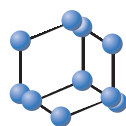


## RESEARCH ARTICLE

BENTHAM  
SCIENCE

## Preparation of Nanoparticles Including Antisolvent Drugs by the Combination of Roll Milling and High-pressure Homogenization



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## ARTICLE HISTORY

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**Abstract: Description:** Design methods of nanoparticle formulations are divided into break-down methods and build-up methods. The former is further divided into dry and wet processes. For drug nanoparticle preparations, the wet process is generally employed, and organic solvents are used in most formulations.

**Method:** In this study, we investigate the preparation of nifedipine (IB) and griseofulvin (GF) nanoparticles without using organic solvent. Both IB and GF nanoparticles, with a mean particle size of approximately 50 nm, were prepared without organic solvent by employing a combination of roll milling and high-pressure homogenization.

**Result:** The X-ray diffraction peak of the IB and GF samples prepared by roll milling was present at a position (2θ) identical to that of IB and GF crystals, indicating that no peak shift was induced by interaction with phospholipids.

**Conclusion:** These findings demonstrate that most IB and GF nanoparticles exist as crystals in phospholipids.

**Keywords:** Nanoparticles, without organic solvent, roll milling, high-pressure homogenization, antisolvent drugs, X-ray diffraction peak, DSC.

## 1. INTRODUCTION

The improvement of the dissolution rate of poorly water-soluble drugs is an important and growing area of research in the field of pharmaceuticals. Conventional methods of improving the dissolution rate that have been employed are the hot-melt extrusion method [1, 2] and/or comminuting with water-soluble polymers [3, 4].

Nanotechnology has recently been employed in a variety of fields. Mere pulverization of drug particles themselves is an effective method where expansion of the specific surface area facilitates dissolution of the drug particles. To date, many methods have been developed for micronizing such particles [5, 6]. In addition to improving the solubility of the drug due to an increase in the drug particle's surface area, drugs micronized to the nano-order may also be directly delivered through intestinal Peyer's patches [7, 8].

Methods for preparing nanoparticles are broadly classified into break-down methods and build-up methods [9, 10]. In the former, particles are ground to a nano-order size by either a dry [11-13] or a wet [14, 15] process. Regarding the dry processes, co-grinding methods with adjuvants (polymers, such as polyvinyl pyrrolidone and microcrystalline

cellulose, and water-soluble substances, such as sugars, sugar alcohol, and amino acids) have been employed [16, 17].

The wet processes have smaller limitations regarding particle size, compared to the dry process by employing, for example, high-pressure homogenization [18, 19]. Such wet grinding methods are capable of micronizing drug particles to sizes in the range of several hundreds of nanometers. However, most of these methods involve the use of organic solvents [20-22].

Accumulation of residual solvents in the body tissue, and environmental pollution by liquid wastes is a matter of concern with regard to the utilization of organic solvents. Additionally, the number of super-antisolvent drugs, which are hard to dissolve in both water and organic solvent, are currently increasing. We believe that mechanical pulverization methods are an attractive means of overcoming these problems. We have previously reported the first example of a method for pulverizing nifedipine nanoparticle preparations without employing organic solvents, such as ethanol, in any stage of the process [23]. The aim of the present study is to demonstrate the viability of pulverization of drugs by combining previously reported methods. Therefore, we investigated the pulverization of other antisolvent drugs by the combination of a dry process (co-grinding by a roll mill) and a wet process (high-pressure homogenization). To date, we have posited that the crystal state transforms to an amor-

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phous state after treatment with ethanol or roll mixture with a carrier (polymers, sugars, sugar alcohol, or amino acids). However, the X-ray powder diffraction patterns of nifedipine did indicate tiny peaks of the nifedipine crystal after being co-ground by a roll mill. The question arises if this is specific to this drug or if other antisolvent drug exhibits similar behavior. This result could represent the possibility that nifedipine was subsistent as crystals inside the nanoparticles. Therefore, we also investigated crystal condition using X-ray powder diffraction. For model drugs Ibuprofen (IB) and Griseofulvin (GF), were used. IB, a non-steroidal anti-inflammatory drug, and GF, an antifungal drug, are both most commonly administered orally.

## 2. MATERIALS AND METHOD

### 2.1. Materials

Hydrogenated soybean phosphatidylcholine (HSPC, COATSOME<sup>®</sup> NC-21) and dipalmitoyl phosphatidylglycerol (DPPG, COATSOME<sup>®</sup> MGLS-6060) were purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). Ibuprofen and griseofulvin (JPXIV) were provided by Nippon Fine Chemical Co., Ltd. (Osaka, Japan). Ethanol (reagent grade) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Gelatin powder was provided by Nitta Gelatin incorporation (Tokyo, Japan).

All reagents were used as received. Water was purified by ion exchange prior to use.

### 2.2. Preparation of IB (or GF)-Phospholipid (HSPC-DPPG) Mixture

Thirty milligrams of IB (or GF) and 1000 mg of phospholipids (HSPC: DPPG = 4:1 molar ratio) were added to a mortar and physically mixed. The mixture was subsequently co-ground in a roll mill (EXAKT50, Nagase Screen Printing Research Co., Ltd.) and mixed-ground at a rotational velocity ratio of 1:2.5:5.8 a total of 5 times, during which the sample mostly adhered to, but partially fell from, the roller. The mill was stopped at every 5th spin, and the fallen sample was collected. The co-grinding cycle was repeated.

### 2.3. Preparation of IB (or GF)-Phosphatidylcholin Mixture Nanoparticle Suspension

A 20:1000 IB (or GF)-phospholipid mixture (HSPC: DPPG = 4:1 molar ratio), prepared by roll milling, was dispersed in 200 mL of purified water and premixed using a Speed Stabilizer (10,000 rpm, KINEMATICA Co.) at 9000 rpm for 10 min. This premixed suspension was placed in a high-pressure homogenizer (NV -200-D\*\*\*, Yoshida Kikai Co., Ltd.; max pressure = 200 MPa) and subjected to 10 homogenization cycles. Premixed suspensions used as comparative controls were prepared as described below.

To prepare control samples by the ethanol treatment method, 20 mg of IB (or GF) and 1000 mg of phospholipid (HSPC: DPPG = 4:1 molar ratio) were dissolved in 2 mL of ethanol in a water bath at 80°C. Consequently, the ethanol evaporated. The mixture was subsequently dispersed in 200 mL of purified water and premixed using a speed stabilizer.

To prepare control samples by sonication samples were directly dispersed in 200 mL of purified water and sonicated for 20 min. The sonicated suspension was premixed using a speed stabilizer.

To prepared control samples with no treatment samples were directly dispersed in 200 mL of purified water and premixed using a speed stabilizer.

## 3. EXPERIMENTAL

### 3.1. Nanoparticle Size Measurement

The mean particle sizes of nanoparticles prepared by high-pressure homogenization using selected numbers of rotations were measured at room temperature using an electrophoretic light scattering photometer (zetalyzer Nano S, Malvern Instrument Ltd.) at a fixed angle of 90°. The particle sizes were analyzed based on weight distribution. The nanoparticle suspension was analyzed without dilution.

### 3.2. X-ray Powder Diffraction

IB (or GF) and phospholipids were weighed at 20:1000 (weight ratio) and mixed in a mortar for 2 min followed by co-grinding in a roll mill for 5 cycles. For physical mixing, phospholipids were processed by roll milling for 5 min. Unprocessed IB (or GF) were mixed using a mortar and pestle for 5 min. The prepared samples were subjected to X-ray powder diffraction using CuK $\alpha$  radiation at 40 kV and 30 mA at room temperature using an X-ray powder diffractometer (PW1825 generator, PHILIPS). Diffraction patterns were collected from a diffraction angle ( $2\theta$ ) of 2 to 30° at a scan rate of 5°/min.

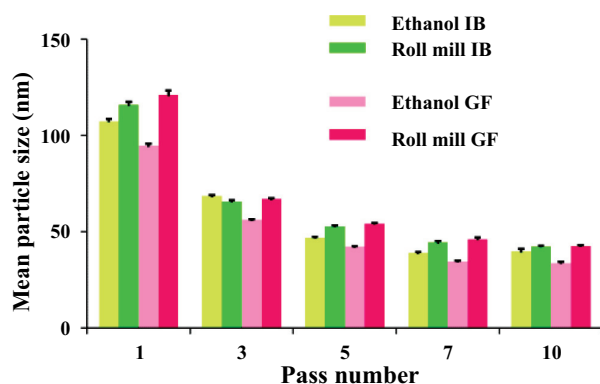
### 3.3. Thermal Analysis of HSPC Containing IB (or GF)

HSPC or IB (or GF) compounds, prepared by roll milling, were analyzed by differential scanning calorimetry (DSC) using a DSC-50 (Shimadzu) differential scanning calorimeter. Each sample (8.0 mg) was weighed in a pan covered with a plate cover (Open Plate Cover, Shimadzu Co.) and subsequently sealed. The samples were heated at a rate of 10°C/min over a temperature range of 40 to 100°C.

## 4. RESULTS AND DISCUSSION

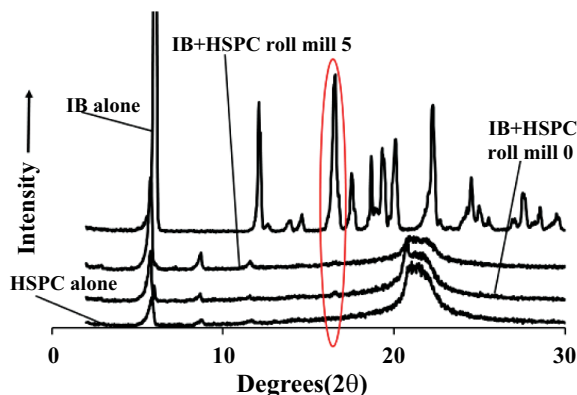
The number of super-antisolvent drugs that are sparingly soluble in water and organic solvents is increasing. It is advantageous to pulverize such compounds mechanically because organic solvent is not essential to create nanoparticles. We have previously reported that the preparation of nifedipine nanoparticles can be achieved without organic solvent by employing a combination roll milling and high-pressure homogenization [23].

The sizes of nanoparticles prepared by a combination of high-pressure homogenization and co-grinding by roll milling, and those prepared by ethanol pretreatment are shown in Fig. (1). The mean size of all the drug-containing nanoparticles decreased with an increasing pass number. The mean size of nanoparticles including IB, pretreated with roll

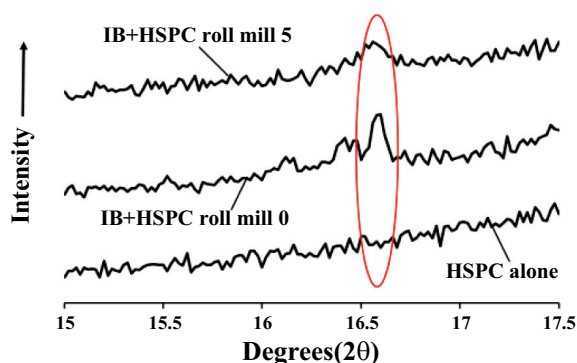


**Fig. (1).** Impact of pass number on the mean size of IB (or GF)-phospholipid nanoparticles prepared by a combination of roll milling and high-pressure homogenization. Each bar represents the mean of three measurements. Roll mill: IB (or GF)-phospholipid (HSPC: DPPG = 4:1) mixture was prepared by roll milling and high-pressure homogenization. IB (or GF)-phospholipid mixture was dispersed in purified water. The dispersed suspension was premixed. The premixed suspension was homogenized by high-pressure homogenization. Ethanol: a IB (or GF) and phospholipid (HSPC: DPPG = 4:1, molar ratio) mixture was dissolved in ethanol. The ethanol evaporated. The IB (or GF) -phospholipid mixture was dispersed in purified water. The dispersed suspension was premixed. The premixed suspension was homogenized by high-pressure homogenization.

milling, was determined to be 42.3 nm at pass number of 10, while the mean particle size of nanoparticles including IB pretreated with ethanol was determined to be 40.0 nm at a pass number of 10. However, the mean particle size of nanoparticles including GF, pretreated with roll milling, was determined to be 42.5 nm at a pass number of 10, while the mean particle size of nanoparticles including GF, prepared using ethanol pretreatment, was determined to be 34.7 nm, at a pass number of 10. Hence, nanoparticles prepared by a combination roll milling and high-pressure homogenization can reduce particle sizes comparable to those achieved by ethanol pretreatment. These findings indicate that co-grinding by roll milling is as effective as ethanol treatment for both drugs studied herein.

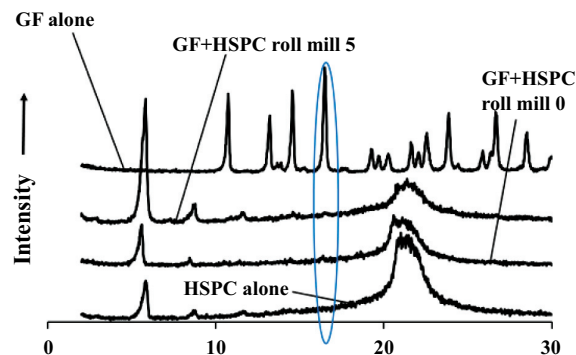


**Fig. (2a).** X-ray powder diffraction patterns of main components IB-phospholipid prepared by roll milling. IB + HSPC roll mill 5: IB and phospholipid (IB: HSPC = 1: 50) were ground by roll milling. IB + HSPC roll mill 0: Physical mixture of IB and phospholipid (IB: HSPC = 1: 50).

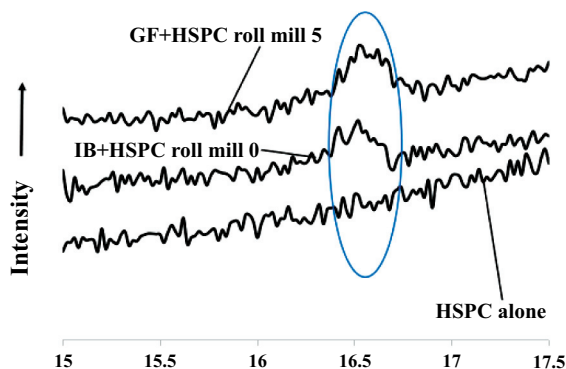


**Fig. (2b).** Magnification of Fig. 2a. X-ray powder diffraction patterns of main components IB-phospholipid prepared by roll milling.

The X-ray powder diffraction pattern of the IB-phospholipid mixture prepared by roll milling is shown in Fig. (2a). The pattern of the IB-phospholipid roll milled mixture was almost identical to the diffraction pattern of HSPC alone (*i.e.*, the physical mixture of IB and phospholipid). However, a small difference was observed between 16 and 17°. To further investigate this detail the powder X-ray diffraction pattern was expanded between 15 and 17.5°, as shown in Fig. (2b). IB-specific diffraction peaks of the physical mixture sample and the sample co-ground by roll milling, appeared at 16.51°. However, diffraction peaks were not observed at 16.51° for HSPC alone.



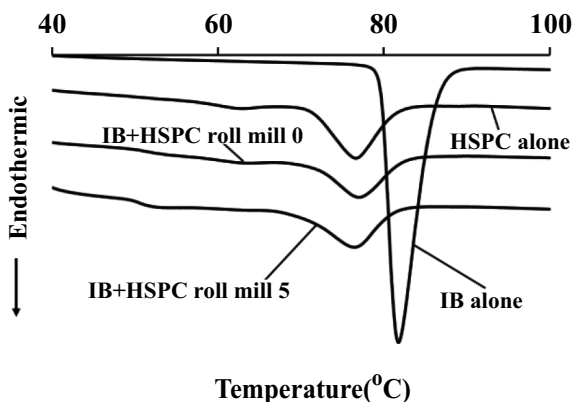
**Fig. (3a).** X-ray powder diffraction patterns of main components GF-phospholipid prepared by roll milling. GF + HSPC roll mill 5: GF and phospholipid (GF: HSPC = 1: 50) were ground by roll milling. GF + HSPC roll mill 0: Physical mixture of GF and phospholipid (GF: HSPC = 1: 50).



**Fig. (3b).** Magnification of Fig. 3a. X-ray powder diffraction patterns of main components IB-phospholipid prepared by roll milling.

The GF mixture was also investigated. The X-ray diffraction powder pattern of the GF-phospholipid mixture prepared by roll milling is shown in Fig. (3a). The pattern of the GF-phospholipid roll milled mixture was almost identical to the diffraction pattern of HSPC alone (*i.e.*, the physical mixture of GF and phospholipid). However, a small difference was observed between 16 and 17°. To further investigate this detail the X-ray powder diffraction pattern was expanded in the range between 15 to 17.5°, as shown in Fig. (3b). GF-specific diffraction peaks of the physical mixture sample and the sample co-ground by roll milling, appeared at 16.47°. However, diffraction peaks were not observed at 16.47° for HSPC alone. Chowdary *et al.* have previously reported that the crystal form of poorly soluble drugs disappeared after co-grinding with a carrier, and that formation of the amorphous state allowed for the preparation of a solid dispersion [23]. The patterns from both our IB mixture and our GF mixture samples appear to be different from the patterns from the diffraction patterns reported by Chowdary *et al.*

The data presented above show that no shift in the IB and GF peaks was induced by interaction with phospholipid, indicating that both IB and GF mostly remained in the crystalline form in the phospholipid. The crystals may not have been crushed until they entered the amorphous state, rather being merely dispersed in the phospholipid as particles smaller than the roller slits. Kamiya *et al.* have reported that nifedipine remained in the crystalline form in phospholipid [24]. It is possible that other antisolvent drugs also exist in the crystalline form in phospholipid. Recently, it has been pointed out that the resolvability of an amorphous drug decreases steadily over time, resulting from transformation from the amorphous form to crystalline form in a long-term storage. In the present study, we did not consider it necessary to investigate long-term storage.

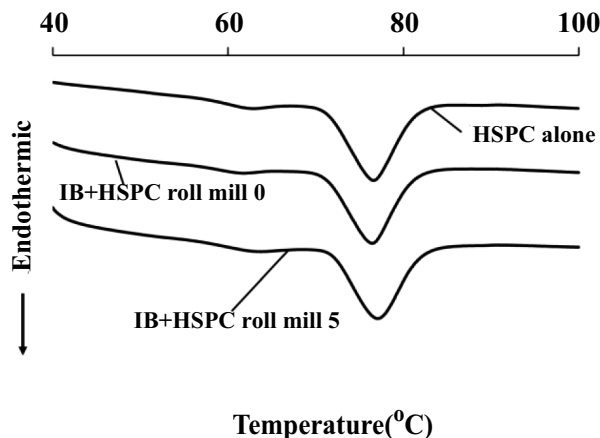


**Fig. (4).** DSC curves of the main components IB-phospholipid prepared by roll milling. IB + HSPC roll mill 5: IB and phospholipid (IB: HSPC = 1: 50) were ground by roll milling. IB + HSPC roll mill 0: Physical mixture of IB and phospholipid (IB: HSPC = 1: 50).

Fig. (4) shows the characteristic DSC thermographs acquired from IB and HSPC mixture preprocessed by roll milling. The three samples (IB and HSPC mixture pretreated by roll milling, IB and HSPC mixture prepared with ethanol pretreatment, and HSPC alone) gave similar DSC curves. The DSC curve of IB alone showed a peak between 78 to 81.8°C. This

endothermic peak is assigned to the melting of IB (the melting point of IB is 76°C). In contrast, no endothermic peak was observed in the other samples, except in IB alone at 76°C. For IB in the crystal state, a small peak is predicted to emerge at approximately 76°C. Another possibility is that IB: HSPC ratio is too low for detection.

McMullen *et al.* have reported a decreasing phospholipid transition temperature with increasing cholesterol concentration [25, 26]. The phase transition temperature of phospholipid appears to change as a result of an interaction between phospholipid and the drugs. However, no phase transition temperature was detected. Hence, we propose that the IB: HSPC ratio is too low for this to be detected.



**Fig. (5).** DSC curves of the main components GF-phospholipid prepared by roll milling. GF + HSPC roll mill 5: GF and phospholipid (GF: HSPC = 1: 50) were ground by roll milling. GF + HSPC roll mill 0: Physical mixture of GF and phospholipid (GF: HSPC = 1: 50).

Fig. (5) shows the characteristic DSC thermographs obtained from GF and HSPC compounds. All samples gave similar DSC traces. Moreover, the DSC curves of HSPC including GF are almost identical to the DSC curves of HSPC alone. The DSC curve of GF alone is not shown as melting point of GF is high (220°C). There was no phase transition temperature. Consequently, the GF: HSPC ratio is also considered to be too low for detection.

Based on the above findings, IB-phospholipid, and GF-phospholipid, nanoparticles suspensions can be prepared without organic solvent.

## CONCLUSION

The results are summarized as follows:

- (1) The mean particle size of IB-phospholipid and GF-phospholipid mixtures prepared by roll mill co-grinding and subsequent high-pressure homogenization at a pass number of 10 was about 42.3 nm and 42.5 nm, respectively.
- (2) A small difference in the diffraction peaks was observed between IB-phospholipid (or GF-phospholipid) mixtures prepared by roll mill co-grinding and HSPC alone. These findings suggested that IB and GF were mostly present as crystals in the phospholipid.

- (3) The DSC curves of IB-phospholipid and GF-phospholipid mixtures prepared by roll mill co-grinding, and the physical mixtures, are almost identical.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

#### HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

#### CONSENT FOR PUBLICATION

Not applicable.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

#### ACKNOWLEDGEMENTS

Declared none.

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