Effect of Mannitol on Nucleation and Crystal Growth of Amorphous Flavonoids: Implications on the Formation of Nanocrystalline Solid Dispersion

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Received 24 April 2015; revised 16 June 2015; accepted 26 June 2015

Published online 16 July 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24586

ABSTRACT: In this work, we studied crystallization kinetics of amorphous hesperetin (HRN) and naringenin (NRN) alone, and in 1:1 proportion with mannitol at T_g + 15 K. Crystallization rate of NRN was found to be significantly higher than HRN. Mannitol accelerated crystallization of HRN as well as NRN. NRN exhibited higher crystallization rate than HRN, in presence of mannitol, as well. Finke-Watzky model was used to deconvolute the crystallization kinetics data into nucleation and crystal growth rate constant. HRN alone had 9.56×10^9 times faster nucleation rate and 1.88 times slower crystal growth than NRN alone. Mannitol increased nucleation and crystal growth rate of HRN as well as NRN. In presence of mannitol, HRN possessed 1.34×10^{10} times faster nucleation rate and 1.70 times slower crystal growth rate than NRN. Differences in crystallization behavior of HRN and NRN were explained by their thermodynamic properties.
 J Pharm Sci 104:3789–3797, 2015
Keywords: amorphous; glass transition; crystallization; solid dispersion; thermodynamics; Avrami modelling; Finke-Watzky model

INTRODUCTION

In the recent years, an increasing number of compounds, with poor oral bioavailability, are appearing in the drug development process. Oral bioavailability may be limited because of the poor aqueous solubility and/or dissolution kinetics. Various strategies have been adopted to enhance the oral bioavailability of these compounds.[1–3] In recent years, nanocrystalline drug delivery systems have been extensively used for this purpose. Drug nanocrystals predominantly provide improvement in dissolution kinetics, whereas apparent solubility is affected only marginally.[4] Drug nanocrystals are usually generated by top-down and bottom-up methods. The top-down method involves high-pressure homogenization[5,6] and media/pearl milling[7] whereas bottom-up method includes nanoprecipitation.[8,9]

Recently, our laboratory has developed a novel, spray drying based, bottom-up technology to generate nanocrystalline solid dispersions (NSDs) of poorly water soluble drugs using small molecule excipients. Latter induce crystallization in the drug phase and include GRAS listed excipients like mannitol, stearic acid, cetostearyl alcohol, and sorbitol. Spray drying process conditions were optimized to generate drug nanocrystals. Briefly, a solution of drug and excipient in common solvent(s) was spray dried to obtain discrete particles of 2–50 μm size. Each particle consisted of drug nanocrystals in the range of 10–1000 nm, embedded in the matrix of excipient.[10] Generation of drug nanocrystals in the matrix of excipient is expected to be influenced by inherent crystallization behavior of the drug from its amorphous state and the influence of excipient on this phenomenon. Preliminary observations in our laboratory had indicated formation of intermediate amorphous state, before formation of nanocrystals.[11]

The aim of the present work was to study the crystallization kinetics of amorphous hesperetin (HRN) and naringenin (NRN), alone and in presence of crystallization-inducing excipient. HRN and NRN were selected as model compounds because of similarity in their chemical structures (Fig. 1). Mannitol was selected as a model crystallization-inducing excipient. Crystallization kinetics of HRN and NRN at their respective T_g + 15 K value were studied alone and in presence of mannitol with 1:1 ratio. The crystallization kinetics data were deconvoluted to determine nucleation and crystal growth rates of as such drugs and in presence of mannitol. Thermodynamic properties of HRN and NRN were studied to explain inherent differences in the crystallization behavior of HRN and NRN, from their amorphous state.

MATERIALS AND METHODS

Materials

Hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone) 98% and NRN (4′,5,7-trihydroxyflavanone) 98% were purchased from Sigma–Aldrich (Gillingham, Dorset, UK). β-(D) mannitol was supplied by Lobachemie Pvt. Ltd. (Mumbai, Maharashtra, India). All other materials and solvents were of analytical grade.

Powder X-Ray Diffraction

Powder X-ray diffraction (PXRD) patterns of samples were recorded at room temperature on Bruker's D8 Advance diffractometer (Karlsruhe, West Germany) Cu Kα radiation (1.54 A), at 40 kV, 40 mA passing through nickel filter with divergence slit (0.5°), antiscattering slit (0.5°), and receiving slit (1 mm). The diffractometer was equipped with a 2θ compensating slit,

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This article contains supplementary material available from the authors upon request or via the Internet at http://wileylibrary.com.

Journal of Pharmaceutical Sciences, Vol. 104, 3789–3797 (2015)

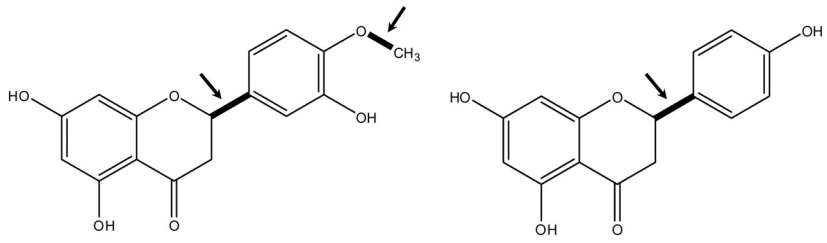

Figure 1. Chemical structures of HRN and NRN. Rotatable bonds are indicated by arrows [determined with a web-based molecular descriptor calculator (http://www.molinspiration.com) using chemical structure inputs].

and was calibrated for accuracy of peak positions and peak broadening with corundum. Samples were subjected to PXRD analysis in continuous reflection mode with a step size of 0.01° and step time of 1 s over an angular range of 3°–40° 2θ. Three hundred milligrams powder mixture was loaded in a 25 mm holder made of poly methyl methacrylate and pressed by a clean glass slide to ensure co-planarity of the powder surface with the surface of the holder. Obtained diffractograms were analyzed with DIFFRAC^{plus} EVA (version 9.0) diffraction software.

Differential Scanning Calorimetry

All differential scanning calorimetry (DSC) experiments were conducted on DSC Q2000 (TA Instruments, New Castle, Delaware, USA) equipped with a refrigerated cooling system and operating with Universal Analysis 2000 software version 4.5 A. The sample cell was purged with dry nitrogen at a flow rate of 50 mL/min. The DSC instrument was calibrated for temperature and heat flow using high purity Indium and Zinc standard. Additionally, specific heat calibration was carried out using Sapphire. All the measurements were performed in triplicates. Glass transition temperature (T_g) and melting temperature (T_m) have been reported as onset values, whereas crystallization temperature (T_c) has been reported as midpoint.

Generation of NSDs

Nanocrystalline solid dispersions of HRN–mannitol and NRN–mannitol system were prepared using laboratory scale spray dryer (LU228 Model; Labultima Ltd., Mumbai, India). Details of spray drying technology can found in literature[12,13] and details regarding Labultima U228 spray drying model can be found on the homepage (http://www.labultima.com/lu228.htm). Mixture of drug (HRN or NRN) and mannitol in 1:1 proportion was dissolved in methanol and water (7:3), to prepare a final composition of 2.0% (w/v) of drug and mannitol in a solvent mixture. The solution was spray dried at an inlet temperatures of 333, 343, 353, and 363 K, feed rate of 3 mL/min, air atomization pressure of 0.95 kg/cm^2 and vacuum of 100 mm of water column. The outlet temperature for all experiments was around 333 K. The spray dryer used in our studies allows control of inlet temperature only. The outlet temperature apart from being dependent on inlet temperature, is also affected by spray rate, solid content, and evaporative cooling. As the powder travels from drying chamber to cyclone (collector pot), where outlet temperature probe is located; it can be concluded that, drying took place at a temperature higher than the T_g of drug–mannitol quench cooled sample. NSDs of HRN–mannitol and NRN–mannitol system were labeled as HRN-M and NRN-M, respectively.

Preparation of Amorphous Form

Crystalline drug samples (7–10 mg) were heated in DSC pans up to 523 and 543 K for HRN and NRN, respectively, for melting the samples, at a heating rate of 20 K/min. The samples were held isothermally at these temperatures for 1 min and cooled back immediately to 298 K at a cooling rate of 20 K/min.

Further, physical mixtures of HRN–mannitol and NRN–mannitol were prepared in 1:1 proportion by geometric mixing. These physical mixtures were quench cooled using procedure mentioned above for HRN and NRN. The quench cooled samples were labeled as HRN-M-QC and NRN-M-QC, respectively. HPLC assay established that no degradation occurred during the preparation of amorphous forms.

Isothermal Crystallization Kinetics

Amorphous samples prepared by *in situ* quench cooling were annealed at T_g + 15 K (363 and 373 K for HRN and NRN, respectively) for 7 h. Samples were analyzed in DSC at specific time points with heating rate of 20 K/min for measurement of heat capacity shift (ΔC_p) in the T_g region. T_g and associated enthalpy relaxation make true estimation of C_p difficult. Modulated DSC (MDSC) could provide a viable alternative by allowing deconvolution of T_g and enthalpy relaxation. However, MDSC could contribute an error by providing a higher temporal stay, because of the slow heating rate employed. Thus, we chose conventional DSC over MDSC as it suited the objective of the study. All the experiments were performed in triplicate in a dehumidifier equipped room at humidity below 15 ± 5% RH. DSC experiments for crystallization kinetics analysis were also performed for HRN-M-QC and NRN-M-QC. Samples were annealed at T_g + 15 K of 347 and 355 K, for HRN-M-QC and NRN-M-QC, respectively. A temperature of T_g + 15 K for crystallization kinetics study was selected by considering T_g as a reference point where relaxation time is 100 s.

Heat Capacity (C_p) Measurement

Differential scanning calorimetry instrument was calibrated for T_{zero} heat flow, cell constant, and baseline by Sapphire disc and Indium standards. C_p calibration was performed by Sapphire in a DSC pan for the temperature range and experimental conditions to be used for MDSC analysis of samples as mentioned below. Amorphous and crystalline samples of drugs were analyzed by MDSC in a crimped DSC pan. The parameters for MDSC were as follows- heating rate: 1 K/min, modulation amplitude: ±0.159 K and modulation period: 60 s. The samples were scanned from 323 to 373 K considering the T_g range for HRN and NRN. Amorphous samples (7–10 mg) were