TRANSFORMATIONS OF CRYSTALLINE SUGARS UPON MILLING

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In this paper we compare the solid-state transformations upon room temperature milling of four sugars: lactose, trehalose, mannitol and sorbitol. The two disaccharides (lactose and trehalose) are found to undergo a direct transformation from crystal to glass while the two sugar alcohols (mannitol and sorbitol) are found to undergo polymorphic transformations. The origin of these different behaviors is discussed and ascribed to the relative position of the glass transition temperature (T_g) of the amorphous states with respect to the milling temperature. This point was also finely studied through co-milling experiments of lactose and mannitol. These two compounds having their glass transition respectively above and below room temperature the T_g of the mixture can be conveniently tuned on either side of the milling temperature by varying the concentrations.

Keywords: disordered solids, glasses, machining, milling, thermal properties of amorphous solids and glasses

Introduction

The possibility to transform the physical state of solids by mechanical milling is well known. This possibility is for instance widely used in metallurgy in order to produced amorphous metallic alloys with low glass transition temperatures [1–3]. Because of their weaker elastic constants molecular material are even more sensitive to milling than metallic compounds. However, they have been much less studied. The pharmaceutical literature abounds of examples of amorphisation and polymorphic transformations induced during the milling of drugs and excipients. Many of them have been reported by Boldyrev *et al.* [4]. The perfect control of these transformations would be highly valuable since it would avoid the formation of undesired physical states like weakly soluble forms or unstable amorphous state. Moreover it would open new formulation routes able to place drugs in highly active therapeutic states unreachable by the usual formulation routes. Co-milling is also actually used to obtain co-crystals of pharmaceutical ingredients [5] which are generally both more soluble than pure crystalline forms and more stable than the amorphous states. Despite the great number of observed effects of milling, the nature of the end product of milling is highly unpredictable and the physical parameters which drive the transformation are not yet clearly identified.

In this paper we contribute to this research field by investigating the transformations upon milling of several sugars widely used in the pharmaceutical industry. Four sugars have been studied: two disaccharides (anhydrous trehalose and lactose) and two sugar alcohols (sorbitol and maltitol). These sugars mainly differ by the position of the glass transition temperature (T_g) of their liquid-state with respect to the milling temperature (i.e. room temperature). We have thus taken advantage of this difference to investigate the role of the glass transition in the nature of the transformations undergone upon milling. The role of T_g could also be finely studied through co-milling experiments of lactose and mannitol. Since these two compounds have their glass transition temperatures respectively above and below room temperature, the glass transition temperature of the mixture can be continuously tuned on either side of the milling temperature by varying the concentrations.

Experimental

Crystalline anhydrous α-lactose was obtained by dehydration of crystalline monohydrate α-lactose purchased from Sigma. Dehydration was performed by blowing dry gaseous methanol through 20 g of a lactose monohydrate during 3 h.

Crystalline α–α anhydrous trehalose, mannitol-β and sorbitol-Γ were purchased from Fluka. Their purity are respectively 99.9, 99.9 and 99.5% and they were used without further purification.

Ball-milling experiments were performed in a high energy planetary mill (Pulverisette 7-Fritsch) at room temperature. Samples (1 g of powder) were sealed in zirconium vials of 45 cm^3 volume with seven balls (∅=15 mm) of the same materials so that

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the ball to sample mass ratio was 75:1. The rotation speed of the solar disk was set to 400 t min^{-1} which corresponds to an average acceleration of the milling balls of 5 g. To avoid any overheating of the samples, milling periods were alternated with pause periods. The apparatus was specially modified to mill hygroscopic samples in a controlled atmosphere. Prior to the milling procedure, the vials were flushed with dry nitrogen gas in order to prevent hydration of the powder during the experiments.

The powder X-ray diffraction (XRD) experiments were performed with an Inel CPS 120 diffractometer (λ_{CuK_α}=1.540 Å) equipped with a 120° curved position sensitive detector coupled to a 4096 channel analyser. The samples were placed into Lindemann glass capillaries ($\varnothing$=5 mm).

Differential scanning calorimetry (DSC) and temperature modulated DSC (TMDSC) experiments were performed with the DSC 2920 instrument from TA. For modulated DSC experiments, a modulation of ±0.796°C min^{-1} was applied. During all the measurements the sample was placed in an open cell (container with no cover) and was flushed with highly pure nitrogen gas. Temperature and enthalpy readings were calibrated using pure indium at the same scan rates used in the experiments.

Results and discussion

Milling of crystalline anhydrous disaccharide trehalose

At room temperature, this sugar is totally amorphized after a 20 h milling process. Figure 1 demonstrates that, not only the compound is X-ray amorphous, but also really glassy since a glass transition is found to occur in the DSC scan at T_g=120°C. This indicates that the sample has undergone a direct transformation

from crystal to glass upon milling. The T_g value is similar to that obtained upon heating the quenched melt. However, while the quenched liquid does not recrystallized upon heating, the glassy state obtained upon milling recrystallizes totally for the same heating rate. This difference is generally attributed to the existence, in the milled sample, of residual nuclei of the starting crystalline phase which have survived to the milling and which trigger the crystallization upon heating. These remaining nuclei disappear for further milling of the apparently amorphous sample. Figure 1b shows that after a 100 h milling process any trace of crystallization could be detected upon heating while the C_p jump at T_g persists. This indicates that fully amorphous trehalose with thermal properties similar to those of the quenched liquid can be reached through a long enough milling process.

Milling of crystalline anhydrous disaccharide lactose

Much interesting information concerning the solid-state amorphization process upon milling could be obtained through the study of lactose. Anhydrous lactose presents at the molecular level two isomeric forms (α and β). A conversion (mutarotation) from α to β is known to occur in a variety of liquid solvents [6]. This results in a rich variety of crystalline forms [7]: anhydrous α [8, 9] and β [10] pure forms and several α/β defined molecular compounds [11–13]. However it has been reported that the mutarotation is not observed when heating the stable anhydrous α crystalline form [14]. We showed [15] that a quasi pure α-anomeric form of lactose in an amorphous state can be obtained by ball milling of the α crystalline form under dry nitrogen atmosphere. Figure 2 demonstrates that the milled compound is X-ray amorphous and has also a real glassy character since a glass transi-
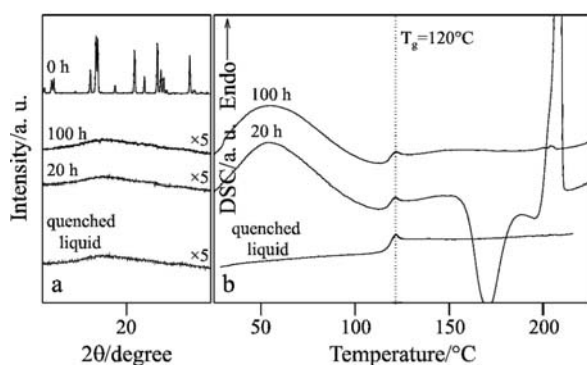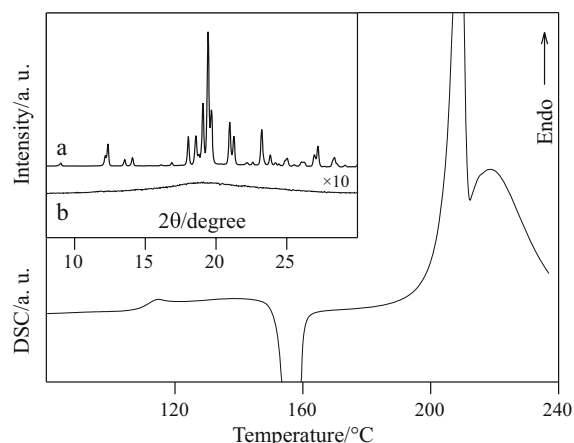

Fig. 1 a – X-ray diffraction patterns recorded at room temperature for the quenched liquid trehalose, and initially crystalline trehalose milled during 0, 20 and 100 h; b – DSC heating curves (5°C min^{-1}) for the quenched liquid trehalose and initially crystalline trehalose milled during 20 and 100 h

Fig. 2 DSC heating curves (5°C min^{-1}) of crystalline lactose after 30 h of milling treatment. The inset shows X-ray diffraction patterns of lactose recorded at room temperature a – before milling and b – after the 30 h milling treatment

tion at $T_g \cong 110°C$ (heating rate=5°C min^{-1}) is found to occur on the DSC scan upon heating the milled material. The mutarotation reaction of lactose in the solid-state after amorphization by milling could be quantitatively measured in situ by solid-state ^{13}C NMR. This allowed to characterize finely the nature of the amorphous state and also to get an indication about the temperature which has been really reached upon milling. It thus offers the opportunity to test the hypothesis that the amorphization would be the result of local melting.

Upon heating the shear amorphized anhydrous lactose, the mutarotation was seen to occur progressively. The evolution of the β-lactose fraction upon heating is shown in Fig. 3. The fraction increases with temperature to reach the value of 50% just above T_g at about 132°C. This value remains unchanged when further heating and also when cooling down the sample before recrystallization could occur. In this case, an amorphous α/β lactose is obtained. Several important information emerge from this set of experiments.

- It is possible to amorphize (to vitrify) lactose directly at low temperature by a dynamical shearing process.

- The glass which is obtained by this route is in a pure α anomeric form. It thus differs structurally from those which are obtained by cooling the metastable liquid below T_g, by lyophilisation or also by spray drying which all give rise to a nearly equimolar amorphous mixture of α and β lactose. It must be noticed that quenching the liquid itself could only produce a caramelized black sample. Milling is the only process able to produce amorphous α-lactose which has the appearance of a white powder of a non chemically damaged compound.
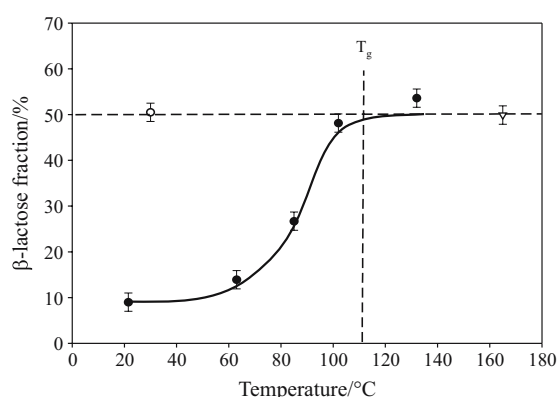

Fig. 3 Temperature evolution of the β-lactose fraction in the amorphous state measured by ^{13}C NMR (filled circles) in the course of a heating process from rT to 132°C. The down triangle corresponds to the α/β ratio after recrystallization towards the 1:1 α/β compound upon further heating. The open circle corresponds to the α/β ratio after cooling again the amorphous sample down to rT. The solid line is a guide for the eye

- Importantly, the anomeric purity of the glass obtained by milling indicates that the amorphization is not associated to a local heating/quenching process as it is often invoked to explain this kind of amorphization. Neither the melting point nor the glass transition temperature were reached during the milling process since temperature higher than T_g would have triggered unavoidably the mutarotational kinetics in solid-state lactose. The mutarotation which is a local chemical process is thus expected to be strongly coupled to the cooperative structural relaxations in the amorphous state. The latter relaxation which becomes more rapid on heating up to T_g was however never fast enough to trigger the chemical process. The structural modification was thus really generated by dynamical shearing occurring far below the melting point. The process appears to be able to generate an amorphous glass which differs from that obtained by a thermal quench. This glass is also strongly out of equilibrium since it can not be recovered after a thermal excursion at a higher temperature.

Milling of crystalline D-sorbitol [16]

Figure 4 shows that milling the crystalline form Γ of *D*-sorbitol during 10 h at room temperature (i.e. above T_g=0°C) promotes a progressive conversion to the metastable crystalline form A. The X-ray analysis of the nanostructure evolution upon milling has shown a clear reconstruction of the ultimate crystallites. In the first stage of milling (t<1 h) a broadening of the diffraction peaks reveals a stage of nanostructuration of the crystallites. This is confirmed by the decrease of the melting temperature of the Γ phase according to the Gibbs Thomson law. Then the peaks of the new polymorph appear (t>2 h) and are also broad. After a long enough milling, the Bragg peaks of the appearing new form progressively become very sharp. This shows that milling is able that time to promote a total reconstruction of the new long range order on a large microstructural scale. It is not totally clear if the transformation between the two polymorphs involves or not a transient amorphization stage. An amorphization is suggested by the fact that some transport of matter is necessary for the crystalline reconstruction to operate. However there is no direct evidence of such a stage through the appearance of an amorphous halo in the X-ray pictures or through a glass transition event in the thermograms. This is certainly a very important point to elucidate in order to know if a direct solid state reconstruction induced by milling is possible. If not, a mechanism of nucleation and growth of crystals in the metastable liquid would prevail. In such a case it would be necessary to
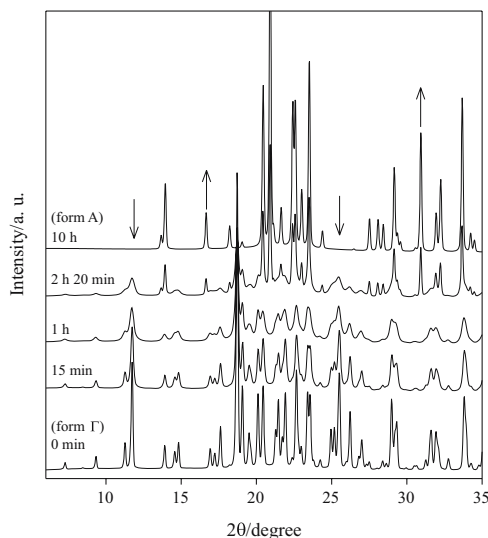
Fig. 4 Evolution of the X-ray diffraction patterns of sorbitol (Γ crystalline form) during a 10 h milling process. It shows the progressive conversion of the Γ phase towards the A phase

accommodate the very weak fraction of liquid that may be assumed from experiments with the slowness of the kinetics of the process.

Milling of crystalline mannitol [17]

Mannitol has at least three crystalline polymorphic varieties [18]. As sketched in the inset of Fig. 5b these phases are named δ, α and β as a function of increasing stability at room temperature. Figures 5a and b represent the evolution of the diffraction pattern during milling of the phases β and δ, respectively. In both cases, the X-ray diffraction pattern of the final state is characteristic of the phase α which is the phase of intermediate physical stability. Over and above the identity of the final states this result demonstrates that milling can drive the system either to a more stable or to a less stable state. In both cases it induces a total crystalline reconstruction. However, in both cases the form α obtained by milling slowly reverses toward the stable crystalline form β within a few days after the end of the milling treatment.

Mechanical alloying of molecular compounds

In this set of milling experiments we have co-milled crystalline powders of α-lactose and mannitol (form β). The thermodynamic characteristics and behaviors upon milling of these two compounds have been described above. They are in particular characterized by different values of their glass transition temperatures which are respectively above and below the milling temperature (room temperature). The practical objective of this investigation is to test
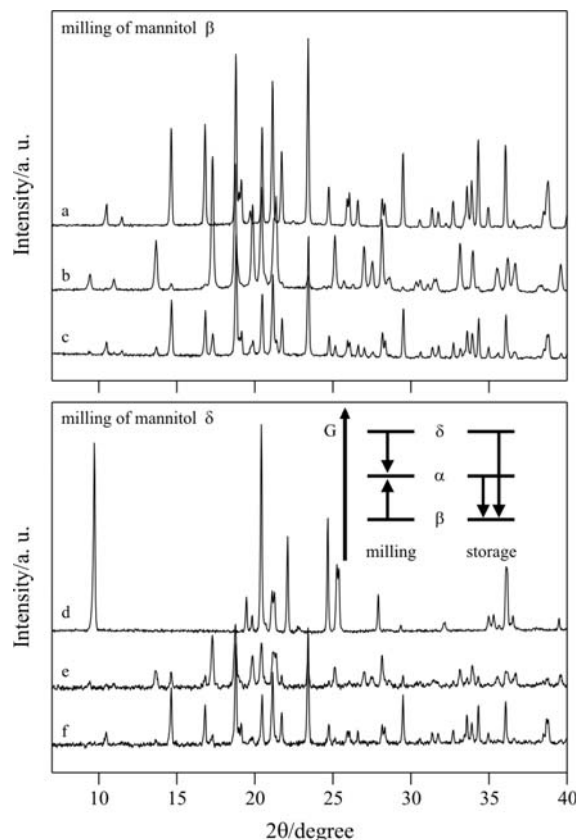

Fig. 5 X-ray diffraction patterns of mannitol recorded at room temperature: a, b and c – correspond respectively to mannitol β before milling, after milling (3 h), and after a subsequent *rT* storage (8 days). d, e and f – correspond respectively to mannitol δ before milling, after milling (3 h), and after a subsequent *rT* storage (1 day). The inset sketches the relative stability of the forms δ, α and β according to their Gibbs free enthalpy *G*

the possibility to directly prepare molecular alloys in the solid state i.e. with no melting of the initial compound. An other objective is to further investigate the effect of temperature on the end product of milling by changing the composition of the mixtures and thus the expected value of T_g of a possible glassy alloy with regard to room temperature.

Figure 6a shows X-ray diffraction patterns of lactose/mannitol mixtures for different mannitol fractions (X_{man}) and after 12 h of co-milling. For $0.00<X_{man}<0.39$ the co-milled samples appear clearly X-ray amorphous suggesting a total amorphization of the sample. On the other hand, for $X_{man}>0.39$ increasing Bragg peaks persists in the X-ray diffraction patterns. These Bragg peaks do not disappear for longer milling times and they are characteristic of the form α of mannitol which is that obtained when milling the form α of mannitol. This indicates that for concentrations of mannitol higher than $X_{man}=0.39$ the amorphization of lactose is total while that of mannitol is only partial. All these samples were also

characterized in the course of TMDSC heating (5°C min^{-1}) experiments. Figure 6b shows the reversible part of the TMDSC signal for the X-ray amorphous samples i.e. for $0.00<X_{man}<0.39$. The results clearly reveal a single glass transition located between those of the pure compounds. Such a single glass transition indicates that the amorphous mixed sample obtained by milling is characterized by a single relaxation process. The mixing of the two kinds of molecules has thus really been performed at the molecular level, giving rise to a homogeneous glass solution formed in non equilibrium conditions. The glass transition temperature of these homogeneous solutions decreases for increasing mannitol concentrations revealing an increasing mobility and thus the plasticizing effect of the mannitol molecules. For X_{man} higher than 0.39 the decrease of T_g get arrested and the amplitude of the C_p jump progressively vanishes as crystalline mannitol α develops to the detriment of the amorphous glass solution. An interesting point is that the previous behaviour starts when the T_g of the alloy approach 60°C which is close to the milling temperature. This temperature, while not accurately known, is obviously located a few degrees above room temperature because of the heat given off by the milling device itself and by the chocks of the balls in the milling jars. In these conditions, the molecular mobility of the alloy is expected to become high enough to trigger the crystallisation of the chemical component which is responsible for the plasticization of the alloy during the co-milling process. Further amorphization of mannitol is thus probably counterbalanced by a rapid

recrystallization towards the form α leading to a steady state concentration of mannitol in the glass solution. This mechanism is coherent with the fact that the phase α is known to arise systematically from the recrystallization of the undercooled liquid mannitol.

Conclusions

From the results presented in this paper, it appears that the nature of the transformation induced by milling depends on the relative position of the glass transition of the milled compound with respect to the milling temperature. In particular, an amorphisation is observed for milling performed at low enough temperature below T_g while a polymorphic transformation occurs for milling performed above T_g. This suggests that the whole set of experiments can be interpreted in the framework of the driven material concept proposed by Martin and Bellon [1]: at low enough milling temperature the thermally activated diffusion coefficients are expected to be less important than the ballistic ones imposed by the shocks during milling. As a result the effective temperature (T_{eff}) under milling becomes higher than the equilibrium melting temperature. This may explain the observed amorphization process. In the case of the amorphization of lactose which can be explained in the same way we have however seen that this temperature is only 'effective' in the sense that it has not been really felt by the compound since no mutarotational changes could be detected. It is more difficult to explain the observed conversion between crystalline polymorphic varieties in the frame of the initial version of the driven material model. For all cases which display such a transformation – i.e. sorbitol, mannitol – there is no direct equilibrium solid state transition at temperatures higher than that of milling. One can thus not expect to reach such a transition by increasing the effective temperature T_{eff}. A simple view would be to suspect an evolution of T_{eff} according to which the first step would be an easy amorphization immediately followed by a very rapid recrystallization toward a stable or metastable polymorph. Some trace of transient amophization was already detected in the course of the polymorphic transformation of indomethacine [19] and fananserine [20] upon milling. On the other hand, such amorphization traces could not be detected during the transformation of mannitol and sorbitol upon milling. In this case the generalized version of the driven material model by Enrique and Bellon [21] which predicts the possibility to create specific steady state patterns could certainly be used to explain the change of the structural and microstructural states under milling.
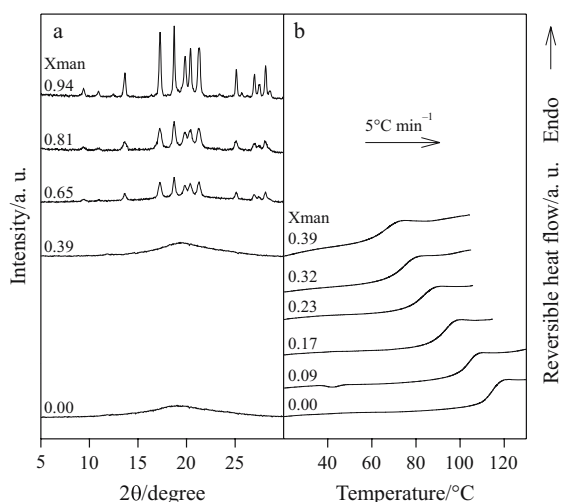

Fig. 6 a – X-ray diffraction patterns recorded at room temperature for different mixtures lactose/mannitol co-milled during 12 h. b – reversible part of the TMDSC signal recorded upon heating (5°C min^{-1}) different mixtures lactose/mannitol co-milled during 12 h. The molar fraction of mannitol for each mixture is reported on the left hand side of the scans

Acknowledgements

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Boldyrev's Special Chapter has finished.