Journal of Controlled Release 256 (2017) 193–202

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Mechanism-based selection of stabilization strategy for amorphous formulations: Insights into crystallization pathways

Khadijah Edueng[a,b], Denny Mahlin[a], Per Larsson[a], Christel A.S. Bergström[a,*]

[a] Department of Pharmacy, Uppsala University, Uppsala Biomedical Centre, P.O Box 580, SE-75123 Uppsala, Sweden
[b] Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Istana, 25200 Bandar Indera Mahkota, Kuantan, Pahang, Malaysia

ARTICLE INFO

Keywords:
Amorphous
Crystallization
Solid-state
Dissolution
Stabilization
Polymer
Supersaturation

ABSTRACT

We developed a step-by-step experimental protocol using differential scanning calorimetry (DSC), dynamic vapour sorption (DVS), polarized light microscopy (PLM) and a small-scale dissolution apparatus (μDISS Profiler) to investigate the mechanism (solid-to-solid or solution-mediated) by which crystallization of amorphous drugs occurs upon dissolution. This protocol then guided how to stabilize the amorphous formulation. Indapamide, metolazone, glibenclamide and glipizide were selected as model drugs and HPMC (Pharmacoat 606) and PVP (K30) as stabilizing polymers. Spray-dried amorphous indapamide, metolazone and glibenclamide crystallized via solution-mediated nucleation while glipizide suffered from solid-to-solid crystallization. The addition of 0.001%–0.01% (w/v) HPMC into the dissolution medium successfully prevented the crystallization of supersaturated solutions of indapamide and metolazone whereas it only reduced the crystallization rate for glibenclamide. Amorphous solid dispersion (ASD) formulation of glipizide and PVP K30, at a ratio of 50:50% (w/w) reduced but did not completely eliminate the solid-to-solid crystallization of glipizide even though the overall dissolution rate was enhanced both in the absence and presence of HPMC. Raman spectroscopy indicated the formation of a glipizide polymorph in the dissolution medium with higher solubility than the stable polymorph. As a complementary technique, molecular dynamics (MD) simulations of indapamide and glibenclamide with HPMC was performed. It was revealed that hydrogen bonding patterns of the two drugs with HPMC differed significantly, suggesting that hydrogen bonding may play a role in the greater stabilizing effect on supersaturation of indapamide, compared to glibenclamide.

1. Introduction

Limited aqueous solubility is one of the major factors associated with poor oral bioavailability and erratic effects *in vivo* [1]. A formulation route that has received much attention is the production of poorly soluble drugs in their amorphous form, mainly by formulating them as amorphous solid dispersion (ASD) with one or more excipient (s). An increasing number of studies focus on understanding the formulation systems with regard to physicochemical properties of the components, i.e., the active pharmaceutical ingredients, (APIs) and excipients; molecular interactions between these components; and processes during the dissolution *in vitro* and *in vivo* [1–4]. Better knowledge on formulations has set a strong platform for ASD to be regarded as a viable strategy for overcoming solubility problems. This was proven by an increasing number of pharmaceutical products based on ASD technologies that have gained approval from the FDA within the past years [5].

However, amorphous systems are associated with instability problems. Although the instability is thermodynamically driven by the free-energy difference between the amorphous and crystalline states, the propensity for transformation from the former to the latter is strongly linked to kinetic factors such as nucleation probability and molecular mobility in the amorphous state. The transformation leads to the loss of the solubility advantage conferred by amorphization [6]. Crystallization of amorphous system can occur in the solid-state (e.g. during processing, handling and storage) and during dissolution. Upon dissolution, the crystallization of an amorphous system occurs through either solid-to-solid or solution-mediated transformation [6,7]. In solid-to-solid transformation, the increase in temperature or water sorption increases the molecular mobility of the amorphous system which may lead to an increase in the crystallization rate [6]. In contrast solution-mediated crystallization requires a supersaturated solution [6–9]. Both solid-to-solid and solution-mediated transformations start with nucleation in which stable nuclei are formed, followed by crystal growth

* Corresponding author at: Department of Pharmacy, Uppsala University, Box 580, SE-751 23 Uppsala, Sweden.
E-mail addresses: khadijah.edueng@farmaci.uu.se (K. Edueng), denny.mahlin@farmaci.uu.se (D. Mahlin), per.larsson@farmaci.uu.se (P. Larsson), christel.bergstrom@farmaci.uu.se (C.A.S. Bergström).

http://dx.doi.org/10.1016/j.jconrel.2017.04.015
Received 17 January 2017; Received in revised form 9 April 2017; Accepted 10 April 2017
Available online 12 April 2017

[6–8]. The formation of stable nuclei is the rate-limiting step, as it requires overcoming a nucleation barrier, but subsequent crystal growth can be rapid, and is accelerated by increasing temperature [8].

Theoretically, the driving force for nucleation depends on the free concentration of the drug available in the medium and the level of supersaturation controls the rate of crystal growth. The higher the free concentration of drug, the greater the probability for the nucleation to take place [10]. Crystallization by either mechanism (solid-to-solid and solution-mediated transformation) may be reduced, or even inhibited, by the addition of excipients (usually polymers).

The crystallization behaviour of amorphous formulations in the solid and dissolved states can be studied by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), dynamic vapour sorption (DVS), polarized light microscopy (PLM), powder x-ray diffraction (XRPD), Raman spectroscopy, and dissolution assays [7,8,11–13]. Few years ago, Alonzo and co-workers introduced the use of different experimental techniques including XRPD, microscopy, Raman spectroscopy and small-scale dissolution apparatus to study the behaviour of amorphous systems during dissolution [7] and the effect of polymers on their dissolution and precipitation behaviour [14]. In a more recent study, Mah *et al.* reported the superiority of DSC compared to Raman spectroscopy in predicting the physical stability and dissolution performance of milled amorphous glibenclamide [15]. Despite the extensive use of these techniques, they have not been combined as a standardized experimental protocol especially to predict crystallization during dissolution and guide the design of amorphous formulations. The addition and selection of polymer(s) to stabilize amorphous formulations is often based on trial-and-error experiments, usually without any sound scientific rationales [16–18]. Typically, the performance of amorphous formulations is evaluated by analyzing the dissolution profile in combination with investigations of the physical stability. The dissolution profile i.e., dissolution rate, degree of supersaturation and area under the dissolution curve, are affected by recrystallization. Thus, dissolution can be compromised if the amorphous drug recrystallizes. In this study, we developed a standardized experimental method to reveal the crystallization mechanisms that impact the dissolution profile of a particular amorphous drug. A combination of small-scale solid characterization methods and an *in vitro* dissolution assay under non-sink condition were used to differentiate between solid-to-solid and solution-mediated crystallization occurring during dissolution. The molecular interactions between the drug and polymer were further studied by molecular dynamic (MD) simulations to understand the specific mechanisms by which the polymer inhibits crystallization of supersaturated system.

2. Materials and methods

2.1. Materials

Glibenclamide, glipizide, PVP K30, sodium hydroxide (purity ≥ 98%), sodium phosphate (purity ≥ 99%), sodium chloride (purity ≥ 99%) and dimethyl sulfoxide (purity ≥ 99.9%) were purchased from Sigma-Aldrich (Germany). Indapamide was purchased from Tokyo Chemical Industry Co. Ltd., (Tokyo, Japan) and metolazone from APIChem (China). Hydroxypropylmethyl cellulose (HPMC) grade 606 was obtained from Shin Etsu (Tokyo, Japan). Acetone (purity ≥ 99.8%) was obtained from Merck (Germany) and ethanol (purity ≥ 99.7%) from Solveco (Sweden). Phosphorus pentoxide was purchased from VWR Chemicals (Leuven, Belgium). The chemical structures of the four model drugs are depicted in Fig. 1 and their physicochemical properties summarized in Table 1.

2.2. Methods

In this study, we employed different experimental techniques in a step-wise approach as shown in Fig. 2. The detailed protocol of each technique is explained below.

2.2.1. Preparation of amorphous drug and amorphous solid dispersion

The crystalline drugs were transformed to their amorphous form by spray drying using a Büchi Mini Spray Dryer B-290 (Switzerland). The spray-drying parameters used throughout the study were: inlet temperature (55 °C), aspiration rate (75%) and pump rate (4 mL/min). The solutions of pure drug for spray drying were prepared by dissolving the drug powder in 10:90% (w/w) acetone and ethanol. The amount of drug powder was kept at ≤ 75% of its solubility to diminish the risk of any non-dissolved, crystalline drug in the solvent mixture. The amorphous solid dispersion (ASD) solution for spray drying was prepared by dissolving the drug powder and PVP (K30) in the same solvent mixture of acetone and ethanol. The final weight ratio of the dissolved compounds was 50:50 (drug/PVP). The spray dried (SD) neat drugs and ASD were stored in a vacuumed desiccator containing phosphorus pentoxide until further analyses. The amorphous nature of spray dried SD drugs and ASD was confirmed by DSC and PLM immediately after spray drying and before proceeding with experiments and analyses for stored materials.

2.2.2. Solid state characterization

2.2.2.1. DSC. A DSC Q2000 Differential Scanning Calorimeter (TA Instrument Co., USA) was used to analyse the thermal behaviour of the unprocessed crystalline drugs, SD neat drugs, and ASD, before and after humidity exposure. Depending on the sensitivity required for the analyses, either standard DSC or modulated DSC was used. The DSC cell was calibrated with indium (melting temperature, T_m = 156.59 °C and heat of fusion, H_f = 28.57 J/g) and purged with 50 mL/min of nitrogen. Detailed protocols for the DSC and modulated DSC (MDSC) are provided below.

2.2.2.2. Conventional DSC. For unprocessed crystalline as well as for SD drugs, 1–5 mg of sample was weighed into an aluminium pan that was sealed with an aluminium lid containing pin holes. An initial heat-cool-heat cycle was used for SD samples (both neat drug and ASD) to remove residual solvent: the sample was first equilibrated at 0 °C, heated at 10 °C/min to 110 °C and held for 5 min, then cooled to 0 °C at 10 °C/min. For thermal analysis, the sample was then equilibrated at 0 °C, and thereafter heated at 10 °C/min to 30–50 °C above its melting point. From the resulting thermograms, the melting temperature (T_m) was determined for the unprocessed crystalline sample. The glass transition temperature (T_g), crystallization temperature (T_c) and melting temperature (T_m) were determined for SD neat amorphous drug and ASD samples. Onset values are reported.

2.2.2.3. MDSC. To determine some of the T_g values and to separate overlapping thermal events, increased sensitivity was needed and MDSC was therefore used. The sample was equilibrated at 0 °C, modulated at ± 0.5 °C every 60 s, and heated at 1 °C/min to a temperature 30–50 °C above its melting point. T_g was determined from the reversible heat flow signal.

2.2.2.4. Polarized light microscopy (PLM). Images of SD neat drugs and ASD were collected using an Olympus BX51 microscope (Tokyo, Japan) at three time points: (i) immediately after obtaining the spray dried material; (ii) 24 h post exposure in the DVS chamber (98% RH, see Section 2.2.3); and (iii) 4 h post dissolution at 37 °C (see Section 2.2.4). Samples were dispersed in olive oil for better image quality and clarity, except for the post dissolution samples. These were already dispersed in the dissolution media and therefore analyzed directly.

2.2.3. Exposure to high humidity

The solid-to-solid crystallization of the SD neat amorphous drugs and ASDs were investigated by DVS (DVS Advantage, Surface Measurement System Ltd., UK). Approximately 1.5–2.0 mg of samples