

Research Article

Preparation and Characterization of High Purity Anhydrous β -Lactose from α -Lactose Monohydrate at Mild Temperature

Ana L. López-Pablos ^{1,2}, César C. Leyva-Porras ³, Macrina B. Silva-Cázares ^{1,2},
Francisco E. Longoria-Rodríguez ⁴, Sergio A. Pérez-García ⁴,
Ángel A. Vértiz-Hernández,² and María Z. Saavedra-Leos ²

¹Doctorado Institucional en Ingeniería y Ciencia de Materiales (DICIM), Sierra Leona 530, Col. Lomas, 2a. Sección, 78210 San Luis Potosí, SLP, Mexico

²Coordinación Académica Región Altiplano, Universidad Autónoma de San Luis Potosí, Carretera a Cedral Km. 5+600, Ejido San José de las Trojes, 78700 Matehuala, SLP, Mexico

³Centro de Investigación en Materiales Avanzados S.C. (CIMAV), Miguel de Cervantes No. 120, Complejo Industrial Chihuahua, 31136 Chihuahua, CHIH, Mexico

⁴Centro de Investigación de Materiales Avanzados S.C. (CIMAV-Mty), Alianza Norte No. 202, Parque de Investigación e Innovación Tecnológica (PIIT), 66600 Apodaca, NL, Mexico

Correspondence should be addressed to María Z. Saavedra-Leos; zenaida.saavedra@uaslp.mx

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Lactose is a disaccharide of importance in humans dietary, food products, and the pharmaceutical industry. From the existing isomeric forms, β -lactose is rarely found in nature. Thus, in this work, a simple methodology to obtain anhydrous β -lactose (β L) from α -lactose monohydrate (α L·H₂O) is presented. The α L·H₂O powder was dispersed into a basic alcoholic solution (72 hours), at controlled conditions of temperature (27, 29, 31, and 32°C), without stirring. The slurry was dried at room temperature and characterized. Fourier transform infrared spectroscopy showed the formation of β L for the samples prepared at 29 and 32°C. Raman spectroscopy confirmed this result and suggested the occurrence of crystalline β L. Rietveld refinement of the X-ray diffraction patterns was employed to identify and quantify the composition of the isomers. The samples prepared at 29 and 31°C showed the formation of pure β L, while those at 27 and 32°C showed the presence of α L·H₂O and a mixture of the two isomers, respectively. The morphology of the powders was studied by scanning electron microscopy, observing the formation of irregular shape α L·H₂O particles and axe-like β L particles. Clearly, with this methodology, it was possible to obtain pure, crystalline, and anhydrous β L at mild temperature.

1. Introduction

Lactose (4-O- β -D-galactopyranosyl-D-glucopyranose) is found exclusively in the milk of mammals and is composed of molecules of galactose and glucose, linked by a β -glycosidic bond (1,4). Since the anomeric carbon of glucose is free, this disaccharide exhibits the features of reducing sugars; that is, the chain configuration is opened by the formation of an aldehyde group [1]. Lactose is used as an additive in many foods, besides being an excipient of numerous drugs. For example, in the food industry, it is used as a commercial

additive because of its texture, flavor, and adhesive properties [2]. In the pharmaceutical industry, lactose is employed as excipient in more than 20% of the total of prescription drugs and in 65% of the over-the-counter medications [3]. When used as excipient in drugs, lactose is the substance where the active ingredient is dispersed and preserved in a stable state [4, 5]. This disaccharide exists in two isomeric forms, α -lactose, and β -lactose, which shows the phenomenon of mutarotation, that is, the interconversion of the stereocenters [6]. Lactose exhibits three polymorphs (anhydrous forms) and a solvate (hydrated form): anhydrous β -lactose (β L),

TABLE 1: Summary of the methodologies reported for the conversion of $\alpha\text{L}\cdot\text{H}_2\text{O}$ into βL .

Methodology	Results	Characterization technique	Reference
<i>Starting material:</i> $\alpha\text{L}\cdot\text{H}_2\text{O}$. <i>Solvent:</i> sodium hydroxide in methanol. <i>Temperature:</i> room temperature, and boiling temperature of methanol. <i>Reaction time (h):</i> 2–10. <i>Post processing:</i> filtering and vacuum drying.	Crystalline βL , purity of 93.6%.	Gas-liquid chromatography	[12]
<i>Starting material:</i> $\alpha\text{L}\cdot\text{H}_2\text{O}$. <i>Solvent:</i> potassium methoxide in methanol. <i>Temperature:</i> room temperature, and boiling temperature of methanol. <i>Reaction time (h):</i> 0.7–24. <i>Post processing:</i> filtering, washing, and vacuum drying by 16 h.	Crystalline βL , purity of 98%.	Differential scanning calorimetry (DSC)	[13]
<i>Starting material:</i> $\alpha\text{L}\cdot\text{H}_2\text{O}$. <i>Solvent:</i> potassium methoxide or potassium hydroxide in methanol. <i>Temperature:</i> room temperature, and boiling temperature of methanol. <i>Reaction time (h):</i> 3–140. <i>Post processing:</i> filtering, washing, and vacuum drying by 16 h.	Crystalline βL , purity of 95%.	Gas-liquid chromatography	[15]

unstable hygroscopic anhydrous α -lactose ($\alpha\text{L}_{\text{H}}$), stable anhydrous α -lactose ($\alpha\text{L}_{\text{S}}$), and α -lactose monohydrate ($\alpha\text{L}\cdot\text{H}_2\text{O}$) [3, 7]. In addition to these polymorphs, there are another two which are classified based on the solid state, crystalline anhydrous mixture of α - and β -lactose and amorphous anhydrous mixture of α - and β -lactose [8]. The study of the polymorphs of lactose is of great interest because several physical properties, such as solubility, density, stability, shelf life, dissolution rate, and bioavailability, depend on the crystalline structure [9]. Several works focused on the study of the crystalline forms have been concerned with the following: (i) the properties characterization of polymorphs with the aim of predicting their stability and (ii) the preparation of the different polymorphs from $\alpha\text{L}\cdot\text{H}_2\text{O}$, especially the βL , since it is thermodynamically stable, just after the $\alpha\text{L}\cdot\text{H}_2\text{O}$. For example, Kirk et al. (2007) provided a guide for identifying the physical and chemical characteristics of lactose polymorphs [10]. Figura and Epple (1995) performed a study on the thermal and physical characterization of the different polymorphs of lactose [11]. Olano and Ríos (1978) and Parrish et al. (1979) reported obtaining the βL from the $\alpha\text{L}\cdot\text{H}_2\text{O}$ in alkaline alcoholic solutions, with conversions of 90–93% and 98%, respectively [12, 13]. In addition, authors such as Olano (1978) obtained the βL with a purity of 90% [14]. More recently, Chen et al. (2015) and Kirk et al. (2007) have also contributed to obtaining the βL , based on the methodology reported elsewhere [9, 10]. Table 1 summarizes the methodologies reported for the conversion of $\alpha\text{L}\cdot\text{H}_2\text{O}$ into βL . Although there are a large number of works reported which are focused on the preparation and characterization of the polymorphs of lactose, to the best of our knowledge, a high conversion of the $\alpha\text{L}\cdot\text{H}_2\text{O}$ into βL has not been reported so far. Therefore, in the present work, a simple methodology to obtain a

relatively high conversion of βL (>99.9%), as well as the physicochemical and thermal characterization, is reported. This methodology allows obtaining βL in an alcoholic solution in a narrow range of temperature, while avoiding the stirring and vacuum-drying steps.

2. Experimental

2.1. Materials. α -Lactose monohydrate ($\alpha\text{L}\cdot\text{H}_2\text{O}$, purity $\geq 99\%$, Sigma) was employed as the starting material and precursor of the βL , while sodium hydroxide (>98%, Macron Fine Chemicals) and methanol (HPLC grade, Sigma-Aldrich) were used in the preparation of the inorganic salt alcoholic solution.

2.2. Preparation of β -Lactose. Figure 1 shows a schematic representation of the methodologies described by Olano and Ríos [12] and Parrish et al. [13]. In these methodologies, the $\alpha\text{L}\cdot\text{H}_2\text{O}$ was used as initial reagent and dissolved in a basic methanol solution employed as the reacting media for the conversion of this polymorph into anhydrous βL . However, Parrish et al. carried out a preliminary treatment in anhydrous sodium acetate solution and acetic anhydride in order to produce two intermediates (α - and β -lactose octaacetates), which according to these authors it was required to achieve higher yields. Figure 1 also summarizes the steps followed in this work. The βL was prepared in batches, in which the α -lactose monohydrate was placed in a 0.2% (wt%) sodium hydroxide methanol solution, in a 1:10 ratio (α -lactose to methanol solution). Each batch was placed in an incubator at 27, 29, 31, and 32°C, respectively, for 72 hours without agitation. After the reaction time, the solutions were filtered and washed and the wet powder was dried at room temperature in a desiccator. According to Garnier et al.

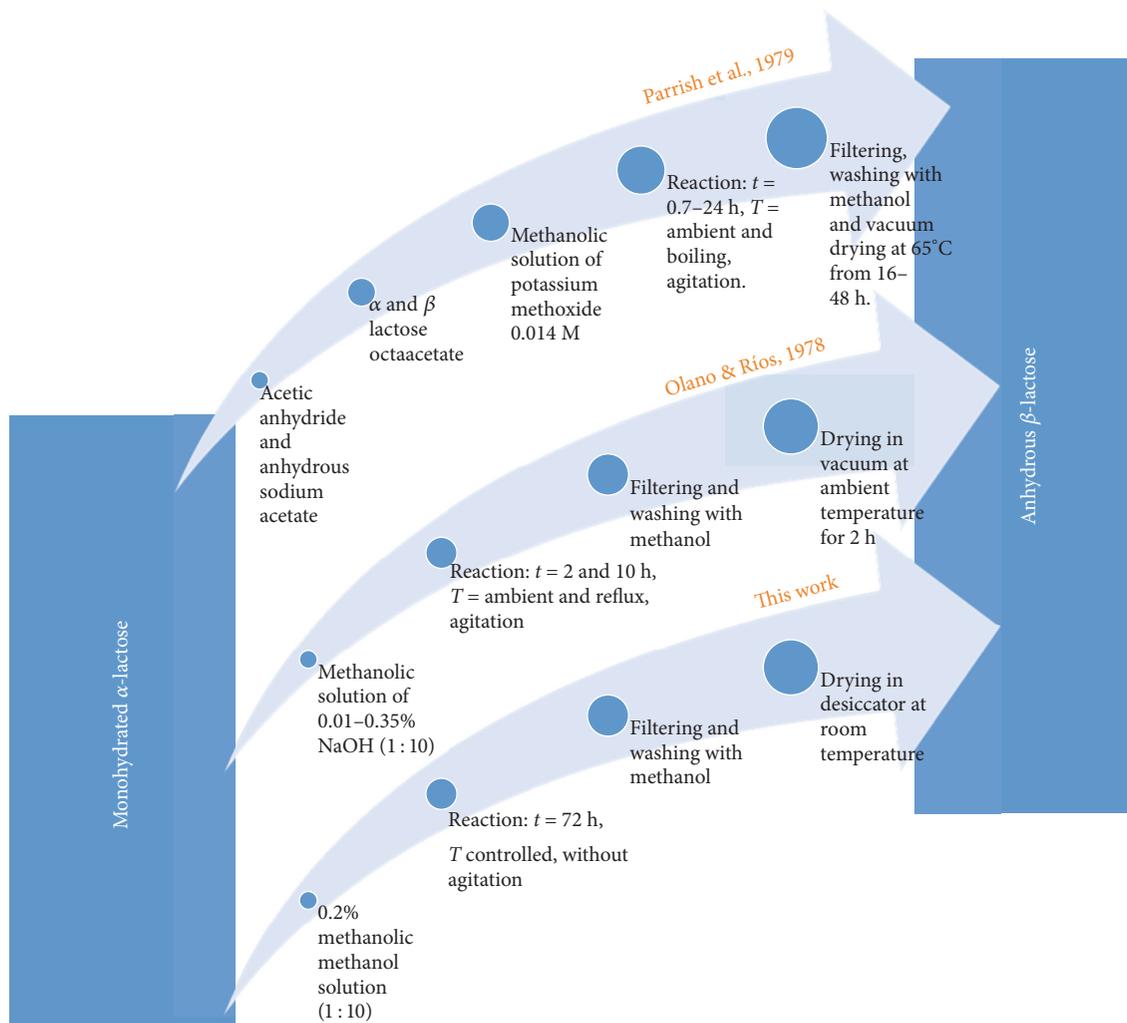


FIGURE 1: Schematic representation of the methodologies reported by Parrish et al. (1979), Olano & Ríos (1978), and this work.

(2002), static conditions, that is, without stirring the solution, promote the nucleation and growth of β -lactose crystals in the solution [16]. The methodology implemented in this work overcomes the stirring and vacuum-drying steps, which are required to obtain the β -lactose.

2.3. Physicochemical Characterization. The dried powders were characterized by Fourier transform infrared (FTIR) spectroscopy, in a Thermo Scientific Nicolet iS50 FTIR spectrophotometer, coupled to a Smart Orbit accessory with total attenuated reflectance (ATR) with a diamond crystal. Data were in a range of 4000 to 600 cm^{-1} and processed by Omnic 9.3.32 software. Raman spectroscopy was carried out using a Horiba LabRam HR Evolution instrument with a Jobin Yvon spectrometer. A red laser of He-Ne with a wavelength of 633 nm was used. An Olympus BAXFM-ILHS microscope with a $100\times$ objective lens was used to focus the laser beam on the sample. The Raman spectra were collected in a range of 50 to 3350 cm^{-1} .

2.4. β -Lactose Purity by X-Ray Diffraction. The characterization of the crystalline phases present within the sample

was carried out using the X-ray diffraction (XRD) technique, using an Emyrean diffractometer, PANalytical, with Cu radiation ($K\alpha = 15.405\text{ \AA}$). Patterns were recorded in the range of 2θ of 5 – 90° , with a step size of 0.016° and 59.69 s per step. For the XRD analysis, the solid sample was placed in a zero-background sample holder of amorphous silicon. The semiquantitative analysis of the crystalline phases present within the material was carried out following the Rietveld method, using the HighScore Plus Software version 3.0.5 (PANalytical B.V.). The Rietveld method consists of adjusting the experimental intensities corresponding to the X-ray diffraction pattern according to a theoretical model, using the least squares method, until the best fit between both is achieved. The diffraction pattern calculated is based on a model that includes structural, microstructural, and instrumental information. The quantity to be minimized in Rietveld refinement of the diffraction pattern is the residual (S_y), which is described according to the following:

$$S_y = \sum iW_i (y_i - y_{\text{cal}})^2, \quad (1)$$

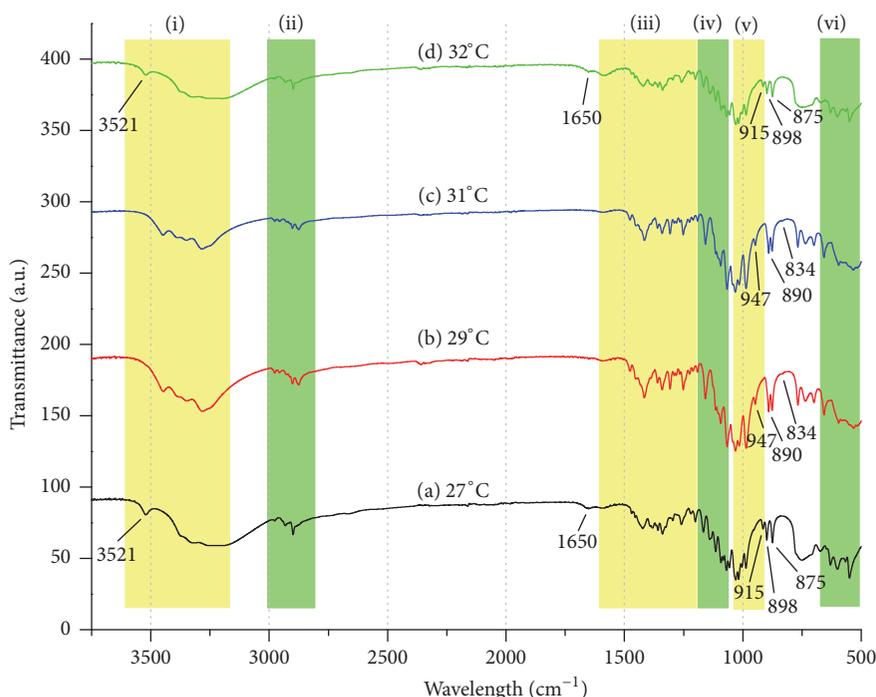


FIGURE 2: FTIR spectra of samples prepared at different temperatures. Temperature is indicated on each curve.

where $W_i = 1/y_i$, y_i is the experimental intensity at the i th step, y_{cal} is the calculated intensity at the i th step, and the sum is done over all the data point of the diffraction pattern [17].

2.5. Thermal Analysis. Thermogravimetric (TGA) and differential thermal analyses (DTA) were carried out in a simultaneous TGA-DTA SDT Q600 (TA Instruments, USA). Baseline was calibrated with Indium with a melting temperature of 156.6°C and melting enthalpy of 28.47 J/g. Samples of 5–10 mg were encapsulated in standard aluminum pans. Thermograms were recorded at a heating rate of 5°C/min over a range of 25–500°C. Using the Universal Analysis 2000© software, several features from the curves were identified: mass loss (% w/w), melting temperature (T_m), and degradation temperature (T_d). A modulated DSC Q200 (TA Instruments, USA) equipped with an RCS90 cooling system was employed for accurately determining the different thermal events such as the glass transition temperature (T_g), melting (T_m), and degradation (T_d). Instrument was calibrated with Indium for melting temperature and enthalpy, while Sapphire was used as the standard for heat capacity (C_p). Samples ranging in 5–10 mg were encapsulated in Tzero® aluminum pans. Thermograms were acquired at a temperature range of –90 to 250°C, with a modulation period of 40 s and amplitude of 1.5°C. Each experiment was repeated three times.

2.6. Scanning Electron Microscopy (SEM). A scanning electron microscope JSM-6010/LV (JEOL, Japan) operated at 15 kV in low vacuum mode was employed for observing the morphology of the powders. A portion of each sample was manually dispersed on a carbon film and observed without any further treatment.

3. Results and Discussion

3.1. Chemical Characterization of Lactose Polymorphs by FTIR. Samples prepared at different temperatures were analyzed using the Fourier transform infrared spectrometry (FTIR), to elucidate the formation of the polymorph. The resulting spectra are presented in Figure 2, where the colored rectangles differentiate the characteristic vibrations of the lactose molecule: (i) the symmetric stretching vibration of the hydroxyl groups (OH) between 3600–3200 cm^{-1} ; (ii) the stretching vibrations of the CH groups presented in the glucose and galactose molecules at 3000–2800 cm^{-1} ; (iii) the rocking and wagging vibrations of the CH, OH, and CH_2 groups at 1600–1200 cm^{-1} ; (iv) the flexion vibration of the glycosidic bond (C–O–C) at 1200–1070 cm^{-1} ; (v) the stretching of the CO of the glycosidic bond at 1142–953 cm^{-1} ; (vi) the deformation vibrations of the glycosidic bond at 633–477 cm^{-1} . Additionally, the spectra from samples prepared at 27 and 32°C (Figure 2 (a) and (d)) showed the characteristic bands of $\alpha\text{L}\cdot\text{H}_2\text{O}$ at 915, 898, and 874 cm^{-1} , while those from samples prepared at 29 and 31°C (Figure 2 (b) and (c)) displayed the distinctive bands of βL at 947, 890, and 834 cm^{-1} . According to Listioghadi et al. (2009) the bands located at 3600–3200 cm^{-1} correspond to the stretching of the OH groups of the lactose molecule, while the vibrations at 1650 cm^{-1} are related to the flexion of the OH groups of the crystallized water molecules [18]. Conversely, Kirk et al. (2007) reported that the bands at 3500 and 1654 cm^{-1} , corresponded to the vibrations and distortion of the OH groups in water molecules, respectively [10]. The FTIR spectra reported in Figure 2 (a) and (d) showed the

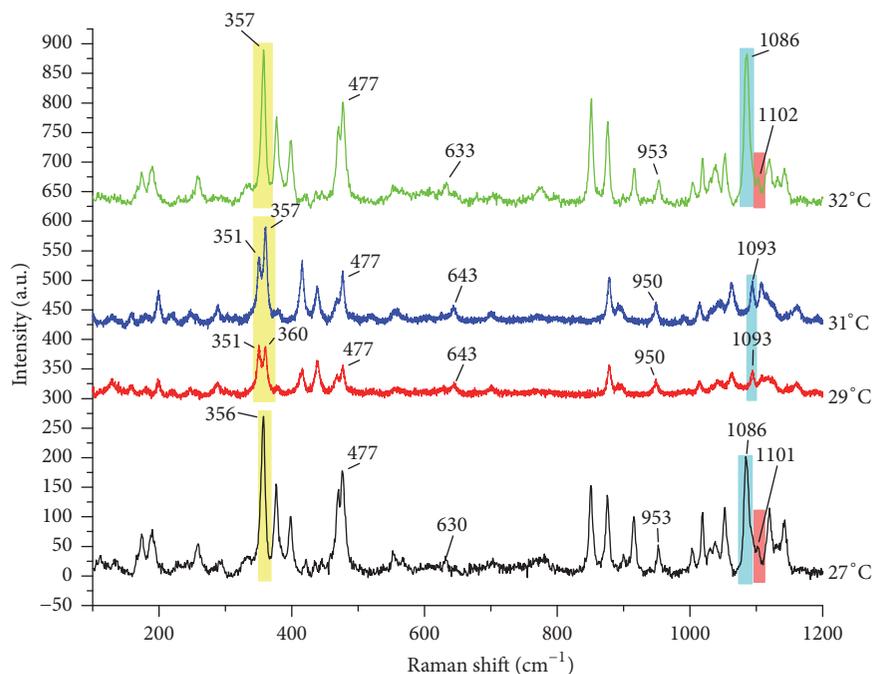


FIGURE 3: Raman spectra obtained for the samples prepared at the different temperatures, which is indicated on each curve.

presence of a broad band at 3500 cm^{-1} and a weak band at 1650 cm^{-1} , while the spectra for Figure 2 (b) and (c) showed the absence of the bands. Thus, these observations confirmed the formation of anhydrous βL for the samples prepared at 29 and 32°C . The spectra reported herein are similar to those reported by the British Pharmacopoeia [19] and Norris and Greenstreet [20], for $\alpha\text{L}\cdot\text{H}_2\text{O}$, and βL , respectively.

Furthermore, Listiohadi et al. [18] employed FTIR technique for differentiate the crystalline lactose from the amorphous. They observed differences in the FTIR spectra between the amorphous and crystalline lactose; that is, the crystalline lactose shows well-defined vibrations peaks, while, in the amorphous lactose, the peaks are broad and less intense [18]. Based on this, all the reported FTIR spectra showed well-defined and sharp vibration peaks, suggesting the formation of crystalline $\alpha\text{L}\cdot\text{H}_2\text{O}$ at 27 and 32°C and crystalline βL at 29 and 31°C .

3.2. Raman Spectroscopy. Raman spectroscopy is a characterization technique sensible to the chemical environment of a molecule; that is, with this tool it is possible to distinguish differences in the composition and the way atoms are bonded [21]. Figure 3 shows the Raman spectra obtained for the samples prepared at the different temperatures. All the spectra showed several sharp bands characteristic of the disaccharides. According to Wiercigroch et al. (2017), the lactose molecule presents many vibrations modes in the wavenumber range up to 800 cm^{-1} , corresponding to the torsion of the ring and the deformation of O-C-C, O-C-O, and C-C-O, while the high intensity of the vibration mode about 357 cm^{-1} is attributed to the twist-like vibrations of the COOH and HOH groups [22]. Kirk et al. (2007) concluded that Raman spectroscopy could be employed

to differentiate the anomeric conformation of lactose by comparing the intensity of the vibration mode at 1100 cm^{-1} [10]. They observed the presence of a high intensity vibration mode at 1100 cm^{-1} in the $\alpha\text{L}\cdot\text{H}_2\text{O}$ and the absence of this mode in the βL . Wiercigroch et al. (2017) explained that the vibration at 1100 cm^{-1} was caused by the rotation of the C-O-C bond, which allows the formation of the anomeric bond [22]. Together with the vibration at 1100 cm^{-1} , the vibration at 357 cm^{-1} must be presented, which is observed as a single peak in the $\alpha\text{L}\cdot\text{H}_2\text{O}$ and as a double peak in the βL [10]. The Raman spectra reported in this work showed the presence of a sharp vibration mode at 1086 cm^{-1} for the samples prepared at 27 and 32°C and the less intense vibration mode about 1100 cm^{-1} for the samples prepared at 29 and 31°C . The vibration mode at 357 cm^{-1} was observed as a single peak in the samples prepared at 27 and 32°C and as a high intensity double peak in the samples prepared at 29 and 31°C . Evidently, these observations indicate the formation of the βL at the intermediate temperatures. In addition, the vibration about $1086\text{--}1100\text{ cm}^{-1}$ can be employed to identify the microstructure of lactose, that is, crystalline or amorphous, where the presence of the band indicates a crystalline structure [23]. All the samples showed a vibration band at $1086\text{--}1100\text{ cm}^{-1}$, suggesting the occurrence of crystalline $\alpha\text{L}\cdot\text{H}_2\text{O}$ and βL . The vibrations modes at 477 and 633 cm^{-1} were assigned to the deformation of the C-C-O group in the glycosidic bond [22]. According to Márquez et al. (2015) and Rodríguez Júnior et al. (2016), the vibration modes at 953 and 1142 cm^{-1} are originated from the stretching of the CO in the glycosidic bond [24, 25].

3.3. Polymorph Identification and Composition Determination by XRD. XRD technique and the Rietveld method were

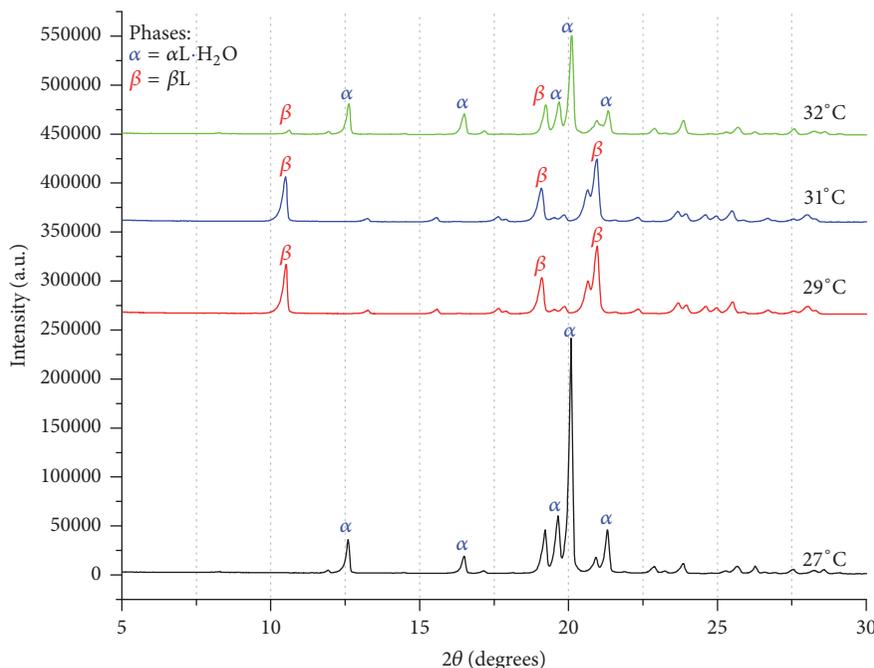


FIGURE 4: XRD diffractograms of the powders prepared at 27, 29, 31, and 32°C.

employed for identifying the distinct polymorphs (α and β) and determining the purity of the lactose powders. Figure 4 shows the diffractograms of the powders prepared at 27, 29, 31, and 32°C. Overall, lactose samples showed well-defined and sharp peaks, typically observed in crystalline materials. The corresponding diffractograms of samples prepared at 27 and 32°C showed the distinctive reflections of the α -lactose monohydrate polymorph located at 2θ angle of 12.5, 16.4, 19.5, and 20.5°. All the reflections matched with those of α -lactose, reported by the international center for diffraction data (ICDD) in the powder diffraction file PDF 00-024-1783 [26]. The results agree with those presented by Gombás et al. (2003), who studied the accuracy of the XRD technique, in the quantification of the crystallinity degree in amorphous-crystalline mixtures of lactose [27]. The samples prepared at 29 and 31°C showed the peaks corresponding to the anhydrous β -lactose at 10.5, 19.0, and 21.0°. For β -lactose, the distinctive diffraction is at 2θ of 10.5° [10]. These reflections matched with those reported for the β -lactose in the PDF 00-029-1758 [28]. XRD analysis was also employed to determine the purity of the powdered samples, quantifying the relative percentage of each phase by the Rietveld method. Results are summarized in Table 2. The composition of the samples prepared at 27°C was 100% of α -lactose monohydrate, while that of sample prepared at 32°C showed a mixture of lactose (α and β). Samples prepared at 29 and 31°C showed a composition of pure β -lactose. These results suggest the complete conversion of the α L·H₂O into crystalline β L at temperatures of 29 and 31°C.

The cell parameters and zero shift values were calculated from the Rietveld refinement and reported in Table 3. As observed, there are no significant differences in the cell parameters of the samples synthesized. This indicated that the

TABLE 2: Relative percentage of each phase determined by the Rietveld method.

Preparation temperature (°C)	Composition	R_{exp}	R_{profile}	R_{Bragg}
27	100% α L·H ₂ O	2.09	8.3	6.41
29	100% β L	2.05	13.0	6.07
31	100% β L	2.03	9.1	6.93
32	92% α L·H ₂ O 8% β L	2.06	9.8	7.6

overall crystal dimensions are maintained constant. However, the zero shift showed a slight increment between the samples synthesized at 29°C and the others synthesized at 27, 31, and 32°C. This may contribute to the displacement observed in some of the reflections of the samples. Additionally, since the displacement observed in the diffractograms was not systematically presented in all the reflections, authors consider the possibility of molecular deformations in some of the crystal planes, which also may contribute to the slight shift observed. The formation of a solid solution was not considered to explain the displacement of some of the reflections, because the samples of β -lactose synthesized at 29 and 31°C were anhydrous, and water is necessary for the formation of the solid solution.

3.4. Thermal Characterization by Simultaneous Thermal Analysis (TGA-DSC) and Modulated Differential Scanning Calorimetry (MDSC). Figure 5 shows the TGA-DSC thermograms of the samples prepared at 27, 29, 31, and 32°C. Each graph shows two curves plotted as a function of temperature, corresponding to the weight loss (TGA) and

TABLE 3: Cell parameters and zero shift calculated from the Rietveld refinement.

Parameters	29°C	31°C	32°C	27°C	
	β -Lactose	β -Lactose	β -Lactose	α -Lactose	α -Lactose
a (Å)	4.96	4.96	4.95	4.82	4.83
b (Å)	13.36	13.35	13.34	21.62	21.61
c (Å)	10.86	10.85	10.85	7.78	7.78
alpha (degrees)	90	90	90	90	90
beta (degrees)	91.57	91.57	91.57	105.96	105.98
gamma (degrees)	90	90	90	90	90
Zero shift (degrees)	0.02	0.06		0.075	0.06

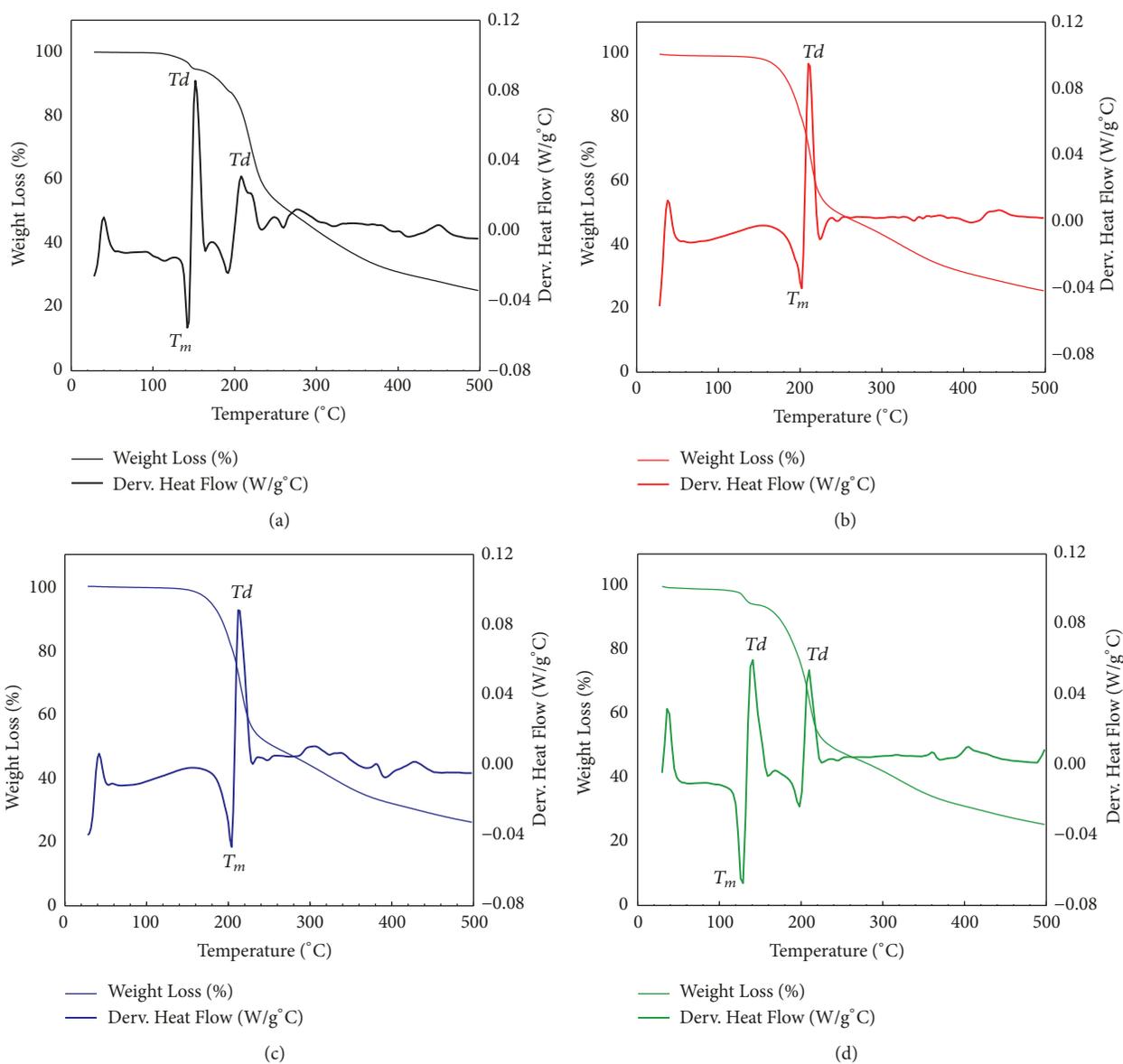


FIGURE 5: Simultaneous TGA-DSC of the samples synthesized at (a) 27, (b) 29, (c) 31, and (d) 32°C.

the derivative of the heat flow (DSC). The α L-H₂O samples prepared at 27 and 32°C (Figures 5(a) and 5(d)) showed several weight losses observed at 100–150°C, 150–200°C, and 220°C. Usually, the first loss is related to the evaporation of water chemically bonded to the lactose molecule. However, the other two weight losses cannot be assigned deliberately to the thermal decomposition of lactose or to the individual decomposition of molecules that compose it (glucose and galactose). The DSC curve showed two thermal events at 140 and 190°C, related to the melting (T_m) of lactose, followed by the degradation (T_d), observed as the abrupt increase of the signal at 150 and 210°C, respectively. On the other hand, the β L samples prepared at 29 and 31°C (Figure 5(b) and (c)) showed a constant baseline up to 170°C, indicating that effectively the β L is anhydrous. In the range of 170–220°C, there is a second mass loss (about 40% of weight loss) corresponding to the thermal decomposition of the β L, which may be understood as the breaking of the glycosidic bond and the subsequent evolution of water. Above 220°C, the glucose and galactose molecules thermally degraded, forming the products derived from the combustion of the organic matter. These observations were confirmed by the DSC curve, which showed a single melting event at 202°C, followed by the degradation of the molecule at 210°C [9, 11, 18, 27]. Clearly, these results are useful for identifying the thermal events of the distinct lactose polymorphs.

Modulated differential scanning calorimetry (MDSC) is an extension of conventional DSC and is a technique widely employed for the thermal characterization of biopolymers [29–31]. In this sense, the MDSC is preferred over conventional DSC because it is more sensitive for detecting time-dependent transitions such as the glass transition temperature and other complex thermal events [29]. Figure 6 shows the MDSC thermograms of the samples prepared at 27, 29, 31, and 32°C. In the α L-H₂O obtained at 27°C (Figure 6(a)), three distinctive endotherms were observed at 145, 185, and 210°C. The first corresponds to the melting of glucose units, the second to the melting of an intermediate compound, and the third to the melting of lactose molecules. The α L-H₂O obtained at 32°C (Figure 6(d)) showed also three thermal events, but the temperatures were presented at lower values, 120, 155, and 200°C. The samples containing pure β L anhydrous prepared at 29 and 31°C (Figure 6 (b) and (c)) showed a single endotherm at 205 and 200°C, respectively. This thermal event corresponded to the melting of the β L molecules. However, the sample prepared at 31°C presented a small endotherm located about 175°C, suggesting the melting of an intermediate molecule formed as a byproduct.

Regarding the determination of T_g , the MDSC thermograms were acquired in the range of temperature of –90 to 290°C. Usually T_g of saccharides, disaccharides, and carbohydrate polymers is observed in this range of temperature and particularly in temperatures below 50°C. Thus, the samples analyzed herein showed a continuous line from –90 to 50°C, indicating the absence of the glass transition temperature. This observation is in agreement with the results of Raman and XRD and confirms that the microstructure of the samples is fully crystalline.

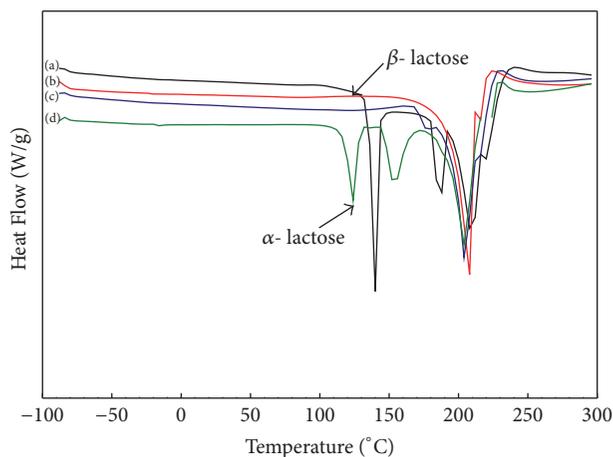


FIGURE 6: MDSC thermograms of the samples prepared at 27, 29, 31, and 32°C.

3.5. Morphological Characterization by SEM. A scanning electron microscope (SEM) was employed to analyze the morphology and particle size lactose powders. Figure 7 shows the SEM micrographs of the samples prepared at 27, 29, 31, and 32°C. The α L-H₂O samples prepared at 27 and 32°C (Figures 7(a) and 7(d)) exhibited an irregular morphology and particle size in the range of 10–100 μ m. On the surface of the big particles some small particles were observed, identified as whisker-like crystals. According to Garnier et al. (2002), these crystals are formed during the crystallization of the α -lactose suspended in methanol solution, without altering the initial morphology and composition [16]. The micrographs of the anhydrous β L samples prepared at 29 and 31°C (Figures 7(b) and 7(c)) showed similar characteristics. The most notorious feature was the smaller particle size (1–10 μ m) when compared with the α L-H₂O particles. Additionally, the β L anhydrous particles showed axe-type morphology with a smooth surface. Carpin et al. (2016) observed that the crystallization of β L polymorph in alcohol produced curved needle shape particles [3].

A possible explanation of the narrow operating conditions, the polymorphic transformation, and particle size decrease found herein is based on the work reported by Petit and Coquerel (1996) [32]. They studied the dehydration mechanisms of copper-8-hydroxyquinoline dihydrate to obtain the anhydrous form. According to them, the polymorphic transformations and solvate decompositions may follow a continuous mechanism or by nucleation and growth. In the continuous mechanism, the transition is carried out by the progressive reorientation and translation of the molecules in the crystal. In the nucleation and growth mechanism, the transitions are carried out on specific sites of the crystal and the progressive propagation of the crystal. Additionally, Garnier et al. (2002) reported the dehydration mechanism and the crystallization behavior of α -lactose monohydrate to obtain stable anhydrous α -lactose, hygroscopic α -lactose, and β -lactose [16]. They found that despite the dehydration conditions, that is, temperature and type of solvent, the evolution of the water molecules occurs in a cooperative way,

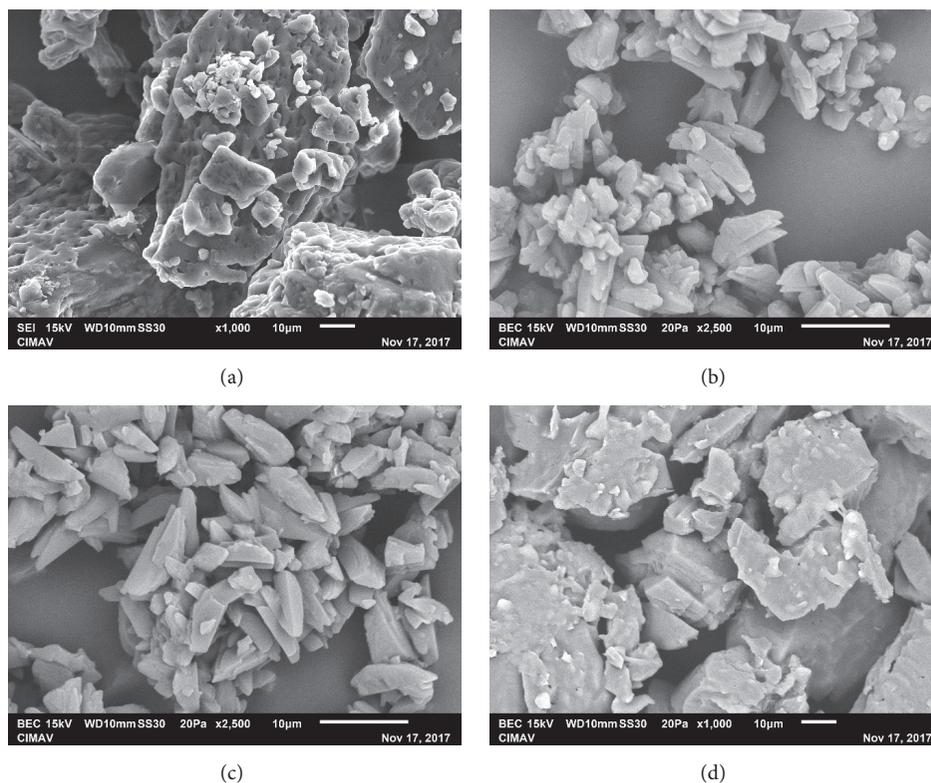


FIGURE 7: SEM micrographs of the samples prepared at (a) 27, (b) 29, (c) 31, and (d) 32°C.

through channels parallel to the c axis, providing stability to the monohydrate molecule. Therefore, the dehydration mechanism of α -lactose monohydrate obeys a continuous model, and the evolution of water from the crystal involves the decrease in the molecular volume, which was observed as the decrease in the particle size of the β -lactose.

4. Conclusions

The methodology proposed in this work for obtaining pure anhydrous β -lactose (β L) involved the static reaction (without stirring) of α -lactose monohydrate (α L \cdot H₂O) in a basic alcoholic solution at controlled conditions of temperature. By Fourier transform infrared (FTIR) and Raman spectroscopies, it was found that the full conversion of α L \cdot H₂O into anhydrous β L proceeds only in a narrow temperature range of 29–31°C. Although the reaction time was longer than in other reported works, the obtained β L composition was pure as determined by the Rietveld refinement of the X-ray diffraction (XRD) patterns. Thermal analysis (simultaneous TGA-DSC, and MDSC) confirmed these results, after observing a single melting event at 204°C, followed by the thermal degradation at 210°C. The thermal characterization of the samples prepared at 27 and 32°C showed the formation of mixtures of α L \cdot H₂O and β L, since two melting events were observed at 146 and 200°C. The observation of the powders by scanning electron microscopy showed a regular axe-like morphology with a smooth surface for the β L samples, while α L \cdot H₂O showed irregular shape particles with sizes of several

tens of microns. Clearly, with this simple methodology, it was possible to obtain pure, crystalline, and anhydrous β L at mild conditions of temperature.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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