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# Establishment of a solvent map for formation of crystalline and amorphous paclitaxel by solvent evaporation process

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**Abstract**−This study intended to establish a solvent map for formation of crystalline and amorphous paclitaxel by a solvent evaporation process. Crystalline paclitaxel was produced by evaporation with polar solvents (acetone, acetonitrile, ethanol, isobutyl alcohol, methanol, methyl ethyl ketone, and n-butyl alcohol) having a polarity index above 4.00. On the other hand, amorphous paclitaxel was produced by evaporation with non-polar solvents (methylene chloride, n-butyl chloride, and toluene) having a polarity index of about 4.00 or lower. The formation of paclitaxel was very closely associated with the polarity index of the organic solvent used in the solvent evaporation process. In the case of crystalline paclitaxel, the higher the polarity index and the lower the viscosity of the organic solvent (n-butyl alcohol, methyl ethyl ketone, and acetonitrile), the higher the degree of crystallinity. In the case of amorphous paclitaxel, the shape and size of particles varied according to the solvent (methylene chloride, n-butyl chloride, and toluene) used in the solvent evaporation process.

Key words: Paclitaxel, Formation Control, Solvent Evaporation Process, Solvent Map, Polarity Index

## INTRODUCTION

Paclitaxel, a diterpenoid anticancer agent discovered in the bark of the yew tree, is one of the most effective anticancer drugs at present. Its chemical composition was revealed in 1971 [1]. The anticancer mechanism of paclitaxel, which differs from those of other anticancer drugs, was revealed in 1979 to be the restriction of cancer cell division in the mitotic phase with relatively low toxicity and high activity [2-4]. Preclinical testing of paclitaxel began in 1977, followed by toxicity studies in 1980, phase I clinical testing in 1983, phase II in 1985, and phase III in 1990. It was approved by the Food and Drug Administration as a treatment for ovarian cancer in 1992, breast cancer in 1994, Kaposi's sarcoma in 1997, and non-small cell lung cancer in 1999. Paclitaxel is currently the most widely used anticancer drug [5-7]. The demand for this drug is expected to increase steadily; its indications, which currently include acute rheumatoid arthritis and Alzheimer's disease, are expanding continuously, and clinical tests for combined prescription with other treatment methods are being conducted [7]. The main methods of paclitaxel production include direct extraction from the yew tree, semi-synthesis involving the chemical combination of side chains after obtaining a precursor from the leaves of the yew tree, and plant cell culture from the main bioreactor after inducing callus from the yew tree and performing a seed culture [8-10]. Among these methods, plant cell culture can stably mass produce paclitaxel of consistent quality in a bioreactor without being affected by such external factors as climate and environment.

For an active pharmaceutical ingredient (API), the specifications for the final purified product are highly varied and strict. The formation (i.e., amorphous or crystalline) of an API is a very important specification, having a great impact on the solubility, dissolution rate, uniformity of drug dispersion, and bioavailability of the final drug product during the formulation process [11-13]. In this respect, the development of a method that easily enables formation control of a purified API can be very useful for the production of the final drug product, especially in the formulation process [14,15]. However, an efficient method of easily and conveniently controlling the formation of paclitaxel as an API has not yet been developed, and related studies have provided insufficient information. Liggins et al. developed a method to control the formation of solid-state paclitaxel, but it is not practical for large-scale production due to the very high temperature that it requires [16]. Moreover, the large amount of heat used in some of the reported schemes can cause degradation of the drug. More recently, a simple method was developed that can control the formation of finally purified paclitaxel by treatment with a solvent [17,18]. This solvent-induced method, however, cannot yet be widely applied because information on the effects of various organic solvents on paclitaxel formation is lacking. Therefore, the present study examined the possibility of controlling paclitaxel formation using various organic solvents in the evaporation process, and attempted to establish for the first time a solvent map for formation control of paclitaxel. If the solvent map thus established for paclitaxel could be applied to formation control of many APIs, it would expected to have a ripple effect in terms of economic as well as academic impacts.

## MATERIALS AND METHODS

### 1. Plant Materials and Culture Conditions

A suspension of cells originating from *Taxus chinensis* was maintained in darkness at 24.8 °C with shaking at 150 rpm. The cells were cultured in modified Gamborg's B5 medium supplemented with 30 g/L sucrose, 10 mM naphthalene acetic acid, 0.2 mM 6-benzylaminopurine, 1 g/L casein hydrolysate, and 1 g/L 2-(N-morpholino) ethanesulfonic acid. Cell cultures were transferred to fresh

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medium every 2 weeks. During prolonged culture for production purposes, 4 mM $AgNO_3$ was added at the initiation of culture as an elicitor, and maltose (1 and 2%, w/v) was added to the medium at days 7 and 21, respectively [19]. Following cultivation, biomass was recovered using a decanter (CA150 Clarifying Decanter; Westfalia) and a high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa-Laval). The biomass was provided by Samyang Genex Company, South Korea.

**2. Preparation of Paclitaxel for Solvent Evaporation Process**

A purified paclitaxel was prepared from biomass obtained from *T. chinensis* cultures using the following steps. (i) Biomass was mixed with methanol and stirred at room temperature for 30 min. The mixture was filtered under vacuum in a Buchner funnel through filter paper, where the biomass was preferably added to methanol at a ratio of 100%. Extraction was repeated at least four times. Each methanol extract was collected, pooled and concentrated at a temperature of 40 °C under reduced pressure to reduce the volume of the methanol extract to 30% of the original. (ii) Methylene chloride (25% of concentrated methanol extract) was added and liquid-liquid extraction was performed three times for 30 min. During the extraction, polar impurities were dissolved in the methanol layer, which eventually formed the upper phase. After this layer was removed, the methylene chloride layer containing paclitaxel was collected and concentrated/dried under reduced pressure. (iii) Dried crude extract was dissolved in methylene chloride at a ratio of 20% (v/w) and sylopute (Fuji Silysia Chemical Ltd., Japan), an adsorbent, was added at a ratio of 50% (w/w). The mixture was agitated for 30 min at 40 °C, then filtered. The filtrate was dried at 30 °C under reduced pressure and subjected to a hexane precipitation process. (iv) The filtrate obtained in the adsorbent treatment step was added to n-hexane to obtain a precipitate, which was dissolved in methanol, dropped into distilled water and left to stand at 0 °C for three days to obtain a paclitaxel precipitate. After precipitation, the paclitaxel was filtered and dried at 30 °C under reduced pressure for HPLC purification. (v) Purification consisted of HPLC on a column packed with ODS (C18; Shiseido), and HPLC on a column packed with silica (Shiseido) [19]. Purified paclitaxel was dried to constant weight at ambient temperature in a vacuum oven equipped with an external pump.

**3. Paclitaxel Analysis**

Dried residue was redissolved in methanol for quantitative analysis using an HPLC system (Waters, USA) with a Capcell Pak C18 column (250 mm×4.6 mm; Shiseido). Elution was performed in a gradient using a distilled water-acetonitrile mixture varying from 65 : 35 to 35 : 65 within 40 min (flow rate=1.0 mL/min). The injection volume was 20 mL, and the effluent was monitored at 227 nm with a UV detector. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich and used as a standard [20].

**4. SEM and XRD Analysis**

The formation of paclitaxel was observed by scanning electron microscopy (MIRA LMH; Tescan, Czech Republic) of an approximately 1 mg sample with an accelerating voltage of 10-15 kV. Paclitaxel samples were also analyzed using an x-ray diffractometer (SMD 3000; SCINCO, Italy) operated by the WINHRD 3000 program at 40 kV and 40 mA with a range of 2-theta from 5 to 85°.

**5. Solvent Evaporation Method**

Zero point three (0.3) grams of purified paclitaxel (purity: >98%) was dissolved in 5 mL of various organic or mixed solvents, which

**Table 1. Characteristics of solvents and their effect on the formation of paclitaxel**

| Solvent | Formation of paclitaxel | Ref. | Boiling point [°C] | Vapor pressure [Torr] | Polarity index | Viscosity [cP] |
|---|---|---|---|---|---|---|
| Acetone | Crystalline | This study | 56.05 | 184.72 | 5.10 | 0.32 |
| Acetone/water (70/30, v/v) | Crystalline | [15] | - | - | 6.63* | - |
| Acetonitrile | Crystalline | This study | 81.65 | 71.56 | 5.80 | 0.36 |
| Acetonitrile/water (90/10, v/v) | Crystalline | [15] | - | - | 6.24* | - |
| Ethanol | Crystalline | This study | 78.29 | 44.21 | 5.20 | 1.14 |
| Ethyl acetate | Crystalline | [17] | 77.11 | 73.56 | 4.40 | 0.44 |
| Isobutyl alcohol | Crystalline | This study | 107.89 | 7.62 | 4.00 | 3.86 |
| Methanol | Crystalline | This study | 64.55 | 96.92 | 5.10 | 0.55 |
| Methanol/water (90/10, v/v) | Crystalline | [18] | - | - | 5.61* | - |
| Methanol/water (98/2, v/v) | Crystalline | [18] | - | - | 5.20* | - |
| Methyl ethyl ketone | Crystalline | This study | 79.59 | 73.05 | 4.70 | 0.41 |
| n-Butyl alcohol | Crystalline | This study | 117.73 | 4.87 | 3.90 | 2.98 |
| Acetonitrile/hexane (1/2, v/v) | Amorphous | [18] | - | - | 2.00* | - |
| Chloroform | Amorphous | [17] | 61.17 | 158.93 | 4.10 | 0.54 |
| Methyl t-butyl ether | Amorphous | [18] | 55.15 | 204.47 | 2.50 | 0.27 |
| Methylene chloride | Amorphous | This study | 39.85 | 353.24 | 3.10 | 0.44 |
| Methylene chloride/methanol (98/2, v/v) | Amorphous | [18] | - | - | 3.14* | - |
| n-Butyl chloride | Amorphous | This study | 78.45 | 80.53 | 1.00 | 0.45 |
| Pentane | Amorphous | [18] | 36.06 | 427.66 | 0.00 | 0.22 |
| Toluene | Amorphous | This study | 110.63 | 21.75 | 2.40 | 0.59 |

*Polarity index was calculated using the formula $\phi=\phi_1x_1+\phi_2x_2$, where $\phi_1$ and $\phi_2$ are the polarity indices of solvents 1 and 2, respectively, and $x_1$ and $x_2$ are the volume fractions of solvents 1 and 2, respectively

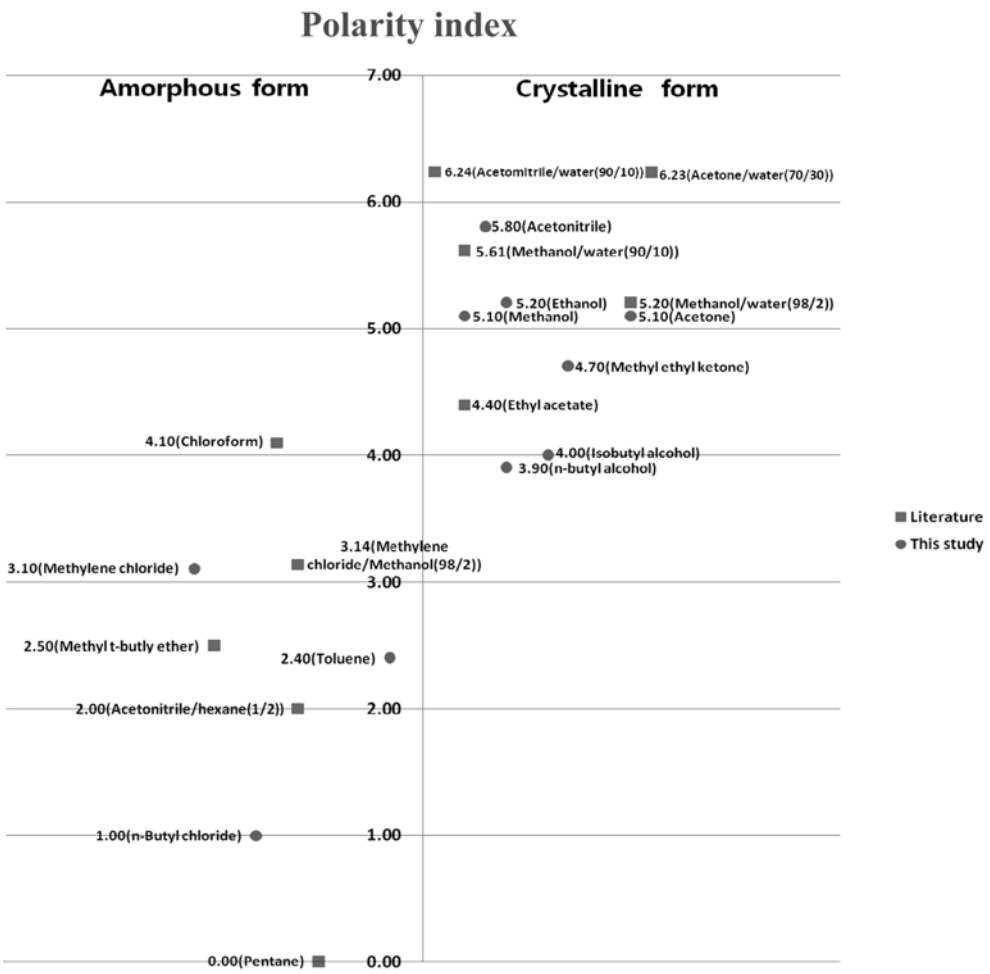


**Fig. 1. Solvent map for formation control of paclitaxel according to polarity index.**

were then dried in a vacuum dryer (UP-2000; EYELA, Japan) at 760 mm Hg and 35 °C for 24 h. All of the organic solvents were of HPLC grade (purity: >90%) and were obtained from Sigma-Aldrich. Since the paclitaxel product cannot be used as a drug if it has been degraded during the solvent evaporation process, the degree of degradation was evaluated by comparing the purity of the product as determined by HPLC before and after solvent evaporation. The formation of dried paclitaxel was determined by SEM and XRD after solvent evaporation.

**6. Analysis of Effect of Evaporation Rate**

To determine the effect of the evaporation rate on paclitaxel crystal shape, solvent evaporation was conducted with acetonitrile at different temperatures (30, 40, and 50 °C). The sample was evaporated in a vacuum dryer for 24 h at each temperature, then analyzed by XRD.

## RESULTS AND DISCUSSION

**1. Establishment of Solvent Map for Formation Control of Paclitaxel**

The properties of the various organic solvents used in the solvent evaporation process and their effects on paclitaxel formation are summarized in Table 1. Carbon tetrachloride was excluded from

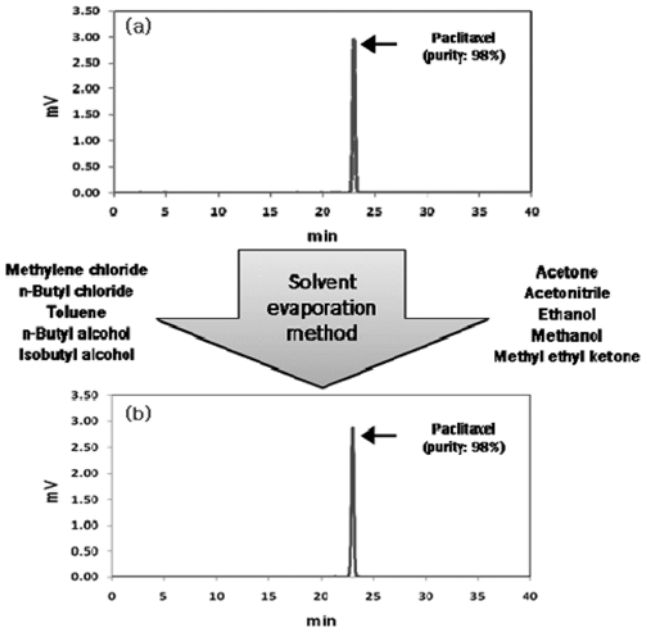


**Fig. 2. Effect of solvents on paclitaxel purity during solvent evaporation: before solvent evaporation (a); after solvent evaporation (b).**

the organic solvents used in this study because it is classified as class 1 (solvents that should be avoided in pharmaceutical products) by the International Conference on Harmonisation (ICH) Q3C Guidance [21]. When solvent evaporation was performed with dioxane, ethylene dichloride, methyl isobutyl ketone, methyl n-propyl ketone, and pyridine, formation was difficult to control due to coevaporation [22,23]; the paclitaxel solute was evaporated together with the solvent, resulting in the loss of a large volume of the product. In addition, 2-methoxyethanol, chlorobenzene, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide, N-methylpyrrolidone, n-butyl acetate, and propylene carbonate were difficult to apply to the solvent evaporation process due to a very high boiling point (>120 °C). Crystalline paclitaxel could be produced using acetone, acetonitrile, ethanol, isobutyl alcohol, methanol, methyl ethyl ketone, and n-butyl alcohol. Amorphous paclitaxel could be produced with methylene chloride, n-butyl chloride, and toluene. According to the results shown in Table 1, paclitaxel formation is very closely associated with the polarity index of the organic solvent used in the solvent evaporation process. Thus, these data were used to establish the solvent map shown in Fig. 1. In particular, amorphous paclitaxel was produced using non-polar solvents with a polarity index of about 4.00 or lower whereas crystalline paclitaxel was produced using polar solvents with a polarity index of over 4.00. This result suggests that paclitaxel formation can be easily and conveniently controlled by selecting a solvent with a particular polarity index for use in the evaporation process. On the other hand, almost no correlation was found between formation and the boiling point, vapor pressure, and viscosity of the organic solvent used. In addition, the degradation of paclitaxel during the solvent evaporation process was examined by analyzing the purity of the paclitaxel via HPLC before and after

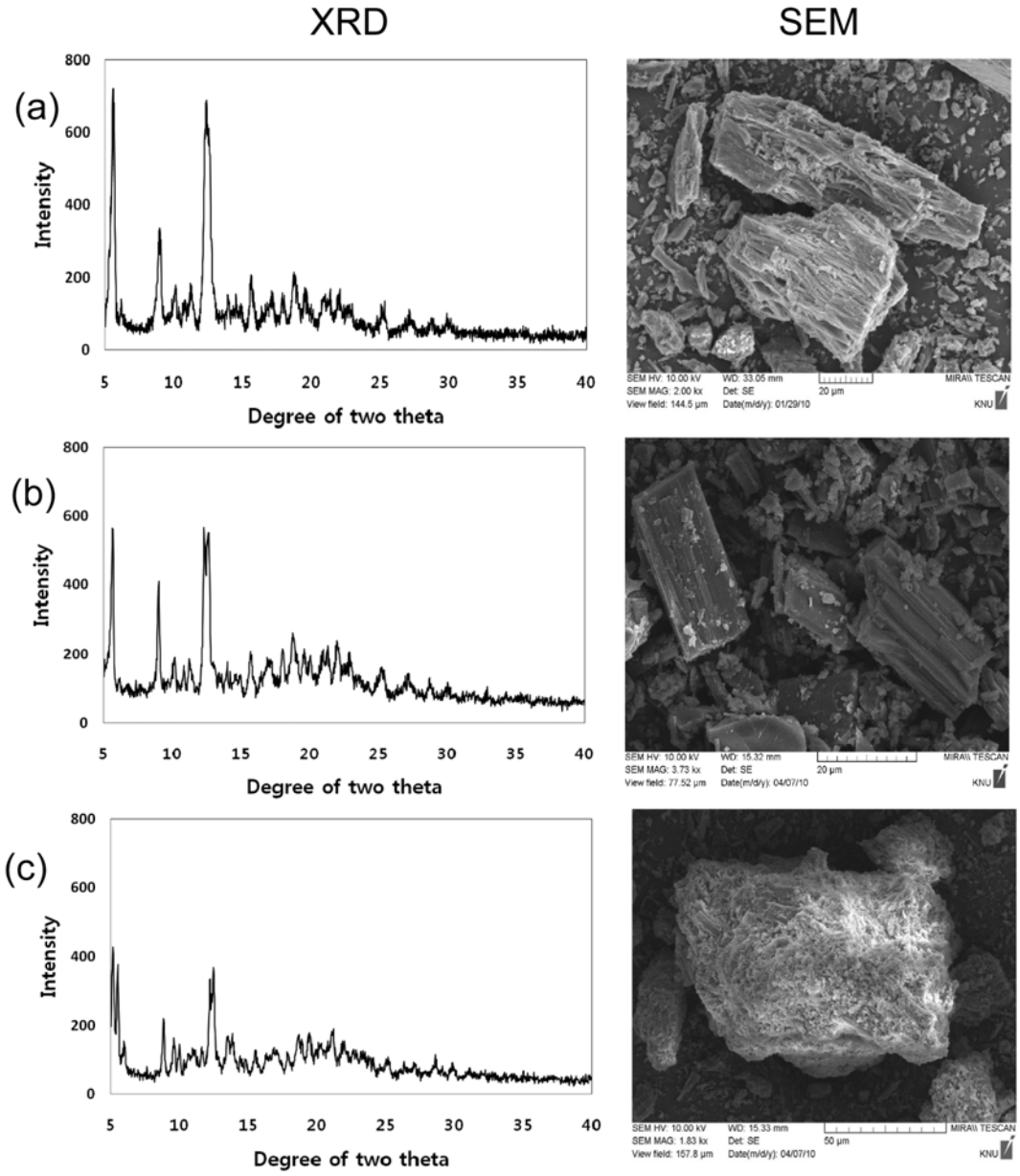


**Fig. 3. XRD patterns and SEM images for paclitaxel crystals in various solvents: acetonitrile (polarity index: 5.80) (a); methyl ethyl ketone (polarity index: 4.70) (b); n-butyl alcohol (polarity index: 3.90) (c).**

solvent evaporation. As illustrated in Fig. 2, the purity of paclitaxel remained unchanged. Thus, each of the organic solvents used in the solvent evaporation process herein can be safely applied to the formation control of the drug product paclitaxel.

**2. Analysis of the Characteristics of Crystalline Paclitaxel**

Crystalline paclitaxel shows peaks at $2\theta$ values of 5.6, 9.1, 10.4, 12.7, and 21.1° in the XRD pattern [18,24]. Among the organic solvents used in the solvent evaporation process, acetonitrile, methyl ethyl ketone, and n-butyl alcohol caused differences in the degree of crystallinity of paclitaxel as revealed by a comparison of XRD patterns (Fig. 3). Peak size generally increased in order of n-butyl alcohol, methyl ethyl ketone, and acetonitrile. The higher the polarity index and the lower the viscosity, the higher the degree of crystallinity by comparing the XRD peak size [25,26]. In contrast, no correlation was found with boiling point and vapor pressure. This result agrees with that for nevirapine, an API with a very low solubility in water; the higher the polarity index of the solvent, the higher the degree of crystallinity [27]. Since crystal shape may vary according to the evaporation rate of a solvent [28], the effect of this parameter was examined using acetonitrile at different temperatures (30, 40, and 50 °C), which can affect the evaporation rate. Almost no change in the degree of crystallinity due to differences in evaporation rate was evident in the XRD pattern (data not shown).

**3. Analysis of the Characteristics of Amorphous Paclitaxel**

In the case of amorphous paclitaxel, no meaningful peaks were found in the XRD pattern. Methylene chloride, n-butyl chloride,

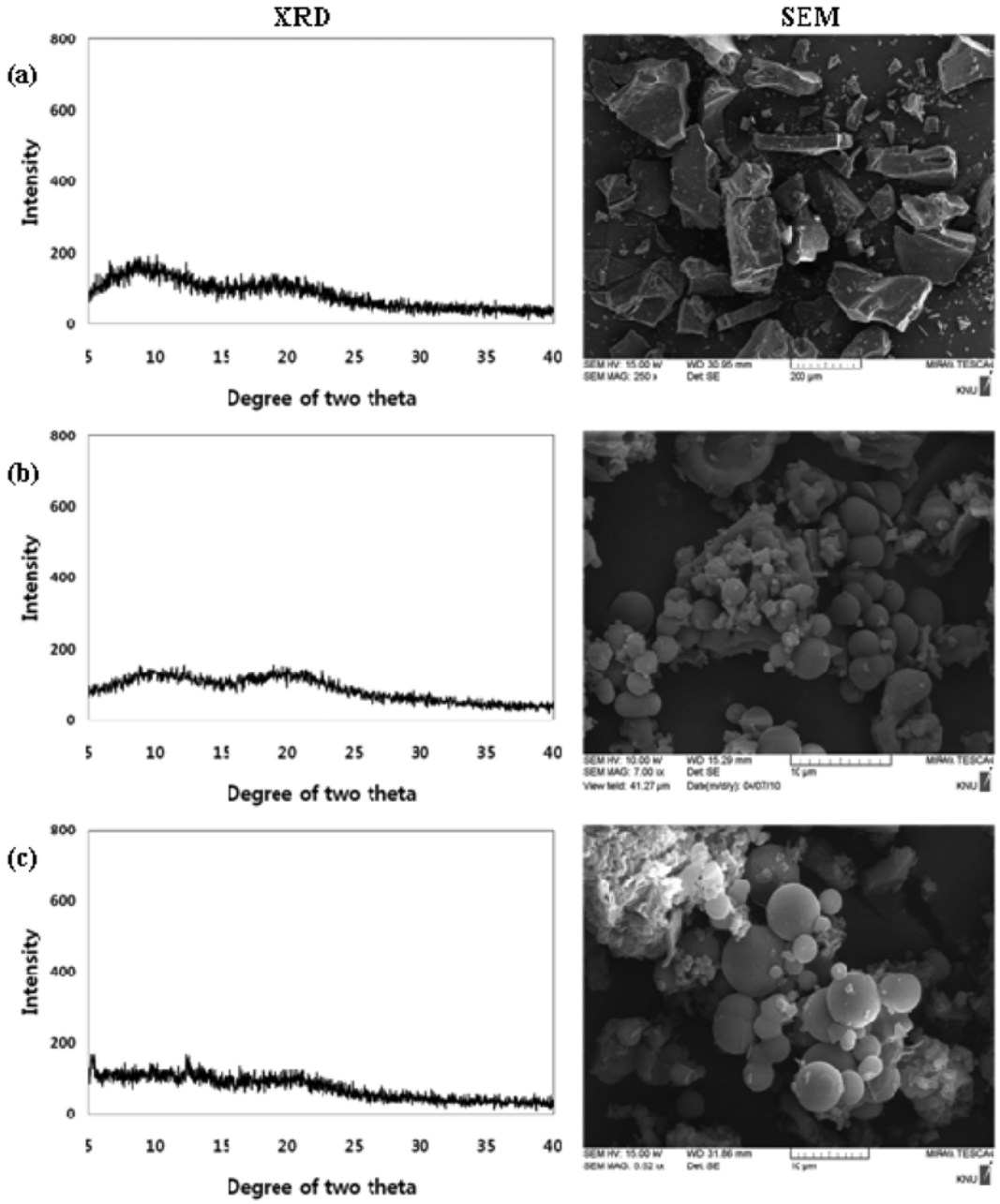


**Fig. 4. XRD patterns and SEM images of amorphous paclitaxel particles in various solvents: methylene chloride (a); n-butyl chloride (b); toluene (c).**

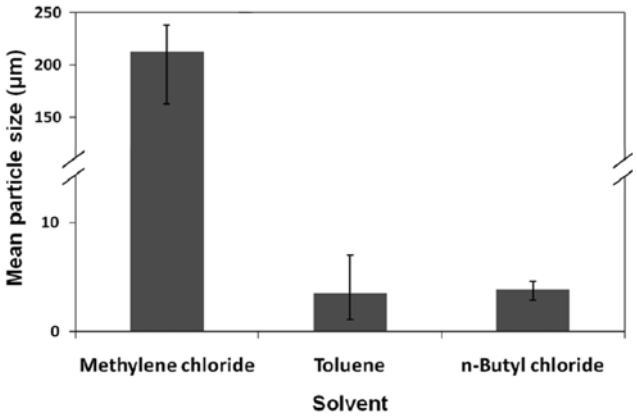


**Fig. 5. Effect of solvents on the mean particle size of amorphous paclitaxel in the solvent evaporation process.**

and toluene generated XRD patterns indicating typical amorphous forms of paclitaxel, and there was no difference between solvents (Fig. 4). On the other hand, SEM revealed differences between different solvents in the shapes and sizes of the amorphous forms. The shape of amorphous paclitaxel particles resulting from the solvent evaporation process with methylene chloride was irregular whereas it was spherical with n-butyl chloride and toluene. Furthermore, the size of amorphous paclitaxel particles was relatively large at approximately 200 μm or more and its size distribution was broad with methylene chloride, whereas it was relatively small at approximately 5 μm or less with n-butyl chloride and toluene (Fig. 5). This phenomenon appears to be associated with the degree of supersaturation of paclitaxel in the organic solvent used in the solvent evaporation process. For APIs, smaller final products have higher utilization value because small size is advantageous in terms of removal of residual solvent and solubility [29,30].

## CONCLUSIONS

A solvent map was established for formation control of the anticancer agent paclitaxel by a solvent evaporation process. The degree of crystallinity and the shape and size of amorphous paclitaxel particles were examined by SEM and XRD. The formation of paclitaxel was very closely associated with the polarity index of the solvent used in the solvent evaporation process. Amorphous paclitaxel was produced using non-polar solvents with a polarity index of about 4.00 or lower, whereas crystalline paclitaxel was produced using polar solvents with a polarity index over 4.00. The higher the polarity index and the lower the viscosity of the organic solvent, the higher the degree of crystallinity. The shape and size of amorphous paclitaxel varied according to the solvent used in the solvent evaporation process. Using methylene chloride, the particles were irregularly shaped, their size was relatively large (approximately 200 μm or larger) and the size distribution was broad, whereas using n-butyl chloride and toluene, the particles were spherical and their size was very small at approximately 5 μm or less.

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