FREEZE-DRIED NANOCRYSTAL DISPERSION OF NOVEL DEUTERATED PYRAZOLOQUINOLINONE LIGAND (DK-I-56-1): PROCESS PARAMETERS AND CRYOPROTECTANT SELECTION THROUGH STABILITY STUDY

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Introduction

Nanocrystal dispersions are considered as the universal formulation strategy for brick dust substances. To overcome the stability issues during storage, nanocrystal dispersions are usually solidified by freeze-drying (lyophilization). For the particle size preservation during this process it is necessary to add cryoprotectants/ lyoprotectants, while to ensure good structure of the cake, bulking agents are often included in formulations, as well [1,2]. However, in nanocrystalline dispersions the combination of these excipients is not much investigated.

Nanocrystals of DK-I-56-1 (7-methoxy-2-(4-methoxy-d3-phenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one), patent protected pyrazoloquinolinone ligand, have been developed recently, and characterized in terms of physicochemical properties and pharmacokinetics after intraperitoneal administration in mice. These formulations were stable for three weeks [3]. Our aim in this study was to improve the stability by freeze-drying, using different ratios of cryoprotectants (sucrose, trehalose) and bulking agent (mannitol).

Methods

Nanocrystal dispersions stabilized by polysorbate 80 and poloxamer 407 were prepared by wet ball milling [3]. After addition of mannitol (M), sucrose (S), or trehalose (T) alone or in combination samples were freeze- dried. Two processes were applied: (1) freezing at -80 °C (3 h), primary drying at -10 °C, 0.340 mbar, secondary drying at 25 °C (24 h) or (2) freezing at -50 °C (3 h), primary drying at -45 °C, 0.2 mbar (21 h), secondary drying at 20 °C (30 h). Samples were stored in crimped vials at 25 °C (lyophilization 1) or 2-8 ºC (lyophilization 2) for three months.

Characterization included: particle size (z-ave, Zetasizer Nano ZS, Malvern Instruments, UK), differential scanning calorimetry (DSC1; Mettler Toledo, Switzerland), powder X-ray diffraction (Rigaku Smartlab X-ray Diffractometer) and polarized light microscopy (PLM, Carl Zeiss ApoTome Imager Z1 microscope Zeiss, Germany).

Redispersibility index (RDI) was calculated as z-ave (before)/z-ave (after) and expressed in percentages.

Results

1. Screening phase

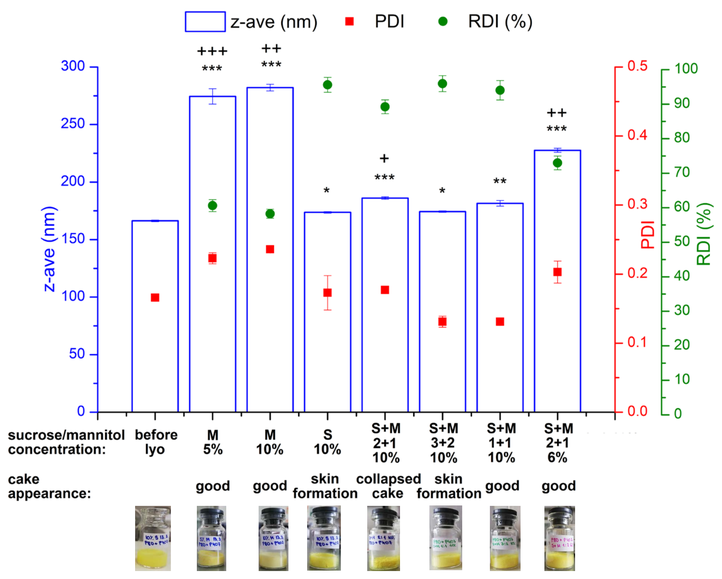
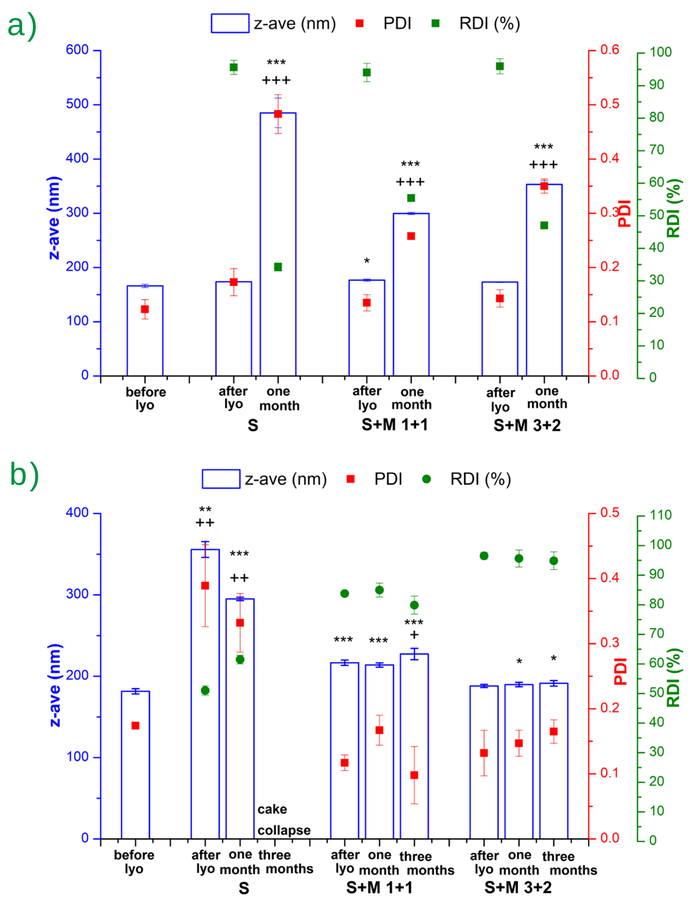


Figure 1: Mean hydrodynamic diameter (z-ave), polydispersity index (PDI) and redispersibiliy index (RDI) after lyophilization with different sucrose/mannitol ratios (z-ave: *, ** and *** for p<0.05, 0.01 and 0.001, respectively, PDI: +, ++ and +++ for p<0.05, 0.01 and 0.001, respectively).

- ✓ 10% of the total stabilizer concentration was needed for the particle size preservation (the achieved RDI was above 95%)
- ✓ Cakes with sucrose alone or in its combination with mannitol in ratio 1:1 or 3:2 were with satisfied appearance

2. Stability study



- ✓ With primary drying at -10 °C aggregation occurred after one month storage
- ✓ With primary drying at -45 °C particle size remained almost unchanged in formulation S+M 3+2 (RDI 95%) or got slightly higher in T+M 3+2 (RDI 90%) after three months storage.

Figure 2: Mean hydrodynamic diameter (z-ave), polydispersity index (PDI) and redispersibiliy index (RDI) right after lyophilization 1 (a) or lyophilizatiorn 2 with sucrose (b) and trehalose (c), and after storage (z-ave: *, ** and *** for p<0.05, 0.01 and 0.001, respectively, PDI: +, ++ and +++ for p<0.05, 0.01 and 0.001, respectively).

3. Physical-state analysis

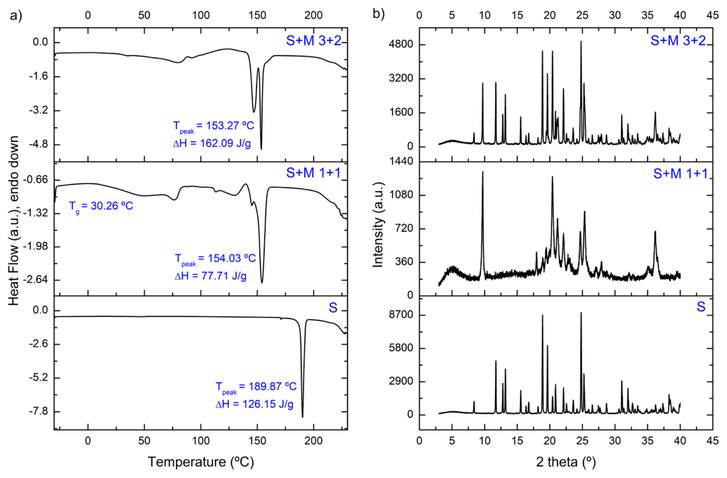


Figure 3: DSC (a) and XRPD (b) analysis of the freeze dried samples with sucrose as cryoprotectant (lyophilization 2).

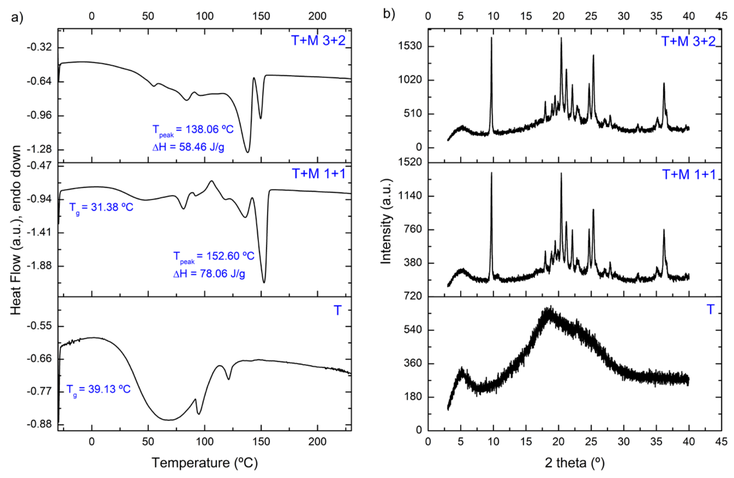


Figure 4: DSC (a) and XRPD (b) analysis of the freeze dried samples with trehalose as cryoprotectant (lyophilization 2).

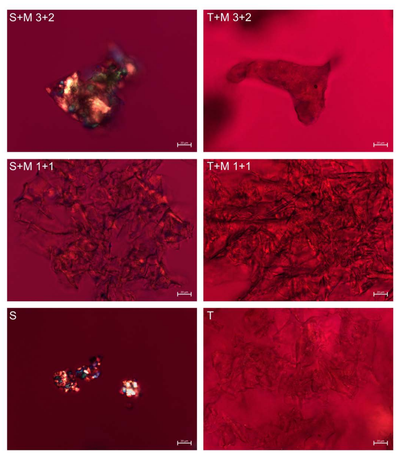


Figure 5: PLM images of the freeze dried samples (lyophilization 2).

- ✓ Sucrose was completely crystalline when used alone, trehalose was amorphus.
- ✓ Mannitol was with low crystallinity, δ polymorphic form.

Conclusion

Freeze-drying is an important technique for the improvement of nanocrystals stability. The selection of cryoprotectant and bulking agent ratio beside process parameters (primary drying at -45 ºC) was crucial to obtain freeze-dried samples with good stability. Sucrose or trehalose in combination with mannitol (ratio 3+2) in total concentration 10% successfully hindered aggregation, thus prolonging the stability to 3 months at 2-8 ºC.

References

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