

Crystallisation of α -lactose monohydrate from DMSO solutions: Influence of β -lactose

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Abstract

In this study, the dimethyl sulfoxide (DMSO)-lactose system has been used to study the effect of β -lactose on the morphology of α -lactose monohydrate crystals. DMSO was used as the solvent as it greatly reduces the rate of mutarotation of α -lactose to β -lactose. It is shown that as the β -content of the solution increases, the crystal shape starts increasing in the **a** and **b** directions, whereas the major growth occurs in the **c** direction at low levels of β -lactose. The morphology of the α -lactose monohydrate crystal calculated by molecular modelling is in good agreement with that of the crystals grown in the presence of low β -lactose concentrations. Atomic force microscopy has revealed growth spirals and unit cell high steps on the (020) face of crystals grown in the presence of low β -anomer concentration.

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1. Introduction

Lactose, the characteristic sugar of milk, is produced from whey, a by-product of cheese making. Its presence in whey constitutes a significant pollution problem for dairy factories, whilst at the same time, a market exists for high purity crystalline lactose in the pharmaceutical industry.

Lactose is a disaccharide consisting of one residue each of D-glucose and D-galactose joined by a β -1,4-glycosidic linkage. The glucose moiety has an anomeric centre (C1), and thus there are two anomers: α - and β -lactose, as shown in Figure 1. An important difference from most other crystal growth systems is that an aqueous lactose solution is a pseudo-ternary system: molecules of both α and β forms of lactose are present, which tend to equilibrate by mutarotation (1). Thus, in water, α -lactose hydrate crystals never grow from a pure environment: their growth environment always contains β -lactose, with an equilibrium β to α ratio of between 1.25-1.64, depending on the temperature and concentration.

It is well known that lactose crystallises very slowly compared to other sugars (1). Van Kreveld (2) claimed that β -lactose selectively blocks the growth of individual faces of α -lactose resulting in low growth rates and the characteristic tomahawk morphology (see Figure 2). However, Nickerson and Moore (3) claimed that the β -anomer did not affect the growth rate of α -lactose monohydrate crystals.

Definitive experiments to investigate the effect of β -lactose on the growth rates of individual faces have not been performed to date, and some questions still remain as to the exact nature of the effect of β -lactose on α -lactose growth (4).

The major difficulty in studying the crystallisation of α -lactose is that the rate of mutarotation in water is fast. Hence, it is not possible to obtain aqueous solutions of lactose with a β -lactose concentration other than the equilibrium value for the period of time necessary for the crystal growth rate experiments. Because DMSO is an aprotic solvent, mutarotation is slow, and therefore it is possible to have only one anomeric form of lactose in DMSO solution

for a reasonable period of time. By diluting a solution of lactose in DMSO with ethanol, it is possible to prepare supersaturated solutions (5).

In this study, DMSO has been used as a solvent to investigate the effect of β -lactose on the morphology of α -lactose monohydrate crystals. The growth morphology of a pure α -lactose monohydrate crystal was predicted using the MARVIN program (6). Atomic force microscopy was used to image the (020) faces of the crystals grown in DMSO.

2. Experimental

2.1 Materials

Pharmaceutical grade α -lactose monohydrate powder (Sigma Aldrich) was used in the experiments. The DMSO (Mallinckrodt) used was HPLC grade and kept over 4Å molecular sieves (Ajax Laboratory Chemicals). Dry pyridine (Alltech) and TMSIM (N-Trimethylsilylimidazole) (Alltech) were used to derivatise lactose for GC analysis.

2.2 Gas Chromatography of Lactose

The relative amounts of α -and β -lactose in DMSO solution were determined by gas chromatography using trimethylsilyl (TMS) derivatives prepared by reaction with TMSIM in the presence of pyridine (5). TMSIM is highly reactive and preferentially trimethylsilylates hydroxyl groups producing a stable derivative. The instrument used was a Hewlett Packard 5890A Gas Chromatograph with a non-polar capillary column (BP 5) and a FID detector. Operating conditions were: column temperature: 250°C, detector temperature: 280°C, injector temperature: 260°C, carrier gas: hydrogen at 25 psi, split ratio: 1:60 and injection volume: 1 μ l. The run time used was 20 minutes, and the components typically eluted after 11 and 16 minutes for α -and β -lactose, respectively.

2.3 Rate of Mutarotation

A 39% (w/w) lactose in DMSO solution was prepared by heating the solution to approximately 80 °C until all the lactose dissolved. Sampling commenced when the solution had cooled to 30 °C. The samples were withdrawn and then diluted with DMSO (1:10) to lower the viscosity. Samples taken were immediately derivitised for GC to determine the α to β ratio. The solution was kept at 30 °C during the experiment.

2.4 Preparation of α -Lactose Monohydrate Crystals in DMSO

The initial solubility of α -lactose monohydrate in DMSO at 30 °C was found to be 40g/100g DMSO. Solutions with a supersaturation ratio of 1.65 in DMSO were prepared by adding 22.13 g of α -lactose monohydrate to 33.45 g of DMSO and heating the solution to approximately 80 °C until all the solid dissolved, followed by slow cooling to 30 °C. Aliquots (5 ml) were removed at regular intervals, and the β -lactose contents were determined by GC. Precipitation from each aliquot was induced by the addition of dry ethanol. Results are given here for solutions with β -lactose levels of approximately 10, 25 and 40 wt/wt %.

Precipitation under these conditions produced rough crystals that were mainly agglomerated. To produce crystals suitable for AFM work, the prism shaped crystals were produced from solutions of 2.5 g α -lactose monohydrate in 25 ml DMSO by addition of ethanol. The crystals were washed with saturated DMSO solution to remove fine particles on the crystal surface and then with saturated water solution to remove viscous DMSO-lactose solution on the crystal surfaces prior to imaging.

2.5 Atomic Force Microscopy

Images were obtained using a Digital Instrument Nanoscope E AFM, operated in the contact mode, in air, using Si₃N₄ 200μ triangular tip cantilevers with a spring constant of 0.12 N/m. All the images were collected in the height mode.

3. Results and Discussion

3.1 The rate of mutarotation

The calculated mutarotation rate constant of α-lactose monohydrate in DMSO is $1.47 \times 10^{-4} \text{ min}^{-1}$ compared to $3.00 \times 10^{-2} \text{ min}^{-1}$ (7) in water, under the same conditions. Thus, the rate of mutarotation in DMSO is 200 times slower than in water under comparable conditions of temperature and supersaturation. The β-lactose content increases by approximately 0.5 % per hour (Figure 3). This shows that DMSO can be used to produce a stable, desired ratio of α- to β-lactose in solution, as opposed to water where mutarotation proceeds rapidly on the crystallisation time scale.

3.2 The effect of β-lactose on the morphology of α-lactose monohydrate crystals

Figure 4 shows optical micrographs of the α-lactose monohydrate crystals grown from DMSO in the presence of 10, 25 and 40 % β-lactose concentrations. When the β-lactose content is 10 %, the major growth occurs in the **c** direction, producing prism shaped crystals (with most rapid growth on the $\{0\bar{1}1\}$ and $\{10\bar{1}\}$ families of faces), with some growth in the **a** direction as well. As the β-lactose content reaches 40 %, growth in **c** direction decreases and growth in the **b** direction becomes significant, resulting in pyramid shaped crystals.

It is well known that β-lactose selectively blocks the growth of the $(0\bar{2}0)$ face, resulting in growth only in the **b** direction and not the **-b** direction(1). The results described here show that the β-lactose significantly influences the growth of the (011) face as well. As the β-lactose content of the solution increases, the growth in the **c** direction drastically decreases. The crystals poisoned in this direction grow in the **b** direction. This explains the slow rate of

lactose crystallisation compared to other disaccharides. Since the fastest growing face in a pure α -lactose solution is poisoned by the β anomer, the slowest growing face, (020) becomes the fastest growing face in a solution containing a mixture of the anomers, resulting in overall low growth rates.

3.3 Molecular modelling

3.3.1 Reproducing the structural parameters of α -lactose monohydrate

Before the energetics and structures of α -lactose monohydrate surfaces can be simulated, a set of potential parameters which reproduce the α -lactose monohydrate crystal structure needs to be chosen. Although there are many potential sets applicable to sugars available in the literature, we trialed the CVFF forcefield (8) first, primarily because of its simplicity. The lattice minimisation with the CVFF forcefield was performed using the GULP program (9) and the calculated and experimental lattice parameters (10) are listed in Table 1. The table shows that the CVFF forcefield is sufficiently well parameterised to reproduce the α -lactose monohydrate crystal structure with maximum deviation between the calculated and experimental parameters being 2.86%.

3.3.2 Calculating crystal morphology

The morphologies of a large number of organic crystals have been successfully predicted using the attachment energy methodology (11). In this model, the attachment energy of the face (hkl), (i.e. the energy liberated when a slice of thickness d_{hkl} is added to the growing crystal) is calculated for all faces with large interplanar spacings. The attachment energy is assumed to be proportional to the growth rate of that face and inversely proportional to its morphological importance. The calculated morphology can be visualised by constructing a Wulff plot (12). Morphology calculated in this manner is called the growth morphology. A recent refinement of this morphology is to allow the surface layers to find their minimum

energy configurations before calculating the attachment energies (13). Although this has been found to have profound effects on inorganic crystal morphology, the few examples of organic surface relaxation in the literature show that the effect of this on the morphology of organic crystals is small but not insignificant (14).

The relaxed and non-relaxed attachment energies of the 15 faces of the α -lactose monohydrate with the largest interplanar spacings, calculated using the MARVIN surface simulation code (6), are tabulated in Table 2. Although the changes in attachment energy are generally small, surface relaxation predicts a difference between the $(0\bar{2}0)$ and (020) faces, (020) having the slower growth rate, thus being the larger face. The non-relaxed and relaxed morphologies of α -lactose monohydrate crystal (Figure 5) are very similar, both producing tablet shaped crystals with dominant (020) and $(0\bar{2}0)$ faces. Closer inspection shows that the (001) face becomes more prominent upon relaxation. The optical micrographs show that crystals grown in DMSO with the lowest of 10 % β -lactose content, are also tablets, in agreement with the predicted morphology (Figure 6). Furthermore, the measured interfacial angles of the prism shaped α -lactose monohydrate crystal are in good agreement with the calculated angles. For example, the calculated angle between the (011) and the (111) face is 142.79° , while the angle measured from the micrograph is $143^\circ \pm 1^\circ$. Similarly, the calculated interfacial angle between the (011) and the (100) plane is 105.97° , while the measured angle is $105^\circ \pm 1^\circ$. Note, however, that the crystal is too thin to determine whether the large number of side faces in the predicted morphology are experimentally observed.

Visser and Bennema (15) have previously calculated the morphology of the α -lactose monohydrate crystal using several methods; the predicted morphologies were different from the observed tomahawk morphology (see Figure 2). It was stated that the discrepancy between observed and predicted morphologies was a result of the growth inhibition action of β -lactose. However, the predicted morphology was not in good agreement with the tablet

shaped crystals grown in the presence of low β -lactose either. In the present work, the calculated morphology of α -lactose monohydrate is in good agreement with that of the crystals grown in the presence of low β -lactose concentrations, although the grown crystals are longer in the **c** direction.

3.4 AFM images of α -lactose monohydrate grown in DMSO

AFM images of the (020) face of a crystal grown in a DMSO solution containing 10 % β -lactose show that growth occurs by the lateral addition of growth layers (Figure 7 (a)). The height of the growth layers is approximately 2 nm, which corresponds to the height of the unit cell in the **b** direction. It was also observed that unit cell height steps splits into two 1 nm steps corresponding to one lactose molecule in the **b** direction and further work is underway to investigate the step splitting.

Visser (16) has investigated the mechanisms of crystal growth of α -lactose monohydrate crystals by measuring the growth rates of lactose at various temperatures and supersaturations, and concluded that at low supersaturations the growth mechanism of the (020) face is by spiral growth. Figure 7 (b) shows a growth spiral imaged on the (020) face of α -lactose monohydrate crystal grown in DMSO-lactose solution.

4. Conclusions

DMSO is an excellent solvent for lactose. The solubility of α -lactose monohydrate in DMSO at 30 °C is 40g/100g DMSO, which is much higher than its solubility in water, (ie 9.93g/100g water). The slowness of mutarotation in DMSO allows us to prepare saturated solutions with a fixed, chosen β -lactose content, but it is not possible to obtain highly supersaturated solutions with less than 10 % β -lactose, since supersaturation requires heating the solution to

high temperatures (where the rate of mutarotation becomes rapid). At 30°C, however, the lactose-DMSO system can easily be used for growth rate experiments since the increase in β -lactose content is less than 0.5 % per hour.

This work shows that β -lactose significantly influences the morphology of α -lactose monohydrate crystals grown from DMSO solution. At low concentrations of β -lactose, the fastest growing face is the $(0\bar{1}1)$ face resulting in long thin prismatic crystals. At higher β -lactose concentrations, the main growth occurs in the **b** direction, and the (020) face becomes the fastest growing face (since the $(0\bar{1}1)$ face is blocked by β -lactose), producing pyramid and tomahawk shaped crystals.

Crystals grown in supersaturated DMSO or water exhibit the same morphology, providing there are similar the β -lactose concentrations in the solutions. This shows that neither DMSO nor water, being highly polar solvents, directly determine the morphology of the lactose crystals, but it is rather the β -lactose present in the solutions which determines the morphology.

Molecular modelling calculations predict a morphology for α -lactose monohydrate which is in good agreement with the observed morphology for crystals grown in the presence of relatively low β -lactose concentrations.

The AFM images show that the growth mechanism of the (020) face is by spiral growth. The growth on the (020) face of α -lactose monohydrate crystal occurs by addition of growth units to steps emanating from one or more spiral sources. Work is underway to obtain further AFM data under a wide range of β -lactose concentrations; and to improve the molecular modelling studies, by docking β -lactose onto various faces of a α -lactose monohydrate crystal.

5. Acknowledgments

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Figure captions

Figure 1. The molecular structure of (a) α -lactose and (b) β -lactose

Figure 2. Miller indices of tomahawk shaped α -lactose monohydrate crystal (Walstra and Jennes, 1984).

Figure 3. The rate of mutarotation of α -lactose monohydrate in DMSO at 30 °C.

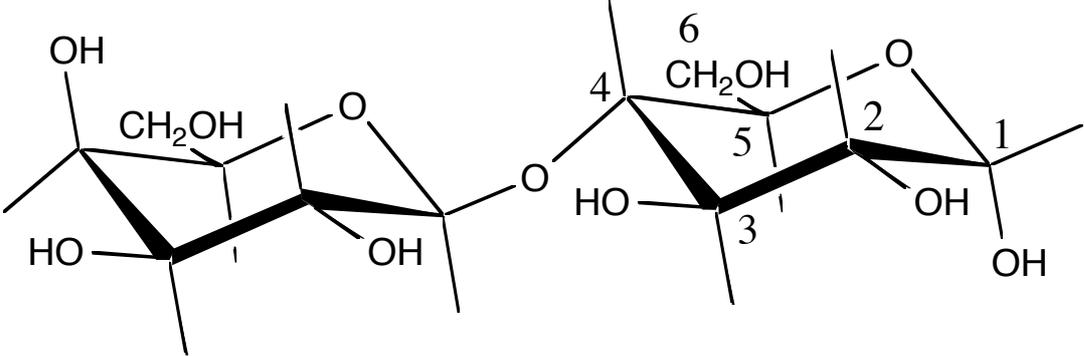
Figure 4. α -lactose monohydrate crystals precipitated from DMSO solutions by the addition of ethanol in the presence of a) 10% , b) 25 % , c) 40%, β -lactose.

Figure 5. The (a) non- relaxed, and (b) relaxed morphologies of α -lactose monohydrate crystal predicted by MARVIN.

Figure 6. The (a) experimental and (b) predicted morphologies of α -lactose monohydrate crystal.

Figure 7. AFM images of the (010) face of prism-shaped α -lactose monohydrate crystals grown in the presence of 10 % β -lactose in DMSO solution, showing (a) growth steps parallel to $[0\bar{1}1]$, scan length: 1.6 μm and Δz_{max} : 50 nm and (b) a growth spiral, scan length: 2 μm and Δz_{max} : 30 nm

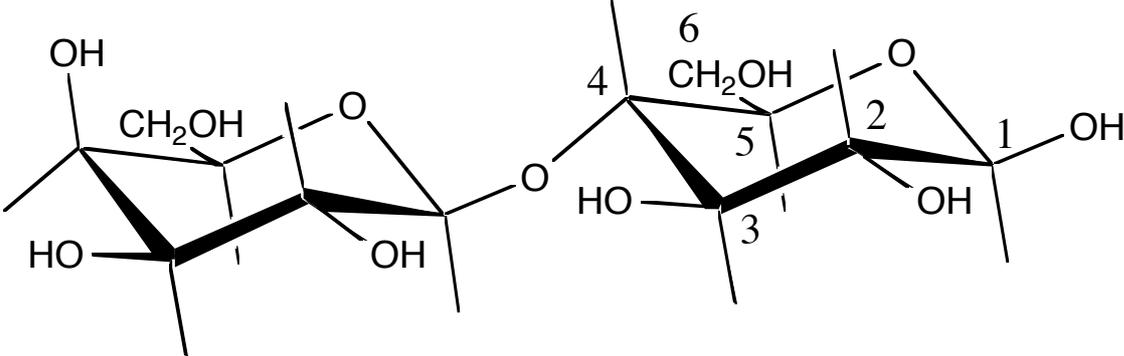
(a)



D-galactose

D-glucose

(b)



D-galactose

D-glucose

Figure 1.

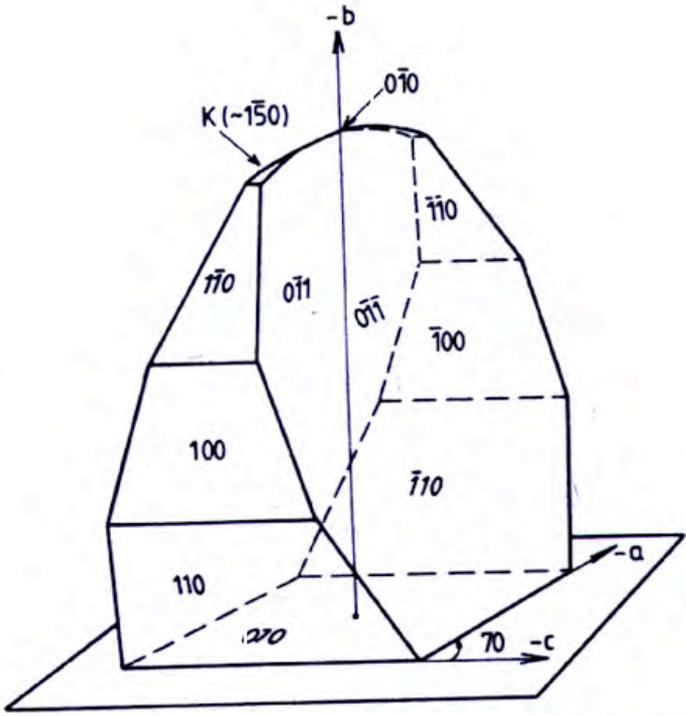


Figure 2.

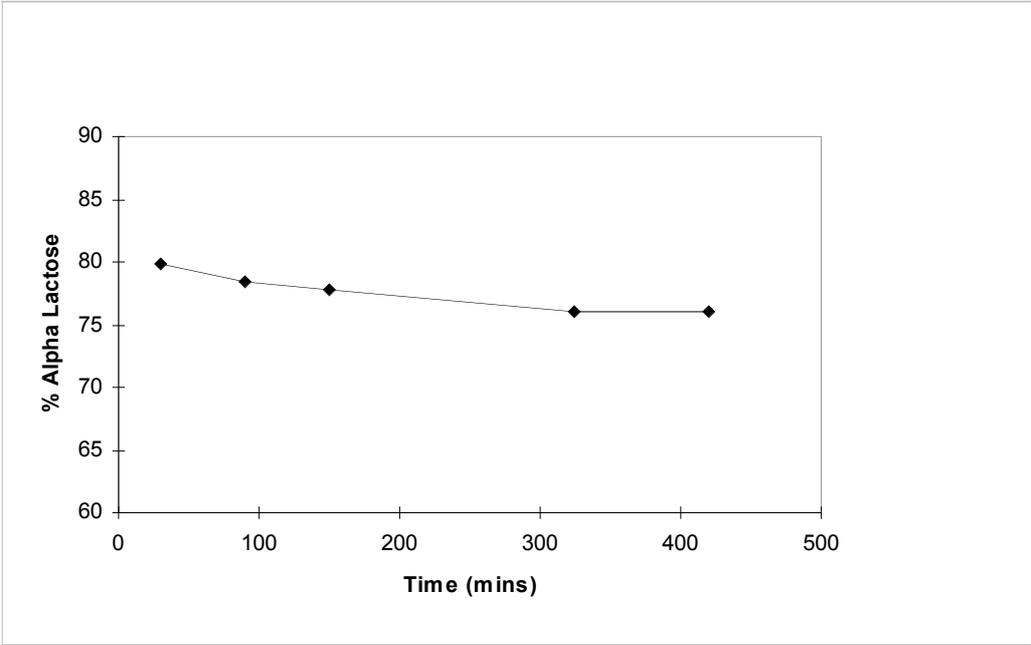
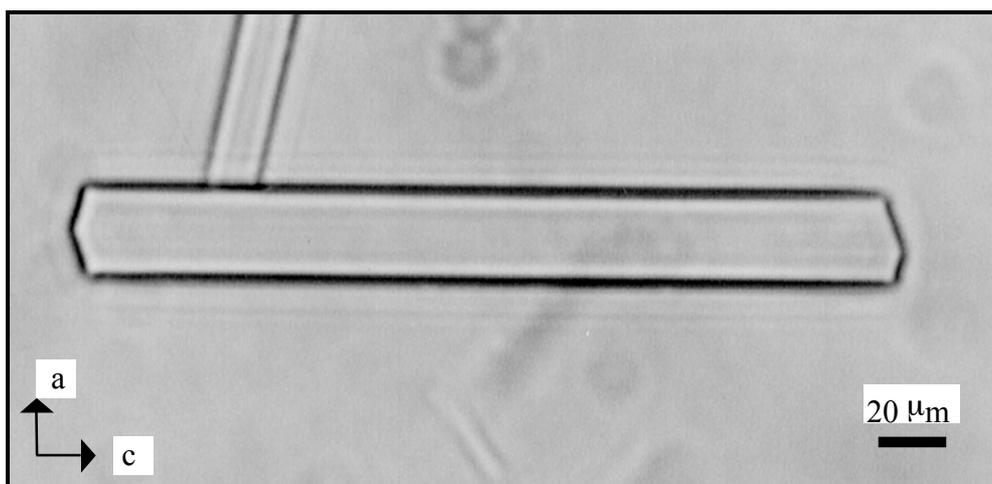
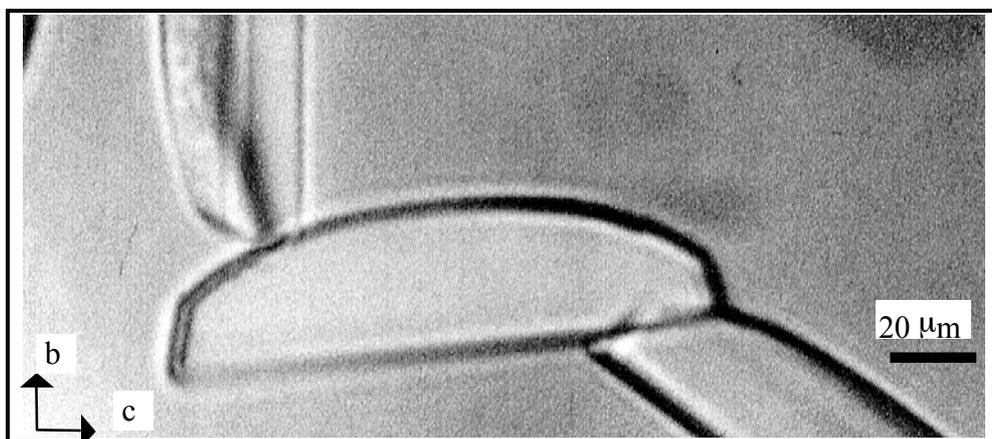


Figure 3.

(a)



(b)



(c)

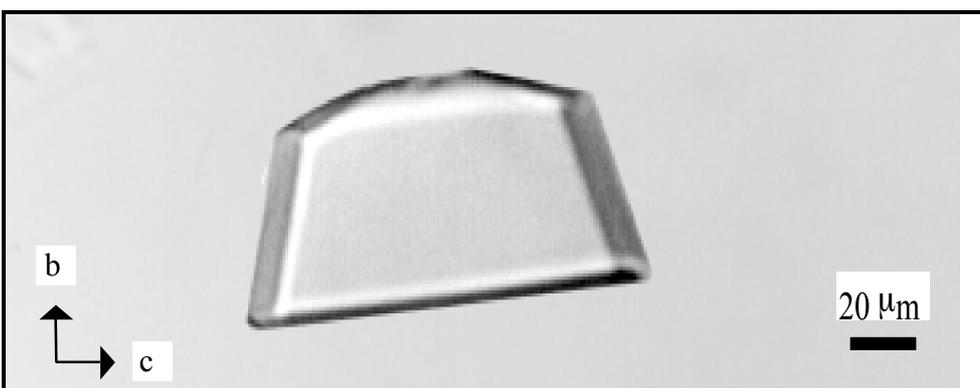
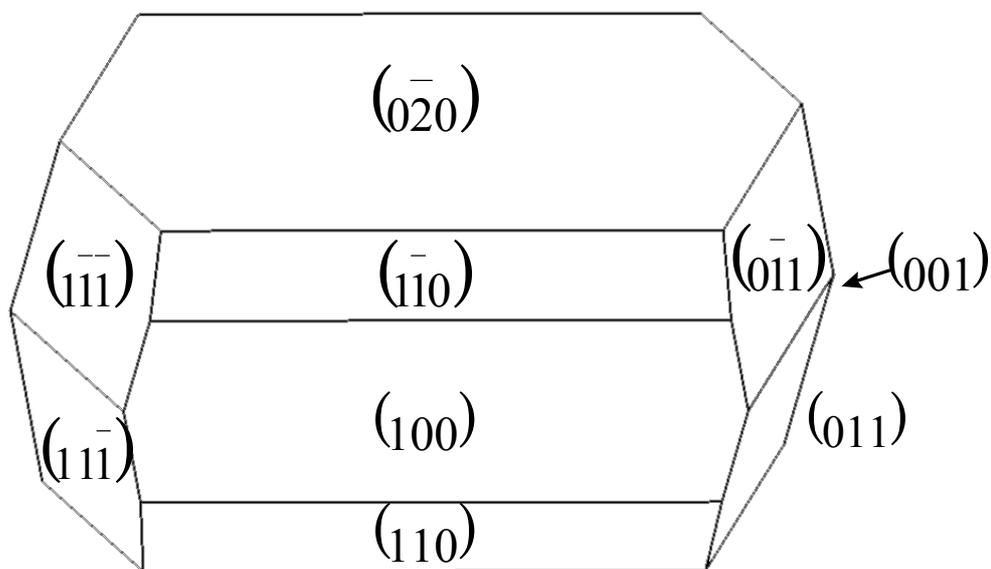


Figure 4.

(a)



(b)

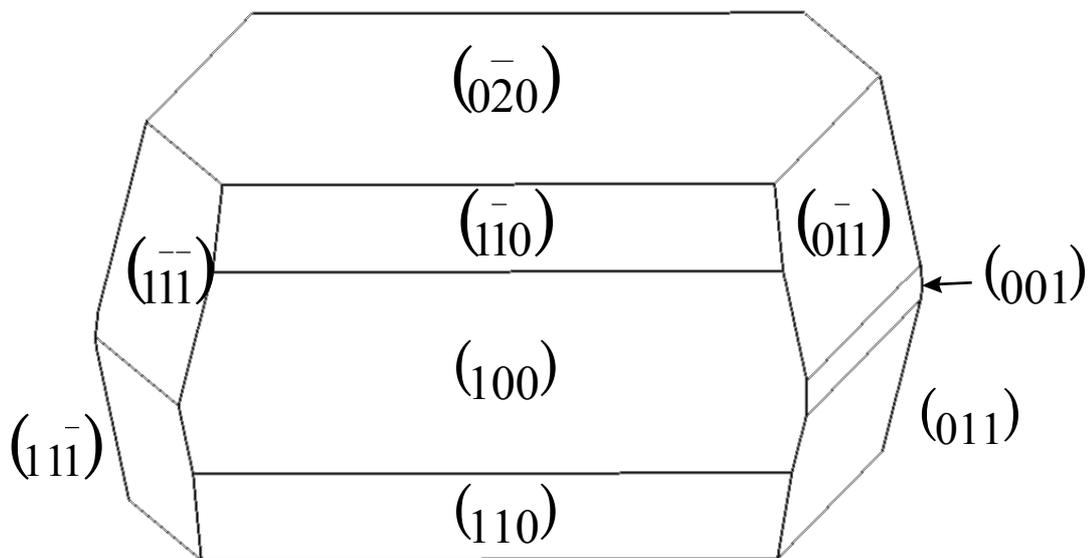
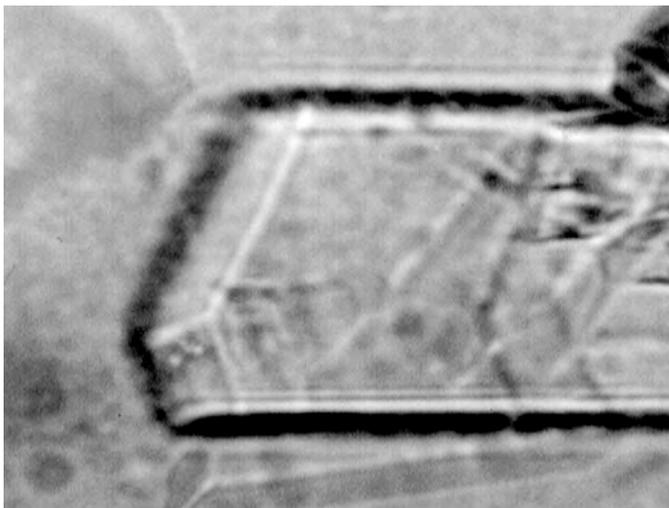


Figure 5

(a)



(b)

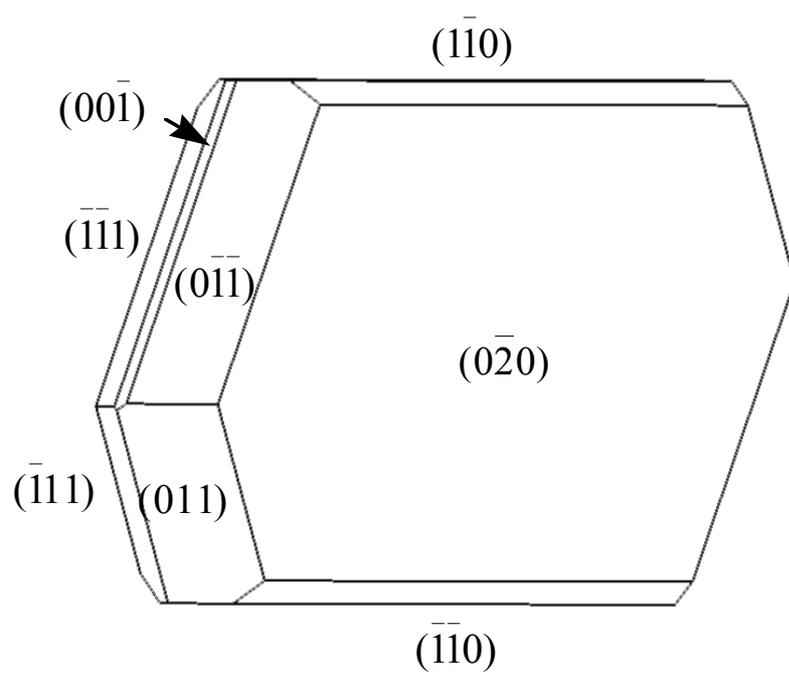
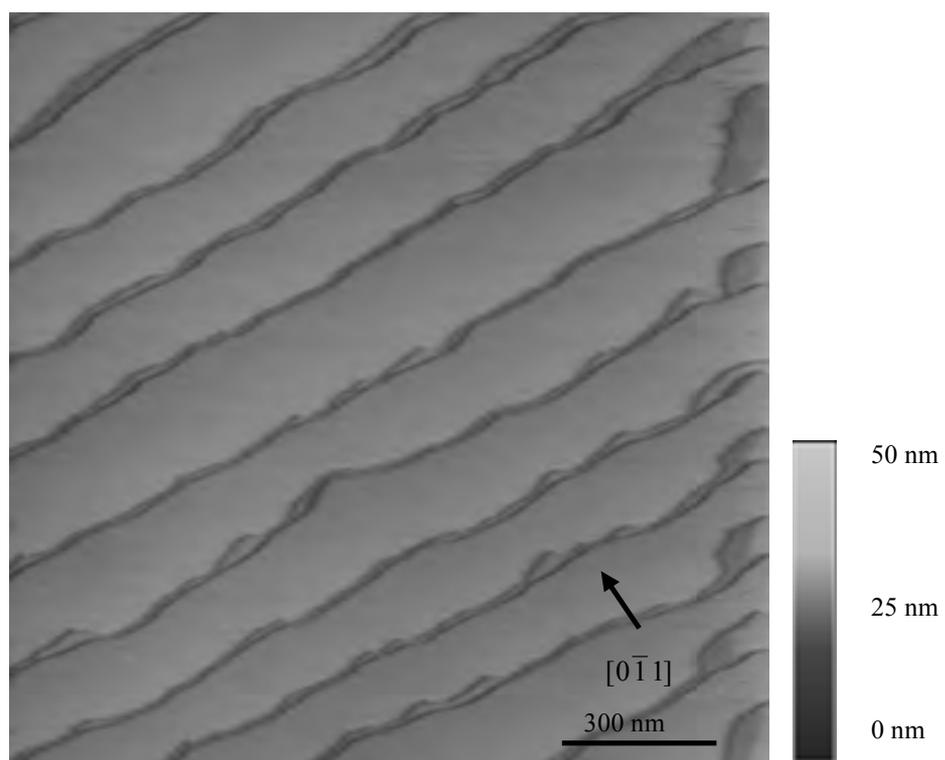


Figure 6

(a)



(b)

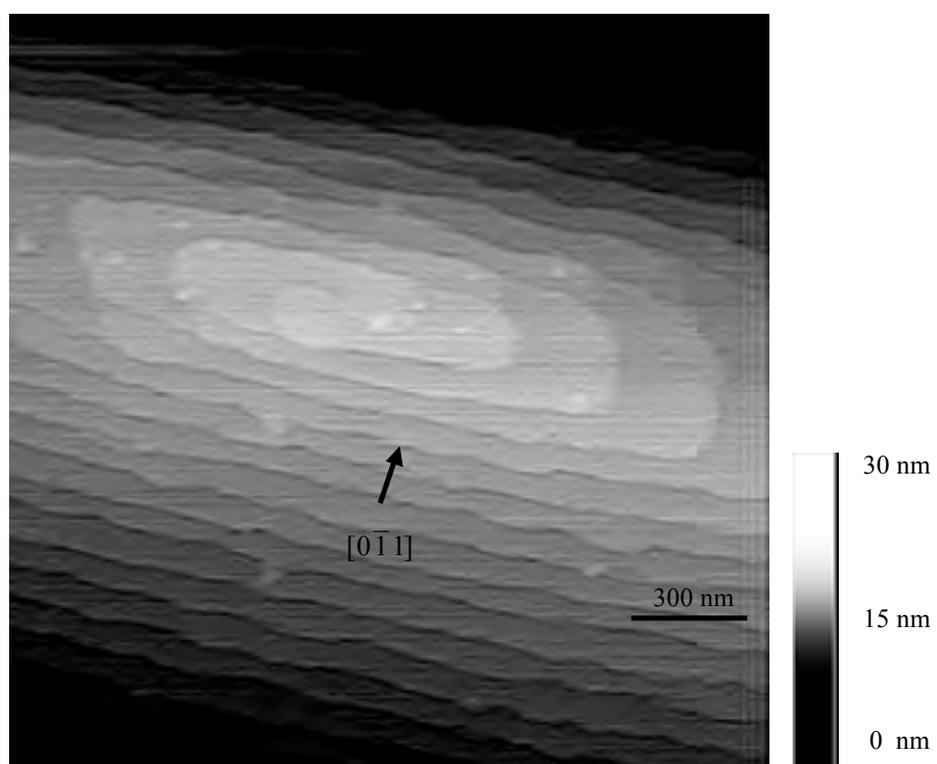


Figure 7.

Table 1

Experimental (10) and calculated lattice parameters of α -lactose monohydrate crystal

	a (Å)	b (Å)	c (Å)	β (°)
Experimental	7.982	21.652	4.824	109.57
Calculated	7.759	21.474	4.860	108.35
Difference (%)	2.86	0.80	-0.07	1.11

Table 2. Calculated attachment energies for the faces of α -lactose monohydrate crystal

Attachment Energy (eV/mol)			Difference in Attachment Energies (%)
Face	Non-relaxed	Relaxed	
(020)	-0.479	-0.510	3.051
($\bar{0}\bar{2}0$)	-0.479	-0.524	4.467
(100)	-0.849	-0.833	-0.861
(110)	-0.885	-0.868	-0.938
($\bar{1}\bar{1}0$)	-0.885	-0.888	0.161
(011)	-0.968	-0.955	-0.639
($0\bar{1}\bar{1}$)	-0.968	-0.937	-1.528
(120)	-0.970	-0.981	0.525
($\bar{1}\bar{2}0$)	-0.970	-0.966	-0.184
(001)	-0.989	-0.956	-1.582
(130)	-1.038	-1.066	1.298
($\bar{1}\bar{3}0$)	-1.066	-1.082	0.725
($10\bar{1}$)	-1.398	-1.452	1.819
($1\bar{1}\bar{1}$)	-1.165	-1.100	1.591
($\bar{1}\bar{1}\bar{1}$)	-1.065	-1.075	0.467