CRYSTALLINITY OF SUGARS DRIED WITH SUPERCRITICAL CO_2

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Aqueous solutions of various concentrations of sucrose, trehalose and mannitol, commonly used as pharmaceutical excipients, were dried by supercritical fluid drying with a co-current flow of supercritical carbon dioxide enriched with ethanol in a pressurised precipitation vessel, while keeping all other process conditions constant. The dry powders were recovered from a filter after pressure release. The residual water content was measured and the particle morphology and crystallinity were observed. Sucrose and mannitol were readily crystallised, but the trehalose was amorphous. The effect of viscosity and of hydrate formation is discussed.

1. Introduction

Supercritical fluids (SCF) are increasingly considered promising for the production of pharmaceutical formulations in dry powder form. Pharmaceutical formulations are generally composed of a large fraction of sugars or polyols (sugar-alcohols) having the function of stabilisers and/or bulking agents [1]. Up to now, only limited efforts have been put towards the comprehension of the crystallisation behaviour of sugars in SCF processes.

In the present study, sucrose, trehalose and mannitol were compared for their crystallisation behaviour in a SCF spraying process. We selected these compounds because they are commonly used as stabilisers in protein formulations. In freeze-dried formulations, sucrose and trehalose usually are amorphous. Mannitol is generally crystalline and used as bulking agent to form a porous structure to facilitate drying.

Only few studies on the SCF-drying of sugars and polyols have been previously performed. The studies related to the compounds selected here are listed in Table 1. In these studies, both the supercritical carbon dioxide (SC-CO_2) and modifier, when applicable, and the solution were injected simultaneously into a pressurised vessel through a nozzle. Mixing of the liquid and the SCF drying medium occurs then at the nozzle, similarly to the process used in this study, and the solute precipitates. According to these earlier studies, crystalline sucrose and mannitol, and amorphous trehalose powders could be produced. Direct comparison of the crystallisation

characteristics between sugars and polyols was not possible as the process conditions - nozzle, solvent, modifier, pressure, and temperature - were all different (see Table 1).

Table 1 Supercritical spray-drying of sugars and polyols

Proteins Sugars	Solvent Modifier*	Nozzle	Temperature (°C) Pressure (bar)	Morphology Particle size	Reference
Sucrose	H_2O EtOH*	3-way concentric	60°C 250 bar	Crystalline	[2]
Trehalose	H_2O EtOH*	3-way concentric	70°C 270 bar	Spongy spheres Smooth surfaces Amorphous	[2]
Trehalose	H_2O IPA*	Multi- channel	40ºC 100 bar	2.3-2.8 μm	[3]
Mannitol	DMSO+MeOH	2-way concentric	40ºC 180 bar	Long fibrous- like	[4]

Abbreviations used in the table: DMSO, dimethyl sulphoxide; EtOH, ethanol; IPA, isopropyl alcohol; MeOH, methanol

*Modifiers are added to the SC-CO_2 in order to increase the solubility of the solvent in the SCF phase.

While determining the applicability of SC-CO_2 for the production of sugar and polyol powders, the physico-chemical properties of these compounds are expected to influence the processibility, and the properties of the final product. Following are some general crystallisation characteristics of the studied compounds:

Sucrose crystallises easily from aqueous solution and its amorphous preparation is difficult even by lyophilisation [5]. Also, the induction time for the amorphous to crystalline transformation of sucrose can take place within 3 to 4 hours (33% relative humidity, 25ºC), once nucleation has occurred [6].

Trehalose has two generally accepted crystal forms: a dihydrate form [7] stable at room temperature and an anhydrate obtained from the hydrated form [8]. Among others, amorphous trehalose can be produced by freeze drying, spray-drying, and dehydration of trehalose dihydrate [9].

Mannitol has a strong tendency to crystallise, even from its amorphous state [10].

One of the advantages of SCF-drying is the ability to form fine, free-flowing particles. The powders are characterised according to their water content, particle characteristics and crystallinity. These characteristics affect, among others, the stickiness, flow ability and compressibility of the powders. The aim of this study was to investigate the applicability of SCF-drying to produce dry sugar and polyol powders, and to determine which physico-chemical properties of the compounds influence the powder characteristics.

2. Experimental Section

Materials
D-saccharose (Riedel-deHaen), D-trehalose (Sigma), and D-mannitol (Fluka) of European Pharmacopoeia grade were used as received. Technical grade ethanol (100%) was used and carbon dioxide (grade 3.5) was purchased from Hoek Loos, Schiedam, The Netherlands.

Supercritical drying equipment
In the experimental set-up (see Figure 1), the CO_2 was supplied by a diaphragm pump (Lewa) and mixed in a T-mixer with an ethanol flow added from a piston pump (Gilson). This mixture was then directly fed to the atomisation device through the outside ring of a two-fluid coaxial nozzle, together with the solution that was added using a syringe pump (Isco) through the internal tube of the nozzle. The pressure in the vessel was controlled using the exit valve of the vessel. The sugar or polyol powder was collected in a 4-litre vessel and recovered once the pressure was released. Operating conditions are described in the next section.

Drying procedure
After the particle formation vessel was pressurised and stabilised at the desired temperature (37ºC) and pressure (100 bar) with the selected flow rates (10 kg/hr CO_2 and 25 ml/min ethanol), 25 g of pressurised aqueous solution (see Table 2 for composition) was sprayed into the vessel at a rate of 0.5 ml/min. After completion of the spraying process, the vessel was flushed with sufficient CO_2 to remove the residual ethanol before depressurisation and product recovery.

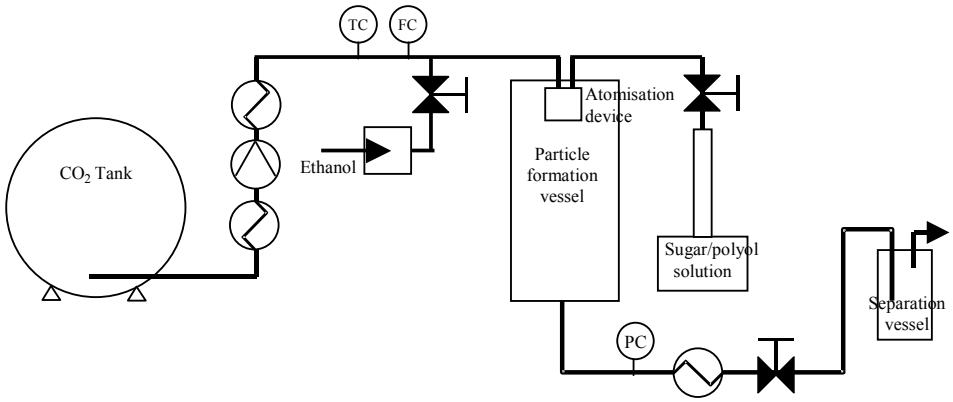


Figure 1 : Basic scheme of the experimental set-up

Scanning electron microscopy
Scanning electron microscopy (Jeol JSM-5400) images were used to examine the morphology of particles. Samples were gold sputtered before scanning.

Residual water content
Karl-Fisher titration (Metrohm 756KF) was performed as per manufacturer instructions.

X-ray powder diffraction (XRPD)
XRPD was performed using a D8Discover X-ray Diffractometer with General Area Detector Diffraction System (Bruker AXS). Diffraction profiles were used to confirm the amorphous or crystalline state of the powders.

Differential scanning calorimetry (DSC)
DSC was performed on a Q-1000 calorimeter, TA instruments. 5-10 mg of dry powder were sealed hermetically in aluminium pans. Samples were heated at a rate of 10°C/min to verify the observation of a glass transition temperature.

3. Results and Discussion

Powder crystallinity and residual water content, and particle size and morphological descriptions are included in Table 2. Additional details and discussion are in the following text.

Table 2 Particle size and morphology description, and powder residual water content

Sugar / Polyol	Solution concentration (w/w)	Crystalline powder	% Residual water content	Morphology	Size (μm)
Sucrose	10%	Y	4.7	Fragments	1-10
	Saturated @ 37ºC	Y	4.0	Spheroid, rough surface	20-60
Trehalose	10%	N	2.4	Sphere, smooth surface	5-40
	Saturated @ 37ºC	N	2.3	Spheroid, smooth surface	1-60
Mannitol	10%	Y	0.5	Needle Sphere, rough surface	1-3W, >50L 5-30
	Saturated @ 37ºC	Y	0.7	Needle Sphere, spherulite	0.25-1W, >50L 10-30

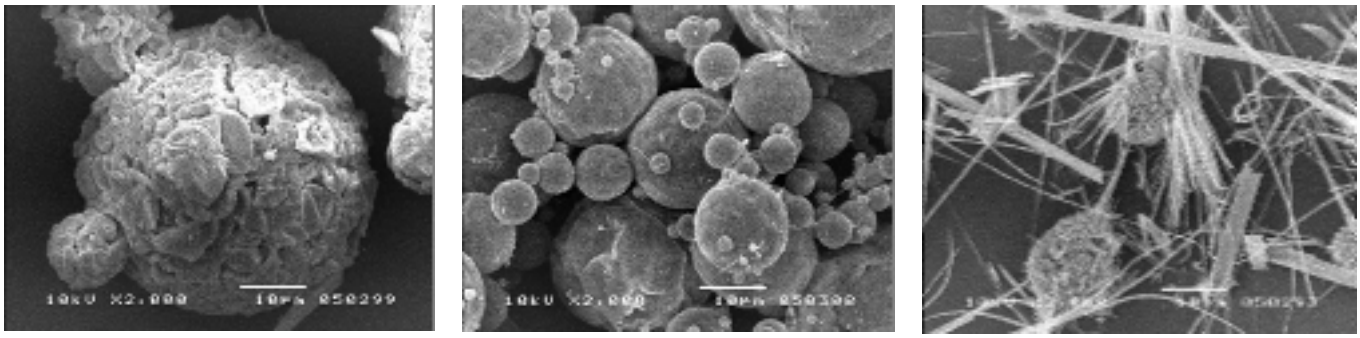
Crystallinity
Trehalose, produced from saturated or 10% concentration solutions, was amorphous. The amorphous character was confirmed by XRPD (no diffraction peaks were observed) and by DSC analysis (a glass transition temperature was measured). In contrast, sucrose and mannitol powders produced from saturated or 10% concentration solutions were shown to be crystalline. These observations of crystalline and amorphous powders were comparable to what was previously obtained by diverse researchers using other process conditions (Table 1).

Residual water content
In general, the residual water content in dry pharmaceutical products should be below 2%. The residual water content (Table 2) of mannitol SCF-dried powders was consistently below 0.7 %.

However, trehalose and sucrose powders had higher residual water contents. The predilection of trehalose to produce a dihydrate structure could be related to the higher water contents obtained for that sugar (2.3-2.4 %). Also, polyol solutions are generally less viscous than sugar solutions at the same concentration [11]. The high viscosity of the sugar solutions could also explain the high residual water content because of the poor diffusion of the water out of the particles. Moreover, the viscosity of a saturated sucrose solution is more than 3 times higher than that of a saturated trehalose solution, which may explain the high (4.0-4.7 %) residual content of sucrose.

Particle size and morphology

The types of particles produced from the different compounds are diverse. Morphological descriptions and sizes of the particles produced are included in Table 2, and SEM pictures are shown in Figure 2.



Sucrose Trehalose Mannitol

Figure 2 SEM pictures of particles produced from saturated solutions

During the spraying process, the viscosity of the sprayed solution has a tremendous effect on the break up of the spraying jet into drops, a high viscosity hampering the process [12]. The high viscosity of the saturated sucrose solution compared to the 10% sucrose solution prevented the break up of the primary droplet of the jet into smaller droplets resulting in larger particles formed from the concentrated solution. This trend was not as clear with trehalose, which has a lower viscosity than sucrose. Compared to trehalose, sucrose droplets have the tendency to remain sticky even at very low residual water content. This explains why sucrose particles were more agglomerated. The particle formation of mannitol seemed not to be affected by the solution viscosity, but by the rate at which supersaturation is achieved and at which nucleation can occur. The three-dimensional organisation of molecules in a crystal produces rough surfaces on the particles, like for sucrose and mannitol.

4. Conclusions

Links between the viscosity and the possibility of hydrate formation of sucrose, trehalose and mannitol, and the type of particles and the crystallinity of the powders produced using SCF as drying medium could be recognised. The residual water content of powders of the hydrate forming trehalose and the highly viscous sucrose was higher than that of the low viscosity, fast

crystallising mannitol. Overall, the crystalline or amorphous forms of the powder produced were in agreement with both the standard crystallisation pattern of these sugars and polyol, and with what has been previously observed by SCF processing. Further research will be required to better understand the individual contributions of each of these and other physico-chemical properties.

In conclusion, SCF technologies have the potential to produce dry sugar and polyol powders, especially for components that allow crystalline morphologies. The addition of crystallisation inhibitors needs to be investigated if amorphous sugar is required for the formulation.

5. References

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