Solid state epimerisation in α-lactose monohydrate by optical DSC and NMR

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Introduction

- Research conducted on lactose as an excipient is critical towards understanding the chemical, physical and morphological behaviour of those formulations containing lactose.
- The reason behind this interest is due to lactose being one of the safest (Jawad et al., 2012) and stable excipients used in drug formulations (tablets, capsules or DPI's) (Pifferi et al., 1999).
- Lactose, however, is a stereo-isomer that can exist in two different forms known as α and β anomers and this processes in known as epimerisation (Yaylayan et al., 1993). When considering chemical changes such as epimerisation, it is rarely noted that the change of anomeric content could arise instantaneously with re-crystallisation or individually.
- There are several analytical techniques used to analyse lactose (Altamimi et al., 2019) such as Differential Scanning Colometry (DSC), which is particularly useful to explore a variety of parameters such as purity, melting point, decompositions process, polymorphic changes (Giron, 2001), enthalpy and degree of crystallinity (Gombás et al., 2002). However, most reports in literature address changes between 160 -190ºC as mainly re-crystallisation (Caron et al., 2011) and lack thermal instability of lactose.

Objectives:

- Explore the epimer content and stability of lactose powders
- Examine visual changes in lactose using DSC hyphened photo microscopy

Methods

Materials: Hermetic aluminium pan and lid (TA Instruments) , 99.999% pure Indium (onset melting point 156.8°C) (Alfa Aesar, USA), DSC Q20 (TA instruments, USA). α-lactose monohydrate ≥99 % (Sigma Aldrich) and β-lactose acquired (ACROS Organics).

DSC: lactose samples were carefully weighed (2-3 mg) and placed inside a pin-holed hermetic pan. The DSC was previously calibrated with indium. The procedure followed Equilibration at 25 ºC and ramped to 250 ºC at a rate of 10 ºC /min. The purge gas of 50cm^3/min dry nitrogen. Samples were stopped at specific temperatures (160°C and 190°C). After allowing the sample to cool down (≈ 2 minutes), it was then transferred to a glass vial prior to NMR analysis. Optical DSC 2500 (microscope equipped) was used to capture visual changes of Lactose during thermal analysis (figure 1).

Figure 1: DSC-hyphenated with microscope imaging.

NMR analysis was performed by dissolving the lactose samples obtained from the DSC with 0.7 mL Dimethyl sulphoxide-D6 (DMSO) 99,8% + 0,03% tetramethyl saline (TMS) (VWR International) then transferred to 400 MHz Wilmad NMR , using proton NMR and 16 scans, the spectra was then analysed using Bruker Topspin 3.5 software. The region between 6.3 and 6.7 ppm of the chemical shifts represented α and β epimers, respectively . Since mutarotation was time dependant, the samples were less then 20min in solution.

Results

DSC thermogram of α-lactose monohydrate and β-lactose (as received) initially shows a loss of hydrate at 144.3 (±0.3) followed by the regions of interest (160-190°C) and finally a recorded melting point of 213.3 (±0.4). Whereas β lactose shows one peak that represents the melting point 234.2 (±0.2) (Figure 2). This is due to the anhydrous nature of the powder.

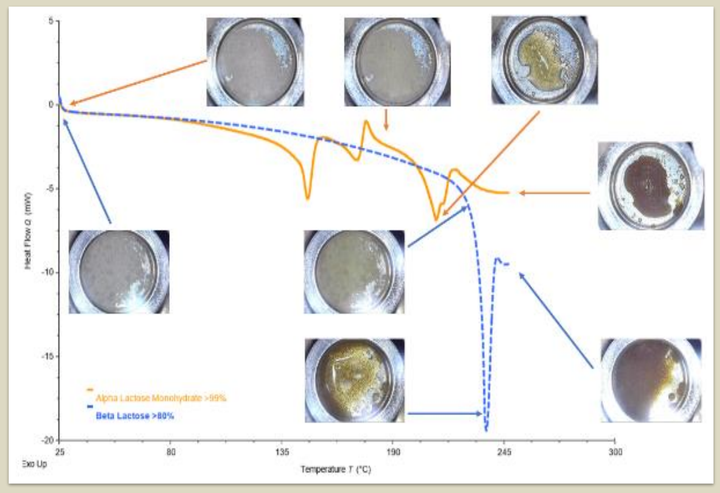

Figure 2: Thermogram overlay between α-lactose monohydrate and β-lactose with optical images at different stages

Optical images were taken at these different stages. What was interesting is the discolouration occurring while melting that could be a results of degradation.

After stopping the DSC analysis at the selected temperatures, the enlarged NMR spectrum (figure 3). Shows an increase of peak integration of β- epimer from 3.7 ±1%t 11.6±0.9% and 29.7±0.8% anomer (*P<0.05*) at temperature 25, 160 and 190 °C respectively (Figure 4).

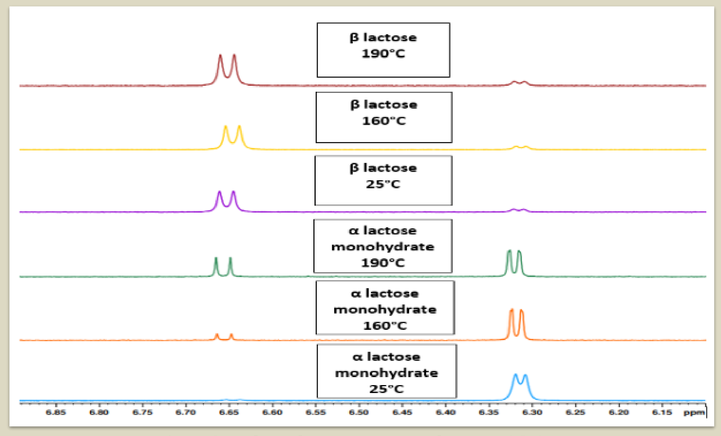

Figure 3: Enlarged NMR spectrum between 6.3-6.7 ppm

Epimer content of β lactose on the other hand remained the same throughout the all temperature. (The temperatures selected to stop the analysis were related to the changes of peak behaviour of α-lactose monohydrate between 160°C-190°C.

Previous work showed that epimerisation results from exposure to water in solution, (Jawad et al., 2012) and humidity and heat in solid state (Altamimi et al., 2017). These findings were separate, but specific to one type of lactose which was β lactose .

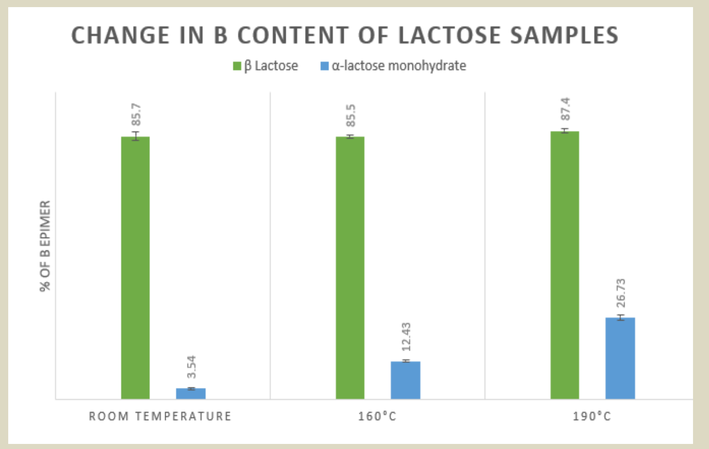

Figure 4: Epimer composition of lactose at different temperatures

Conclusion

- Better understanding of chemical stability of lactose
- Avoid 160 °C during manufacturing process of lactose
- Discolouration indicates degradation during melting point.
- Region between 160-190 °C is epimerisation and not only recrystallisation
- Water molecule plays vital part in epimerisation

Future work

- Future work would involve investigating the kinetics of α-lactose monohydrate epimerisation.
- Identification of degradation products
- Affect of epimer instability on medicine
- Stability study on lactose powders
- Quality control testing

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