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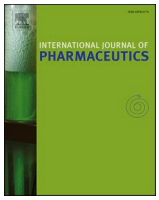

Tableting behavior of freeze and spray-dried excipients in pharmaceutical formulations

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ABSTRACT

Most of biopharmaceuticals, in their liquid form, are prone to instabilities during storage. In order to improve their stability, lyophilization is the most commonly used drying technique in the pharmaceutical industry. In addition, certain applications of biopharmaceutical products can be considered by oral administration and tablets are the most frequent solid pharmaceutical dosage form used for oral route. Thus, the tableting properties of freeze-dried products used as cryo and lyoprotectant could be a key element for future pharmaceutical developments and applications. In this study, we investigated the properties that might play a particular role in the specific compaction behavior of freeze-dried excipients. The tableting properties of freeze-dried trehalose, lactose and mannitol were investigated and compared to other forms of these excipients (spray-dried, commercial crystalline and commercial crystalline milled powders). The obtained results showed a specific behavior in terms of compressibility, tabletability and brittleness for the amorphous powders obtained after freeze-drying. The comparison with the other powders showed that this specific tableting behavior is linked to both the specific texture and the physical state (amorphization) of these freeze-dried powders.

1. Introduction

The pharmaceutical industry is witnessing a growing interest in developing tablets containing biopharmaceutical molecules, including monoclonal antibodies, proteins, and RNA. Since the discovery of insulin in 1921 (Vecchio et al., 2018), therapeutic peptides and proteins have become a major focus of research, leading to significant therapeutic innovations (Haddadzadegan et al., 2022). Traditionally administered through parenteral routes, biomolecules face stability challenges in liquid form, often requiring low-temperature storage. Alternative administration routes like oral or buccal are gaining attention due to advantages such as better patient compliance and improved drug delivery. Tablets, the most common oral dosage form, are preferred despite biomolecules being initially in liquid form. Various drying techniques, such as freeze-drying, are explored to obtain solid forms for tablet production.

In order to stabilize the biomolecules during the drying processes, formulations should contain cryo and lyoprotectants like sucrose and trehalose (non-reducing sugars) or lactose (a reducing sugar); surfactants like polysorbate 20 and polysorbate 80; bulking agents like mannitol (a polyol) or glycine (an amino acid) and buffer salts to adjust the pH and the tonicity (Bjelošević et al., 2020). Currently, tableting properties of such freeze-dried (FD) excipients have not been extensively described in the literature. Since development of oral route for biopharmaceutics could be the goal of many studies, it is important to investigate the behavior of these products, during and after compaction.

The mechanical properties of a powder can be changed depending on many factors. For example, Persson et al. have demonstrated that the compaction end point parameters, the compactibility and tabletability rate parameters increased with the decrease in particle size of α-lactose monohydrate (Persson et al., 2022). Tomar et al. have found that moisture content may affects the physical and chemical properties of the product during compaction, and their results showed that in order to obtain satisfactory hardness for tablets, temperature and humidity must be maintained in a specific limit (Tomar et al., 2017). Since FD excipients have a particular particle size and shape, a specific residual moisture and could be amorphous or crystalline, it is important to consider those aspects in a study on their tableting and mechanical properties.

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Hsein et al. investigated the physico-chemical and mechanical tableting properties of freeze-dried trehalose and showed that the latter presents very specific properties in terms of compressibility and tabletability in comparison with crystalline trehalose (Hsein et al., 2023). Compressibility profiles highlighted that FD trehalose is less compressible than crystalline trehalose at low pressures, and more compressible when pressure increases. On tablettabilty profiles of FD trehalose, it was observed that tablets with high tensile strength (1.5 MPa) could be obtained under very low pressures (25 MPa). The authors also demonstrated that FD trehalose has a high propensity to brittle fracture and that its brittleness was among the highest when compared to classical pharmaceutical excipients found in the literature (Hiestand et al., 1977; Hsein et al., 2023; Meynard et al., 2022). Therefore, our study aims to identify the critical factors influencing these behaviors. Following the changes observed in physical structure and texture of FD trehalose compared to native crystalline trehalose powder, we conducted experiments with other FD and crystalline excipients, including lactose (amorphous after freeze-drying) and mannitol (crystalline even after freeze-drying). By comparing these excipients, we sought to understand the impact of physical structure on the tableting properties of FD powders. Additionally, spray-drying was performed on all three excipients to create amorphous trehalose and lactose, as well as crystalline mannitol, with distinct spherical particle textures. These differences in texture aimed to elucidate the effect of powder texture on tableting properties while keeping the physical structure constant. Lastly, we ground the crystalline powders to assess the influence of texture on tableting properties.

2. Material and methods

Three excipients were used for freeze-drying, spray-drying, milling and for direct compression. Trehalose dihydrate (Biohale) and lactose (Supertab 30 Gr) were obtained from DFE Pharma (Goch, Germany), mannitol from Cooper (Melun, France) and magnesium stearate (Ligamed MF-2-V) from Peter Greven (Bad Münstereifel, Nordrhein-Westafalen, Germany).

2.1. Powder manufacturing processes

2.1.1. Freeze-drying

Freeze-dried samples were produced using a cryotec® pilot freeze-dryer (Montpellier, France). 6 mL of aqueous solutions of trehalose dihydrate, lactose or mannitol (5 % w/v in water) were put in a 20 mL vial and transferred in the freeze-dryer. The volume of the solution corresponds to a height of 15 mm. For each of the excipients, a total of 90 vials were freeze-dried at once. Thermocouples were used during freeze-drying cycles to monitor the shelves and product temperatures and all cycles (Fig. A1 supplementary data) were carried out as follow:

Freezing step: the samples were frozen by a cooling ramp of −1°C/min, from 25 °C to −50 °C. The final temperature of −50 °C was held for 1 h 30 min to make sure that all the samples were frozen.

Primary drying: at the end of the freezing step, the shelves temperature was risen to 25 °C at a rate of +1 °C/min and the chamber pressure was decreased to 0.1 mbar. This temperature setpoint was held until the pressure values of capacitance and pirani manometers gauges came together. Then, the primary drying time was dependent on the number of vials placed in the chamber. The primary drying time for all three excipients and the total cycle time were respectively always around 21 h and 28 h.

Secondary drying: At the end of the primary drying step, a temperature of 25 °C and a chamber pressure of 0.1 mbar were maintained for 3 to 4 h. Afterwards samples were sealed under vacuum, and then stored at ambient temperature. For all three excipients, cakes had the same global aspect: a height of 1.5 cm with no sign of collapse (Fig. A2 supplementary data).

2.1.2. Spray-drying

Aqueous solutions of trehalose dihydrate, lactose or mannitol (10 % w/v) were spray-dried (SD) on a Büchi laboratory spray dryer (Büchi Mini Spray Dryer B-191, Buchegg, Switzerland). The liquid feed rate was 8.2 mL/min, the atomizing-air flow was 0.7 m^3/h and the aspiration vacuum was around 40 mbar. T_{inlet} was set on 110 °C based on the work of Adler and Lee (Adler and Lee, 1999), where trehalose was spray-dried in the presence of lactate dehydrogenase using different process conditions (T_{inlet} of 110 °C was found to be the optimal process condition to preserve enzymatic activity). During the spray-drying, T_{outlet} could not be controlled but it could be measured. For a T_{inlet} of 110 °C, T_{outlet} was around 60 °C for all the three excipients used. The yield obtained using this process was dependent on the excipient used (powder present on the wall surface of the cyclone was not considered as part of the yield), and will be presented in the results section.

2.1.3. Milling

Crystalline trehalose, lactose and mannitol were ground using a vibration mill (Restsch vibration mill MM 2000, Düsseldorf, Germany), in dry conditions. 6 g of crystalline powder were introduced with one grinding steel ball (20 mm ∅) in the steel grinding jar (nominal volume of 25 mL) and ground for 8 min at a vibrational frequency of 26.6 Hz (1600 RPM).

2.1.4. Tablet manufacturing

All tablets were manufactured on a compaction simulator Styl'One Evolution (Medelpharm, Beynost, France) which is a single station press equipped with displacement and force sensors associated with the upper and lower punches (accuracy 10 N).

For each of the three excipients, FD, SD, commercial crystalline powder (CCP) and commercial crystalline milled powder (CCMP) were compressed. SD powders, CCP and CCMP were directly compressed without prior preparation. FD cakes were gently ground using a mortar and a pestle to obtain a powder (each FD vial was intended to form one tablet and contained approximately 250 mg of powder) prior to compression, in a room where relative humidity was maintained between 30 and 40 %.

The compactions were done in a room where the temperatures were between 23 °C and 25 °C with a relative humidity between 30 % and 40 %. 250 mg of powder was weighted and then set manually in the die, as fast as possible to limit water uptake. The default cycle of the machine was used at the lowest speed possible (which corresponds to a cycle time around 2 s). Furthermore, an external lubrification system using magnesium stearate was used to lubricate the punches and the die.

Two types of euro B punches were used in this work. Except for the determination of brittle fracture index, flat-faced round punches with a 10 mm diameter were used. As developed previously in (Mazel et al., 2016), brittle fracture index was obtained from flattened disc geometry tablets (punches with a flattened geometry, Fig. A3 in supplementary data). The range of compaction pressure used for all experiments was from 12.5 MPa to 250 MPa. Depending on the tensile strength obtained with the different products, the lowest compaction pressure chosen was the one giving a tablet with a sufficient cohesion to be handled.

2.2. Physico-chemical characterization of the powders

2.2.1. Karl fisher titration

The residual water content of FD and SD samples was measured by the Karl Fisher titration method using a titrator compact 20S (Mettler Toledo; Greinfensee, Switzerland). Anhydrous methanol (extra dry methanol, 99 %, Acroseal®) was used to dissolve the samples. Before titration, a blank measurement on methanol was done. FD cakes were directly dispersed in 6 mL methanol in their original vials. 1 mL of the mixture was added into the reaction vessel, and the water content of the samples was obtained at the end of the titration after subtracting the blank's water content. Since all the SD powder was collected in the same

vial, samples were weighted (10–20 mg), and directly added to the reaction vessel, without dispersing in methanol. Water content was also obtained at the end of the titration. Each experiment was carried out in triplicate and water content was expressed in % w/w.

2.2.2. Differential scanning calorimetry (DSC)

DSC measurements were performed on all powders (FD, SD, CCP and CCMP) using a DSC3 instrument (Mettler Toledo, Switzerland) equipped with a refrigerated cooling system (RCS). About 10 mg of powders were weighted and sealed in a 100 µL aluminum pan in a hermetically sealed glove box (relative humidity = 7 %, temperature = 25 °C) and were heated from 10 °C to 230 °C at a heating rate of 10 °C/min. During all of the experiments, an empty aluminum pan similar to those containing the sample was used as a reference.

2.2.3. X-ray diffraction (XRD)

Diffractograms of all samples were assessed using a benchtop powder X-ray diffractometer (MiniFlex2, Rigaku, Japan) with Cu Kα radiation (30 kV, 15 mA). Each scan was performed in the 2θ range from 0 to 60° with a step size of 0.1° and a scan speed of 5°/min.

2.2.4. Scanning electron microscopy (SEM)

The morphology of all powders (FD powders, SD powders, CCP and CCMP) and tablets were studied by SEM using a tabletop microscope (Hitachi TM3000, Japan). A sharp blade was used to cut thin slices of the FD samples. The cut FD samples, intact tablets, tablets broken diametrically and all other powders (SD, CCP et CCMP) were then fixed to aluminum stubs with double-sided carbon tapes in order to visualize their morphology.

2.2.5. Particle sizing

The size distributions of SD powders, CCP and CCMP were measured using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Panalytical, United Kingdom). Air injection pressures were adjusted from 2 to 3 bar depending on the analyzed powder.

2.2.6. Density measurements

Pycnometric density (ρ_{pycno}) of all powders was measured using a helium pycnometer (AccuPyc II 1340, Micromeritics, Norcross, GA, USA) in triplicates, with a 1 cm^3 cell using ten purges followed by ten measurements. Cycle and purge fill pressures were both set at 19.5 psig and the end equilibration rate was set at 0.0050 psig/min.

2.2.7. Specific surface area (SSA) measurements

Five-point Brunauer, Emmett and Teller (BET) measurements were done using a Surface Area and Porosity Analyzer instrument (ASAP 2020, Micromeritics, Norcross, USA). Sample outgazing was performed at a temperature of 60 °C until a pressure of 10^{-3} mmHg was reached. The measurements were performed at a relative nitrogen pressure range P/P_0 from 0.05 to 0.2 (5 values of P/P_0) and at a temperature of – 196 °C. For all measurements, it was controlled that the R^2 value of the BET linear fit was higher than 0.999 and the specific surface area was calculated according to the BET equation. For FD samples, multiple cakes were combined (6–8) to achieve total area >1 m^2 for accurate measurements. For all other powders (SD powder, CCP and CCMP), a sufficient quantity was weighted for accurate analysis. All samples were rapidly prepared in a low relative humidity room (30 %). Each measurement was carried out in triplicate.

2.3. Tablet characterization

2.3.1. Porosity and elastic recovery

In-die and out-of die porosities were calculated using Eq. (1):

$$\varepsilon = 1 - \frac{\rho_{apparent}}{\rho_{pycno}} \quad (1)$$

where $\rho_{apparent}$ is the apparent density calculated using the tablet's volume.

For in-die porosity, $\rho_{apparent}$ was calculated using V_{min}, the minimal volume under pressure measured directly with the compaction simulator. In the case of out-of die porosity $\rho_{apparent}$ was calculated using V_f, the final volume of the tablet, obtained from the tablet's diameter and thickness after ejection from the die. ρ_{pycno} refers to the material density measured by helium pycnometry.

Elastic recovery (% ER) could then be determined using Eq. (2):

$$\%ER = \frac{V_f - V_{min}}{V_{min}} \quad (2)$$

2.3.2. Tablets strength properties

In order to draw tabletability profiles, diametral compression tests were performed on 10 mm cylindrical tablets using a texture analyzer equipped with a force sensor (500 ± 0.1 N) (TA.HD, Stable microsystems, Surrey, United Kingdom). Speed and acquisition frequency were respectively 0.1 $mm{\cdot}s^{-1}$ and 500 Hz. Each kind of tablet was tested in triplicate, the breaking force F (N) was measured and was then used to calculate the tensile strength (σ) using Eq. (3) (Fell and Newton, 1970):

$$\sigma = \frac{2F}{\pi D h} \quad (3)$$

where D and h are respectively the tablet diameter and thickness.

Tablet Brittle Fracture Index (BFI) of all the tablets produced was also determined. BFI is used to assess the sensitivity to a stress concentration of tablets and tablet brittleness (Hiestand et al., 1977; Mazel and Tchoreloff, 2023). BFI determination requires two kinds of tablets, with and without a center hole. The tests were performed by diametral compression, using 11 mm tablets with a flattened geometry (Croquelois et al., 2017). Holes of 1 mm diameter were drilled at the center of the tablets as described by Croquelois et al (Croquelois et al., 2017). For each excipient, the tests were performed in triplicate. BFI could then be calculated using Eq. (4):

$$BFI = \frac{1}{2}\left(\frac{\sigma}{\sigma_h} - 1\right) \quad (4)$$

where σ and σ_h are the apparent tensile strengths of tablets without and with a centered hole, respectively.

3. Results and discussion

3.1. Powder's physico-chemical properties

3.1.1. FD powders

Cakes visual appearance for trehalose, lactose and mannitol was elegant and no collapse nor cracks were detected even if the shelf temperature used for primary drying during the freeze-drying cycles of all three excipients was quite high (25 °C), in order to achieve fast cycles.

The residual moisture (RM) content was measured in all FD samples using Karl Fisher titration method. Trehalose and lactose contained respectively a RM of 1.00 ± 0.02 % and 1.00 ± 0.05 % which is coherent for future applications since a RM neighboring 1 % is required for stabilization of biopharmaceutics (Schneid et al., 2011). RM couldn't be determined for FD mannitol because of its poor solubility in methanol, but it is expected that it has a low RM after freeze-drying because of its low hygroscopicity (Vanhoorne et al., 2020). Characterization of FD trehalose, lactose and mannitol was then carried out with XRD measurements to analyze their physical structure (crystalline or amorphous) after freeze-drying. XRD results showed that after freeze-drying, trehalose and lactose were totally amorphous (Fig. 1 A and C) since no peaks were detected on the diffractogram, while mannitol remained crystalline (Fig. 1 E), and was essentially formed by the β polymorph with a minority of the α one. DSC consolidated the XRD results. FD trehalose

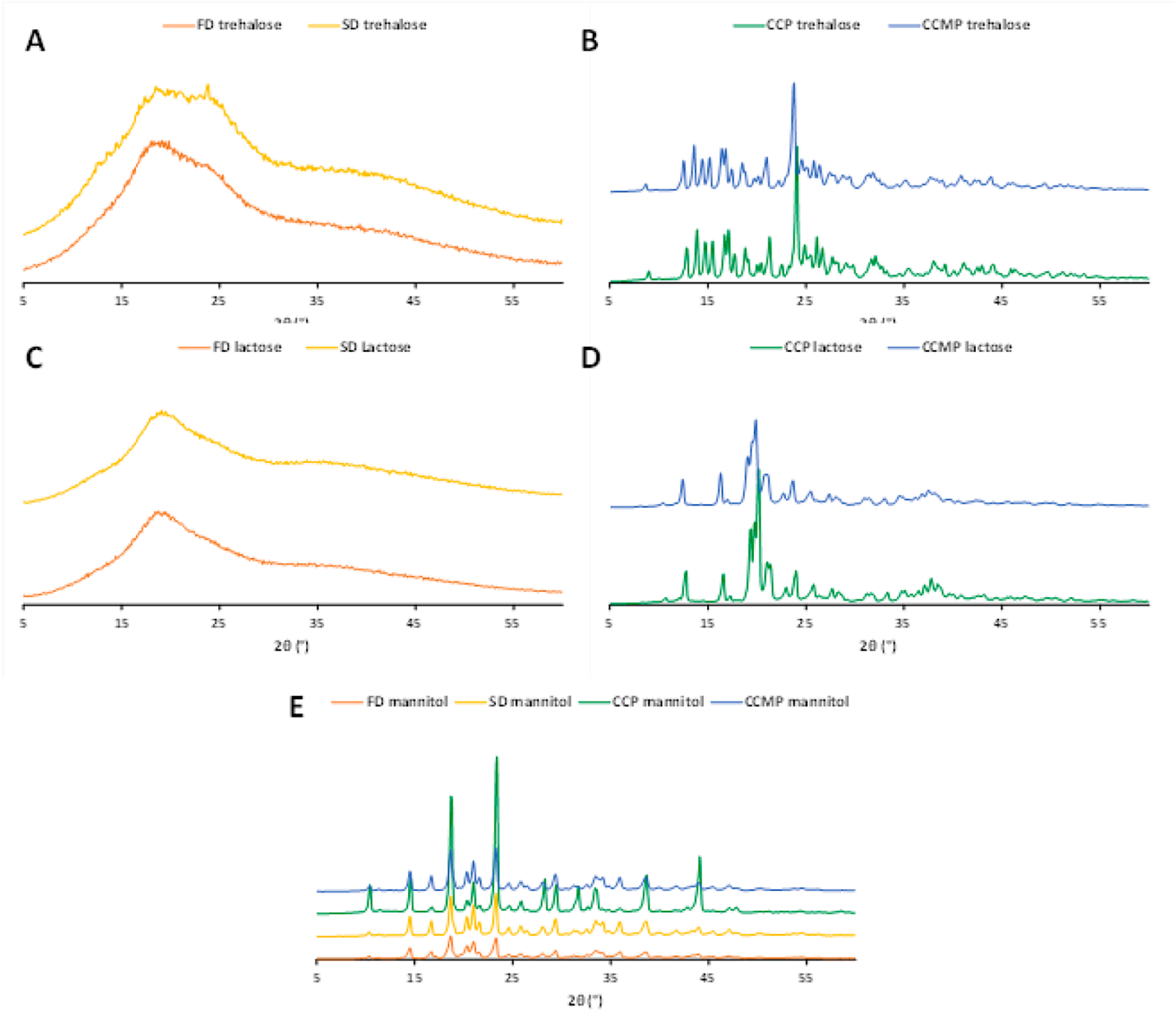

Fig. 1. XRD patterns of (A) FD and SD trehalose, (B) CCP and CCMP trehalose, (C) FD and SD lactose, (D) CCP and CCMP lactose and (E) FD, SD, CCP and CCMP mannitol.

and lactose are characterized by glass transitions, while FD mannitol does not exhibit any T_g. Experimental T_g values obtained for FD trehalose and lactose are given in Table 1. T_g of dry amorphous trehalose and lactose are given in the literature at values of 120 and 107 °C respectively (Haque and Roos, 2006; Roe and Labuza, 2005). But it is well known that T_g values decrease with the increase of the RM (Haque and Roos, 2006; Roe and Labuza, 2005). Then, the experimental T_g values obtained are consistent with the corresponding RM content as described in the literature (Haque and Roos, 2006; Roe and Labuza, 2005).

Cakes morphologies were assessed by SEM for each material to visualize their internal and external morphologies. Viewed from above, trehalose (Fig. 2A) and lactose (Fig. 2B) cakes surfaces presented similar honeycomb structures and pore sizes, while mannitol presented a different surface texture, more like a smooth surface with less pores (Fig. 2 C). Cross sections imaging showed for trehalose and lactose the same honeycomb structures constituted of very smooth leaflets (Fig. 2 D, E). In the case of mannitol, a slight difference was seen with a texture made up of rougher and more irregular leaflets (Fig. 2 F). These differences of appearance may be linked to the fact that trehalose and lactose are amorphous after freeze-drying while mannitol crystallizes.

Finally, the SSA and pycnometric densities of FD powders were measured and are presented in Table 1. Rambhalta et al. have demonstrated that the SSA could change depending on freeze-drying cycle parameters and especially depending on the nucleation temperature during freezing (Rambhatla et al., 2004). They also demonstrated that crystalline FD mannitol has a SSA much higher than amorphous FD sucrose. For the conditions chosen for our freeze-drying cycle, FD trehalose and lactose are characterized by quite similar SSA (respectively 0.950 ± 0.060 m^2/g and 1.060 ± 0.018 m^2/g), while mannitol presented a higher one (2.780 ± 0.084 m^2/g) (Table 1). Those results are consistent with Rambhaltas' work. The pycnometric density measurements of FD trehalose and lactose gave relatively low values in regard to the one obtained with their crystalline forms (Table 1). This is an expected result since the true density of a material decreases after amorphization, as observed in other studies (Bookwala et al., 2020; Bourduche et al., 2020). On the contrary, the pycnometric density was practically the same for FD and CCP mannitol since FD mannitol remained crystalline after freeze-drying.

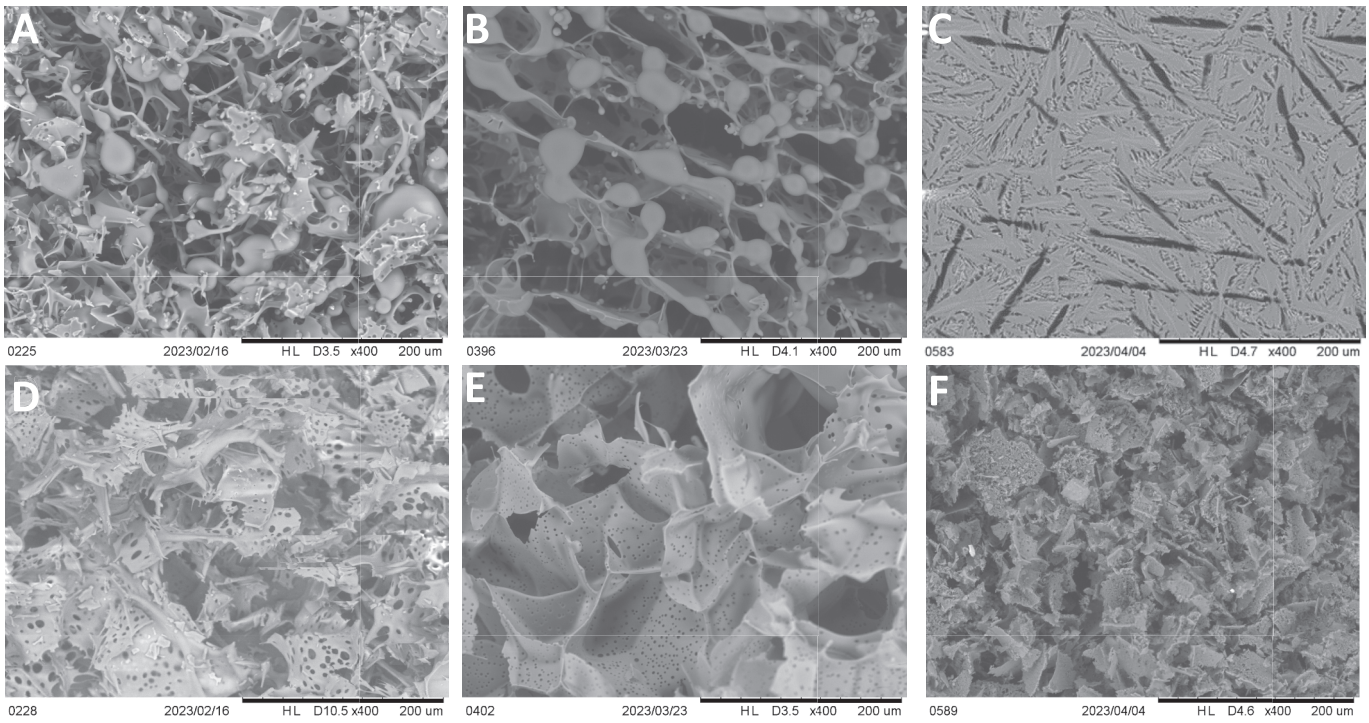

Fig. 2. SEM imaging of FD trehalose (A and D), FD lactose (B and E) and FD mannitol (C and F) (A, B and C are seen from above, D, E and F are cross sections).

3.1.2. SD powders

Exactly the same parameters were used for all three excipients during the spray-drying cycles. When T_{inlet} was set at 110 °C, T_{outlet} was always measured between 58 and 60 °C. Trehalose and lactose powders gave a yield of about 50 %, lower than mannitol which gave a yield of about 85 %.

XRD measurements showed that SD trehalose was mostly amorphous (Fig. 1 A). It could be noted the conservation of only one small peak at $2\theta = 23.8°$ which corresponds to the most intense peak seen on crystalline trehalose diffractogram (Megarry et al., 2011). The diffractogram obtained with SD lactose showed that it was completely amorphous immediately after spray-drying (Fig. 1 C). Finally, results showed that mannitol was completely crystalline after spray-drying (Fig. 1 E), and that the peaks profile was corresponding to those of β-mannitol polymorph (Cares-Pacheco et al., 2014). Water titration by Karl Fisher technique showed that the SD powders presented higher RM than the FD ones. SD trehalose presented a RM of 4 ± 0.6 % and lactose of 5 ± 0.3 %. Crystalline mannitol is known to be non-hygroscopic, and because of its non-solubility in methanol, its RM wasn't measured. DSC measurements showed no T_g for SD mannitol, but showed lower T_g values for SD trehalose and lactose compared to those obtained for FD powders (results shown in Table 1). Here too, since T_g depends on the % RM, these results are consistent with those found in the literature at the same RM content as explained in the case of FD powders (Haque and Roos, 2006; Roe and Labuza, 2005).

SD powder morphologies and size distributions were evaluated by SEM (Fig. 3) and by laser diffraction particle size analyzer (Table 1). As expected, all three SD excipients exhibit the same spherical morphology with a particle diameter ranging between 1 μm and 10 μm with a predominance of a medium size particle at around 3–6 μm as shown in Fig. 3. Particle size distribution confirmed these results. Table 1 shows that 50 % of particles have a diameter smaller than 4.2, 5.2 and 3.5 μm (D_{50}) respectively for SD trehalose, lactose and mannitol.

Pycnometric densities were obtained, and higher values were noted for SD trehalose and lactose, compared to the FD ones but lower when it comes to compare SD mannitol to the FD one (Table 1). These results could be somehow linked to the differences in the structure of the powders.

SSA results for SD powders showed the same tendency as the FD ones. Amorphous SD trehalose and lactose have also a similar SSA, respectively 1.390 ± 0.037 and 1.160 ± 0.042 m^2/g, which are much lower than SD crystalline mannitol with a SSA of 2.370 ± 0.074 m^2/g.

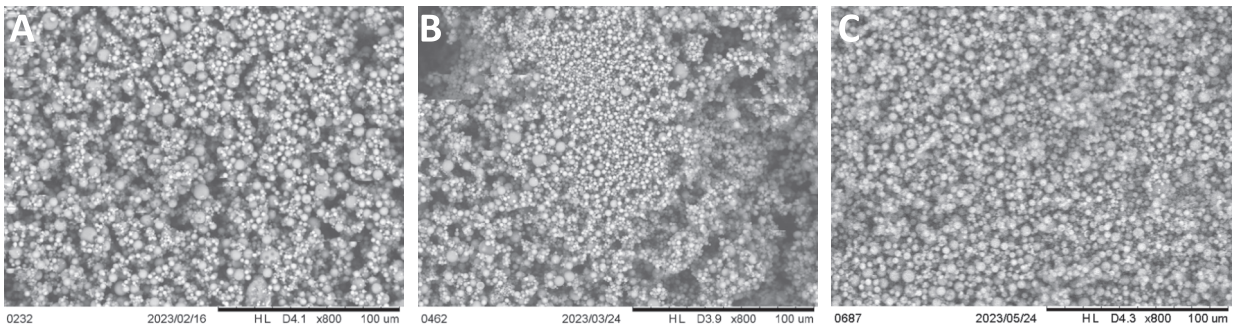

Fig. 3. SEM imaging of SD trehalose (A), SD lactose (B) and SD mannitol (C).

Table 1
Physico-chemical characterization of all three excipients used in this study.

Excipient	Experimental T_g	% RM	Pycnometric density (g/cm^3)	SSA (m^2/g)	Physical structure (XRD)	Particle size distribution D_v(50) (μm)
FD trehalose	100 ± 1.5 °C	1 ± 0.02 %	1.45 ± 0.02	0.95 ± 0.060	Amorphous	–
FD lactose	96 ± 0.9 °C	1 ± 0.05 %	1.39 ± 0.008	1.06 ± 0.018	Amorphous	–
FD Mannitol			1.49 ± 0.01	2.78 ± 0.084	Crystalline	–
SD trehalose	65 ± 2.7 °C	4 ± 0.6 %	1.51 ± 0.002	1.39 ± 0.037	Amorphous	4.2
SD lactose	69 ± 0.2 °C	5 ± 0.3 %	1.51 ± 0.006	1.16 ± 0.042	Amorphous	5.2
SD Mannitol	–	–	1.43 ± 0.011	2.37 ± 0.074	Crystalline	3.5
Trehalose CCP	–	–	1.51 ± 0.004	1.64 ± 0.231	Crystalline	285
Lactose CCP	–	–	1.53 ± 0.0003	0.45 ± 0.099	Crystalline	100
Mannitol CCP	–	–	1.49 ± 0.001	0.23 ± 0.058	Crystalline	69.6
Trehalose CCMP	–	–	1.53 ± 0.0008	3.63 ± 0.287	Crystalline	6.3
Lactose CCMP	–	–	1.55 ± 0.001	3.39 ± 0.090	Crystalline	6.8
Mannitol CCMP	–	–	1.50 ± 0.0006	3.57 ± 0.280	Crystalline	5.2

3.1.3. CCP and CCMP powders

Particle size distribution (Table 1), and SEM imaging (Fig. 4) showed that as expected, milling reduced particle size and increased the SSA (Table 1). Table 1 shows that the obtained CCMP powders exhibited the highest SSA values regardless of the products.

Milling of raw powders was only done in the goal of changing their texture, without amorphization, to evaluate the impact of the texture on the tableting properties. All crystalline powders were analyzed by DSC and XRD. DSC was done to prove that milling did not amorphize the raw powders. As expected, all CCP and CCMP did not show any T_g on DSC graphs. XRD measurements were then performed to be sure that milling did not result in a change of the basic polymorphic form of CCP. The diffractograms of each of the ground and unground excipients showed that no polymorphic changes occurred upon milling (Fig. 1 B, D, E), which means that the grinding, in our experimental conditions, did not lead to any change in the physical structure of the products.

3.2. Compaction properties of the tested powders

3.2.1. Compressibility

At first, all excipients compaction behaviors were assessed by the study of their compressibility profiles. Knowing that moisture could influence compaction behavior (Elkhider et al., 2007) and since trehalose and lactose's FD and SD powders are hygroscopic, compression was performed rapidly after vials opening in a room with a relative humidity around 35 %. CCP and CCMP powders were also compressed in the same conditions. Compressibility profiles were evaluated by drawing the evolution of in-die porosity as a function of the applied pressure (Fig. 5).

It can be noted that some of the profiles present apparent negative porosities at high pressure. Two main phenomena can explain these results. First, due to the compressibility of the solid itself, the particle density of the powder in fact increases along with the applied pressure (Wünsch et al., 2019). As generally done in the literature, this effect was not considered in the porosity calculation made in this study. At high pressure this underestimation of the particle density can lead to apparent negative porosity, as seen at high pressures for FD amorphous products (Fig. 5 A, B). Another phenomena could be related with the amorphous nature of the FD products. As amorphous products don't have a well-defined density (unlike crystalline powders), this density can change (increase) during compression, and this can also lead to an apparent negative porosity at high pressure when considering the powder pycnometric density to calculate the porosity of the tablets.

FD trehalose and lactose in-die compressibility profiles seemed to exhibit a quite similar evolution. As shown in Fig. 5 A and 5B, at low

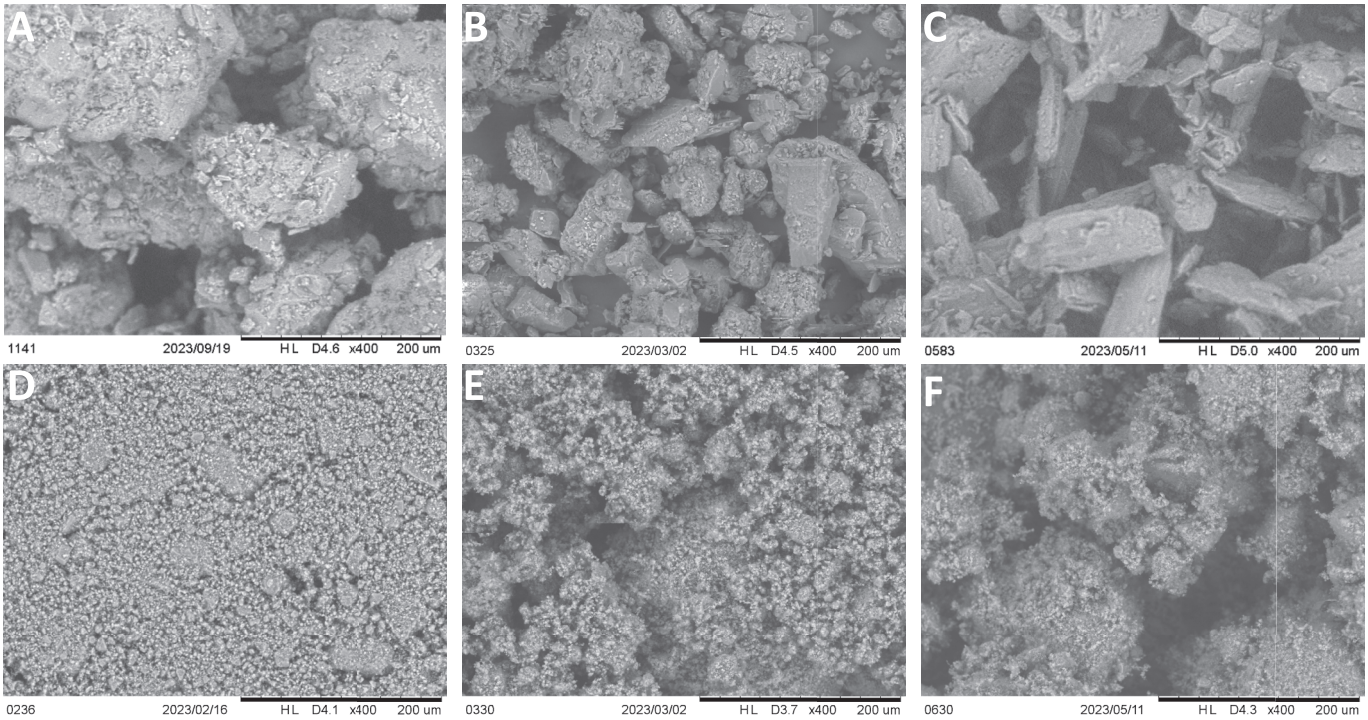

Fig. 4. SEM imaging of CCP trehalose, lactose, and mannitol (respectively A, B and C) and CCMP trehalose, lactose, and mannitol (respectively D, E and F).

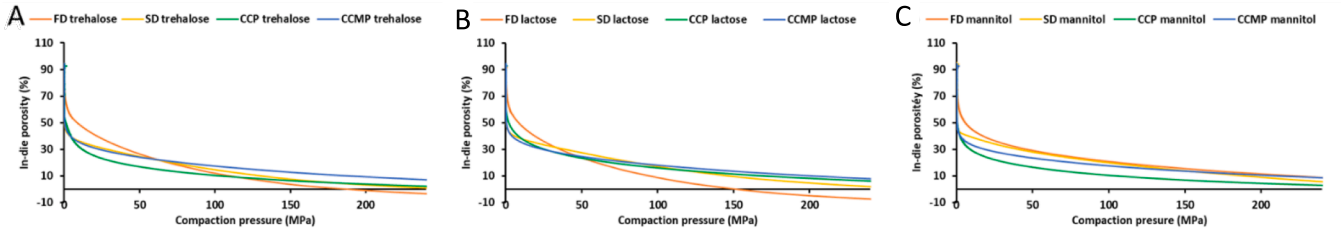

Fig. 5. In-die compressibility profiles of FD, SD, CCP and CCMP powders.

pressure (until 50–60 MPa) the in-die porosities of FD trehalose and lactose are greater than those of SD, CCP and CCMP powders. But, at pressure higher than 50 MPa, these FD powders give lower in-die porosity than others. Mannitol seemed to have a different behavior since the in-die porosity never became below than other powders and no negative porosities were observed (Fig. 5 C).

Considering the three SD compressibility profiles, the same shape was observed that clearly differs from all other powders. Fig. 5 A, B and C show that their porosity rapidly decreases at near zero pressure, until reaching approximately 40 %, then suddenly the curve deviation change. At maximum pressure, porosity of SD powders is higher than the one of FD powders for trehalose and lactose, while it is not the case for mannitol. It is known that SD powders morphology and mechanical properties are outlet temperature dependent (Littringer et al., 2013). When spray-dried at relatively low temperatures (67 °C) like in our study, spherical, rough and hollow particles are formed (Littringer et al., 2013) with entrapped gas inside. Furthermore, moisture content in SD particles is higher than freeze-dried and crystalline powders. Then, difference in texture and high percentage of RM (since water works as a plasticizer) can explain why the mechanical properties during compression are so different from the freeze-dried or crystalline powders.

When comparing CCP and CCMP compressibility profiles, it appears that CCP gives always lower in-die porosities than CCMP which is coherent with the literature (Persson et al., 2022). This is a little less marked in the case of lactose. These results could be explained by the fact that smaller particles of CCMP have more interactions one to the other and are thus more cohesive, and as such, the powder bed develops greater resistance to the compression resulting in a higher in-die porosity.

The results above show that compressibility profiles are impacted by the powders texture, but that it is also linked to their physical structures. Indeed, as expected, for all powders tested, when their texture changes, their compressibility profiles are also modified. Furthermore, the obtained results show that when FD and SD powders are amorphous, particular compressibility profiles are obtained when compared with crystalline powders even if these are obtained by different drying process.

SEM imaging of tablets helped in understanding these results. At low pressures (in a range of 12.5–75 MPa), SEM imaging of SD and FD tablet surfaces showed that the particles morphologies observed in the powders, spherical particles and randomly oriented sheets respectively, were conserved (Fig. 6 A, C, E and G) and high porosity was also observed. When the pressure was further increased, particles started to lose their shapes to form smoother surfaces with less porosity, until reaching the maximum pressure, where close to no porosity was observed (Fig. 6 B, D, F and H). The exact same feature was observed when CCP and CCMP were compressed.

Moreover, as reported in the Hsein et al. work, FD trehalose presents a specific compressibility profile different from the crystalline one (Hsein et al., 2023). In this study, it is also shown that amorphous FD lactose had the exact same behavior than FD trehalose while it is not the case for crystalline FD mannitol. These results show that the particular behavior of FD trehalose and lactose is tightly linked to the amorphous properties of the powders. Furthermore, when excipients were FD, SD or even milled, leading to a change of their physical structure, texture or both, the compressibility profile of each excipient is altered, and this phenomenon is clearly linked to both the texture and the physical structure of the powders.

3.2.2. *Elastic recovery (ER)*

Elastic recovery is an important tablet property to be investigated. Fig. 7 represents the ER profiles as a function of applied pressures for all trehalose, lactose and mannitol powders. Hsein et al showed that FD trehalose has a specific ER profile different from crystalline trehalose (Hsein et al., 2023). Results for FD trehalose show that at low pressures, ER is the highest, and when pressure starts to increase, ER decreases gradually, until a pressure around 100 MPa, where it stabilizes to become pressure independent. ER profiles were different when it comes to crystalline trehalose (CCP and CCMP powders) since an increase in pressure, results in an increase in the ER before to stabilize and to become independent of pressure.

In addition, FD lactose, FD mannitol, and SD trehalose, SD lactose and SD mannitol exhibited the same behavior than FD trehalose. Nevertheless, the profile is a little less marked for FD and SD mannitol. That means that whether amorphous or crystalline, it is the specific texture of SD and FD powders that is responsible for such a behavior (Fig. 7 A, B, C).

For lactose and mannitol, CCP and CCMP exhibited similar profiles than the one described above for trehalose. One interesting point is that, after milling, all CCMP had lower ER than CCP for any given pressure. That means that for these powders, the smaller the particle size, the lower the ER.

3.2.3. *Plastic energy*

Plastic energy (PE) is the total energy given to the powder during the compaction cycle. It can be normalized by the volume of solid compacted to allow comparison of products with different material densities (Wünsch et al., 2021).

Hsein et al have shown that PE of FD trehalose is higher than those of crystalline trehalose (Hsein et al., 2023). Here too, the same tendency was found. FD trehalose, FD lactose and FD mannitol have all three a higher PE, at any given pressure when compared to crystalline powders (Fig. 8 A, B and C). In the case of mannitol, we observed an impact of freeze-drying on the plastic energy (texture effect), but FD and SD mannitol, which are both crystalline, have the same plastic energies. However, in the case where FD and SD powders are amorphous (trehalose and lactose), a higher plastic energy was found for the FD powders compared to the SD ones, demonstrating a significant impact of the texture of amorphous FD powders on plastic energy.

3.2.4. *Tabletability*

The diametral compression tests were done 24 h after compression, in triplicate to evaluate the reproducibility. The tensile strength of tablets was then calculated and the tabletability profiles were drawn for all the tested products (Fig. 9 A, B, C). It is generally considered that a

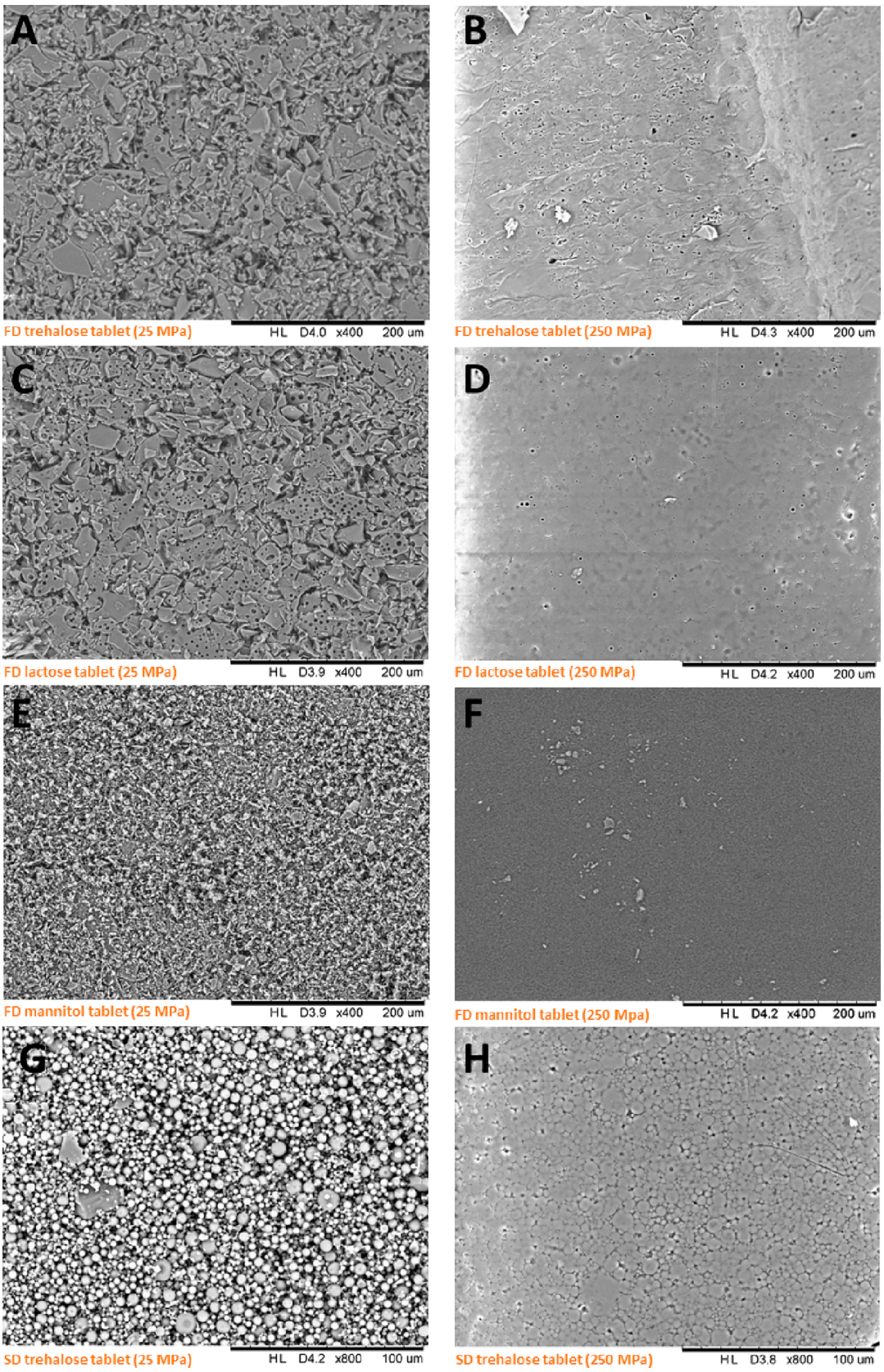

Fig. 6. SEM imaging of freeze-dried and spray dried tableted powders at different pressures.

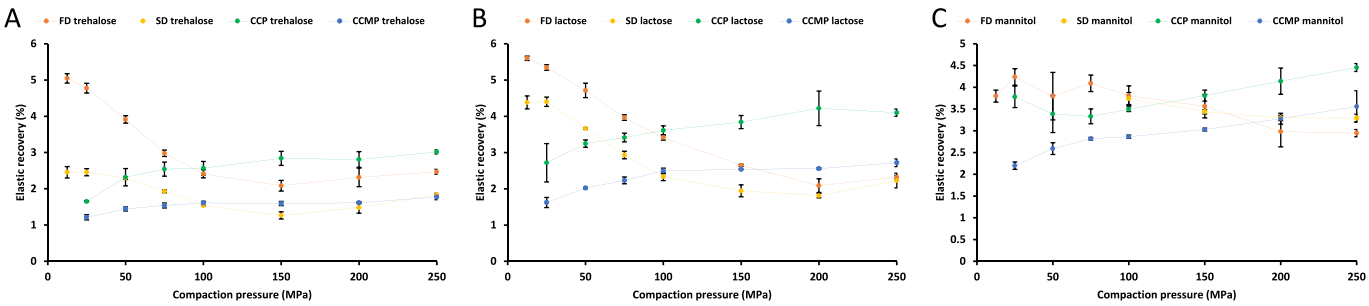

Fig. 7. Elastic recovery profiles of all trehalose powders (A), lactose powders (B) and mannitol powders (C).

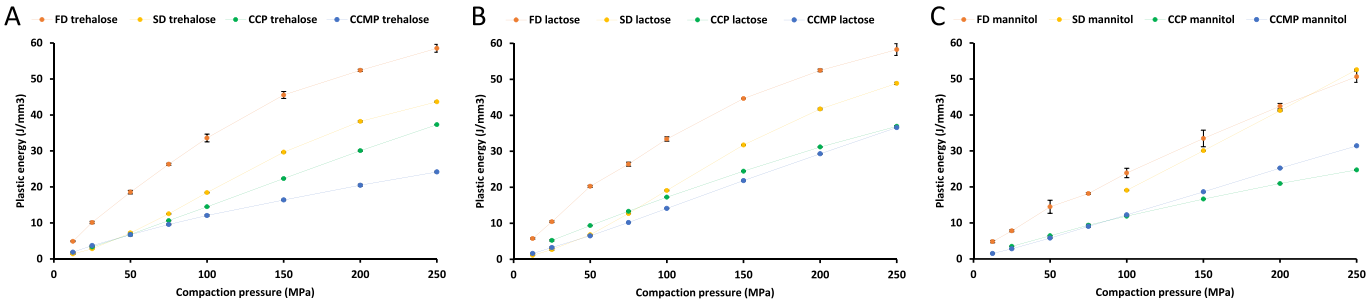

Fig. 8. Plastic energy profiles of FD, SD, CCP and CCMP powders; (A) trehalose, (B) lactose and (C) mannitol.

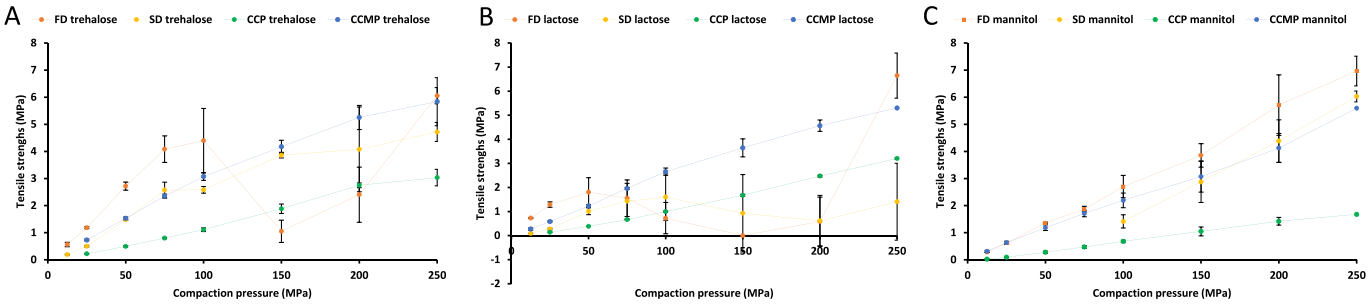

Fig. 9. Tabletability profiles of FD, SD, CCP and CCMP powders (A, B and C: test done 24 h after compression, D and E: test done immediately after compression).

tensile strength higher than 2 MPa is sufficient to ensure mechanical resistance to maintain the physical integrity of tablets both during the whole production process and till the delivery to the patient (Pitt and Heasley, 2013).

As illustrated in Fig. 9A, B, and C for all three excipients, it was consistently observed that the tensile strength and thus the tabletability profile of CCMP is greater than that of CCP following the milling process. Such a result was expected regarding the differences in particles size distribution of the two kinds of products (Persson et al., 2022).

In the same manner, at low pressures for trehalose and lactose, and at all the pressure range for mannitol, SD powder's tabletability profiles are similar to the CCMP (Fig. 9 A, B and C). That could be linked to the fact that SD powders and CCMP have almost identical particle size.

More interestingly, at very low pressures, amorphous FD trehalose and FD lactose exhibited high tensile strength since a value of more than 2 MPa is obtained from 50 MPa for FD lactose and even about 25 MPa for FD trehalose. These values are significantly higher compared to those observed with the SD, CCP and CCMP powders of trehalose and lactose. Conversely, crystalline FD mannitol exhibited similar tensile strengths compared to SD and CCMP mannitol. These results showed one more time the effect of the amorphous FD texture compared to other forms of the same products in terms of tablet's mechanical properties.

Another interesting behavior is seen when it comes to amorphous FD and SD powders (trehalose and lactose). Fig. 9A and B show that when pressure increases, measurements variability highly increased for FD and SD trehalose and lactose and that we obtain a drastic decrease of the tensile strength in the range 100–200 MPa. This behavior was not detected in the case of crystalline FD and SD mannitol. This variability could probably be explained by the fact that amorphous tablets develop numerous cracks immediately after compression (Fig. 10 A), and during storage (Fig. 10 B) when the pressure was increased. These defects make the diametrical compression test unreliable and thus variability in the tensile strength results increases. Interestingly, at higher pressures (250 MPa) it was possible to obtain tablets with high tensile strength for both FD trehalose and lactose. Explanation of this phenomenon is complicated with the present results. A more in-depth study of the mechanism of development of the cracks in the tablets would be needed to better understand this point, but this is beyond the scope of the present work.

Conversely, crystalline mannitol tablets showed no signs of cracking even for FD mannitol.

It could be then concluded from these results that while the tabletability effect is likely associated with the amorphous FD powders (texture-structure), the effect on tensile strength variability when the compaction pressure is increased appears to be more related to its amorphous nature, independently from its texture. In fact, the range of compression pressure of interest when considering amorphous FD powders (trehalose and lactose) is at remarkably low-pressure levels (25–75 MPa) and should not exceed a hundred of MPa to avoid the risk

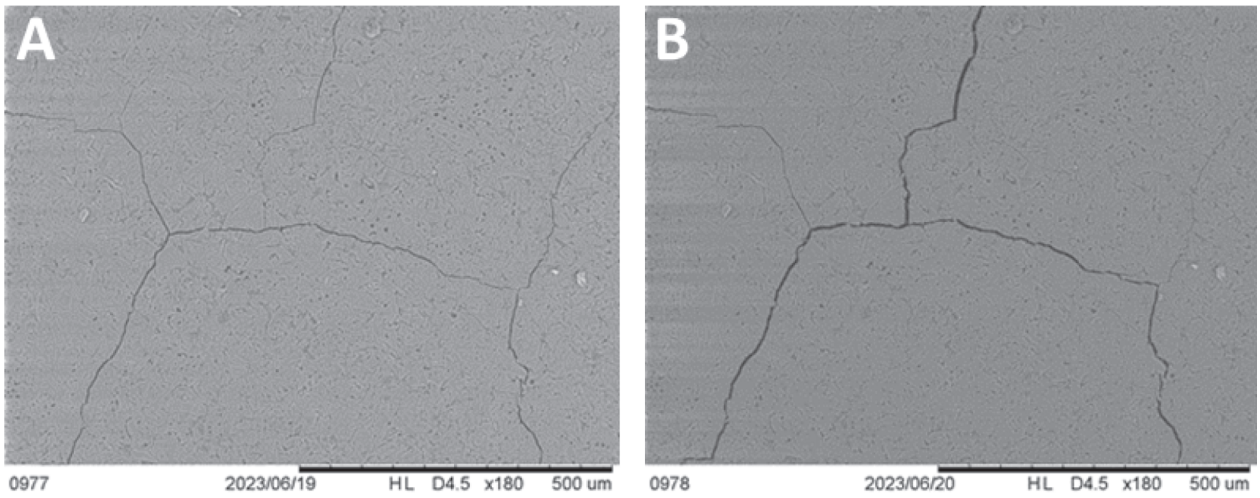

Fig. 10. SEM imaging of trehalose tablets obtained at a pressure of 150 MPa (A) after compression (B) 18 h after compression.

of occurrence of cracks.

3.2.5. *Brittle fracture index (BFI)*

The BFI is an indicator that makes it possible to assess the sensitivity of a tablet to a stress concentration which is linked with its brittleness (Hiestand et al., 1977; Mazel and Tchoreloff, 2023). It is well known that the BFI values are pressure dependent and are more elevated when pressure is high (Croquelois et al., 2020; Hiestand et al., 1977).

The test was done for each material and powder on tablets obtained at a pressure where they were sufficiently resistant and where the tensile strength results were mostly reproducible. All perforated tablets were visualized with the SEM, before diametral compression to be sure that no cracks were created because of the drilling. When cracks were seen during imaging, BFI tests couldn't be performed. CCMP trehalose, whether compacted at 75 MPa or 150 MPa, showed cracks after perforation, which made it impossible to perform the BFI determinations. Moreover, it wasn't possible to compress all SD powders at the same pressure in order to compare them one to the other. Then, SD powders for each excipient were compressed at the best as possible pressure in terms of reproducibility and some CCMP excipients tablets were produced at those exact same pressures in order to compare SD and CCMP powders (Table 2).

When it comes to compare the BFI values (Table 2) of SD lactose and lactose CCMP (respectively 0.48 ± 0.12 and 0.38 ± 0.02) both compressed at 50 MPa, there is no significant difference. The BFI values of SD mannitol and mannitol CCMP compressed at 150 MPa (respectively 0.95 ± 0.44 and 1.20 ± 0.21) are also in the same order of magnitude. Knowing that for both excipients the CCMP and SD powders have small sized particles, and knowing that SD lactose is amorphous while SD mannitol is crystalline, physical structure doesn't seem to have an influence on the BFI in this case.

Hsein et al. results showed that FD trehalose has a much higher BFI than crystalline trehalose (Hsein et al., 2023). Our results are consistent with this previous work since we obtained the exact same trend with FD trehalose's BFI of 1.90 ± 0.17 at a 25 MPa compaction pressure while CCP trehalose's BFI is only 0.83 ± 0.01 at a 250 MPa compaction pressure (Table 2). The same behavior is observed when comparing FD lactose (BFI of 1.60 ± 0.49 at a pressure of 25 MPa) to lactose CCP (BFI of 0.50 ± 0.03 at a pressure of 250 MPa). Knowing that BFI values increase with higher pressures, the fact that the BFI of FD lactose tablets obtained at a 25 MPa pressure is higher than the one of CCP trehalose compressed at 250 MPa, means that FD lactose as FD trehalose are much more brittle than CCP lactose and trehalose. Whereas when comparing the BFI of crystalline FD mannitol (0.41 ± 0.18) compressed at 25 MPa to the BFI of CCP mannitol compressed at 250 MPa (0.34 ± 0.02), even if it shows that FD mannitol is more brittle than CCP mannitol, the effect is much less than in the case of trehalose and lactose.

Based on the results described above, one more time, the specific texture of amorphous FD powders seems to play an important role when it comes to tablet's brittleness.

Table 2
Experimental BFI values of all three excipients.

Excipient	Compaction pressure	Calculated Brittle Fracture Index (BFI)
FD trehalose	25 MPa	1.90 ± 0.17
FD lactose	25 MPa	1.60 ± 0.49
FD mannitol	25 MPa	0.41 ± 0.18
SD trehalose	75 MPa	0.52 ± 0.17
SD lactose	50 MPa	0.48 ± 0.12
SD mannitol	150 MPa	0.95 ± 0.44
CCP trehalose	250 MPa	0.83 ± 0.01
CCP lactose	250 MPa	0.50 ± 0.03
CCP mannitol	250 MPa	0.34 ± 0.02
CCMP lactose	50 MPa	0.38 ± 0.02
CCMP mannitol	150 MPa	1.20 ± 0.21

4. Conclusion

According to all the results described above, it was shown that the texture and the physical structure, both have singular or combined impacts on the tableting properties of the studied powders.

This study showed that FD trehalose has indeed the same particular behavior in terms of tableting properties when compared to Hsein et al.'s previous results. In addition, lactose, which was also amorphous after freeze-drying behaved very similarly. It seems therefore that the particular texture of amorphous FD powder is the main reason behind those specific tableting properties and that this behavior is not limited to the chemical structure of trehalose.

Furthermore, the only property obtained that was completely independent from the texture, and that was only influenced by the physical state of the powder (amorphous or crystalline) is the high variability of tensile strength measurements at relatively high pressures. This is due to cracks developed in the tablet after compression. This is a problem that could be easily overcome when it comes to compress amorphous FD powders since these powders have a high tensile strength at very low pressures. It is thus possible to compress these powders using a very low pressure (<50 MPa) since no signs of defects are seen but the tensile

strength is high enough and the test's reproducibility is at its best.

If this work made it possible to better understand the behavior of freeze-dried products during compaction, further is needed to understand the mechanisms that can explain, at the microscopic level, the trends demonstrated. In conclusion, these findings represent the initial phase in the journey towards industrialization. As previously stated, the ultimate objective of this research is to achieve the development of tablets made of freeze-dried powders containing biomolecules. We established that these powders can be compressed at a relatively low pressure to yield tablets with sufficient cohesion. Nevertheless, manual compression is currently employed due to the poor flow of the FD powders. Next challenge would thus be to improve the processability of the powder to make it suitable for industrial processes.

CRediT authorship contribution statement

Charbel Madi: Writing – original draft, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Project administration. **Hassana Hsein:** Writing – review & editing, Conceptualization, Methodology, validation, Visualization, Project administration, Supervision. **Virginie Busignies:** Writing – review & editing, Validation, Supervision. **Pierre Tchoreloff:** Writing – review & editing, Validation, Supervision. **Vincent Mazel:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.124059.

References

Adler, M., Lee, G., 1999. Stability and surface activity of lactate dehydrogenase in spray-dried trehalose. J. Pharmaceut. Sci. 88, 199–208. https://doi.org/10.1021/js980321x.

Bjelošević, M., Zvonar Pobirk, A., Planinšek, O., Ahlin Grabnar, P., 2020. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. Int. J. Pharm. 576, 119029 https://doi.org/10.1016/j.ijpharm.2020.119029.

Bookwala, M., DeBoyace, K., Buckner, I.S., Wildfong, P.L., 2020. Predicting density of amorphous solid materials using molecular dynamics simulation. AAPS PharmSciTech 21, 1–11.

Bourduche, F., Sanchez-Ballester, N.M., Bataille, B., Lefèvre, P., Sharkawi, T., 2020. Structure-property relationship of amorphous maltitol as tableting excipient. AAPS PharmSciTech 21, 1–12.

Cares-Pacheco, M.G., Vaca-Medina, G., Calvet, R., Espitalier, F., Letourneau, J.-J., Rouilly, A., Rodier, E., 2014. Physicochemical characterization of d-mannitol polymorphs: the challenging surface energy determination by inverse gas chromatography in the infinite dilution region. Int. J. Pharm. 475, 69–81. https://doi.org/10.1016/j.ijpharm.2014.08.029.

Croquelois, B., Girardot, J., Kopp, J.B., Cazautets, C., Tchoreloff, P., Mazel, V., 2017. Breaking pharmaceutical tablets with a hole: Reevaluation of the stress concentration factor and influence of the hole size. Powder Technol. 317, 126–132. https://doi.org/10.1016/j.powtec.2017.04.033.

Croquelois, B., Girardot, J., Kopp, J.B., Tchoreloff, P., Mazel, V., 2020. Quantification of tablet sensitivity to a stress concentration: Generalization of Hiestand's approach and link with the microstructure. Powder Technol. 369, 176–183. https://doi.org/10.1016/j.powtec.2020.05.002.

Elkhider, N., Chan, K.A., Kazarian, S.G., 2007. Effect of moisture and pressure on tablet compaction studied with FTIR spectroscopic imaging. J. Pharm. Sci. 96, 351–360.

Fell, J., Newton, J., 1970. Determination of tablet strength by the diametral-compression test. J. Pharm. Sci. 59, 688–691.

Haddadzadegan, S., Dorkoosh, F., Bernkop-Schnürch, A., 2022. Oral delivery of therapeutic peptides and proteins: technology landscape of lipid-based nanocarriers. Adv. Drug Deliv. Rev. 182, 114097 https://doi.org/10.1016/j.addr.2021.114097.

Haque, M.K., Roos, Y.H., 2006. Differences in the physical state and thermal behavior of spray-dried and freeze-dried lactose and lactose/protein mixtures. Innov. Food Sci. Emerg. Technol. 7, 62–73. https://doi.org/10.1016/j.ifset.2004.12.004.

Hiestand, E.N., Wells, J.E., Peot, C.B., Ochs, J.F., 1977. Physical processes of tableting. J. Pharm. Sci. 66, 510–519. https://doi.org/10.1002/jps.2600660413.

Hsein, H., Madi, C., Mazel, V., Tchoreloff, P., Busignies, V., 2023. Tableting properties of freeze-dried trehalose: physico-chemical and mechanical investigation. Int. J. Pharm. https://doi.org/10.1016/j.ijpharm.2023.123598.

Littringer, E.M., Noisternig, M.F., Mescher, A., Schroettner, H., Walzel, P., Griesser, U.J., Urbanetz, N.A., 2013. The morphology and various densities of spray dried mannitol. Powder Technol. 246, 193–200. https://doi.org/10.1016/j.powtec.2013.05.004.

Mazel, V., Guerard, S., Croquelois, B., Kopp, J.B., Girardot, J., Diarra, H., Busignies, V., Tchoreloff, P., 2016. Reevaluation of the diametral compression test for tablets using the flattened disc geometry. Int. J. Pharm. 513, 669–677. https://doi.org/10.1016/j.ijpharm.2016.09.088.

Mazel, V., Tchoreloff, P., 2023. Indices for the brittleness of pharmaceutical tablets: a reassessment. Int. J. Pharm. 645, 123364 https://doi.org/10.1016/j.ijpharm.2023.123364.

Megarry, A.J., Booth, J., Burley, J., 2011. Amorphous trehalose dihydrate by cryogenic milling. Carbohydr. Res. 346, 1061–1064. https://doi.org/10.1016/j.carres.2011.03.011.

Meynard, J., Amado-Becker, F., Tchoreloff, P., Mazel, V., 2022. On the complexity of predicting tablet capping. Int. J. Pharm. 623, 121949 https://doi.org/10.1016/j.ijpharm.2022.121949.

Persson, A.-S., Pazesh, S., Alderborn, G., 2022. Tabletability and compactibility of α-lactose monohydrate powders of different particle size. I. Experimental comparison. Pharmaceut. Dev. Technol. 27, 319–330. https://doi.org/10.1080/10837450.2022.2051550.

Pitt, K.G., Heasley, M.G., 2013. Determination of the tensile strength of elongated tablets. In: Powder Technology, Special Issue: 5th International Granulation Workshop Granulation across the length scale 2011 238, 169–175. doi:10.1016/j.powtec.2011.12.060.

Rambhatla, S., Ramot, R., Bhugra, C., Pikal, M.J., 2004. Heat and mass transfer scale-up issues during freeze drying: II. Control and characterization of the degree of supercooling. AAPS PharmSciTech 5, 54–62. https://doi.org/10.1208/pt050458.

Roe, K.D., Labuza, T.P., 2005. Glass transition and crystallization of amorphous trehalose-sucrose mixtures. Int. J. of Food Properties 8, 559–574. https://doi.org/10.1080/10942910500269824.

Schneid, S.C., Gieseler, H., Kessler, W.J., Luthra, S.A., Pikal, M.J., 2011. Optimization of the secondary drying step in freeze drying using TDLAS technology. AAPS PharmSciTech 12, 379–387.

Tomar, M., Sinha, A.R., Singh, A.K., 2017. Process and development of co-processed excipient silicified microcrystalline cellulose and manufacture paracetamol tablet by direct compression. Int. J. Pharm. Sci. Rev. Res 42, 191–196.

Vanhoorne, V., Almey, R., De Beer, T., Vervaet, C., 2020. Delta-mannitol to enable continuous twin-screw granulation of a highly dosed, poorly compactable formulation. Int. J. Pharm. 583, 119374 https://doi.org/10.1016/j.ijpharm.2020.119374.

Vecchio, I., Tornali, C., Bragazzi, N.L., Martini, M., 2018. The discovery of insulin: an important milestone in the history of medicine. Front. Endocrinol. 9, 613. https://doi.org/10.3389/fendo.2018.00613.

Wünsch, I., Finke, J.H., John, E., Juhnke, M., Kwade, A., 2019. A mathematical approach to consider solid compressibility in the compression of pharmaceutical powders. Pharmaceutics 11, 121. https://doi.org/10.3390/pharmaceutics11030121.

Wünsch, I., Michel, S., Finke, J.H., John, E., Juhnke, M., Kwade, A., 2021. How can single particle compression and nanoindentation contribute to the understanding of pharmaceutical powder compression? Eur. J. Pharm. Biopharm. 165, 203–218. https://doi.org/10.1016/j.ejpb.2021.05.009.