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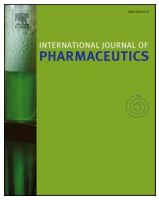

Tableting properties of freeze-dried trehalose: Physico-chemical and mechanical investigation

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ABSTRACT

Freeze-drying of biopharmaceutical products is the method of choice in order to improve their stability and storage conditions. Such freeze-dried products are usually intended for parenteral route administration. However, many biopharmaceutical materials administered by parenteral route are used to treat local diseases particularly in the gastro-intestinal tract. Therefore, many studies concentrate nowadays their effort on developing alternative dosage forms to deliver biopharmaceutical molecules by the oral route. Tablets are the most popular solid pharmaceutical dosage form used for oral administration since they present many advantages, but poor informations are available on the possibility of tableting freeze-dried powders. In this study, we evaluate the compaction behavior of freeze-dried trehalose powder since trehalose is one of the most used cryo and lyoprotectant for the lyophilisation of biopharmaceutical entities. Results show that freeze-dried trehalose powder can be tableted while remaining amorphous and the obtained compacts present very specific properties in terms of compressibility, tabletability, brittleness and viscoelasticity compared to the crystalline trehalose and compared to classical pharmaceutical excipients.

1. Introduction

Biopharmaceutical molecules represent nowadays a major therapeutic class and their use gained lots of attention in the last decades since they cover a large panel of therapeutic indications such as cancer, auto-immune disorders, neurological diseases or metabolic disorders (Kesik-Brodacka, 2018). Indeed, the COVID 19 pandemic has boosted the biopharmaceutical market with the development of vaccines and monoclonal antibodies against SARS CoV2 virus (Jaworski, 2021).

Biopharmaceutical active substances such as therapeutic proteins, vaccines or cell and gene therapies are usually obtained and prepared in liquid dosage forms intended for parenteral administration. However, one major challenge in using biopharmaceutical molecules refers to their complex and sensitive macromolecular structure making them prone to physicochemical instabilities such as hydrolysis, deamidation, aggregation or degradation when formulated as aqueous solution (Butreddy et al., 2021). Therefore, dry solid state may be preferred in order to improve their stability. Amongst the different drying strategies, freeze-drying is the method of choice. Freeze-dried drug delivery systems present better biological stability and allow for better shipping, storage and handling conditions (Liu & Zhou, 2021; Sharma et al., 2021). Process control of freeze-drying implies both the choice of formulation and process parameters and is essential to preserve biopharmaceutical molecules and to achieve the required product quality attributes (Hsein et al., 2022). Formulation strategies mainly consist in adding cryo and lyoprotectors such as sucrose and trehalose, surfactants such as polysorbate 20 or 80, polyols such as mannitol or glycine, or choosing buffer salts to adjust the pH (Bjelošević et al., 2020). Amongst these excipients, the non-reducing sugar trehalose is the most widely used as stabilizer both in commercial biopharmaceutical products and in research papers. The stabilization mechanisms of trehalose during freeze-drying are generally explained by the water replacement theory and by the formation of amorphous materiallimiting molecular mobility (Ohtake & Wang, 2011; Olsson et al., 2016).

Moreover, obtaining dry products opens the way to the development of other dosage forms than those dedicated to the parenteral administration. Then, developing oral route for biopharmaceutics has been the goal of many studies in the last decades although it still remains highly challenging since the absorption of such hydrophilic and high molecular weight molecules is very limited (Haddadzadegan et al., 2022; Homayun

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et al., 2019; Madani et al., 2020; Olorunsola et al., 2023; Vass et al., 2019). However, the oral administration of these molecules for the local treatment of some gastrointestinal pathologies may be an interesting option. Nevertheless, this administration will require additional formulation steps to protect the biomolecules from degradation by gastric acidity and by the gastrointestinal tract proteases (Choonara et al., 2014; Homayun et al., 2019). For oral administration, tablets are the most popular solid pharmaceutical dosage form since they present many advantages such as being easy to take and improving patient compliance. Other advantages from industrial standpoint, are the low cost of production and above all, the absence of cold chain requirements and sterility issues (Zhong et al., 2018). However, producing a tablet by powder compaction from a freeze-dried (FD) solid requires additional knowledge and very little information is available about the tableting behavior of these powders. Therefore, the aim of this study is to investigate by a systematic approach, the compaction behavior of FD trehalose and finally, to conclude on a possible use in tablet formulations. Then, a detailed analysis of the physico-chemical and mechanical properties of FD trehalose tablets is carried out and discussed in comparison with crystalline trehalose and traditional pharmaceutical excipients commonly used in direct compression processes.

2. Material and methods

2.1. Materials

Trehalose dihydrate used for freeze-drying was generously given by DFE Pharma (Goch, Germany) and the one used for direct compression was generously given by Nagase (Kyoto, Japan). Three other excipients were used to make tablets: anhydrous calcium phosphate (ACP) (Anhydrous Emcompress®, JRS Pharma, Rosenberg, Germany), granulated lactose monohydrate (GLac) (Tablettose® 80, Meggle, Wasserburg, Germany) and microcrystalline cellulose (MCC) (Vivapur® 12, JRS Pharma Rosenberg, Germany). Magnesium stearate (Ligamed MF-2-V) was obtained from Peter Greven (Bad Münstereifel, Nordrhein-Westfalen, Germany).

2.2. Freeze-drying

Freeze-drying was performed using a Cryotec® pilot freeze-dryer (Montpellier, France). To perform lyophilization, 6 mL of trehalose solutions (5 % w/v in water) were filled in 20 mL glass tubing vials, partially stoppered with rubber stopper and placed on the same shelf of the freeze-dryer. The freeze-drying cycle was carried out as follow, using thermocouples to monitor the product temperature all over the freeze-drying process:

- The freezing step started by cooling shelves to 5 °C with a cooling rate of −1°C/min and held at 5 °C for 30 min. Shelves were then cooled again to −5°C with a cooling rate of −1°C/min and held at −5°C for 30 min. This step was followed by a final cooling ramp down to −50 °C with a cooling rate of −1°C/min. The final temperature of – 50 °C was held for 2 h.
- During the primary drying step, shelves were heated to −20 °C at + 1 °C/min heating rate and the chamber pressure was decreased to 0.1 mbar. Shelves temperature was hold to −20 °C until the convergence of the pressure values of pirani and capacitance manometers gauges was observed.
- To perform the secondary drying step, shelves temperatures were raised to + 25 °C at + 0.1 °C/min ramp rate then held isothermally for 12 h at a chamber pressure of 0.06 mbar.

At the end of the freeze-drying cycle, vials were closed under vacuum in the freeze dryer before unloading.

2.3. Tableting

Tableting was performed using a “Styl’One” evolution tableting press (Medelpharm, Beynost, France) which is a single station press. The press is equipped with force sensor (accuracy 10 N) and the displacements of the punches are monitored with an accuracy of 0.01 mm.

Before compression, FD cakes (one vial corresponded to one tablet) were gently ground with a mortar and a pestle. All sample preparation steps were performed in 2 min to limit water uptake and all tablets were produced in a room where the relative humidity was set between 45 and 55 % RH with a temperature of 22 °C.

Two kinds of flat-faced euro B punches were used. One set was round with a diameter of 10 mm diameter. The other was a special set that made it possible to obtain a so-called flattened disc geometry (Mazel et al., 2016). The tablets obtained with this geometry had a diameter of 11 mm with a flat end of 30°. For all the experiments the compressed powder was put manually in the die. An external lubrication system using magnesium stearate was used to lubricate the punches and the die. Compression pressures from 12.5 MPa to 250 MPa were applied to make tablets (250 mg) using the force-driven mode of Analis software. FD powders were compacted at a low and a high compression speeds corresponding to cycle times around 2 s and 70 ms respectively. The other excipients were only compacted at the low speed for comparison purposes.

2.4. Physico-chemical characterizations

2.4.1. Residual moisture content of FD samples

Residual moisture content in the samples was evaluated by Karl Fisher Titration method using a titrator compact 20S (Mettler Toledo, Greifensee, Switzerland) with anhydrous methanol as the sample solvent (extra dry methanol (99.9 %, Acroseal®). Cakes were dispersed in 6 mL of anhydrous methanol and then 1 mL was titrated with Riedel-de Haen Hydranal Composite 2 reagent (Hoechst Celanese Corp., Germany) until the endpoint was reached. Experiments were carried out in triplicate and the water content in % w/w was expressed as mean ± SD.

2.4.2. Thermal behavior

DSC experiments were performed in 40 µL aluminum crucibles hermetically sealed using a DSC3 instrument (Mettler Toledo, Switzerland) equipped with a refrigerated cooling system (RCS). The glass transition temperatures of the maximally freeze-concentrated solution (T'_g) was determined on 30 µL of liquid formulations by applying the following cycle: a temperature decrease from 25 to −50 °C at −1°C/min, followed by an isotherm at −50 °C for 10 min and finally heating of the sample from −50 °C to 25 °C at + 10 °C/min. For the determination of the glass temperature (T_g) of solid samples (lyophilized samples and tablets) about 5 to 10 mg of the samples were placed in the crucibles and sealed. DSC was then carried out by an initial isotherm at 10 °C followed by a ramp of temperature from 10 to 220 °C at a rate of + 10 °C/min. In all experiments, an empty aluminum crucible crimped in the same manner as the sample pans was used as reference. T_g and T'_g correspond to the midpoint of the glass transition in the heating scan of each DSC experiment.

2.4.3. Scanning electron microscopy

The morphologies of FD samples and tablets were studied by scanning electron microscopy (SEM) using the analyze mode (Hitachi TM3000, Japan). For FD samples, a sharp blade was used to cut a thin slice along the cylindrical axis of the cake. For tablets, samples were either directly fixed to aluminium stubs with double-sided carbon tape or broken diametrically in order to respectively visualize the external and internal morphologies of the tablets.

2.4.4. DRX

The FD samples and tablets were analyzed with 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 scan speed of 5°/min.

2.5. Compaction behavior

2.5.1. Tablet's porosity and elastic recovery

Porosities (in-die and out-of-die) and elastic recovery (% ERT) were calculated using equations (1) and (2) respectively.

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

$$\boldsymbol{ERT}(\%) = \frac{\boldsymbol{Vf - Vmin}}{\boldsymbol{Vmin}} \times 100 \quad (2)$$

Apparent density ($\rho_{apparent}$) was calculated from tablet's volume (minimal volume under pressure, V_{min} for in-die determination or final volume of tablets, V_f for out-of-die measurement). Powder's pycnometric densities (ρ_{pycno}) were evaluated using a helium pycnometer (Accupyc II 1340, Micromeritics, Mérignac) with a 1 cm^3 cell and a cycle of 20 purges and 20 measurements.

2.5.2. Heckel parameter

In die data were then used to apply Heckel model, in order to determine Heckel parameter (P_y). P_y was obtained from the plot of the natural logarithm of the reciprocal of in-die porosity as a function of compression pressure (Heckel, 1961; Hersey & Rees, 1970). On this plot, a linear regression was performed on its linear part where R^2 was greater than 0.99.

Py was used to estimate the strain-rate sensitivity (SRS) which makes it possible to characterize the influence of compaction speed on material deformation (Roberts & Rowe, 1985). The equation of SRS index is:

$$SRS = \frac{P_{y2} - P_{y1}}{P_{y2}} \times 100 \quad (3)$$

where P_{y1} and P_{y2} are the mean yield pressure for compression cycle of 2000 ms and 70 ms, respectively.

2.5.3. Tablets' mechanical properties

Diametral compression tests were performed using a TA.HD plus texture analyzer (Stable microsystems, Surrey, United Kingdom) at a constant speed of 0.1 $mm.s^{-1}$ and an acquisition frequency of 500 Hz. The tests were performed in triplicate for each condition (compaction pressure and formulation). The tensile strengths (σ) of the tested tablets were then calculated as follows (Fell and Newton, 1970):

$$\sigma(\mathbf{MPa}) = \frac{2\mathbf{F}}{\pi \mathbf{Dh}} \quad (4)$$

with F the force (N) required to break the tablet diametrically, h and D the tablet's thickness and diameter (mm).

Tablets' brittle fracture index (BFI) were determined using the tablets with the flattened geometry to evaluate the deformation behavior under breakage and the sensitivity to a stress concentration (Croquelois et al., 2017; Hiestand et al., 1977). This approach consists in comparing the apparent tensile strength of a tablet with and without a hole at its center. Holes of 1 mm of diameter were introduced at the center of the tablet using a drill as described elsewhere (Croquelois et al., 2017). The BFI was calculated using the following equation:

$$BFI = 0.5\left(\frac{\sigma}{\sigma_h} - 1\right) \quad (5)$$

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

Finally, in order to characterize the viscoelasticity of the tablets, the damping ratio (ζ_A) was determined using impulse excitation tests following the methodology presented by Meynard et al., 2021.

3. Results and discussion

3.1. Freeze-dried trehalose powder properties

The freeze-drying cycle used in this work was performed at a shelf temperature of −20 °C during primary drying. It is well accepted that the shelf temperature should be adapted to maintain, a product temperature (T_p) below the product's critical formulation temperature (T_c) during the steady state of the primary drying, in order to avoid collapse. Moreover, it is classically accepted that T_c is few degrees above T'_g in the case of an amorphous formulation (Tang & Pikal, 2004). T'_g of the trehalose formulation used in this work was observed at −29 °C. This value is consistent with those found in the literature (Bjelošević et al., 2020; Wang, 2000). A shelf temperature of −20 °C combined with a chamber pressure of 0.1 mbar resulted in a T_p of −30 °C at the steady state of the primary drying. This value is lower than T'_g and correlates with the cake appearance, since elegant cakes (Fig. 1) with a slight shrinkage at the bottom of the vials but without any sign of collapse were obtained (Awotwe Otoo et al., 2014).

Cake morphology was also observed by SEM in order to visualize the internal structure. SEM images showed geometrical prism shaped cells of various sizes similar to a sponge-like matrix (Fig. 2a). The variability in pores dimensions between cells is related to the heterogeneity in the ice crystal sizes formed during freezing. In fact, the shelves ramped freezing method used in this study results in uncontrolled nucleation temperature leading to heterogeneous ice crystals formation. The described foam structure may correspond to "open-celled" foam structures or to "close-celled" with an outlet passageway through which water vapors escape during drying (Liu, 2006). SEM images also showed the presence of holes in the walls between adjacent cells (diameter between 5 and 10 μm). Parker et al (2010) observed the presence of such holes for a sucrose containing protein formulation in both conservative and aggressive freeze-drying cycles (Parker et al., 2010). Therefore, the presence of holes may correspond to local dry layer low resistance which would allow water vapor flow through the wall cells during drying (Searles et al., 2001).

FD trehalose cakes were further characterized in order to study their physico-chemical properties. The residual moisture content of FD trehalose measured by Karl Fischer titration method was determined at 0.52 ± 0.06 %. This value is coherent for future applications since a residual moisture content lower than 1 % is often required for the stabilization of biopharmaceutics (Schneid et al., 2011) due to the observation that many degradation reactions are slowed down below this level (Horn et al., 2018). However, overdrying may lead to protein destabilization. Therefore, the process parameters of the freeze-drying cycle can be optimized as a function of the optimal residual moisture stability of the incorporated biomolecule (Schneid et al., 2011; Hsein et al., 2022).

DRX and DSC techniques were used to analyze the physical state and the chemical structure of trehalose FD powders. DRX showed a crystalline state for trehalose before freeze-drying and an amorphous state at the end of the freeze-drying process (Fig. 3). The amorphous state of trehalose after freeze-drying is consistent with the literature and is the main reason for using trehalose as a highly efficient stabilizers during

Fig. 1. Macroscopic aspect of freeze-dried trehalose cake.

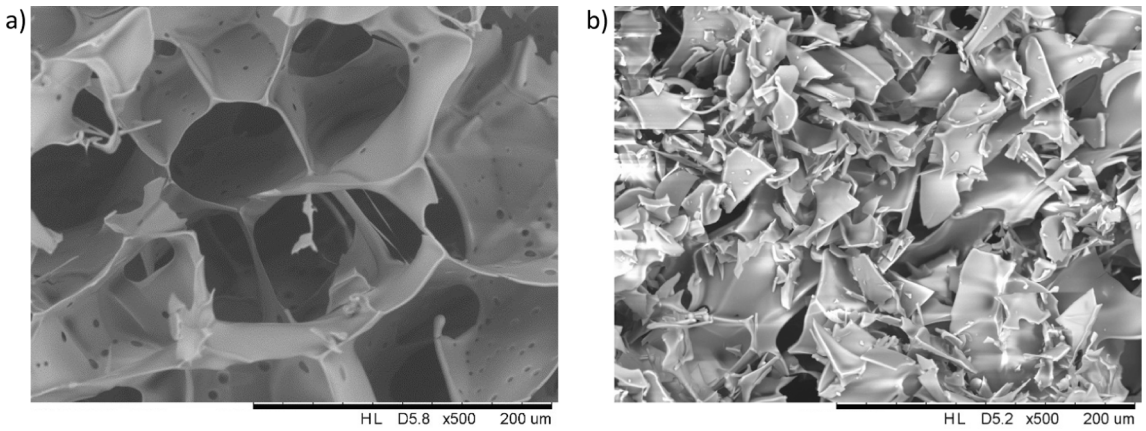

Fig. 2. SEM images of freeze-dried trehalose cakes (a) with sample preparation conserving the sponge like structure, (b) with sample preparation disrupting the freeze-dried cake structure (500× magnification).

the lyophilization process of biological molecules and the following storage (Crowe et al., 1996; Sundaramurthi & Suryanarayanan, 2010).

The DRX results were consolidated by DSC measurements which showed a glass transition temperature for FD trehalose powder (T_g = 103 ± 3 °C), a feature of an amorphous material. This characteristic has to be taken into consideration for FD bioproducts since the glass transition temperatures are related to the physical stability of an amorphous formulation and determine the storage temperature and stability. Below the T_g, biopharmaceuticals should be stable due to limited molecular mobility and high viscosity while above T_g, the molecular mobility increases, thus decreasing stability during storage. It is often suggested that the FD amorphous product should be kept at 50 °C below its T_g during the storage (even if this rule of thumb must be taken with precaution because storage temperature also depends on the formulation) (Duddu et al., 1997; Liu, 2006). In fact, 50 °C below the Tg is often estimated to be the Kauzmann temperature. It is assumed that below the Kauzmann temperature, any translational molecular mobility is completely inhibited. It means that for a given formulation, the biological is optimally vitrified and there also optimally stabilized. Moreover, since water acts as plasticizer, the T_g value decreases when water content increases. That is the reason why, all vials were closed under vacuum in the freeze dryer chamber and opened just before tableting.

Finally, in the conditions of the chosen freeze-drying cycle, FD trehalose presented a pycnometric density of 1.496 ± 0.009 $g.cm^{-3}$ while the crystalline trehalose had a true density of 1.513 ± 0.005 $g.cm^{-3}$. This slight decrease in true density due to amorphization is an expected result (Bookwala et al., 2020) and was also observed by (Bourduche et al., 2020) for maltitol and by (Stange et al., 2013) for sucrose as well as by (Hancock et al., 2002) for a drug substance.

3.2. Physico-chemical properties of FD trehalose tablets

The tablets obtained from FD trehalose powder presented interesting physico-chemical characteristics. First, the DRX and DSC results showed that trehalose remained amorphous after compaction but a decrease in T_g values was observed (T_g in the range of 65 to 78 °C as a function of tested tablet). The decrease in T_g value is most probably due to water uptake during the sample grinding and during the die filling and tableting (tablet moisture content around 4 % as determined by Karl Fischer titration method). A better control of environmental conditions e.g. lower ambient humidity could probably help maintaining higher T_g values if necessary for improving long term stability. The absence of crystallization regardless of the applied pressure is an important indicator which is of great interest for future applications when biomolecules such as proteins will be added to the formulation. These results are in accordance with the results obtained by Eriksson et al (2002) who demonstrated that FD trehalose remained amorphous after tableting when compacts were conserved at 20 °C/0% RH. In addition, they showed that the activity of alkaline phosphatase used as a model protein was only conserved when trehalose tablets remained amorphous (storage at 20 °C/0% RH). A significant or total loss of the protein activity was observed when crystallization occurred respectively for tablets stored at 60 °C/0% RH or when FD samples were preconditioned at 33 % or 45 % RH before tableting (Eriksson et al., 2002).

3.3. Compaction behavior of freeze-dried trehalose powders

3.3.1. Compressibility

Compaction behavior of FD trehalose powders was first evaluated by studying the compressibility profiles. In order to study these properties, the moisture content of the FD powder was an important parameter to take into consideration. Since water acts as a plasticizer, the T_g value of amorphous FD trehalose decreases when water content increases which compromise the physical stability of the produced tablets. In addition, it is established that moisture may influence the compaction behavior of powder and tablets properties such as tablet strength and dissolution rate (Elkhider et al., 2007). Therefore, all vials were closed under vacuum and were stored for maximum two days before tableting. In addition, FD cakes were gently ground using a mortar and a pestle and then quickly filled manually in the die.

Compressibility of FD trehalose was evaluated by plotting the evolution of porosity as a function of applied pressure in-die and out-of-die. Out-of-die compressibility profile (Fig. 4) shows a reduction of porosity to 50 % at an applied pressure of 12.5 MPa and to nearly 1 % at 250 MPa. As expected, in-die compressibility profile showed lower porosity values reaching −1% at 250 MPa since in-die profile includes tablet elastic deformation (Ilić et al., 2013). We can note that this profile is specific of trehalose in the amorphous FD form. Indeed, in-die and out-of die compressibility profiles showed that crystalline trehalose presents significantly lower porosity values specifically for compaction pressures from 12.5 to 100 MPa indicating that trehalose in the crystalline form is more compressible than the FD amorphous form at low pressures. In parallel to compressibility, the internal morphology of the tablets was observed with SEM (Fig. 5a–h). SEM images of broken FD trehalose tablets showed randomly oriented sheets for applied pressures from 12.5 to 75 MPa indicating that the sheet structure of FD powders was conserved after tableting. High porosity inside the tablets was also observed in this range of pressures and the visible porosity decreased with increasing pressure. These observations consolidate the values obtained for out-of-die porosity as a function of axial pressure presented on Fig. 4. It should be noted that above a pressure of 100 MPa, the morphology changes and one can observed that particles start to merge

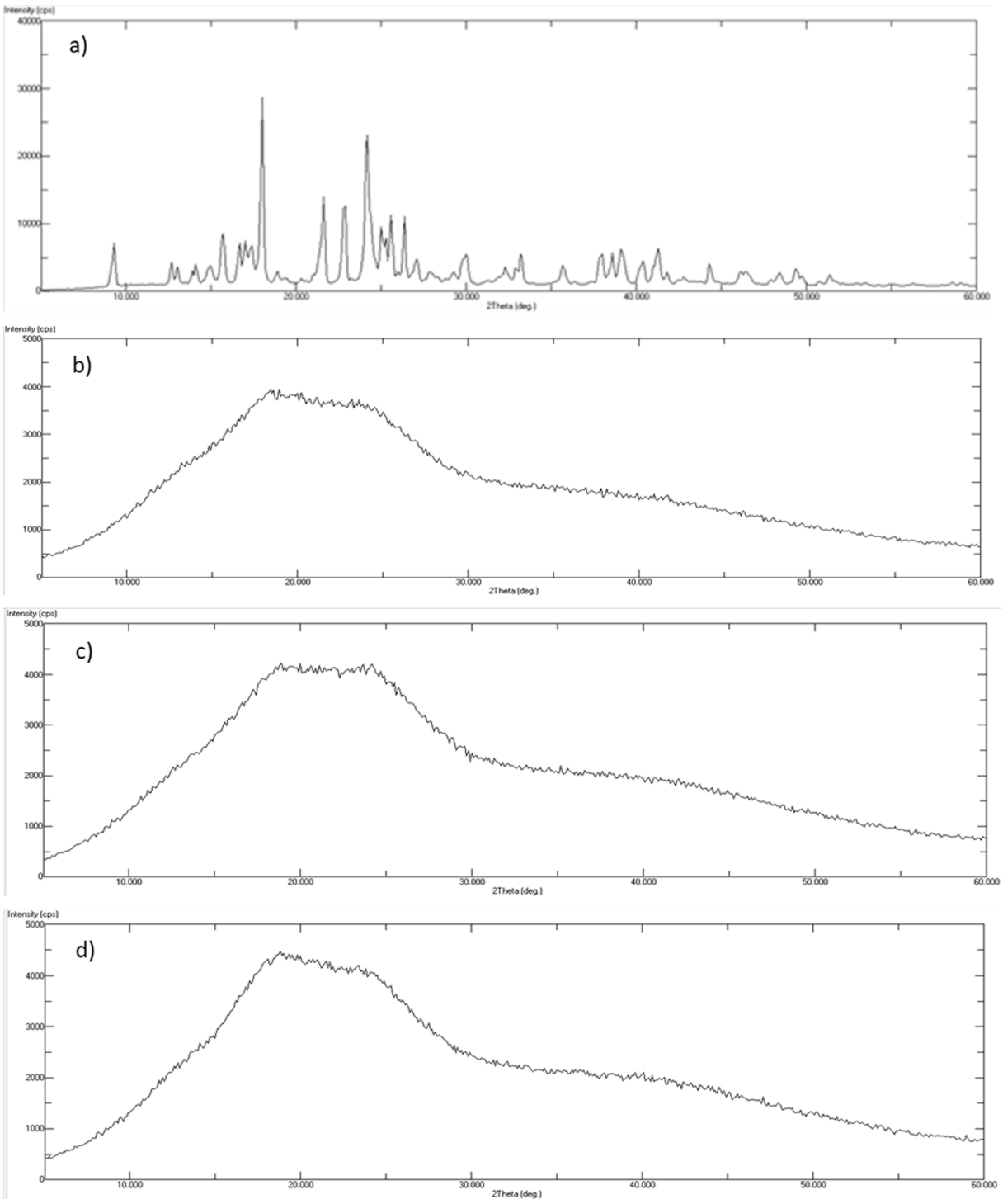

Fig. 3. DRX diffractograms of trehalose powder before freeze-drying (a), FD trehalose powder (b), FD trehalose tablets obtained at compaction pressure of 50 MPa (c) and 150 MPa (d).

resulting in a decrease in tablet's porosity. The preservation of the FD powder morphology inside the tablet as shown in Fig. 5 in addition to the amorphous solid state of tableted FD trehalose (paragraph 3.2) can be an asset for the protection of the structure of incorporated biomolecules.

In a second step, FD trehalose compressibility profile was compared to classical pharmaceutical excipients exhibiting different deformation mechanisms and to some amorphous excipients. Chosen classical pharmaceutical excipients were microcrystalline cellulose, anhydrous calcium phosphate, and granulated lactose monohydrate. FD trehalose exhibited out-of die compressibility profile quite similar to microcrystalline cellulose while crystalline trehalose's compressibility profile was closer to lactose (Fig. 4).

To understand the influence of the compression speed on compressibility for FD trehalose, the strain rate sensitivity was calculated. Results showed a SRS value of 20 % suggesting a sensitivity to compaction speed observed for a plastic deformation mechanism (Roberts & Rowe, 1985). This value of SRS was the highest between the tested excipients, characterized by SRSs of 12.8, 12.7, 5.5 and 3.5 for crystalline trehalose, MCC, Lac and aCP respectively. It was also

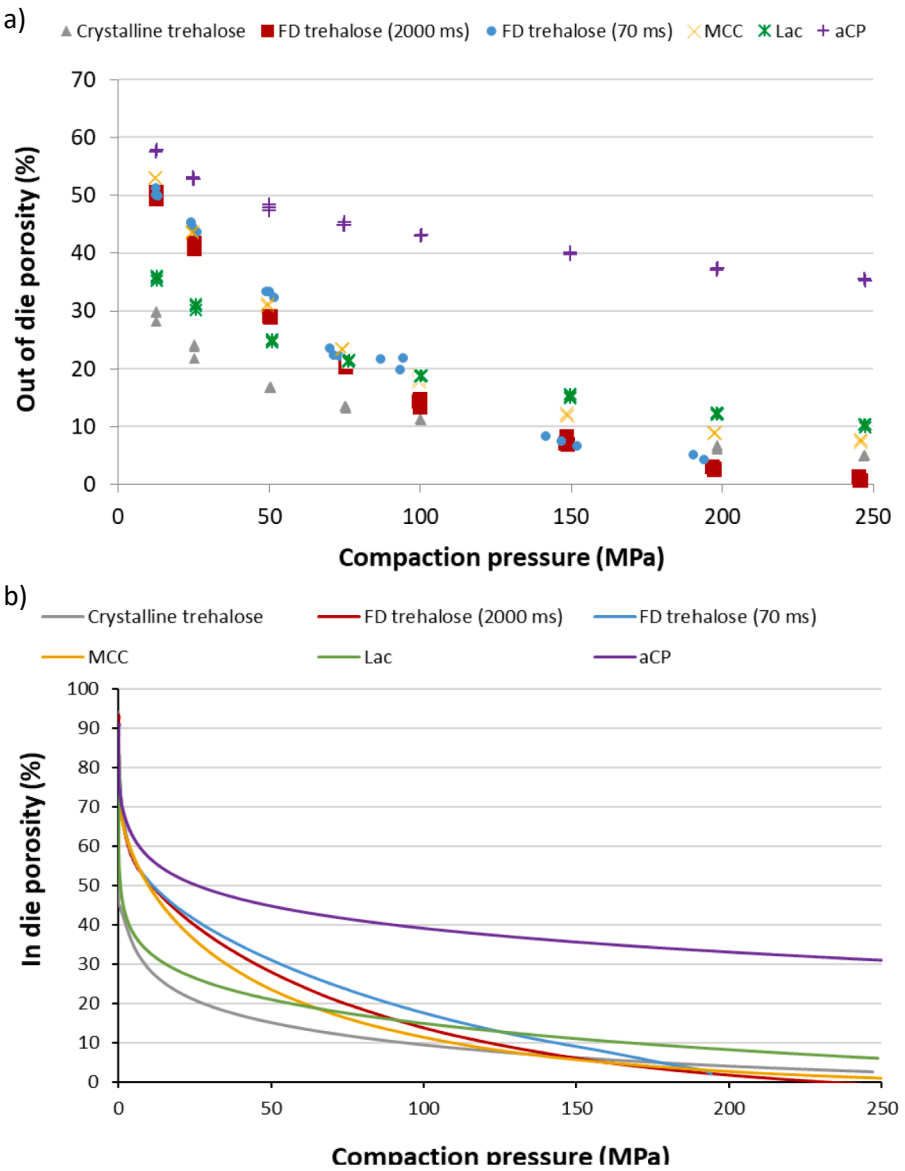

Fig. 4. Out-of-die and in-die compressibility profiles showing the evolution of the out-of-die (a) and the in-die porosity (b) as a function of applied axial compaction pressure. For FD (freeze-dried) trehalose, 2000 ms and 70 ms refer to tableting cycle time, MCC refers to microcrystalline cellulose, Lac refers to lactose monohydrate and aCP refers to anhydrous calcium phosphate.

interesting to observe the differences between the in-die compressibility curves obtained at the two different speeds for FD trehalose. In fact, the curves were superimposed at low pressures, separated at intermediate pressures but tend to overlap again around 200 MPa, which correspond to a pressure were the porosity of the tablet is very low. This kind of behavior is coherent with a viscoplastic behavior (Desbois et al., 2022).

3.3.2. *Plastic energy*

The plastic energy considered is the total energy given to the powder during the compaction cycle (i.e the total area under the curve force vs thickness). The values were directly calculated by the Analis software. As recommended by Wünsch et al. (Wünsch et al., 2021), the energy was normalized by the volume of solid compacted which makes it possible to compare products with different pycnometric densities. This energy is one of the signature of the compaction behavior of a product and it was also recently suggested that a low plastic energy could be related with problems like capping (Meynard et al., 2022; Nakamura et al., 2012). Of course, the plastic energy depends on the pressure used so comparison must be done for tablets made under the same pressure. The values of the compaction energies obtained for the different products for tablets made at 150 MPa are presented in Table 1. Whereas crystalline trehalose had a low plastic energy, similar to Lac, FD trehalose had the highest plastic energy of all the studied products. As previously in the case of compressibility, the freeze-drying process has a huge impact on the plastic energy of trehalose.

3.3.3. *Tabletability*

The tabletability profiles were studied by measuring the tensile strength of tablets obtained at different pressures. Tensile strength (TS) is a crucial tablet property since tablets should reach the patient intact, without any mechanical damage such as breaking, chipping or capping (Yohannes & Abebe, 2021). It is generally considered that tablet TS higher than 1.7 MPa will be sufficient to ensure adequate mechanical resistance to maintain the physical integrity of the tablet throughout the whole process of production, from compaction to packaging. TS as low as 1 MPa could be adequate for small-scale production where the tablets aren't exposed to significant mechanical forces (Mccormick, 2005; Pitt & Heasley, 2013). As shown in Fig. 6, FD trehalose tablets can be

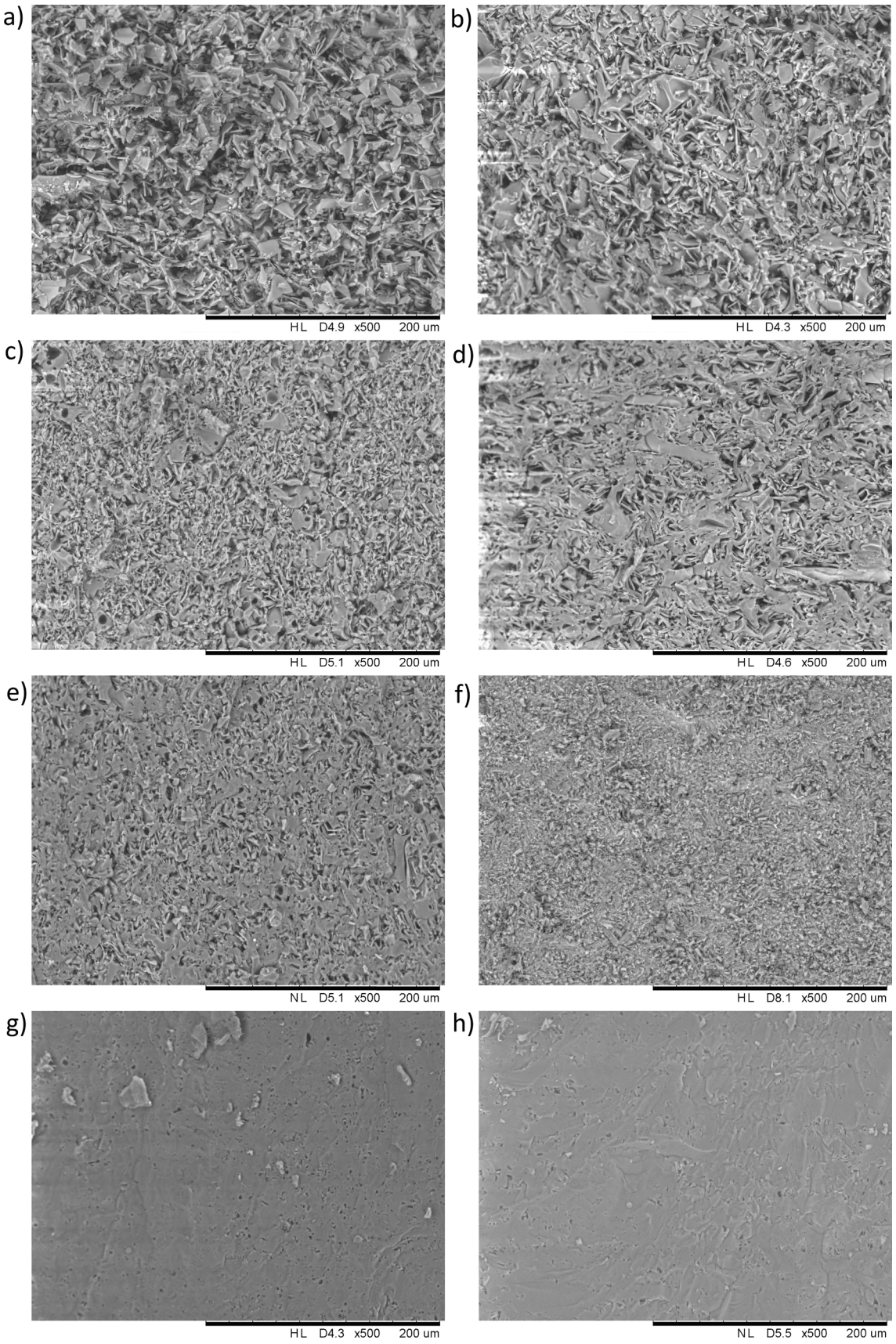

Fig. 5. SEM images of freeze-dried trehalose tablets obtained at 12.5, 25, 50, 75, 100, 150, 200 and 250 MPa respectively (a–h) showing their internal structure (500× magnification).

Table 1
Plastic energy during the compaction cycle (at 150 MPa) for the tested excipients.

Plastic energy ($J.cm^{-3}$)				
FD trehalose	Crystalline trehalose	MCC	Lac	aCP
45.6 ± 0.4	16.4 ± 0.2	38.5 ± 0.1	18.7 ± 0.0	37.1 ± 0.5

obtained under very low compaction pressures with TS of 1.5 MPa and 2.8 MPa for compaction pressure of 25 MPa and 50 MPa respectively. The comparison with the other products studied showed that the tabletability profile is very different from crystallized trehalose. On the contrary it is very close to the one of MCC.

Nevertheless, the behavior of FD trehalose tablets under the test is very different from the one obtained for MCC tablets, both obtained under a compaction pressure of 50 MPa, as shown in Fig. 7. It is well-known that under diametral compression MCC tablets behave in a rather ductile manner, i.e. that the tablet does not behave as an elastic solid until the failure (Gong & Sun, 2015; Mazel & Tchoreloff, 2023; Procopio et al., 2003). This can be observed from the force displacement curves obtained during the test. MCC tablet did not present sudden failure but rather a slow rounding of the curve (Fig. 7). On the contrary FD trehalose presented curves that corresponds to a brittle failure, i.e. to a failure in the elastic domain (Fig. 7). In addition, the FD trehalose tablets obtained under high compression pressures (higher than 75 or 150 MPa depending on the compression speed) exhibited a non conventionnal breakage pattern during the diametral compression test. In fact, not all tablets broke diametrically which led to a decrease in TS as the compression pressure increased and to significant variability in the results obtained.

The tabletability behavior was specific to trehalose in the amorphous FD form since crystalline trehalose necessitated the application of a high compaction pressures in order to obtain compacts with sufficient TS (tablet strength of 1.44 ± 0.02 MPa and 1.79 ± 0.04 MPa at 150 MPa

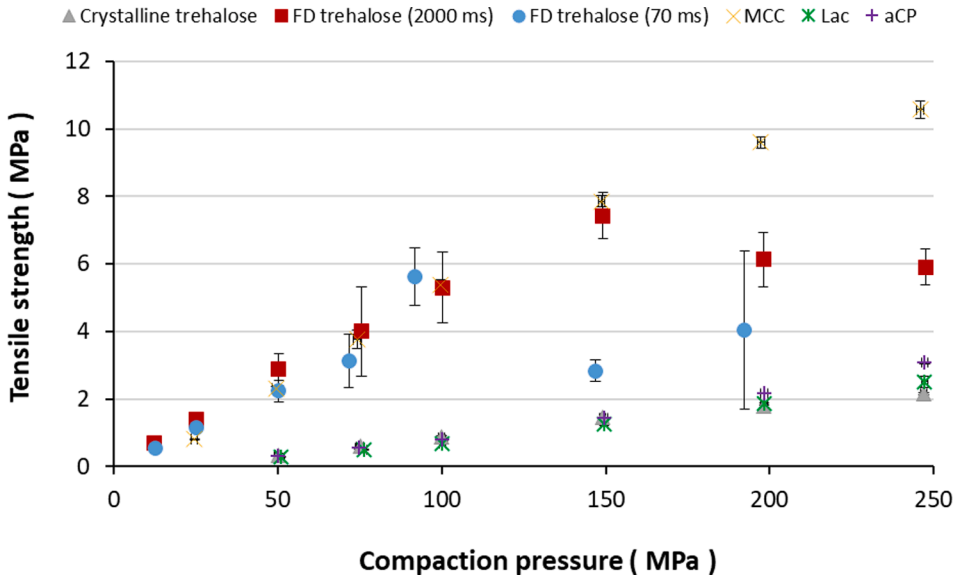

Fig. 6. Tabletability profiles showing the evolution of tensile strength as a function of applied axial compaction pressure. For FD (freeze-dried) trehalose, 2000 ms and 70 ms refer to tableting cycle time, MCC refers to microcrystalline cellulose, Lac refers to lactose monohydrate and aCP refers to anhydrous calcium phosphate.

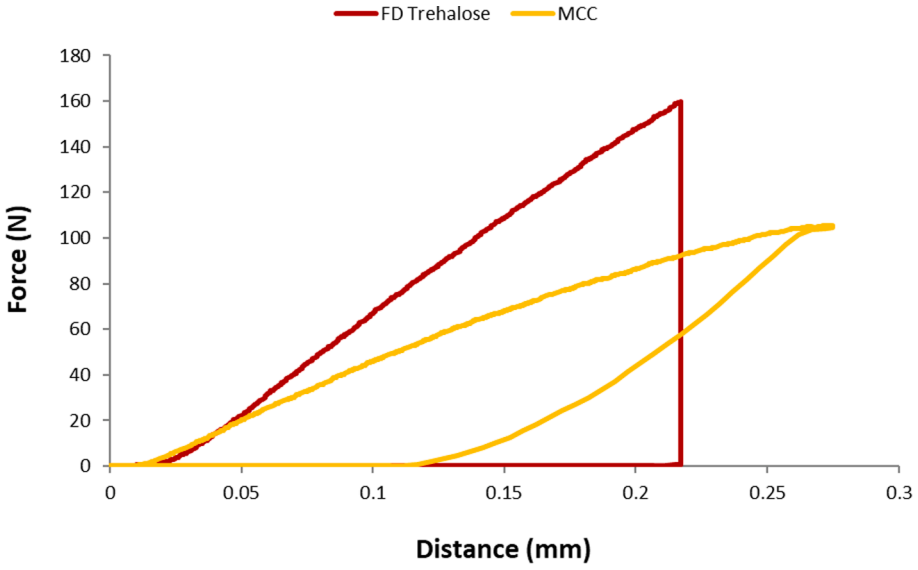

Fig. 7. Force displacement curves obtained during the diametral compression test for FD (freeze-dried) trehalose and MCC (microcrystalline cellulose) tablets obtained at 50 MPa compaction pressure.

and 200 MPa compaction pressure respectively). This difference can be either due to the amorphous state of trehalose or to the specific texture of the FD powder. Indeed, Bourduche et al. (2020) demonstrated that amorphous maltitol presented good cohesion at low compaction pressure unlike to crystalline maltitol (Bourduche et al., 2020).

Finally, the impact of the compaction speed on tabletability profiles was evaluated for some compaction pressure values (25 to 250 MPa for 2 % speed and 25 to 200 MPa for 100 % speed). Considering the variability of the TS obtained it was difficult to see a real difference on the results obtained at low pressures up to 100 MPa. For the two kinetic conditions, trehalose FD showed an extremely cohesive behavior in this range of low compaction pressures. Nevertheless, for high speed compression, TS droped sharply at 150 MPa and at 200 MPa and lamination was observed on the tablets. To counterbalance this lamination effect, tests have been carried out by adding a precompression step. It was found that using a precompression made it was possible to avoid lamination. This defects might thus be classified as lamination type I according to the classification proposed by Mazel et al. (Mazel & Tchoreloff, 2022) i.e. to a lamination due to air entrapment. The drop in TS at 150 MPa could therefore be due to internal defect that are related to air entrapment most probably linked to the sheet texture of the FD powders.

3.3.4. Brittle fracture index

The brittle Fracture index was introduced by Hiestand to characterize the ability of a material to relieve stresses at points of stress concentration and is supposed to be linked with the brittleness of the tablet (Hiestand et al., 1977; Mazel & Tchoreloff, 2023). FD trehalose BFI was determined by testing tablets obtained at the pressure of interest, 25 MPa (tensile strength about 1.5 MPa), and gave a value of 1.12 indicating a high propensity to brittle fracture. This is coherent with the brittleness behavior observed in the previous part of the failure curves. This value is among the highest values that can be found in the literature (Meynard et al., 2022; Okor et al., 1998). It should be noted that MCC tablets obtained under the same pressure gave a very low BFI (Table 2) which is well-reported in the literature and is also coherent with its ductile failure behavior. No comparison can be done with other excipients compressed at 25 MPa compaction pressure, since obtained tablets at 25 MPa are too friable and no hole can be done into the tablets. In order to compare the FD trehalose BFI to the other excipients (crystalline trehalose, Lac and aCP), BFI was measured on tablets prepared at 150 MPa compaction pressure. It can be noted that the BFI normally increases with the pressure (Croquelois et al., 2020). Results showed (Table 2) that BFI values for MCC, Lac and aCP are far below the values of 1.12 obtained for FD trehalose. The BFI value of the FD trehalose was also significantly increased compared to the crystalline form. Then, trehalose in the FD form was the only tested excipient which presented a so high brittleness.

The brittleness of FD trehalose tablets was also observed during the failure test through the failure patterns observed using high speed camera. One example is given in Fig. 8. As it can be seen the central fracture, which is expected in diametral compression test, was present but other failure were also seen and tablets finally failed with multiple fracture pattern. This observation gived a visual confirmation of the high brittleness of the tablets and could also explain the variability observed in the tensile strength measurement of the FD trehalose tablets obtained at high compression pressures.

Table 2
Brittle fracture index (BFI) of tested excipients at different compaction pressure.

	Compaction pressure (MPa)	BFI
FD trehalose	25	1.12
Crystalline Trehalose	150	0.20
MCC	25	0.09
	150	0.18
Lac	150	0.23
aCP	150	0.18

3.3.5. Elastic recovery

Elastic recovery of tablets is also an important feature used in compaction characterization. Indeed, a high elastic recovery is often linked with adverse phenomena like capping or chipping (Hiestand et al., 1977). The elastic recovery as a function of applied axial pressure is presented in Fig. 9 for FD, crystalline trehalose, MCC, aCP and Lac. Below 150 MPa, results obtained on FD trehalose tablets showed that the elastic recovery decreaseed with increasing compaction pressure (from 6 % at 12.5 MPa to 2 % at 150 MPa) and that these values were dependent on the speed of the compression cycle. Then no changes were observed between 150 and 250 MPa. This behavior was different from the one observed for the other products studied. MCC also presented an initial decrease of the elastic recovery, but the values are much higher than those of FD trehalose. It should be pointed out that crystalline trehalose presented a very low elastic recovery around 2 % which is similar to the one obtained for FD trehalose at high pressures (above 150 MPa). These results may be also linked with the differences observed on the in-die versus out-of die compressibility curves.

3.3.6. Viscoelasticity

Viscoelasticity, is one of the components of the strain rate sensitivity. It is also well-known that amorphous products are usually more prone to present viscoelasticity (Alcoutlabi & Martinez-vega, 1999; Deshmukh et al., 2020). This property was studied by measuring the damping ratio based on impulse excitation technique (Meynard et al., 2021). Results are shown in Fig. 10. Whereas crystalline trehalose presented low viscolelasticity like Lac and aCP, FD trehalose gave rise to a viscoelastic behavior with a damping ratio similar to the one of MCC. This increase in the viscoelastic behavior after freeze-drying can be attributed to the amorphization and might also partially explain the influence of compaction speed on the compressibility and the elastic recovery of FD trehalose.

4. Conclusion

This study demonstrated that FD trehalose powder is characterized by a specific and interesting compaction behavior which is different from the other classical excipients. FD trehalose compressibility and tabletability results have shown a behavior that seems to be similar to the one of MCC. The large value of the SRS and the presence of a significant viscoelastic behavior seems to point toward the same direction. Nevertheless, contrary to MCC tablet which behave like a ductile material during breaking tests, the tablets obtained for FD trehalose were very brittle which can be seen from the failure profile obtained during diametral compression but also by considering the BFI value which was very high even compared with results found in the literature.

Another point that differentiate strongly FD trehalose from MCC was the elastic recovery obtained after compression. The elastic recovery of FD trehalose tablets decreased with the pressure and reached values around 2 % which are very low. It is also interesting to note that the amorphous structure of FD trehalose was not affected by the compaction process.

Interestingly, FD trehalose exhibited also a compaction behavior very different from its crystalline form.

This behavior is likely to be linked with the amorphous state of the FD trehalose but also to the microstructure obtained. Nevertheless, further is needed to understand this link.

Finally, this study is to our knowledge, the first one presenting a complete evaluation of the compaction behavior of FD trehalose and demonstrating that it is possible to tablet FD trehalose powder at low compaction pressures which can be of great interest for biopharmaceutical applications since this will limit the mechanical stress undergone by the molecules.

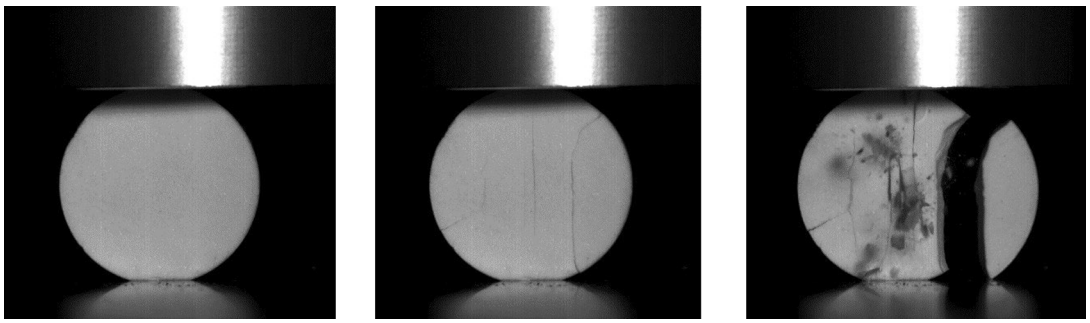

Fig. 8. Different failure patterns observed on freeze-dried trehalose tablets (50 MPa) during the diametral compression test showing the high propensity to brittle fracture.

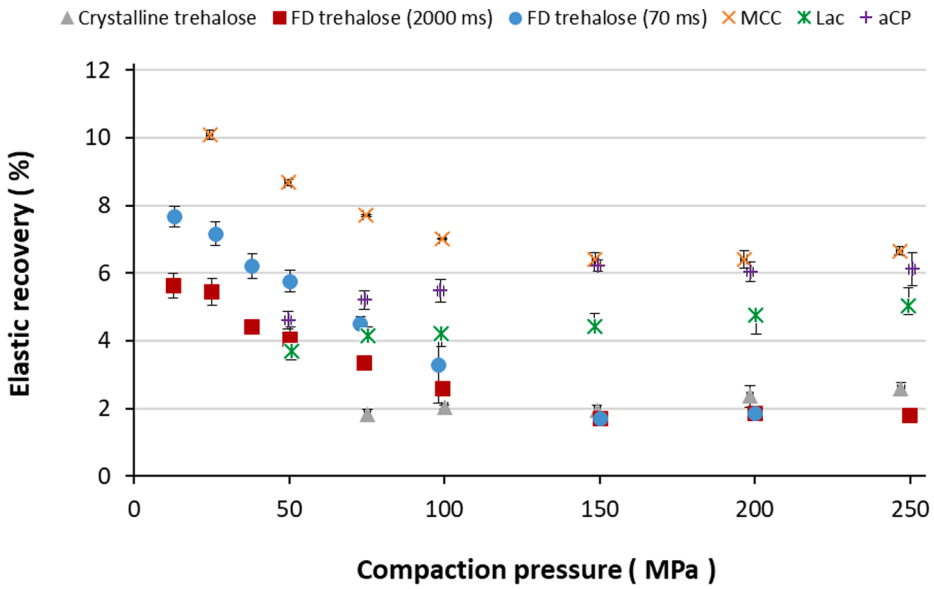

Fig. 9. Evolution of the elastic recovery as a function of axial compaction pressure (for each compaction pressure, mean +/− SD, n = 3). For FD (freeze-dried) trehalose, 2000 ms and 70 ms refer to tableting cycle time, MCC refers to microcrystalline cellulose, Lac refers to lactose monohydrate and aCP refers to anhydrous calcium phosphate.

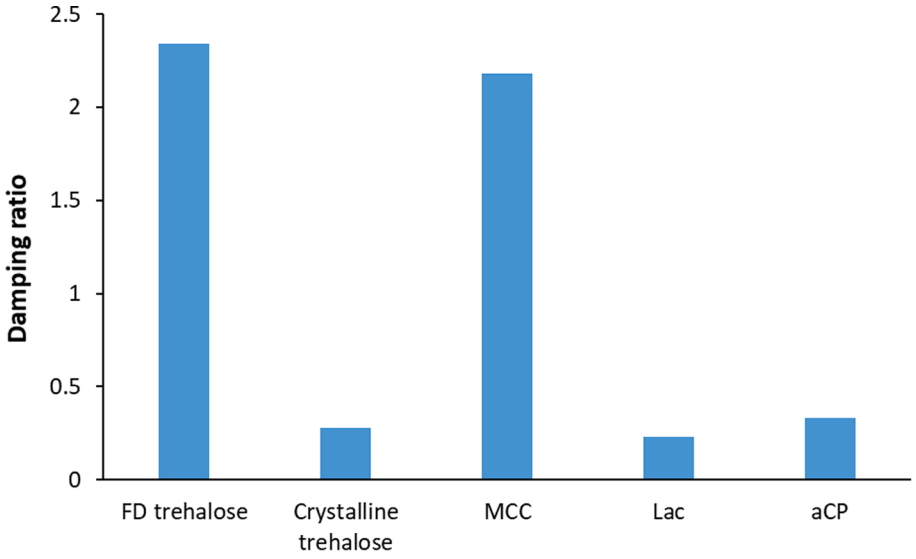

Fig. 10. Damping ratio determined on trehalose and other tested excipients at 150 MPa compaction pressure. FD trehalose refers to freeze-dried trehalose, MCC refers to microcrystalline cellulose, Lac refers to lactose monohydrate and aCP refers to anhydrous calcium phosphate.

CRediT authorship contribution statement

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

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.

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