International Journal of Pharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Differential effect of buffering agents on the crystallization of gemcitabine hydrochloride in frozen solutions

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ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 6 May 2014
Accepted 8 May 2014
Available online xxx

PubChem classifications:
Gemcitabine hydrochloride (PubChem CID: 60749)
Citric acid (PubChem CID: 311)
Malic acid (PubChem CID: 525)
Succinic acid (PubChem CID: 1110)
Tartaric acid (PubChem CID: 875)

Keywords:
Crystallization tendency
Unfrozen water
Calorimetry (DSC)
Low temperature XRD
Supersaturation
Lyophilization

ABSTRACT

The purpose of this study was to evaluate the differential effect of buffering agents on the crystallization of gemcitabine hydrochloride (GHCl) in frozen solutions. Four buffering agents, viz. citric acid (CA), malic acid (MA), succinic acid (SA) and tartaric acid (TA) were selected and their effect on GHCl crystallization was monitored using standard DSC and low temperature XRD. Onset of GHCl crystallization during heating run in DSC was measured to compare the differential effect of buffering agents. Glass transition temperature (T_g'), unfrozen water content in the freeze concentrate and crystallization propensity of the buffering agents was also determined for mechanistic understanding of the underlying effects. CA and MA inhibited while SA facilitated crystallization of GHCl even at 25 mM concentration. Increasing the concentration enhanced their effect. However, TA inhibited GHCl crystallization at concentrations <100 mM and facilitated it at concentrations ≥ 100 mM. Lyophilization of GHCl with either SA or TA yielded elegant cakes, while CA and MA caused collapse. T_g' failed to explain the inhibitory effects of CA, MA and TA as all buffering agents lowered the T_g' of the system. Differential effect of buffering agents on GHCl crystallization could be explained by consideration of two opposing factors: (i) their own crystallization tendency and (ii) unfrozen water content in the freeze concentrate. In conclusion, it was established that API crystallization in frozen solution is affected by the type and concentration of the buffering agents.

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1. Introduction

Lyophilization (also known as freeze drying) is a common unit operation utilized for drying of thermolabile pharmaceuticals including small molecules and peptides (Nail et al., 2002; Wang, 2000). It involves three steps – (i) cooling of the aqueous solution to well below the freezing point of water (freezing), (ii) removal of ice by sublimation under reduced pressure (primary drying), and (iii) removal of sorbed water (secondary drying) at elevated temperatures (usually around 40 °C) (Tang and Pikal, 2004). In most lyophilized formulations, excipients are incorporated to enhance the performance and stability of the lyophilized product. Various excipients utilized for lyophilization of small molecules include bulking agents, solubilizing agents, collapse temperature modifiers and buffering agents (Baheti et al., 2010). Buffering agents are primarily used to control pH for improving the stability during processing, storage and reconstitution. Usually, buffer capacity and buffer catalysis are considered for selection of buffering agents in pharmaceutical preparations (Flynn, 1979). Additionally, for lyophilized products, buffer specific parameters like volatility, crystallization during freezing and impact on critical process temperature are also to be considered, as they have serious implications on processing and quality of lyophilized products (Shalaev, 2005; Shalaev and Gatlin, 2010).

Crystallization of active pharmaceutical ingredients (APIs) during freezing is advantageous as it increases the critical process temperature, thereby facilitating faster primary drying (Korey and Schwartz, 1989; Pyne and Suryanarayanan, 2003; Rodríguez-hornedo and Murphy, 1999; Schwegman et al., 2005; Sundaramurthi et al., 2012; Tang and Pikal, 2004; Telang and Suryanarayanan, 2005). Additionally, crystallization of API during freezing usually yields a crystalline product after drying, thereby improving product shelf life. However, presence of excipients can alter the crystallization behavior of API and this has important

Abbreviations: API, active pharmaceutical ingredient; CA, citric acid; DSC, differential scanning calorimetry; GHCl, gemcitabine hydrochloride; MA, malic acid; RT, room temperature; SA, succinic acid; TA, tartaric acid; XRD, X-ray diffractometry.

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http://dx.doi.org/10.1016/j.ijpharm.2014.05.008
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Please cite this article in press as: Patel, M., et al., Differential effect of buffering agents on the crystallization of gemcitabine hydrochloride in frozen solutions, Int J Pharmaceut (2014), http://dx.doi.org/10.1016/j.ijpharm.2014.05.008

implications on products primarily consisting of lyophilized API (Pyne and Suryanarayanan, 2003; Sundaramurthi and Suryanarayanan, 2010; Sundaramurthi and Suryanarayanan, 2011a; Telang and Suryanarayanan, 2005). We hypothesized that addition of buffering agents can affect the crystallization of APIs during freezing. We focused our work on assessment of the effect of carboxylic acid buffering agents on the crystallization behavior of a small molecule API, gemcitabine hydrochloride (GHCl) in frozen solutions. Studies dealing with the effect of buffering agents on critical temperature, stability and excipient crystallization have been published (Cavatur et al., 2002; Izutsu and Aoyagi, 2005; Izutsu et al., 2004; Izutsu et al., 2007; Sundaramurthi and Suryanarayanan, 2010; Telang et al., 2003). However, influence of buffering agents on API crystallization remains largely ignored. Only a couple of studies showing the effect of glycine, a commonly used bulking agent (which can be used as a buffering agent as well) in enhancing API crystallization have also been reported (Pyne and Suryanarayanan, 2003; Telang and Suryanarayanan, 2005). To the best of our knowledge, no such report exists in literature wherein effect of different buffering agents on the crystallization behavior of an API in frozen solution, has been compared.

GHCl was selected for the current investigation as preliminary investigations using sub-ambient differential scanning calorimetry (DSC) revealed that its crystallization kinetics can be conveniently monitored during experimental timeframe. GHCl, an anticancer drug, is available in market as lyophilized powder for injection, under the brand name Gemzar® (Eli Lilly and company, USA) (Grindey and Hertel, 1995). Four carboxylic acids viz. citric acid (CA), malic acid (MA), succinic acid (SA) and tartaric acid (TA) were selected as buffering agents. These buffering agents have been recently explored by several groups for their possible potential for lyophilized formulations (Shalaev et al., 2002; Sundaramurthi and Suryanarayanan, 2011b). Solutions containing GHCl and buffering agents were frozen *in situ* in DSC under controlled conditions and their thermal behavior during subsequent heating run was monitored to assess GHCl crystallization. DSC results were confirmed by conducting selected experiments in low temperature X-ray diffractometry (XRD) and actual lyophilization cycle.

2. Materials and methods

2.1. Materials

GHCl was received as gratis sample (>99.8% purity) from Hetero Labs Limited (India) and was used without further purification. CA and MA were purchased from Merck Specialties Pvt. Ltd. (India). SA, TA and HPLC grade water were purchased from Fluka Analytical (Austria), Himedia Laboratories Pvt. Ltd. (India) and Fisher Scientific India Pvt. Ltd. (India), respectively.

2.2. Differential scanning calorimetry (DSC)

DSC (model Q2000; TA Instruments, USA; data analysis with Universal Analysis®, version 4.5A) equipped with a refrigerated cooling accessory, RCS90, was used. Dry nitrogen, at 50 mL/min, was used as the purge gas. About 15 mg of the solution was weighed in T_{zero} aluminum pan and hermetically sealed. The solutions were cooled to −80 °C at 17 °C/min, held for 15 min, and finally heated to room temperature (RT) at 1 °C/min. All the analyses were conducted in triplicate and mean onset temperatures have been reported throughout the text.

The DSC T_{zero} calibration was performed in heat-cool mode by running two experiments, one without samples or pans (baseline) and the second using sapphire disks provided by the instrument manufacturer (without pans, weight approximately 100 mg). High purity standard of indium was used to calibrate the cell constant and temperature.

Deconvolution of the DSC endotherms was performed with PeakFit® (version 4.12, Systat Software Inc., San Jose, USA), as previously described (Alfonso et al., 2001; Elsabee and Prankerd, 1992a,b).

Freezable water content was determined from the enthalpy of crystallization (ΔH; J/g) of ice during the cooling run, obtained by integrating the area under the crystallization exotherm, as previously described (Kumar et al., 2011a,b).

2.3. X-ray diffraction (XRD)

XRD scan was recorded using Bruker's D8 Advance Diffractometer (Karlsruhe, West Germany) equipped with a 2θ compensating slit, using Cu-Kα radiation (1.54 Å) at 40 kV and 40 mA passing through a nickel filter. The instrument was connected to a heating/cooling stage (Anton Paar TTK 450 temperature stage) with a working temperature range of −190 °C–300 °C, using Paar Physica liquid nitrogen suction equipment to achieve sub-ambient temperatures. Aqueous solutions (100 μL) were placed in the sample holder. XRD patterns were obtained by scanning over an angular range of 13°–33° 2θ with a step size of 0.05° and a dwell time of 0.8 s. The samples were maintained under isothermal conditions at selected temperatures during the XRD runs. Diffractograms were analyzed using DIFFRACplus EVA (version 9.0) diffraction software. The specific details are provided in the text and relevant figure legends.

2.4. Statistical analysis

Tests of significance were carried out by 1-way ANOVA using SigmaStat® for Windows version 2.03 (SPSS Inc.) followed by the Tukey–Kramer multiple comparison test.

2.5. Lyophilization

Solutions containing GHCl (30 mg/mL) and buffering agents at 100 mM were lyophilized to compare the cake characteristics. Solutions, passed through 0.22 μ filters, were filled into 5 mL glass vials (3 mL fill volume), and transferred to a bench top laboratory freeze-dryer (VirTis® Advantage™, SP Scientific, Gardiner, New York). Vials were covered with gray butyl 2-pronged rubber stoppers (Fisher Scientific India Pvt. Ltd., India). Samples were cooled to a shelf temperature of −60 °C and held isothermally for 2 h. Cooling rate was 1 °C/min. Then, vacuum (200 mTorr) was applied and primary drying was conducted in three steps of −40 °C for 4 h, −30 °C for 6 h and −20 °C for 14 h. Secondary drying was conducted at 25 °C for 6 h, with a vacuum of 200 mTorr. Temperature ramp of 0.5 °C/min was employed during the drying stages.

2.6. Moisture content determination

The total moisture content of the lyophilized cakes was determined by Karl-Fischer Titrimetry (Titrino KF 794 Metrohm SA, CH-9100 Herisau, Switzerland,). Approximately 100 mg of the sample was weighed and titrated with Karl Fischer reagent in the presence of methanol. All the samples were analyzed in triplicate.

3. Results

3.1. Solid state characterization

The 'as-received' GHCl was characterized by XRD and DSC. XRD pattern of GHCl showed characteristic peaks at 2θ values of 9.6°,

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