Correlation between molecular dynamics and physical stability of two milled anhydrous sugars: lactose and sucrose

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Abstract

The process of milling often results in amorphization and the physical stability of amorphous phase is linked with its molecular dynamics. This study focuses on a propensity of two disaccharides (lactose and sucrose) to amorphize on ball milling and the stability of the resultant amorphous phase. The amorphous content in milled sugars is estimated by Differential Scanning Calorimetry (DSC)and the stability was measured in terms of the tendency to recrystallize by Broadband Dielectric Spectroscopy (BDS).The results show that the amorphous content increases with milling time and is greater for lactose than sucrose. At the same degree of amorphization, sucrose devitrifies at temperature ~15 °C higher than lactose, indicating higher stability. The molecular dynamics (beta relaxation process), suggest that milled sucrose is more stable with higher activation energy (~9 kJ mol^{-1}) than that of lactose. The moisture content of amorphous phase also impacts its molecular dynamics in terms of increase in activation energy as the moisture decrease with increasing the milling times. The study suggests a greater stability of amorphous sucrose and susceptibility of milled lactose to recrystallize, however, on extended milling when the moisture content decreases, lactose was seen to become relatively more stable.

Keywords: Amorphous, molecular dynamics, stability, milling, relaxation time.

Abbreviations: Broadband Dielectric Spectroscopy (BDS), Differential scanning calorimetry (DSC).Relaxation time (τ), Activation energy (E$_a$), Fröhlich parameter B(T)

1 Introduction

Amorphous solid are produced in pharmaceutical industry by quench cooling of melts,(Angell, 1995) freeze drying,(Liu *et al.*, 2002) spray drying,(Gharsallaoui *et al.*, 2007) precipitation from solution,(Li *et al.*, 2000) vapor condensation(Kearns *et al.*, 2007) and melt extrusion(Crowley *et al.*, 2007).However, certain processes (including milling, a common process used for particle size reduction)causes the unwanted generation of amorphous phase(Willart *et al.*, 2004).

The amorphous phase has the advantage of increasing the super-saturated solubility and dissolution rate of poorly soluble drug substances owing to its high surface free energy and enormous surface area (Dudognon *et al.*, 2006; Hancock *et al.*, 2000; Murdande *et al.*, 2010). At the same time, this phase is metastable and may ultimately revert back (i.e. recrystallize) to its original crystalline form, or even a different crystalline form, thus compromising the stability and efficacy of the product during its shelf life(Fisher, 2007). The amorphous phase produced during milling is probably the most unstable because of the presence of residual crystallites that act as nuclei for crystallization. Therefore the benefits of milling could only be achieved until the post-milling crystallization is prevented, for example by co-milling with an agent that stabilizes the amorphous phase.

The physical stability of amorphous phase is related to its molecular mobility which itself is defined by glass transition temperature (T_g), the temperature above which long range co-operative molecular mobility is manifest owing to the high internal energy and specific volume, thereby creating opportunities for the material to crystallize.

There is a range of techniques available for studying the molecular mobility and thus stability of the amorphous phase. Parameters such as structural relaxation time, enthalpy relaxation time, glass transition and/or fragility index (obtained from dielectric spectroscopy, DSC or isothermal micro-calorimetry techniques) have been used to provide certain measures of molecular mobility(Aso *et al.*, 2000; Graeser *et al.*, 2009; Kawai *et al.*, 2005; Pokharkar *et al.*, 2006; Rodrigues *et al.*, 2013).Recently, T_g of β-relaxation (Kissi *et al.*, 2018) and THz spectroscopy (Sibik *et al.*, 2015; Sibik *et al.*, 2016)have also been employed to study molecular mobility and crystallization dynamics of amorphous phase. Interestingly, it has also shown that there are certain energy barriers behind this molecular dynamics of the disordered amorphous phase (Ruggiero *et al.*, 2017).

In dielectric studies the dynamics of amorphous materials is usually described by primary and secondary relaxation processes; the former represents the structural (α-) relaxation process which is observed in all disordered materials while later can be described as Johari-Goldstein (β- relaxation) and non- Johari- Goldstein (sometimes termed as γ-relaxation)(K. Kaminski *et al.*, 2008).Of these processes, it is the structural relaxation (in terms of the characteristics of α-relaxation) that is expected to reflect a degree of freedom/ molecular mobility that underpin the crystallization process (and hence instability) of materials containing an amorphous phase(Kolodziejczyk *et al.*, 2013; Rodrigues *et al.*, 2013).There are many studies in literature that show the correlation between molecular dynamics and crystallization; these are discussed below.

Zhou et al.,(2002) in their study on amorphous pharmaceuticals demonstrated that the molecular mobility along with the configurational entropy are important parameters in determining the physical stability of the amorphous phase(Zhou *et al.*, 2002). Whereas, Bughra et.al, (2007) from the correlation between crystallization onset time and dielectric relaxation time (of the α- process at temperatures above T_g)suggested that the molecular mobility can be used for the prediction of stability(Bhugra *et al.*, 2007). Another study has suggested the involvement of some other factor in addition to global mobility i.e. α- process that predicts the instability in amorphous systems (Bhardwaj *et al.*, 2013).

In spite of relation of α- process to instability of amorphous phase, its exploitation is compromised, as it is sometimes difficult to clearly observe the structural relaxation in some materials (i.e. sugars) at temperatures above T_g due to the contribution from ionic conductivity in quenched systems(K Kaminski *et al.*, 2008). It was also ascertained by Ermolina et al. (2011), that the structural relaxation of freeze dried amorphous disaccharides is masked by the percolation of protons and dc conductivity within the hydration shell on the extensive surface area of such material(Ermolina *et al.*, 2011).

Therefore, various attempts have been made to find some other features of the dielectric relaxation behavior that may have some relation with the structural relaxation, and might therefore be used to predict the stability of the amorphous phase.

Alie et al., (2004), in their dielectric relaxation study of a quench cooled amorphous phase of a proprietary drug molecule, has suggested that it is the beta relaxation process (the Johari-Goldstein β-relaxation) not alpha that defines the specific molecular dynamics and underpin the physical stability of the amorphous phase (Alie *et al.*, 2004).This Johari-Goldstein process can be clearly identified by subtraction of dc conductivity and annealing(Bhardwaj *et al.*, 2011).

There are many other studies that demonstrate the faster secondary relaxation has in fact a close relation to the structural relaxation. Evidence for this comes from Paluch et al, (2003)who showed that the β- process deviates from Arrhenius behavior in a similar way to the α-process(Paluch *et al.*, 2003) while Ngai et.al.2004 have shown that the activation energy of this process correlates well with the dynamic glass transition which is determined from the α-relaxation, when τ ~ 100 s(Ngai *et al.*, 2004). In the other studies Kaminski et. al., 2011 and Ermolina et. al., 2011 have shown that the β- process mimics the features of structural relaxation in terms of its response to pressure (i.e. shift towards lower frequencies by increasing pressure) or moisture (the activation energy of the beta process decreases with increasing the moisture) respectively (Ermolina *et al.*, 2011; K Kaminski *et al.*, 2006). Hence the beta relaxation might be considered in some way as a precursor of structural relaxation, possibly in the sense that the cooperative unit of the beta process represents the minimal scale length for the otherwise more long range cooperative process. Certainly, it has been suggested that the activation energy of the beta process is the indicator of flexibility of the molecule, i.e. the greater the value of the energy barrier, the more inflexible is the molecule(K. Kaminski *et al.*, 2012) i.e. fewer its degrees of freedom.

Another fact concerning the dielectric studies of amorphous phase is that in the majority (~95%) of these cases the amorphous phase was usually produced by quench cooling of the melt; a procedure which is not commonly applied in the pharmaceutical industry as it may degrade the material. Considering the fact that the method of preparation influences the molecular mobility of the amorphous phase as previously stated(Surana *et al.*, 2004)there is a need to study the specific molecular dynamics of amorphous phases, as they are generated by unwantedly during milling in order to have any chance to understand and even predict the stability of such materials after milling. +9

Another important aspect to consider when milling crystalline materials is the fact that, the amorphous phase inevitably contains some quantity of moisture (either generated from within the material in case of crystalline hydrates or adsorbed from the environment). A recent study on ball milled indapamide(Wojnarowska *et al.*, 2013) has suggested the presence of a water relaxation peak rather than the typical relaxation processes of amorphous phase. The distribution of this water affects the molecular dynamics of the amorphous phase and is therefore important in defining the stability of this phase. Therefore the cooperative molecular dynamics of this amorphous phase, as modulated by the so called 'plasticization' effect of water (Gupta *et al.*, 2013) may be of greater relevance to the stability of amorphous system obtained by milling than on quench cooled systems.

In this work, our aim is to quantify the impact of milling on the amorphization of two anhydrous disaccharides (lactose and sucrose) and to elucidate the molecular mechanisms underlying the stability of the amorphous phases. In the first part of the study DSC was used to investigate the extent of amorphization of crystalline materials as a function of milling times and the relative stability of the amorphous phase determined via an evaluation of the onset temperatures

for recrystallization. In the second part of this study, an assessment of possible contribution of the molecular dynamics was made. For this purpose BDS (in terms of activation energies for the gamma and beta processes) was employed. The results from DSC experiments were compared to the onset temperature of recrystallization obtained by BDS to establish whether its parameters can be used, in part to predict the relative stability of milled amorphous sugars. This may help to establish the approaches for the prediction of the stability of milled pharmaceuticals, in general.

2 Materials and Methods

Lactose and sucrose used in this study were purchased from Fluka (UK) and used as received from the supplier. Lactose occurs as a white crystalline powder with finely divided particles (<100 µm) while sucrose was presented as a coarse crystalline material with the particle size <500 µm.

Note: The lactose used in this study was of a commercial grade and contained mainly beta lactose with a small proportion of α-lactose (as indicated by THz spectra, unpublished data), therefore wherever the word lactose is used in this study means the anhydrous beta lactose.

2.1 Ball Milling

Milling of both lactose (particle size <100 µm) and sucrose (particle size 400-500 µm) was performed at ambient conditions (40-45% RH and 20-22 °C temperature) in a vibratory MM 301 ball mill (Retsch, Germany) equipped with two 50 ml stainless steel cylindrical jars each containing one stainless steel ball of 25 mm diameter. A weight of 2.5 g of sugar was loaded into each jar, which were then closed tightly and clamped in position of the mill. The mill was operated at frequency of 18 Hz for 15, 30, 45, 60 min. The freshly milled samples were used for thermal and dielectric analysis.

Note: The mill frequency of 18 Hz was selected after an initial screening of three speeds i.e. 15, 18 and 25 Hz based on the fact that this frequency had a significant impact on crystallinity without a marked increase in the temperature of the mill(Kwan *et al.*, 2004). The temperature of the milled material was ~28-40 °C, estimated by insertion of a thermocouple probe into the powder immediately after milling.

2.2 Thermogravimetric analysis (TGA)

TGA of the un-milled and milled materials (immediately post milling) was carried out in a TGA instrument(Perkin Elmer, US) with auto-sampler system according to the method used previously in literature(Hurtta *et al.*, 2004).The empty aluminium pan was loaded into the TGA instrument and tarred. Then the sample was placed in the pan covered with the ventilated lid and returned back to the instrument. The weight of sample was recorded in the temperature range from 25 °C to 250 °C at the heating rate of 20 °C min^{-1}.

2.3 Differential Scanning Calorimetry (DSC)

DSC experiments were performed according to method described in literature(Hurtta *et al.*, 2004) using a Jade DSC (Perkin Elmer, US) fitted with disc of chromel alloy as thermocouple. The heating rate and heat flow were calibrated at 20 °C min^{-1} using indium and zinc standards. Approximately 7 mg of each material was loaded in 50 µL aluminum

pans and closed with perforated lids, to allow the evaporation of water, and then loaded in the DSC instrument by the auto-sampler. All samples were measured over the temperature range 25 to 250 °C, at a heating rate of 20 °C min^{-1}, applying the calibration acquired at 20 °C min^{-1}. The recrystallization temperature was reported as the onset of the exothermic peak while the melting temperature was taken as the midpoint of the endothermic peaks.

2.4   Broadband Dielectric Spectroscopy

The dielectric measurements of un-milled and milled sugars were performed at ambient pressure in a Novocontrol Alpha broadband dielectric spectrometer (BDS) (Novocontrol, GMBH Germany)(K. Kaminski *et al.*, 2008).The powder sample was placed in layer between two circular gold plated electrodes of the sample cell. The diameter of the sample was taken from the internal dimension of the cell, i.e. 20 mm and the thickness of sample layer was ~1.0 mm. The cell was then mounted in the sample holder and dielectric spectra recorded at frequencies between 0.1 Hz and 10 MHz and at discrete temperatures from −120 to +100 °C with 5 °C increments. The temperature was controlled (with stability of ± 0.1 °C) by a Novocontrol Quattro system. The data were then analyzed by proprietary fitting software *(Z-View*by Scribner Associates).

3   Results and Discussion

Part I: Amorphization of sugars

Data from DSC results were used to determine % amorphous content in the milled samples (Section 3.3) based on the enthalpies of crystallization and melting events.

3.1   DSC Results of Un-milled and Milled Lactose

The DSC curve of un-milled lactose (Fig. 1a) shows a small desorption endotherm at ~140 °C (peak A) indicating the presence of a small quantity of lactose monohydrate. This peak is followed by a shoulder at ~210 °C (indicated as B), representing the melting of anhydrous α-lactose and the next large endotherm at ~238 °C (peak C) represents the melting of anhydrous beta lactose (Listiohadi *et al.*, 2009). DSC curves of milled lactose (Fig. 1b) show that the principle melting peak (peak E) shifts towards lower temperatures with milling time (i.e. from ~238 °C for the un-milled lactose to ~222 °C for the 60 min milled sample). The crystallization peaks were also observed in milled samples of lactose however, the onset temperature (T_{dv}) of this peak does not have any trend and occur at temperatures between 45 and 90 °C as shown by irregular dotted line D in Fig. 1b.

The values of enthalpy of crystallization and melting of milled anhydrous lactose show a gradual increase in the enthalpy of crystallization long with a decrease in the enthalpy of melting (Table 1). These trends indicate the loss in crystallinity of lactose on milling.

Table 1: Temperatures and enthalpies of crystallization and melting for lactose at different milling times.

Milling time (min)	Crystallization Peak		Melting Peak	
	Onset temp. (°C)	ΔH (J g^{-1})	Peak temp. (°C)	ΔH (J g^{-1})
0	-	-	238.1	173.2
5	-	-	234.0	125.9
10	63.1	20.8	231.6	119.5
15	48.0	26.1	230.1	113.9
30	85.3	37.6	226.5	97.8
45	57.9	42.9	226.2	105.3
60	88.19	41.8	222.1	96.9

3.2   DSC Results of Un-milled and Milled Sucrose

The DSC curve of un-milled sucrose (Fig.2a)shows a single endotherm at ~192 °C (peak A), corresponding to the melting of sucrose (Beckett *et al.*, 2006).In milled samples (5 to 60 min milling times), the melting endotherms appear at almost same temperature (192 ± 4 °C) as the un-milled one peak C indicated by straight line in Fig.2b. Additionally, the exothermic peaks representing the crystallization of amorphous phase are also observed (Peak B in Figure 2b). It is worth to note that the onset temperature for the crystallization (summarized in Table 2) decreases from ~49 °C observed for the 10 min milled sample to ~42 °C measured for the 15 min milled sample. Thereafter, it increases gradually with milling time and reaches to ~57 °C in 60 min milled sample of sucrose.
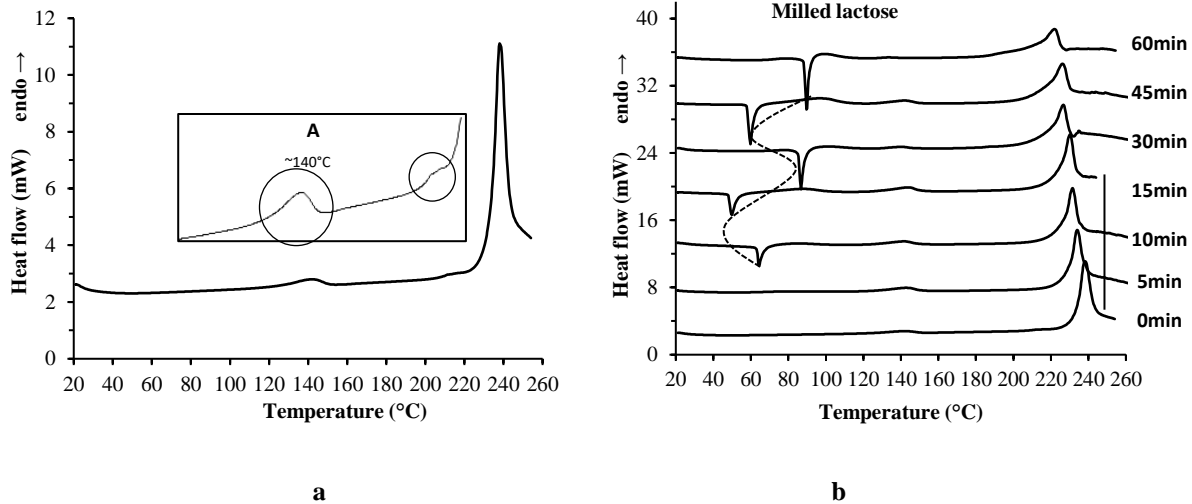

a

b

Fig.1: DSC curves of a) un-milled lactose showing the two small endotherms A and B in the inset and a melting endotherm C b) DSC curves of milled lactose, the vertical lines show the melting endotherm (peak 'E') appears at lower temperatures in longer milled samples. The exotherm (peak D, highlighted by dotted line) represent the recrystallization of the amorphous phase.
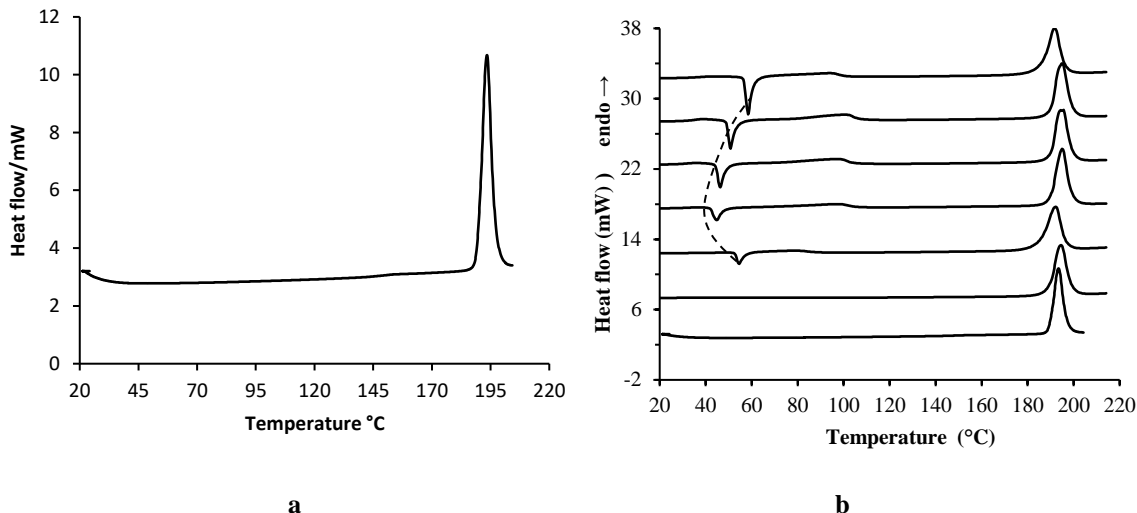
a   **b**

Fig.2: DSC curves of a) un-milled sucrose showing the only melting peak 'A' b) milled sucrose for different milling times. The curved dotted line 'B' indicates the shifts of crystallization with increasing the milling time whilethe straight line'C' indicates almost no change in melting temperature with milling.

Table 2: Temperatures and enthalpies of crystallization and melting for milled sucrose at different milling times.

Milling time (min)	Crystalization Peak		Melting Peak	
	Onset temp. (°C)	ΔH (J g^{-1})	Peak temp. (°C)	ΔH (J g^{-1})
0	-	-	193.4	125.7
5	-	-	194.4	113.3
10	48.7	18.4	193.7	115.3
15	42.0	18.5	195.0	120.2
30	44.4	28.6	195.7	116.0
45	49.0	33.2	195.0	119.3
60	56.6	40.4	191.5	110.7

The milled samples show an increase in the enthalpy of crystallizationfrom ~20 to ~40 Jg^{-1} (Table 2)as the milling time increases from 10 min to 60 min, with the enthalpy of the melting remaining almost constant at 120±5 Jg^{-1}. The increase in the enthalpy of crystallization indicates an increase in the amorphization of sucrose with increased milling time. The zero value for the crystallization enthalpy in the 5 min milled sample represents a failure, in the DSC methodology to detect what was presumably a level of amorphous content that was below the limit of detection of the instrument.

The crystallization temperature is irregular dependent on the milling time for both sugars as it is stochastic process, and is dependent on number of different factors running in parallel during milling process. Number and size of crystallites, temperature, and moisture content all are responsible for variation in crystallization temperature.

3.3 Amorphous content in milled sugars

The residual crystalline content in milled samples of both sugars was estimated by the method taken from (Blaine, 2010; Smith *et al.*, 2015). This involves the enthalpies of crystallization and melting for pure crystalline and milled material using the following equation:

$$X_c(\%) = \frac{\Delta H_m - \Delta H_c}{\Delta H_m (100\% \; crystalline)} \times 100 \quad (1)$$

where X_c is crystalline content, ΔH_m and ΔH_c are enthalpy of melting and enthalpy of crystallization of each sugar. The amorphous content was then determined by applying Equation 2.

$$X_a(\%) = 100 - X_c(\%) \quad (2)$$

Fig.3shows the estimates for the amorphous content of the milled sugar as determined from the above calculation. The total amount of amorphous content produced in lactose was approximately three times larger than in sucrose and has reached a plateau (~60%) at ~ 30 min milling time, whereas for sucrose the amorphization of the material continues to increase upto60 min of milling.
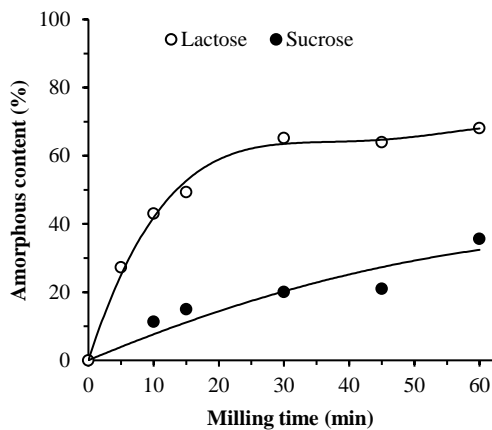

Fig.3: Amorphous content in milled lactose and sucrose as calculated from the enthalpies of crystallization and melting obtained from DSC results.

Part II: Factors impacting the amorphous phase stability

In this part of study, various factors which may impact the physical stability of the amorphous phase was investigated including the moisture content (Section 3.4) and the molecular dynamics (Section 3.5& 3.6).

3.4 Moisture content in milled sugars

Given that the dielectric response of the amorphous phase of disaccharides is known to depend on moisture content (Ermolina *et al.*, 2011) then it would be interesting to estimate the moisture content within the amorphous phase only. On the basis that the amorphous phase has the highest surface area, one might expect that the vast majority of the water in the system would be associated with that phase rather than with the residual crystalline material. Given that assumption then one can normalise the total % moisture content of the system (from TGA results) to the % content of

the amorphous phase (obtained from DSC results) to derive an assessment of the % moisture within the amorphous phase (Equation 3).

$$m_a = m_{surface} x \frac{100}{A\%}(3)$$

where m_a represents the moisture linked to amorphous phase, $m_{surface}$ is the total surface moisture of sample and A% is the percentage amorphous content (determined from DSC results Fig.3).Figure 4shows the results of the calculation of moisture linked with the amorphous phase. In the case of lactose, there is a clear decrease in moisture content of the amorphous phase with increased milling time. In the case of sucrose the trend is less clear with a lot of scatter in the data, however, line of best fit suggesting a gradual decrease in moisture content within the amorphous phase as the milling time increases.

Note: The scatter in moisture content data for the amorphous phase of sucrose is an issue associated with small sample size and an inherent variability in moisture content that results in part from the lack of any suitable method to prevent any phase transitions and/or subsequent loss or gain of moisture. This would be a challenge in itself as one cannot easily control and replicate the humidity and temperature inside the ball mill. These factors introduce random and uncontrollable variation in the data which meant that one could not achieve a definite trend even after few repeats. We therefore have to take the data set as a whole to consider whether the amorphous content and moisture increases or decreases with milling time.
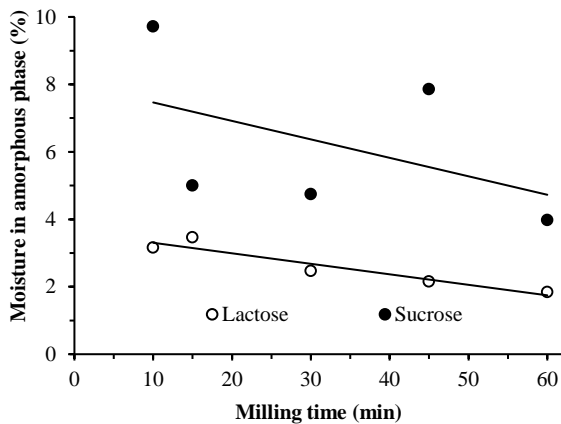

Fig.4: Moisture linked with amorphous phase of milled lactose and sucrose calculated on the basis of total moisture and amorphous content estimated for each milled sample.

These observations are not surprising, given that extended milling times result in longer periods of time at elevated temperature with higher surface area exposed (of milled sugars) and hence more time for moisture to dissipate from the material.

The collective data of both milled sugars suggest that there is more moisture at the beginning than at the end of milling and it may not be linear, and the moisture linked with amorphous phase (which is calculated on the basis of an assumption that all moisture is linked with amorphous phase) is high at the beginning and lower at the end.

There may be one of these three scenarios:

i) Moisture content associated with the amorphous phase increases as the amorphous content increases with milling

ii) Moisture content associated with the amorphous phase remains the same during milling

iii) Moisture content associated with the amorphous phase decreases with milling time

In the following sections, the dielectric data in terms of molecular dynamics of amorphous phase was analyzed to understand which one of these three scenarios and to collaborate what might be otherwise a weak inference that can be made from the DSC data.

3.5 Dielectric Relaxation Processes in Un-milled Sugars

The dielectric loss spectra of un-milled sucrose is almost flat at sub-zero temperature (Fig.5b) indicating the absence of any low temperature process, however for lactose in the same region there are two small processes (as shown by the dotted circle in Fig.5a). At higher temperatures both sugars display a large process which is probably associated with the percolation of protonic charges through water present on the hard crystalline surface of these un-milled sugars. This is followed by what appears to be the dc conductivity of both sugars as the temperature increases above 50 °C (the percolation process and dc conductivity are indicated by the arrow heads in Fig.5a & b).

3.6 Dielectric Relaxation Processes in Milled Sugars

In contrast to the un-milled sugars, the dielectric spectra for the milled samples (*e.g.*60 min milled) display two secondary relaxation processes in the low temperature window (< 0 °C) represented as relaxation process 1 (RP1) and relaxation process 2 (RP2) (shown by the arrows on Fig. 6a & b respectively). These processes are the secondary relaxations (γ and β processes) of the amorphous phase. Now one would then expect to observe the structural relaxation (i.e. α-process) at higher temperatures.

In the high temperature range (> 0 °C), there are two processes in case of sucrose, RP3 which appears as a shoulder in the narrow temperature range followed by a large, sharp spike (RP4), while in case of lactose there is a merged process represented as RP3/4.

The shoulder that is observed in case of sucrose in a narrow temperature range is in fact α-process which merges with the sharp spikes in dielectric loss associated with the dc conductivity of these samples (Fig. 6b). The likely explanation for these spikes in milled sucrose is that, the material has passed through the glass transition (i.e. the structural relaxation phase) and now with enhanced mobility in the system, there is an opportunity for crystallization and/or collapse (liquefaction).
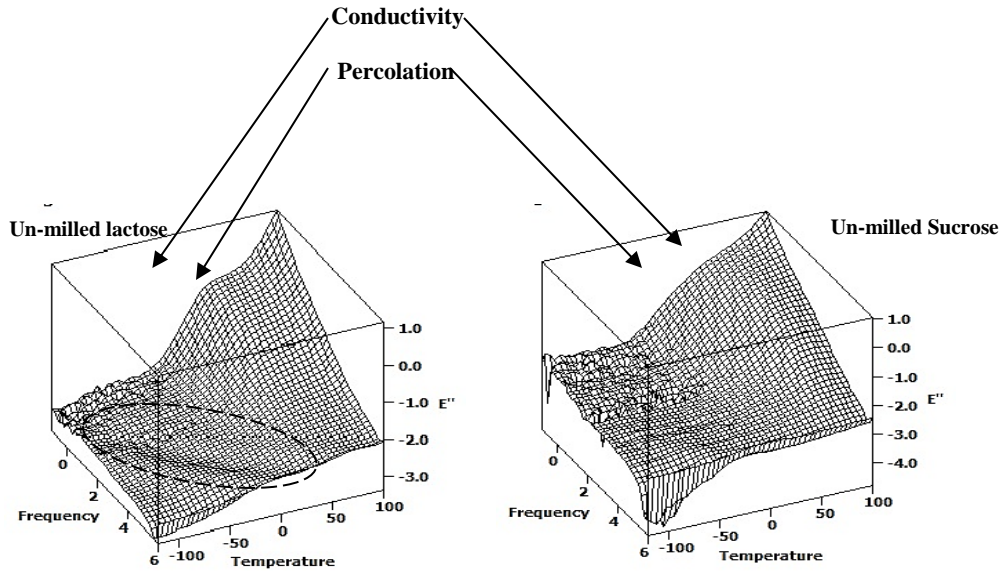
Fig.5: 3D dielectric loss spectra of a) un-milled anhydrous lactose and b) sucrose, showing the plot of imaginary permittivity against temperature (°C) and log frequency (Hz). The spectrum of lactose shows a low temperature process highlighted by dotted circle.
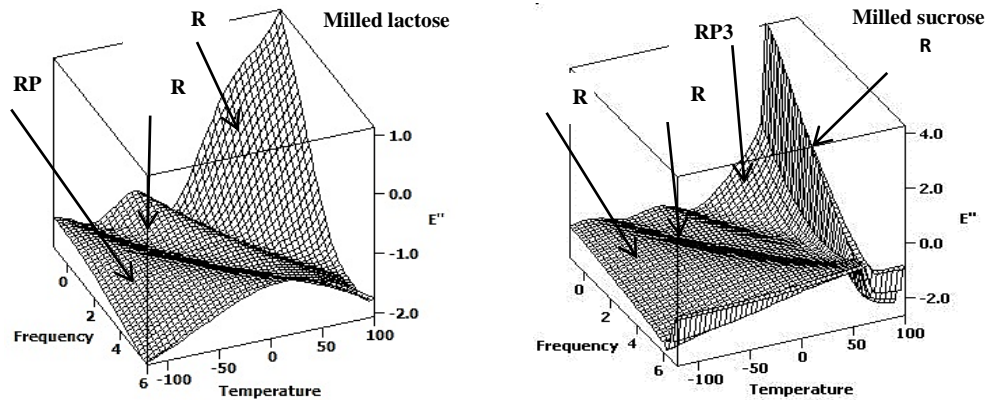

Fig.6: 3D dielectric spectra showing the plot of imaginary permittivity against temperature (°C) and log frequency (Hz) of a) 60 min milled lactose and b) 60 min milled sucrose. Both plots show four relaxation processes (RP) as highlighted by arrow heads. RP1 and RP2 are well separated in both milled sugars, RP3 and RP4 are also well separated in case of milled sucrose but appear as a merged single huge wing in case of milled lactose.

Given that we observed the material at the end of the measurement cycle and that it appears, to all intents and purposes, as a structurally 'intact' matrix then we presume this conductivity spike is more than likely to be associated with crystallization rather than collapse; a process which is observed in case of hydrated sugar(Darcy *et al.*, 1997).

The single high temperature process of lactose, which appears as a large wing towards low frequency, unlike to the two separate processes for sucrose, probably represents a merged structural relaxation and conductivity as already reported in case of freeze dried lactose (Ermolina *et al.*, 2011).

The characteristics of amorphous phase of milled sugars and its instability is more easily recognized, and possibly understood, by analyzing the dielectric spectra in terms of their temperature slices (t-slices) through the response surface at a specific frequency (Fig.7).

The t-slices of **milled lactose** at low frequency (0.1Hz) show that there are two secondary relaxation (γ and β) processes of amorphous phase towards lower temperature and followed by a broad process between -20 and 90 °C. This broad process again can be split into two processes, with one starting at ~ 0 °C (line 'A' in Fig. 7a) which is probably the structural relaxation, followed by the appearance of conductivity towards high temperatures (line 'B' in Figure 7a).The point where the high temperature wing takes the plateau of dielectric signals probably represents recrystallization. In short time milled lactose samples, this plateau in dielectric signals occurs at higher temperature while in long milled samples it occurs at relatively lower temperature. It is also worth to note that, this event occurs between 30 °C and 80 °C which is close to the recrystallization temperature observed in DSC (48 to 88 °C, Figure 1b). The small differences in recrystallization temperature from both techniques might be due to i) the variation in the rate of heating and ii) different geometry and size of samples in both techniques (Megarry *et al.*, 2014).

Unlike lactose where it is masked under conductivity, the structural relaxation in **milled sucrose** is observed as a shoulder in all milled samples in the temperature range of -10 °C to 45 °C (Fig. 7b). This is followed by the appearance of huge and sharp peaks (spikes) that start at ~50 °C and shift towards higher temperature with milling time.(Fig. 7b) These spikes occur between 50 °C to 70 °C and represent the recrystallization of amorphous phase with temperature very much similar to that observed in DSC results (see Figure 2b).
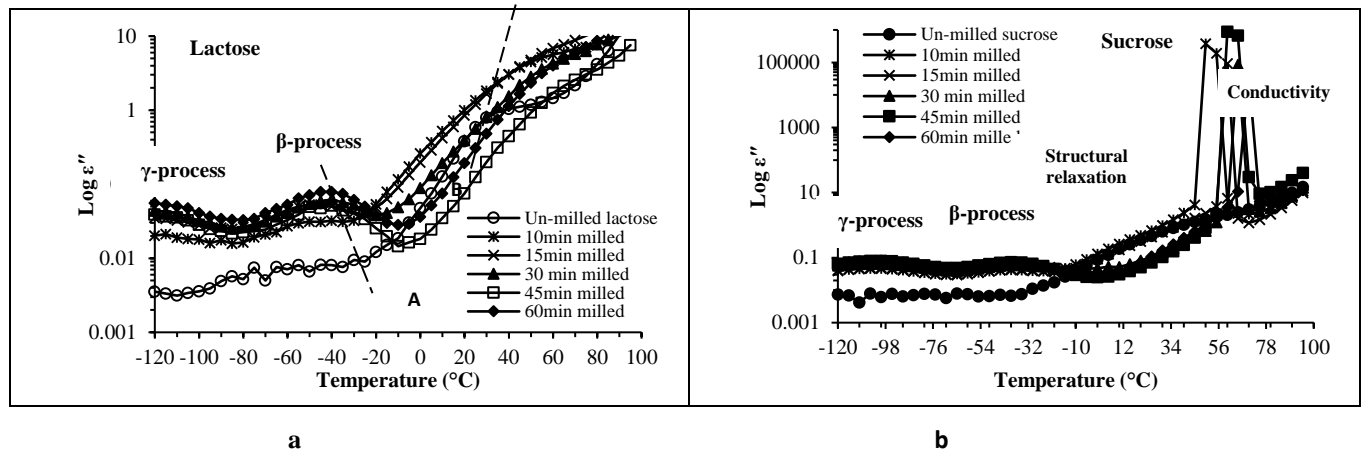

a   b

Fig. 7: Temperature slices (plots of imaginary permittivity against temperature) at 0.1 Hz, frequencies from dielectric spectra of a) milled lactose and b)milled sucrose. The four processes have been labelled as γ, β, structural relaxation and conductivity. The dotted lines 'A' and 'B' in figure 'a' indicates the start point of structural α -relaxation and conductivity, (at increased temperature) respectively.
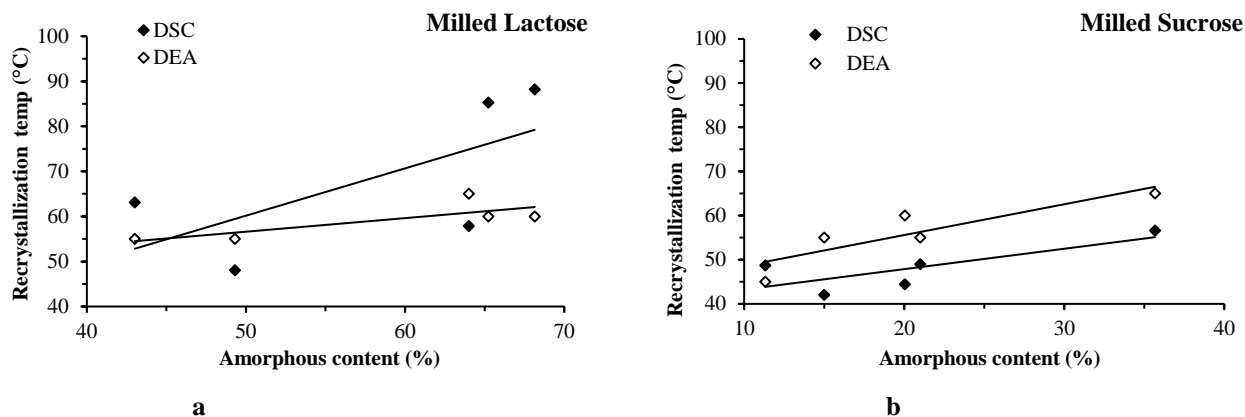
Fig.8: Recrystallization (onset) temperature as a function of amorphous content (a &b) for both lactose and sucrose as measured from DSC and Dielectric Analysis (DEA).

Recrystallization temperatures (from both the DSC and BDS data) have been plotted as a function of amorphous content (Fig. 8a & b). Although the data is somewhat scattered but there appears to be a tendency for the recrystallization temperature to increase with amorphous content and vice versa. This is in agreement with the literature evidence that seeding of amorphous sugars (i.e. presence of crystalline components which is obvious in milled materials) not only reduces the recrystallization temperature but also the induction time of appearance of such peak(Saleki-Gerhardt *et al.*, 1994).

The possible explanation of this is, at early milling times, where amorphous content is low the enormous surface to volume ratio (due to size reduction) provide opportunities for early recrystallization so the onset temperature for recrystallization is low. On further milling, when the area of the hard crystalline surface decreases, there are fewer nucleation sites and hence the recrystallization is delayed until the higher temperatures are reached on heating.

Therefore, the recrystallization temperatures will depend on the opportunities for the formation of stable nuclei and then growth. The former, in the case of heterogeneous nucleation (as present here) is dependent on the availability of nucleation sites (interfacial area and surface energies) whereas the latter is considered to be underpinned by diffusion process i.e. molecular degrees of freedom in the amorphous phase and the energetics (bond energies) of the constituent molecules(Yu, 2001).

The comparison of the stability of each amorphous phase (i.e. that in sucrose vs that in lactose) based on the recrystallization temperatures between materials is only valid if one considers similar physical states in terms of the numbers of nucleation sites. Whilst we recognize that the exact quantification of sites is unachievable with the technologies used in this study, it may be that an approximation and therefore comparison between materials may be founded upon a comparison of those lactose and sucrose samples with a similar ratio of amorphous to crystalline phases.

Given that the DSC results of recrystallization of lactose are more scattered (owing to the small sample size ~7-8 mg and fast heating rate i.e. 20°C min^{-1} that gives less time to crystalize), indicating less reliability in the data, whereas in

contrast there are more obvious trends in the BDS data (more stable to manipulation with relatively large sample size ~300mg and slow scan rate i.e. ~0.3 °C min^{-1}) whereby the recrystallization temperature increases in a more systematic (monotonic) way with the amorphous content, it was decided to focus on the BDS values of recrystallization temperature in our efforts to understand the relative instability of the amorphous phase in each milled sugar. The BDS values of recrystallization of both sugars are now plotted on a single graph (Fig. 9). To this end sucrose data was extrapolated to the 50% amorphous point in order to compare its recrystallization temperatures with lactose with similar amorphization (i.e. 50%). Fig.9 shows that sucrose has the recrystallization temperature of ~75 °C which is higher than that of lactose (~50 °C) with similar amorphous content. Such recrystallization temperature (74 °C)of partially amorphous milled sucrose was previously reported with ~30% amorphous content(Saleki-Gerhardt *et al.*, 1994).
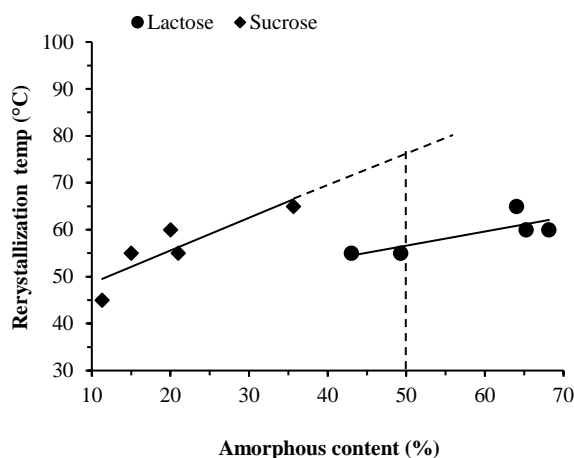

Fig.9: Graphs showing the changes in rerystallization (onset) temperature as a function of amorphous content for both lactose and sucrose as measured byBroadband Dielectric Spectroscopy (BDS). The data of amorphous content of sucrose was extrapolated as shown by the dotted line to show recrystalization temperature at 50% amorphization.

In summary, the behavior of amorphous phase of milled sugars and its recrystallization indicate the greater stability of sucrose as compared with lactose after milling. This fact is consistent with the previous observations as reported in (K Kaminski *et al.*, 2008).

From the observation made so far about the recrystallization/instability of amorphous phase of milled sugars, it seems that the instability is governed by the number of nucleation sites (first phase in recrystallization) but actually it is also a function of the growth phase which is under pinned by molecular dynamics, and the potential role of water as plasticizing materials. Therefore, the potential role of water and the parameters of the molecular dynamics that determines the recrystallization of amorphous phase will be discussed below.

In order to show the effect of moisture on the recrystallization of both sugars, the recrystallization temperature (from BDS data) against moisture was plotted in Fig. 10.The graph clearly demonstrates that the recrystallization temperature decreases with moisture content for both sugars, which is consistent with the idea that water facilitates the diffusion and growth of crystalline solid i.e. the well-known plasticization effect of moisture(Heljo *et al.*, 2012; Saleki-Gerhardt

et al., 1994). The data also shows that the recrystallization temperatures are similar in both sets of milled sugars (being in the range of 55 – 65 °C) in spite of different moisture content. It is evident that sucrose can tolerate higher moisture contents (5-8%), which is a further indication that the amorphous phase of sucrose is more stable than lactose.
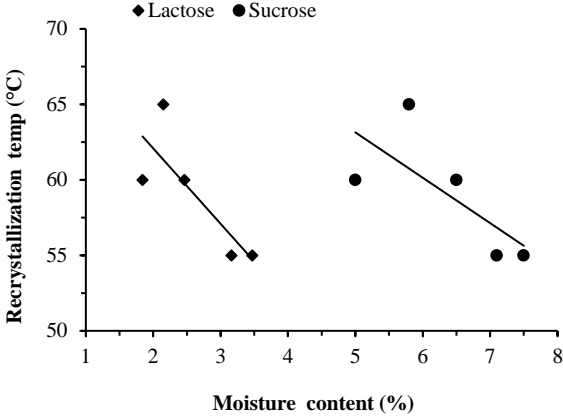

Fig. 10: Graph showing the change in recrysatllization temperature (onset) with moisture content of amorphous phase of both milled sugars.

A detailed analysis of the dielectric relaxation processes was then performed in order to analyze the stability of amorphous phase at molecular level. Since the α-relaxation is not distinguishable in the dielectric spectra particularly of milled lactose, as stated earlier, the dynamics of amorphous phase of the milled sugars was estimated from γ and β-relaxation processes. The molecular dynamics of amorphous phase in terms of relaxation parameter (activation energy) was obtained by fitting each process in the relaxation spectrum with a Haviliak-Negami function (Equation 4).

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{[1 + (i\omega\tau)^\alpha]^\beta} \mathbf{(4)}$$

Where ε^*, ε_s and ε_∞ are complex, static and infinity permittivity, respectively. τ is relaxation time while α and β are exponents for symmetry and broadness of spectra.

The plot of relaxation time (obtained from fitting or from peak frequencies) against the reciprocal of temperature for both the γ and β-processes of milled lactose and sucrose Fig. 11) is linear, indicating the temperature dependence in term of Arrhenius behavior. The gradients of these plots for the γ-process do not change on milling (Fig. 11a &c) however there is a little variation for β-process, see Fig.11b& d), indicating that the β-process is more influenced by the milling of these sugars than the γ-process. The activation energies of both γ and β-processes are estimated from the gradient of these plots, by applying Arrhenius relation (Equation 5).

$$\tau = \tau_0 e^{-Ea/RT} \qquad (5)$$

where τ is relaxation time, τ_0 is pre-exponential factor corresponding to limit of t at T->∞; E_a represents the activation energy, T is the temperature in Kelvin and R is the universal gas constant.

The activation energy for both processes are presented in Table 3. The results for β-process for sucrose show gradual decrease in activation energy as a function of milling time, whereas for lactose the results are almost constant with

milling time (excepting 60 min). The disparity reflects the variation in moisture as it was seen in Fig 4, the range of moisture variation for amorphous phase of lactose is much less (i.e. from 2 to 3.5%), whereas for sucrose this value much higher and is between 4 and 10%. Such behavior reflects better moisture sorption properties of sucrose than lactose(Hackl *et al.*, 2016).
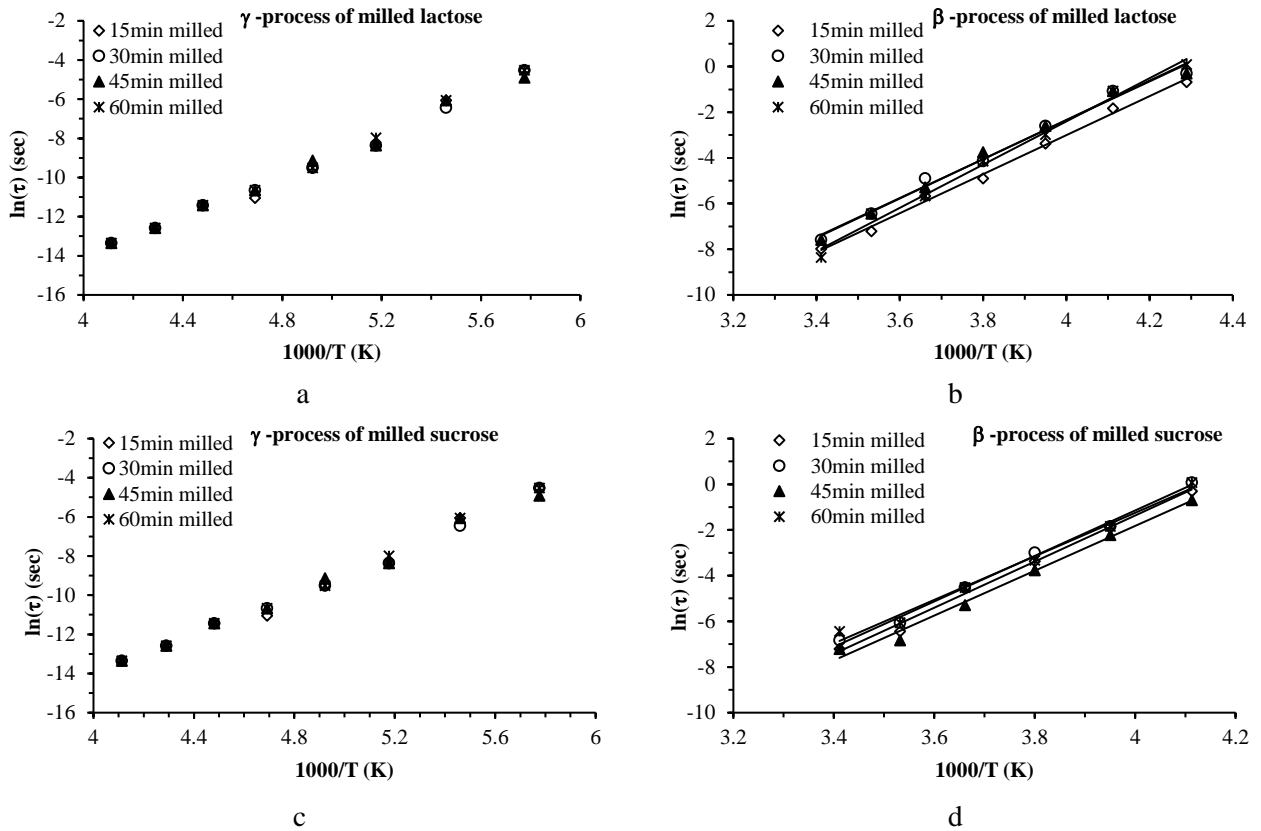

Fig.11: The Arrhenius plots of relaxation time against inverse of temperature for 15, 30, 45 and 60 min milled samples of lactose and sucrose. Figures a &c show the plots of γ-process while Figures b& d show the β-process of both milled sugars, respectively.

The values of activation energy of the γ-process of all milled lactose samples are ~44±1.5 kJ mol^{-1} (Table 3) which is very close to the values reported for this process in amorphous lactose i.e. ~42 kJ mol^{-1}.(K. Kaminski *et al.*, 2008)For all milled samples of sucrose, the activation energy of γ-process is between 50-52 kJ mol^{-1} (Table 3) which is in agreement with that in amorphous sucrose.(K. Kaminski *et al.*, 2008)The activation energies of β-relaxation process of milled lactose samples (i.e. 71-79 kJ mol^{-1}) are in agreement with the data reported for amorphous lactose (72 kJ mol^{-1})(K. Kaminski *et al.*, 2008).Activation energy values for milled sucrose samples(79-84 kJ mol^{-1}) are lower than the results published for amorphous sucrose (98 kJ mol^{-1})(K. Kaminski *et al.*, 2008).The most likely reason is the moisture sorption during sample preparation due to enlarged surface area of milled samples and intrinsic hydrophilic property of sucrose (Hackl *et al.*, 2016).The comparison of the activation energies of both milled sugars with that from freeze dried and super cooled sugars represents that amorphous phase is almost the same regardless of processing methods(Wojnarowska *et al.*, 2010).Also, note that the activation energies for both secondary relaxations in milled

sucrose are higher than that in milled lactose which potentially indicates the higher stability of sucrose as compared to lactose.

Table 3: Values of activation energy for milled lactose and sucrose. (E$_a$= energy of activation).

Milling time (min)	Lactose		Sucrose	
	E$_a$ (γ) (kJmol^{-1})	E$_a$ (β) (kJmol^{-1})	E$_a$ (γ) (kJmol^{-1})	E$_a$ (β) (kJmol^{-1})
15	44 ± 1.6	71 ± 3.8	51 ± 0.5	84 ± 8.4
30	43 ± 1.1	71 ± 5.9	50 ± 0.5	83 ± 4.8
45	43 ± 1.2	72 ± 5.7	50 ± 0.5	82 ± 7.0
60	44 ± 1.0	79 ± 5.9	52 ± 0.7	79 ± 7.6

It is also worth noting that the activation energy β-relaxation processes for sucrose slightly decreases with the milling time, whereas this parameter is insignificantly increases for lactose.

The relatively high values of activation energy for β-relaxation of milled sucrose in comparison with milled lactose may be explained in terms of fewer degrees of freedom/flexibility in sucrose (pyranose form) amorphous state compared with the lactose (furanose form) amorphous state. These observations coincide well with our recrystallization results (Section 3.5) that the milled sucrose recrystallizes at higher temperature than milled lactose. Therefore, the molecular dynamics study of amorphous phase of both milled sugars suggest that there is well link between the recrystallization tendency and molecular dynamics as also shown by a recent studies (Bhardwaj *et al.*, 2012; Kothari *et al.*, 2014).

In order to demonstrate how the β-relaxation in the amorphous phase is influenced by the moisture associated with that phase, we have used the data of amorphous lactose from Ermolina et. al (Ermolina *et al.*, 2011), where it was shown that the activation energy was seen to increase at low moisture contents but was almost constant at intermediate moisture (5-7%) (no such data was available for amorphous sucrose). A linear fit (Fig. 12) to the activation energies for amorphous lactose which had been hydrated between 2 to 5 % moisture suggests that the activation energy for lactose in this study could decrease by 1.5 kJ mol^{-1} per 1 % moisture which is consistent with our observations (Fig.12) in which the activation energy decreases by 1.5 kJ mol^{-1} over a 1% decrease in moisture (from 3% to 2%). Moreover, the discontinuity in activation energy at low moisture content (i.e. Extended milling times) in which it was seen to ramp to 79 kJ mol^{-1} was also seen in Ermolina et al, (2011) in which the activation energy was ~90 kJ mol^{-1} at the lowest moisture content in that study (i.e. 1%). Thus, the moisture concentration (i.e. the water content normalized to the amorphous phase content) impacts the molecular dynamics in terms of increase in activation energy as the moisture decrease with increasing the milling times (Fig. 12).
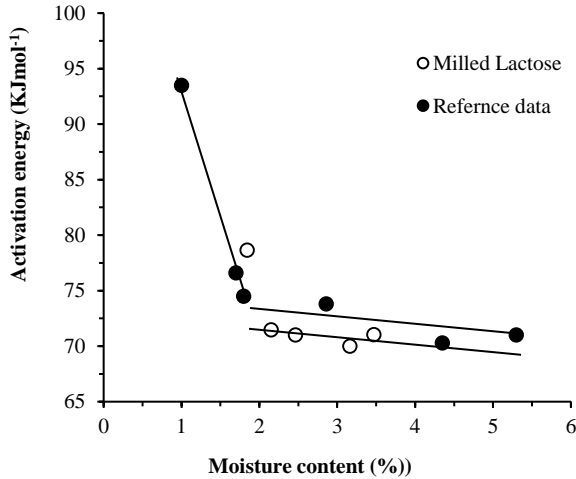
Fig. 12: Activation energies against moisture content of amorphous lactose, filled circles corrosponds to the reference data taken from Ermolina etal, 2011;while open circles show data of milled lactose from this study. The two lines which demonstrate the inflection close to 2% moisture are reproduced on the graph to illustrate a similarity of the data observed from two very different systems.

A consideration of the molecular packing may also be helpful in understanding the molecular mobility within the amorphous phase.(Yu, 2001)This may be achieved to some degree by studying the temperature dependency of the Fröhlich parameter B(T). To this end, B(T) was estimated by applying the Equation 6, which included the values of dielectric strength ($\Delta\varepsilon$) and high frequency permittivity (ε_∞), obtained from the fitting of relaxation data.

$$B(T) = T \cdot \frac{\Delta\varepsilon\,(2\,\Delta\varepsilon + 3\,\varepsilon_\infty)}{\Delta\varepsilon + \varepsilon_\infty} \quad (6)$$

The values of B(T) obtained for the 60 min milled samples were plotted against temperature (Fig. 13). The error bars referred thehighest and lowest values of B(T). Figures13a & b show that the such plots for both sugars are almost constant towards lower temperatures (<-10 °C) suggesting a non-correlated disposition of the dipoles. However, an apparent increase in the dependency of B(T) on temperature is observed above 0 °C suggests an anti-parallel alignment of the relaxing dipoles.(Frohlich)This alteration from non-correlated to anti-parallel correlation is a feature of either the residual water in the milled sugars or some other dipoles of the system. This fact remains unclear however the presence of common feature of non-correlated dipole in both sugars suggests that there might be some common system in both sugars. Given that the 60min milled samples of both sugars have low moisture content (as described above),they may represent a non-dipole heterogeneous system having only short range interaction similar to as described by Gutina et.al.,2003 in porous glasses.(Gutina *et al.*, 2003)In short, both milled sugars have similar molecular arrangements. However, the fact that the higher values of the Fröhlich parameter in lactose than sucrose might support the suggestion that the amorphous lactose in the milled samples has greater degrees of freedom and hence lower stability than in sucrose.
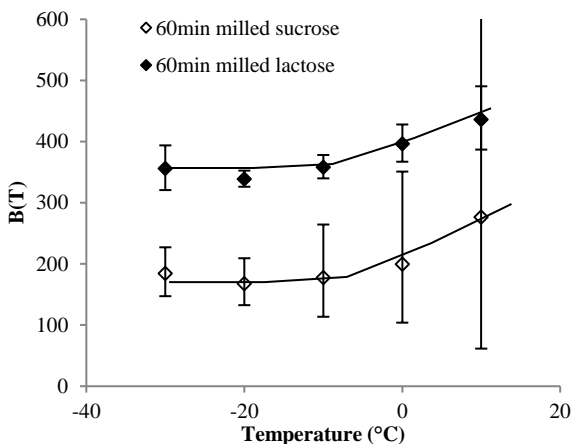
Fig. 13: Frohlich parameters, B(T), for 60 min milled lactose and sucrose. The shape of the line drawn through the Frohlich parameter for lactose (where the error bars are smaller and hence the trend is more obvious) has been over-laid onto the Frohlich parameter for sucrose to demonstrate that there might be an increase in molecular degrees of freedom as the temperature increases above -10 °C.

In summary, BDS indicated that milled lactose devitrifies at slightly lower temperature than the milled sucrose. This was related to the relatively lower activation energies of milled lactose than milled sucrose. The most probable reasons for this include the difference in molecular packing because of the fundamental differences in the molecular structure of both sugars but most probably it is the influence of moisture that causes lactose to recrystallize earlier than sucrose. However, long time milled lactose having higher activation energy is more stable as compared with the short milled ones.

4   Conclusion

The molecular dynamics of amorphous sugars produced by ball milling at various milling times was analyzed in terms of dielectric relaxation parameters of Johari-Goldstein β process and crystallization kinetics. The results indicate that the sucrose is more stable and it has fewer propensities for crystallization than lactose after milling.

The study also indicates that the long-time milled sugar samples are more stable as they crystalize at higher temperatures. One of the possible reasons for such behavior is that moisture content (acting as a plasticizer) in the amorphous phase decreases with an increase of milling time. Another reason could be related to the nucleation process that controls the overall instability of the amorphous phase. In the long milled samples, there are fewer nucleation sites therefore the chance for crystallization is reduced.

5   Acknowledgements

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