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RAPID COMMUNICATION

Characterization of solvent induced crystalline and amorphous homoharringtonine

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Abstract−The morphologies of homoharringtonine were conveniently controlled by solvent treatment and the morphologies of homoharringtonine were characterized by XRD and SEM. Crystalline homoharringtonine was simply made by dissolving homoharringtonine in a special polar solvent containing a small amount of water (methanol/water= 98/2, v/v). On the other hand, amorphous homoharringtonine was made by dissolving homoharringtonine in relatively non-polar solvent (methylene chloride/methanol=98/2, v/v). Amorphous homoharringtonine, with a fine particle size, was obtained by dissolving in methylene chloride/methanol (98/2, v/v), following by spray drying. Also, residual solvents, methanol and methylene chloride, were easily removed to less than concentration limit in ICH (International Conference on Harmonisation) guidance by spray drying and successive drying in a vacuum oven. Amorphous homoharringtonine was more soluble in water than crystalline homoharringtonine, and the water content of amorphous homoharringtonine was less changeable than crystalline homoharringtonine at given humidity (95 RH%) during storage. This information is very useful for production and quality control of pharmaceuticals in the commercialization step.

Key words: Crystalline and Amorphous Homoharringtonine, Solubility, Water Content, Residual Solvent, Spray Drying

INTRODUCTION

Homoharringtonine (HHT) is a plant alkaloid isolated from the genus *Cephalotaxus*. It is an alkyl-substituted succinic acid ester of cephalotaxine [1,2] and possesses antileukemic activity [3-6]. Several researchers have investigated the antineoplastic mechanism of HHT and related alkaloids; all have concluded that these drugs inhibit protein biosynthesis in a dose- and time-dependent manner by acting on the ribosomes of cancer cells. HHT and congeners cause the breakdown of polyribosomes to monosomes, the release of completed globin chains, and delayed inhibition of the initiation of protein synthesis without affecting chain elongation. HHT has been tested clinically in advanced breast cancer, acute myelogenous leukemia, myelodysplastic syndrome (MDS), and MDS evolving to acute myeloid leukemia [7-11]. Although the chemical synthesis of cephalotaxine and its esters has been reported, extraction from plants is still the major source of HHT [12,13].

The solid-state properties of active pharmaceutical ingredients (APIs) are very important information in the production, quality control, and formulation of APIs for purposes of commercialization [14,15]. Many drugs have different applications according to characteristics of their different solid states. Sometimes, the solubility of a drug in water or other solvents is largely dependent on its solid properties [16,17]. Moreover, the stability of the drug may have a close relationship with the inter-conversion 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. A systematic study is needed to find a practical way to control the morphologies of pharmacologically active materials for drug products. In this study, we first tried to control the morphology of HHT and prepare morphologically different HHT using solvent treatment not exposing the system to severe processing conditions. We also report the solubility in water and water content of preparing HHT. An additional powerful and efficient method, spray drying, was used to remove the residual solvents in HHT. This information is very useful for production, quality control, and formulation of HHT for purposes of commercialization.

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MATERIALS AND METHODS

1. Materials and Preparation of the Samples

Homoharringtonine (HHT) was purchased from Taihua Natural Plant Pharmaceutical Co. (Guilin, China) and used without further purification. All solvents used in this study were HPLC grade. Homoharringtonine was dried to constant weight at ambient temperature in a vacuum oven equipped with an external pump. Three samples (sample A, B, and C) were formed from the solutions of HHT. For samples A and B, HHT was dissolved in methanol/water (98/2, v/v) solution and methylene chloride/methanol (98/2, v/v) solution, and these samples were dried by evaporation. For sample C, HHT was dissolved in methylene chloride/methanol (98/2, v/v) solution, which is the same solvent with sample B, and this sample was dried by spray drying. These samples were further dried to constant weight at 60 °C in a vacuum oven equipped with an external pump. Samples A, B, and C were characterized and their morphologies were determined. In the case of sample C, the HHT was dissolved in methylene chloride/methanol (98/2, v/v) solution, and the solution was loaded onto a spray dryer (SD-1000, EYELA, Japan), which was heated to an inlet temperature 70 °C.

2. Analysis of SEM (Scanning Electron Microscopy) and XRD (X-ray Diffractometer)

The morphology of HHT was analyzed by scanning electron microscopy (JSM-6335F, Jeol, Japan) and X-ray diffractometer (D/

WAX-3B, Rigaku, Japan). The measurements of XRD were performed in the 5 to 40° 2θ range at a rate of 2°2θ/min using CuKα radiation (45 kV, 40 mA) as X-ray source. The amount of each sample was about 50 mg.

3. Karl Fischer Titration

Water content was determined with a Karl Fischer Titrator (658 KF, Wetrohm, Swiss). To remove the residual moisture in the solvent, it was carried out with KF-reagent and the end point was setup. The titrant was standardized with 20 μL injections of distilled water. The amount of each sample was about 20 mg.

4. Dissolution Test

Solubility measurements of prepared samples were carried out with HPLC (Waters) using C18 column (4.6×250 mm, 5 μm, Shiseido). The elution was performed with a methanol/0.1 M ammonium formate gradient from 25 : 75 (v/v) to 45 : 55 (v/v) at a flow rate of 1.0 mL/min. Approximately 1 mg of each HHT was weighed, put into 50 mL volumetric flask, filled with water, and incubated at 37 °C with shaking for 20 h. Approximately 2 mL of each sample filtered with 0.4 μm filter.

5. Analysis of Residual Solvents

Gas chromatography system (GC2014ATFSP, Shimadzu, Japan) with DB-5 column (0.32 mm ID×30 m, 0.25 μm film) was used for quantitative analysis of residual solvents (methylene chloride and methanol). The conditions were as follows: FID with a temperature program of 40 °C to 100 °C (10 °C/min), hold for 2 min, up to 300 °C (30 °C/min) fold for 15 min, flow rate 3 mL/min with helium.

RESULTS AND DISCUSSION

1. Effect of Solvents and Drying Methods on HHT Morphology

The morphologies of HHT were conveniently controlled by solvent treatment, and the morphologies of HHT were characterized by XRD and SEM. Crystalline HHT was simply made by dissolving HHT in a special polar solvent containing a small amount of

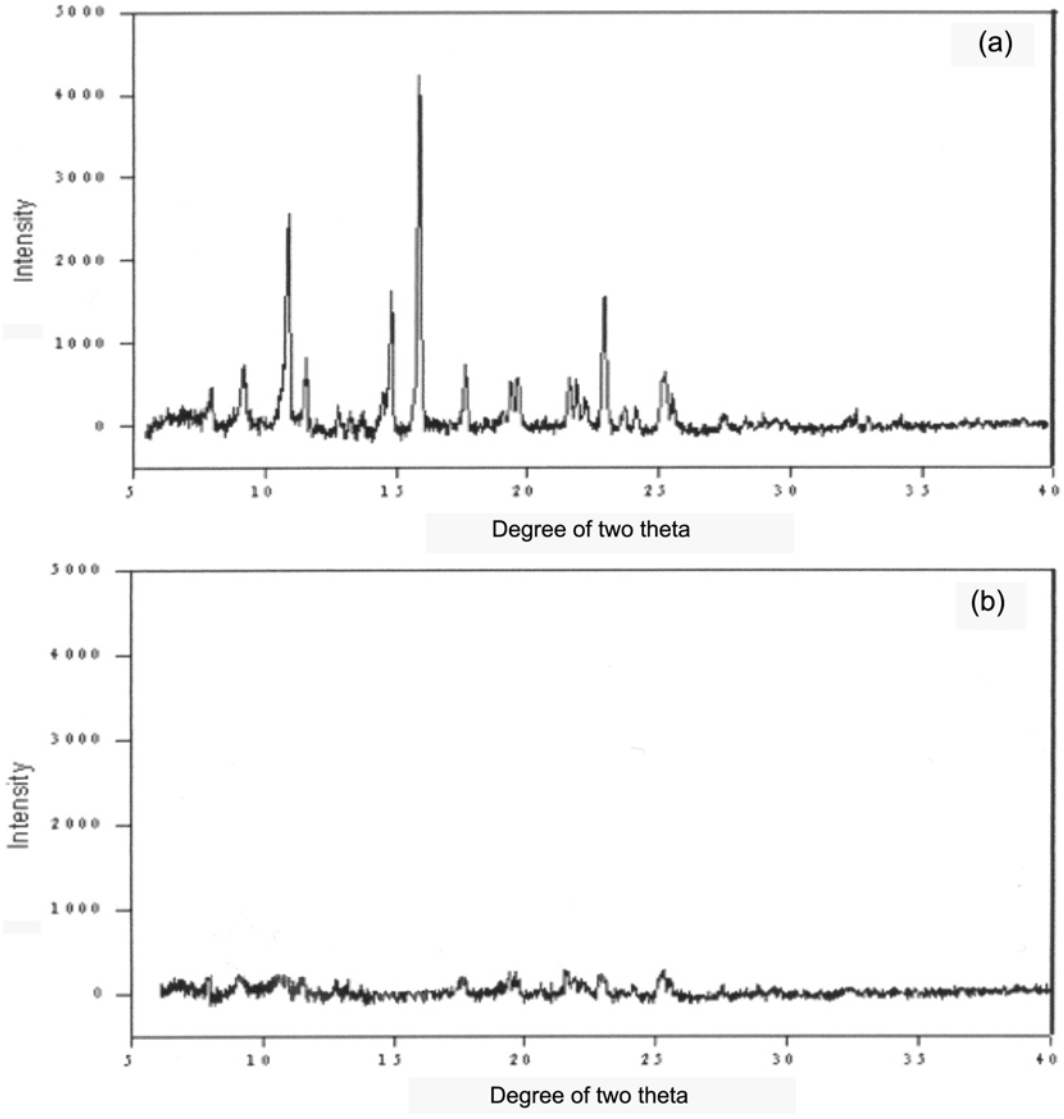

Fig. 1. XRD patterns of crystalline homoharringtonine from methanol/water (98/2, v/v) (a), amorphous homoharringtonine from methylene chloride/methanol (98/2, v/v) (b), respectively.

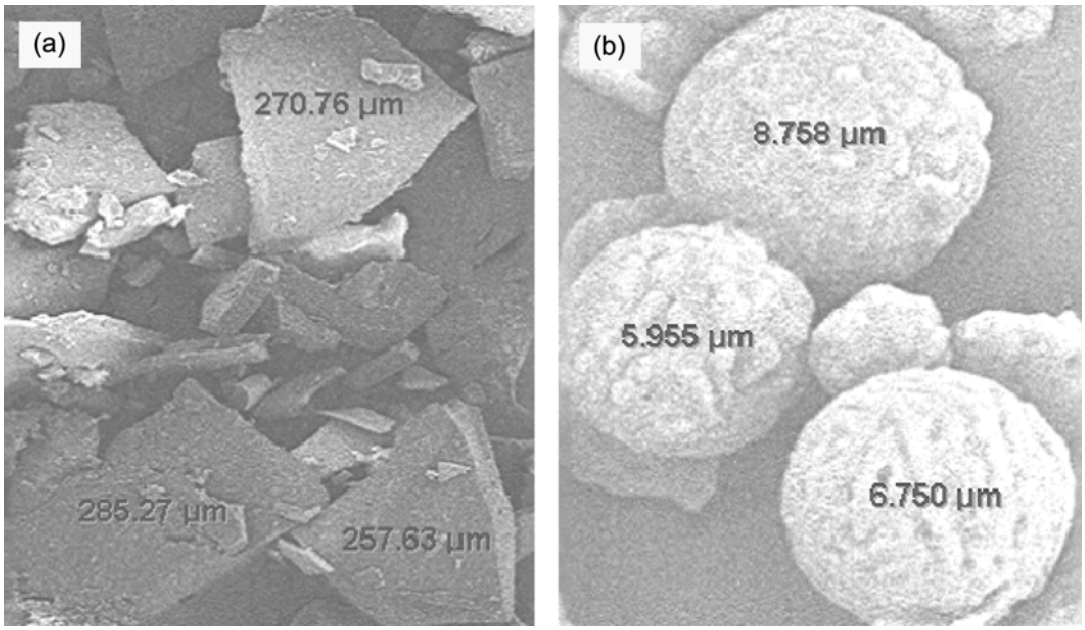

Fig. 2. Pictures for morphology of homoharringtonine analyzed by SEM: amorphous homoharringtonine from methylene chloride/methanol (98/2, v/v) and evaporation (a); amorphous homoharringtonine from methylene chloride/methanol (98/2, v/v) and spray drying (b), respectively.

Table 1. The effect of drying method on residual solvents in homoharringtonine

Residual solvent*	Rotary evaporation	Spray drying
Methanol (ppm)	1,180	1,160
Methylene chloride (ppm)	1,040	250

*The methanol and methylene chloride belong to class 2 in ICH guidance Q3C [18], and they should be limited in pharmaceutical products
*The limited concentration of methanol and methylene chloride by ICH is 3,000 ppm and 600 ppm, respectively

water (methanol/water=98/2, v/v) as shown in Fig. 1(a). On the other hand, amorphous HHT was made by dissolving HHT in relatively non-polar solvent (methylene chloride/methanol=98/2, v/v) (Fig. 1(b)). The features of the amorphous forms were compared by SEM analysis (Fig. 2). The HHT of the amorphous form, which was obtained by dissolving HHT in methylene chloride/methanol (98/2, v/v) solution followed by rotary evaporation, was seen to be of too large a particle size as analyzed by SEM (Fig. 2(a)); also, the removal of residual solvent, especially methylene chloride, was very difficult (Table 1). Amorphous HHT, with a fine particle size (Fig. 2(b)), was obtained by dissolving HHT in the same solvent system, methylene chloride/methanol (98/2, v/v), followed by spray drying. Residual methylene chloride was easily removed to less than 250 ppm, which is below the concentration limit (600 ppm) for APIs (active pharmaceutical ingredients) required by ICH (International Conference on Harmonisation) guidance Q3C [18], by spray drying and successive drying in a vacuum oven on a temperature of 60 °C for 72 h (Table 1).

2. Effect of HHT Morphology on Water Solubility and Water Content

The amorphous form of APIs has received considerable atten-

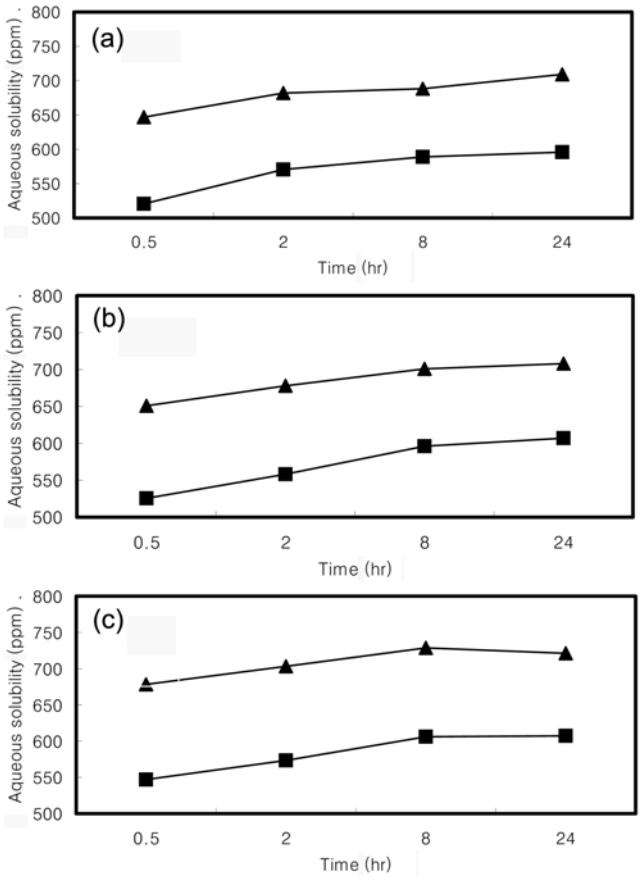

Fig. 3. Experimental aqueous solubility profiles for crystalline and amorphous homoharingtonine at 5 °C (a), 25 °C (b), and 45 °C (c): crystalline homoharringtonine from methanol/water (98/2, v/v) (■); amorphous homoharringtonine from methylene chloride/methanol (98/2, v/v) (▲), respectively.

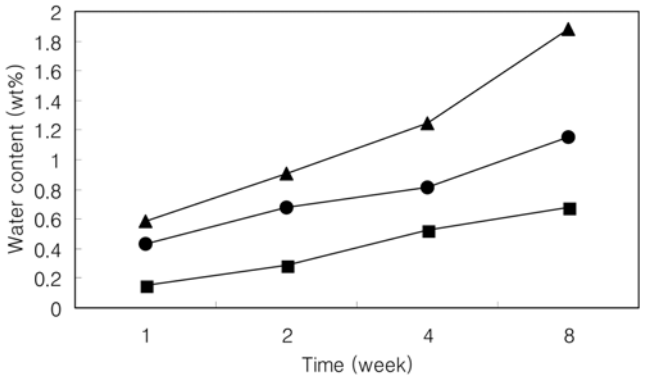

Fig. 4. The change of water content during storage at given humidity (RH95%): crystalline homoharringtonine from methanol/water (98/2, v/v) and evaporation (▲); amorphous homoharringtonine methylene chloride/methanol (98/2, v/v) and evaporation (●); amorphous homoharringtonine methylene chloride/methanol (98/2, v/v) and spray drying (■), respectively.

tion because it should provide the biggest advantage in terms of solubility and bioavailability [19]. The property of APIs, especially solubility, is critical in a formulation [20]. The solubility advantage of the amorphous form compared with the most stable crystalline form was predicted to be between 1.1- and 24-fold [19]. The solubilities of amorphous HHT were consistently greater than those of the crystalline form at various temperatures and times as shown in Fig. 3. The solubility of amorphous HHT was as much as 1.2-fold greater than the crystalline form at given temperatures. As the temperature increased, the solubility of HHT increased. Also, the increasing level of solubility between amorphous and crystalline form at high temperature (25-45 °C) was greater than that of solubility at low temperature (5-25 °C). Fig. 4 shows the initial water content of crystalline HHT (sample A) and amorphous HHT (sample B, C) for 3.373 wt%, 2.334 wt%, and 1.310 wt%, respectively. This means the crystalline form absorbed water from the atmosphere, quickly. It could be recognized that the crystalline form absorbed more water than the amorphous form at all given humidity (95 RH%) in storage conditions. So, the crystalline form with its initial water content of 3.373 wt% showed very hard to hold under certain water content for formulation of drug because its water content was extremely changeable during storage. This information is very useful for production, quality control, and formulation of HHT for purposes of commercialization.

CONCLUSIONS

We found that solvent treatment of HHT was a convenient method to control the morphologies of HHT. Crystalline HHT was made by dissolving HHT in a special polar solvent containing a small amount of water (methanol/water=98/2, v/v). On the other hand, amorphous HHT was made by dissolving HHT in relatively nonpolar solvent (methylene chloride/methanol=98/2, v/v). Amorphous HHT, with a fine particle size, was obtained by dissolving in methylene chloride/methanol (98/2, v/v), followed by spray drying. Residual methylene chloride and methanol were easily removed to less than concentration limit in ICH (International Conference on Harmonisation) guidance by spray drying and successive drying in a vacuum oven. The water solubilities of amorphous HHT were consistently greater than those of the crystalline form at various temperatures (5-45 °C). The hygroscopic property of crystalline form absorbed more water than amorphous form at all given humidity (95 RH%) during storage.

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