Antisolvent Crystallization Using Ionic Liquids As Solvent and Antisolvent for Polymorphic Design of Active Pharmaceutical Ingredient

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ABSTRACT: The applicability of ionic liquids to the polymorphic design of the active pharmaceutical ingredient adefovir dipivoxil (AD) was investigated in the case of antisolvent crystallization. When using 1-allyl-3-ethylimidazolium tetrafluoroborate (AEImBF4) as the solvent and 1-butyl-2,3-dimethylimidazolium tetrafluoroborate (BDMImBF4) as the antisolvent (AEImBF4/BDMImBF4), a new form (NF) of AD crystal was obtained at a crystallization temperature below 50 °C and initial solute concentration of 25.6 mg/mL. However, when using 1-allyl-3-ethylimidazolium tetrafluoroborate/1,3-diallylimidazolium tetrafluoroborate (AEImBF4/AAlmBF4) and 1-allyl-3-ethylimidazolium tetrafluoroborate/1-ethyl-3-methylimidazolium ethylsulfate (AEImBF4/EMImEtSO4), the conventional form-I polymorph was directly crystallized. No crystallization occurred in the ionic solutions of 1-allyl-3-ethylimidazolium tetrafluoroborate/1-allyl-3-ethylimidazolium bromide (AEImBF4/AEImBr) and 1-allyl-3-ethylimidazolium tetrafluoroborate/1,3-diallylimidazolium bromide (AEImBF4/AAlmBr). According to a spectroscopic analysis, the polymorphs were predominantly determined by the preorientation of the solute molecules in the ionic liquid solution (AEImBF4/BDMImBF4), and this preorientation varied according to the crystallization temperature. Thus, the pure form-X polymorph was nucleated at a crystallization temperature above 80 °C. Plus, the polymorphic nucleation depending on the crystallization temperature was also combined with the crystallization concentration. Thus, the minimum crystallization temperature for the nucleation of the pure form-X polymorph was reduced to 40 °C when decreasing the crystallization concentration to 15 mg/mL. The relationship between the polymorphic nucleation and the crystallization conditions was effectively predicted by a polymorphic supersaturation level diagram ($S_{NF} - S_{form-X}$ diagram), where the formation of the metastable polymorph (NF crystals) was favored at a high supersaturation level ($S_{NF} > 2.1$), while the stable polymorph (form-X crystals) was preferentially nucleated at a low supersaturation level ($S_{NF} < 1.6$). Differential scanning calorimetry thermal analysis confirmed that the NF polymorph was the metastable phase and the form-X polymorph was the stable phase, and there was a monotropic relationship between the two polymorphs.

INTRODUCTION

Polymorphisms refer to the variety of crystal structures created by different interactions between the same molecules in a solid state. It is well-known that the physiochemical properties of crystals, such as their solubility, structural stability, dissolution rate, density, and melting temperature, depend predominantly on their polymorphs. Thus, the polymorph design of active pharmaceutical ingredients (APIs) is generally considered one of the most important factors in drug development and production as it determines the bioavailability of the drug and its shelf life.

Therefore, over the last few decades, many methods, including solution crystallization from single or mixed solvents, supercritical crystallization, seeding strategies, capillary crystallization, polymer-induced heteronucleation, heteronucleation on substrates, and laser-induced nucleation, have all been developed to help design and control the polymorphs of APIs. For all these methods, the common motive is to induce distinct molecular packing arrangements for different polymorphs, and the most popular method for the polymorphic design of APIs is solution crystallization due to the variety of possible crystallization methods and conditions, where the crystallization methods include cooling, antisolvent, salting-out, melt, evaporation, vacuum, and reaction methods, while the crystallization conditions include solvent/antisolvent species, the solution composition, temperature, additive, supersaturation, cooling rate, agitation, and composition, all of which can modify the molecular interaction between API molecules during the formation of crystals. Among these conditions, solvent/antisolvent species and the solution composition are often considered the most decisive factors determining the polymorphs in crystallization. For example, the polymorphs of diflunisal are primarily controlled by varying the polarity of the solvent, where a polymorph with form-III
crystals is produced when using polar solvents of methanol or ethanol, whereas different polymorphs with form-I and form-IV crystals are generated when using nonpolar solvents of toluene, chloroform, or carbon tetrachloride. Similarly, polymorphic varieties of dipyrididiazooine and erythromycin have also been observed when varying the solvent during crystallization.12,13

Meanwhile, in the case of the BPT ester (2-(3-cyano-4-(2-methylpropoxy) phenyl)-4-methylthiazole-5-carboxylate) and peptide derivative talirerin, the polymorph depends on the supersaturation of the crystallization,14–16 where a high supersaturation level favors the generation of a metastable polymorph, while a low supersaturation level nucleates a stable polymorph. Plus, for talirerin, conformational polymorphs have been observed when using a methanol fraction in the solvent mixture.16 Furthermore, the water fraction in a water–organic mixture solvent has also been found to be a critical factor determining the pseudopolymorphs of APIs due to the variation of the water activity in the mixture solvent.17–21 Also, the cooling rate was considered a determining factor for the polymorphs.22

On the basis of previous studies using various organic solutions to create polymorphs of APIs, it would appear that different solutions induce different interactions between API molecules, thereby generating different polymorphs in the crystallization. Thus, the application of ionic liquids to polymorphic design also needs to be considered, as they might induce distinct intermolecular interactions compared to conventional organic solutions. Ionic liquids refer to liquid salts with dissociated cations and anions below 100 °C.23 Yet, despite their tunable physicochemical properties according to the proper selection of cations and anions, surprisingly few studies have explored the use of ionic liquids for polymorphic design. Zhao et al.24 used the ionic liquids 1-butyl-3-methylimidazolium bromide (BMIImBr) and 1-dodecyl-3-methylimidazolium bromide (DMImBr) as additives to produce polymorphic change in calcium carbonate crystallization. As a result, calcite was obtained without the ionic additives, while aragonite was crystallized when using the ionic liquid additives due to their micelle formation in the aqueous solution. Also, the crystal size of the aragonite was found to depend on the alky chain length of the ionic liquid additives. That is, smaller aragonite was produced when the ionic liquid had a longer chain length (DMImBr) owing to the stronger inhibition effect on crystal growth. In our previous study,25 the polymorphic design of the pharmaceutical ingredient adefovir dipivoxil (AD) was achieved when using an ionic liquid as the solvent in the crystallization. Because of a unique interaction between the ionic liquid solvent and the solute, two new polymorphs were generated.

In the case of protein crystallization, ethylammonium nitrate (EAN) was used as an ionic liquid additive for the salting-out crystallization of lysozyme by Garlitz et al.26 Plus, Li et al.27 used 1-butyl-3-methylimidazolium tetrafluoroborrate (BMIImBF4) as an ionic liquid additive for lysozyme crystallization to modify the crystal morphology and crystal structure. Because of the strong variation of the ionic shape of the ionic liquid, the dipole of the charged groups in lysozyme was distinctly changed compared to that induced by conventional salt ions, resulting in a different packing arrangement of lysozyme in the crystals. In addition, ionic liquid additives have also been applied to obtain high quality proteins for X-ray crystallography28,29 and synthesize nanocrystals of barium sulfate, gold, and zinc sulfide.30–32

However, in most previous studies, ionic liquids have always been used as crystallization additives to change the morphology, size, and structure of protein and inorganic crystals. Accordingly, the present study investigated the applicability of ionic liquids as a solvent and antisolvent during crystallization for the polymorphic design of APIs. Thus, based on the 10 known polymorphs of AD (Figure 1), as summarized in Table 1, antisolvent crystallization using an ionic liquid solvent and ionic liquid antisolvent was attempted to create a polymorphic design that has not been achieved when using conventional organic solvents and antisolvents. To control the polymorphism, the ionic liquids, crystallization temperature, and initial solute concentration were all varied. In addition, the polymorphic nucleation was explained in terms of the supersaturation level depending on the crystallization conditions.

### Experimental Procedures

**Materials.** The active pharmaceutical ingredient adefovir dipivoxil (AD; purity higher than 99.9%), supplied from a pharmaceutical company (Dahee Chem. Co., Korea), was used without further purification. For the antisolvent crystallization, ionic liquids of 1-allyl-3-ethylimidazolium tetrafluoroborrate (AEImBF4), 1-butyl-2,3-dimethylimidazolium tetrafluoroborrate (BDMImBF4), 1,3-diallylimidazolium tetrafluoroborrate (AAImBF4), 1-ethyl-3-methylimidazolium ethylsulfate (EMImEtSO4), 1-allyl-3-ethylimidazolium bromide (AEImBr), and 1,3-diallylimidazolium bromide (AAImBr) were purchased from Kanto Chem. Co. (Japan), where AEImBr was used as the crystallization solvent, while BDMImBF4, AAImBF4, EMImEtSO4, AEImBr, and AAImBr were all used as antisolvents (Figure 2).

**Crystallization.** An initial AD solution of 64 mg/mL was prepared with AEImBF4 as the ionic liquid solvent. Then, 2 mL of the AD solution was mixed with 3 mL of an ionic liquid antisolvent, resulting in an initial solute concentration of 25.6 mg/mL in the mixture. To induce the antisolvent crystallization of AD, the mixture was placed in a temperature bath for 24 h. Meanwhile, the design of new AD polymorphs was investigated by varying the ionic liquid antisolvent among BDMImBF4, AAImBF4, EMImEtSO4, AEImBr, and AAImBr. In addition, the crystallization temperature was varied from 25 to 100 °C and the initial solute concentration was adjusted from 15 mg/mL to 25.6 mg/mL. However, the volume ratio between the ionic liquid solvent and the ionic liquid antisolvent was always fixed at 2:3. Thus, the variation of the initial solute concentration was fulfilled by adjusting the AD solution concentration. For example, the initial solute concentration of 15 mg/mL was prepared with an AD solution of 37.5 mg/mL. That is, 2 mL of a 37.5 mg/mL AD solution was mixed with 3 mL of the ionic liquid antisolvent, resulting in a 15 mg/mL initial
crystallization concentration. To analyze the polymorphisms following crystallization, the AD crystals were washed with hexane and stored in a desiccator after drying at room temperature.

■ RESULTS AND DISCUSSION

Analysis. Powder XRD (Cu–Kα ray (1.54056 Å), M18XHF-SRA, Mac Science, Japan), DSC (Q100, TA Instrument, U.S.A.), and FT-IR (Spectrum I, Perkin-Elmer, U.S.A.) were all used to investigate the AD polymorphisms. Plus, TGA (Q5000, TA Instrument, U.S.A.) was used to analyze the solvate form of the AD crystals. The thermal stability of the AD molecules in the ionic liquids was examined using a liquid chromatography–mass spectrometer (Agilent 6410B, RRLC system, Agilent, U.S.A.), while the solubility of the AD in the ionic liquid mixtures was measured by high pressure liquid chromatography (Agilent 1100, Agilent Technology, U.S.A.) using a C18 column with a mobile phase of water/methanol/CAN (5:4:1 v/v/v).

The antisolvent crystallization of AD was performed at room temperature (25 °C) using AEImBF4 as the ionic liquid solvent and BDMImBF4, AAImBF4, EMImEtSO4, AEImBr, or AAImBr as the ionic liquid antisolvent. When using BDMImBF4 as the ionic liquid antisolvent in the crystallization, the resulting crystals were shaped as thick and wide rectangles (Figure 3a). Meanwhile, needle-shaped crystals were obtained when using AAImBF4 or EMImEtSO4 as the ionic liquid antisolvent in the

<table>
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<tr>
<td>Form-I</td>
<td>anhydrate (C20H32N5O8P)</td>
<td>cooling</td>
<td>acetone + di-n-butyl ether</td>
<td>Gilead Sciences (U.S.A.)</td>
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<td>Form-II</td>
<td>dihydrate (C20H32N5O8P·2H2O)</td>
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<td>methanol/water</td>
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<tr>
<td>Form-III</td>
<td>solvate (C20H32N5O8P·CH3OH)</td>
<td>evaporation</td>
<td>methanol</td>
<td>Gilead Sciences (U.S.A.)</td>
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<td>Form-IV</td>
<td>salt form (C20H32N5O8P·C6H4O)</td>
<td>cooling</td>
<td>isopropanol + fumaric acid</td>
<td>Gilead Sciences (U.S.A.)</td>
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<td>Form-V</td>
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<tr>
<td>Form-VI</td>
<td>monohydrate (C20H32N5O8P·H2O)</td>
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<td>AEImBF4(IL)/water</td>
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<td>Form-IX</td>
<td>hemihydrate (C20H32N5O8P·0.5H2O)</td>
<td>drowning-out</td>
<td>AEImBF4(IL)/water</td>
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</tr>
<tr>
<td>Form-X</td>
<td>anhydrate (C20H32N5O8P)</td>
<td>drowning-out</td>
<td>DMC/Hex.</td>
<td>Kyung hee Univ. (Korea)</td>
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Figure 2. Molecular structures of ionic liquids used as (a) solvent and (b–f) antisolvents in antisolvent crystallization of adefovir dipivoxil.
crystallization (Figure 3b,c). While a needle shape is a typical morphology for AD crystals consisting of form-I, form-VIII, form-IX, and form-X polymorphs, the thick and wide rectangular-shaped AD crystals were distinct from any previously reported polymorphic crystal morphologies. Unfortunately, no crystallization was induced when using AEImBr or AAImBr as the ionic liquid antisolvent in the crystallization.

According to the microscopic images of the crystal shapes, the ionic liquid antisolvents appeared to provide a unique solution environment for distinct crystal structural formation during the crystallization. Thus, the polymorphisms of the AD crystals were examined using powder XRD and DSC, as shown in Figure 4. According to the XRD spectra, the crystal structure obtained when using BDMImBF$_4$ as the ionic liquid antisolvent demonstrated unique characteristic peaks ($2\theta = 8.3^\circ$, $9.3^\circ$, $10.9^\circ$, $12.6^\circ$, $16.3^\circ$, $16.9^\circ$, $19.6^\circ$, $21.3^\circ$, $21.8^\circ$, $24.4^\circ$, $25.7^\circ$, $27.8^\circ$, $30^\circ$) that were clearly different from the XRD patterns for any previous AD polymorphs, thereby indicating a new AD polymorph (called NF in the present study) (Figure 4a).

However, the crystals obtained when using AAImBF$_4$ or EMImEtSO$_4$ as the ionic liquid antisolvent were confirmed as the most stable AD polymorph, known as form-I. Meanwhile, DSC scanning found that the NF crystals exhibited a unique thermal profile, including the exothermic and endothermic peaks at 80 and $97^\circ$C, respectively. The exothermic peak at $80^\circ$C was due to the phase transition of the NF crystals to a stable form in a solid state, while the high endothermic peak at $97^\circ$C indicated the melting point of the stable crystal form. Interestingly, it should be mentioned that the melting point at $97^\circ$C is the same as the melting temperature of the form-X polymorph, as reported in our previous study.33

The above phase transition of the NF crystals to the stable form in the solid state was confirmed by XRD spectral pattern for the crystals before and after the solid state transition, as shown in Figure 5a. The XRD pattern of the crystals at point "a"
Thus, it apparently resulted in a single broad endothermic peak at 97 °C. However, when reducing the thermal scanning speed below 0.1 °C/min, this facilitated recrystallization of the melt phase into form-I crystals, which then finally melted at 102 °C. Thus, the slow thermal scanning resulted in two sharp melting peaks for the form-X and form-I crystals at 97 and 102 °C, respectively. Therefore, the slow thermal profile inferred that the NF crystals were transformed to form-X crystals and then finally to form-I crystals due to a difference in the free energy levels. Also, the two exothermic peaks resulting from the solid state transformation and recrystallization at around 80 and 100 °C, respectively, implied monotropic systems among the NF, form-X, and form-I polymorphs.

The influence of the crystallization temperature on the polymorphic design was also investigated, as shown in Figure 6. In the crystallization using AEImBF4/BDMImBF4 as the solvent/antisolvent, respectively, the wide rectangular-shaped NF crystals at a crystallization temperature of 25 °C shifted to needle-shaped crystals when increasing the crystallization temperature (Figure 6a,b). Thus, the crystallization at a crystallization temperature of 70 °C produced a mixture of rectangular and needle-shaped crystals (Figure 6b), while a crystallization temperature of 90 °C produced only needle-shaped crystals (Figure 6c). When the polymorphic change according to the crystallization temperature was examined using XRD (Figure 6d), the XRD patterns revealed that the characteristic peaks of the crystals obtained at a crystallization temperature of 90 °C corresponded exactly to those of the form-X polymorph, while the XRD spectrum for the crystals obtained at a crystallization temperature of 70 °C included the characteristic peaks for both the NF and form-X polymorphs, which was consistent with the morphological shift according to the crystallization temperature.

The polymorphic fractions of the NF and form-X polymorphs according to the crystallization temperature were quantitatively estimated using the FTIR spectroscopic spectra, as unique and distinctive absorption peaks for the form-X and NF polymorphs occurred at a C==N vibrational frequency of 1680 cm⁻¹ and 1660 cm⁻¹, respectively, due to the different intermolecular strength in the different molecular packing structures. Thus, on the basis of a standard calibration using the characteristic peaks of the FTIR, the polymorphic fractions according to the crystallization temperature are summarized in Figure 7. At a crystallization temperature below 50 °C, only the NF polymorph was produced. Then, the form-X polymorph began to be generated at a crystallization temperature above 50 °C and was uniquely nucleated at a crystallization temperature above 80 °C. Therefore, these experimental results agreed well with the above microscopic and XRD measurements. Also, the results indicated that a high temperature favored the formation of the stable form-X polymorph. The reason for this will be discussed in a later section on the control of polymorphic nucleation by the supersaturation level.

Using the spectroscopic analysis, the mechanism of the polymorphic formation of AD crystals according to the crystallization temperature was investigated, as shown in Figure 8. According to a crystallographic study, the molecular packing of AD polymorphs is mainly determined by the intermolecular hydrogen bonds between the functional groups of the AD molecules, such as NH2, C==O, and CH3. For example, in the case of the form-X crystals, the asymmetric interaction of the diester group in the AD molecule resulted in dual C==O absorption peaks at 1750 and 1730 cm⁻¹.
8a). Plus, the dual absorption peaks at around 1400 and 1380 cm\(^{-1}\) were caused by the asymmetric interaction of two methyl groups (CH\(_3\)) in the molecules of the form-X crystals. The C=N absorption peak at 1680 cm\(^{-1}\), which was a slight red-shift from the free absorption peak at 1690 cm\(^{-1}\), also indicated a weak interaction between the molecules in the form-X polymorph. This absorption peak pattern was highly similar to that for form-I crystals, possibly due to a similar molecular arrangement in the respective structures.\(^{33}\) The similarity in the spectroscopic spectra between the form-X and form-I polymorphs was also reflected in the powder XRD patterns for the two polymorphs.\(^{33}\) Meanwhile, the absorption peak pattern for the NF polymorph was quite distinct from that for the form-X polymorph, with single absorption peaks occurring at 1745 and 1390 cm\(^{-1}\) for C=O and CH\(_3\), respectively, due to symmetric interactions. Also, the absorption peak for C=N was measured at 1660 cm\(^{-1}\), implying a stronger hydrogen bonding in the NF polymorph than in the form-X polymorph. Thus, since the absorption of C=N was clearly distinctive between the two polymorphs, this was used as the characteristic peaks to estimate the polymorphic fractions in the crystal mixtures, as mentioned above (Figure 7).

Figure 6. Morphology and XRD analysis of AD crystals obtained at various crystallization temperatures when using AEImBF\(_4\) and BDMImBF\(_4\) as ionic liquid solvent and antisolvent, respectively. (a–c) Morphology of AD crystals and (d) powder X-ray diffraction patterns of AD crystals.

Figure 7. Variation of polymorphic fractions of NF and form-X crystals according to crystallization temperature. The initial solute concentration was fixed at 25.6 mg/mL.

According to Davey et al.,\(^{35,36}\) polymorphic crystal formation is primarily predetermined by the solution, as the intermolecular interactions of the solute molecules are preoriented by the solution conditions. That is, different solutions induce different hydrogen bondings between molecules, resulting in different molecular packing of different polymorphs during crystallization. Thus, a popular method of polymorphic design is to adjust the solution conditions during crystallization by

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varying the solvent and antisolvent species, temperature, and additives. In the present study, as shown in Figure 8b, the preorientation of the AD molecules in a saturated ionic liquid solution when varying the crystallization temperature was confirmed using spectroscopy (FTIR-ATR). At a low crystallization temperature of 25°C, one stretching vibration peak appeared at 1745 cm⁻¹ due to the symmetric intermolecular interaction of the diester (two C–Os) group in the AD molecule, which was similar to the intermolecular interaction in the NF polymorph. However, at a high crystallization temperature of 90°C, two stretching vibration peaks at 1757 and 1747 cm⁻¹ were consistently observed, as with the form-X polymorph, due to the asymmetric intermolecular interaction of the diester (two C–Os) group in the AD molecule. In addition, the lower wavenumber for the C–N absorption peak at 25°C indicated a stronger intermolecular interaction than at 90°C, which was consistent with the wavenumber shift for the solid state polymorphs. Thus, the spectroscopic spectra for the solution and solid state AD confirmed that the AD crystal polymorphs were predetermined according to the preorientation of the molecules in the ionic liquid solution.

The influence of the initial solute concentration on the polymorphic nucleation was also investigated, as shown in Figure 9. According to Ostwald’s Rule of Stage, a labile supersaturated solution crystallizes the metastable phase of crystals in order to minimize a possible change in the free energy. The metastable crystals are then transformed to the most stable phase through each possible polymorphic structure. Thus, as demonstrated in the solute concentration–temperature diagram of the polymorphic nucleation (Figure 9), an initial solute concentration above 20 mg/mL was high enough to crystallize only metastable (NF) crystals at a crystallization temperature of 25°C. Then, when decreasing the initial solute concentration to 15 mg/mL, a polymorphic mixture of metastable (NF) and stable (form-X) crystals was simultaneously nucleated due to the reduced supersaturation level. However, when increasing the crystallization temperature to 60°C, a polymeric mixture of NF and form-X crystals, rather than a pure polymorph of NF crystals, was formed, even at the highest initial solute concentration of 25.6 mg/mL. The formation of pure form-X crystals was then achieved by reducing the initial solute concentration to 20 mg/mL. Therefore, these experimental results inferred that the polymorphic nucleation was determined by the supersaturation level (S), defined as the ratio of the initial solute concentration to the equilibrium concentration (solubility), which varied according to the crystallization temperature and concentration. Thus, a high supersaturation level was favorable for the nucleation of the metastable polymorph (NF), whereas a low supersaturation level resulted in the direct nucleation of the stable form-X polymorph. Consequently, increasing the crystallization temperature at a fixed initial solute concentration decreased the supersaturation level due to an increase in the solubility of the NF and form-X crystals (Figure 9), thereby favoring the nucleation of the stable form-X polymorph. This then explains why pure form-X crystals were obtained at a high crystallization temperature above 80°C, even with a high initial solute concentration of 25.6 mg/mL, as shown above in Figure 7. Furthermore, the crystallization temperature for the nucleation of pure form-X crystals was reduced to 40°C when decreasing the initial solute concentration to 15 mg/mL. Here, the polymorphic nucleation along with the crystallization temperature and concentration was monitored using FTIR, as shown in the Supporting Information (Figure S-1).

The relationship between the supersaturation level and the polymorphic nucleation was further investigated using the polymorphic supersaturation level diagram shown in Figure 10. Here, the polymorphic supersaturation levels are defined as the
ratio of the initial solute concentration to the polymorph solubility. Thus, the ratio of the initial solute concentration to the stable solubility (solubility of form-X crystals) is referred to as the supersaturation level of the stable polymorph ($S_{form-X}$), while the ratio of the initial solute concentration to the metastable solubility (solubility of NF polymorph) is referred to as the supersaturation level of the metastable polymorph ($S_{NF}$). According to the $S_{NF}$=$S_{form-X}$ diagram of polymorphic nucleation, it was found that no crystallization occurred when $S_{form-X}$ was lower than 1.8 due to the metastable state of the supersaturated solution. However, when $S_{form-X}$ was above 1.8, this was high enough to induce the crystallization of form-X crystals. Meanwhile, for the NF polymorph, no nucleation occurred when $S_{NF}$ was below 1.6, meaning only form-X crystals were obtained. However, when $S_{NF}$ was above 1.6, the supersaturation level was high enough to induce the metastable polymorph, resulting in the simultaneous crystallization of both NF and form-X crystals. Finally, when $S_{NF}$ was higher than 2.1, only NF crystals were nucleated. Therefore, the $S_{NF}$=$S_{form-X}$ diagram revealed that the polymorphic nucleation was predominantly controlled by the supersaturation level and also quantitatively predictable in terms of the supersaturation level.

In our previous study, AD molecules were found to be thermally unstable in a solution due to their weak phosphate bonds, making them easily hydrolyzed or decomposed even at a moderate temperature in a conventional organic and water solvent. As shown in Figure 11, AD molecules in a conventional organic solvent/antisolvent mixture (50/50 vol%), which is typically used for the antisolvent crystallization of AD, were easily decomposed at an elevated temperature. As such, in the AC/IPE mixture, the AD molecules began to decompose at 50 °C (Supporting Information S-2). Plus, similar decomposition occurred in organic mixtures of MeOH/IPE and DCM/Hex at around 50–60 °C. In contrast, no decomposition of the AD molecules occurred in the ionic liquid mixture of AEImBF$_4$/BDMImBF$_4$ even at a high temperature of 100 °C, as the ionic liquids protected the weak phosphor bonds of the AD molecules from decomposition, thereby thermally stabilizing the AD molecules. As a result, pure AD crystals were crystallized out from the AEImBF$_4$/BDMImBF$_4$ mixture, as proven by a mass spectroscopic analysis (Supporting Information S-2). The thermostability induced by the ionic liquids also agreed well with previous studies, where ionic liquids have been found to stabilize proteins by protecting against degradation by hydrolysis, meaning that the protein activity in an ionic liquid-water mixture has been found to last more than 6 months in contrast to a week in pure water. In addition, ionic liquids provide proteins with thermostability, which allows proteins in an ionic liquid–water mixture to unfold up to 100 °C. Thus, AD molecules are highly protected from hydrolysis at a high temperature in a water–ionic liquid mixture due to the thermostability effect of the ionic liquid. Therefore, the use of ionic liquids as solvents and antisolvents for crystallization can facilitate a wide variation of crystallization temperatures for the polymorphic design of pharmaceutical ingredients.

**CONCLUSION**

When using ionic liquids as the solvent and antisolvent, it was demonstrated that antisolvent crystallization could successfully produce a new AD polymorph. This capability of antisolvent crystallization to design a new crystal polymorph (NF) was due to unique intermolecular interactions of the AD molecules induced by the ionic liquids (AEImBF$_4$/BDMImBF$_4$) that are not achievable with conventional organic solvents/antisolvents. However, the crystallization using AEImBF$_4$/AAImBF$_4$ and AEImBF$_4$/EMImEtSO$_4$ produced form-I crystals, which is a typical polymorph that can be obtained with conventional organic solvents and antisolvents. No crystals were formed in the crystallization using AEImBF$_4$/AEImBr and AEImBF$_4$/AAImBr.

XRD and DSC analyses confirmed that the crystals produced in the AEIMBF$_4$/BDMImBF$_4$ solution had a different crystal structure and thermal behavior from other AD polymorphs. In addition, a DSC thermal scan revealed that the NF polymorph shifted in a solid state to form-X and then finally to form-I when increasing the temperature, indicating a monotropic relationship. From the spectroscopic spectra, the AD polymorphs were found to be predetermined by the AD molecular orientation in the ionic liquid solution, plus the preorientation of the AD molecules in the ionic liquid solution also varied according to the temperature. As such, at a low temperature, the AD molecules in the ionic liquid solution were preoriented to the NF polymorph, resulting in the nucleation of NF crystals in the crystallization. However, this preorientation in the ionic liquid solution shifted to the nucleation of the form-X polymorph when increasing the temperature. Fur-
thermore, the nucleation of the form-X polymorph was favored with a low crystallization concentration, while the nucleation of the NF polymorph was favored with a high crystallization concentration. The polymorphic nucleation according to the crystallization temperature and initial solute concentration was also investigated in terms of the supersaturation level. According to a polymorphic supersaturation level diagram \( S_{NF} - S_{form-X} \), the metastable NF polymorph was preferably nucleated at a high supersaturation level \( S_{NF} > 2.1 \), whereas only the stable form-X polymorph was generated when reducing the supersaturation level \( S_{NF} < 1.6 \). Plus, a high crystallization temperature was advantageous for the formation of the stable polymorph, as the elevated solubility reduced the supersaturation level. Finally, due to the strong thermal stabilization of the ionic liquids, the AD molecules remained perfectly stabilized up to 100 °C, whereas they are easily decomposed in conventional organic solvent mixtures, even at a low temperature of 50 °C. Thus, the advantage of a wider temperature range when using ionic liquids may help with polymorphic design.

**ASSOCIATED CONTENT**

**Supporting Information**

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**Notes**

The authors declare no competing financial interest.

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