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RAPID COMMUNICATION

Removal of residual chloroform from amorphous paclitaxel pretreated by alcohol

Hoe-Jong Kang and Jin-Hyun Kim†

Department of Chemical Engineering, Kongju National University, Cheonan 31080, Korea

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Abstract−A drying method that can effectively remove residual solvents from chloroform-induced amorphous paclitaxel was developed. Simple rotary evaporation with alcohol (methanol or ethanol) pretreatment was sufficient to remove residual chloroform and alcohol concentrations below the ICH limits (60 ppm for chloroform, 3,000 ppm for methanol, and 5,000 ppm for ethanol). In addition, SEM analysis and ultrasonic treatment showed that residual solvent removal is related to the porous structure of the sample due to the high vapor pressure of the chloroform-alcohol mixture and the hydrogen bonding between chloroform and alcohol.

Keywords: Amorphous Paclitaxel, Residual Solvent, Removal, Pretreatment with Alcohol, Drying

INTRODUCTION

Paclitaxel is an anticancer compound most widely used in the treatment of breast cancer, ovarian cancer, head and neck cancer, Kaposi's sarcoma, and non-small lung cancer [1]. It can be produced from the yew tree by extraction, semi-synthesis, and plant cell culture [2-4]. Recently, plant cell culture techniques have received attention for stable mass production of paclitaxel [5]. For the purified paclitaxel to be used as an active pharmaceutical ingredient (API), various specifications have to be satisfied [6,7]. In particular, the morphology of an API is a critical factor that significantly affects the solubility and permeability of the drug during the formulation process [8,9]. Paclitaxel is classified as a class IV compound in the Biopharmaceutics Classification System (BCS) according to drug characteristics, and its bioavailability is highly limited due to the drug's low dissolution rate and intestinal permeability [10]. Morphology control of low-bioavailability API drugs in mass production in oral solid dosage forms is important in terms of their utilization. In 1997, morphology control of solid-state paclitaxel by heat treatment was developed but deemed impractical as a high-temperature manufacturing process [11]. A solvent-induced method was later developed to overcome problems of high-temperature treatment with existing methods [8,12].

According to the International Conference on Harmonisation (ICH) Q3C guidelines, the residual solvent concentrations of manufactured APIs are strictly regulated [6,13]. The allowed concentration of a residual solvent in a drug product is determined by the solvent's degree of toxicity (Class 1>Class 2>Class 3), and any concentration exceeding this limit must be removed. Various drying methods have been introduced to satisfy specified values for API concentration limits. In general, rotary evaporation, vacuum drying, and microwave-assisted drying are used for removing residual solvents from paclitaxel [7,8,14]. Characteristics for these drying methods differ according to time, operating condition, efficiency, equipment cost, and installation space. Rotary evaporation is widely used because it is relatively simple to operate and economically efficient [14].

For the manufacture of amorphous paclitaxel, the solvent-induced method uses the non-polar solvents methylene chloride, pentane, acetonitrile/hexane (1 : 2, v/v), and chloroform [14]. Of these solvents, methylene chloride, pentane, acetonitrile, and hexane are conventional residual solvents used in drying methods that easily satisfy the ICH-specified values, while chloroform exceeds the ICH-set limit [6,14,15]. Chloroform is classified as an ICH Class 2 solvent and is strictly limited to less than the allowable concentration [13]. In addition, among the solvents used to prepare amorphous paclitaxel by the solvent-induced method, chloroform has the lowest allowable concentration (ICH-specified value: 60 ppm) for the residual solvent [14]. Therefore, there is a need to develop a method that efficiently removes residual chloroform from chloroform-induced amorphous paclitaxel. According to a recent study, a method that effectively removes residual methylene chloride by pre-treating the sample with ethanol has been introduced [15,16]. From this idea, we tried to develop a method that effectively removes residual chloroform from the chloroform-induced amorphous paclitaxel through pretreatment with alcohol (methanol or ethanol). In other words, we present a method that removes residual chloroform by applying simple rotary evaporation to the alcohol-pretreated sample. In addition, we investigated the relationship between alcohol pretreatment and residual solvent removal through SEM analysis and an ultrasonic treatment. Such results can be useful for effective removal and observation of residual solvent in the API mass production process.

MATERIALS AND METHODS

1. Paclitaxel Production and Purification

Plant cell culture was used on the cell line derived from the *Taxus chinensis* leaf for the production of paclitaxel. The suspension cells were maintained in darkness at 24 °C with shaking at 150 rpm in

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[†] To whom correspondence should be addressed.
E-mail: jinhyun@kongju.ac.kr

a Gambor's B5 medium. The culture medium was replaced with fresh medium every two weeks. Maltose (1-2%, w/v) and elicitor ($AgNO_3$, 4 μM) were added for culture and production [2]. After culturing, plant cells (biomass) were collected from the culture medium using a centrifuge. First, solid-liquid extraction was carried out with a biomass/methanol ratio of 1 : 1 (w/v), followed by liquid-liquid extraction with addition of methylene chloride (25% of the extract). Paclitaxel was collected from the lower layer of methylene chloride through phase separation and concentrated/dried. The dried crude extract was treated with an adsorbent (Sylopute) and filtered. The filtrate was dried and subjected to hexane precipitation and fractional precipitation to obtain paclitaxel precipitate. Final purification was performed using ODS (C18)-HPLC and Silica-HPLC [17]. The isolation and purification process of the sample is described in detail in previous studies [6,17].

2. Manufacturing and Drying of Amorphous Paclitaxel

First, 1.0 g of sample (purity of purified paclitaxel: 92.7%) was dissolved in 20 mL of chloroform and then dried using a rotary evaporator (CCA-1100, EYELA, Japan) to prepare chloroform-induced amorphous paclitaxel. The morphology of the prepared samples was confirmed by SEM and XRD analysis [14]. To remove the residual solvent from the sample, we carried out rotary evaporation under reduced pressure at different drying temperatures (25, 35, and 45 °C). Methanol (5 mL, 10 mL, 20 mL) or ethanol (5 mL, 10 mL) was added when the concentration of residual chloroform remained constant (30 min of drying), and further rotary evaporation was performed. The dried samples were dissolved in dimethylacetamide and the residual chloroform and alcohol concentrations were measured by GC analysis. We also used SEM (MIRAII LMH, Tescan Czech) analysis to examine the surface of the dried sample. Accelerating voltages were maintained between 10 kV to 15 kV, and particle surface was observed by varying the magnification of each sample. The amount of sample taken for analysis was about 1.0 mg. In addition, to investigate the effect of hydrogen bond breakage between chloroform and alcohol, we used the 40 kHz ultrasound cleaner (JAC-4020, KODO, Korea) (ultrasound power: 530 W) to carry out ultrasonic treatment during rotary evaporation. The bottle containing the sample was placed in an ultrasonic cleaning bath and rotary evaporated. For this, 5 mL of alcohol (methanol) had been added to the sample at a drying temperature of 25 °C.

3. Residual Solvent and Paclitaxel Analysis

The residual chloroform and alcohol contents were analyzed using an HP-5 column (25 m, 0.33 mm film, 0.20 mm ID) and gas chromatograph (YL6500GC, Young-rin, Korea) equipped with a flame ionization detector (FID). The separation temperature in the column was 40-250 °C, and programmed at a rate of 18 °C/min [14]. The flow rate of the carrier gas (helium) was 0.7 mL/min. The limits of detection (LOD) of chloroform, methanol, and ethanol were 1.0, 0.5 and 0.5 ppm, respectively. Paclitaxel content was analyzed using the HPLC system (SCL-10AVP, Shimadzu, Japan) equipped with Capcell Pak C_{18} columns (250 mm×4.6 mm; Shiseido, Japan) [18]. The mobile phase was an acetonitrile and distilled water mixture (65/35 to 35/65, v/v, gradient mode) at a flow rate of 1.0 mL/min. The sample injection volume was 20 mL and detected by a UV detector at 227 nm. As a standard sample, we used the Sigma-Aldrich product (purity: 99.0%).

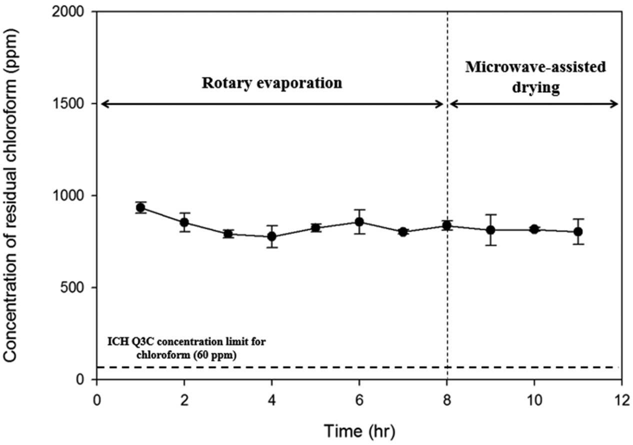


Fig. 1. Change of the concentration of residual chloroform through drying time in rotary evaporated and microwave-assisted dried amorphous paclitaxel samples prepared by the chloroform-induced method.

RESULTS AND DISCUSSION

In general, amorphous paclitaxel is more effective in terms of drug dissolution rate, dispersion uniformity, and oral bioavailability compared to crystalline paclitaxel [19]. A solvent-induced method, which can easily control the paclitaxel morphology, has been developed, but there is a lack of research on the efficient removal of the residual solvent from the amorphous paclitaxel. According to the literature, the removal method of residual methylene chloride, pentane, acetonitrile, and hexane is relatively well established, but there is no study on the residual chloroform removal method [6,14,15]. In particular, no drying methods have been reported that can reduce the residual chloroform concentration below the IHC-specified value (60 ppm). To investigate the possibility of removing the residual solvent (chloroform) from chloroform-induced amorphous paclitaxel, samples were first subjected to simple rotary evaporation (8 hr, 45 °C, reduced pressure) and then microwave-assisted drying (3 hr, 55 °C, 300 W). As shown in Fig. 1, a residual chloroform concentration remained at 820 ppm with almost no change after 11 hr of drying. According to ICH Q3C guidelines, the residual chloroform concentration is strictly limited to less than 60 ppm [13]. As a result, conventional rotary evaporation and microwave-assisted drying did not meet the ICH limit (60 ppm) for residual chloroform concentration. Therefore, in this study, we developed a drying method that incorporated an alcohol pretreatment to remove residual chloroform effectively. First, 1.0 g of the purified paclitaxel sample was dissolved in 20 mL of chloroform and dried (temperature: 25, 35, 45 °C) using a rotary evaporator. Then, we added methanol (5 mL, 10 mL, 20 mL) or ethanol (5 mL, 10 mL) when there was no further change in the concentration of residual chloroform (30 min of drying). As shown in Fig. 2, the residual chloroform concentration (800-1,100 ppm) remained almost constant at all conditions until the addition of alcohol (methanol or ethanol), at which point it took 10 to 20 min of drying for the residual chloroform to be completely removed. With a relatively high methanol addition (20 mL) and low drying temperature (25 °C), more operating time

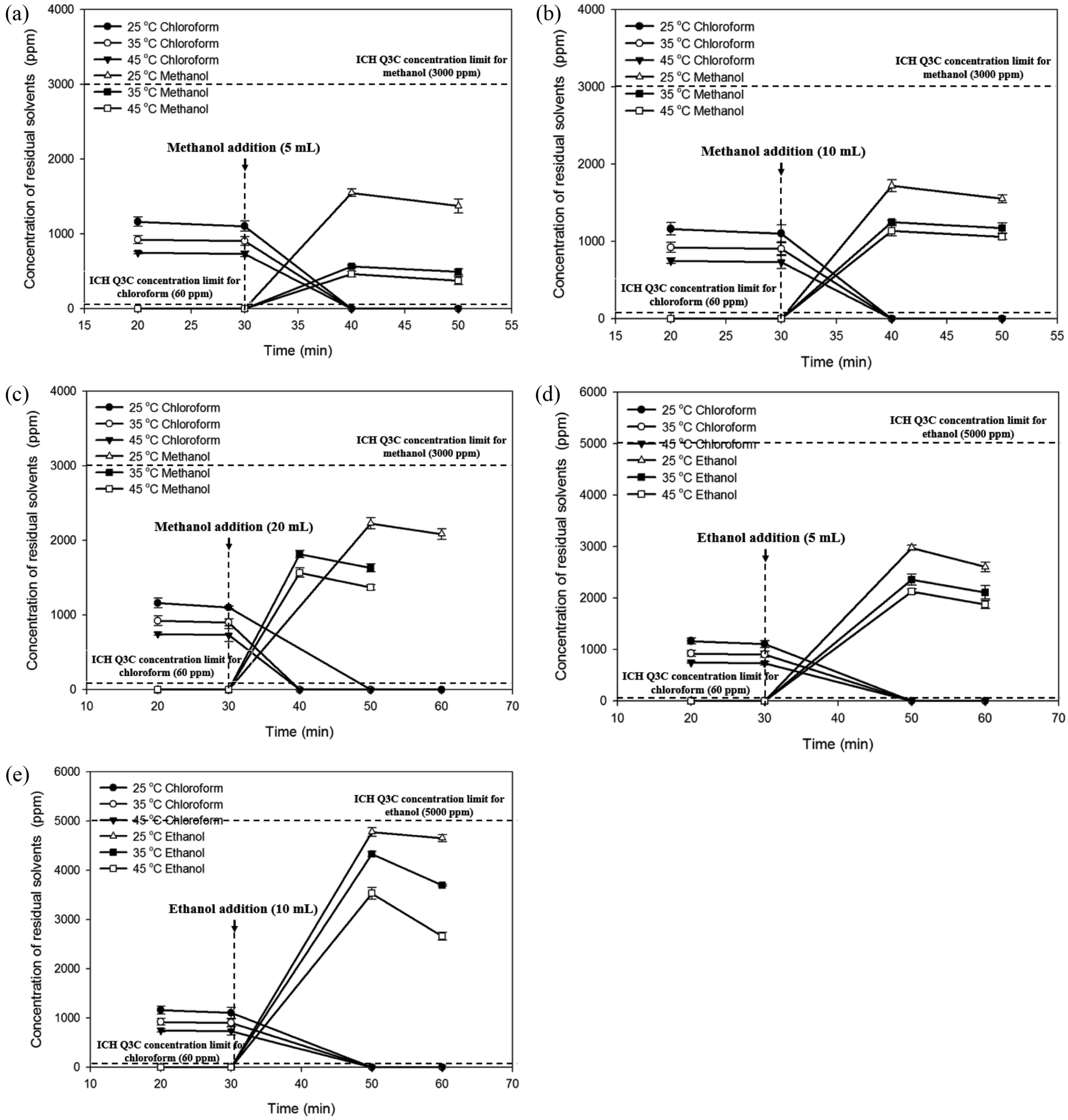


Fig. 2. Effect of evaporation time on residual solvent concentrations in rotary evaporated sample pre-treated with alcohol (methanol or ethanol) at different addition amounts. (a) Methanol 5 mL, (b) methanol 10 mL, (c) methanol 20 mL, (d) ethanol 5 mL, (e) ethanol 10 mL.

(~20min) was required for sample drying. At the same addition amount, a longer drying time was required in ethanol than in methanol because of their boiling point difference. The residual chloroform concentration of chloroform-induced amorphous paclitaxel was sufficiently lowered to the ICH-specified value (60 ppm) by simple rotary evaporation with alcohol pretreatment. In addition, the concentrations of residual methanol and ethanol in all experimental conditions were 374-2,231 ppm and 1,800-4,700 ppm, satisfying the ICH-specified values (methanol<3,000 ppm and ethanol<5,000 ppm). These results are similar to previous studies on the removal of residual methylene chloride in APIs (paclitaxel, homoharringtonine) by ethanol pretreatment [15,16].

As shown in Fig. 3, a mechanism for removing residual solvent by methanol pretreatment during drying was proposed. A chloroform-methanol mixture has a high vapor pressure as well as hydrogen bond networks between the hydroxyl oxygen of methanol and the hydrogen of chloroform [20-22]. The vapor pressures of pure chloroform, methanol, and ethanol at 20 °C are 158.9, 96.9, and 44.2 torr [8]. During drying, the sample surface is damaged and a porous structure is formed due to the high vapor pressure, pro-

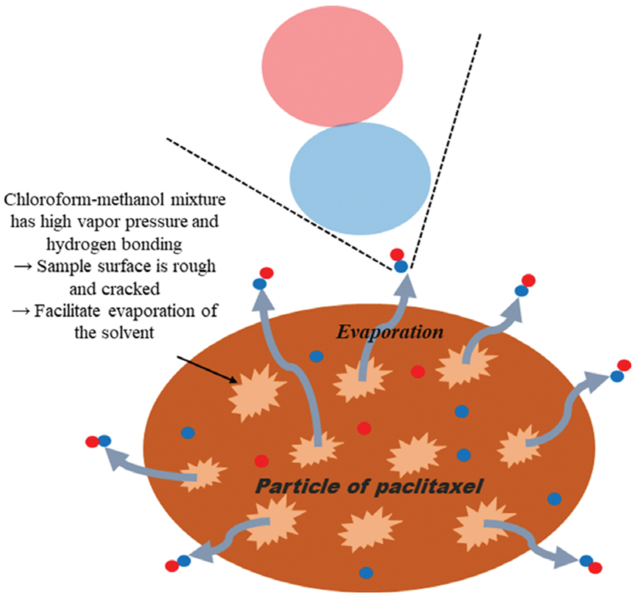


Fig. 3. A schematic diagram of the removal mechanism of residual solvents from a sample pre-treated with methanol.

moting the evaporation of residual chloroform and hydrogen-bond forming methanol. To confirm this, ultrasound, which is widely used for breaking hydrogen bonds, was introduced [23,24]. The effect of breaking hydrogen bonds between chloroform and methanol on the removal of residual solvents by ultrasonic treatment during drying was investigated. As shown in Fig. 4, the residual chloroform was completely removed after 10 min of drying without ultrasound after the methanol pretreatment, and the residual methanol was also sufficiently removed to the ICH-value limit (<3,000 ppm). When ultrasound was added, the residual chloroform was completely removed after 10 min of drying; however, residual methanol still remained over the ICH acceptable level (>3,000 ppm). That is, the breakage of hydrogen bonds by ultrasonic waves limited the evaporation of methanol with a relatively low vapor pressure and high boiling temperature. The surface of the dried sample was examined by SEM analysis. The surface of the dried sample before pretreatment with methanol at 25, 35, and 45 °C is shown in Fig. 5(a), (c) and (e); the surface was smooth and no pores were observed. Thus, the surface of the sample was case-hardened, which made the formation of pores difficult, leading to insufficient removal of the residual solvents [14,15]. On the other hand,

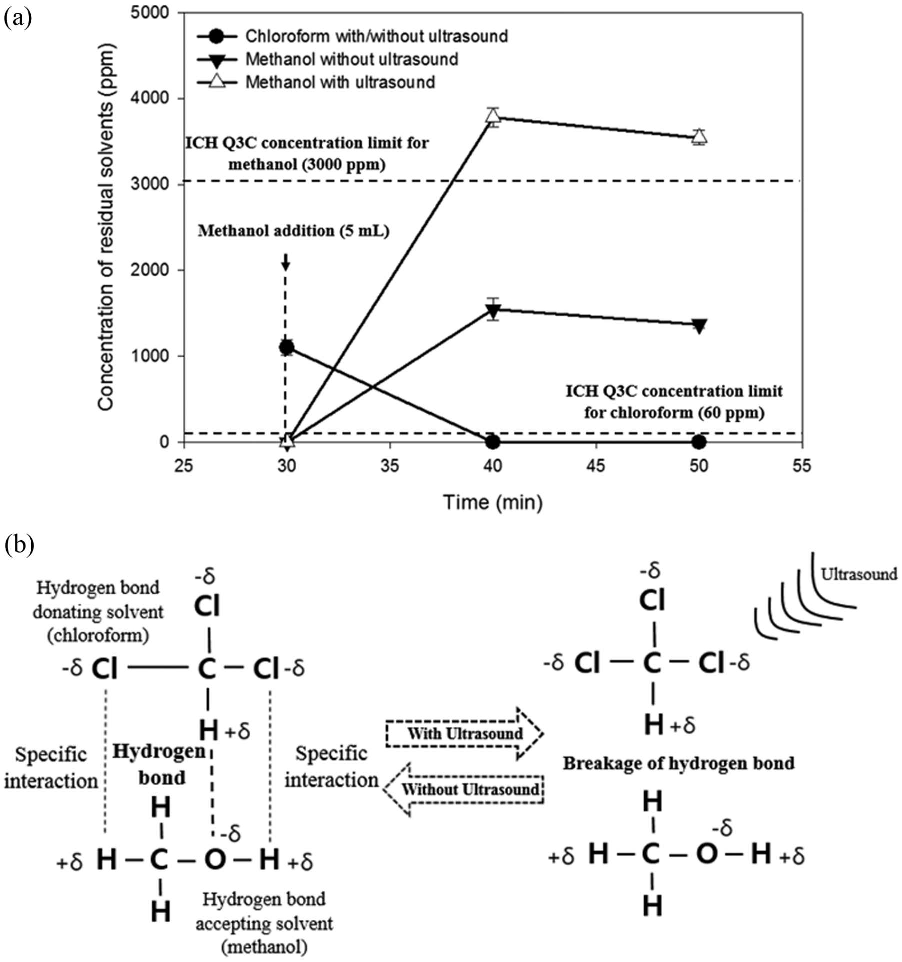
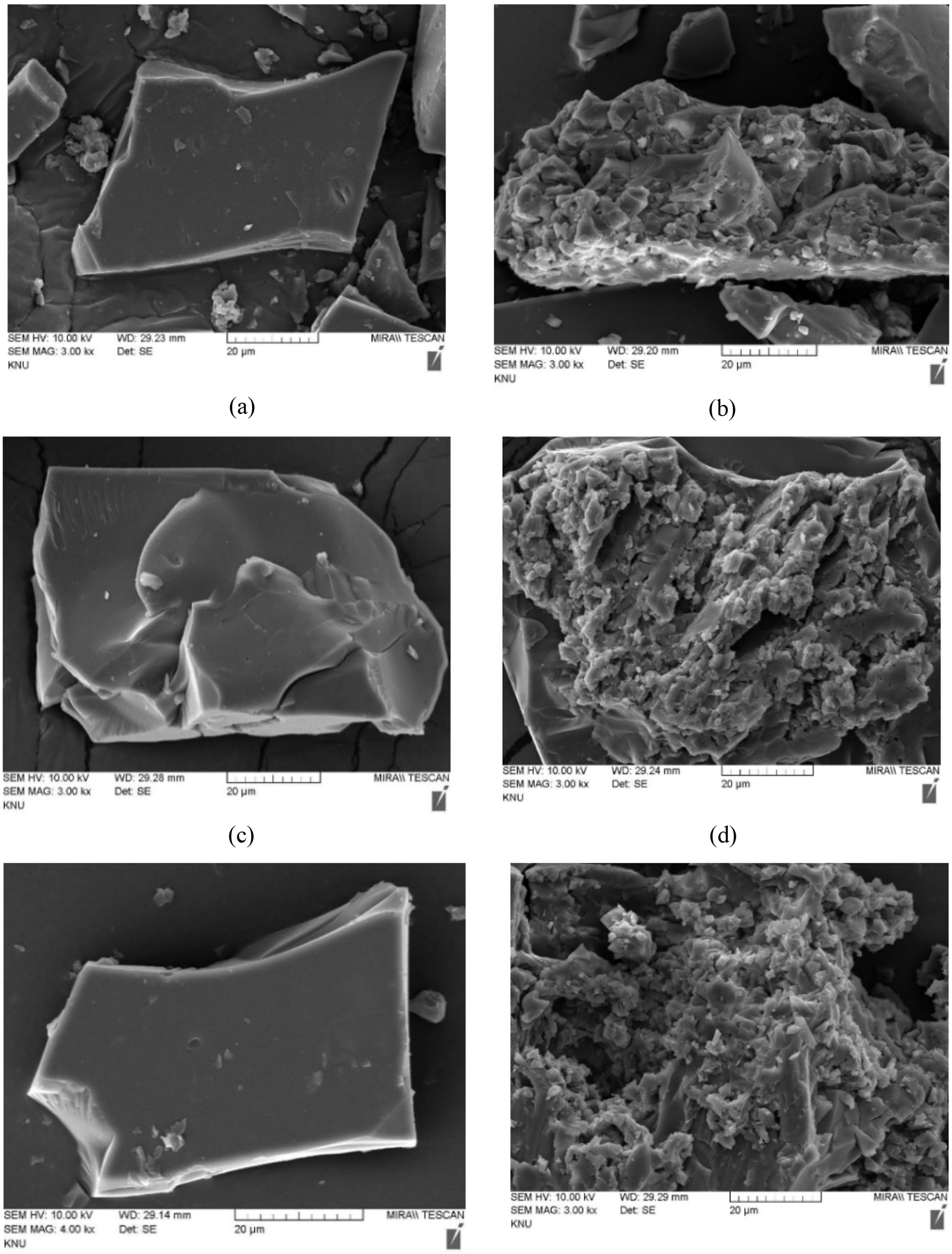


Fig. 4. Effect of ultrasound on the removal of residual solvents. (a) Change of the concentration of residual solvents through drying time in rotary evaporated samples pre-treated with methanol (5 mL) at 25 °C, (b) illustration showing the influence of ultrasound upon hydrogen bond networks between chloroform and methanol.



(a) (b)

(c) (d)

(e) (f)

Fig. 5. SEM images of paclitaxel samples dried by rotary evaporation using different methods. (a) No pretreatment with methanol and drying at 25 °C, (b) pretreatment with methanol (5 mL) and drying at 25 °C, (c) no pretreatment with methanol and drying at 35 °C, (d) pretreatment with methanol (5 mL) and drying at 35 °C, (e) no pretreatment with methanol and drying at 45 °C, (f) pretreatment with methanol (5 mL) and drying at 45 °C.

the surface of the dried sample after pretreatment with methanol at 25, 35, and 45 °C was rough and porous as shown in Fig. 5(b), (d), and (f). This suggests that the structure of the surface of the sample was damaged due to the high vapor pressure of the chloroform-methanol mixture, and this porous structure promoted the removal of the residual solvents.

CONCLUSIONS

We developed an alcohol pretreatment drying method to effectively remove residual solvents from chloroform-induced amorphous paclitaxel. The drying mechanism was also investigated by SEM and ultrasonic treatment. The chloroform was completely removed by simple rotary evaporation for 10-20 min after alcohol (methanol or ethanol) pretreatment at different drying temperatures (25, 35, 45 °C) and different alcohol mixture levels (5-20 mL of methanol or 5-10 mL of ethanol). In addition, the residual methanol and ethanol concentrations met the ICH-specified values (methanol<3,000 ppm, ethanol<5,000 ppm). The result of the SEM analysis showed that the surface of the dried sample pretreated with methanol was rough and cracked, and this porous structure promotes the evaporation of residual solvents. In addition, when the hydrogen bonds between chloroform and methanol were cleaved by ultrasonic treatment, the relatively volatile residual chloroform easily evaporated, while the evaporation of residual methanol was limited. From these results, it was found that the residual solvents were effectively removed due to high vapor pressure and hydrogen bonding properties of the chloroform-alcohol mixture by alcohol pretreatment.

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