetry (DSC). Among these four solid states, only three solid forms are considered to be stable under ambient condition. We will refer to them as dihydrated, anhydrous, and amorphous paclitaxel according to the paper.[1] In the paper, they described the methods to control the morphologies of paclitaxel, in which they used some severe experimental conditions. These methods, however, are not suitable for industrial application because they are not practical for large-scale production. Moreover, the large amount of heat used in some of their schemes can cause degradation of the drug. Therefore, a systematic study is needed to find a new way to control the morphologies of paclitaxel, considering the importance of drug. In this study, we tried to control the morphology of paclitaxel and prepare morphologically different paclitaxels using several common solvents not exposing the system to high temperature. The resulting morphologies were identified by comparing X-ray powder diffraction (XRPD) pattern as well as differential scanning calorimetry (DSC) data with previous ones.[1] The additional powerful and simple methods, solid NMR and infrared spectroscopy (IR) were used to characterize the solid state of paclitaxel. Despite the recent increasing utilization of these methods for characterization of solid drugs, this is the first study to use these methods for solid-state paclitaxels.

Experimental Section

Materials and preparation of the samples. Paclitaxel was purchased from Hauser, Boulder, Co. and used without further purification. All solvents used in this study were HPLC grade. Paclitaxel was dried to constant weight at ambient temperature in a vacuum oven equipped with an external pump.

To investigate the crucial factor to control the morphologies of paclitaxel, the effects of the properties of solvents were closely examined by producing and characterizing three samples (sample I, II, and III) using different solvent systems. First, a re-crystallization method was applied to get different crystalline morphologies of paclitaxel (sample I and sample II). To make sample I, paclitaxel was dissolved in methanol and water was added gradually. The nearly saturated paclitaxel solution was left in the refrigerator at -20 °C. After 24 hours in that condition, needle type precipitation was formed in the bottle. The precipitation was separated from the solvent by filtration. Successive vacuum drying was applied to remove residual solvent. To make sample II, similar procedures were used. In this case, hexane was added to the methanol solution instead of water. The same procedures were utilized for re-crystallization and separation of the re-crystallized precipitation. In the case of sample III, the paclitaxel was dissolved in methylene chloride and the solvent was allowed to evaporate. Successive vacuum drying was then used to remove residual solvent. The effects of several other solvents systems covering wide range of polarities including methanol/water (80/20, v/v), methanol, acetone, diethyl ether, ethyl acetate, and chloroform were investigated in terms of the resulting morphologies of paclitaxel. A complete dissolution of paclitaxel in each solvent, as in the case of sample III, the solution was left in ambient condition to allow evaporation of the solvents. Successive vacuum drying was then applied to remove the residual solvents.

X-ray powder diffraction. The X-ray powder diffraction (XRPD) patterns for sample I, II, and III were obtained with a model XRD-2000 X-ray powder diffractometer, Rigaku (Japan). The measurements were performed in the 5 to 25° 2θ range at a rate of 0.5°2θ/min using Cu$K\alpha$ radiation (45 kV, 40 mA) as X-ray source. The amount of each sample

was about 50 mg.

Thermal analysis. Differential scanning calorimetry (DSC) measurements of prepared samples were carried out with the model of DSC-7, Perkin-Elmer (USA) calibrated with indium. Approximately 5 mg of the sample was placed on the aluminum pans for each measurement. The cell was purged with nitrogen flowing at 40 mL/min. All measurements were made in the range of 25 °C to 300 °C with a scan rate of 20 °C/min.

Solid-state NMR spectroscopy. Cross polarization/magic angle spinning (CP/MAS) ^{13}C solid state NMR experiments were carried out with Bruker DSX-300 NMR spectrometer (Germany) operating at 75.6 MHz. Standard pulse sequences and phase programs supplied by Bruker with NMR spectrometer were used to obtain CP/MAS ^{13}C NMR spectra. For each sample, approximately 250 mg sample was spun at about 5 KHz in a 4 mm rotor. Cross polarization was achieved with contact time of 1 ms. This process was followed by data acquisition over 35 ms with high power proton decoupling. A three second relaxation delay was used. The number of transients varied from 1 to 2000, depending on sample properties. Spectra were referenced to adamantane, using glycine as a secondary reference (carbonyl signal of glycine was 176.04 ppm).

Infrared spectroscopy. Fourier transform Infrared (FT-IR) spectroscopy was carried out with FTS 185, BIO-RAD (USA). IR spectra of sample were obtained using KBr pellet prepared with a press after careful grinding of each sample with KBr. Spectral width was 500-4000 cm^{-1} and spectral resolution was 2 cm^{-1}.

Results and Discussion

First of all, to identify the morphologies of the prepared samples, XRPD and DSC data of the samples were compared with those of the previous study.[1] In comparison with the previous study, it can be easily noticed that XRPD patterns of sample I and II were identical to those of dihydrated and anhydrous paclitaxel,[1] respectively. The only difference was the improved S/N ratio of our data which may be attributed to the quantity of samples. For sample III, we could see no meaningful peaks in the XRPD patterns. Therefore, sample III is amorphous state paclitaxel. DSC analysis of the above samples also gave us the same results. The thermal events of three different samples having different solid-states in this study are shown in Table 1 along with comparison with the data in the previous study. From Table 1, we can see fairly good correspondence of thermal events between two samples of a given morphology of paclitaxel. All these results indicate that sample I, sample II, and sample III can be identified as dihydrated, anhydrous, and amorphous paclitaxel.

Above experimental results suggest that the properties of solvents in the process can be decisive factors in determining the resulting morphologies of solid paclitaxel. This consideration may have some relationship with several other studies of solution structures of paclitaxel in different solvents including interesting topics such as the hydrophobic collapse model.[9] To examine more closely the effect of solvent polarities on the morphologies of paclitaxel, we took the same measurements on the samples resulting from evaporating the solvents from the solutions made by several different solvents including methanol/water (80/20, v/v), methanol, acetone, diethyl ether, ethyl acetate and chloroform. In above solvent treatments, we found that we could get different morphologies of paclitaxel using different solvents. We got dihydrated paclitaxel by dissolving paclitaxel in methanol/water (80/20, v/v) followed by successive evaporating of the solvents. However, when we used only methanol, we got mixed morphologies of paclitaxel made of both the dihydrated and amorphous forms. Anhydrous paclitaxel was made by evaporating respective solvent after dissolving in acetone, diethyl ether and ethyl acetate. Dissolving paclitaxel in chloroform and succesive evaporation of the solvent resulted in an amorphous form. The results were summarized in Table 2.

Recently, spectroscopic studies on pharmaceutical products are growing.[10,11] Particularly, solid-state NMR is recognized as powerful techniques to characterize the morphologies of drugs.[12,13,14] In this study, we tried to characterize three morphologically different paclitaxels using spectroscopic methods such as solid state NMR and FT-IR. Figure 1 shows the

Table 2. The effect of solvents on the morphologies of paclitaxel

Solvent	Morphology
Methanol+water (8+2)	Dihydrated
Methanol	Dihydrated + Amorphous
Acetone	Anhydrous
Diethyl ether	Anhydrous
Ethyl acetate	Anhydrous
Chloroform	Amorphous
Methylene chloride	Amorphous

Table 1. Summary of thermal events observed by DSC for paclitaxels made in present and previous study

Samples		Peak I (°C)	Peak II (°C)	Glass transition (°C)	Solid-Solid Transition (°C)	Melting Transition (°C)
Present Study	Sample I	80	117	–	165	220
	Sample II	–	–	–	–	220
	Sample III	–	–	150	–	–
Previous Study (Ref. 1)	Dihydrated	85	119	–	168	221
	Anhydrous	–	–	–	–	223
	Amorphous	–	–	153	–	–

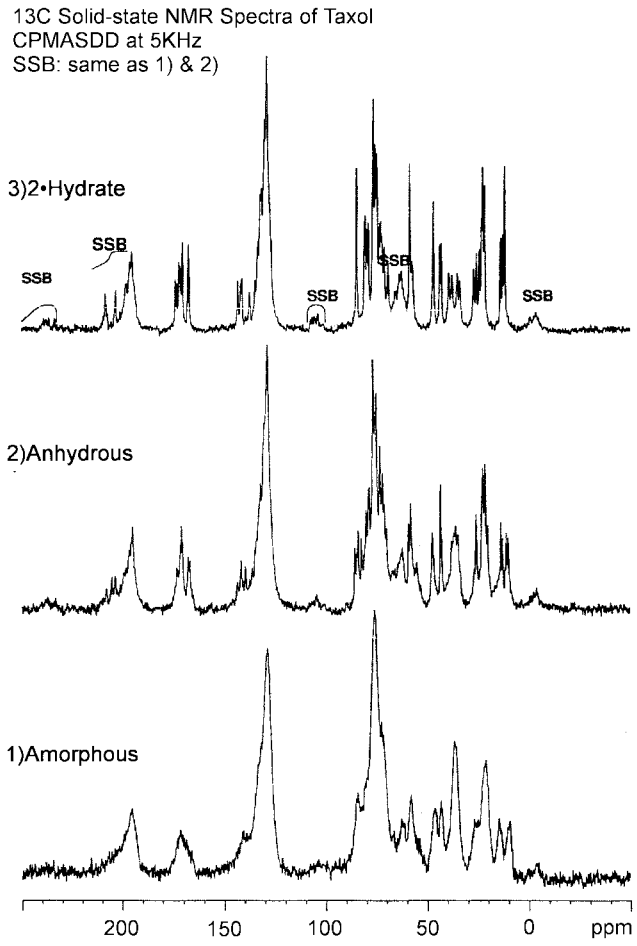

Figure 1. The ^{13}C CP/MAS Solid state NMR spectra of dihydrated, anhydrous and amorphous paclitaxels. SSB means spinning side band.

solid-state ^{13}C-NMR spectra of dihydrated, anhydrous and amorphous paclitaxels. The spectrum of dihydrated paclitaxel shows sharper and more distinctive peaks for all kinds of carbon resonance than the other forms. The NMR peaks for anhydrous paclitaxel are sharper than those of amorphous paclitaxel. In addition to the sharpness, we can see the pattern of peak splitting is also different from sample to sample. It can be recognized that the changing in crystal packing results in significantly different magnetic environments for some of the carbon nuclei and causes the change in peak patterns.

Infrared spectroscopy is also quite useful for the analysis of solids, because it can be performed on small amounts of the solid substance[3,10,13] with relatively easy operation. In this study, we found that it also could be used to determine the morphologies of paclitaxel solids. Figure 2 shows the spectra of dihydrated, anhydrous, and amorphous paclitaxel. Clearly these forms have different spectra in several regions. For example, dihydrated and anhydrous paclitaxel show two carbonyl absorptions near 1720 cm^{-1}, whereas the amorphous form shows only single carbonyl absorption. Other absorption peaks (near 3000, 1650 and 1522 cm^{-1}) also show different characteristic patterns to be used to discriminate different polymorphs of paclitaxel.

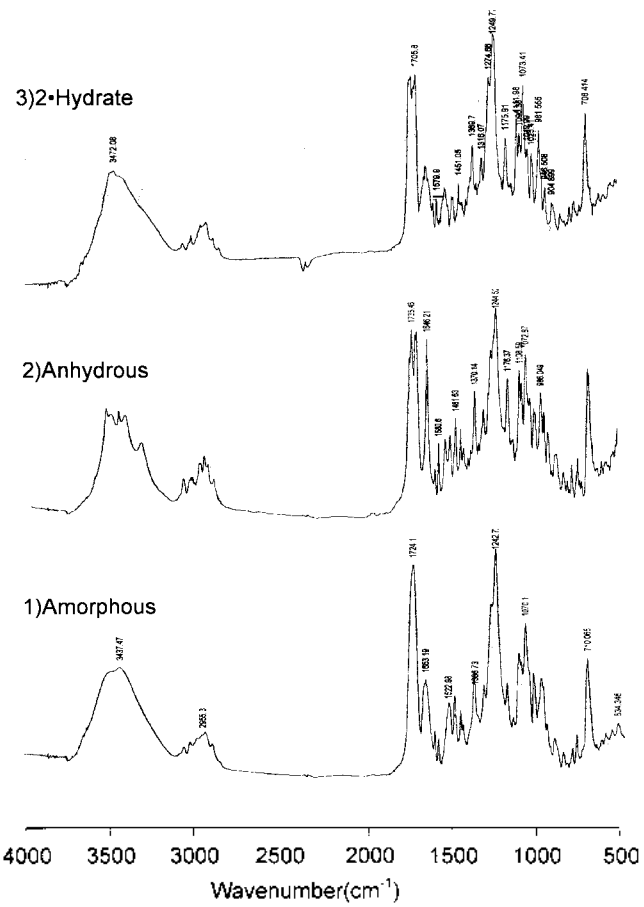

Figure 2. The IR spectra of dihydrated, anhydrous, and amorphous paclitaxels.

Concluding Remarks

Though the controls of morphologies of solid-state paclitaxel have been suggested in previous study,[1] those are considered not to be practical because of the severe processing conditions. In this study, we found that solvent treatment of paclitaxel was a convenient method to control the morphologies of paclitaxel. Methylene chloride was an efficient solvent to change the paclitaxel morphology into an amorphous form. Anhydrous paclitaxel was simply made by using relatively anhydrous non-polar solvents. In a similar way, dihydrated paclitaxel was made by dissolving paclitaxel in methanol containing a small amount of water. As convenient methods to characterize morphologies of paclitaxel, solid state NMR and FT-IR were newly introduced. This study shows that solid-state NMR and FT-IR are convenient methods to characterize the morphology of paclitaxel.

References

1. Liggins, R. T.; Hunter, W. L.; Burt, H. M. *J. Pharm. Sci.* **1997**, *86*, 1458.
2. Byrn, S. R.; Pfeiffer, R. R.; Ganey, M.; Hoiberg, C.; Poochikian, G. *Pharm. Res.* **1995**, *12*, 945.
3. Byrn, S. R.; Pfeiffer, R. R.; Stephenson, G.; Grant, D. J.

W.; Gleason, W. B. *Chem. Mater.* **1994**, *6*, 1148.
4. Giordano, F.; Gazzaniga, A.; Moyano, J. R.; Ventura, P.; Zanol, M.; Peveri, T.; Carima, L. *J. Pharm. Sci.* **1998**, *87*, 333.
5. Caira, N. R.; Zanol, M.; Peveri, T.; Gazzaniga, A.; Giordano, F. *J. Pharm. Sci.* **1998**, *87*, 1608.
6. Gao, P. *Pharm. Res.* **1998**, *15*, 1425.
7. Reutzel, S. M.; Russell, V. A. *J. Pharm. Sci.* **1998**, *87*, 1568.
8. Suryanarayanan, R.; Wiedmann, T. S. *Pharm. Res.* **1990**, *7*, 184.
9. Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Mitscher, L. A. *J. Am.Chem. Soc.* **1993**, *115*, 11650.
10. Cassanas, A. T.; Nurit, J.; Pauvert, B.; Bouassab, A.; Rambaud, J.; Chevallet, P. *J. Pharm. Sci.* **1994**, *83*, 1437.
11. Middleton, D. A.; LeDuff, C. S.; Berst, F.; Reid, D. G. *J. Pharm. Sci.* **1997**, *86*, 1400.
12. Byrn, S. R.; Gray, G.; Pfeiffer, R. R.; Frye, J. *J. Pharm. Sci.* **1985**, *74*, 565.
13. Bugay, D. E. *Pharm. Res.* **1993**, *10*, 317.
14. Stephenson, G. A.; Stowell, J. G.; Toma, P. H.; Pfeiffer, R. R.; Byrn, S. R. *J. Pharm. Sci.* **1997**, *86*, 1239.