Polymer-Directed Crystallization of Atorvastatin

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Received 29 February 2012; revised 14 April 2012; accepted 2 May 2012

Published online 24 May 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23206

ABSTRACT: Living organisms secrete minerals composed of peptides and proteins, resulting in "mesocrystals" of three-dimensional-assembled composite structures. Recently, this biomimetic polymer-directed crystallization technique has been widely applied to inorganic materials, although it has seldom been used with drugs. In this study, the technique was applied to the drowning-out crystallization of atorvastatin using various polymers. Nucleation and growth at optimized conditions successfully produced composite crystals with significant polymer contents and unusual characteristics. Atorvastatin composite crystals containing polyethylene glycol, polyacrylic acid, polyethylene imine, and chitosan showed a markedly decreased melting point and heat of fusion, improved stability, and sustained-release patterns. The use of hydroxypropyl cellulose yielded a unique combination of enhanced *in vitro* release and improved drug stability under a forced degradation condition. The formation hypothesis of unique mesocrystal structures was strongly supported by an X-ray diffraction pattern and substantial melting point reduction. This polymer-directed crystallization technique offers a novel and effective way, different from the solid dispersion approach, to engineer the release, stability, and processability of drug crystals. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:2941–2951, 2012

Keywords: drying; crystallization; polymer-directed crystallization; particle size; mesocrystals; composite particles; polymers; crystal engineering

INTRODUCTION

Engineering of crystal structure (including polymorphism), behavior, and size has continually been an essential research subject and is a critical determining factor of the bioavailability, stability, and processability of drugs.[1–7] Crystallization is normally controlled through the use of nucleation agents, stabilizers, and cosolvents.[8] Crystal nucleation and growth are very sensitive processes, and many of their aspects are still unknown.[2,9–11]

The existence of polymers or peptides dissolved in a crystallizing solution can trigger a nonclassical pathway, producing "mesocrystals" with three-dimensional (3D)-ordered superstructures of crystallites.[12] This nonclassical crystallization pathway is often called "polymer-directed crystallization." Mesocrystals, mesoscopically (1–1000 nm) structured crystals, were first synthesized many decades ago but were not recognized because of their identical scattering patterns and behaviors to those of single crystals. With advances in analytical methods, detailed nonclassical crystallization pathways, such as the oriented self-assembly mechanism of small single crystals, were recently elucidated, offering new opportunities for materials design.

The nonclassical crystallization pathway has been employed to generate inorganic materials with controlled morphology and self-assembled composite superstructures. According to the widely accepted mechanisms, a peptide or polymer interacts with crystallizing molecules in solution, and this interaction results in the nucleation of structured composite particles of polymers and crystallizing molecules, followed by the formation of their self-assembled structures.

Biomimetic mineralization produced via polymer-directed crystallization has been intensively investigated in the field of morphosynthesis,[13] through which living organisms secrete inorganic materials in the forms of skeletons, shells, and teeth that have remarkable properties.[14] Gower and Tirrell[14] first reported the fine droplet formation of a polymer-induced liquid precursor phase for $CaCO_3$/polypeptide systems. $CaCO_3$ has been the most common material

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Journal of Pharmaceutical Sciences, Vol. 101, 2941–2951 (2012)

for this type of research, and only recently organic materials, such as D,L-glutamic acid, have been attempted.[8,15,16]

The polymer-directed crystallization approach for pharmaceutically active ingredients has rarely been reported, with only a few similar investigations using heterogeneous nuclei or surfactants.[10,17] During the crystallization of drugs, dissolved polymers can be treated as crystal growth inhibitors, which is important for the resuspendability of solid dispersions.[18] Raghavan et al.[19] used polymers for the crystallization of hydrocortisone acetate and observed delayed nucleation and modified crystal habits. However, it is not clear whether composite particles of polymers and drugs have been produced. The mesocrystal formation mechanism in bio-inspired mineralization has been utilized for the formation of nanosized ibuprofen platelets with improved dissolution, although a polymeric material was not used.[17] In that study, Lee and Zhang[17] used a small molecular surfactant, sodium dodecyl sulfate (not a polymer), to engineer ibuprofen crystals via a distinctly different method from the common crystallization approaches.

In this study, we systematically investigated the usefulness of the polymer-directed crystallization technique for drugs. Using atorvastatin calcium (ATC) as a model drug, various different polymers were used to modify the nucleation and growth pathway of drowning-out crystallization. The crystal habits and structures of the resulting crystals were examined with respect to *in vitro* release and drug stability.

Atorvastatin calcium, which acts as a synthetic lipid-lowering agent by inhibiting an early rate-limiting step in cholesterol biosynthesis, is a selective inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase.[20] ATC is a white crystalline powder that is slightly soluble in distilled water and pH 7 phosphate buffer.[21] The absolute bioavailability of ATC is approximately 12% after a 40 mg oral dose.[20] Therefore, improving the bioavailability of ATC, while retaining its stability using particle size and crystal engineering, is critical in the development of ATC formulations.[6,21–23]

MATERIALS AND METHODS

Materials

Atorvastatin calcium (Generic Lipitor) was kindly provided by Amorepacific (Seoul, Republic of Korea) and was used without purification. Polyethylene glycol (PEG; Mw = 2000 g/mol), hydroxypropyl cellulose (HPC; Mw = 80,000 g/mol), polyacrylic acid (PAA; Mw = 1800 g/mol), polyethylene imine (PEI; Mw = 600 g/mol), and polyethylene oxide-polypropylene oxide triblock (PEO–*b*-PPO–*b*- PEO) (PLU; Pluronic® F127) were purchased from Sigma–Aldrich (St. Louis, Missouri). The elastin-like peptide, ELPE [(GVGVP GVGVP G**E**GVP GVGVP GVGVP GVGVP)$_4$(GVGVP)], was purchased from Cosmos Genetech (Seoul, Republic of Korea), and chitosan (CTS; Mw < 3000 g/mol) purchased from Kitto Life (Kyeongki, Republic of Korea) was used. High-performance liquid chromatography (HPLC)-grade water (J.T. Baker, Phillipsburg, New Jersey) was used without purification. Methanol (99.8%, HPLC grade) was purchased from Samchun Pure Chemical (Pyongtack, Gyeonggi, Republic of Korea), and was used as a solvent for drowning-out crystallization.

Crystallization of ATC

Atorvastatin calcium was first dissolved in methanol (5 wt %), and the weight ratio of polymer–ATC was fixed at 1:9. A 96-well plate (polypropylene, 0.3 mL; Bioneer, Seoul, Republic of Korea) was used for drowning-out crystallization using methanol as a solvent and water as a nonsolvent.[4] The volume ratios of ATC solution–water were 1:9, 2:8, 3:7, 5:5, 7:3, 8:2, 9:1, and the final volume of the solvent and nonsolvent mixture was 0.22 mL. A thermal gradient system (MyGenie 96 Gradient Thermal Block; Bioneer) was used to screen the initial crystallization conditions by changing the volume ratio of water–methanol (row) and the temperature (30°C–50°C, column). After mixing with nonsolvent for 1 min (Vortex; Scientific Industries, New York, USA), crystallization occurred at a constant temperature (±0.3°C) for 24 h. Unless otherwise stated, the temperature and the volume ratio of ATC solution–water were 30°C and 1:9, respectively. For detailed sample characterization, 5 mL scale crystallization was also performed according to the same procedure.

Solubility Analysis of ATC

The amount of dissolved ATC was traced using ultraviolet (UV)–visible spectroscopy (V-550; JASCO, Hachioji, Japan). The absorption peak at 237 nm was quantified for the concentration values of ATC. After an equilibration time of 24 h at room temperature (RT) (2 mL water with an excess amount of ATC), the transparent supernatant of 0.01 mL was diluted 100 times with methanol and used for UV–visible characterization. ATC particles were excluded by a 0.45 μm filter membrane.

To understand the crystallization mechanism and the effects of the polymer, the concentration of ATC in solution was measured as a function of time with the continuous injection of a nonsolvent. The 50 mL ATC–methanol solution (5 wt %) was first prepared and mixed with a polymer (polymer–ATC = 1:9) by vortexing (Vortex; Scientific Industries) for 3 min and magnetic stirring for 12 h at RT. The nonsolvent was then injected at a constant feed rate, 1.2 mL/h, using