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ARTICLE TYPE

The role of DMSO: water mixture on the crystallization of α-Lactose monohydrate (α-LM) single crystals with desired morphology

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In this study, DMSO – water solvent mixtures (from 10 to 90% v/v) were used for the growth of alpha-lactose monohydrate (α-LM) single crystals. In the mixture of solvents (i.e.) DMSO and water in the ratio from 9D:1W to 1D:9W, the solubility of α-LM in these solvent mixtures show a parabolic relationship. While adopting two crystallization methods, slow and fast evaporation, the growth rate depends on the evaporation of water in water rich solutions. On the other hand, in DMSO rich solutions it depends on the absorption of moisture that reduces the solubility of α-LM. The water absorption nature of DMSO and evaporation nature of water at 33 °C induced an important increase in the mean crystal size, which can be explained by taking into account the role of solvent on the growth in the environment. The bulk single crystals of α-LM with tomahawk morphology having different sizes were grown by slow evaporation. Among these 9 mixing compositions, 9D:1W and 1D:9W mixtures only produced industrially preferred needle like morphology of α-LM with narrow crystal size distribution while adopting the fast evaporation. Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) analyses confirm the form of crystallization of pure α-LM with different morphologies obtained by fast and slow evaporation methods. The percentages of α-L present in the grown crystals having different morphology were determined using automatic digital polarimeter. The fast evaporation method employed led to the achievement of crystals with desired morphology, narrow crystal size distribution, shorter nucleation period and high purity compared to slow evaporation method.

1. Introduction

Lactose exists in two isomeric forms, alpha (α-L) and beta (β-L) lactose, which differs only in one special position of hydroxyl group on the first carbon of glucose moiety [1- 4]. In aqueous solution, both α- L and β-L will mutually transform from one to the other to maintain an equilibrium ratio between them by changing the position of the hydroxyl group, a peculiar activity known as 'mutarotation'. α-L crystallizes only below 93.5° C as a monohydrate whereas; β-L crystallizes above 93.5° C as anhydrous form [5-8]. Generally alpha –Lactose crystallizes in three different forms: alpha lactose monohydrate (α-LM), stable alpha lactose anhydrous (α-L_S) and hygroscopic alpha lactose anhydrous (α-L_H). α-L_H is thermodynamically unstable at ambient environmental conditions as it readily absorbs moisture from the atmospheric air [9,10]. α-L_S is relatively more stable compared to α-L_H. α-LM is the only thermodynamically stable form among these three crystal forms. Hence it is widely used as a more suitable candidate for the dry powder inhaler (DPI) applications [11, 12]. One of the challengeable criteria in the crystallization of α-LM is the achievement of narrow crystal size distribution (CSD). It has been reported that in order to achieve the desired morphology of α-LM with narrow CSD, the antisolvent crystallization of α-LM at higher concentration of alcohol in aqueous solution is considerably important. But β-L crystals will be dominantly nucleated at high concentration of alcohol [13-15]. Hence in order to obtain the desired morphology of pure α-LM with narrow CSD for DPI application, it is essential to control the nucleation of β-L in mother solution. It was reported that the rate of mutarotation of α-L and β-L is comparatively low in dimethylsulfoxide (DMSO) compared to water [16]. The presence of β-L within the solution acts as a habit modifier to α-LM and it leads to the growth of tomahawk shaped α-LM crystals [16, 17]. The size and morphology of the nucleated α-LM crystals are important in pharmaceutical industry because even a small change in these attributes influences the flowability, compactability and aerosol performance [18-21]. Hence, the solutions with nine mixing compositions from 9D:1W to 1D:9W were chosen for the growth of α-LM by two different crystallization methods such as slow evaporation and fast evaporation. The morphology of the nucleated α-LM single crystals were analysed by optical microscopy. The grown crystals were subjected to PXRD and DSC analyses and the percentage of α -L present in the nucleated crystals were calculated using the polarimetric analysis.

2. Experimental procedure

α- Lactose Monohydrate (α-LM) and dimethylsulfoxide (DMSO) from Merck and laboratory double distilled water were used in all experiments. The α-LM powder was individually dissolved in known volume of solvent mixtures such as 90% DMSO and 10% water (9D:1W), 80% DMSO and 20% water (8D:2W), 70% DMSO and 30% water (7D:3W), 60% DMSO and 40% water (6D:4W), 50% DMSO and 50% water (5D:5W), 40% DMSO and 60% water (4D:6W), 30% DMSO and 70% water (3D:7W), 20% DMSO and 80% water (2D:8W) and 10% DMSO and 90% water (1D:9W). The solvent mixtures with α-LM were taken in round bottom flasks at temperature of 33°C kept in the constant temperature bath with continuous stirring at the rate of 250 rpm for 24 h. After reaching the equilibrium, the solutions were filtered with Whatmann no.41 filter sheets and 1g of the solutions were pipetted out on petridishes and subjected to fast evaporation. In these experiments, size and shape of the nucleated crystals were carefully analysed through an Olympus Stereo Zoom research microscope SZX16 attached with a Jenoptik progress (CT3) digital camera. The weights of these solutions were measured at constant intervals of time to understand the hygroscopic nature of DMSO. The remaining 9g of solutions were poured into clean crystallization beakers, covered with perforated parafilm, and kept in a water bath maintained at constant temperature of 33 °C. The solutions were left for growth and continuously monitored for the occurrence of nucleation. Weights of these solutions were measured at every 24h till the end of the experiments. The procedure was same for all the experiments. The nucleated crystals were confirmed by subjecting them to PXRD and DSC analyses. The percentage of α -L present in the nucleated crystals were calculated using the automatic digital polarimeter.

3. Result and discussion

3.1. Solubility and slow evaporation

The equilibrium solubility of alpha-lactose monohydrate in 9D:1W, 8D:2W, 7D:3W, 6D:4W, 5D:5W, 4D:6W, 3D:7W, 2D:8W and 1D:9W were found to be 3.3 g,1.6 g, 0.8 g, 0.7 g, 0.6 g, 0.8 g, 1.0 g, 1.5 g and 2.3 g per 10 mL respectively. The solubility of all solutions was measured at 33 °C. When concentration of DMSO is increased in water, the percentage of water molecules which hydrogen bonded to themselves is substantially reduced compared to pure water due to a large proportion of the hydrogens available for bonding with the lone pairs of the DMSO [22]. When DMSO and water are in equal ratio, the hydrogen bonding interactions between water and DMSO are stronger than that of the α-LM and DMSO or α-LM and water, hence the solubility of α-LM is less in this combination (5D:5W). Whereas, if the concentration of any one among the two solvents is higher i.e., 6D:4W or 4D:6W then the interaction between the two solvents will be less than that observed in equal ratios. Moreover, in these unequal ratios the constituent solvent with dominating concentration exerts more influence on the solubility of α-LM than the one with lesser concentration. The variation in the solubility of α-LM at different volumes of solvent mixtures is shown in figure.1.

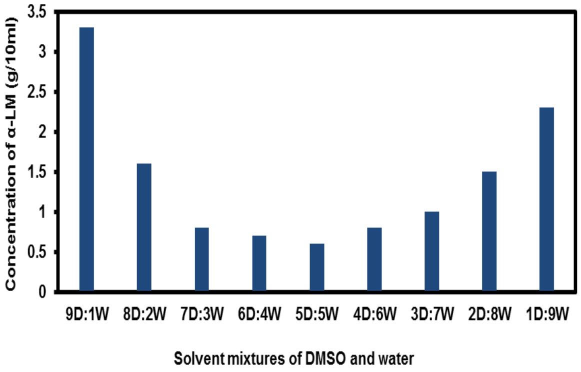

Fig.1 Variation in the solubility of α-LM in the solvent mixtures of DMSO and water (9D: 1W to 1D: 9W)

It was reported that DMSO absorbs water and gets diluted to a concentration of 66 – 67% than the initial concentration when exposed to atmosphere as the bonding between DMSO and water is 1.33 times stronger than the bonding between two water molecules [23]. Hence, the 9g of α-LM solution was weighed at constant intervals of every 24 h and the percentage of water molecules present in it was determined. The obtained results indicate that the percentage of water molecules gets increased with time in DMSO rich solutions. This maximum increase depends on the moisture absorption capability of DMSO at a particular concentrations in the mixture solvents whereas, the percentage of water molecules get decreased with time in water rich solutions. The maximum decrease depends on evaporation capability of water that in turn depends on the concentration of water in mixture solvents and the temperature difference between the solution and ambient. The obtained results are shown in figure 2. From the figure 1, it is clear that the solubility of α-LM reduces when the percentage of water molecules increases in DMSO. This solubility reduction is increased until the water molecules reach the same proportion of DMSO in the solution. Hence the solubility of α-LM in DMSO rich solutions (9D:1W and 8D:2W) reduced when the moisture absorption from atmospheric air takes place. The solubility reduction of α-LM generated the necessary supersaturation for the nucleation of α-LM crystals and the crystals were grown until the absorption proceeds further. With 14.78% and 22.07% of moisture absorption, the induction time of α-LM nucleated in 9D: 1W and 8D:2W solvent mixtures are 15 and 34 days respectively. The nucleated crystals were allowed for further growth and on attaining a reasonable size the α-LM crystals with the traditional tomahawk morphology were harvested. But in 7D:3W, 6D:4W solvent mixtures, the nucleation was not observed even after 2 months as the solubility of α-LM is very low compared to other DMSO rich solutions. This might also be due to the fluctuation in the driving force resulted by the alternative water absorption and evaporation in these solvent mixtures. On the other hand, the solutions having high water content (5D:5W, 4D:6W, 3D:7W, 2D:8W and 1D:9W), solvent evaporates slowly and hence the excess solute concentration generates the nucleation of α-LM crystals.

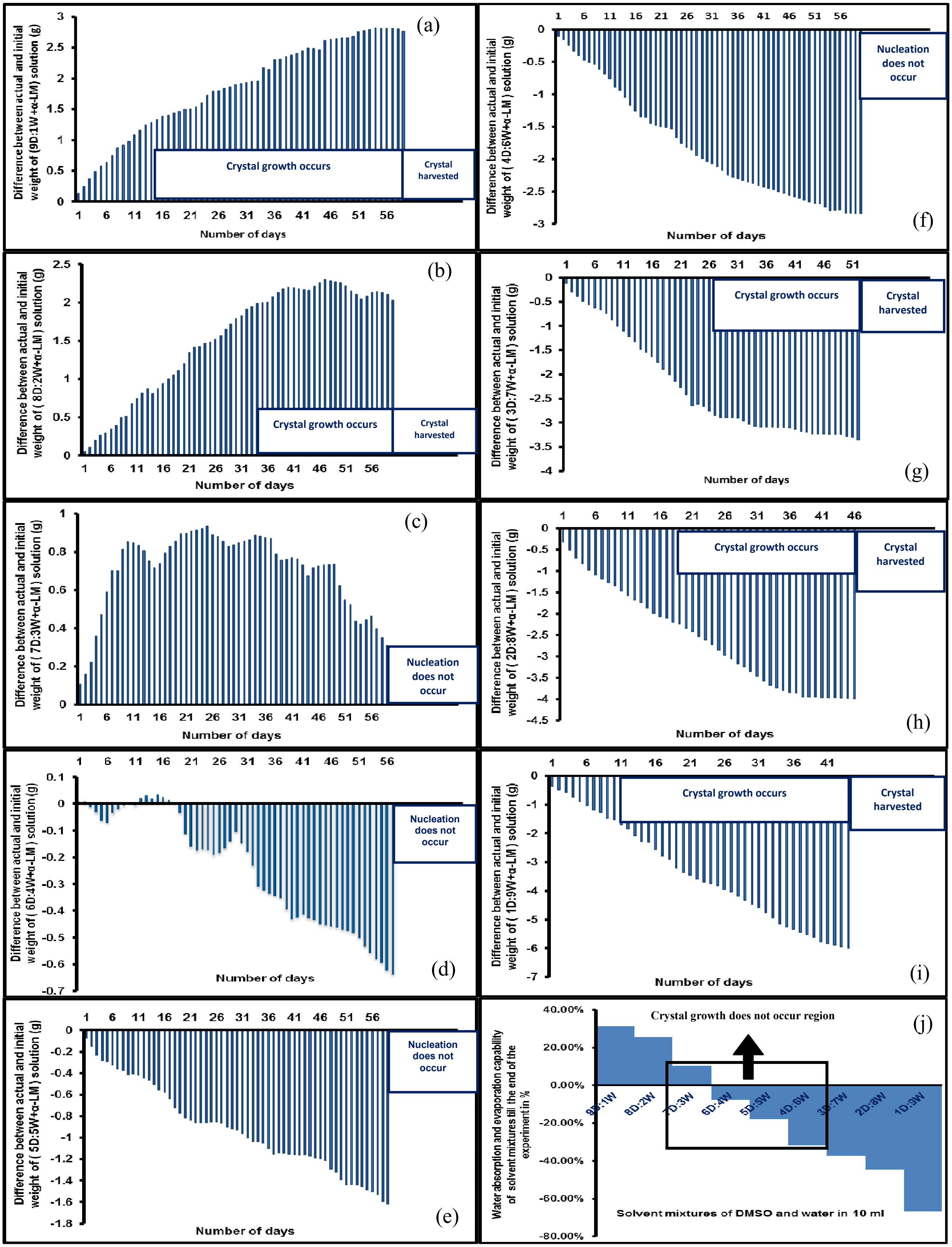

Fig.2 The observed weight difference of solutions at constant time intervals by slow evaporation method

Thus, at 17.01%, 24.95% and 32.17% of water evaporation, the induction time of α-LM nucleated in 1D:9W, 2D:8W and 3D:7W are 11, 19 and 28 days respectively. The nucleated crystals were left for further growth and harvested. In 4D:6W, 5D:5W solvent mixtures, the concentration of α-LM is well below the equilibrium level and hence high evaporation rate is needed to initiate the formation of critical nuclei than that at other water rich solutions (3D:7W, 2D:8W, 1D:9W). But in these solvent mixtures, water evaporation is low compared to other water rich solutions and hence nucleation was not occurred even after the time span of 2 months. From the above observations it is clear that, α-LM crystals nucleated only in DMSO rich solutions such as 9D:1W, 8D:2W and in water rich solution mixtures such as 1D:9W, 2D:8W, 3D:7W and the intermediate solvent mixtures are not suitable for the growth of α-LM crystals by slow evaporation. The photographs given below (Fig. 3) shows the α-LM crystals with tomahawk morphology that was formed in 9D:1W, 8D:2W, 3D:7W, 2D:8W and 1D:9W by slow evaporation.

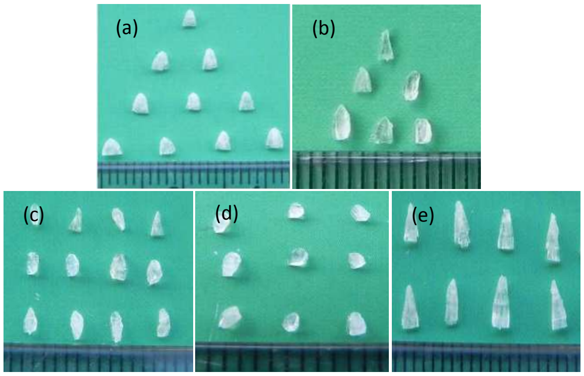

Fig.3 α-LM crystals grown from (a) 9D:1W, (b) 8D:2W, (c) 3D:7W, (d) 2D:8W and (e) 1D:9W solutions

3.2. Fast evaporation

After reaching equilibrium solubility, the filtrate of 1 g was taken in a petridish. The same procedure was repeated for all the experiments i.e., at the combinations of 9D:1W, 8D:2W, 7D:3W, 6D:4W, 5D:5W, 4D:6W, 3D:7W, 2D:8W and 1D:9W solvent mixtures. The weights of the solutions were measured at the interval of every 30 min for 9D:1W, 8D:2W, 3D:7W, 2D:8W and 1D:9W solutions. The growth rate of α-LM was very low in 4D:6W, 5D:5W, 6D:4W and 7D:3W solvent mixtures. Hence, depends upon the growth rate, the weight of the solutions were measured at the interval of every 1h for 4D:6W and at the interval of every 2h for 7D:3W, 6D:4W and 5D:5W mixture solvents. In the case of DMSO rich solution, the growth rate is highly dependent on the rate of moisture absorption which reduces the solubility of α-LM. This effect is a consequence of decrease in interactions between DMSO and solute molecules on the growing crystal face as a result of the increase in interactions between DMSO and water molecules (moisture). During fast evaporation, the reduction in solubility was found to be high as a result of high moisture absorption compared to that in the slow evaporation method and the results are shown in figure 4.

3.2.1. The effect of moisture absorption and water evaporation rates on induction period and the nucleation of α-LM crystals

The nucleation were occurred in the case of DMSO rich solutions i.e., 9D:1W and 8D:2W where the moisture absorption rates were 8.92% and 19.8% at the induction period of 49 min and 8.5 h respectively. Whereas, in 7D:3W, 6D:4W solvent mixtures, alternate moisture absorption and water evaporation rates were observed and hence due to this effect alternative increase and decrease in the weight of the solution is noticed. As a result the high moisture absorption rate of 12.77% and 4.8% in 7D:3W and 6D:4W respectively, were not sufficient enough to induce nucleation within the mother liquor by generating the required supersaturation. Hence eventually, the nucleation was induced only by the evaporation of solvent that occurs at the evaporation rates of 38.81% and 41.40% respectively. It is obvious from Fig. 4c and 4d that the alternative moisture absorption and water evaporation delay the nucleation of α-LM crystals to certain extent, i.e., until attaining the induction period of 68 h for 7D: 3W, 60 h for 6D: 4W solution mixtures. As a result, the metastable state was found to be wider in these solvent mixtures compared to other solvent mixtures. In the case of 5D:5W, 4D:6W, 3D:7W, 2D:8W and 1D:9W solvent mixtures, the threshold evaporation rates 45.62%, 39.43%, 31.39%, 24.56% and 10.14% led to nucleation at the induction period of 54 h, 24 h, 5.5 h, 3.5 h and 34 min respectively. It is obvious from Fig. 4a and 4i that the rate of absorption was high in 9D:1W solvent mixture and rate of evaporation was high in 1D:9W solvent mixture and hence the metastable state becomes narrowed in these solvent mixtures compared to others. Hence it is worth to note that in 9D: 1W and 1D:9W solvent mixtures are favourable for nucleation and growth of α-LM crystals with desired morphology.

3.2.2. Morphology and growth of the nucleated α-LM crystals

The α-LM crystals nucleated in the solvent mixture of 9D:1W exhibited needle like morphology with the (010) face as prominent (Fig.5.1). In the case of 8D:2W solvent mixture, three different morphologies of α-LM crystals were nucleated such as needle with the (010) face as prominent and trapezoidal with the (010), (100) and (0-11) faces which grow at approximately equivalent rates and triangle with (0-11) as prominent face were observed (Fig.S1). In rest of the solvent mixtures such as 7D: 3W, 6D: 4W, 5D:5W and 4D:6W, α-LM crystals were noticed with their traditional tomahawk morphology with (0-11) as prominent faces (Fig.S2, S3, S4 and S5). In the solution mixtures of 3D:7W and 2D:8W, α-LM crystals with mixed morphologies of parallelogram with (010) as prominent and trapezoidal with (010),(100) and (0-11) faces, which grows at approximately equivalent rates and tomahawk with (0-11) as prominent face were observed (Fig. S6 and S7). Finally, at the solution mixture of 1D:9W, needle like α-LM crystals with (010) as prominent face was observed (Fig.5.2).

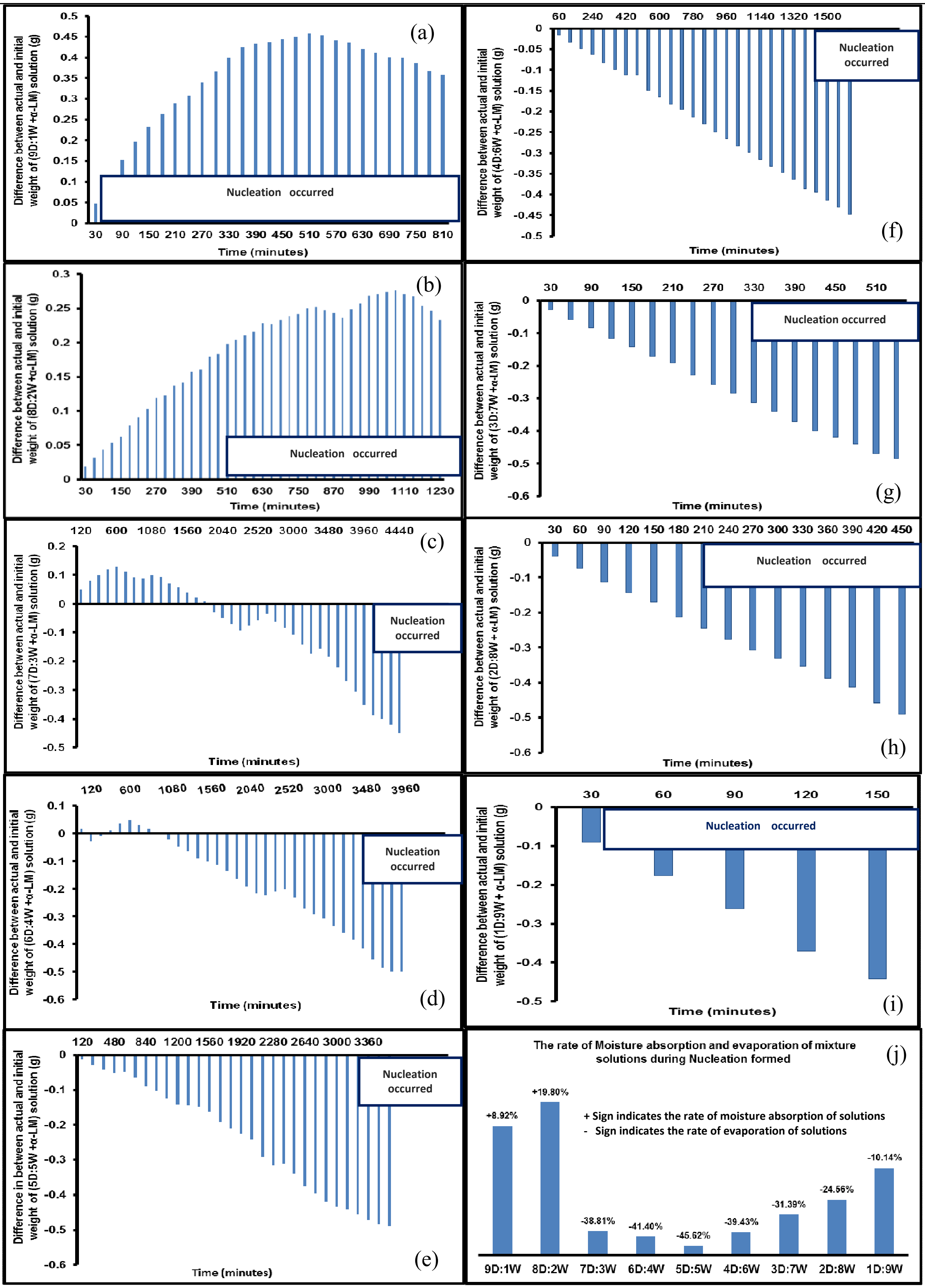

Fig.4 The observed weight difference of solutions at constant time intervals by fast evaporation method

Three different morphologies of α-LM crystals were observed from 1g of solution subjected to fast evaporation. The morphological variations formed an account of the asymmetric nature of growth of α-LM when different ratio of solvent mixtures induce different growth rate in solutions. From these figures it could be observed that the industrially preferred needle like morphology of α-LM has been achieved in the solution mixture of 9D:1W and 1D:9W through the fast evaporation. However, in the same ratio of solvent mixtures (9D:1W and 1D:9W) α-LM crystals with tomahawk like morphology was noticed during slow evaporation. It has been stated by previous report that the influence of β-L on α-LM could be observed in needle like morphology [13-15]. Hence, in order to estimate the purity of α-LM crystals obtained with needle like morphology in the solution mixtures of 9D:1W and 1D:9W, the nucleated crystals were subjected to PXRD and DSC analysis. Furthermore, the percentage of α-L present in the nucleated crystals was also calculated using the polarimetric analysis. The above estimation was also carried out for tomahawk shaped α-LM crystals and the obtained results were compared.

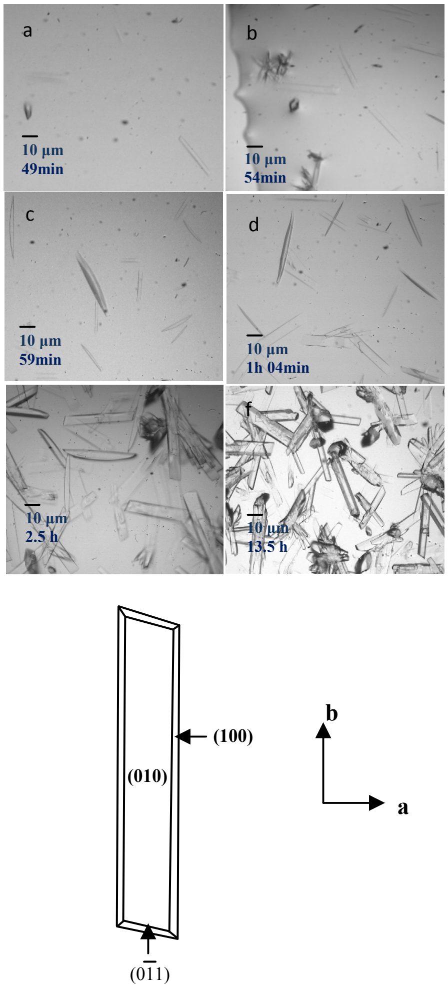

Fig.5.1The α-LM crystals obtained with needle like morphology in 9D:1W solvent mixture by fast evaporation

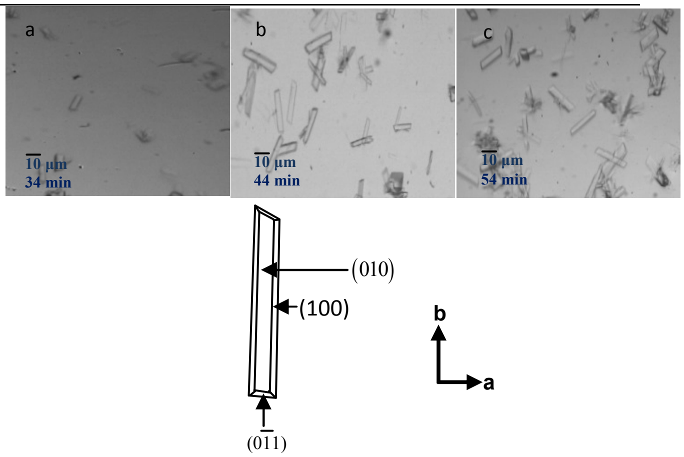

Fig.5.2 The needle like morphology of α-LM crystals obtained in 1D:9W solvent mixture by fast evaporation

3.3. Powder X-ray diffraction (PXRD) study

The characteristic 2θ peak of the α-LM crystals nucleated with needle like morphology in 9D:1W and 1D:9W by fast evaporation method and α-LM crystals with tomahawk morphology formed in 9D:1W and 1D:9W by slow evaporation were observed at 20°2θ (Fig. 6). It is the characteristic peak for α-LM. The X- ray diffraction data of α-LM obtained from the Cambridge Structural Database [24 (a)] concurs with our experimental result. The obtained result confirms that the α-LM crystals having different morphologies are pure in their crystalline form [24- 28]. The primary characteristic peak of β-L and α-L anhydrous is 10.5° and 18° 2θ respectively [24 (b) and (c)] and these peaks are not present in this study. Hence the crystals do not have both anhydrous forms of α-L and β-L characteristics. The intensities of each diffraction peak are different for crystals grown from fast and slow evaporation those having different morphologies. It is due to the total area of each crystal face is varied with crystal habit [29].

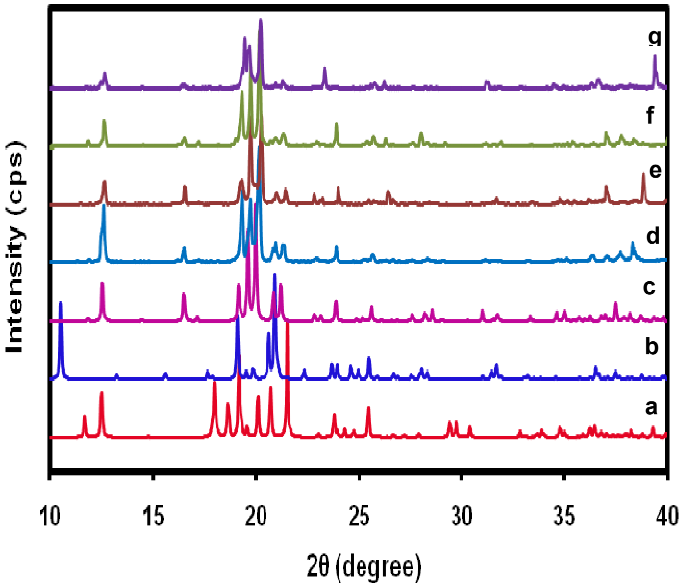

Fig.6 Powder X-ray diffraction patterns of α-LM grown from 9D: 1W and 1D: 9W mixtures by fast and slow evaporation

Hence the intensity of the PXRD peak depends on the shape of the crystals. The presence of preferred orientation especially for the needle shaped crystals cause a variation in intensities and the missing of certain theoretical peaks in figure 6. Fig 6 (a), (b) and (c) are the reference PXRD patterns of anhydrous α-L, β-L and α-LM obtained from the Cambridge Structural Database [24 (a), (b) and (c)]. Fig 6 (d) and (e) are the experimental PXRD patterns of α-LM crystals with needle and tomahawk morphology from 1D:9W solution. Fig 6 (f) and (g) are the experimental PXRD patterns of α-LM crystals with needle and tomahawk morphology from 9D:1W.

3.4. Differential Scanning Calorimetry (DSC) analysis

The DSC thermograms recorded for α-LM crystals with needle like morphology obtained by fast evaporation method and tomahawk morphology obtained by slow evaporation are given in figure 7. From the recorded DSC thermograms it is clear that, the α-LM crystals having different morphologies are in their pure form without any incorporation of β-L [30-32]. The recorded thermograms shows clear endothermic peak at 140.7-148.9°C corresponding to the removal of water from α-LM crystals and another endothermic peak is melt of α anomer at 209.5 – 210.9°C. It indicates that crystallized samples are only α-LM. The doublet (split) endothermic peaks [Fig. 7(c) and (d)] presented at corresponding dehydration event is due to the loosely bounded hydrate in the grown crystalline samples especially for α-LM crystals with needle and tomahawk morphology from 1D:9W solution [30-32].

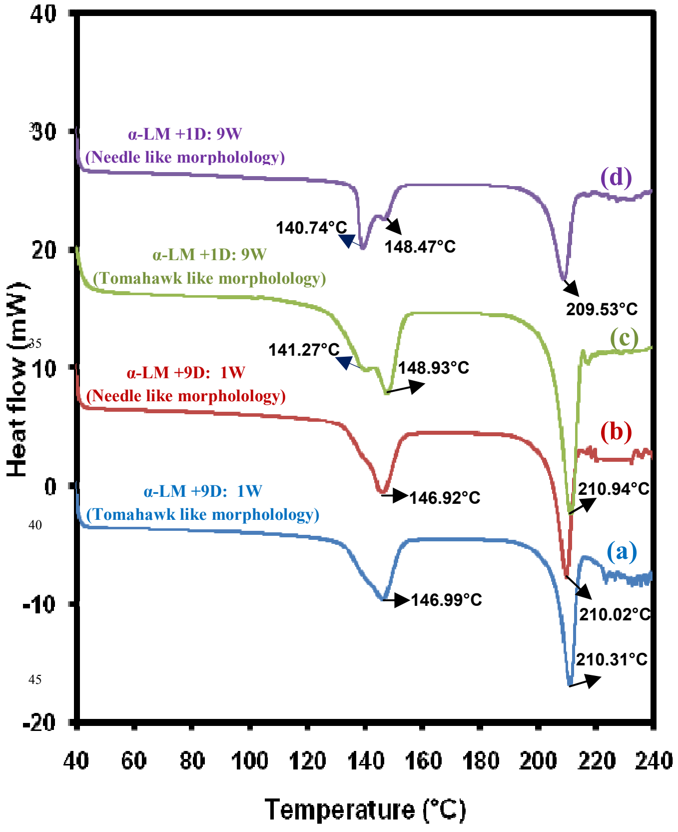

Fig.7 Differential Scanning Calorimetry thermogram of α-LM grown from 9D:1W and 1D:9W mixtures by fast and slow evaporation

3.5. Purity estimation of the nucleated α-LM crystals by automatic digital polarimeter

The initial and final specific rotation reading was noticed based on experimental procedure and these details are given in ESI. The percentage of α-L in the solution was calculated using the equation developed by Sharp and Droop [33] shown below and the obtained results are presented in table 1.

$$\% \alpha = 101.12 [(I/F)-0.635]$$

Table 1: Measured specific rotations of α-LM crystals dissolved in water

Type of crystals	Initial specific rotation	Final specific rotation	%α
The α-LM crystals with needle like morphology in 9D: 1W (fast evaporation)	**86.7**	**53.6**	**99.35**
The α-LM crystals with tomahawk morphology in 9D: 1W (slow evaporation)	**86.2**	**53.4**	**99.02**
The α-LM crystals with needle like morphology in 1D: 9W (fast evaporation)	**84.9**	**52.9**	**98.07**
The α-LM crystals with tomahawk morphology in 1D: 9W (slow evaporation)	**85.3**	**53.0**	**98.53**

Results of this study clearly indicate that the grown α-LM crystals both needles and tomahawk morphology obtained through slow evaporation as well as fast evaporation methods have high level of purity

4. Conclusion

The solubility of α-LM in the solvent mixtures of DMSO and water in the ratio from 9D:1W to 1D:9W showed an altered trend. During slow evaporation at 33°C, the supersaturation generated within the mother liquor was low in all combination of solvent mixtures and it was not suffice to produce nucleation of α-LM with desired morphology and hence α-LM crystals with tomahawk morphology were harvested. When the saturated solutions were allowed for fast evaporation at 33°C, the supersaturation generated within the mother liquor was high. Increasing the supersaturation in different ways in different ratios of DMSO and water induced different morphology of α-LM crystals. However, among the 9 mixing compositions selected 9D:1W and 1D:9W mixing compositions only produced industrially preferred needle like morphology of α-LM, due to the result of the narrowing down of the metastable state in these mixture compositions when compared to other solvent mixtures. The α-LM crystals with needle like morphology that was formed in 9D:1W and 1D:9W solvent mixtures by fast evaporation and the α-LM crystals with tomahawk morphology that was formed in 9D:1W and 1D:9W solvent mixtures by slow evaporation were subjected to PXRD and DSC analyses and they were confirmed as original form of pure α-LM. The purity of α-LM crystals was determined through studying the specific rotations of their aqueous solutions by automatic digital polarimeter. The obtained results indicate that the quality of α-LM crystals was not affected by their different morphology in these solvent mixtures. It is believed from our experimental observations that induction period plays a vital role in attaining the desired morphology of α-LM single crystals for serving its purpose in pharmaceutical applications.

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Notes and references

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†Electronic Supplementary Information (ESI):

Powder X-ray diffraction (PXRD) study

Samples were analysed with a Bruker D8 Advance X-ray powder diffractometer, with CuKα radiation of wavelength 1.5406 Å, at current of 40 mA and voltage of 40 kV. The digitized patterns were recorded at 25 °C by step scanning method between 10 and 40° 2θ with the step size of 0.05° and step time of 1.0 s.

Differential Scanning Calorimetry (DSC) analysis

Thermal analysis of the nucleated α-LM crystals was performed using TA instrument Q 20 series Differential Scanning Calorimeter (DSC). Sample of 2 mg was taken in an aluminium pan and the DSC theromograms were recorded at a heating rate of 10 °C /min in temperature range from 40 to 240 °C under nitrogen atmosphere.

Purity estimation of the nucleated α-LM crystals by automatic digital polarimeter

The specific rotations of the α-LM solutions were measured at 25°C using ATAGO AP-300, automatic digital polarimeter. The first specific optical rotation reading was taken after 4 min from the reaction had started (i.e., dissolution of α- LM in DD water) because the 0.1 g of grown α-LM crystals which were ground to powder form take some more time to dissolve completely in 100 ml of water. The first 10 readings were plotted against the time and readings were noticed at the time interval of every 1 min. The line was drawn through these points, and it was extrapolated to zero time, that is the time of adding the 0.1 g powder in 100 ml water, in order to obtain the initial specific rotation (I). The final (equilibrium) reading was taken after the time span of 24 h. The final equilibrium rotation is considered as the final specific rotation.

Morphology and growth of the nucleated α-LM crystals

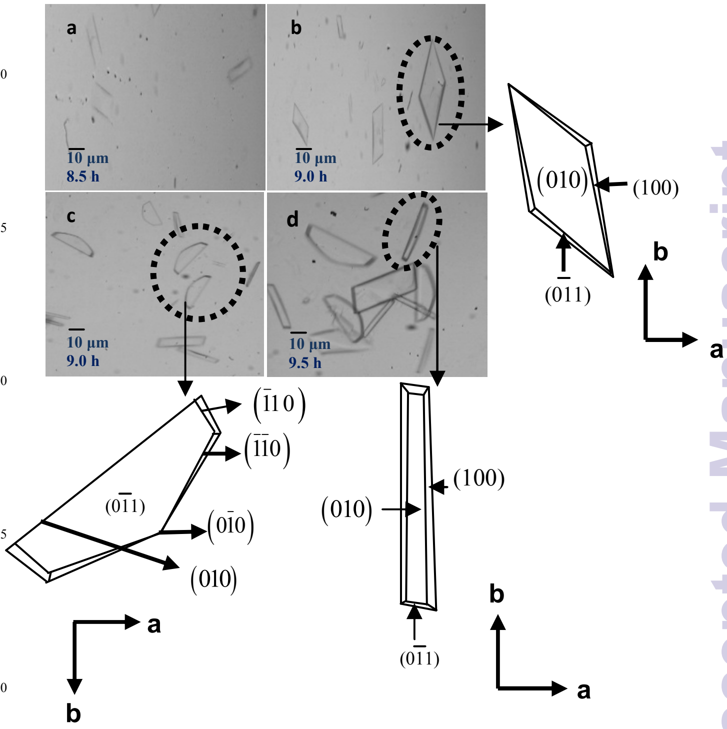

Fig.S1 The α-LM crystals obtained with needle, trapezoidal and triangular like morphology in 8D:2W solvent mixture by fast evaporation

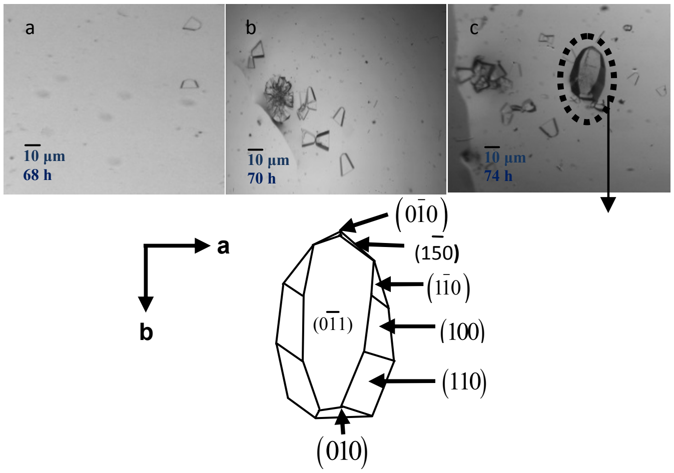

Fig.S2 The tomahawk morphology of α-LM crystals obtained in 7D:3W solvent mixture by fast evaporation

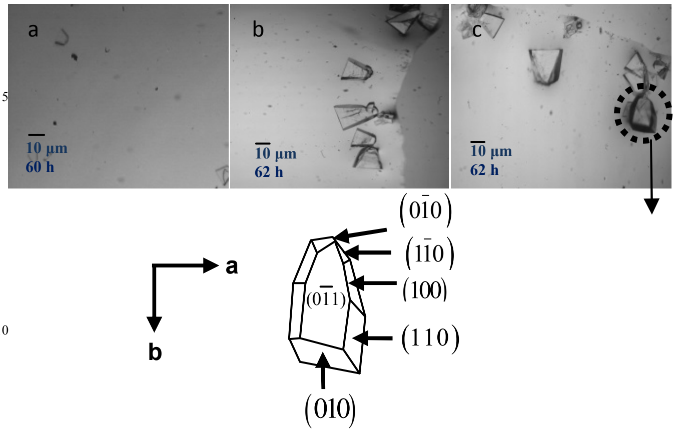

Fig. S3 The tomahawk morphology of α-LM crystals obtained in 6D:4W solvent mixture by fast evaporationn

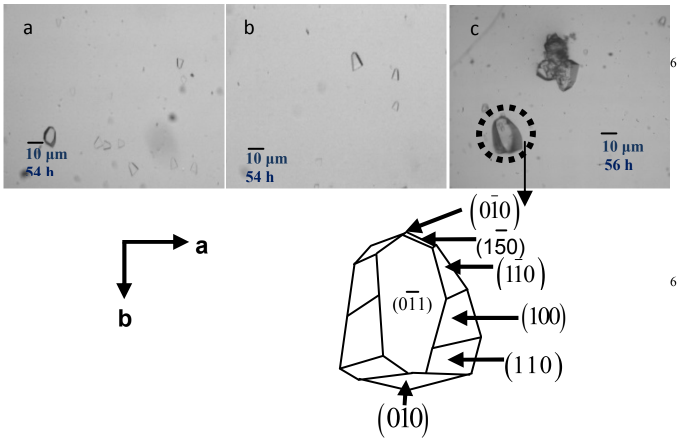

Fig. S4 The tomahawk morphology of α-LM crystals obtained in 5D:5W solvent mixture by fast evaporation

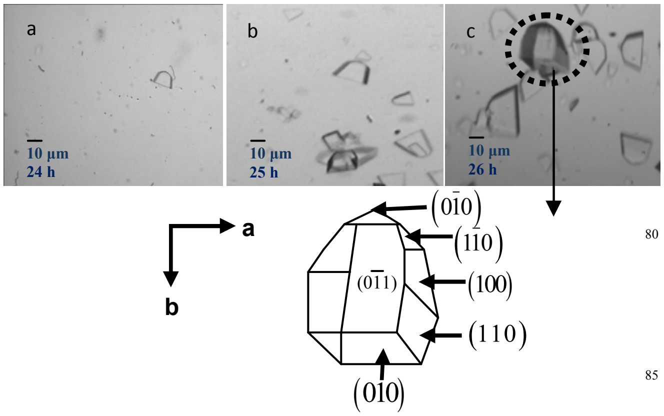

Fig. S5 The tomahawk morphology of α-LM crystals obtained in 4D:6W solvent mixture by fast evaporation

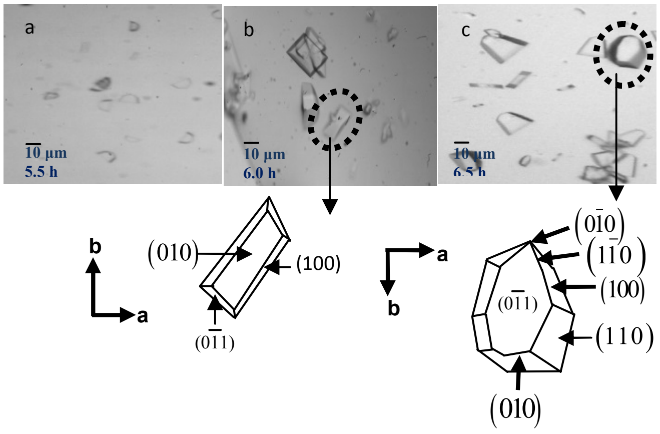

Fig. S6 The α-LM crystals obtained with parallelogram, trapezoidal and tomahawk like morphology in 3D:7W solvent mixtures by fast evaporation

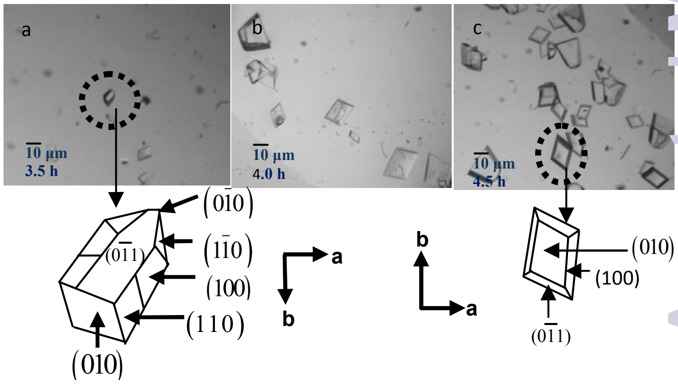

Fig. S7 The α-LM crystals obtained with parallelogram, trapezoidal and tomahawk like morphology in 2D:8W solvent mixtures by fast evaporation

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