Preparation of Amorphous Composite Particles of Drugs with Ursodeoxycholic Acid as Preclinical Formulations

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We studied the possibility of using ursodeoxycholic acid (UDCA) as an excipient to create an amorphous composite that can be administered to animals in preclinical studies of experimental drugs. Three UDCA-based amorphous samples composed of nifedipine (NIF), indomethacin (IND), and naproxen (NAP) were found by screening. The UDCA-based formulations were adjudged amorphous by solid-state analysis using X-ray powder diffraction and differential scanning calorimetry. In addition, amorphous samples of NIF–UDCA, IND–UDCA, and NAP–UDCA did not crystallize while in 1% methyl cellulose (MC) solution for 120 min, although an amorphous solid dispersion of NIF–poly(vinylpyrrolidone) (PVP) crystallized rapidly. The low hygroscopicity of UDCA helps NIF maintain an amorphous state in 1% MC solution. The UDCA-based amorphous composites can be administered as suspended formulations to animals in preclinical studies.

Key words preclinical formulation; spray-drying; hygroscopicity; amorphous composite; physical stability

Introduction
According to recent reports, many compounds discovered and synthesized by the pharmaceutical industry are not highly water soluble because of high-throughput screening. Indeed, up to 75% of active pharmaceutical ingredient (API) candidates in pipeline have been evaluated poorly water soluble.2) Because candidate drugs must obtain a large exposure in toxicity studies, efforts are made to develop formulations with better water solubility to administer to animals.3–5) In toxicity studies, there are two approaches and issues for improving exposure. First, the use of organic solvents or surfactants would help improve solubility and exposure of preclinical formulations. However, for long-term toxicity studies, the type and quantity of organic solvents and surfactants used are restricted for safety reasons.3–5) Second, powders used to change the solid state, such as crystalline salt,6,7) cocrystals,8,9) and amorphous solid dispersion,10,11) have been used to overcome low solubility. However, in general, these powders have been suspended in aqueous solution when administered to animals. Therefore the exposure of the APIs may not be improved because these solid-state forms easily convert to free crystalline form in an aqueous vehicle. It is important that the solid state not change in aqueous media, at least until the suspension is administered to animals.

Amorphization of APIs is one technique for overcoming the low-solubility issue, because an amorphous solid state has higher free energy than the crystalline state. However, the amorphous state is thermodynamically unstable relative to the crystalline state;12) therefore a purely amorphous API is rarely used.13) The use of amorphous solid dispersion (ASD) formulations with hydrophilic polymers may be a preferable way to achieve a higher physicochemically stable amorphous state;14) because the interaction between the API molecule and the polymer can prevent crystallization. ASD formulations that include nifedipine with polymers have been reported,12,15) because nifedipine (NIF) in the amorphous state is easily converted to a crystalline state.15) Poly(vinylpyrrolidone) (PVP) has been recommended as a hydrophilic polymer that prevents nucleation and crystal growth of NIF because of strong interactions between it and NIF.12,16) However, preparation and evaluation of the ASD suspension in an aqueous vehicle have not been studied for use in drug pre-clinical studies.

Recently, a new approach to stabilizing the amorphous state has been studied.17–19) This co-amorphous system, which incorporates an API with a small-molecule compound instead of a polymer, can be roughly classified as an API–API system or an API–excipient system. However, the former classification applies only when combination therapeutics is necessary, whereas the API–excipient system has been considered more useful in the development of new APIs. Many studies have reported that API–excipient system improved solubility, dissolution rate, and physical stability.20,21) To disseminate a formulation that uses the API–excipient system, further study of product development with respect to the manufacturing process and regulatory requirements is necessary.21) For instance, most of the many reported API–excipient systems that use amino acids22,23) have been prepared via ball milling. Ball milling is an excellent method for preparing a small amount of sample, but not so for obtaining homogeneous samples in large-scale production. Although hot melting and spray dry-
ing are used to prepare amorphous composites in large-scale production, the techniques cannot be applied to an API–amino acid system because amino acids have a high melting point and low solubility in volatile solvents.

The physicochemical properties of ursodeoxycholic acid (UDCA), a human bile acid, have been previously reported.\(^{24}\) UDCA crystallizes into a complex structure with other compounds,\(^{25}\) similar to cholic acid (CA).\(^ {26}\) In addition, the preparation and characterization of amorphous UDCA have been studied.\(^ {27-29}\) There have been few studies on the usefulness of amorphous formulation using UDCA. In this study, we investigated the amorphous composite of UDCA for use in a preclinical formulation, and used indomethacin (IND), naproxen (NAP), and NIF as model compounds that are Biopharmaceutics Classification System (BCS) class II compounds (high permeability and low solubility).\(^ {30,31}\) A screening method that uses evaporation was developed to optimize the API-to-excipient ratio. The solid-state properties and physical stability of the amorphous composite in an aqueous vehicle were evaluated by X-ray powder diffraction (XRPD) and Raman spectroscopy.

**Experimental**

**Materials** IND, NAP, NIF, and UDCA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) [Figs. 1(a)–(d), respectively]. PVP was purchased from BASF (Ludwigshafen, Germany) and methyl cellulose (MC) from Shinetsu Chemical Industry Company, Ltd. (Tokyo, Japan). All solvents and other chemicals used were purchased from various commercial suppliers.

**Solvent-Casting Method for Amorphous Screening** NIF and UDCA (mole ratio \(1 : 0.5, 1 : 1, 1 : 2, 1 : 5\)) were dissolved in ethanol for a 50-mg/mL concentration of NIF. The ethanol solutions of IND–UDCA and NAP–UDCA were prepared in the same manner. A 120-\(\mu\)L portion of each ethanol solution was dispensed onto a glass plate heated to 60°C using a hot-plate (C-MAG HS4, IKA, Staufen, Germany). Dried samples were stored at 40°C and 0% relative humidity (RH) and at 40°C and 75% RH for 1, 3, and 7 d. The initial and stored samples were analyzed by XRPD.

**Preparation of Samples via Spray Drying** NIF and UDCA (mole ratio \(1 : 2\)) were dissolved in ethanol/water (1 : 1) for a 2-mg/mL concentration of NIF. IND–UDCA and NAP–UDCA ethanol/water solutions were similarly prepared. These ethanol/water solutions were spray-dried using a B-290 mini spray dryer (Büchi, Flawil, Switzerland) at an inlet temperature of 110°C, a solution feeding rate of 6 mL/min, and a nitrogen flow rate of 7.5 m\(^3\)/h.

NIF and PVP (weight ratio \(1 : 2\)) were dissolved in ethanol by stirring at 60°C for a 33-mg/mL concentration of NIF. The ethanol solution was spray-dried using a B-290 mini spray dryer at an inlet temperature of 70°C, a solution feeding rate of 6 mL/min, and a nitrogen flow rate of 7.5 m\(^3\)/h. The obtained solid was dried overnight using a VT220 vacuum dryer (Kusumoto Chemicals, Tokyo, Japan) at 25°C.

**Preparation of Suspension in 1% MC Solution** MC (5 g)
was weighed and water (500 mL) added to a beaker. While stirring the water with a magnetic stir, the weighed MC was added to a final concentration of 1% MC solution. Samples (20 mg) of each powder were placed in individual vials to which the 1% MC solution (0.4 mL) was added. The vials were sonicated to make dispersed suspensions. Each suspension was filtered through stainless-steel mesh (Sankeishoji, Ltd., Shizuoka, Japan) and each solid state evaluated by XRPD.

**Solubility Measurement of NIF–UDCA and NIF–PVP in 1% MC Solution** The concentration of NIF in 1% MC solution was measured by Waters Alliance HPLC system with a Waters 2795 separation module and a Waters 2487 dual λ UV/Vis detector. NIF was detected using the absorbance at 235 nm. The samples were obtained by Millipore Centrifugal Filters (ULTRAFREE-MC-LG, polytetrafluoroethylene (PTFE) 0.2 µm). The solubility measurement was diluted to twice their volume; diluted samples were injected into an Imtakt Cadenza CD-C18 column (3 µm, 3.0 × 50 mm). Separations were conducted using a mixture of water and acetonitrile containing 0.1% trifluoroacetic acid as the mobile phase at 40°C and a gradient of 1 mL/min, as follows: 0–1.5 min with 60% acetonitrile and 1.5–3 min with 95% acetonitrile.

**Evaluation of Physical Stability** The NIF–UDCA and NIF–PVP samples prepared using spray drying were stored at 25°C and 0, 75, and 100% RH and at 40°C and 0, 75, and 100% RH for 4 weeks. Stored samples were analyzed by XRPD to evaluate transformation of the solid state.

**Calculation of Molecular Volume** Three-dimensional chemical structure models of IND, NAP, and NIF were obtained from single-crystal structure (INDMET, COYRUD, and BICCIZ, respectively) using ConQuest version 1.19 (Cambridge Crystallographic Data Centre, Cambridge, U.K.). The molecular volume of each compound was calculated by Discovery Studio Client version 17.2 (BIOVIA, San Diego, CA, U.S.A.).

**XRPD Analysis** All samples were analyzed by Miniflex 600 powder X-ray diffractometer (Rigaku Corporation, Tokyo, Japan). XRPD patterns in the range 2θ = 5–30° were collected in a continuous scan at a scan speed of 5°/min and a step size 0.04°.

### Table 1. Summary of Physical Stability of Amorphous Composites Prepared by Solvent Casting

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ratio</th>
<th>Initial</th>
<th>1d</th>
<th>3d</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Stability conditions</td>
<td>0% RH at 25°C</td>
<td>100% RH at 40°C</td>
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<tr>
<td>IND–UDCA</td>
<td>1:0.5</td>
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<td>Amorphous</td>
<td>Amorphous</td>
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<td></td>
<td>1:1</td>
<td>Amorphous</td>
<td>Amorphous</td>
<td>Amorphous</td>
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<td></td>
<td>1:2</td>
<td>Amorphous</td>
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<td>Amorphous</td>
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<tr>
<td></td>
<td>1:5</td>
<td>Amorphous</td>
<td>—</td>
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<tr>
<td>NAP–UDCA</td>
<td>1:0.5</td>
<td>Amorphous</td>
<td>NAP</td>
<td>NAP</td>
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<tr>
<td></td>
<td>1:1</td>
<td>Amorphous</td>
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<td></td>
<td>1:2</td>
<td>Amorphous</td>
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<tr>
<td></td>
<td>1:5</td>
<td>Amorphous</td>
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<tr>
<td>NIF–UDCA</td>
<td>1:0.5</td>
<td>NIF</td>
<td>—</td>
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<td></td>
<td>1:1</td>
<td>NIF</td>
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<td></td>
<td>1:5</td>
<td>Amorphous</td>
<td>NIF, UDCA</td>
<td>NIF, UDCA</td>
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</table>

**Fig. 3. Thermal Profiles Obtained by Modulated DSC**
Differential Scanning Calorimetry (DSC) Thermal analysis was performed using a Q2000 modulated DSC (TA Instruments, New Castle, DE, U.S.A.) to measure the glass transition temperature ($T_g$). The samples were placed in an aluminum pan and heated to 200°C at 5°C/min with modulation of ±1.00°C every 60 s under a constant flow of nitrogen at a rate 50 mL/min. The measured $T_g$ value of each spray-dried sample was compared with the $T_g$ value calculated using the Gordon-Taylor ideal mixing equation 35):

$$T_g = \frac{\omega_1 T_{g1} + \omega_2 T_{g2}}{\omega_1 + \omega_2}$$

where $\omega_1$ and $\omega_2$ are the weight fractions of NIF and UDCA, respectively; $T_{g1}$ and $T_{g2}$ are their $T_g$ values, respectively; and $\rho_1$ and $\rho_2$ are the densities of NIF and UDCA, respectively, calculated from the crystal structure data. We used the reported values of 42°C and 100°C for $T_{g1}$ and $T_{g2}$.

### Figures

**Fig. 4.** Raman Spectra: (i) 900–700 cm$^{-1}$; (ii) 1630–1520 cm$^{-1}$; (iii) 1800–1600 cm$^{-1}$

(a) UDCA crystal, (b) NIF crystal, (c) physical mixture of NIF and UDCA crystals, (d) NIF–UDCA.

**Fig. 5.** XRPD Patterns of NIF–UDCA or NIF–PVP: (i) NIF–UDCA, Storage Temperature = 25°C; (ii) NIF–UDCA, Storage Temperature = 40°C; (iii) NIF–UDCA, Storage Temperature = 25°C

(i) (a) NIF crystal, (b) UDCA crystal, (c) 0% RH/4 weeks, (d) 75% RH/4 weeks, (e) 100% RH/4 weeks. (ii) (a) NIF crystal, (b) UDCA crystal, (c) 0% RH/1 week, (d) 0% RH/4 weeks, (e) 75% RH/1 week, (f) 75% RH/4 weeks, (g) 100% RH/4 weeks. (iii) (a) NIF crystal, (b) PVP, (c) 0% RH/4 weeks, (d) 100% RH/3 days.
Raman Spectroscopy  Raman spectroscopy was performed using a RamanRxn™ analyzer (Kaiser Optical Systems Inc., Ann Arbor, MI, U.S.A.) with a 785-nm excitation laser. The spot obtained using a 10-fold objective lens was measured to be approximately 50 µm in diameter. Raman spectra were acquired with 1-s exposure and four accumulated measurements.

Dynamic Vapor Sorption (DVS)  Dynamic vapor sorption at 25°C was measured by DVS-1 Intrinsic water sorption analyzer (Surface Measure Systems, London, U.K.). The data were collected over a RH range of 0–95% with an equilibrium criterion of <0.0002%/min weight change.

Field Emission Scanning Electron Microscopy (FE-SEM)  The morphology of the powders was determined by FE-SEM (SU8010, Hitachi High Technologies, Tokyo, Japan). Samples were mounted on aluminum stubs and coated with approximately 3-nm layer of platinum using an E-1045 ion sputter coater (Hitachi High Technologies). FE-SEM images were obtained using secondary electrons and backscattered electrons at an acceleration voltage 1.0 kV.

Results and Discussion

Solvent Casting Results  Figure 2 shows the XRPD patterns of the initial samples of NIF–UDCA, IND–UDCA, and NAP–UDCA prepared by solvent-casting method. There were no diffraction peaks attributed to the crystalline phase, except those for NIF–UDCA (1:0.5) and NIF–UDCA (1:1), indicating that the prepared samples, except NIF–UDCA (1:0.5) and NIF–UDCA (1:1), were amorphous.

The physical stability of the samples prepared by solvent casting was assessed; results are presented in Table 1. Although IND, NAP, and NIF crystallize immediately without excipients, their crystallization would be inhibited by UDCA. A diffraction peak was not observed for the IND–UDCA sample at 0% RH for 3 d, while diffraction peaks from crystalline IND and UDCA were observed at 100% RH for 3 d. For the NAP–UDCA sample, NAP and UDCA instantly crystallized when the mole ratio of UDCA to NAP was low. On the other hand, NAP–UDCA (1:2) maintained the amorphous state for 1 d. Although NIF–UDCA (1:2) maintained the amorphous phase for 3 d, the NIF–UDCA (1:5) amorphous formulation transformed into crystalline NIF and UDCA after 1 d. Either compound may crystallize in the amorphous composite system between low molecular weight compounds. The compound could promote to crystallize as a seed of nucleation in the presence of excessive amount of compound. If the excessive amount of UDCA exists in the composite system, it could induce the crystallization of UDCA as a seed of nucleation. NIF may crystallize by decreasing the number of UDCA molecules that form amorphous composite. In general, if the amorphous solid dispersions have a higher ratio of polymers, it is difficult for the APIs to crystallize because $T_g$ tends to be higher.

![SEM Images of NIF–UDCA and NIF–PVP](image)

![DVS Profiles](image)
Thus a large amount of polymer is needed to prevent crystallization. On the other hand, it is important to determine the optimal ratio of API to UDCA for making physically stable amorphous composites. We confirmed that the solvent-casting method can be used to screen the formulation and the ratio in small amounts. The time and sample are limited on the early drug-discovery stage. The solvent-casting method used in the screening was based on evaporation and easy to scale-up.

Characterization of Spray-Dried Samples

IND–UDCA, NAP–UDCA, and NIF–UDCA were prepared using a spray dryer at a molecular ratio of 1 : 2. The weight ratio of NIF/UDCA was almost 1 : 2 because the molecular weights of NIF and UDCA are 346.3 and 392.5 g/mol, respectively. NIF–PVP was also created at a weight ratio of 1 : 2.

All initial solid states of the spray-dried samples were found by XRPD to be amorphous (data not shown). Spray-dried samples were confirmed to be in a single amorphous phase because a single Tg for each sample was measured using modulated DSC (mDSC) (Fig. 3). The results demonstrate that the preparation of amorphous composites, with UDCA as an excipient, by spray drying was successful. For a complex crystalline system, guest chemicals can be included in the framework of UDCA or CA, which are host compounds. In addition, there is a limitation for the molecular volumes of the guest chemicals that CA derivative can include in crystal structure. The framework of crystalline CA can contain guest chemicals with a molecular volume of <220 Å³. Moreover, the void formed by the framework of UDCA is less flexible than that of CA. If UDCA-based amorphous composites have same interactions between UDCA and others such as the crystalline inclusion complex, XRPD will observe diffraction peaks because the guest compounds are between the layers formed by the framework of UDCA. The molecular volumes of IND, NAP, and NIF were calculated 272, 187, and 269 Å³, respectively, using Discovery Studio software. IND, NAP, and NIF may interact separately with UDCA in the crystalline inclusion complex. The UDCA-based amorphous might include larger-size molecules than that of crystalline inclusion complex.

Comparison between NIF–UDCA and NIF–PVP

NIF–PVP is a conventional amorphous solid dispersion that includes a polymer; hence it is conceptualized that NIF molecules are dispersed in the chains of the PVP. The Raman spectra of NIF–UDCA had several different peaks (Fig. 4). According to a previous study on the wavenumbers from Raman spectra of NIF, the peaks at 1575 and 810 cm⁻¹ ([Figs. 4(i) and (ii)] were derived from NH scissors and a CH ring. In addition, it is thought that the peak at 1717 cm⁻¹ [Fig. 4(iii)] derives from the carbonyl group in UDCA. The Raman spectra of NIF–UDCA indicate that there was interaction between NH in NIF and COOH in UDCA because the peaks of their functional groups were shifted. Although NIF is a basic
compound ($pK_a = 2.17$)\(^{38}\) and UDCA an acidic compound ($pK_a = 5.1$),\(^{24}\) this interaction is not an ionized interaction between NIF and UDCA because $\Delta pK_a < 0$ (base-acid). mDSC measured $T_g$ of NIF–UDCA to be 75°C and the Gordon–Taylor equation calculated the theoretical $T_g$ of NIF–UDCA to be 78.6°C, which is almost equal to the measured $T_g$. NIF–UDCA was in a single amorphous phase because there was no diffraction peak in the XRPD analysis of the sample and mDSC showed the sample to have a single $T_g$. The hypothetical $T_g$ value does not correspond to the measured $T_g$ value when there is a strong interaction, e.g., an ionic interaction, between the API and excipient.\(^{39}\) Therefore NIF and UDCA in the molecular state are mixed without a strong interaction such as that in polymer-based amorphous composites.

The result of the study on the physical stability of NIF–UDCA is shown in Fig. 5. No diffraction peaks caused by crystals were observed when the sample was at 25°C over 4 weeks irrespective of the humidity level. Although NIF–UDCA remained in the amorphous state when at 40°C and 0% RH for 4 weeks, both crystalline peaks of NIF and UDCA appeared when the humidity was high. On the other hand, the crystalline peak of NIF in NIF–PVP was detected when it was at 25°C and 100% RH for 3 d, and the NIF–PVP particles changed from spherical to shapeless when under 100% RH for 1 d (Fig. 6). NIF–PVP showed a higher absorption of water than NIF–UDCA in the DVS profile (Fig. 7). These results suggest that not only the shape of NIF–PVP particles changed, but also their molecular state because of high hygroscopicity. The hygroscopicity of PVP is probably reflected in the properties of NIF–PVP because PVP is a major component in solid dispersion. Although NIF–PVP was estimated more stable than NIF–UDCA with respect to $T_g$, physical stability analysis suggests that NIF–UDCA has good physical stability. In general, absorbed water affects significantly on $T_g$ of amorphous solids because the $T_g$ of water is very low ($\sim -138$°C).\(^{40}\) Hygroscopic amorphous solids of NIF–PVP decreased the $T_g$ owing to the absorbed water in high-humidity conditions. It is difficult to predict the physical stability of the amorphous state in high humidity using the $T_g$ value, particularly in solid dispersions with hydrophilic polymers. This is a disadvantage for polymer-based solid dispersion because many hydrophilic polymers are highly hygroscopic.

**Solid-State Analysis of Amorphous Samples in 1% MC Solution**

A 1% MC solution is usually used to administer drugs to animals in preclinical studies.\(^{3,4}\) Figure 8 shows changes in XRPD patterns of solids filtered from suspension in 1% MC solution, and Fig. 9 shows the Raman spectra of the samples based on UDCA. Crystallization of NIF from NIF–PVP solid dispersion occurred immediately after suspension in 1% MC solution, although PVP is a good polymer for stabilizing the amorphous state of NIF. By contrast, the three UDCA-based amorphous composites remained in the amorphous state after 120 min. The solubility of NIF in 1% MC solution is summarized in Fig. 10. The concentration of NIF in the NIF–PVP solid dispersion decreased immediately after suspension of NIF–PVP in 1% MC solution, although PVP is a good polymer for stabilizing the amorphous state of NIF. By contrast, the concentration of NIF in NIF–UDCA remained in supersaturated state. The concentration of NIF in NIF–UDCA showed about 2.2 times higher solubility than that in NIF–PVP.

**Conclusion**

This study suggests that UDCA is a good excipient that improves physical stability of amorphous APIs. The solvent-
casting method was demonstrated a valid process for screening amorphous formulations that do not contain polymers for during preclinical studies. We found that the optimal API-to-UDCA ratio is 1:2 and UDCA-based amorphous composites could be prepared on a large scale using the spray-drying method. UDCA-based amorphous composites showed higher physical stability than PVP-based solid dispersion, and, in particular, the effect of water was greatly different between the two types of composites. This is a disadvantage for solid dispersions using hydrophilic polymers because many polymers such as PVP are highly hygroscopic. On the other hand, UDCA-based amorphous composites were not hygroscopic in high-humidity conditions. Moreover, UDCA could maintain supersaturation of NIF in 1% MC solution for 120 min, although PVP could not inhibit crystallization of NIF.

UDCA-based amorphous composites may include molecules larger than the crystalline inclusion complex. Additionally, the amorphous state of UDCA-based amorphous composites can be maintained in aqueous suspension because they absorb less water than polymer-based solid dispersions. Therefore UDCA-based amorphous composites can be administered to animals as suspended formulations in preclinical evaluations of experimental drugs. UDCA is an useful excipient for amorphous formulations that do not contain polymers instead of hydrophilic polymers.

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Conflict of Interest The authors declare no conflict of interest.

References