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Dehydration of Lactose Monohydrate: Analytical and Physical Characterization

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ABSTRACT

Dehydration of hydrates of pharmaceutical inactive/active ingredients (pharmaceutical hydrates) may easily occur during storage or manufacturing. Loss of water may have little effect on the crystal lattice, produce less hydrated forms or possibly amorphous form. Characterizing the effects of water loss on crystal hydrate forms is important for understanding the behavior of hydrates throughout the manufacturing and storage processes. This study shows that exposure of the monohydrate form of Lactose to gentle heating (145°C) results in the loss of 1 mole of bound water. Upon removal of the bound water, the crystal lattice adjusts producing a distinct phase characterized by powder X-ray diffraction, thermal, IR, BET specific surface area, and PSD data. Adjustment of the crystal lattice appears to compromise crystal integrity and can result in reduced crystallite, particle sizes and increase in surface area. The evidences of adjustment in crystal integrity and water movement from particle also showed using hot stage microscopy (HSM).

Keywords: dehydration; X-ray diffractometry; Lactose; Monohydrate; FTIR; BET specific surface area, PSD; thermal analysis; HSM.

INTRODUCTION

Environmental variables encountered during the manufacturing process may affect the formation of different crystalline states of hydration for the inactive ingredient .These different solid forms or hydrates can possess different physical properties such as differences in solubility, stability, and particle habit. Water in inactive ingredient hydrates can be classified into three different structural classes that include those residing in isolated lattice sites, lattice channel sites, or ion-coordinated sites(2,3). In isolated lattice sites, water molecules are isolated from other water

molecules due to contact with drug molecules. Water molecules forming lattice channel sites are in contact with other water molecules of adjoining unit cells along an axis of a unit cell. It has been shown that some channel water containing hydrates may undergo dehydration under conditions of low relative humidity (RH) or pick up water under conditions of high RH (3). Ion-coordinated water participates in an ion water bond which usually is much stronger than any hydrogen bonds present. In addition to the formation of different hydrates, a single hydrate form of an API/inactive may contain more than one structural class of water(2). Lactose is mainly reported in three different forms are Monohydrate, anhydrous and amorphous(6). Upon the removal of bound water from Lactose Monohydrate thermal and spectroscopic data indicate the lattice undergoes an adjustment and the crystal integrity appears to be compromised. This study describes the formation of dehydrated Lactose by removing 1 mole of bound water with heat and discusses the associated physical and chemical changes that occur. These changes are found to be reversible, and the crystal lattice return to its original state as the sample rehydrates over a period of time.

MATERIALS AND METHODS

Materials

The FlowLac 100 is a spray-dried α-lactose monohydrate was sourced from MEGGLE Pharma excipients and technology commercial supplier and used as received. The dehydrated material was prepared by heating monohydrate on hot plate for 10 minutes at 150°C. Polymorphic transformation also observed under the hot stage microscope by heating the sample from 100°C to 170°C.

Analytical Methodology

Thermal Analysis

Simultaneous thermogravimetry(TGA) and Differential Scanning Calorimetry(DSC) analysis thermograms were generated using a METTLER TOLEDO. Approximately 5 mg sample were scanned under a dry nitrogen purge from 25 to 250°C at 10°C/min. Microscopic images were obtained by mounting a microscope with a video feed hot stage above the quartz glass window. Acceptable depth of field and focus was achieved using top illumination, a 10X objective on the microscopy, and 1.0X magnification in the eyepieces and camera feed.

Infrared Spectroscopy

Fourier transform infrared spectra (FTIR) were obtained using a Perkin Elmer spectrum one spectrometer. Sample was prepared using KBr pallet method and scanned in the range from 400 to 4000 cm-1, with a resolution of 4cm $^{-1}$.

Powder X-Ray Diffraction (XRD)

X-ray powder diffraction was performed on the samples using the Bruker D8 advance X-ray diffractometer. The instrument was equipped with a 2.2 kW Cu anode X-ray tube, high temperature stage, and high-speed position sensitive detector (PSD). Cu Ka radiation (Wavelength = 1.5418A °) was used to obtain all powder diffraction patterns. A nickel filter was placed in the receiving path of the X-rays to remove the Kb radiation. Lactose monohydrate material was mounted and analyzed on a front loading sample holder, without any special sample preparation. Environmental conditions for the analysis were manipulated to facilitate drying the

sample without removing it from the instrument. The sample was dehydrated by heating the material from 30°C to 170°C with 2 minutes gap interval between the scans. After dehydration, it was allowed to cool to room temperature and again scanned. All scans were performed over the range of 5–40° 2 theta, at a 0.01 step size for 0.1 s/step.

Hot stage Microscopy (HSM)

Images were obtained using a LEICA DMLM Polarizing Light Microscope (PLM) with JVC digital color video camera having METTLER TOLEDO FP 82 hot stage movable attachment. Slides were prepared by mixing the powdered sample with low viscosity silicon oil, and the resulting dispersion placed between a clean glass slide and cover slip. Each prepared slide was examined using bright field and slightly uncrossed polarized light using a LEICA HC Plan 10X / 20 eye piece and 10X objective.

Particle Size Distribution (PSD)

Particle size distribution of the Monohydrate and dehydrated forms of Lactose were determined using laser diffraction technique. Malvern Mastersizer 2000 instrument were used and analysis were performed using dry method. Approximately 1gm of each sample was transferred in dried and clean sample feeder tray, vibration feed rate 50% and dispersive air pressure 2 bar were applied. For each sample two measurements were performed to calculate the average particle size.

BET specific surface area analysis

Specific surface area of Lactose monohydrate and dehydrates sample were measured by using QuadraSorb SI BET automated surface area and pore size analyser. Analysis was performed using nitrogen gas as an adsorbate with multi-point model.

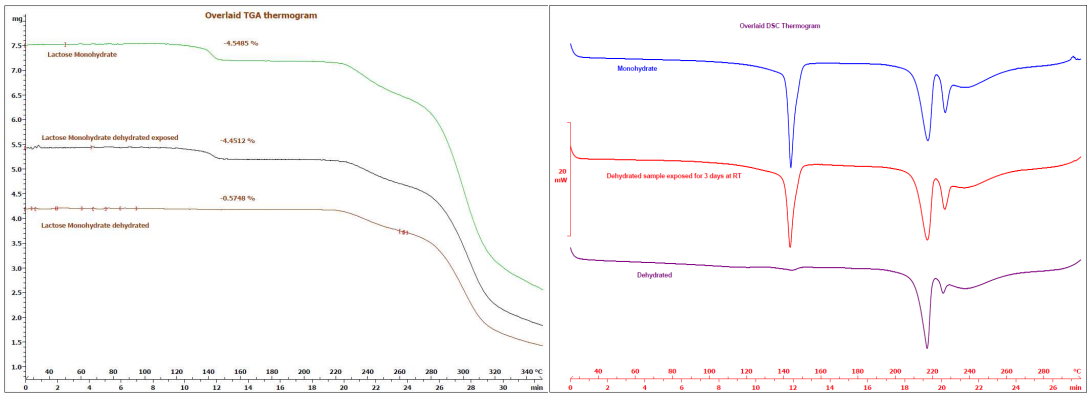
RESULTS AND DISCUSSION

Thermal Analysis

Comparing hydrated Lactose with dehydrated Lactose via thermal analysis clearly illustrates that heating results in loss of 1 mole of bound water. Representative thermograms are provided below in Figure 1. The only difference in the TGA curves (Fig. 1A) is the mass loss of about 4.5% observed from 120°C to 160°C in the hydrate sample that is almost missing from the dehydrated sample. This value is in agreement with theoretical value for 1 mole of water in Lactose monohydrate (5%). The dehydration was assigned to loss of bound water based upon the temperature of the water loss (above the boiling point of water). The dehydrated sample yielded a almost flat baseline through 140°C to 160°C with small weight loss, over the same temperature range that a broad endotherm due to dehydration is observed for the hydrate sample by DSC, which is almost absent in dehydrated sample.

Infrared Spectroscopy is particularly sensitive to the polar functional groups observed in Figure 2. The infrared spectrum of the dehydrated form of Lactose compared to spectra from the monohydrate and the material exhibit significant differences. (4) A comparison of infrared spectra from the monohydrate and dehydrated forms of Lactose is illustrated in Figure 2A and 2B. There is significant change in the O_H stretch region (3000 to 3600/cm) as well as

throughout the spectra indicating significant differences in hydrogen bonding characteristics within the crystal lattice between the two different hydration states.

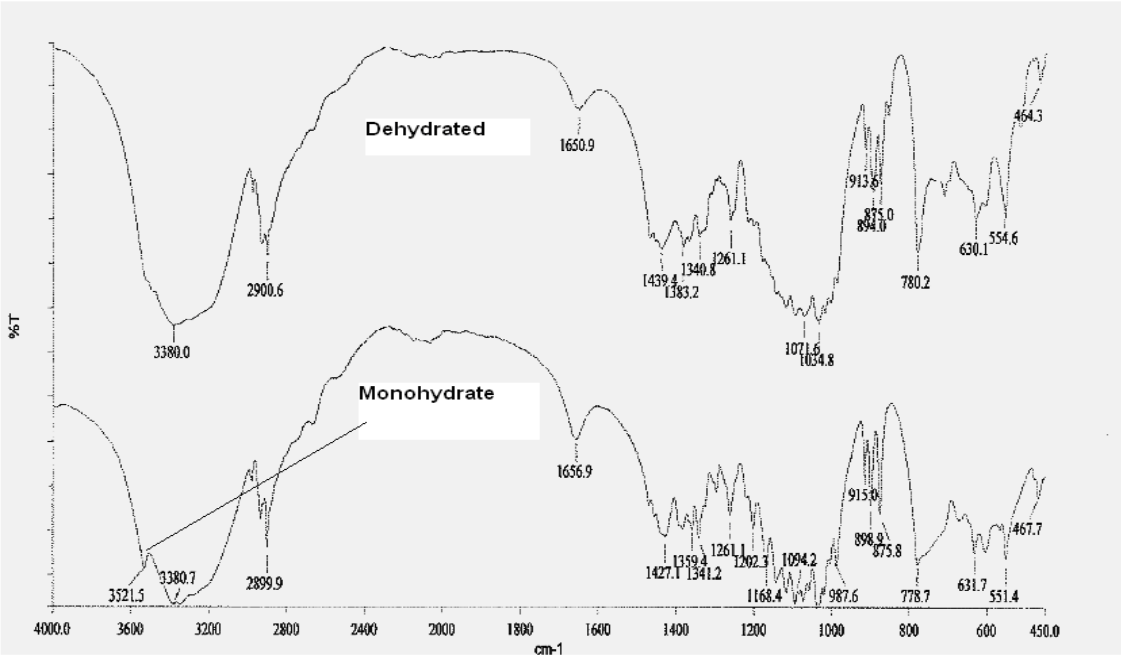


A. TGA **B. DSC**

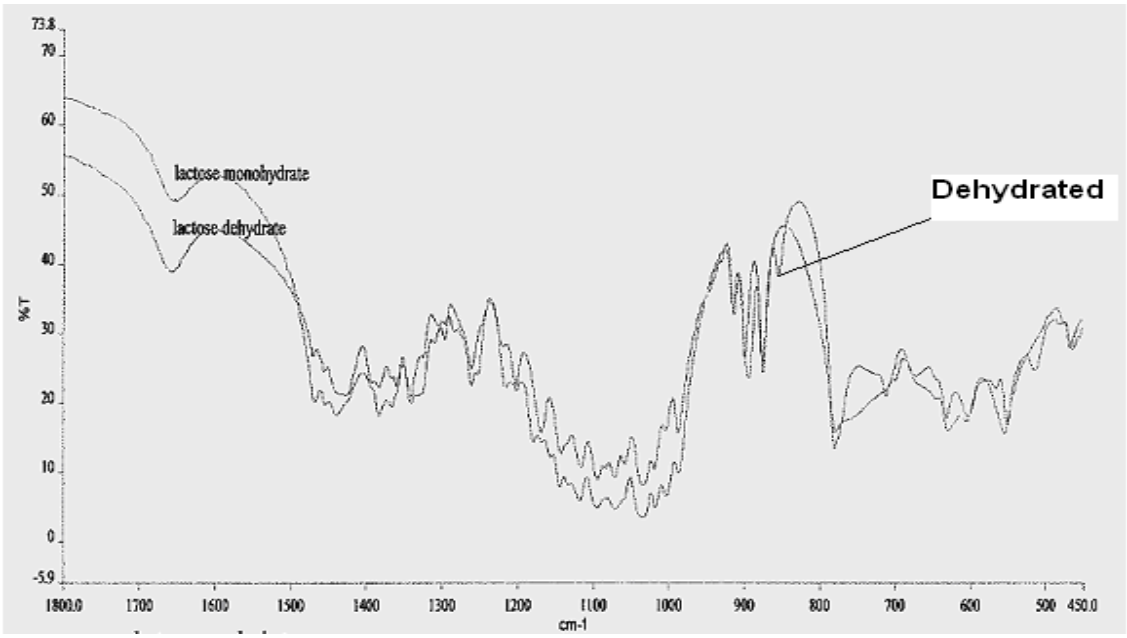
Figure 1. Infrared Spectroscopy

A: Comparison of TGA curves for as such, dehydrated and dehydrated sample exposed at RT for 3 days. Loss of 1 mole of crystal bound water observed at about 140°C in as such and dehydrated exposed sample but not in dehydrated sample.

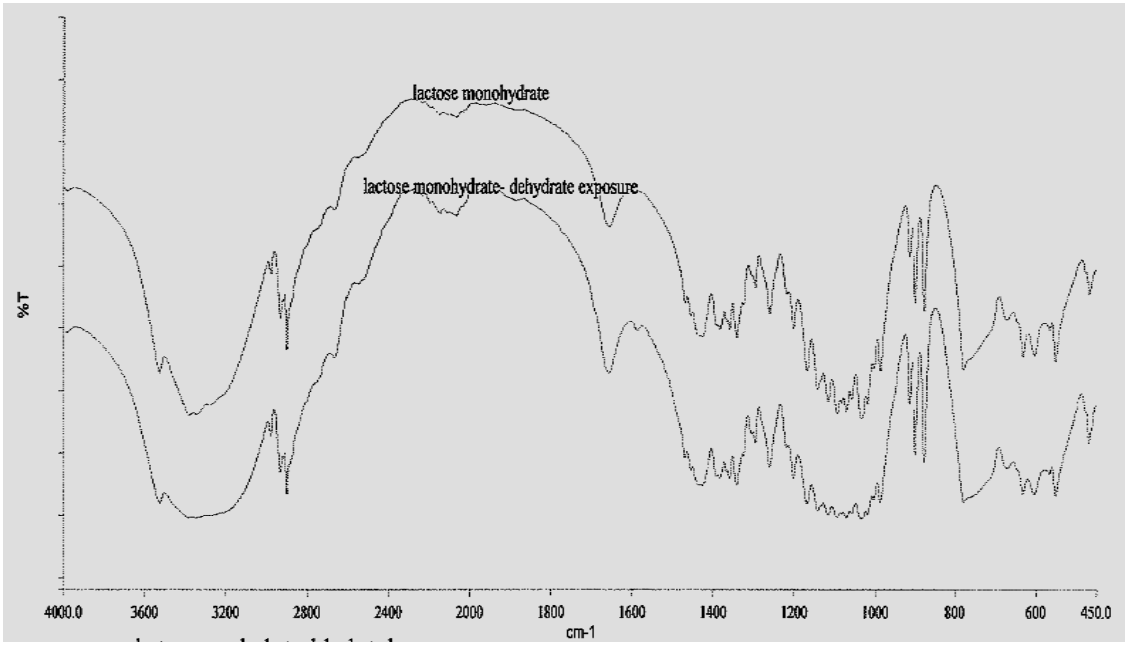
B: Comparison of DSC curves. No sharp dehydration endotherm at about 140°C in dehydrated sample w.r.t. monohydrate and dehydrated sample exposed at RT for 3 days.



A



B



C

Figure 2. A and B: Infrared spectra of the Monohydrate and dehydrated forms of Lactose shows differences. C. Infrared spectra of the Monohydrate and dehydrated forms exposed at RT for 3 days of Lactose shows similarity.

The monohydrate form exhibits a sharp, distinct, O_H stretch peaks at 3521.5/cm. The shape and location of these peaks are indicative of constrained water in the crystal lattice. Dehydration of the monohydrate form results in the disappearance of the peak at 3521.5/cm producing spectral evidence indicating water was removed from crystal lattice. Upon rehydration for three days, water is coming back to the same position and FT-IR spectra are well matches with that of

monohydrate spectra shown in figure 2C. Changes observed upon dehydration of monohydrate show shifting and changes in relative peak intensities in this spectral region from 800 to 900/cm.

X-Ray Diffraction

X-ray powder diffraction clearly discriminates between the fully hydrated and dehydrated forms of Lactose monohydrate as shown in Figure 3A. Upon the loss of 1 mole of water from the crystal system, the lattice undergoes a transition. This transition results in the formation of a new lattice configuration, which is distinct from commercially available anhydrous form(Fig. 3B). By slowly heating a fully hydrated sample on a hot stage X-ray diffraction stage (Fig. 3C) it was demonstrated that the removal of the bound water causes a spontaneous lattice readjustment corresponding to the X-ray signature of the dehydrated monohydrate. As a result, when a bulk sample is dried, the characteristic peaks for the dehydrated form grow as the corresponding peaks for the fully hydrated form decrease and disappear. These results indicate that the population of dehydrated form increases relative to the fully hydrated monohydrate.

Changes in the morphology associated with dehydration may be responsible for the crystal fracturing reported in the thermal analysis section and subsequently discussed in the microscopy and particle size sections of this report. Once dehydrated, the material will rehydrate under ambient conditions (50% RH at 25°C) over a period of time Fig. 3A. The only effect of the dehydration process is a reduction in crystal size, which is confirmed by particle size distribution and BET Surface area analysis data.

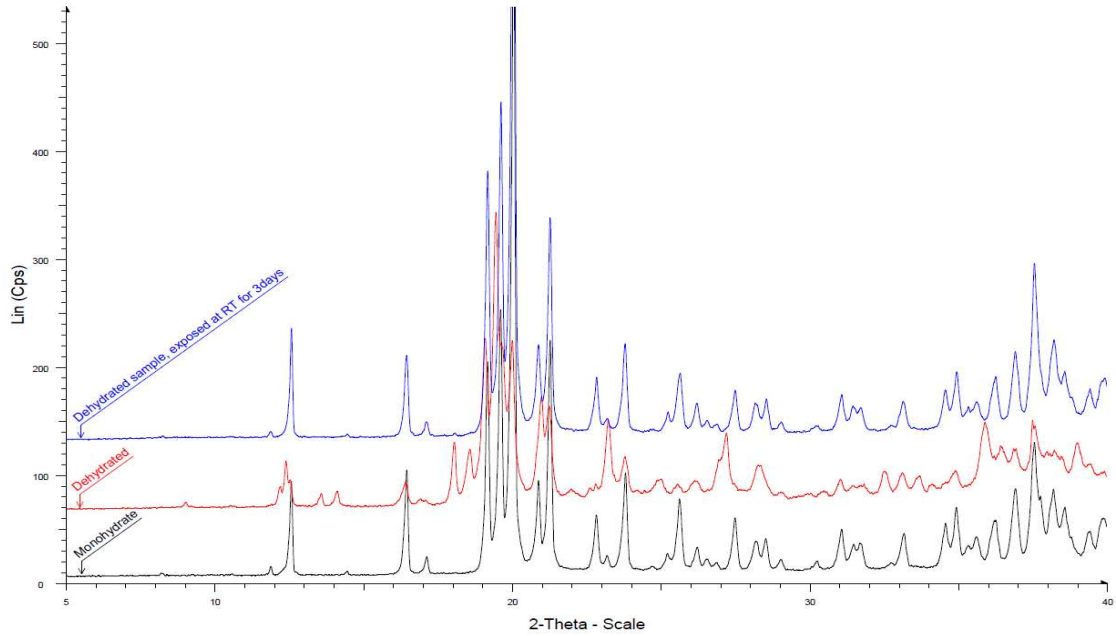


Figure 3A. Overlaid diffraction pattern Lactose monohydrate, dehydrated and dehydrated sample exposed at RT for 3 days.

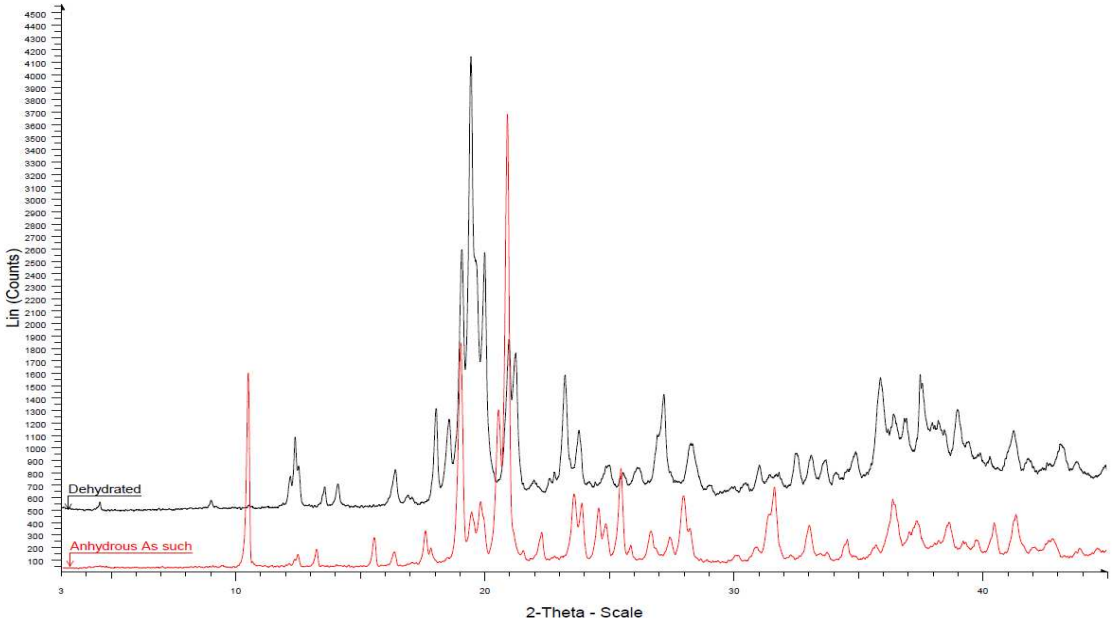


Figure 3B. Diffractogram of Dehydrated Lactose (Top) shows distinct pattern when compare with commercially available Lactose Anhydrous (bottom).

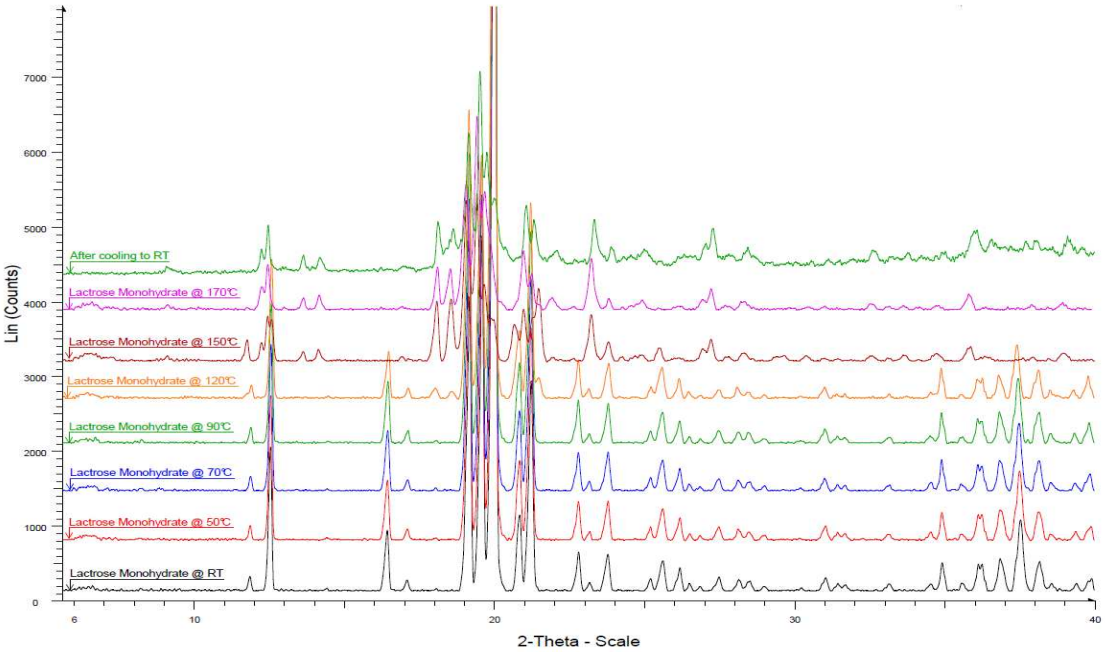


Figure 3C. Lactose Monohydrate (bottom) undergoing the complete transition to the dehydrated form (top last but one) while being slowly heated on the hot stage XRD system and dehydrated sample cooled at room temperature and room relative humidity does not come back to Monohydrate immediately (top).

Particle Size/ Hot stage light Microscopy

Comparison of fully hydrated monohydrate to the same lot of material after dehydration illustrates the impact that drying has upon the particle size shown in table-1 and the structural

integrity of the crystals (Fig. 4). Brightfield and polarized light images of monohydrate show merely cubic crystals with high birefringence crystals, which appear to be colorful. Images of the same material following dehydrattion, show crystals that appear colorless and deep dark shown in figure 4A. A mole of water is oozing out of Lactose monohydrate particle during dehydration also shown in the figure 4B. The external dehydrated material using hot plat is also studied under the hot stage microscope and observed that there is no water oozing out of particles. The same material was exposed for three days at room temperature (25°C) and room relative humidity (50%RH) and again studied under the hot stage microscope and observed that the water is oozing out of particles shown in figure 4C.

Table-1 PSD Results for Lactose monohydrate, anhydrous and dehydrated sample

S. No.	Sample	PSD		
		D10	D50	D90
01.	Monohydrate	80.26	151.29	257.045
02.	Monohydrate Dehydrate	67.55	145.15	254.37

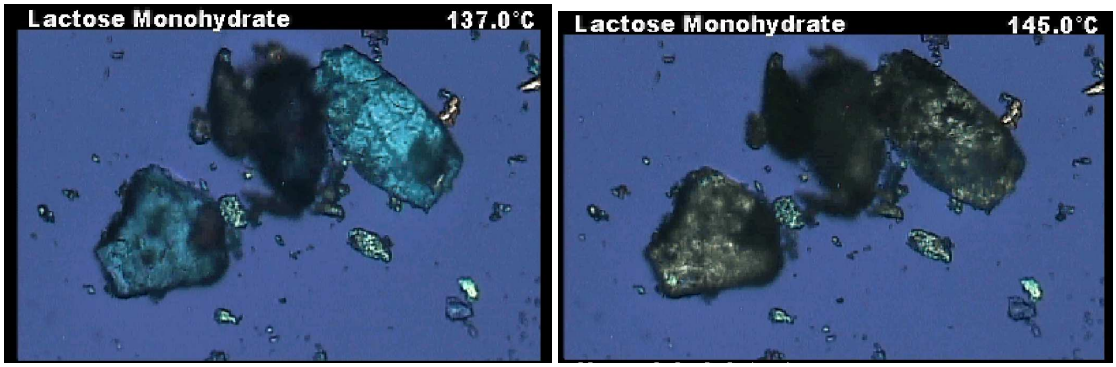


Fig-A **a** **b**

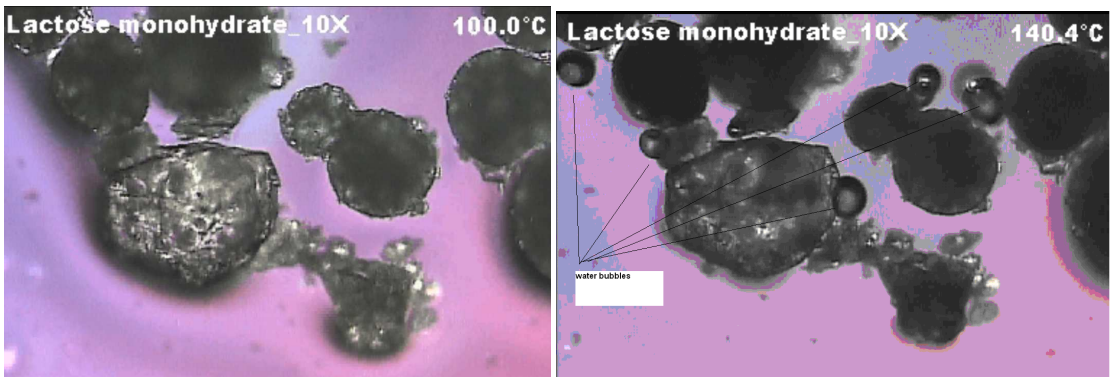
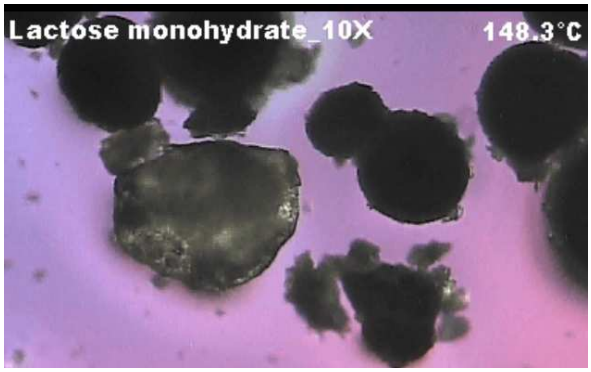


Fig-B **a** **b**



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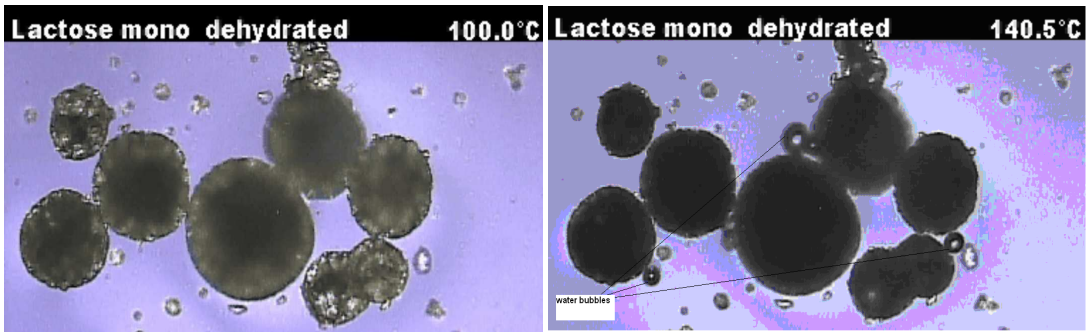

Fig-C **a** **b**

Figure 4.
Fig-A: Lactose Monohydrate a. before dehydration, b. after dehydration
Fig-B:Lactose Monohydrate a. before dehydration, b. Water oozing out during dehydration, c. after dehydration
Fig-C: Lactose Monohydrate a. after dehydration exposed at RT for 3days, b. Water molecules oozing out during dehydration

BET specific surface area analysis

Decrease in the particle size increases the specific surface area, and at the same time dehydration leads to increase the porosity.

Surface area of Lactose monohydrate and dehydrates sample were measured using QuadraSorb SI BET automated surface area and pore size analyser. Results are reported in table-2

Table-2 BET Specific surface area results for Lactose monohydrate, anhydrous and dehydrated sample

S. No.	Sample	BET Surface area
01.	Monohydrate	0.184 m2/g
02.	Dehydrated Monohydrate	0.272 m2/g

CONCLUSION

Dehydration of crystal hydrates can create new less hydrated crystal forms or even create amorphous forms. Removal of 1 mole of bound water from Lactose monohydrate results in the creation of a new anhydrous form which is different from existing commercially available anhydrous form and does not revert back to the monohydrate under appropriate conditions

immediatly. Evidence for creation of this new phase includes a unique X-ray diffraction pattern. Dehydrated Lactose also displays different behavior in the DSC than the monohydrate form. This behavior includes a sharper and more intense endotherm for removal of the one mole of water from the crystal lattice of hydrated Lactose relative to the lattice of dehydrated one. Changes in the crystal lattice for the dehydrated form affects how this one mole of water is bound to the lattice. Adjustment of the lattice affects the mechanical strength of the dehydrated crystal form which readily fractures producing crystals with smaller particle size. This reduction in particle size is evident from particle size data. Understanding this physical consequence of drying displays the importance of complete characterization of an excipient under various environmental conditions of humidity and temperature. The dehydration process is a one-state process that is evident from X-ray diffraction data recorded while slowly heating the sample to drive off 1 mole of crystal bound water. Similar experiments were performed by IR spectroscopy. Transformation from the monohydrate to the dehydrated state occurs in one step as evident from disappearance of peaks in the powder pattern consistent with the monohydrate form concurrent with the appearance of peaks consistent with the dehydrated form. As shown in this study, dehydration of the desired form results in a new unique signature. Without the knowledge that this signature indicates over drying, the result may be interpreted as identification of an undesirable form rather than evidence that the desired form was somewhat over dried. Likewise, without a complete understanding of this drying phenomenon, it is possible to obtain erroneous results during routine analysis if care is not taken to control the exposure of the excipient form to environmental or testing conditions that promote dehydration. In conclusion, dehydration of pharmaceutically accepted excipient hydrates can easily occur under different storage conditions with varying temperature and RH. Crystal hydrate forms also can undergo dehydration or rehydration during manufacturing processes such as drying, milling, or tabletting. In this study, conditions required for the dehydration of Lactose monohydrate have been described, and the dehydrated crystal form has been extensively characterized. Complete characterization of dehydration and rehydration processes is necessary for a thorough understanding of the performance of crystal hydrate forms of inactive pharmaceutically accepted ingredients or excipients used in pharmaceutical manufacturing.

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