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Morphological Changes of $\alpha$-Lactose Monohydrate ($\alpha$-LM) Single Crystals under Different Crystallization Conditions Using Polar Protopic and Aprotic Solvents

K.Vinodhini$^{1, a}$, K.Srinivasan$^{1, a}$

1 – Crystal Growth Laboratory, Department of Physics, School of physical sciences, Bharathiar University, Coimbatore, Tamil Nadu, India
a – nivas_5@yahoo.com

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ABSTRACT. In this study, polar protic (water) and aprotic (DMSO) solvents were used to analyse the morphological changes of alpha-lactose monohydrate ($\alpha$-LM) single crystals. The solubility of $\alpha$-LM determined in DMSO by gravimetric method is nearly 2.5 times higher than that found in water at 33 °C. Surprisingly, the weight of the solution increases with time in DMSO as it is highly hygroscopic, whereas the weight of the solution decreases with time in water as it evaporates. Also it is found that the supersaturation of aqueous solution gradually increases by evaporation of solvent, whereas the supersaturation of DMSO solution gradually increases by moisture absorption which reduced the solubility of $\alpha$-LM. The increasing level of supersaturation in multiple ways in DMSO and water at 33°C induces the mean crystal size, which can be explained by taking into account the role of solvent in the growth environment. The form of grown crystals was confirmed by powder x-ray diffraction (PXRD) and differential scanning calorimetry (DSC) study.

1. Introduction. In aqueous lactose solution, both $\alpha$ and $\beta$-Lactose forms change into one another continuously in reversible equilibrium called mutarotation [1, 2]. When $\alpha$-LM dissolves in water, it readily undergoes mutarotation to yield the $\beta$-isomer. DMSO an aprotic solvent reduces the rate of mutarotation of $\alpha$ to $\beta$-Lactose in solution [3]. For this reason, in this present study, DMSO and water were used as solvent for growth of $\alpha$-LM at 33 °C. But there are always interactions of $\beta$-L on the nucleated $\alpha$-LM crystals that are unavoidable as reported by many other researchers. Size and shape of the nucleated $\alpha$-LM crystals act as an important parameters in the formulation of drug products used both orally and in the form of an inhaler because a small change in this attributes influence blending and mixing, caking, compatibility, flow ability, and aerosol performance. So the changes in the morphology of $\alpha$-LM single crystals under different crystallization conditions using different solvents were investigated. Different habits of $\alpha$-LM crystals were harvested from DMSO and water by slow and fast evaporation methods. These grown crystals were subjected to powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) study.

2. Experimental procedure. At 33°C, the solubility of $\alpha$-LM were determined in different solvents, water as polar protic and dimethyl sulfoxide (DMSO) as polar aprotic by gravimetric method. The $\alpha$-LM powder was individually dissolved in DMSO and water with constant stirring at the rate of 250 rpm for 24 hrs. After reaching the equilibrium solubility, the solutions were filtered with Whatmann filter sheets and then 9 ml solutions were only collected, in order to reduce the weight variations of these filtered solutions. Thus the obtained solutions were taken in beakers with a perforated parafilm and kept for growth in water bath maintained at constant temperature of 33 °C. Solutions placed in the beakers were weighed for every 24 hrs to measure the total mass of solution.
The above procedure was repeated again to individually observe the microscopic images of the nucleated α-LM crystals by fast evaporation. For this experiment, 500μl of the solutions were pipetted out on petri dishes and then the size and shape of the nucleated crystals were carefully analysed through microscope.

3. Result and discussion

3.1. Slow evaporation method

3.1.1. Growth of α-LM in DMSO

The equilibrium solubility of α-LM in DMSO was found to be 6.8 g per 10 ml. From this solution, 9 g of the solutions were adopted by slow evaporation method. The initial weight of DMSO solution increased from 9 g to 12.16 g until the grown crystals were harvested as DMSO is highly hygroscopic. DMSO absorbs water and gets diluted to a concentration of 66 to 67 % than the initial concentration when exposed to room temperature as the DMSO - water bond is 1.33 times stronger than the water - water bond [4]. Hence, the hygroscopic nature of DMSO naturally helps for the growth of α-LM because the solubility of α-LM in DMSO that diluted with water is always low [5]. When the interesting phenomenon of moisture absorbance is occurred to DMSO solution, another interesting phenomenon of colloidal creamy structure is formed within overnight. The photographs shown below (Figs.1a and 1b) illustrate the formation of creamy structure (milky white).

![Photograph of formation of creamy structure (milky white), a) The microscopic image of creamy structure (milky white), b) The microscopic image of nucleated α-LM crystals after the disappearance of milky white, d) Photograph of the grown macroscopic α-LM crystals from DMSO by slow evaporation method](image)

After a few days, this creamy structure disappeared and then clears up again because of the occurrence of nucleated crystals in solution. The microscopic image in Fig.1c, illustrates the disappearance of milkiness. Although it is an interesting phenomenon, its nature is as so far unknown. If it were α-LM, there seems no reason why it clears up again. It may be anhydrous α-L, but it could not be confirmed through PXRD and DSC analyses because the anhydrous α-L is transferred into α-LM very quickly. Hence, in DMSO solution nucleation was formed after a long time because there was time delay between the time of clearing the milky white and the time when the visible crystallization of lactose takes place. The photograph (Fig. 1d) shows crystals of α-LM grown in DMSO by slow evaporation

3.1.2 Growth of α-LM in water

The equilibrium solubility of α-LM in water was found to be 2.7 g per 10 ml respectively. From this solution, 9 g of the solutions were adopted by slow evaporation method. The weight of the water solution decreased from initial weight of 9 g to 2.021 g until the grown crystals were harvested due to the evaporation of water. The induction period of α-LM in water was lower than that in DMSO. The Figures (2a and 2b) show the microscopic images of nucleated α-LM by slow evaporation. The Fig (2c) shows macroscopic photograph of α-LM crystals grown in water by slow evaporation.

Hence, increasing the supersaturation in different ways in different solvents at 33 °C induced a significant increase in the mean crystal size. During the slow evaporation process, meta stable zone is larger in lactose solution when compared to other sugar solutions because of the interconversion of α-L and β-L and vice versa [6]. Hence the interactions of β-L on the nucleated α-L crystals are
unavoidable. Due to this reason all the nucleated \( \alpha \)-LM crystals are formed with tomahawk morphology as reported by many other researchers.

![Fig. 2. a) – b) The microscopic images of nucleated \( \alpha \)-LM crystals, c) Photograph of the grown macroscopic \( \alpha \)-LM crystals from water by slow evaporation method.](image)

3.2. Fast evaporation method

3.2.1. Growth of \( \alpha \)-LM in DMSO and water

During the fast evaporation process, solutions get supersaturated more rapidly and also time available for interconversion is comparatively low. So \( \alpha \)-LM crystals nucleate within a short period of time and the interaction of \( \beta \)-L on the different growth faces of \( \alpha \)-LM is significantly low. As a result, the growth of (010) face significantly increased and yielded more symmetrically needle-like crystals. It can be clearly visualized from 500\( \mu \)l solution that was observed from prepared DMSO and water solution as shown in Fig 3(a), (b), (c) & (d). In fast evaporation method also it was found that the induction period of \( \alpha \)-LM in water was lower than that in DMSO because there was delay of several minutes between the time of milky white formation and then disappearance again and finally visible crystallization takes place. This interesting phenomenon is shown in fig (3a and 3b )

![Fig. 3. The needle like morphology of \( \alpha \)-LM single crystals grown from DMSO (a, b) and water (c, d)](image)

Fig. 3, a) and b) show the crystals formed through transitory milky white after 7.30 and 8.30 hrs respectively with DMSO. Fig. 3, c) and d) show the crystals formed after 16 and 20 minutes respectively with water.
The desired needle-like morphology of α-LM crystals has been achieved only by fast evaporation method. Hence, in order to estimate the purity of α-LM crystals obtained with needle-like morphology in DMSO and water, the nucleated crystals were subjected to PXRD and DSC analyses. Fig. 4a and 4b show the PXRD patterns of α-LM crystals grown from DMSO and water by fast evaporation method. The characteristic PXRD peak for α-LM was observed at 20° 2θ. Fig. 5a and 5b show the Differential scanning calorimetry curve of α-LM crystals grown from DMSO and water by fast evaporation method. The DSC results also show that the grown crystals are α-LM. The endothermic peak observed at 148.54-157.47 °C obtained indicates the removal of water of crystallization from α-LM crystals, whereas the endothermic peak observed at 204.56-211.02 shows the melting point of α-LM crystals. The obtained results by above estimation confirmed the form of crystallization of pure α-LM. Figs. 4a and 4b show the experimental PXRD and DSC analyses of α-LM crystals with needle-like morphology grown from DMSO and water.

Summary. The solubility of α-LM in DMSO and water were determined. The α-LM crystals with tomahawk morphology were harvested in DMSO and water by slow evaporation method, whereas the α-LM crystals with needle-like morphology were harvested by fast evaporation method. The fast evaporation method employed led to the achievement of crystals with desired morphology and hence the crystals were subjected to PXRD and DSC analyses. The obtained results confirmed the form of pure α-LM.

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