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RAPID COMMUNICATION

Effect of drying methods on removal of residual solvents from solvent-induced amorphous paclitaxel

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Abstract−We investigated the effect of solvents on the formation of amorphous paclitaxel and proposed an efficient strategy for the removal of residual solvents from solvent-induced amorphous paclitaxel. Amorphous paclitaxel was produced by solvent-induced method using non-polar solvents (methylene chloride, toluene, pentane, methyl t-butyl ether, chloroform, and acetonitrile/hexane (1 : 2, v/v)). The residual pentane and hexane levels easily met the International Conference on Harmonization (ICH)-specified values (5,000 and 290 ppm) by simple rotary evaporation. When the vacuum-dried sample was subjected to microwave-assisted drying, the ICH requirements for methylene chloride (600 ppm) and acetonitrile (410 ppm) were met by drying for 23 hr at 200 W and 3 hr at 200 W, respectively. However, residual toluene, methyl t-butyl ether, and chloroform concentrations did not meet the ICH-specified values (890, 5,000, and 60 ppm). The shape and size of amorphous paclitaxel particles were examined by SEM and XRD.

Keywords: Solvent-induced Amorphous Paclitaxel, Residual Solvent, Removal, Rotary Evaporation, Vacuum Drying, Microwave-assisted Drying

INTRODUCTION

Paclitaxel, a tubulin-binding diterpenoid isolated from the bark of the Pacific yew tree *Taxus brevifolia*, has been one of the most important anticancer agents of recent decades [1,2]. It is widely used clinically for the treatment of ovarian, breast, head and neck, and non-small cell lung cancer. The main production methods of paclitaxel are direct extraction from yew trees, chemical synthesis, and plant cell culture [3-5]. Among these, plant cell culture enables stable mass production of paclitaxel of consistent quality in a bioreactor without the influence of external factors such as climate or environment.

For an active pharmaceutical ingredient (API), the specifications for the final purified product are highly varied and strict. The morphology (amorphous or crystalline) of the APIs is a very important specification, having a great impact on the formulation of the final drug product [6,7]. An amorphous solid often has higher solubility than a crystalline solid, resulting in enhanced dissolution and bioavailability. In addition, paclitaxel is classified as class IV in the biopharmaceutics classification system (BCS), as its water solubility is very low, which limits usage [8]. Thus, the development of a method that easily enables morphology control of a purified API can be very useful for the production of the final drug product, especially in the formulation process. In 1997, Liggins et al. [9] developed a method to control the morphology of solid-state paclitaxel, but it was not practical because the manufacturing conditions included very high temperatures. In 2011, the solvent-induced method was developed to easily control the morphology of purified paclitaxel by solvent treatment [10]. Crystalline paclitaxel was produced by drying with polar solvents (acetone, ethanol, methanol, methyl ethyl ketone, and n-butyl alcohol) having a polarity index above 4.00. On the other hand, amorphous paclitaxel was produced by drying with non-polar solvents (methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, and acetonitrile/hexane (1 : 2 [v/v])) having a polarity index of about 4.00 or lower. The morphology of paclitaxel was very closely associated with the polarity index of the organic solvent used in the solvent drying process [10].

According to the International Conference on Harmonisation (ICH), the concentration of residual solvents is strictly regulated because of their inherent toxicity [11,12]. To meet the requirement of ICH-guideline for these residual solvents, it is critical to utilize a suitable drying method. Supercritical drying and spray drying have been proposed to remove residual solvents [13,14]. However, a drying method using supercritical carbon dioxide requires high-pressure equipment and paclitaxel can decompose due to high operating pressure. In the case of spray drying, there are problems because the operation takes a long time (~24 hr) and a large installation space is required. On the other hand, rotary evaporation and vacuum drying, which are widely used, are simple as well as economical, while microwave-assisted drying has a high rate of heating and facilitates homogeneous drying with smaller equipment. In addition, the thermal efficiency is high and power control is easy, because the microwave energy is directly absorbed by the object that is being heated [15-17]. Because of such advantages, these methods are widely used for drying trees, vegetables, fruits, and ores [18-21]. In the present study, a rotary evaporation, vacuum drying and microwave-assisted drying were sequentially introduced to investigate the changes in the residual solvents concentration of amorphous paclitaxel prepared by the solvent-induced method. Furthermore,

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SEM and XRD analysis were performed to confirm the morphology and size of the dried paclitaxel. Ultimately, we proposed a method to efficiently remove residual solvent from amorphous paclitaxel to meet ICH requirements.

MATERIALS AND METHODS

1. Plant Materials

Taxus chinensis cells were cultured in a modified Gamborg's B5 medium at 24 °C with shaking at 150 rpm in the dark. The cell culture was transferred to a fresh medium every two weeks. During this prolonged culture, 4 mM $AgNO_3$ was added at the initiation as an elicitor, and 1 and 2% (w/v) maltose were added to the medium on day 7 and 21, respectively [22]. After the culturing, plant cells were recovered using a decanter (CA150 Clarifying Decanter; Westphalia, Germany) and a high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa-Laval, Sweden). The recovered plant cells, hereinafter referred to as the biomass, were provided by Samyang Biopharm Company, South Korea.

2. Preparation of Samples for Drying

The purified paclitaxel sample (purity: 98.7%) for this study was prepared from biomass using the following steps: (i) biomass extraction with methanol and liquid-liquid extraction with methylene chloride to obtain a crude extract, (ii) adsorbent Sylopute treatment of the crude extract to remove the tar compounds, (iii) hexane precipitation and fractional precipitation to obtain the paclitaxel precipitate, and (iv) ODS (C18)-HPLC and Silica-HPLC to obtain the purified paclitaxel. The sample preparation process is described in detail in a previous study [23].

3. Drying Method for Amorphous Paclitaxel

The drying was applied to the samples from the Silica-HPLC (mobile phase: methylene chloride/methanol=98/2 [v/v]), which is the final purification process of paclitaxel. The samples, 1 g of paclitaxel (98.7% pure), were dissolved in 20 mL of non-polar solvents such as methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, and acetonitrile/hexane (1 : 2 [v/v]) and then concentrated and dried using a rotary evaporator (CCA-1100, EYELA, Japan) at 45 °C under reduced pressure. Additional drying was performed at 45 °C at 760 mm Hg by gradual vacuum drying (UP-2000, EYELA, Japan) and microwave-assisted drying (2,450 MHz Model 1501, Korea Microwave Instrument Co., Korea). The microwave cavity was W420 mm×D380 mm×H420 mm in size and consisted of cooling fan and thermocouple. A thermocouple was installed to measure temperature changes continuously during drying. The power supplied to the microwave generator (capacity: 1.5 kW) produced temperatures of 45 °C. The possibility of paclitaxel degradation at a high temperature (>50 °C) was also considered [24]. All experiments were performed in triplicate. The concentration of residual solvents in the dried sample was analyzed by GC after being dissolved in dimethylacetamide.

4. Residual Solvent and Paclitaxel Analysis

The concentration of the residual solvents in paclitaxel, including methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, acetonitrile, and hexane, were analyzed using GC (GC-2014; Shimadzu, Japan), with an HP-5 column (0.20 mm ID×25 m, 0.33-μm film) and a flame ionization detector. The separation temperature within the column was programmed to increase from 40 to 250 °C at 18 °C/min. The carrier gas used was helium and the flow rate was 0.7 mL/min [24]. Each sample was analyzed in triplicate. The limits of detection (LOD) for methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, acetonitrile, and hexane were 1.5, 4, 1, 7, 2, 3, and 5 ppm, respectively. The paclitaxel content was analyzed using an HPLC system (SCL-10AVP, Shimadzu, Japan) equipped with a Capcell Pak C18 column (250 mm×4.6 mm; Shiseido). The elution was performed for 40 min on a gradient of distilled water/acetonitrile mixture, with the ratio ranging from 65 : 35 to 35 : 65 (flow rate=1.0 mL/min). The injection volume was 20 mL, and the effluent was monitored using a UV detector at 227 nm [25]. Authentic paclitaxel (purity: 99.5%) was provided by Samyang Biopharm Company and used as the standard.

5. SEM and XRD Analysis

The surface of the paclitaxel particles produced through the solvent-induced method was assessed using a scanning electron microscope (MIRA II LMH, Tescan Czech). The accelerating voltage used was from 10-15 kV. Different magnifications were used for each sample to observe the surface. The amount of sample used for observation was approximately 1 mg. In addition, X-ray diffraction (MiniFlex600, Rigaku, Japan) was used to assess the morphology of the paclitaxel samples. In operating conditions, the angle of diffraction was set as 2θ=3-40° using CuKα (40 kV, 15 mA). The amount of sample used was 50 mg.

RESULTS AND DISCUSSION

1. Removal of Residual Solvents from Solvent-induced Amorphous Paclitaxel

In a previous study [10], the amorphous paclitaxel was easily obtained by solvent-induced method. However, very few studies have been conducted to develop a method to efficiently remove residual solvents from solvent-induced amorphous paclitaxel. Therefore, we explored different methods for efficiently removing residual solvents from amorphous paclitaxel produced using non-polar solvents such as methylene chloride, acetonitrile/hexane (1 : 2 [v/v]), toluene, methyl t-butyl ether, chloroform, and pentane. First, for the sample dried by rotary evaporation, the concentrations of the residual solvents as a function of drying time are shown in Fig. 1. The concentration of hexane (Fig. 1(b)) and pentane (Fig. 1(f)), which were residual solvents in the sample, easily met the ICH specifications (hexane: 290 ppm, pentane: 5,000 ppm) within 1 hr. Hexane was not detected (LOD=5 ppm), while pentane reached the ICH-specified value (5,000 ppm) after 25 min of drying. The concentration of residual methylene chloride (Fig. 1(a)), acetonitrile (Fig. 1(b)), toluene (Fig. 1(c)), methyl t-butyl ether (Fig. 1(d)), and chloroform (Fig. 1(e)) tended to decrease, but was maintained at 30,000 ppm, 8,500 ppm, 7,500 ppm, 22,000 ppm, and 78,000 ppm after 11, 6, 4, 4, and 3 hr of drying, respectively. After rotary evaporation, some solvents did not meet the ICH specifications (methylene chloride: 600 ppm, acetonitrile: 410 ppm, toluene: 890 ppm, methyl t-butyl ether: 5,000 ppm, and chloroform: 60 ppm) [11], and therefore, additional vacuum drying was performed for them. As shown in Fig. 2, the concentration of residual methylene chloride (Fig. 2(a)), acetonitrile (Fig. 2(b)), toluene (Fig. 2(c)), methyl t-

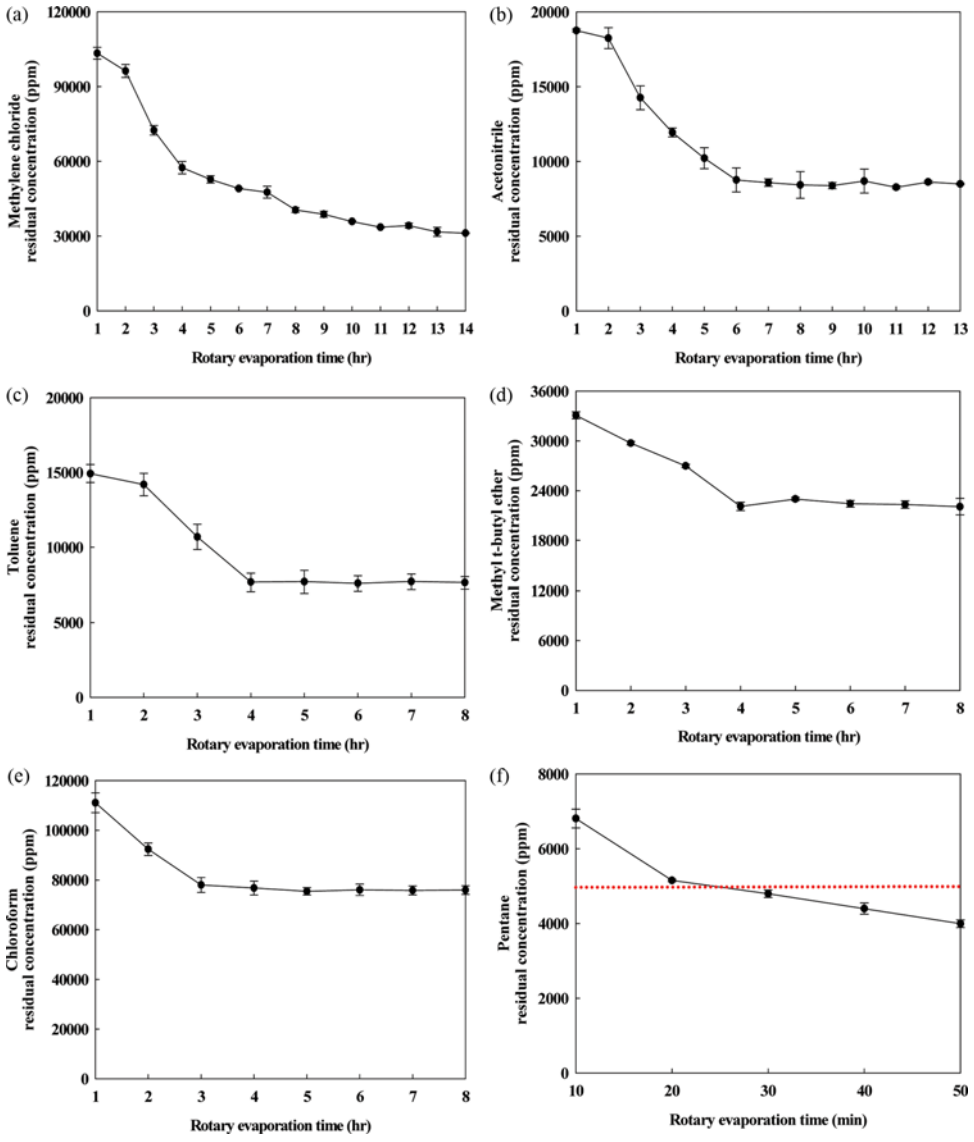

Fig. 1. Effect of evaporation time on the concentration of residual solvents in rotary-evaporated samples pre-treated with methylene chloride (a), acetonitrile/hexane (1 : 2 [v/v]) (b), toluene (c), methyl t-butyl ether (d), chloroform (e), and pentane (f) at 45 °C. The concentration limits of methylene chloride, hexane, acetonitrile, toluene, methyl t-butyl ether, chloroform, and pentane according to the Q3C guidance of ICH are 600, 290, 410, 890, 5,000, 60, and 5,000 ppm, respectively. Dotted line indicates the concentration limit of pentane. No residual hexane (LOD=5 ppm) was detected in (b).

butyl ether (Fig. 2(d)), and chloroform (Fig. 2(e)) in the vacuum-dried samples was 18,000 ppm, 3,500 ppm, 6,000 ppm, 20,000 ppm, and 78,000 ppm, after 8, 8, 6, 5, and 1 hr of drying, respectively. Thus, meeting the ICH specifications was still difficult. This might have been caused by case-hardening, where the surface of the samples undergo solidification before the residual solvents are removed [1,12]. To remove the residual solvents inside the casehardened samples, we used microwave-assisted drying, in which a heating object becomes the heating element for heat transfer to the whole sample [24]. As shown in Fig. 3, after microwave-assisted drying, the concentration of residual methylene chloride (Fig. 3(a)) and acetonitrile (Fig. 3(b)) was under 580 ppm and 400 ppm after 23 and 3 hr of drying, respectively, and thus satisfied the ICH specification. However, the concentration of toluene (Fig. 3(c)), methyl t-butyl ether (Fig. 3(d)), and chloroform (Fig. 3(e)) did not decrease even after 6 hr, and remained at 6,000 ppm, 20,000 ppm, and 70,000 ppm, respectively. The pattern of microwave-assisted drying might be associated with the dielectric constant of the solvents, indicating the storage of electromagnetic radiation, which is radiant energy in the electric field [26,27]. Thus, a solvent that heats up rapidly under microwave radiation typically has a high dielectric constant. The residual concentrations of various solvents according to the drying methods, together with a dielectric constants obtained in previous studies [26-28], are listed in Table 1. Methylene chloride (dielectric constant: 9.10) and acetonitrile/hexane (1 : 2, v/v) (dielectric constant: 9.45) has higher dielectric constant than toluene (dielectric constant: 3.00), methyl t-butyl ether (dielectric constant: 2.60), and chloroform (dielectric constant: 4.80), indicating that these

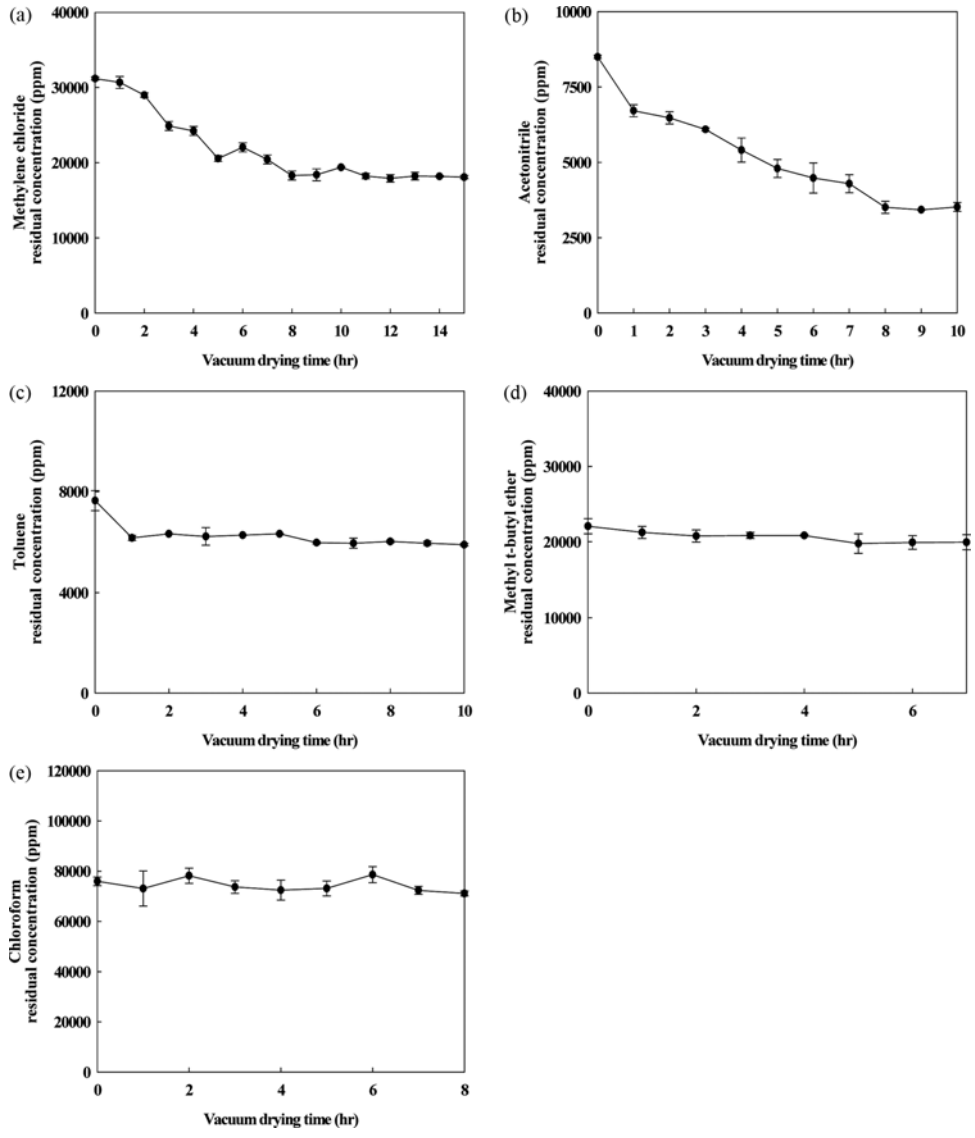

Fig. 2. Effect of vacuum-drying time on the concentration of residual solvents in vacuum dried samples pre-treated with methylene chloride (a), acetonitrile/hexane (1 : 2 [v/v]) (b), toluene (c), methyl t-butyl ether (d), and chloroform (e) at 45 °C. No residual hexane (LOD= 5 ppm) was detected in (b).

solvents can absorb much more microwave energy and can convert it to heat more efficiently than the other solvents. Therefore, it is estimated that the microwave heating rate of methylene chloride and acetonitrile/hexane (1 : 2, v/v) was the highest and thus could provide the highest efficiency in microwave-assisted drying. Based on these results, pentane and hexane were efficiently removed by rotary evaporation alone, and satisfied the ICH specifications (pentane: 5,000 ppm, hexane: 290 ppm). Methylene chloride, acetonitrile, toluene, methyl t-butyl ether, and chloroform did not meet the ICH-specified values, even after additional vacuum drying. Microwave-assisted drying efficiently removed methylene chloride and acetonitrile, and satisfied the ICH specifications (600 ppm and 410 ppm, respectively), while toluene, chloroform, and methyl t-butyl ether could not be reduced sufficiently to meet the ICH-specified values. Thus, pentane-induced amorphous paclitaxel would be most economical and efficient, because the ICH specifications were satisfied after simple rotary evaporation for 1 hr.

2. Morphology and Size of Solvent-induced Amorphous Paclitaxel

The morphology of paclitaxel prepared by solvent-induced method with non-polar solvents (methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, and acetonitrile/hexane [1 : 2; v/v]) was confirmed through XRD analysis (Fig. 4). Amorphous paclitaxel shows no meaningful peaks, whereas crystalline paclitaxel shows peaks at 2θ values of 5.6, 9.1, 10.4, 12.7, and 21.1° in the XRD pattern [10,13]. In the result, the XRD patterns of none of the solvents showed a significant peak, which suggested a typical amorphous form. There was no difference between the peaks of the different solvents. The results of the SEM analysis (Fig. 4) showed that the shape and size of paclitaxel particles were different for the different solvents. When methylene chloride (Fig. 4(a)), acetonitrile/hexane (1 : 2 [v/v]) (Fig. 4(b)), chloroform (Fig. 4(e)), and pen-

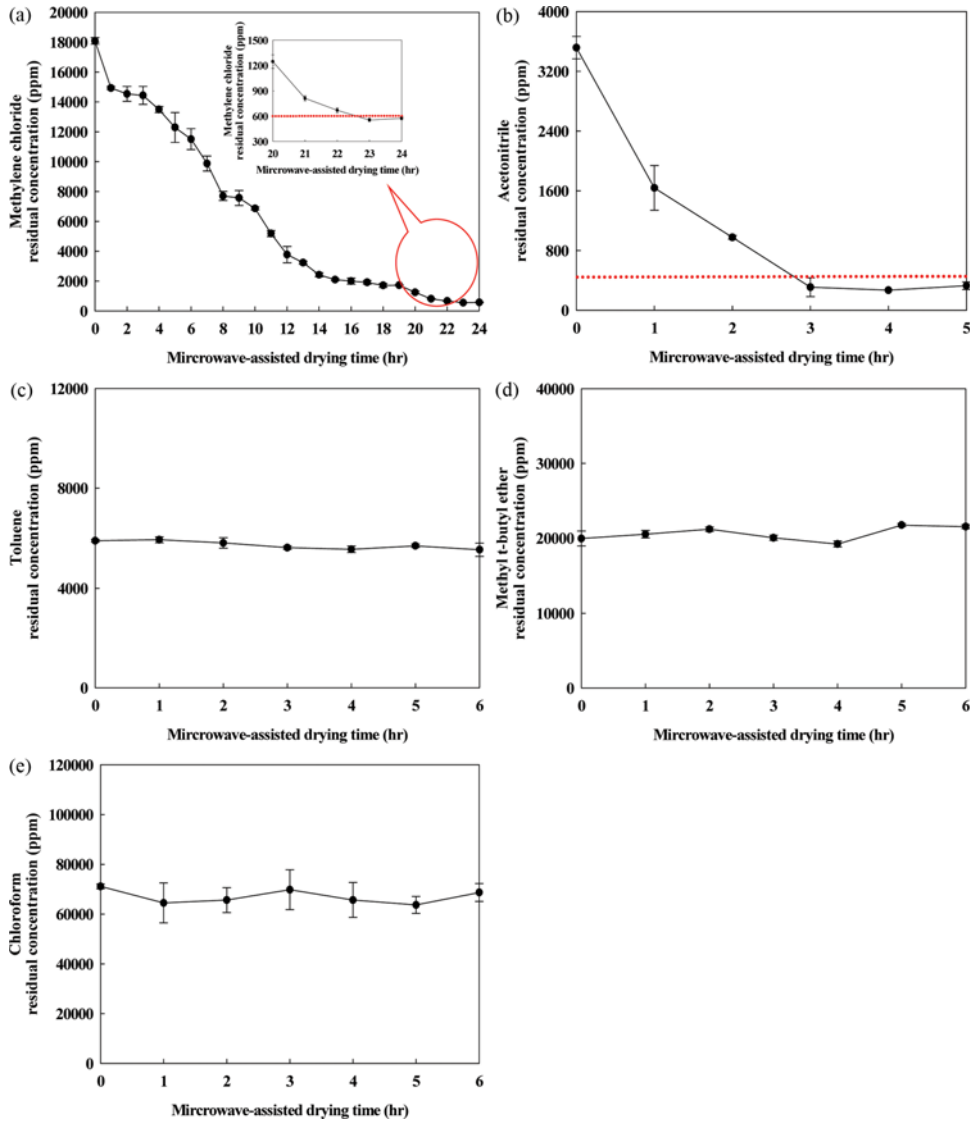

Fig. 3. Effect of microwave-assisted drying time on the concentration of residual solvents in microwave-assisted dried samples pre-treated with methylene chloride (a), acetonitrile/hexane (1 : 2 [v/v]) (b), toluene (c), methyl t-butyl ether (d), and chloroform (e) at 200 W. Dotted line indicates the concentration limit (ppm) of methylene chloride (600 ppm) and acetonitrile (410 ppm) according to the Q3C guidance of ICH.

Table 1. Summary of the residual concentrations, ICH-specified values, and dielectric constants of various solvents and drying methods

Solvent		ICH-specified value (ppm)[a]	Residual concentration (ppm)			Dielectric constant[b]
			Rotary evaporation	Vacuum drying	Microwave-assisted drying	
Toluene		890	7500	6000	6000	3.00
Methyl t-butyl ether		5000	22000	20000	20000	2.60
Methylene chloride		600	30000	18000	550	9.10
Pentane		5000	1000	N/A[c]	N/A	1.80
Chloroform		60	76000	71000	65000	4.80
Acetonitrile/Hexane (1 : 2 [v/v])	Acetonitrile	410	8500	3500	300	9.45
	Hexane	290	N/D[d]	N/D	N/D	

[a]ICH-specified value obtained in ICH guidelines [11]
[b]Dielectric constant obtained in previous studies [26-28]
[c]N/A: not applicable
[d]N/D: not detectable

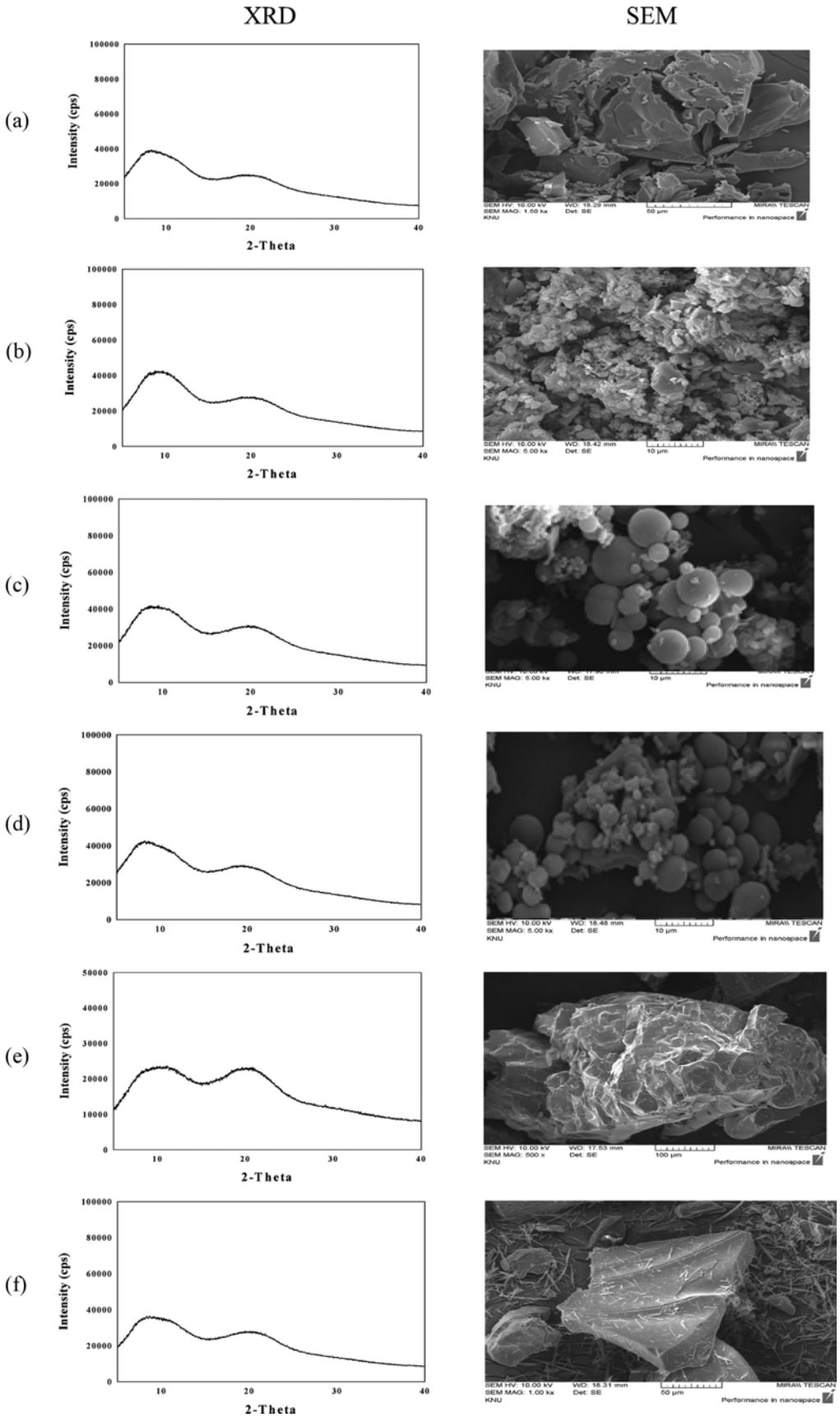

Fig. 4. XRD patterns and SEM images of paclitaxel prepared by solvent-induced method with non-polar solvents. (a) Methylene chloride, (b) acetonitrile/hexane (1 : 2 [v/v]), (c) toluene, (d) methyl t-butyl ether, (e) chloroform, and (f) pentane.

tane (Fig. 4(f)) were used, the morphology of the paclitaxel particles was irregular, whereas the particles were spherical when toluene (Fig. 4(c)) and methyl t-butyl ether (Fig. 4(d)) were used. In addition, when methylene chloride, chloroform, and pentane were used, the paclitaxel particles were bigger than 150 μm and the particle size distribution was wide. However, f the paclitaxel particles were smaller than 10 μm when methyl t-butyl ether, toluene, and acetonitrile/hexane (1 : 2 [v/v]) were used. This difference might have been caused by the supersaturation of paclitaxel in the non-polar solvents used [29-31]. This effect has been reported in previous studies that showed that supersaturation affected the rate of nucleation, and a higher nucleation rate caused smaller particle sizes [10,32]. For APIs, smaller amorphous product has higher utilization value because it is advantageous in terms of removal of residual solvent and improvement of solubility [32]. Thus, amorphous paclitaxel with a smaller particle size can be easily produced by selecting an appropriate organic solvent. Our results can contribute to the development of better techniques to mass-produce other similar alkaloid drugs.

CONCLUSIONS

We investigated efficient drying methods to remove residual solvents from amorphous paclitaxel, which was produced using a solvent-induced method. Among the residual solvents of amorphous paclitaxel produced using non-polar solvents (methylene chloride, pentane, toluene, chloroform, methyl t-butyl ether, and acetonitrile/hexane [1 : 2; v/v]), pentane and hexane were easily removed by simple rotary evaporation within 1 hr, and they met the ICH-specified values (hexane: 290 ppm, pentane: 5,000 ppm). The concentration of methylene chloride, acetonitrile, toluene, methyl t-butyl ether, and chloroform did not meet the ICH-specified values, even after additional vacuum drying. When additional microwave-assisted drying was performed for methylene chloride and acetonitrile, the concentration reached 580 ppm in 23 hr and 400 ppm in 3 hr, respectively, and this was sufficient to satisfy the ICH specifications (methylene chloride: 600 ppm, acetonitrile: 410 ppm). The concentration of toluene, methyl t-butyl ether, and chloroform did not meet the ICH-specified values even after additional microwave-assisted drying. The results of SEM and XRD analyses showed that the morphology of the amorphous paclitaxel was irregular when methylene chloride, pentane, chloroform, and acetonitrile/hexane (1 : 2, v/v) were used, whereas the paclitaxel particles were spherical when toluene and methyl t-butyl ether were used. In addition, the amorphous paclitaxel particles were bigger than 150 μm and the size distribution was wide when methylene chloride, chloroform, and pentane were used, whereas the particles were smaller than 10 μm when toluene, methyl t-butyl ether, and acetonitrile/hexane (1 : 2 [v/v]) were used.

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