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Novel polymorphic form of adefovir dipivoxil derived from polymer-directed crystallization

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Crystallization is an essential step in pharmaceutical processing. The discovery of a non-classical crystallization pathway would be a promising strategy to engineer the properties of drug crystalline particles for specific delivery conditions. Herein, polymer-directed crystallization was successfully employed to modify the characteristics of a model drug, adefovir dipivoxil (AD). Polyacrylic acid (PAA), ethyl cellulose (EC), and hydroxypropyl cellulose were added as active polymers to control the crystallization pathway of AD. Changes in crystal habit were observed in all cases. A novel polymorph was found after the addition of PAA and EC, and was confirmed by XRD and DSC results. In FTIR investigations, the crystals derived from PAA-directed crystallization showed strong interactions between PAA and AD. The polymer content in polymer-directed crystallization-derived powders varied from 7 to 24 wt%, and the presence of polymers lead to sustained release of AD. These results make polymer-directed crystallization a simple and efficient technique to engineer the physical and chemical properties of drug crystals.

1. Introduction

Controlling crystal polymorphism is a major step in drug manufacture because it affects bioavailability, stability, and processability. Modification of crystallization conditions, solvent, crystallization temperature, or extent of super saturation, have traditionally been used to control polymorphism (Price et al. 2002, 2003; Rodriguez-Spong et al. 2004), however these methods are limited by the classical pathway of crystallization. As a result, limited engineering options place limitations on related characteristics such as crystal habit, thermal properties, and release behavior. In classical crystallization, the growth of crystal shapes is based on the integration of atomic or molecular building blocks into energetically favorable sites in growing crystal faces.

Polymer-directed crystallization, which utilizes non-classical pathways of crystallization, has facilitated the discovery of novel polymorphs as well as the growth of single crystals of small molecules (Lang et al. 2002; Price et al. 2005), extended networks (Grzesiak et al. 2007), and inorganic complexes (Grzesial et al. 2007). This method is suitable for the production of both stable and metastable polymorphs because it sheds light on the intermolecular interactions stabilizing the crystal structure. Self-consistent sets of spectroscopic, calorimetric, and powder X-ray diffraction (XRD) data can then be correlated with the corresponding structures, allowing more accurate phase identification (Adam et al. 2007).

The epitaxial nucleation and growth of active pharmaceutical ingredients by heterogeneous surfaces of polymers have been investigated, while homogeneous solutions of polymer and drug have been the subject of investigations of drug solubility (solid dispersion) (Lee et al. 2009, 2010). Polymers dissolved in solutions have been regarded as crystallization inhibitors, which are important in self-emulsifying drug delivery systems and solid dispersions (Gao et al. 2009). Alternatively, in the field of inorganic materials, polymer-directed crystallization using dissolved polymers or peptides is known as a powerful method leading to novel polymorphs or composite polycrystalline particles (mesocrystals) of polymers and inorganic materials such as calcium carbonate (biomimetic mineralization). However, in the field of crystalline drugs, the role of dissolved polymers as active agents directing the crystallization pathway has been investigated through only a few studies using heterogeneous nuclei (or surfactants) (Grzesiak et al. 2006; Lee and Zhang 2008; Raghavan et al. 2001).

In this study, we applied a polymer-directed crystallization technique for homogeneous solutions to a model drug, and evaluated the usefulness of non-classical crystallization methods for engineering the physical and chemical properties of drugs. Conditions for modification of the original crystal polymorphism and *in vitro* release behavior were examined in detail.

Adefovir dipivoxil (AD), which was selected as a model drug, is the popular name of 9-[2-bis(pivaloyloxymethyl) phosphonmethoxyethyl] adenine. It is an orally bioavailable prodrug of 9-[2-(phosphonylmethoxy)ethyl] adenine, which acts as a chain terminator nucleotide analogue and is effective against the human immunodeficiency virus, herpes viruses, Epstein-Barr virus, retroviruses, cytomegalovirus, and other DNA viruses (Yu et al. 1999; Qaqish et al. 2003; Julander et al. 2002).

2. Investigations, results and discussion

2.1. Microscopy

Crystal morphology plays an important role in pharmaceutical processing and in the product development of solid dosage

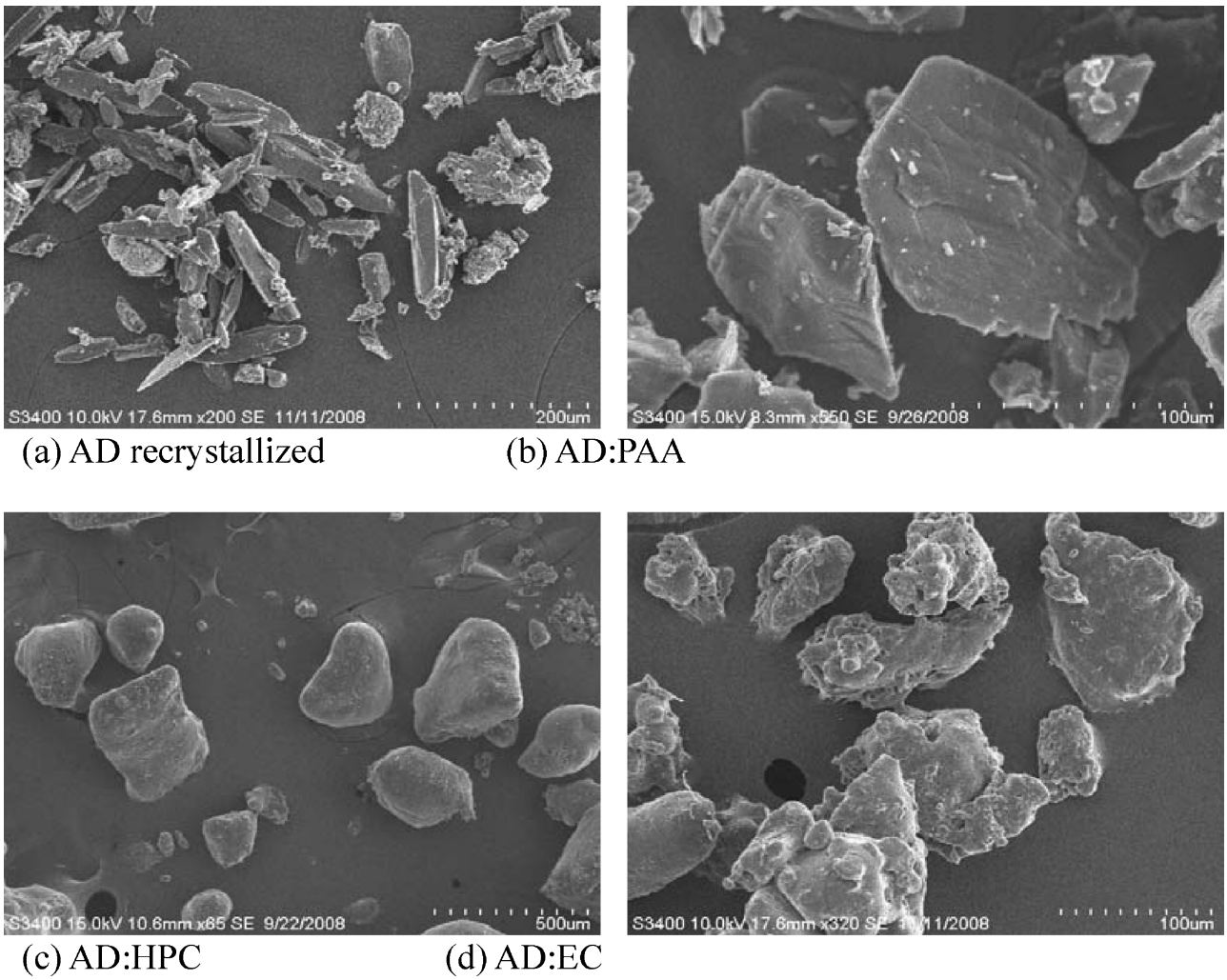


Fig. 1: SEM micrographs of AD crystals prepared by polymer-directed crystallization

forms. Differences in crystal habit strongly influence the particle orientation, flowability, packing, compaction, compressibility and dissolution characteristics of drug powders. Solid-liquid interfacial interactions can alter the roundness of interfaces, change crystal growth kinetics and enhance or inhibit growth at certain crystal faces, resulting in different habits (acicular, plates, tabular, bladed and prismatic) (Tiwary 2001). As a result, we first verified the crystal habit by SEM and OM in our experiments.

Fig. 1 shows the particle morphology of crystallized AD. The roles of the polymers on nucleation, growth, and aggregation into a possible mesocrystal could be presumed. While the pure AD that had recrystallized under the same conditions without polymer was needle-shaped (Fig. 1a), the other particles showed different morphologies depending on the type of polymers that were used in crystallization. The crystals with PAA showed plate-like morphology (Fig. 1b), and those with HPC were nearly spherical. AD crystallized with HPC had relatively smooth surfaces in contrast to the rough surfaces of AD crystallized with PAA (Fig. 1b). As shown in the magnified images of AD crystallized with EC, the particles were irregular (Fig. 1d). As a result, the addition of polymers to the crystallization processes changed the crystal habit, possibly as a result of interfacial tension between the nuclei and solution.

2.2. Crystal structures

XRD is one of the most sensitive and foolproof methods for solid-state characterization, as the results directly reflect the molecular arrangements of crystalline materials (Chao and Vail 1987). Fig. 2 shows the powder XRD results of AD. We expected different polymorphs based on the different morphologies observed in SEM images. Significant changes in XRD patterns were observed in AD crystallized with PAA and EC, in which unique characteristic peaks were identified at 17.2 ° and 24.8 °. This crystal form is novel, since the powder patterns of the precipitated AD crystallized with PAA or EC were not consistent with any previously discovered crystal forms (Goucheng 2008; Arimilli 2004). Most of the peaks in AD crystallized with PAA and EC were at the same positions, however the crystallity seems to be reduced with EC, as the peak broadening was more distinct. The XRD pattern of AD crystallized with HPC was similar to that of AD recrystallized without a polymer (denoted as "AD recrystallized" in Fig. 2). The patterns were the same whether the particles were collected in methanol or ethanol as a solvent (Fig. 2. AD:HPC = 14:1(MeOH)).

2.3. Thermal properties

The DSC thermograms of AD-polymer composite particles that were prepared by polymer-directed crystallization and of AD recrystallized by the same process are shown in Fig. 3. The melting point of recrystallized AD was 75.4 °C, indicating Form II (Goucheng 2008; Arimilli 2004). However, the melting point was increased by the addition of HPC or PAA. In the case of HPC, the melting point was 101.5 °C, indicating Form I (Goucheng 2008; Arimilli 2004). The melting point of AD

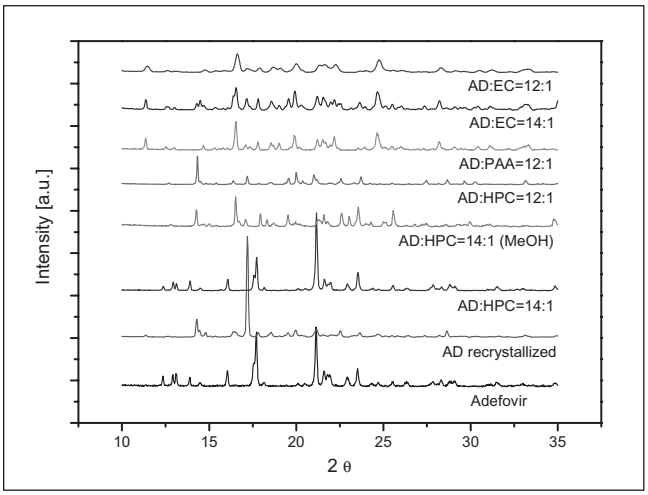


Fig. 2: X-ray diffraction results of AD prepared by polymer-directed crystallization

crystallized with EC was 70.6 °C, similar to Form II, and that of AD crystallized with PAA was 83.5 °C.

The melting point was affected by the polymer. Interestingly, the melting points of AD crystallized with PAA and EC were quite different, although they showed the same XRD patterns. These results were confirmed by repeated experiments and supported by another recent experiment with different drugs. When there are strong interactions between polymer and drug, the modification of the classical crystal nucleation and growth mechanisms can create different polymorphs, crystal habits, and/or unique thermal properties. Both PAA and EC, which influenced crystalline structure as shown in Fig. 2, successfully modified the crystallization mechanism of AD, resulting in the activation of a non-classical crystallization pathway leading to possibly mesocrystals. Strong interactions between polymers and drug molecules, and the self-assembled structures of mesocrystals may significantly depress melting points depending on the strengths of the interactions, which are related to interaction parameters, and the resulting sizes of the crystalline domains. According to theoretical considerations (Bergese et al. 2004) based on Laplace and Gibbs-Duhem equations, the sizes of drug crystallites are inversely proportional to melting temperature depression. Therefore, AD crystallized with EC may have stronger interactions and smaller crystalline domains. The lower melting point of EC may be related to the broadened peaks observed in the XRD results shown in Fig. 2.

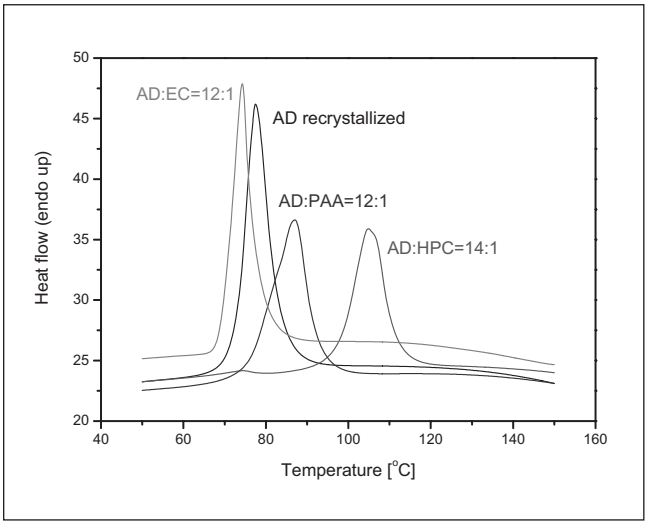


Fig. 3: DSC thermal analysis of AD prepared by polymer-directed crystallization

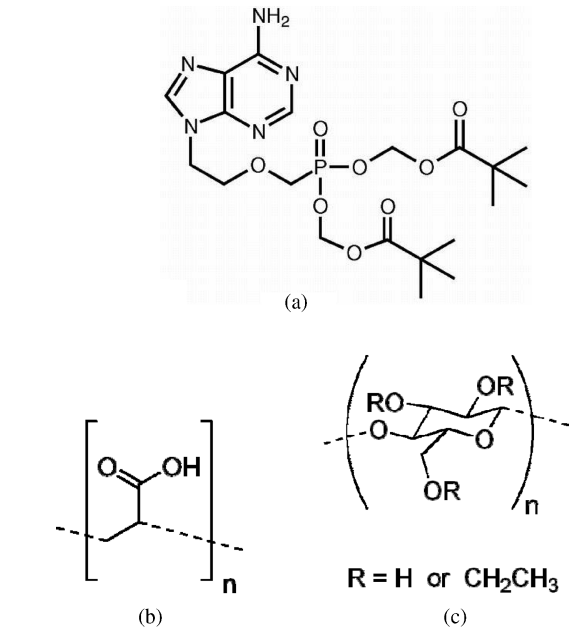


Fig. 4: Chemical structures of A) adefovir dipivoxil, B) polyacrylic acid, and C) ethyl cellulose

2.4. FTIR spectroscopy

FTIR spectroscopy has been successfully used to explore differences in molecular conformations, crystal packing and hydrogen bonding arrangements in different solid-state forms of organic compounds (Brittain 1997). Spectral variations originate from alterations in bonds that exhibit characteristic vibrational frequencies, leading to frequency shifts and splitting in absorption peaks. Fig. 4 shows the chemical structures of AD and polymers used for polymer-directed crystallization, and Table 1 shows an example of specific drug-polymer hydrogen bond interactions (-NH_2). Fig. 5 shows a characteristic C=O stretching band at 1660 cm^{-1} and an NH_2 stretching band at 3460 cm^{-1} in AD crystallized with PAA. As shown in Fig. 5, the peaks were significantly shifted from those of a physical mixture of AD and PAA of the same composition.

These shifts in frequencies may indicate hydrogen bonding between the −C=O group of PAA and –NH_2 group of AD (Table 1). This hydrogen bonding may in turn lead increases in negative charge over the oxygen atom due to shifts of π electrons of the C=O group, resulting in the weakening of its double bond

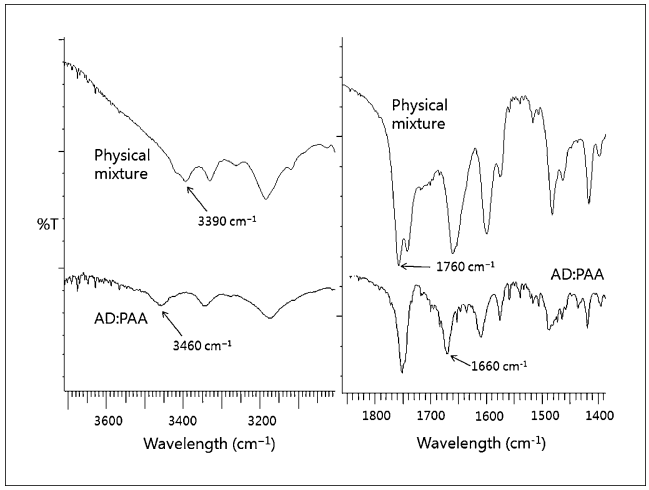
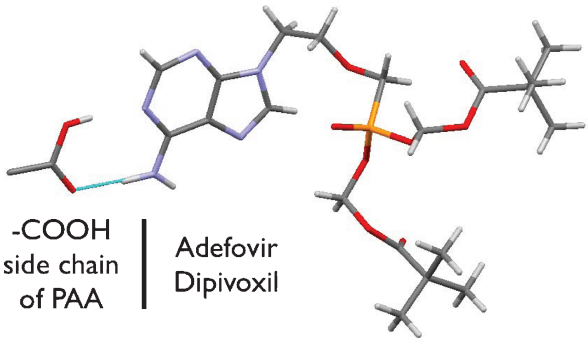


Fig. 5: Investigation of drug-polymer interactions by FTIR. Comparison between the physical mixture (upper) of AD and PAA, and PAA-induced crystallized AD (lower)

Table 1: Proposed hydrogen-bonding sites between AD and polymers

Samples	Amine, -NH_2 (cm^{-1})	Assignment of hydrogen bonding
Crystalline AD	3390	AD-NH–O(H)-AD
AD:EC	3390	AD-NH–O(H)-EC
AD:PAA	3460	AD-NH–O=C-PAA



Proposed structure of PAA and AD complex

character. Hydrogen bonding alters the force constant of C=O as well as of NH_2, thus altering the frequencies of stretching vibrations. The influence of hydrogen bonding may be maximized by nanoscale crystalline domains when their surfaces have strongly adsorbed polymers via hydrogen bonding. Alternatively, the novel polymorph itself may have unique vibrational frequencies different from those of the original AD. However, the following discussion explains why this hypothesis is less plausible.

Unlike the FTIR results of AD crystallized with PAA, AD crystallized with EC or HPC showed no significant peak shifts (data not given). No differences were found in comparison between crystals produced by polymer-directed crystallization and their physical mixture controls, and all the peaks matched the original peaks of AD and polymers. However, AD:EC might still have different interactions, which is not clear in FTIR, since it showed unique XRD results. FTIR may not be sensitive enough to identify interactions through the ether linkage of EC (Fig. 4c).

2.5. Polymer content in composite particles

The polymer content of composite crystalline particles prepared by polymer-directed crystallization is provided in Table 2. As expected from the XRD and melting point depression results of Figs. 2 and 3, AD crystallized with EC showed the greatest polymer content (23.6 wt%) in the composite powders. All other composite powders had relatively low polymer contents between 7.6 – 10.7 wt%.

Although polymers exist as surface adsorbed molecules on primary crystalline domains, the content shown in Table 2 is not negligible. Therefore, if uniformly distributed polymer

Table 2: Amounts of absorbed polymers in AD-polymer composites measured by UV-visible spectroscopy, which were prepared by polymer-directed crystallization

Samples	Polymer content in crystallized solid powder (wt%)	S.D.
Adefovir	0	–
AD:PAA = 12:1	7.6	± 1.3
AD:HPC = 12:1	10.7	± 0.6
AD:EC = 14:1	23.6	± 0.5

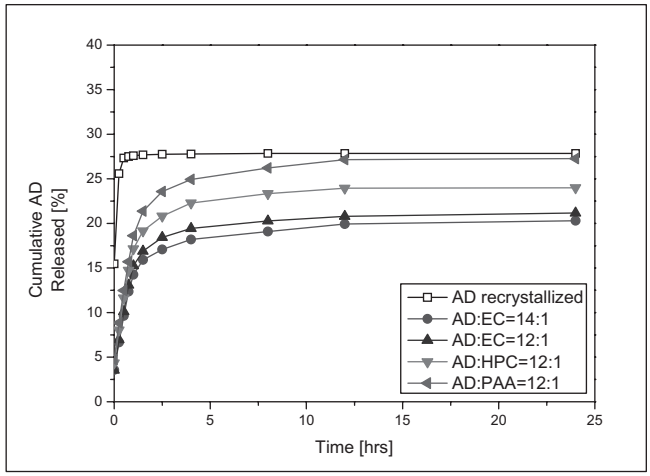


Fig. 6: *In vitro* release behavior of AD prepared by polymer-directed crystallization

layers have formed, their roles in drug dissolution and stability are expected to be significant. Additionally, taste masking, mucoadhesiveness, processability, and compactability may be influenced, making polymer-directed crystallization attractive for the development of novel drug delivery systems.

2.6. In vitro release behavior

When polymer chains strongly interact with AD during crystallization, they can significantly influence the nucleation and growth mechanisms of AD. They can induce dissolved AD molecules to assemble into unique structures, leading to the nucleation of novel polymorphs. The crystallites of novel polymorphs can then form mesocrystals during subsequent growth processes. Without polymorph modification, the growth of small crystallites may be inhibited and induce the assembly of crystallites into mesocrystals. Therefore, polymer-directed crystallization is expected to affect the crystal structures (or morphology) of AD in all size ranges.

Changes in polymorphs, mesocrystal structures, and particle morphology can all influence the release behavior of AD. The *in vitro* release behavior of AD is shown in Fig. 6. Compared to the recrystallized AD case, all the cases show sustained release behavior, possibly indicating the existence of polymer on the surfaces of AD crystallites acting as diffusion barriers. The most sustained release behaviors were observed in AD crystallized with EC at a 14:1 ratio, followed by AD crystallized with EC at a 12:1 ratio, AD crystallized with HPC, and AD crystallized with PAA. This order reflects differences hydrophobicity and amount of polymer. Therefore, the roles of diffusion barriers formed by surface-adsorbed polymers are probably the most critical factors determining the *in vitro* release behaviors of AD mesocrystals after polymer-directed crystallization.

In conclusions, we applied a polymer-directed crystallization technique to AD that led to the formation of unique crystal habits, a novel polymorph, and possible mesocrystals. The crystal habit was influenced by all of the polymers that we tested, however a novel polymorph was found only in AD crystallized with PAA and EC. The molecular interactions between PAA and AD were confirmed using FTIR, but were not detected in AD crystallized with EC and HPC. The polymers, whose contents were 7 to 24 wt% in powders obtained by polymer-directed crystallization, significantly sustained the *in vitro* release behavior of AD. The sustained release behavior that we observed reflected the hydrophobicity of the polymers. These results indicate that polymer-directed crystallization produces unique composite crystals, and that this method is a simple and effective route to modify the properties of crystalline drugs.

3. Experimental

3.1. Materials

Adefovir dipivoxil (AD, GMP) was provided by Amorepacific (Seoul, South Korea) and used without purification. Polyethylene glycol (PEG, Mw 2000 g/mol), hydroxypropyl cellulose (HPC, Mw 10000 g/mol), polyacrylic acid (PAA, Mw 14000 g/mol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl cellulose (EC) was purchased from Junsei Chemical (Tokyo, Japan). HPLC grade water (J.T. Baker, NJ, USA) was used without purification. Ethanol (99.9%, HPLC) was purchased from Samchun Pure Chemical (Pyongtack, Gyeonggi, South Korea) and used as a solvent for drowning-out crystallization.

3.2. Crystallization of AD

AD was dissolved into ethanol (33.3 wt%) at a wt ratio of AD:polymer = 12:1 or 14:1 at room temperature (RT). Crystallization was allowed at 4 °C for 48 h, and crystal particles were filtered and dried under vacuum at RT. PAA, HPC, and EC were used for this cooling crystallization.

3.3. Characterization of particles

After filtering and washing with water, crystallized particles were vacuum dried for more than 24 h at RT. The crystallinity and crystal structure of dried powder were detected with an X-ray powder diffractometer (XDS 2000, Scintag, USA) at a scan rate of 1 degree/min (2θ: $10^\circ - 35^\circ$). An optical microscope (BX-51, Olympus, Japan) and a scanning electron microscope (S-3400, Hitachi, Japan) were used to investigate the size and morphology of particles. The particles were mounted on a carbon tape surface, and Pt-C coated with a carbon coater (Hitachi, Japan) at 6.7 nm/min for 120 s. The thermal properties of powders (9 – 10 mg) were assessed with a differential scanning calorimeter (Pyris 6, Perkin Elmer, USA) at a heating ramp of 4 °C/min in a N_2/air atmosphere (25–180 °C, single scan). Possible interactions between the drug and polymer were studied via FTIR spectroscopy (Magna 750, Nicolet, USA) using the conventional KBr pellet method.

3.4. Solubility analysis of AD

The amount of dissolved AD was traced by UV/visible spectroscopy (V-550, JASCO, Japan). The absorption peak of AD at 260 nm was quantified. After an equilibration time of 24 h, the transparent supernatant of 0.01 mL was diluted 100 times with methanol and used for UV/visible characterization. The polymer contents of dried powders were assessed by UV/visible measurements (three repeated measurements). Crystallized particles were first filtered and dried in a vacuum oven at RT for more than 24 h, and 50 mg of dried powders were dissolved in 10 mL ethanol for measurement, using the same peak at 260 nm.

3.5. In vitro release of AD powders

The *in vitro* release of crystallized AD powders was measured in pH 6 buffer solutions using a dissolution tester (KDT-600, Kuk-Je, Seoul, Korea, Type II USP paddle apparatus) under a sink condition. Powder (750 mg) was put into a buffer solution (300 mL) and stirred at 100 rpm at 37 ± 0.5 °C. UV/visible spectroscopy was used to measure the amount of AD released at time points of 0, 15, 30 and 45 min and 1, 1.5, 2.5, 4, 8, 12, and 24 h. After each sampling (5 mL), fresh 5 mL buffer solution was added to the sample.

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