

Studies on the concentration dependence of specific rotation of Alpha lactose monohydrate (α -LM) aqueous solutions and growth of α -LM single crystals

K. Vinodhini, R. Divya Bharathi and K. Srinivasan*

Crystal Growth Laboratory, Department of Physics, Bharathiar University,
Coimbatore- 641 046, Tamil Nadu, India

*E-mail: nivas_5@yahoo.com

Abstract. Lactose is an optically active substance. As it is one of the reducing sugars, exhibits mutarotation in solution when it dissolves in any solvent. In solution, lactose exists in two isomeric forms, alpha-Lactose (α -L) and beta- lactose (β -L) through the mutarotation reaction. Mutarotation produces a dynamic equilibrium between two isomers in a solution and kinetics of this process determines the growth rate of alpha lactose monohydrate (α -LM) crystals. Since no data were available on the specific rotation of aqueous α -LM solutions at different concentrations at 33 °C, the initial experiments were carried out on the specific rotation of aqueous α -LM solutions at different concentrations at 33 °C. The specific rotations of the solutions were decreased with increasing time through the mutarotation reaction. The initial and final (equilibrium) specific rotations of the solutions were determined by using automatic digital polarimeter. The compositions of α and β -L in all prepared solutions were calculated from initial and final optical rotations by the method of Sharp and Doob. The composition of α -L decreased whereas, the composition of β -L increased in solutions with increasing concentration of α -LM at 33 °C. Experimental results revealed that this method could be easily and safely employed to study the dependence of specific rotation of solutions on their concentration. The effect of β - lactose on the morphology of nucleated α -LM single crystals has been studied at different experimental conditions.

1. Introduction

Alpha-lactose (α -L) and beta-lactose (β -L) are the two isomeric forms of lactose. The literature reports revealed that in aqueous solution, α -L is simultaneously converted into β -L and vice- versa by establishing a reversible equilibrium called mutarotation [1, 2]. The specific optical rotation of α -L in water is +89.4° at 20° C whereas; the specific optical rotation of β -L in water is +35° at 20 °C [3]. The optical rotation of lactose solution will continuously change because of the mutarotation of α -L into β -L and vice versa until the specific rotation of + 55.4° is reached at equilibrium, irrespective of the anomeric form used to prepare the precursor solution [4-6]. The kinetics of this process determines the growth rate of alpha- lactose monohydrate (α -LM) crystals and the presence of β -L acts as a habit modifier [7]. Moreover, the crystallization of α -LM is very slow and is hard to control the size and morphology of the nucleated crystals [8]. Since no data were available on the specific rotation of aqueous α -LM solutions at different concentrations at 33 °C, the initial experiments were carried out



on the specific rotation of aqueous α -LM solutions at different concentrations at 33 °C. The presence of β -L in solution slows down the nucleation and growth of α -LM single crystals to a certain extent. As the β -L isomer control the crystallization of α -LM, it is essential to study the extent of this effect on the morphology of the nucleated α -LM in solution under different experimental conditions. Hence, the effect of β -L on the morphology of the α -LM single crystals has been studied at different experimental conditions.

2. Experimental procedure

2.1. The preparation of different concentration of α -LM solution for specific rotation measurements

α - Lactose monohydrate (α -LM) from Merck and laboratory double distilled (DD) water were used to prepare aqueous α -LM solutions at different concentrations. Different quantities (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 g) of α -LM were individually dissolved in 10 ml water at room temperature (33 °C). They were continuously stirring for 4 hours with magnetic stirring. After the dissolution, these solutions were filtered with Whatman no.41 filter sheets. After the filtration, the solutions were immediately filled in observation tube. The observation tube filled with a sample was placed between two polarising element (polariser and analyser) in the sampling chamber of automatic digital polarimeter, ATAGO AP-300, shown in Figure 1 and then the specific rotations of the α -LM solutions were measured at a time intervals of 10 minutes until 150 minutes (a total of 15 has been made), at 33 °C. These 15, specific rotation readings were plotted against the time. Eventually the compositions of the sample were calculated from the initial and final optical rotations by the method of sharp and Doob [9]. The saturated aqueous solution of α -LM (28.3 g/ 100 ml) was also prepared at 33 °C. This solution was employed to measure specific rotation readings at different time and to analyse the morphology of α -LM at two different crystallization methods (fast and slow evaporation method).



Figure 1. The automatic digital polarimeter

3. Results and discussion

The composition of α -L and β -L in aqueous α -LM solution can be determined by using polarimeter. Since no data were published on specific rotation of the different concentration of aqueous α -LM solutions at room temperatures (33 °C), the initial experiments were designed to measure the optical rotations at a regular time interval of 10 minutes until 150 minutes. It was observed that increasing the time the specific rotation of given solutions were decreased as shown in Figure 2. The results show that the composition of α -Lactose decreased and beta lactose increased in all solutions carried out with increasing time. It is happened until two anomers attain equilibrium because mutarotation produces a dynamic equilibrium between the two isomers in a solution [1, 2], and hence the optical rotation of these solutions were observed at once every 24 hours until 10 days and the maximum constant readings was taken as final optical rotation, F and the first reading of the specific rotation that was

taken after the dissolution was initial specific rotation, I . At lower concentration of aqueous α -LM solution (0.2 g), the initial specific rotation (I) was very high whereas, at higher concentration of aqueous α -LM solution (2.2 g), the initial specific rotation (I) was very low as shown in Figure 2. The α and β -L composition of the different solutions were calculated from the initial and final optical rotations by the method of sharp and Doob as shown in equation (1 and 2) [9]. The percentage of α and β -L present in all prepared solutions is tabulated in table (1).

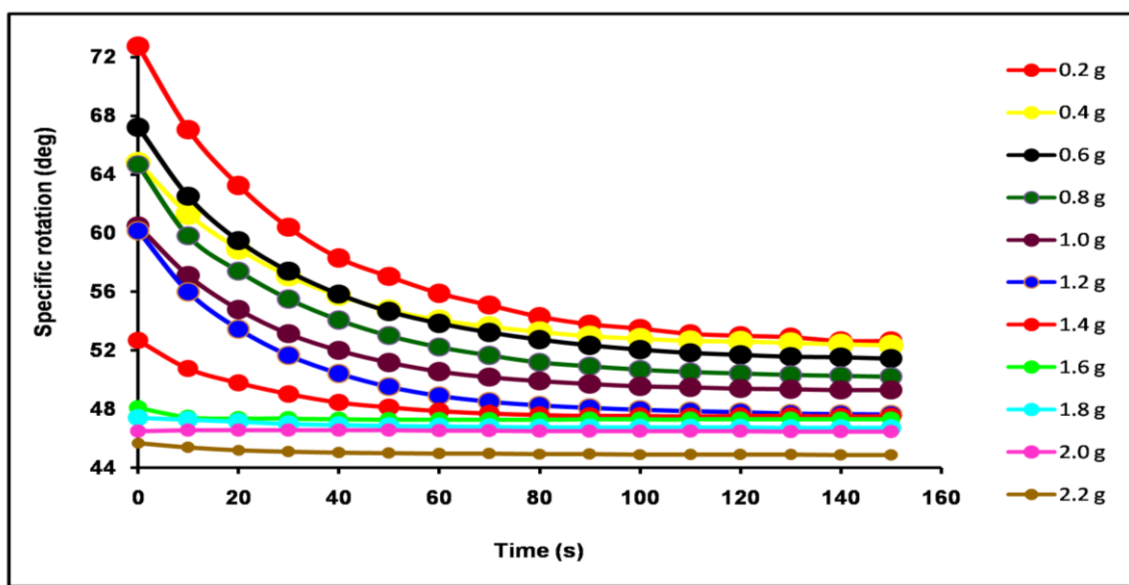


Figure 2. Specific rotation of aqueous α -LM solution at different concentration at 33 °C

$$\% \text{ Alpha} = A0 = (I/F - 0.635) 101.1 \quad (1)$$

$$\% \text{ Beta} = (1 - A0) = (1.624 - I/F) 101.1 \quad (2)$$

Table-1. Percentage of α and β -L present in different concentration of aqueous α -LM solution

% α	% β
79.96	20.04
74.94	25.06
71.41	28.59
68.10	31.90
65.48	34.52
61.25	38.75
58.35	41.65
51.83	48.17
47.31	52.69
41.94	58.06
41.04	58.96

Analyses of the different solutions gave different results depends upon the concentration and from the present experiment, it was investigated that when the concentration of α -LM increases in water (from 2% to 22%), the composition of α -L decreases while β -L increases as shown in Figure 3.

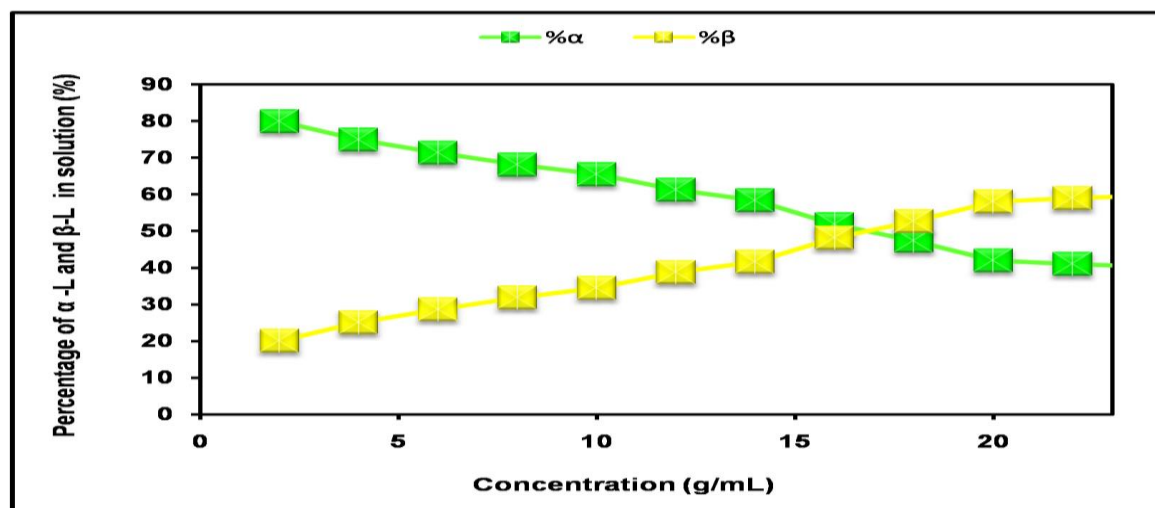


Figure 3. The percentage of α and β -L in different concentration of aqueous α -LM solution

The observed results revealed that this method could be easily and safely employed to study the dependence of specific rotation of solutions on their concentration.

The saturated aqueous solution of α -LM (28.3 g/ 100 ml) was also prepared at 33 °C. This solution was employed to measure specific rotation readings at different time and to analyse the morphology of α -LM at two different crystallization methods.

3.1. Fast and slow evaporation method

The initial specific rotation reading of saturated solution was observed (+50.38 at 33 °C) and then 30 μ L solutions was pipetted out on microscopic slide and observed the needle like morphology of nucleated α -LM crystals by fast evaporation method. It is shown in Figure 4. After 24 hours, the specific rotation readings was +49.77 and hence the Parallelogram like morphology of α -LM were observed from 30 μ L solutions by fast evaporation method as shown in Figure 5. After 48 hours, the specific rotation reading was + 48.96 and hence the tomahawk morphology of α -LM crystals were observed from 30 μ L solutions by fast evaporation method as shown in Figure 6. It was happened by beta-lactose content increases in solution.

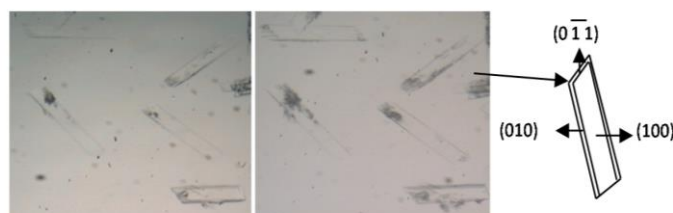


Figure 4. The needle like morphology of α -LM single crystals grown from water after the preparation of saturated solution

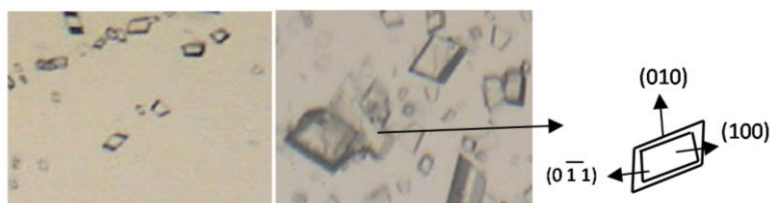


Figure 5. The Parallelogram like morphology of α -LM single crystals grown from water after 24 hours from the prepared saturated solution

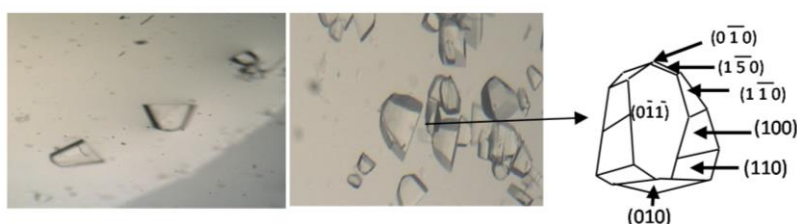


Figure 6. The tomahawk morphology of α -LM single crystals grown from water after 48 hours from the prepared saturated solution

In slow evaporation method, the nucleation was formed after 15 days. Hence, all nucleated crystals having tomahawk morphology.

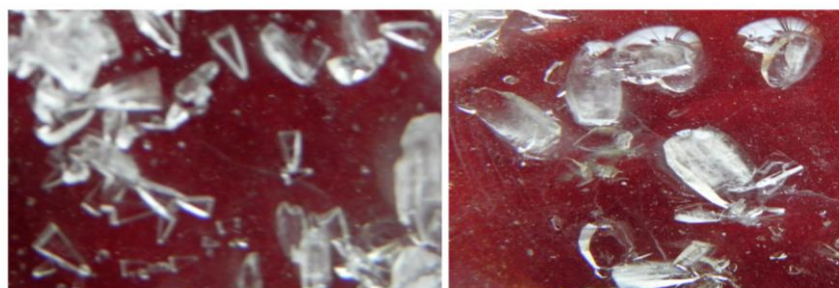


Figure 7. The tomahawk morphology of α -LM single crystals grown from saturated solution by slow evaporation method

4. Conclusion

The specific rotations of all concentration of solutions were decreased with increasing time through the mutarotation reaction of α -LM. The compositions of α and β -L in all prepared solutions were calculated from initial and final optical rotations by the method of Sharp and Doob. The composition of α -L decreased whereas that of β -L increased in solutions with increasing concentration of α -LM solutions. The effect of β -L on the morphology of α -LM single crystals was also studied using different experimental conditions.

Acknowledgements

One of the authors (KS) thankfully acknowledges the financial support provided for this work by University Grants Commission (UGC), New Delhi, India, through a major research project [Grant #42-866/2013 (SR)].

References

1. Paul Finch, *Carbohydrates, Structures, syntheses and dynamics*, Netherland pp.1-339
2. G. Haase and T. A. Nickerson, *Journal of Dairy Science*, 1966 a, 49, pp-127-132
3. P. L. H. McSweeney, P. F. Fox, *Advanced Dairy Chemistry; Lactose, water, salts and vitamins*. New York, 2009, USA, Springer Science.
4. P. F. Fox, T. Uniacke-Lowe, P. L. H. McSweeney, J. A. O, Mahony, *Dairy chemistry and bio chemistry*, New York, 2015, pp.21-68.
5. P. F. Fox, Timothy P. Guinee, Timothy M. Cogan, P. L. H. McSweeney, *Fundamentals of cheese science*, New York, 2015, 1-771.
6. M. Ganzle, G. Hasse, P. Jelen, *Int. Dairy J.* 18 (2008) 685-694.
7. G. Haase and T. A. Nickerson, *Journal of Dairy Science*, 1966 b, 49, 757-761.
8. S. Y. Wong, R. W. Hartel, *J. Food Sci.* 2014, 79, R257-R272.
9. P. F. Sharp and H. Doob, *J. Dairy Sci.*, 1941, 24, 589–602.