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# Prednisolone multicomponent nanoparticle preparation by aerosol solvent extraction system

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ABSTRACT

Prednisolone nanoparticles were prepared in the presence of a hydrophilic polymer and a surfactant by the aerosol solvent extraction system (ASES). A ternary mixture of prednisolone, polyethylene glycol (PEG), and sodium dodecyl sulfate (SDS) dissolved in methanol was sprayed through a nozzle into the reaction vessel filled with supercritical carbon dioxide. After the ASES process was repeated, precipitates of the ternary components were obtained by depressurizing the reaction vessel. When a methanolic solution of prednisolone/PEG 4000/SDS at a weight ratio of 1:6:2 was sprayed under the optimized ASES conditions, the mean particle size of prednisolone obtained after dispersing the precipitates in water was observed to be ca. 230 nm. Prednisolone nanoparticles were not obtained by the binary ASES process for prednisolone, in the presence of either PEG or SDS. Furthermore, ternary cryogenic cogrinding, as well as solvent evaporation, was not effective for the preparation of prednisolone nanoparticles. As the ASES process can be conducted under moderate temperature conditions, the ASES process that was applied to the ternary system appeared to be one of the most promising methods for the preparation of drug nanoparticles using the multicomponent system.



## 1. Introduction

Size reduction of drugs to submicron order is one of the conventional methods used to enhance the dissolution of poorly water-soluble drugs. Many techniques to prepare nanoparticles in the presence of suitable excipients have been reported (Liversidge and Cundy, 1995; El-Shabouri, 2002; Merisko-Liversidge et al., 2003; Lee et al., 2005). We have previously reported that drug nanoparticles are obtained by the cogrinding of a ternary mixture of hydrophobic drug, water-soluble polymer, and surfactant (Itoh et al., 2003; Pongpeerapat et al., 2004; Moribe et al., 2006). Comixing of polymer and surfactant was necessary for the preparation of drug nanoparticles with considerable stability and bioavailability (Chingunpitak et al., 2008a; Pongpeerapat et al., 2008; Shudo et al., 2008). The limitation of this method was that it could not be applied to compounds with low melting point. Cryogenic cogrinding is an alternative method to reduce particle size. However, this technique is not always suitable for effective size reduction.

Supercritical fluid technique is another candidate technique for the preparation of drug nanoparticles. The preparation methods for fine drug particles using supercritical carbon dioxide are classified into two types. One of them is called the rapid extraction method and the other is called the antisolvent method. In the rapid expansion method, supercritical carbon dioxide is used as the solvent. Micro- or nano-sized drug particles can be obtained by spraying the supercritical solution. Rapid expansion of supercritical solution (RESS) is a representative method (Martin et al., 2000; Türk et al., 2002; Sun et al., 2005; Moribe et al., 2005; Shinozaki et al., 2006; Chingunpitak et al., 2008b) for particle preparation. However, the number of drugs to which this technique is applicable is limited because of the poor solubility of most drugs in supercritical carbon dioxide. On the other hand, supercritical carbon dioxide is used as a poor solvent in antisolvent methods. Gas antisolvent or supercritical antisolvent method (Chattopadhyay and Gupta, 2001; Kim et al., 2008), aerosol solvent extraction system (ASES) (Steckel et al., 1997, 2004; Engwicht et al., 1999; Breitenbach et al., 2000), and solution enhanced dispersion by supercritical fluids method (Velaga et al., 2002; Moshashaée et al., 2003; Pyo et al., 2007; Kang et al., 2008) are known as the representative methods for the preparation of micronized particles. Fine particles were obtained by spraying the drug solution into supercritical carbon dioxide. The advantage of these methods is that they can be applied to most hydrophobic drugs. Furthermore, this technique could be utilized under conditions milder than those required to perform the rapid expansion method.

In this study, drug nanoparticles were prepared by ASES process. Prednisolone was used as a model drug to prepare the nanoparticles in the presence of polyethylene glycol (PEG) and sodium

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dodecyl sulphate (SDS). Particle size distribution of the ASES-processed samples and their morphology in aqueous solution were investigated by dynamic light scattering and electron microscopy. The effects of the composition and the preparation method of the particles on the size reduction of prednisolone were investigated.

## 2. Materials and methods

### 2.1. Materials

Prednisolone was obtained from Sigma–Aldrich Japan and used without further purification. PEG 4000 and 20000 were purchased from Wako Pure Chemical Industries Ltd. (Japan) and Nakarai Tesque Inc. (Japan), respectively. SDS was purchased from Wako Pure Chemical Industries Ltd. (Japan). Reagent grade methanol was used as received.

### 2.2. ASES process

Fig. 1 shows the schematic representation of the apparatus used for ASES process (SC sprayer®; Nikkiso Co. Ltd., Japan). The operation of the ASES process was as follows: firstly, a reaction vessel was filled with carbon dioxide; thereafter, both the pressure and the temperature inside the vessel were raised to a supercritical state. Subsequently, the ternary mixture of drug, polymer, and surfactant dissolved in methanol in the proper weight ratio was sprayed through a nozzle into the reaction vessel with a spraying pressure of 20.0 MPa. Although prednisolone was not dissolved in supercritical carbon dioxide, the solvent was miscible in it. Mixing of organic solvent and supercritical carbon dioxide caused a decrease in the solvation power and resulted in the precipitation of the solute through the supersaturation state. After the ASES process was repeated, the precipitated sample was obtained by depressurizing the reaction vessel.

### 2.3. Process parameters used for ASES

The process parameters for ASES, namely the pressure and the temperature of the reaction vessel, were adjusted to 8.0 MPa and 35 °C, respectively. As the melting temperature of PEG was in the range of 50–60 °C, the spraying pressure and temperature was set as low as possible to maintain the supercritical condition. Similar pressure and temperature conditions have been used in previously reported ASES experiments (Engwicht et al., 1999; Steckel et al., 2004). The spray pressure to load the drug solution into the reaction vessel was fixed at 20.0 MPa.

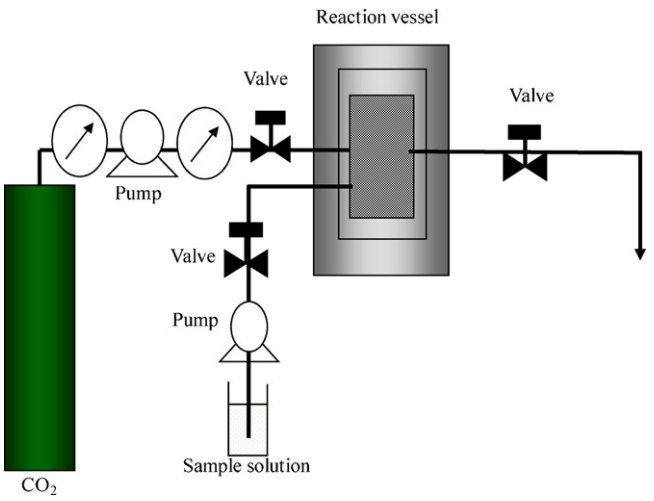


**Fig. 1.** Schematic diagram of an aerosol solvent extraction system (ASES) apparatus.

### 2.4. Preparation of solvent evaporated sample

A physical mixture of prednisolone/PEG 4000/SDS at the weight ratio of 1:6:2 was dissolved in methanol and loaded into a round-bottom flask. Samples were evaporated at 40 °C using a rotary evaporator in order to obtain solvent evaporated samples.

### 2.5. Preparation of cryogenic coground mixture

Cryogenic grinding was performed by loading 3.0 g of the ternary physical mixture in a stainless steel mill chamber and grinding this mixture with a freezing mill (TI-500 ET; CMT Co. Ltd., Japan). Prior to grinding, the mill chamber was gradually exposed to liquid nitrogen until the temperature dropped to −180 °C.

### 2.6. Particle size measurement

Particle size distribution of the ASES-processed sample in water was determined using the dynamic light scattering technique with Microtrac UPA® (Nikkiso Co. Ltd., Japan; measurement range, 0.003–6 μm) or the laser diffraction technique using Microtrac FRA® (Nikkiso Co. Ltd., Japan; measurement range, 0.1–700 μm). Volumetric mean particle size (Mv) was used for comparison.

### 2.7. Scanning electron microscopy (SEM)

The morphology of ASES-treated samples was investigated by cryogenic scanning electron microscopy (SEM) after the samples were dispersed in water. The sample solution was transferred to the specimen holder and frozen in liquid nitrogen. Thereafter, the frozen sample was moved to the cryo-unit, fractured, and moved to the microscope sample stage under vacuum. After sublimation of water, the sample was coated with gold. The secondary electron image was obtained using a scanning electron microscope (JSM-6301F; JEOL, Japan) at the accelerating voltage of 1 kV. After filtering the sample suspension using a 0.1-μm filter, the prednisolone particles were collected; in addition, the dried particles were investigated by SEM (JSM-T330A; JEOL, Japan).

## 3. Results and discussion

### 3.1. Effect of excipients and composition on prednisolone nanoparticle formation by ASES process

Reduction in size of drug particles as a result of the ASES process has been challenged by many researchers (Jeong et al., 2008; Chu et al., 2009); it was more difficult to obtain nanoparticles by spraying the sample into the reaction vessel, due to the lower supersaturation phenomena associated with it, compared with that produced by the rapid expansion method (Engwicht et al., 1999; Steckel et al., 2004). As shown in Fig. 2, the particle size and the distribution of prednisolone particles that were only subjected to ASES were still large compared to the corresponding unprocessed particles. Thus, it is important to control nucleation, crystal growth, and particle agglomeration of prednisolone to prepare nano-sized particles. Precipitation of prednisolone in the presence of excipients in the ASES process is one of the promising methods for the preparation of drug nanoparticles. Excipients are precipitated out with prednisolone by the ASES process. Various hydrophilic polymers and surfactants were surveyed for this purpose. PEG 4000, PEG 20000, and SDS were selected for the experiment.

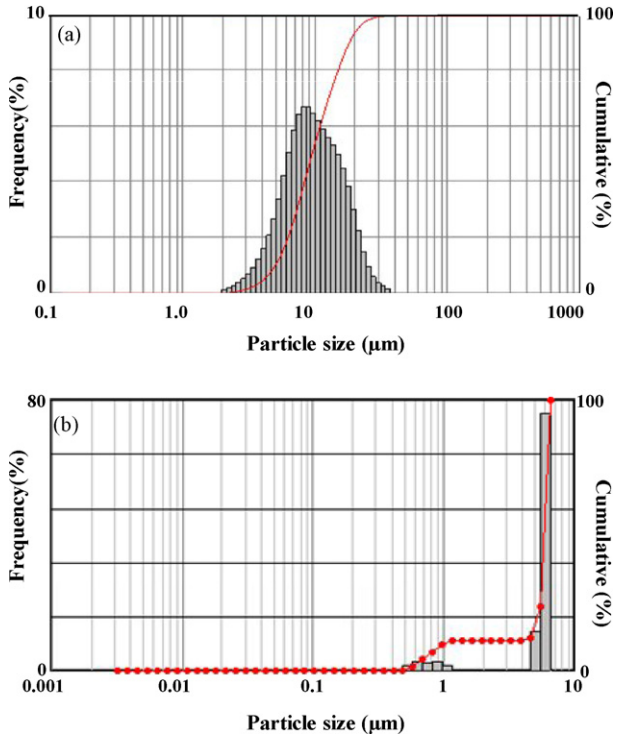


**Fig. 2.** Particle size distribution patterns of (a) unprocessed prednisolone and (b) ASES-processed prednisolone. Particle size distribution was measured (a) using HRA® and (b) UPA®. ASES conditions: concentration of sample solution, 0.5% (w/v); spraying pressure, 20.0 MPa; internal pressure of the reaction vessel, 8.0 MPa.

The important difference of the preparation method of nanoparticle between ASES and grinding is that the former is the build-up process after dissolving the drug in methanol and the latter is the break down process from the drug crystals. It was speculated that a hydrophilic polymer and a surfactant could prevent crystal growth of the drug in ASES process. In the case of grinding, aggregation and agglomeration of the micronized drug was prevented in the presence of excipients as in the case of drug/PVP/SDS ternary system.

Fig. 3 shows the effects of additives on the particle size distribution of ASES-processed prednisolone. When the ASES process was performed at the concentration of 0.5% (w/v) prednisolone, spray pressure of 20.0 MPa, and internal pressure of the reaction vessel of 8.0 MPa, prednisolone nanoparticles with a mean particle size of ca. 230 nm were obtained using prednisolone/PEG 4000/SDS (weight ratio of 1:6:2) ternary mixture (Fig. 3c). Since prednisolone nanoparticle formation was not observed in the cases in which the ASES process was performed with prednisolone/PEG 4000 and prednisolone/SDS binary components, ternary ASES process of prednisolone with PEG 4000 and SDS were essential for the size reduction of the prednisolone particles to submicron order.

Table 1 summarizes the effects of the weight ratio of the ternary components on prednisolone particle size. ASES-processed prednisolone/PEG 4000/SDS samples were prepared at a weight ratio of 1:10:3.3, 1:6:2, and 1:3:1. The concentration of drug in methanol was adjusted to 0.5% (w/v). The ratio of PEG to SDS was fixed at 3:1; this is the same ratio as that employed in our previous experiments (Itoh et al., 2003; Pongpeerapat et al., 2004). Smallest particle size was obtained when the ternary system was used at a weight ratio of 1:6:2 (Batch B). In our previous experiments, drug/polymer/surfactant ternary grinding was conducted at a weight ratio of 1:3:1 for the preparation of drug nanoparticles. Optimum weight ratio of prednisolone/PEG 4000/SDS from the

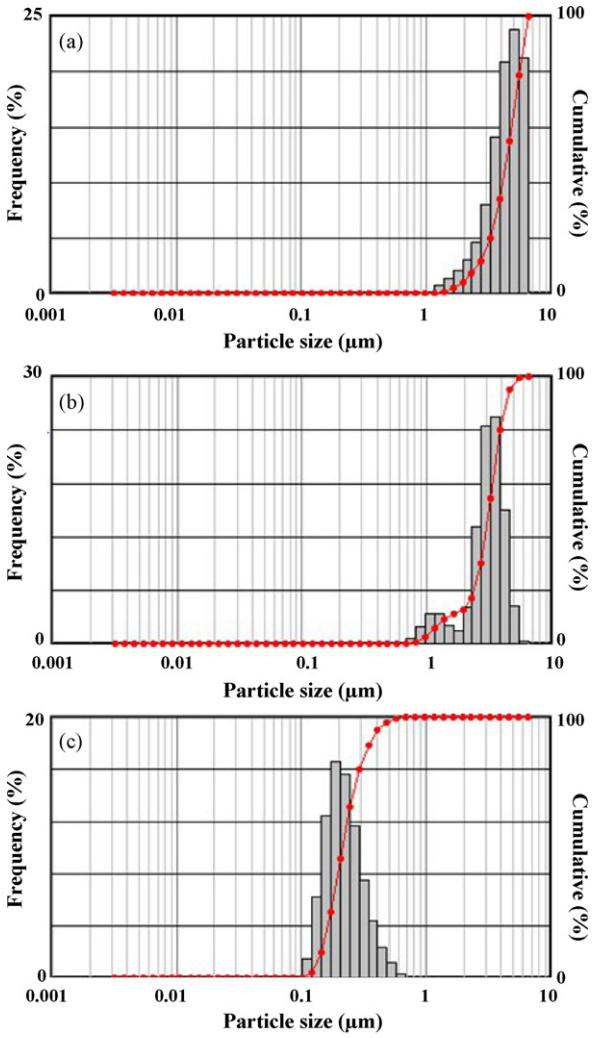


**Fig. 3.** Effect of composition on particle size distribution patterns of prednisolone. (a) Prednisolone/polyethylene glycol (PEG) 4000 (1/6), (b) prednisolone/sodium dodecyl sulfate (SDS) (1/2), (c) prednisolone/PEG 4000/SDS (Batch B, 1:6:2). Particle size was measured using UPA®. ASES conditions: concentration of sample solution, 0.5% (w/v); spraying pressure, 20.0 MPa; internal pressure of the reaction vessel, 8.0 MPa.

perspective of the ASES process, which prevented crystal growth and agglomeration of prednisolone, was different from that considered from the perspective of ternary grinding. An excess amount of excipients was required for the effective prevention of prednisolone crystal growth by the ASES process. When the drug concentration in methanol was adjusted as same, the viscosity of the sample solution due to PEG and SDS should influence the droplet size following spraying, even if the excess amount of excipients is effective for the prevention of crystal growth. The difference in viscosity appeared to be the reason underlying the greater particle size of ASES-processed samples at the weight ratio of 1:10:3.3 than that at 1:6:2. In addition to the weight ratio of the ternary components, the concentration of the sample solution also affected the particle size. When we used prednisolone/PEG 4000/SDS at a weight ratio of 1:6:2, the concentration of the sample solution at 0.5% (w/v) was observed to be sufficient to yield prednisolone nanoparticles to a greater extent than when used at concentrations of 0.3 and 0.7% (w/v). The results indicated that there was an optimum composition and concentration for effective size reduction in prednisolone, which may be associated with the mechanism of prednisolone particle formation using ASES.

**Table 1**
Effect of weight ratio of the ternary components, concentration of the sample solution and PEG molecular weight on mean particle size of prednisolone/PEG/SDS system.

| Batch | Molecular weight of PEG | Prednisolone/PEG/SDS (weight ratio) | Concentration of sample solution (w/v %) | Mean particle size (μm) |
|---|---|---|---|---|
| A | 4000 | 1/10/3.3 | 0.5 | 1.41 |
| B | 4000 | 1/6/2 | 0.5 | 0.23 |
| C | 4000 | 1/3/1 | 0.5 | 1.44 |
| D | 4000 | 1/6/2 | 0.3 | 3.45 |
| E | 4000 | 1/6/2 | 0.7 | 2.85 |
| F | 20000 | 1/6/2 | 0.5 | 0.27 |

*Note*: spraying pressure: 20.0 MPa, pressure inside the reaction vessel: 8.0 MPa.

The optimum composition and concentration determined using prednisolone/PEG 4000/SDS were applicable to the prednisolone/PEG 20000/SDS ternary system (Batch F). Prednisolone nanoparticles of ca. 270 nm were obtained by ASES using the prednisolone/PEG 20000/SDS system. These results implied that it was possible to obtain prednisolone nanoparticles using different molecular weights of PEG under optimized experimental conditions.

### 3.2. Morphology of ASES-treated sample

Fig. 4 shows the morphology of prednisolone/PEG 4000/SDS nanoparticles prepared using ASES following dispersion in water. Prednisolone nanoparticles were not observed in the ASES-treated precipitate, as prednisolone was covered with PEG and SDS. Spherical nanoparticles were observed by cryogenic SEM of the suspension of ASES-treated samples. The size of prednisolone particles was similar to that determined by dynamic light scattering. The prednisolone particles retained on the 0.1-μm membrane filter after the sample solution passed through were observed to still be of the submicron size; however, their morphology was not spherical. The SEM micrograph of particles on a filter was taken after water was removed. In the case of cryogenic SEM, water still remains in the particles. The different condition may reflect on the morphology and shape. Assuming that particle was composed of drug crystals covered with polymer and surfactant, morphology of the crystal shape might be different from spherical.

### 3.3. Comparison of preparation method of fine particles

We prepared prednisolone particles using other preparation methods. As shown in Fig. 5, prednisolone nanoparticles could not be obtained with the solvent evaporation and cryogenic grinding methods. Cryogenic grinding has been conducted as an effective method for the reduction in drug particle size, though drug crystals occasionally change their morphology to amorphous, which may not be suitable for the preparation of nano-sized particles. The elongation of the grinding time was performed for approximately 90 min. However, following this process, further improvement in the preparation of particles was not observed compared with the 30-min ground sample (Mv = 1.59 μm). From the results of powder X-ray diffraction measurement, diffraction peaks originated from prednisolone were observed not only in ASES-processed sample but also in cryogenic ground sample (data not shown). Thus, crystal form of prednisolone did not change by the cryogenic grinding. Effective size reduction of prednisolone through the interaction with PEG may not occur by the cogrinding.

Since drug nanoparticle formation by ASES process was observed to depend on the solubility of ternary components both in organic solvent and supercritical carbon dioxide, the number of drugs and excipients applicable to the ASES method might be restricted. However, the ASES process used in this study was the only method suited for the preparation of prednisolone nanoparticles from the ternary system. Thus, the ASES process was observed to be one among the most promising techniques for the

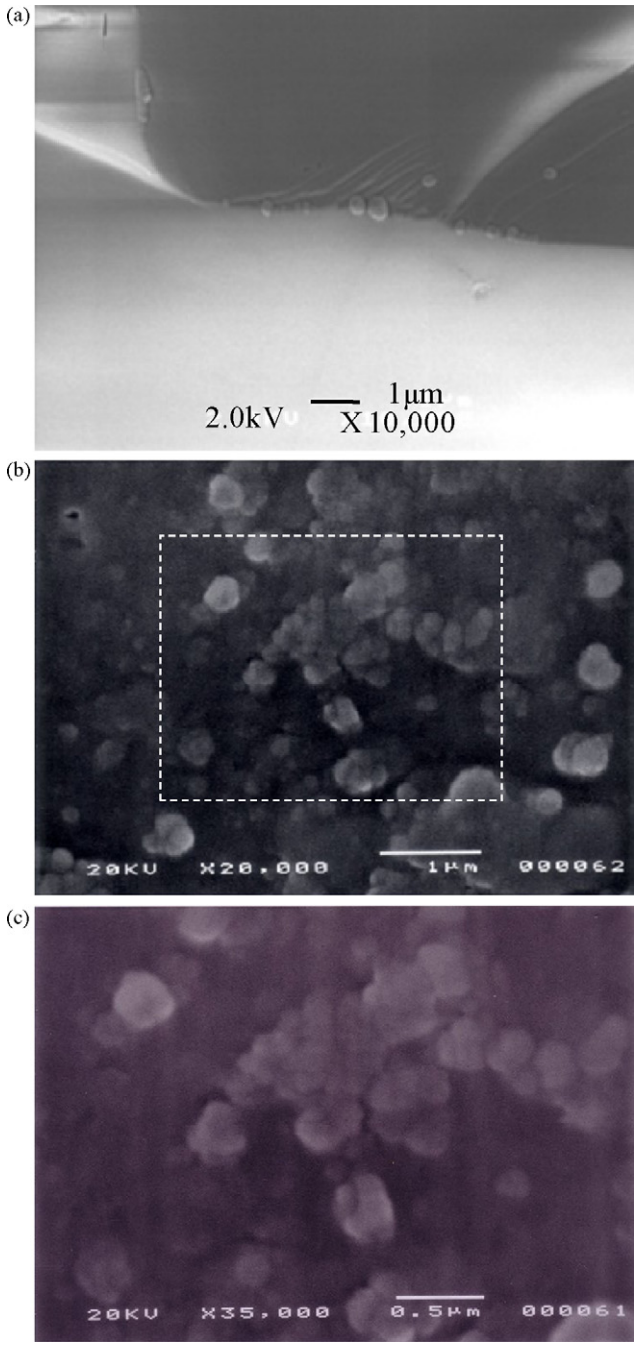


**Fig. 4.** Morphology of prednisolone/PEG 4000/SDS (Batch B, 1:6:2) nanoparticles prepared by ASES process. (a) Cryogenic scanning electron microscopy (SEM) of prednisolone nanosuspension, (b) SEM of prednisolone nanoparticles retained on the 0.1-μm membrane filter after passing the prednisolone suspension, (c) enlargement of a part of (b) shown by the dotted square. ASES conditions: concentration of sample solution, 0.5% (w/v); spraying pressure, 20.0 MPa; internal pressure of the reaction vessel, 8.0 MPa.

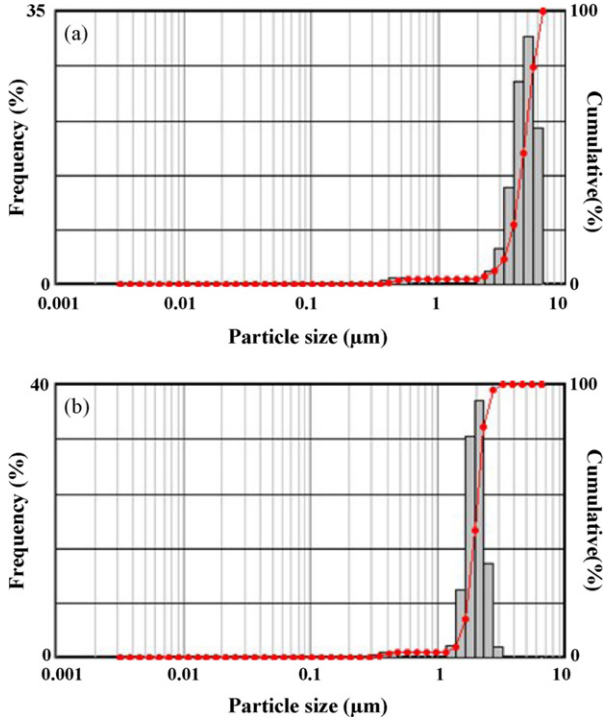


**Fig. 5.** Particle size distribution patterns of the prednisolone/PEG 4000/SDS system. (a) Evaporated sample, (b) cryogenic coground mixture. Weight ratio of prednisolone/PEG 4000/SDS was 1:6:2. Particle size was measured using UPA®. Evaporation conditions: concentration of sample solution, 0.5% (w/v); temperature of water bath, 45 °C. Grinding condition: grinding time, 30 min under liquid $N_2$ atmosphere (−180 °C).

preparation of drug nanoparticles from the ternary component system.

## 4. Conclusion

Prednisolone nanoparticles can be obtained from the prednisolone/PEG 4000/SDS ternary component system by the ASES process. Experimental parameters were observed to be the most important factors determining the prednisolone nanoparticle preparation by this process. Prednisolone/PEG 4000/SDS at the weight ratio of 1:6:2 with a solution concentration of 0.5% (w/v) yielded the smallest particle size of prednisolone of approximately 230 nm. The prednisolone nanoparticles could not be obtained by applying the ASES method of binary components, evaporation, and cryogenic grinding methods. ASES process employing the multicomponent system appears to be an alternative method for the preparation of drug nanoparticles.

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