European Journal of Pharmaceutics and Biopharmaceutics 132 (2018) 146–156

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Phase separation of supersaturated solution created from amorphous solid dispersions: Relevance to oral absorption

Kohsaku Kawakami[a,*], Keiko Sato[a], Mayuko Fukushima[a], Aoi Miyazaki[a], Yuko Yamamura[b], Shinji Sakuma[b]

[a] World Premier International Research Center for Materials Nanoarchitectonics (WPI-MANA), National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
[b] Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan

ARTICLE INFO

Keywords:
Amorphous solid dispersion
Supersaturation
Phase separation
Nanoparticle
In vitro-in vivo correlation

ABSTRACT

Dissolution of amorphous solid dispersions (ASDs) is a complicated process, which may involve phase separation from the supersaturated state and formation of a colloidal phase. However, relevance of the phase separation behavior to oral absorption from ASDs is still not well understood. We investigated phase separation of a supersaturated fenofibrate (FEN) solution in the presence of polymers, in vitro dissolution of FEN ASDs, and their in vivo absorption. The supersaturation behavior was assessed based on turbidity measurement in an artificial supersaturation system, where FEN ethanol solutions were added to aqueous polymer solutions. The phase separation concentration of FEN was ca. 1 μg/mL regardless of the presence/absence of the polymer, which was approximately 10-fold the equilibrium solubility. In the presence of 0.1% Tween 80 in the media, the phase separation concentration depended on the polymer species, presumably due to differences in their inhibitory effect of crystallization. The degrees of supersaturation achieved by the ASDs were similar to those found in the artificial system, suggesting that the artificial system works for comprehending the effect of polymer species on supersaturation ability for designing ASDs. A robust in vitro-in vivo correlation was achieved using the paddle and the flow-through cell methods by employing non-sink and pH-shift conditions. However, the phase separation concentration may rather be a good and simple indicator to estimate the absorption-enhancing ability of the polymeric excipients for ASDs, if the absorption is limited by solubility.

1. Introduction

The pharmaceutical industry is required to deal with many poorly soluble candidates for oral delivery. Amorphous solid dispersion (ASD) is one of the most important formulation technologies for poorly soluble drugs [1–3], as it can provide higher solubility and dissolution rates relative to those of crystalline formulations to form supersaturated states. Formulations that exhibit high levels of supersaturation for long periods are thought to be effective in improving the oral absorption of poorly soluble drugs, provided that they have sufficient membrane permeability [4].

However, it is not possible to predict the oral absorption behavior of ASDs using conventional protocols for dissolution testing. In particular, focusing on the initial dissolution rates does not provide insight into the absorption enhancement [3,5,6] because it does not necessarily correlate with the supersaturation ability of ASDs. Rapid increase in the concentration may rather lead to immediate crystallization [7], because degree of the supersaturation is a dominant factor for the crystallization rate [8]. To observe supersaturation behavior, non-sink conditions must be employed [5,9,10]. The presence of the absorption sink can influence supersaturation behavior dramatically since elimination of drug molecules due to transportation to the acceptor phase reduces the degree of supersaturation, resulting in effective maintenance of the supersaturated state [7]. In this regard, Shi et al. combined the vessel for the paddle method with the flow-through cell (FTC), where the organic phase was placed in the dissolution vessel as an absorption reservoir [11]. The drug fraction in the organic phase exhibited greater correlation with the absorbed amount *in vivo* compared to that observed with the conventional paddle apparatus. However, supersaturation behavior of ASDs may be difficult to investigate in this system because transfer of molecules from the aqueous to the organic phase is achieved without membrane resistance at the interface. Also, dissolution of small amount of octanol in the aqueous phase can influence the dissolution behavior [12]. Kataoka et al. also showed correlation with the in vivo results by

* Corresponding author.
E-mail address: kawakami.kohsaku@nims.go.jp (K. Kawakami).

https://doi.org/10.1016/j.ejpb.2018.09.014
Received 15 May 2018; Received in revised form 16 August 2018; Accepted 21 September 2018
Available online 22 September 2018
0939-6411/

utilizing a dissolution system in which the dissolution vessel was attached to the absorption reservoir via a living membrane [13,14]. Hens et al. applied pH-shift and incorporated a permeation bag to the dissolution system to demonstrate that the apparent increase in solubility did not necessarily lead to an increase in the absorbed fraction, which corresponded highly with the in vivo absorption [15]. The TNO system has been recognized as an ultimate prediction system that mimics the morphology of the human gastrointestinal tract [16,17]. These systems may provide advance understanding of the absorption mechanisms of supersaturable formulations. However, since complicated evaluation systems increase operational variables and thereby decrease reproducibility of the results, the simple dissolution test should not lose its demand.

Supersaturation is a thermodynamically unstable state. When degree of the supersaturation is sufficiently high, immediate crystallization to destabilize the supersaturation is expected. However, modest supersaturation leads to liquid-liquid phase separation in the mechanism of spinodal decomposition [3,5,18,19]. The supersaturation created by ASDs may also be separated into two phases at least in the dissolution tests. Size of the dispersed (concentrated) phase is sometimes very small, such as a few tens or hundreds of nanometers, because of stabilization of the interface by polymeric excipients included in the ASDs. Then, (nano)particles are likely to be formed from this concentrated dispersed phase [20,21]. However, it is not known whether this phenomenon can take place in the gastrointestinal tract. If this event does occur in the gastrointestinal tract, the impact of the phase separation on the absorption process must be clarified. If not, it would be necessary to explore an idea to link this in vitro specific phenomenon with the in vivo transmembrane absorption of drug molecules. In the traditional dissolution test, the solute concentration is determined following filtration or ultracentrifugation of the medium. Since the dispersed phase from supersaturated solutions frequently have comparable size with that of the pore size of the filter, the dispersed phase may or may not be removed during the filtration. It has been suggested that the dispersed phase functions as a drug reservoir [22–24]. If it is the case, it is problematic to remove the particles during the evaluation process. Careful investigation should be required for the phase separation behavior during the dissolution tests in order to achieve in vitro-in vivo correlation for the ASDs.

We utilized fenofibrate (FEN) in this study as a model drug for ASDs. After investigating the phase separation behavior of supersaturated FEN solutions in the presence/absence of polymers, non-sink dissolution tests were performed using paddle and FTC methods. The link between these in vitro results and in vivo absorption was discussed to clarify relevance of the phase separation behavior on the oral absorption of ASDs.

2. Materials and methods

2.1. Materials

Fenofibrate (FEN) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Vinylpyrrolidone-vinyl acetate copolymer (Kollidon VA64, PVPVA) and Tween 80 were provided by BASF (Ludwigshafen am Rhein, Germany) and Nacalai Tesque (Kyoto, Japan), respectively. Eudragit (Poly(methacrylic acid-co-methyl methacrylate)) L100-55 (Eudragit) and hydroxypropyl methylcellulose acetate succinate (HG grade) (HPMCAS) were supplied from Evonik (Essen, Germany) and Shin-Etsu Chemical (Tokyo, Japan), respectively. All compounds were used as supplied.

2.2. Preparation and physical characterization of ASDs

To prepare PVPVA ASD, FEN and PVPVA were mixed at a weight ratio of 3–7 using a mortar and pestle, followed by heating at 100 °C for 30 min in a temperature-controlled oven. The HPMCAS ASD was prepared in the same manner, except that the heating was applied for 1 h. No degradation products were found for the heated formulation in the HPLC gradient analysis, for which the procedure was described in our previous paper in detail [25]. Both ASDs were processed again by the mortar and pestle before use.

Eudragit ASD was prepared by freeze-drying. FEN and Eudragit were dissolved in t-butyl alcohol at 50 °C at a concentration of 3 and 12 mg/mL, respectively, followed by freeze-drying on Elyla FDU-2200 (Tokyo Rikakikai, Tokyo, Japan). The sample shelf was initially cooled using ice prior to freeze-drying, and the drying process was applied for 18 h at 25 °C.

X-ray powder diffraction (XRPD) patterns of the ASDs were acquired on a Rigaku RINT Ultima X-ray Diffraction System (Rigaku Denki, Tokyo, Japan) using CuKα radiation. The voltage and the current were 40 kV and 40 mA, respectively. Data were collected at intervals of 0.02° (2 theta) with a scan speed of 2°/min.

Differential scanning calorimetry (DSC) measurements were performed on a DSC Q2000 (TA Instruments, New Castle, DE, USA), which is calibrated bimonthly using indium and sapphire, at a heating rate of 10 °C/min. Dry nitrogen was used as the inert gas at a flow rate of 50 mL/min. Approximately 5 mg of samples were measured using crimped aluminum pans.

2.3. Phase separation of supersaturated FEN solutions (Artificial supersaturation system)

FEN was dissolved in ethanol at a concentration of 2 or 20 mg/mL, and the appropriate amount of ethanol solution was added to 50 mL of 0.5 wt% polymer solution prepared by 50 mM phosphate buffer (PB, pH 7.0) in the absence or presence of 0.1 wt% of Tween 80. Note that FEN concentration in ethanol (i.e. ethanol concentration in the solutions for measurements) did not influence the supersaturation behaviour. Effect of solvent species was also confirmed to be marginal as reported in the literature [19]. The supersaturated solutions were stirred at approximately 200 rpm for 30 min at 25 or 37 °C and subjected to turbidity measurements at 500 nm on a V-630 spectrophotometer (JASCO Corp, Tokyo, Japan). The stirring period was determined by a preliminary study, where the turbidity reached a plateau after 30 min stirring and was maintained for several hours in the absence of Tween 80. In the presence of Tween 80, since the turbidity had strong time-dependency, the value at 120 min was also read for the study at 25 °C. The resultant solution/suspension was diluted with 50% ethanol aqueous solution to measure the FEN concentration by HPLC using a YMC-Pack Pro C18 column (150 mm × 2.0 mmID, YMC, Kyoto, Japan) at a flow rate of 0.2 mL/min. The mobile phase was a mixture of acetonitrile and water at a ratio of 8/2 (v/v). The injection volume and detection wavelength were 2 μL and 285 nm, respectively. Further details on the quantification procedure can be found elsewhere [26]. The particles formed in the study above were examined using an optical microscope (Olympus BX-51, Tokyo, Japan) equipped with a U-POT polarizer and a U-ANT analyzer.

The supersaturated suspensions were also subjected to particle size and zeta potential measurements on a Delsa Nano C particle analyzer (Beckman Coulter, Brea, CA, USA) with a He-Ne laser (632.8 nm, 35 mW) as the light source. In this study, FEN was dissolved in ethanol at a concentration of 40 mg/mL, and 20 μL of the solution was added to 100 mL of 0.5 wt% polymer solution prepared by 50 mM phosphate buffer (PB, pH 7.0) under stirring at approximately 200 rpm. All measurements were repeated three times. Mean particle size was determined by Cumulant analysis.

The fraction of FEN that passes through the syringe filters from the supersaturated suspensions was examined as follows. FEN was dissolved in ethanol at a concentration of 40 mg/mL, and then 20 μL of the FEN solution was added to 100 mL of 0.5 wt% polymer solution prepared by 50 mM phosphate buffer (pH 7.0). The solutions were stirred at approximately 200 rpm at 25 °C. Aliquots of media (approximately