

Preparation and Dissolution Profiles of the Amorphous, Dihydrated Crystalline, and Anhydrous Crystalline Forms of Paclitaxel

Sang-Hyun Pyo , Jin-Suk Cho , Ho-Joon Choi & Byung-Hee Han

To cite this article: Sang-Hyun Pyo , Jin-Suk Cho , Ho-Joon Choi & Byung-Hee Han (2007) Preparation and Dissolution Profiles of the Amorphous, Dihydrated Crystalline, and Anhydrous Crystalline Forms of Paclitaxel, *Drying Technology*, 25:10, 1759-1767, DOI: [10.1080/07373930701593180](https://doi.org/10.1080/07373930701593180)

To link to this article: <http://dx.doi.org/10.1080/07373930701593180>



Published online: 10 Dec 2007.



Submit your article to this journal [↗](#)



Article views: 109



View related articles [↗](#)



Citing articles: 6 View citing articles [↗](#)

Full Terms & Conditions of access and use can be found at
<http://www.tandfonline.com/action/journalInformation?journalCode=ldrt20>

Preparation and Dissolution Profiles of the Amorphous, Dihydrated Crystalline, and Anhydrous Crystalline Forms of Paclitaxel

Sang-Hyun Pyo,^{1,2} Jin-Suk Cho,¹ Ho-Joon Choi,¹ and Byung-Hee Han²

¹*Samyang Genex Food & Bio Research Center, Yusung-Gu, Daejeon, South Korea*

²*Department of Chemistry, Chungnam National University, Yusung-Gu, Daejeon, South Korea*

The selection of pharmaceutical polymorphisms in the final production step is very important in terms of product recovery, properties, and storage. The amorphous, dihydrated crystalline, and anhydrous crystalline forms of paclitaxel were prepared using precipitation, spray drying, and colloid formation methods. These methods were found to be highly efficient and convenient, giving high recovery, short processing time, and good stability, as compared with conventional methods such as freeze drying, evaporation, recrystallization, and melting. The polymorphic natures of the resulting paclitaxel samples were confirmed by XRPD, IR, TGA, DSC, and SEM. The dissolution rates of the paclitaxel samples were studied in pharmaceutical solvents, which included cotton seed oil, corn oil, tricaprylin, and tributyrin. For each solvent, all of the amorphous paclitaxel samples showed much higher dissolution rates than the dihydrated crystalline, anhydrous crystalline, and commercial forms, and can be used for clinical applications that demand improvements in drug delivery.

Keywords Amorphous paclitaxel; Anhydrous crystalline paclitaxel; Dihydrated crystalline paclitaxel; Dissolution rate; Morphology

INTRODUCTION

Paclitaxel, which is a diterpenoid that was isolated originally from the bark of *Taxus brevifolia*,^[1] is one of the most important anticancer agents; this is due to its unique cytotoxicity mechanism that involves the promotion of the assembly of tubulin and stabilization of the resulting microtubules.^[2–4] Paclitaxel is produced commercially by a semi-synthetic method using 10-deacetylbaccatin III isolated from the leaves of yew trees and also by a plant cell culture method. The selection, in the final production step, of polymorphic products such as the amorphous anhydrous crystalline and hydrated crystalline forms of paclitaxel, is very important with regard to product recovery, properties,

usage, and storage and needs to be controlled with a view to the intended application. Generally, the amorphous form has the advantage of better solubility in pharmaceutical solvents, while the crystalline form is more stable under storage conditions. However, little information is available on the optimal preparation methods. Amorphous paclitaxel can be dissolved in pharmaceutical solvents to increase its clinical efficacy. Paclitaxel, which is a lipophilic anticancer drug, has extremely low solubility in aqueous and most pharmaceutical solvents.^[5] In previous attempts to increase solubility, paclitaxel has been formulated in a vehicle composed of a 50:50 blend of Cremophor EL (CrEL) and absolute ethanol and subsequently diluted with normal saline or dextrose solution (5%) before administration.^[6] However, serious side effects, such as hypersensitivity, neurotoxicity, nephrotoxicity, and extraction of plasticizers from intravenous infusion line catheters, have been noted for this formulation.^[7,8] Thus, alternative delivery systems, which involve liposomes, cyclodextrins, and microspheres, have been examined to increase the solubility of lipophilic drugs.^[9–11] Particle formation methods in the final production step are very important in determining the final product specifications and properties.^[12,13] On the other hand, amorphous paclitaxel can be used to increase the solubility of paclitaxel in pharmaceutical solvents. However, there is little information available on the methods of preparation and the properties of amorphous paclitaxel. In previous reports, amorphous paclitaxel was prepared by heating it up to the melting temperature of 221°C, followed by quench cooling.^[5,14] Protocols that involve evaporation and recrystallization produce large-size particles at low yield.^[15] In the patented invention of Janicki et al.,^[16] amorphous paclitaxel (possibly with crystalline additive) was prepared by freeze drying at –50°C, a system that requires a very low temperature and a long processing time.

The anhydrous crystalline and dihydrated crystalline forms of paclitaxel have better storage properties than the

Correspondence: Sang Hyun Pyo, Department of Chemistry, Chungnam National University, 220 Gung Dong, Yusung Gu, Daejeon 305 764, South Korea; E mail: shpyo@cnu.ac.kr

amorphous form. According to the U.S. patent of Sharma et al.,^[17] hydrated paclitaxel and docetaxel, which is a paclitaxel-derived anticancer agent, can be obtained with a yield of 84% by dissolving and stirring in acetonitrile at 60°C, followed by the addition of water and crystallization.

Unfortunately, these methods use very harsh conditions that can result in product degradation; in addition, they are unsuitable for large-scale production, and they give low yields in the final purification step. Therefore, it is necessary to develop novel large-scale methods for the production of paclitaxel in the amorphous form, as well as in the dihydrate crystalline and anhydrous crystalline forms. In the current study, we describe various methods for obtaining paclitaxel of desired morphology and solubility. These methods, which involve spray drying, precipitation, and colloid formation, are remarkably efficient and convenient, as compared with the conventional methods described above. We expect that improvements in dissolution rates achieved using amorphous fine particles of paclitaxel, as well as new methods for the production of the crystalline form of paclitaxel, will facilitate large-scale production of this drug for clinical applications.

MATERIALS AND METHODS

Materials

Cells of *Taxus chinensis* were cultured in suspension.^[18] Paclitaxel (>99.5% purity) was purified from the cultured cell using solvent extraction, precipitation, ODS-HPLC, and silica-HPLC, as described previously.^[19] Tributyrin, tricaprillin, corn oil, and cotton seed oil were purchased from Sigma Chemical Co. (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade solvents and water were used to prepare paclitaxel specimens with various morphologies for dilution and HPLC analysis.

Methods

Chromatography Analysis

Quantitative analyses of solubility were performed using a HPLC system (HP1090; Hewlett-Packard, Palo Alto, CA) together with a Curosil PFP column (Phenomenex, 4.6 mm × 250 mm, dp = 5 μm). Elution was performed within 30 min with water and acetonitrile using a gradient from 65:35 to 35:65 (flow rate = 1.0 mL/min). The eluent was monitored at 227 nm with a photo diode array detector. The content of residual solvent was analyzed in a GC system (HP-5890; Hewlett-Packard) using a Supelco fused silica capillary column (30 m × 0.53 mm × 5.0 μm film thickness; Sigma-Aldrich, St. Louis, MO).

Spectrometry

X-ray powder diffraction (XRPD) was performed with a Model D/Max3B diffractometer (Rigaku, Tokyo, Japan) in the 5 to 40° 2θ range at a rate of 2° 2θ/min using CuKα radiation (45 kV, 40 mA) as the X-ray source. The appearance

and size of the products were analyzed by scanning electron microscopy (SEM) (JSM-6635F; Jeol, Tokyo, Japan). Fourier transform infrared spectroscopy (FT-IR) was performed with a model FTS-165 spectrometer (Digilab Division, Bio-Rad, Cambridge, MA) using the potassium bromide (KBr) pellet technique.

Thermal Analysis

Thermograms were generated with a differential scanning calorimeter (DSC) (DSC-7; Perkin Elmer, Wellesley, MA), which was calibrated with indium. Approximately 5 mg of each sample was placed on the aluminum pan. The cell was purged with nitrogen at a flow rate of 40 mL/min. All measurements were carried out in the range of 25–300°C with a scan rate of 20°C/min. Thermogravimetric analysis (TGA) was performed with the TGA-7 system (Perkin Elmer, Norwalk, CT). Approximately 5 mg of each sample was weighed and heated to 700°C at a rate of 20°C/min under a nitrogen purge.

Preparation of the Amorphous, Dihydrated Crystalline, and Anhydrous Crystalline Forms of Paclitaxel

The amorphous, dihydrated crystalline, and anhydrous crystalline forms of paclitaxel were prepared by evaporation, precipitation, colloid formation, or spray drying. The first method was carried out using a rotary evaporator (Rotavapor R-124; Büchi, Flawil, Switzerland) in a water bath at 35°C. Paclitaxel was dissolved at a concentration of 10% in dichloromethane, acetone, or ethyl acetate, followed by evaporation under reduced pressure. The paclitaxel that coated the surface of the evaporation flask was recovered and crushed (Table 1, samples A, J, and K).

The second method of preparation used precipitation with two types of solvent. Paclitaxel was dissolved at a concentration of 10% in a solvent such as dichloromethane, tetrahydrofuran (THF), or acetone, and the solution was added slowly to a precipitation solvent such as n-hexane, n-pentane, or water at a 10- to 15-fold ratio, to obtain the precipitate, which was filtered (Table 1, samples B, C, D, E, G, H, and L).

The third method of preparation involved colloid formation. Paclitaxel was dissolved in acetone and evaporated under reduced pressure to remove residual solvents. The colloidal solution was formed by adding acetone at a ratio of 100% (w/v) while rotating the evaporation bowl. Paclitaxel was dispersed (but not dissolved), and the white solution was evaporated using the same method. Fine particles were easily recovered from the evaporation bowl without the coating problem in the first method (Table 1, sample I).

The fourth method involved spray drying (Table 1, sample F).^[20] Paclitaxel was dissolved at a concentration of 10% in dichloromethane and loaded onto a mini spray dryer (B-191; Büchi, Flawil, Switzerland), which was heated to 75°C inlet temperature (Table 1, sample F). The spray drying was carried out at the liquid feed rate

TABLE 1
Comparison of conditions for the preparation of the various morphologies of paclitaxel

| Sample | Solvent condition | | Preparation method | Morphology | Residual solvent (ppm) | |
|--------|-------------------|----------------|--------------------------------|-----------------------|------------------------|-----------|
| | A ^e | B ^f | | | Solvent A | Solvent B |
| A | DCM | | Evaporation ^a | Amorphous | 410.9 | |
| B | DCM | Hexane | Precipitation ^b | Amorphous | 22.2 | 108.3 |
| C | DCM | Pentane | Precipitation ^b | Amorphous | 34.5 | 14.5 |
| D | THF | Hexane | Precipitation ^b | Amorphous | 61.0 | 54.3 |
| E | THF | Pentane | Precipitation ^b | Amorphous | 11.2 | 57.0 |
| F | DCM | | Spray dry ^d | Amorphous | 41.5 | |
| G | THF | Water | Precipitation ^b | Dihydrate crystalline | 415.1 | |
| H | Acetone | Water | Precipitation ^b | Dihydrate crystalline | 11.6 | |
| I | Acetone | | Colloid formation ^c | Anhydrous crystalline | 11.7 | |
| J | Acetone | | Evaporation ^a | Anhydrous crystalline | 377.4 | |
| K | EA | | Evaporation ^a | Anhydrous crystalline | 2,437.6 | |
| L | Acetone | Pentane | Precipitation ^b | Anhydrous crystalline | <10.0 | 131.7 |

^aAfter dissolving in solvent A, the solution was evaporated.

^bAfter dissolving in solvent A, the solution was added to solvent B to obtain the precipitate, which was filtered.

^cAfter formation of the colloid in solvent A, the solution was evaporated.

^dAfter dissolving in solvent A, the solution was loaded onto a spray dryer.

^eSolvent A is the dissolving solvent (DCM, dichloromethane; THF, tetrahydrofuran; EA, ethylacetate).

^fSolvent B is the precipitation solvent.

of 10 mL/min, drying aspiration flow rate of 25 m³/h, and compressed nitrogen spray flow rate of 600 m³/h. To remove residual solvents, the paclitaxel samples obtained using the four different methods were dried in a vacuum dryer for 3 days at 40°C.

Determination of Dissolution Rates

The method used for studying the dissolution rates of paclitaxel was a modified version of that described previously.^[10] Paclitaxel samples from each preparation protocol were added to the selected pharmaceutical solvents, followed by vortexing of the solution for 2 min. The solution was centrifuged at 10,000 × *g* for 10 min, and the supernatant was withdrawn and diluted in methanol or n-propanol, for the HPLC analysis of paclitaxel solubility. The dissolution rate profile of paclitaxel in cotton seed oil was obtained by analysis of samples that were shaken at 150 rpm for 48 h at 20°C.

RESULTS AND DISCUSSION

Preparation and Residual Solvent

Paclitaxel of >99.5% purity, which was obtained from plant cell cultures^[18] and subjected to purification as

described previously,^[19] was used in all the experiments. The comparisons of the different methods of paclitaxel preparation are summarized in Table 1. The properties of these samples were analyzed and confirmed by XRPD, thermal analysis, FT-IR, SEM, and solubility testing. Samples of the various paclitaxel morphologies were obtained by the application of various solvents and methods. Under conditions that used the same solvent, the physicochemical properties, which include particle size, appearance, and amount of residual solvent, differed. In the case of dichloromethane, amorphous paclitaxel was obtained using every protocol. The dihydrated crystalline form of paclitaxel was obtained by precipitation in water after dissolving in either acetone or THF, followed by filtration. Anhydrous crystalline paclitaxel was also obtained by these methods using other solvents, such as acetone and ethylacetate.

The recovery of paclitaxel by evaporation under reduced pressure is much more difficult than recovery by the precipitation method, due to sample coating of the surface of the flask. However, paclitaxel recovery in the precipitation method was easily achieved by simple filtration. In addition, the anhydrous paclitaxel obtained by colloid formation was of small particle size and had lower residual solvent content after vacuum drying. Unlike the other

solvents, the acetone-formed colloidal solution of paclitaxel was evenly dispersed at a paclitaxel concentration of 100% (w/v), and the colloid form of highly concentrated paclitaxel (> 1:1, w/v) was evenly dispersed. The resulting colloidal paclitaxel was easily recovered by evaporation under reduced pressure without product coating of the surface of the flask.

The levels of residual solvents were analyzed after vacuum drying for 3 days (Table 1). Paclitaxel for clinical use should be dried to minimize the amount of residual solvent, in accordance with the International Conference on Harmonization (ICH) guideline Q3C, titled, "Impurities: Residual Solvents, Which Depend on Solvents." Thus, the effective removal of residual solvents is an important processing factor. In samples A, J, and K, which were prepared by the conventional method, there were high levels of residual solvents, although these levels were lower than the recommended levels in the ICH guideline. Samples B, C, D, E, G, H, and L, which were obtained by the precipitation method, tended to have low residual solvent levels, with the exception of sample G. Sample F, which was obtained by the spray-drying method, and sample I, which was obtained by colloid formation, showed low levels of residual solvents. In general, the dihydrated crystalline form contains $4.47 \pm 0.72\%$ (w/w) moisture,^[5] which can be readily removed by simple vacuum drying.

Using the different preparation methods (precipitation, spray drying, and colloid formation), the amorphous, anhydrous crystalline, and dihydrated crystalline form of paclitaxel were obtained with high yields, short processing times, good reproducibility, and high efficacy. In previous studies,^[5,14] amorphous paclitaxel was prepared by heating it to the melting temperature of 221°C, followed by quench cooling, as well as by freeze drying at -50°C. Melting at 221°C constitutes a harsh treatment that may lead to product decomposition, and freeze drying involves subjecting the product to -50°C for long periods of time.

In general, the crystalline forms of paclitaxel and docetaxel have been prepared by dissolving at 40–60°C in a solvent, such as methanol, ethanol, acetone, or acetonitrile, followed by recrystallization for several days at 0–4°C.^[17,21,22] In these methods, paclitaxel becomes oversaturated when subjected to high temperature and stirring for 1 h; i.e., the conditions necessary for high yields after crystallization. However, paclitaxel is unstable, especially in solutions of methanol, ethanol, and acetonitrile, as well as in non-neutral aqueous solutions.^[9] Paclitaxel may be degraded and epimerized under these conditions, which involve dissolving, heating, and stirring at 60°C for at least 1 h. In reality, paclitaxel is rapidly degraded and epimerized to various compounds, such as 10-deacetylpaclitaxel and 7-epipaclitaxel, in methanol and acetonitrile solutions at 50°C (data not shown). Therefore, these methods are not

suitable for large-scale production, give low yields, have poor long-term storage, and can generate degraded paclitaxel due to the high temperatures used. It is very difficult to produce paclitaxel of >99.5% purity using these methods, which have been claimed as unique and useful technologies.^[17,21,22]

Thus, the methods of spray drying, precipitation, and colloid formation are remarkably efficient and convenient techniques when compared with conventional methods, such as evaporation, recrystallization, and melting.

X-Ray Powder Diffraction, Thermal Analysis, and FT-IR Analysis

All of the sample morphologies were confirmed by the XRPD technique; representative spectra are shown in Fig. 1. The XRPD patterns of the amorphous and crystalline forms were significantly different. The pattern of the amorphous form showed a broad line, while many sharp peaks were noted for the dihydrate and anhydrous crystalline forms. Taking into consideration the previous study,^[5] the differences in patterns between dihydrate and anhydrous paclitaxel can be easily confirmed by comparing the peak intensities (e.g., 6.1, 9.5, 13.2, 13.8° 2θ).

The DSC data for all of the samples were obtained between 25 and 250°C (Fig. 2). The DSC thermograms of the amorphous, dihydrated crystalline, and anhydrous crystalline forms were significantly different. These results are in accordance with those of the previous study.^[5] There was no significant single melting endotherm around 223°C, as seen for the anhydrous crystalline form, and there were no significant peaks at 70–140°C, as noted for the dihydrate

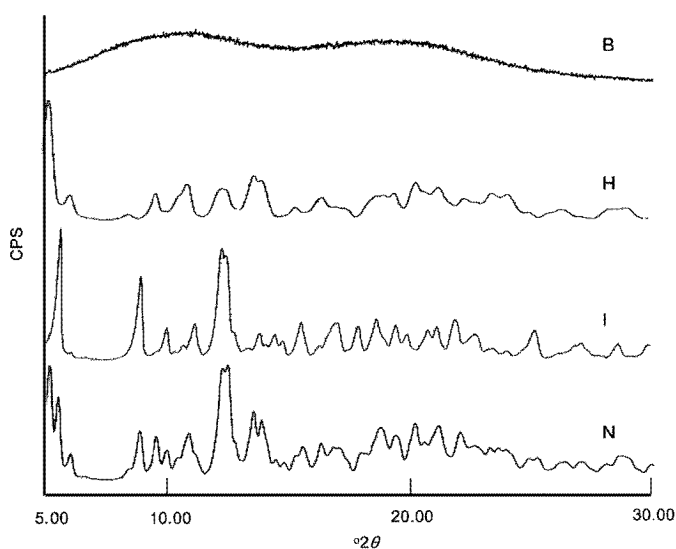


FIG. 1. XRPD analysis of paclitaxel morphologies. Representative spectra are shown for amorphous paclitaxel (B), dihydrated crystalline paclitaxel (H), anhydrous crystalline paclitaxel (I), and mixed crystalline paclitaxel (N).

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.