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# Preparation and stabilization of nifedipine lipid nanoparticles

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**Abstract**

Design methods of nanoparticle formulations are divided into a break-down method and a build-up method. Furthermore, 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. In this study, we investigated the preparation of nifedipine (NI) nanoparticles without using any organic solvent. NI nanoparticles with a mean particle size of approximately 50 nm could be prepared without organic solvent by a combination of roll mixing and high-pressure homogenization. The X-ray diffraction peak of the sample prepared by roll mixing was present at an identical position ($2\theta$) to that of NI crystals, showing that no peak shift was induced by interaction with lipid. These findings clarified that most NI remained as crystals in lipid.

To maintain the particle size of the nanoparticles in suspension for a long time, a method of adding gelatin powder to the NI-lipid nanoparticle suspension, dissolving the mixture by heating, and then solidifying by cooling was investigated. The mean particle size of the sample was about 55 nm, and that after heat-liquefaction of the NI-lipid nanoparticle suspension gelated at 5 °C for 24 h was also about 55 nm, showing that the nanoparticle condition was retained.


*Keywords:* Nanoparticles; Without organic solvent; Roll mixing; Gelatin; High-pressure homogenization

## 1. Introduction

Improvement of water solubility of drugs is widely performed to efficiently deliver poorly water-soluble drugs into the body, and utilization of surfactants (Kim et al., 2001; Kawakami et al., 2004) and co-grinding with water-soluble polymers (Yamada et al., 1999) are employed. Nanotechnology has recently been attracting attention, and establishment of pharmaceutical technologies to micronize drug particles may improve drug solubility (Eyjolfsson, 1999; Law et al., 2003). In addition to improvement of drug solubility due to an increase in the drug particles surface area, drugs micronized to the nano-order may also be directly delivered through the intestinal Payer's patches (Desai et al., 1996).

The design methods of nanoparticle preparations are roughly divided into break-down and build-up methods (Henzie et al., 2006; Rothemund, 2006). The former grinds particles to a nano-order size by the dry (Carrasquillo et al., 2001; Moribe et al., 2005; Young et al., 2005) or wet (Jacobs et al., 2000; Gao and Yao, 2004) process. Regarding the dry process, 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 developed (Adesogan et al., 2004; Williams et al., 2005). The wet process is capable of reducing the limit of particle size, compared to the dry process, for example, high-pressure homogenization (Pupo et al., 2005; Uner et al., 2005). The wet grinding methods are capable of micronizing drug particles to a

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several hundred-nanometer size, but most methods use organic solvents in the process (Sakuma et al., 2002; Santhi et al., 2002; Thirumala et al., 2000).

Accumulation of residual solvents in the body and environmental pollution by liquid wastes are matters of concern regarding the use of organic solvents. To overcome these problems, we investigated nanoparticle preparations without using organic solvents, such as ethanol, in any stage of the process. As methods not involving organic solvents, the fusion method with heating (Sheen et al., 1995) and supercritical fluid method using carbon dioxide as a solvent (Thakur and Gupta, 2006) are available. In this study, we investigated the combination of a dry process, co-grinding by a roll mill, and a wet process, high-pressure homogenization.

For the model drug, a practically insoluble drug, Nifedipine (NI), was used. NI is an antihypertensive drug administered mainly orally.

We previously reported that freeze-drying of nanoparticles allowed reproduction of the nanoparticle condition in suspension after long-term storage. However, for its application to oral drugs, this procedure is disadvantageous in requiring preparation at the time of use and having a high production cost. Therefore, a simple method substituting for the freeze-drying method is necessary. We investigated a method to maintain the dispersed condition of nanoparticles by adding gelatin to nanoparticle dispersion and solidifying the mixture. Semisolid preparation was put to practical use as external preparation such as ointment or cream. Oral jelly formulation has been reported recently, but a main purpose was improvement of compliance of the patient who had dysphagia (Dairaku and Togashi, 2005). In this study, we studied the formulation which became half solidity for the purpose of maintaining nanoparticles for a long term, it is not almost reported till now.

## 2. Materials and methods

### 2.1. Materials

Hydrogenated soybean phosphatidylcholine (COATSOME® NC-21 (HSPC)) and dipalmitoyl phosphatidylglycerol (COATSOME® MGLS-6060 (DPPG)) were purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). Nifedipine (JPXIV, NI) was 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).

The membrane filter (pore size: 100 nm) was purchased from Toyo Roshi Kaisha Ltd. (Tokyo, Japan). All reagents were used as it is. Purified water that had been treated by ion exchange was used.

### 2.2. Preparation of NI-lipid (HSPC–DPPG) mixture

Thirty milligram of NI and 1000 mg of lipid (HSPC: DPPG = 5:1 molar ratio) were added into a mortar, and physically mixed. The mixture was then co-ground in a roll mill (Kodaira Seisakusho Co., Ltd.), and mixed-ground at a rotational velocity ratio of 1:2.5:5.8 for 5 min, in which the sample mostly adhered to, but partially fell from the roller. Thus, the mill was stopped every 30 s, and the fallen sample was collected. This co-grinding cycle was repeated.

### 2.3. Preparation of NI-lipid mixture nanoparticle suspension

A 30:1000 NI-lipid mixture (HSPC:DPPG = 5:1 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 applied to a high-pressure homogenizer (max pressure: 9.5 kg/cm$^2$) (Nanomizer, X form chamber; TOKUSHU KIKA KOGYO, Co.), and processed by 10, 20, 30, or 40 cycles of homogenization. Other premixed suspension used as comparative controls were prepared as described below.

Ethanol treatment method: 30 mg of NI and 1000 mg of lipid (HSPC:DPPG = 5:1 molar ratio) were dissolved with 2 ml of ethanol in a 80 °C water bath, and ethanol was evaporated. The mixture was then dispersed in 200 ml of purified water and premixed using a speed stabilizer.

Sonication method: Samples were directly dispersed in 200 ml of purified water and sonicated for 20 min. The sonicated suspension was premixed using a speed stabilizer.

No treatment: Samples were directly dispersed in 200 ml of purified water and premixed using a speed stabilizer.

### 2.4. Nanoparticle size measurement

The mean particle sizes of nanoparticles prepared by high-pressure homogenization at various numbers of rotations were measured at room temperature using an electrophoretic light scattering photometer (ELS-8000, Otsuka Electronics Co., Ltd.) at a fixed angle of 90°. The particle sizes were analyzed based on weight distribution. The nanoparticle suspension was analyzed without dilution.

### 2.5. Quantification of NI

To measure the ratio of 100 nm or smaller NI particles, nanoparticles were filtered through a membrane filter with a 100-nm pore size, and 1 ml of the filtered nanoparticle suspension was completely dissolved with 5 ml of methanol. The NI content in the methanol/water (=5/1) solution was measured by the absolute calibration curve method using an HPLC (LC-9A, Shimadzu Co.). The column used was LiChroCART 250-4 (Merch Co.). For the calibration curve, NI was dissolved with methanol/water (=5/1) at 5, 10, 20, 40, and 80 μg/ml, and its absorbance was measured. The mobile phase was methanol:water = 5:1 and the absorbance wavelength was 350 nm. The flow rate was 0.6 ml/min.

### 2.6. Powder X-ray diffraction

NI and lipid were weighed at 30:1000 (weight ratio) and mixed in a mortar for 5 min, followed by co-grinding in a roll mill

for 1–5 min. Individual NI and lipid samples were consecutively processed by roll milling for 5 min each. For physical mixing, lipid processed by roll milling for 5 min and unprocessed NI were mixed in a mortar using a pestle for 5 min. The prepared samples were subjected to powder X-ray diffraction with CuKα radiation at 40 kV, 30 mA, and room temperature using a powder X-ray diffractometer (RAD-C, Rigaku Denki Co., Ltd.). The scanning rate was 5°/min, and the diffraction angle ($2\theta$) was 2–30°.

### 2.7. Fourier-transform infrared spectroscopy

Lipid alone (HSPC), the physical mixture of NI and lipid (HSPC), and NI-lipid (HSPC) mixture prepared by roll milling were analyzed by Fourier-transform infrared spectroscopy (FT-IR). In addition, the difference spectra: subtraction of the spectrum of the lipid alone from that of the NI-lipid physical mixture and from that of the NI-lipid roll mill mixture, were calculated. The samples were measured by the diffuse reflection method using an FT-IR spectrometer (IR-Prestige 21, Shimadzu Co.).

### 2.8. Gel solidification of nanoparticle suspension

Gelatin powder (Nitta Gelatin Inc.) was added to the prepared nanoparticle suspension at 1.5% and dissolved in a 70 °C water bath, and the sample was then solidified at 4 °C for 24 h.

## 3. Result and discussion

Photographs of NI:lipid = 30:1000 (weight ratio) after co-grinding are shown in Fig. 1. The sample had a whitish appearance before co-grinding by roll milling (Fig. 1A), but the color changed to homogenous fluorescent yellow after co-grinding, as shown in Fig. 1B, based on which NI was judged to have been sufficiently mixed.

Treatment of premixed suspension before micronization of the drug by high-pressure homogenization is essential to prevent the nozzle of the instrument from clogging. In the untreated

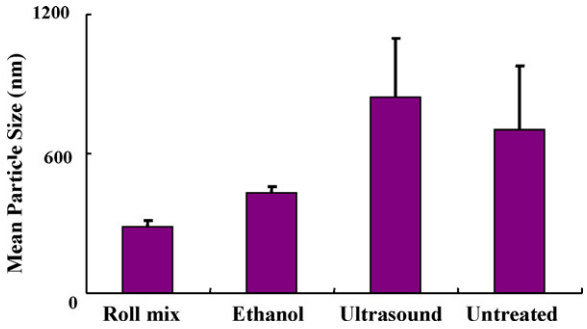


Fig. 2. The mean particle size of premixed suspension prepared by various methods. Each bar represents the mean S.D. of three measurements. Roll milling method: NI-lipid (HSPC:DPPG = 5:1, molar ratio) mixture was prepared by roll milling. NI-lipid mixture was dispersed in purified water. The dispersed suspension was premixed. Ethanol treatment method: NI and lipid (HSPC:DPPG = 5:1, molar ratio) mixture were dissolved in ethanol and evaporated. NI-lipid mixture was dispersed in purified water. The dispersed suspension was premixed. Sonication: NI and lipid were dispersed in purified water directly and sonicated for 20 min. The dispersed suspension was premixed. No treatment: NI and lipid were directly dispersed in purified water. The dispersed suspension was premixed.

suspension, NI crystals were floating on the water surface, and dispersion was heterogeneous, being insufficiently mixed with lipid. In the sonicated premixed suspension, NI alone sedimented downward, and lipid and NI were separated. NI particles in this premixed suspension were too large for micronization by high-pressure homogenization. NI crystalline particles were floating on the water surface because of incomplete dispersion. After sonication, NI crystals were finely dispersed so that the NI crystal surface became wet, which may have induced sedimentation. However, as shown in Fig. 2, the particle size in the dispersion premixed by roll milling was 284.2 nm, which was smaller than the sizes of ethanol-treated, sonicated, and untreated particles, and sufficiently small for application to high-pressure homogenization.

The results of high-pressure homogenization of the sample prepared by co-grinding by roll milling and the ethanol-treated sample are shown in Fig. 3. The mean particle size decreased as the pass number increased in both samples, and the sizes after 40 passes were 53.5 and 56.2 nm, respectively. Regarding the

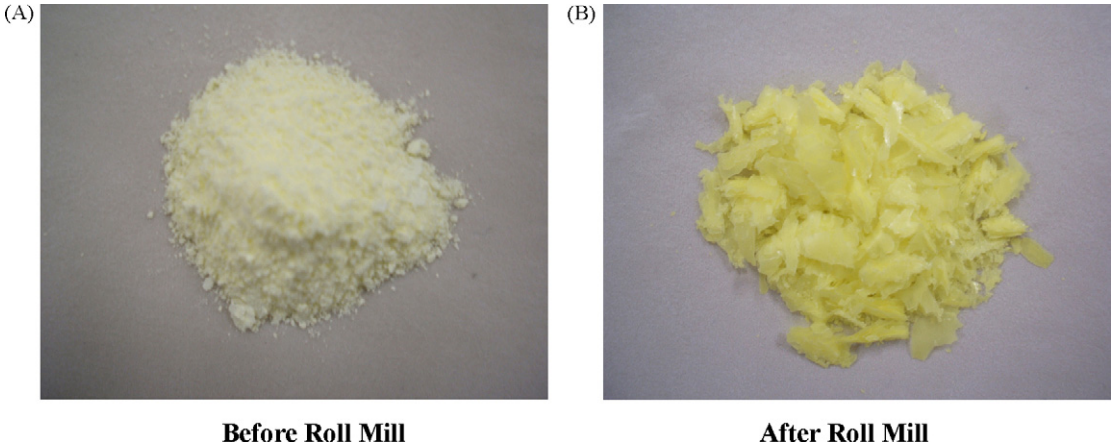


Fig. 1. Photographs of the samples mixed by NI and lipid before/ after co-grinding by roll milling. Panel A: a photograph before co-grinding of NI and lipid. Panel B: a photograph after co-grinding of NI and lipid.

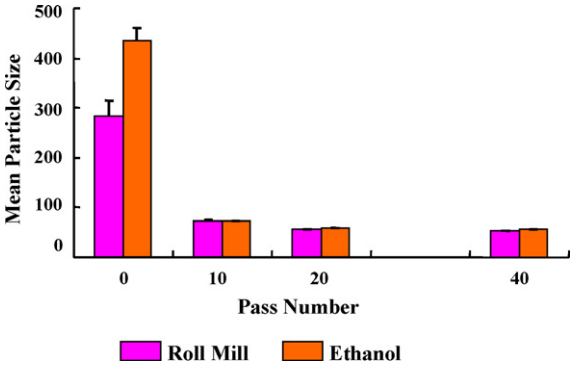


Fig. 3. Influence of pass number on the mean particle size of NI-lipid nanoparticles with high-pressure homogenization. Colum shows the relationship between pass number. Each bar represents the mean S.D. of three measurements. Roll mill: NI-lipid (HSPC:DPPG = 5:1) mixture were prepared by roll milling. NI-lipid mixture was dispersed in purified water. The dispersed suspension was premixed. Premixed suspension was homogenized by high-pressure homogenization. Ethanol: NI and lipid (HSPC:DPPG = 5:1, molar ratio) mixture was dissolved in ethanol and evaporated. NI-lipid mixture was dispersed in purified water. The dispersed suspension was premixed. Premixed suspension was homogenized by high-pressure homogenization.

ratio of NI after 40 passes through a filter with a 100-nm pore size, the concentration after filtration was 92.02% of that before filtration, showing that NI was mostly micronized to 100 nm or smaller. This finding clarified that roll mixing was as effective as ethanol treatment.

The powder X-ray diffraction pattern of the NI-lipid mixture prepared by roll milling is shown in Fig. 4. In the patterns of NI alone, the physical mixture of NI and lipid, and NI-lipid roll mill mixture, NI-specific diffraction peaks appeared at 8.2, 16.2, 19.6, 24.4, and 25.9°. As for the sample co-ground by roll milling, the peaks were reduced, but the pattern was similar to that of the physical mixture. Chowdary et al. reported that the crystal form of poorly soluble drugs disappeared after co-grinding with a carrier, and formation of the amorphous state allowed the preparation of solid dispersion (Chowdary et al., 2003; Itoh et al., 2003). In our study, the X-ray diffraction peak of the sample after mixing was present at the position of NI crystals, and no peak shift was induced by interaction with lipid, showing that NI mostly remained as crystals in the lipid.

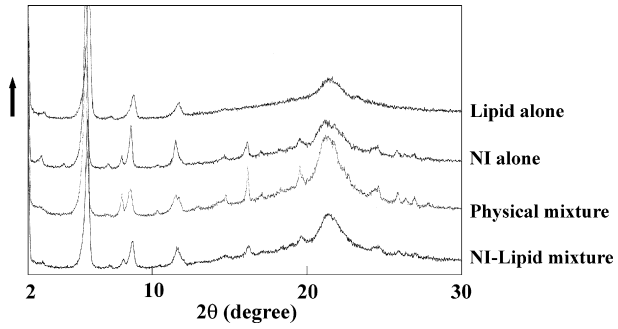


Fig. 4. Powder X-ray diffraction patterns of main components NI-lipid prepared by roll milling. NI-lipid mixture: NI and lipid were ground by roll milling for 5 min.

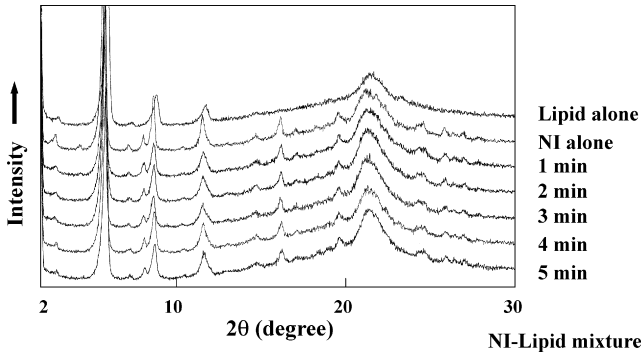


Fig. 5. Changes on powder X-ray diffraction patterns with grinding time of NI-lipid Mixture. 1 min, 2 min, 3 min, 4 min and 5 min: grinding time of NI-lipid (HSPC:DPPG = 5:1) mixture ground by roll mill.

The relationship between the roll mixing time and X-ray diffraction is shown in Fig. 5. The crystalline state of NI was maintained after mixing for 5 min, suggesting that NI crystals were incorporated into the lipid. The crystals may not have been destroyed until they entered the amorphous state, being dispersed in the lipid as particles smaller than the roller slits. Thus, roll mixing for 5 min may be sufficient.

The FT-IR spectrum of the NI-lipid (HSPC) mixture prepared by roll mixing is shown in Fig. 6. The spectra of the NI-lipid roll mixture, NI-lipid physical mixture, and lipid alone were similar because the spectrum of the lipid was strong due to its weight ratio being 25 times that of NI.

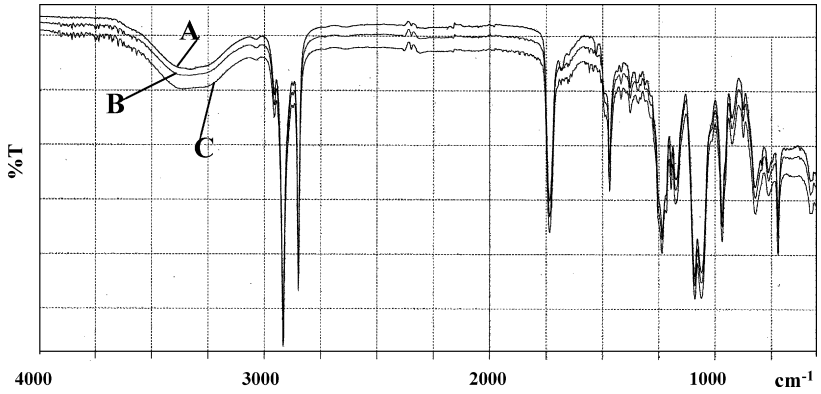


Fig. 6. FT-IR spectra of main components in NI-lipid (HSPC) mixture prepared by roll mill. (A) NI-lipid (HSPC) mixture prepared by roll mill. (B) Physical mixture of NI and lipid (HSPC). (C) Lipid (HSPC) alone.

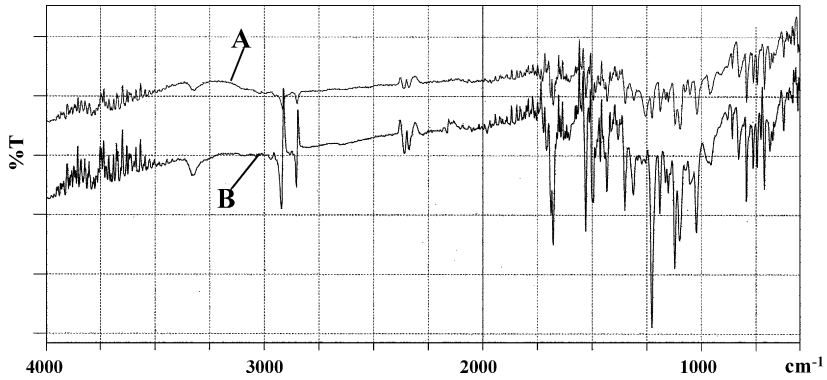


Fig. 7. FT-IR differential spectra NI-lipid mixture prepared and physical mixture of NI and lipid. (A) Differential spectrum on subtraction of lipid alone from NI-lipid mixture. (B) Differential spectrum on subtraction of lipid alone from physical mixture of NI and lipid.

To clarify the FT-IR spectrum of NI alone, the difference spectra were calculated by subtracting the spectrum of the lipid alone from those of the mixture. The results are shown in Fig. 7. The difference spectrum of the roll mill mixture was apparently different from that of the physical mixture in a range of 2800–3000 $cm^{-1}$. The appearance of the peaks outside the regions of C=O and N–H stretching vibrations suggested that partial structural change or new interaction occurred. Saito et al. reported that roll mixing of poorly soluble drugs with carriers altered the drugs to the amorphous state, and induced intermolecular interaction (Saito et al., 2002). However, when NI was mixed with lipid, as shown in the X-ray diffraction pattern in Fig. 4, NI was mostly present as crystals, suggesting that NI interacted with lipid as crystals.

Based on the above findings, NI-lipid nanoparticle suspension can be prepared without organic solvent. The fusion method (Chen et al., 2004) is a typical method not involving organic solvents, but this method may degrade heat-labile drugs, such as protein preparations. This method using roll milling is unlikely to degrade drugs because of the absence of heat treatment, showing its usefulness.

A novel method for the long-term stability of the particle size of the prepared nanoparticle suspension was investigated. Gelatin powder was added to the NI-lipid nanoparticle suspension, dissolved by heating in a water bath, and the mixture was solidified in a refrigerator. This method is called the gelatin solidification method below. Photographs of the NI-lipid nanoparticle suspension and its gelatin-solidified preparation are shown in Fig. 8. Fig. 8A shows the NI-lipid nanoparticle suspension immediately after high-pressure homogenization. Fig. 8B shows the NI-lipid nanoparticle suspension combined with gelatin and cooled in a refrigerator for 24 h. To visually present the solidification, the suspension was slanted in a refrigerator. On macroscopic observation, the dispersion was homogenous and transparent after gelatin addition.

The mean particle size was about 55 nm after gelatin addition to the NI-lipid nanoparticle suspension, and also about 55 nm after liquefaction following 24-h cooling at 5 °C, showing that the nanoparticle condition was maintained (Fig. 9). This gel solidification method using gelatin to maintain the nanoparticle condition is expected to be a novel technique.

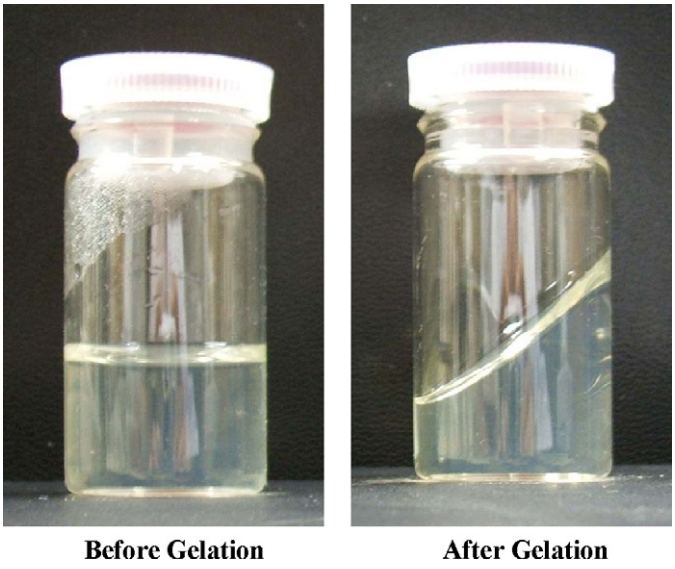


Fig. 8. Photographs of NI-lipid nanoparticle suspension containing gelatin before/ after gelation. (A) NI-lipid (HSPC:DPPG = 5:1) nanoparticle suspension prepared by high-pressure homogenization. (B) Gelated NI-lipid (HSPC:DPPG= 5:1) nanoparticles. Gelatin was dissolved in NI-lipid nanoparticle suspension at 70°; the suspension was refrigerated for 24 h at 5°.

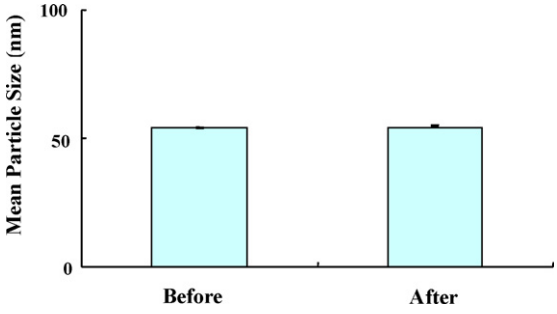


Fig. 9. Effect of gelatin on the mean particle size of NI-lipid nanoparticle suspension before/after gelation. Gelatin concentration in NI-lipid nanoparticle suspension was 1.5% (W/V). Each bar represents the mean S.D. of three determinations.

## 4. Conclusion

The results are summarized as follows:

(1) The mean particle size of NI-lipid mixture prepared by roll mill co-grinding and subsequent high-pressure homogenization at a pass number of 40 was about 55 nm, showing that an NI-lipid nanoparticle suspension could be prepared without organic solvent.
(2) No difference was noted in the X-ray diffraction peak between the NI-lipid mixture prepared by roll mill co-grinding and the physical mixture, or in the NI-specific X-ray diffraction peak between the NI alone and NI-lipid mixture prepared by roll milling for 5 min. These findings suggested that NI was mostly present as crystals in the lipid.
(3) The difference spectra calculated by subtracting the spectrum of the lipid alone from that of the NI-lipid roll mill mixture and that of the NI-lipid physical mixture were apparently different in a range of 2800–3000 $cm^{-1}$, suggesting that intermolecular interaction between NI and lipid occurred.
(4) The mean particle size was about 55 nm before and 24 h after the gelatin solidification of the nanoparticle suspension, suggesting that this gel solidification method is useful for the storage of nanoparticles.

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