Dairy Sci. Technol. 89 (2009) 43–67

DOI: 10.1051/dst:2008027

Available online at:
www.dairy-journal.org

Original article

Thermal analysis of amorphous lactose and α-lactose monohydrate

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Received 15 January 2008 – Accepted 9 June 2008

Abstract – It is common to find that some of the lactose in dairy powders and pharmaceutical tablets is present in the unstable amorphous state. If stored at inappropriate temperatures and humidities amorphous lactose is susceptible to crystallization. The integration of thermal gravimetric analysis (TGA) with single differential thermal analysis (SDTA) provided a descriptive method for a sequential and direct determination of surface water, water of crystallization and amorphous lactose in a single analysis on one sample. Peaks and mass changes on the TGA/SDTA thermograms characteristic of surface water, water of crystallization and amorphous lactose were identified. The content of water of crystallization was used to estimate α-lactose monohydrate. The loss of surface water was indicated on the TGA/SDTA thermograms as weight loss between 40 and 130 °C and the loss of water of crystallization occurred at 153 °C. Amorphous lactose was indicated by an exothermic crystallization peak at 174 °C. The area under the exothermic crystallization peak was linearly related to the proportion of the amorphous lactose in mixtures with α-lactose monohydrate (r = 0.989). This work presented the TGA/SDTA thermograms of lactose samples containing some crystalline forms of lactose and amorphous lactose. The study compared the methods for determining surface water and total water content of lactose accepted by official bodies worldwide with the TGA/SDTA approach. The potential of new methods for qualitatively detecting the amorphous and crystalline forms of lactose by thermochemistry and Fourier transform infra-red (FT-IR) was also explored and compared.

TGA-SDTA / differential thermal analysis / thermal gravimetric analysis / water content / amorphous lactose

摘要 – 无定形乳糖和α-乳糖单水合物的热分析。通常乳糖在乳粉和药片中处于不稳定的无定形状态。如果贮藏温度和湿度不当, 乳糖就会形成结晶。采用热重 (TGA) / 同步差热分析 (SDTA) 法可以连续直接测定样品中的游离水、结晶水和无定形乳糖。从 TGA/SDTA 热重图的峰和质量变化可以判定游离水, 结晶水和无定形乳糖的热特性。根据结晶水的含量可以估算α-乳糖单水合物。从 TGA/SDTA 的热重图上可以看出在 40–130 °C 之间显示的重量损失是游离水的损失, 在 153 °C 时则是结晶水的损失。无定形乳糖于 174 °C 发生结晶而产生一个放热峰。结晶放热峰的峰面积与混合物中无定形乳糖和α-乳糖单水合物的比例呈线性关系 (r = 0.989) 。试验结果证明了在乳糖样品中含有一些结晶乳糖和无定形的乳糖。采用 TGA/SDTA 法测定乳糖总水分含量和游离水含量已经在全世界范围内得到认可。本文还对具有潜在优势的热化学和傅立叶变换红外光谱法测定无定形和结晶乳糖进行了探讨, 并将这两种方法与 TGA/SDTA 方法进行对比。

热重/同步差热分析 / 热分析 / 乳糖 / 水分含量 / 无定形

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Résumé – Analyse thermique de lactose amorphe et de lactose α monohydrate. Généralement une partie du lactose dans les poudres laitières et les comprimés pharmaceutiques se trouve à l'état amorphe instable. S'il est conservé à des températures et humidités inappropriées, le lactose amorphe peut cristalliser. Le couplage de l'analyse thermogravimétrique (TGA) avec l'analyse thermique différentielle (SDTA) a fourni une méthode descriptive pour la détermination séquentielle et directe de l'eau de surface, de l'eau de cristallisation et du lactose amorphe d'un échantillon en analyse. Les pics et les changements de masse sur les thermogrammes TGA/SDTA caractéristiques de l'eau de surface, de l'eau de cristallisation et la teneur en lactose amorphe ont été identifiés. La teneur en eau de cristallisation a été utilisée pour estimer le lactose α monohydrate. La perte d'eau de surface se traduisait sur les thermogrammes TGA/SDTA par la perte de poids entre 40 et 130 °C, et la perte d'eau de cristallisation avait lieu à 153 °C. Le lactose amorphe était indiqué par un pic de cristallisation exothermique à 174 °C. La surface sous le pic de cristallisation exothermique était linéairement reliée à la proportion de lactose amorphe dans les mélanges avec lactose α monohydrate ($r = 0,989$). Ce travail présente les thermogrammes TGA/SDTA d'échantillons de lactose contenant quelques formes cristallines de lactose et de lactose amorphe. L'étude a comparé les méthodes pour déterminer les teneurs en eau de surface et en eau totale du lactose acceptées par les organismes officiels internationaux avec l'approche TGA/SDTA. Le potentiel de nouvelles méthodes pour détecter qualitativement les formes amorphes et cristallines du lactose par thermochimie et spectroscopie infrarouge à transformée de Fourier a également été exploré et comparé.

analyse thermogravimétrique / analyse thermique différentielle / TGA-SDTA / lactose amorphe / teneur en eau

1. INTRODUCTION

Lactose, an important pharmaceutical excipient and ingredient in dairy powders and many food dry mixes, is often associated with sticking and caking possibly because of the presence of amorphous lactose [30]. Amorphous lactose is hygroscopic and is easily formed in bulk lactose powder during manufacturing such as drying and milling [42]. Stickiness and caking is controlled by handling and storing under dry conditions (e.g. at 25 °C at less than 30% humidity [4, 29]). This method is effective when sufficient information on the content of water and the proportions of crystalline and amorphous lactose is known.

The total water content of lactose should consist of sorbed surface water and water of crystallization of α-lactose monohydrate. The surface water forms a few layers of free water molecules and the water of crystallization of lactose exists within the crystal lattice strongly linking together oxygens from four different lactose molecules. While there are independent methods for determining the surface water and total water content of lactose [29], the water of crystallization is often calculated by difference and is subject to errors.

In the dairy industry, the degree of crystallization is often used as a measure of the potential of a lactose powder to cake [43]. This is the proportion of total lactose present as α-lactose monohydrate calculated from the water of crystallization. The higher the degree of crystallization of lactose, the lower is the proportion of other forms of lactose (including the troublesome amorphous lactose) and the less the potential for caking. Commercial lactose and whey powders may contain other crystal forms of lactose, such as α-lactose anhydrous stable, α-lactose anhydrous unstable, β-lactose anhydrous and the compound crystals of β/α-lactose [19, 25], in addition to the more common α-lactose monohydrate. For comprehensive presentation of the degree of crystallization, the proportion of the dehydrated crystal forms of lactose should be included in the total content of crystals. However, methods for full quantitative characterization of the crystal forms of lactose using the

same initial starting material are not readily available in the literature [25]. Fourier transform infra-red (FT-IR) is the reference method for identifying α-lactose monohydrate and β-lactose anhydrous by analyzing their molecular arrangements [1], but it has not been exploited for quantitative analysis. There have been a number of recent publications on methods for direct estimation of amorphous lactose, mostly using advanced instrumentation and related to the use of lactose in the pharmaceutical industry e.g. [12–16, 18, 41, 42]. Yet the pivotal role of amorphous lactose is not reflected in the food and pharmaceutical standards for lactose [1, 6, 44]. Direct analysis of the content of amorphous lactose would complement the data on the degree of crystallization. There is a place for a simple, convenient method for independently measuring on the same sample, amorphous lactose, α-lactose monohydrate (via the water of crystallization) and the surface water. This information should put manufacturers and users of lactose products in a better position to control caking.

Thermal analysis such as differential scanning calorimetry (DSC) was used to measure the heat evolved when amorphous lactose transforms to crystals to estimate the proportion of amorphous lactose [12, 14]. Thermal gravimetric analysis (TGA) [3] and differential thermal analysis (DTA) [36] were used for directly determining the surface water and water of crystallization of lactose and dairy powders. TGA, DTA and DSC are sensitive and the results are often complementary to each other. However the conduct of multiple analyses on separate instruments may mean higher costs and longer time of analysis.

Recent advances in instrumentation of thermal gravimetric analysis integrated with single differential thermal analysis (TGA/SDTA) allow simultaneous analysis of mass and energy changes of a sample when heated at specific temperatures. TGA/SDTA could be a valuable tool for separately and directly determining the surface water, water of crystallization of lactose and amorphous lactose in a single analysis on one sample.

Solution calorimetry has recently been re-examined for the analysis of amorphous material in pharmaceutical products using specialized equipment [16,17]. The change in heat when a substance is dissolved is known as the heat of solution [10]. The heat of solution of lactose is available in earlier [2, 20, 24, 31, 32] and recent papers [16, 17]. A small calorimeter, constructed inexpensively in our laboratory, has been evaluated in this study to estimate amorphous lactose by measuring the heat of solution.

The objectives of this study were to explore TGA/SDTA for an independent and direct determination of water of crystallization, surface water and amorphous lactose in a single analysis, on one sample using one piece of equipment. The TGA/SDTA were compared to some methods accepted in the industry (i.e. Karl Fischer, toluene distillation and the loss on drying) for measuring the total water and surface water content of lactose. The water of crystallization of lactose was used to calculate the content of α-lactose monohydrate. The study also explored FT-IR for the differentiation of amorphous from crystalline lactose and the development of a new thermochemical method for possible determination of amorphous lactose. The TGA/SDTA, FT-IR and thermochemical methods were used to explore the characteristics of some lactose crystals and amorphous lactose.

2. MATERIALS AND METHODS

2.1. Materials

α-Lactose monohydrate (Wyndale™, refined edible grade, 100 mesh;

Lactose NZ) was conditioned at 75% relative humidity at 25 °C for at least 1 month prior to the experiment following the widespread practice of reputable authors [12, 34] to eliminate any incipient amorphous lactose. This sample was used as the reference crystalline material (corresponding to 100 g·100 g^{-1} crystalline lactose [14]). The identity of α-lactose monohydrate was confirmed by FT-IR analysis [1].

Anhydrous lactose Pharmatose DCL21™ (pharmaceutical grade, produced by DMV International, the Netherlands, and supplied by Fernz Specialty Chemicals, Australia), was used as supplied. The anhydrous lactose Pharmatose DCL21 was the readily available commercial crystalline lactose low in α-lactose monohydrate and high in β-lactose content. The sample was confirmed to be mostly β-lactose anhydrous by FT-IR [1] with β- and α-lactose in the proportion of 83.8:16.2 (HPLC). This sample will be referred to as "anhydrous β-lactose" in this paper to distinguish it from the anhydrous forms of α-lactose.

"α-Lactose anhydrous stable" was made by extracting the water of crystallization of α-lactose monohydrate in toluene using the Dean and Stark apparatus for 5 h [19, 23]. "α-Lactose anhydrous unstable" was made by drying α-lactose monohydrate in a vacuum oven at 100 to 120 °C for 16 h [9, 19, 25]. The nature of the α-lactose anhydrous samples was confirmed [28] by exposing 3 g of the samples to 57% relative humidity at 25 °C (in a desiccator with saturated solution of NaBr). The α-lactose anhydrous stable was less hygroscopic at 57% relative humidity absorbing only 1 g·100 g^{-1} moisture in 1 week [9, 28]. The α-lactose anhydrous unstable was very hygroscopic absorbing more than 4 g·100 g^{-1} moisture in less than 1 day at 57% relative humidity [9, 28].

Amorphous lactose was prepared by rapid freezing using liquid nitrogen and freeze-drying of 10 to 20% solutions of lactose at –80 to –70 °C (pressure < 0.1 mbar [4]) for 48 h (Dynavac: FD400/3RH Freeze-drier, Australia). The absence of crystalline lactose in the freeze-dried lactose was identified by examination under a microscope with polarized light [30, 38]. This sample was used as the reference non-crystalline material (corresponding to 100 g·100 g^{-1} amorphous lactose [39]).

Binary mixtures of amorphous lactose and α-lactose monohydrate were prepared by physical mixing to give 0 to 100 g·100 g^{-1} crystalline content by anhydrous weight. For TGA/SDTA, the binary mixtures were prepared directly in TGA/SDTA crucibles (total weight of sample in each crucible was 10 (± 1) mg).

Spray-dried lactose Pharmatose DCL11™ (pharmaceutical grade, produced by DMV International, the Netherlands, and supplied by Fernz Specialty Chemicals, Australia), was used as supplied. The spray-dried lactose was the readily available commercial source for lactose powder, which contained amorphous lactose (specified to contain ca. 5 to 12 g·100 g^{-1} amorphous lactose).

In all cases, preparation and sampling took place in a glove box flushed with dry nitrogen gas. A complete removal of surface water from the crystalline lactose samples after each sample preparation as was done in studies for moisture sorption [4,42] using P_2O_5 was not necessary in this study since the presence of surface water was desirable for testing the methods of analysis for water content. However, it was ensured that the initial presence of water was not sufficient to cause transformation of one lactose form to another during sample storage and absorption of water was avoided by storing below the critical relative humidity of the various forms of crystalline and amorphous lactose [9, 42]. The samples were stable when stored in desiccators over dry silica gel at 25 °C until used.

All other materials used in the analyses were analytical grade.

2.2. Methods

2.2.1. Thermal analysis (TGA/SDTA)

Thermal analysis was conducted using thermal gravimetric analysis integrated with single differential thermal analysis (TGA/SDTA) instrument (model: 851e/LF1100, Mettler-Toledo GmbH, Switzerland) equipped with a robotic arm (model: TSO801RO) for automated sampling. The TGA/SDTA was conducted in four replicates on: α-lactose monohydrate, α-lactose anhydrous stable, α-lactose anhydrous unstable, anhydrous β-lactose DCL21, amorphous lactose of various weights (0.5 to 10 mg), binary mixtures of amorphous lactose and α-lactose monohydrate (to give 0 to 100 g·100 g^{-1} crystalline content by anhydrous weight) and spray-dried lactose DCL11. The total weight of each sample (accurately weighed into open 70 μL aluminum oxide crucibles using the TGA/SDTA microbalance) was 10 (± 1) mg, except for the samples of amorphous lactose of various weights. The powder sample was heated from 25 to 300 °C at a heating rate of 5 °C·min^{-1}, under a nitrogen flush (50 mL·min^{-1}). The instrument measured the change in mass and recorded the temperature profile and was calibrated using indium. Data were analyzed using Star^e Base Software for Windows NT Service Pack 4 (Mettler-Toledo GmbH, Switzerland).

2.2.2. Loss on drying by oven methods (80 °C for 2 h and 120 °C for 16 h) for water content analysis

The loss on drying at 80 °C for 2 h as described in the United States Pharmacopeia [44] and at 120 °C for 16 h as described in the Codex Alimentarius [5] were followed. A Qualtex Solidstat fan-forced oven (model no: OM 24 S2, Watson Victor Ltd., Australia) was used. The analyses were conducted in four replicates on α-lactose monohydrate.

2.2.3. Karl Fischer titration for water content analysis

The Karl Fischer titration method was adapted from the United States Pharmacopeia [44]. The solvent used was a mixture of methanol and formamide (1:1), which had been pre-titrated to dryness with Karl Fischer reagent (Hydranal composite 5, Riedel-de Haën). The Karl Fischer factor was determined by titrations of pure water (25 mg). A sample of α-lactose monohydrate (250 mg) was then introduced into the system and stirred for 10 min before titration. A rapid drop in voltage indicated by the Metrohm Karl Fischer apparatus (UK) was used to determine the titration end point. The analysis was done in six replicates.

2.2.4. Toluene distillation for water content analysis

α-Lactose monohydrate powder (50 g) was subjected to 5 h distillation in dry toluene using a Dean and Stark apparatus [19, 23]. The purity of water collected from the lactose after distillation was confirmed by its refractive index [26]. The analysis was conducted in four replicates.

2.2.5. Thermochemical method using a 0.3 mm and 0.2 mm thermocouple and a thermistor for the analysis of crystalline and amorphous lactose

A system as shown in Figure 1A was prepared. Sample of α-lactose monohydrate, α-lactose anhydrous stable,

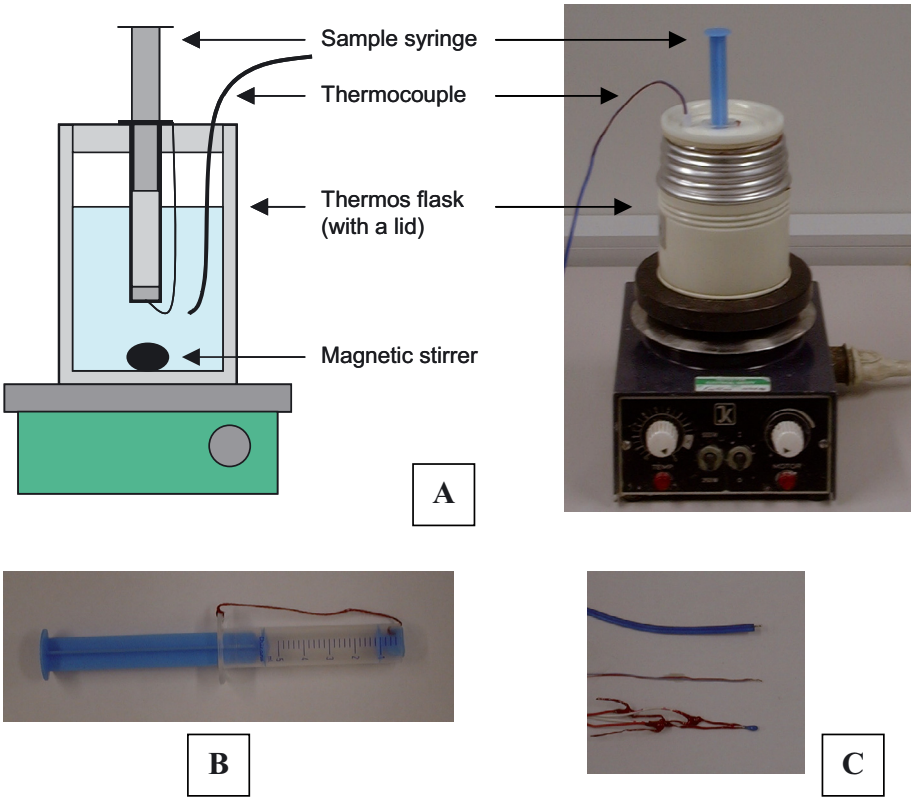

Figure 1. A: Experimental set up for measuring the temperature change of water when lactose dissolved at 25 °C; B: sample syringe; C: temperature sensors (from top to bottom: 0.3 mm thermocouple, 0.2 mm thermocouple and thermistor).

α-lactose anhydrous unstable, anhydrous β-lactose DCL21, binary mixtures of amorphous lactose and α-lactose monohydrate (to give 0 to 100 g·100 g^{-1} crystalline content by anhydrous weight) and spray-dried lactose DCL11, were prepared in syringes shown in Figure 1B. The total weight of sample in each syringe was 1.000 g. Thermocouples with 0.3 mm diameter (of each wire) and 0.2 mm diameter (of each wire) made of copper-constantan (sensitive to 0.01 °C and 0.001 °C respectively) and a thermistor (3000 Resistance, sensitive to 0.0001 °C) were used to measure the temperature change of distilled water (100 mL) when the lactose powder was dissolved at 25 °C. The analysis was conducted in four replicates. Data was recorded using 3497A Hewlett Packard (USA) data acquisition/control unit.

There are three types of heat of solution: the initial heat of solution (produced when the molecules of any form of sugar separate upon dissolution), the heat of passage (produced when a given quantity of one form of the sugar changes in solution to an equivalent quantity of the other form, e.g. mutarotation) and the final heat of solution (the total heat produced when any forms of the dissolved sugar have reached an equilibrium) [20]. In this study, the initial heat of solution was used because

at 20 to 25 °C, the slow mutarotation of lactose [37] prevented the heat of passage from affecting the initial heat of solution [20]. A preliminary study showed that 1 g of α-lactose monohydrate (the least soluble type of lactose) dissolved in 100 mL of water in less than 15 min (at 25 °C).

The initial temperature of water in the flask was recorded for 1 min before the lactose sample was introduced into the water. The temperature change of water when sample had been introduced was recorded for a maximum of 25 min (during this time the lactose mutarotation is negligible [27, 37]). The temperature difference was obtained by subtracting the initial temperature of the water from the temperature of the system at which the lactose had been dissolved and a stable system (no noticeable heat changes) was achieved.

2.2.6. *FT-IR for the identification of crystalline and amorphous lactose*

FT-IR spectra of α-lactose monohydrate, α-lactose anhydrous stable, α-lactose anhydrous unstable, anhydrous β-lactose DCL21 and binary mixtures of amorphous lactose and α-lactose monohydrate (total weight of each sample was ± 2 mg) in dry KBr disks (± 0.3 g) were recorded with Avatar 360 FT-IR E.S.P.™ (Nicolet Instrument Corporation, USA). An average of 32 scans was recorded at a resolution of 4 cm^{-1}. The spectra were analyzed using OMNIC 5.1 software (Nicolet Instrument Corporation, USA). Each sample was analyzed in duplicates.

2.2.7. *Analysis of β/α-anomer proportions in lactose by HPLC*

The proportion of β- and α-lactose anomer was analyzed using high pressure liquid chromatography (HPLC) [27]. The system was made up of a pump (model no. SP8810-020, Spectra-Physics, USA), a C18 column (Aqua 5 μ 125 A, Phenomenex, Australia) 250 mm × 4.6 mm internal diameter and a refractive index detector (Shodex, model: RI se-61, Showa Denko K.K., Japan). The HPLC was run using Delta Chromatography Data Systems software version 5.0 (Digital Solutions Pty. Ltd., Australia). The HPLC analysis was conducted in four replicates on: α-lactose monohydrate, α-lactose anhydrous stable, α-lactose anhydrous unstable, anhydrous β-lactose DCL21 and spray-dried lactose DCL11.

The lactose powder was dissolved rapidly (less than 1 min) into distilled water (lactose concentration was 0.1 to 1%) and injected into the C18 HPLC column (sample size: 20 μL) through a filter (pore size: 0.45 μm). The analysis was carried out at 25 °C with distilled water as the mobile phase at a flow rate of 0.7 (± 0.02) $mL \cdot min^{-1}$. The peak of β-lactose anomer appeared first (retention time ca. 4.10 min) followed by that of α-lactose anomer (ca. 4.37 min). The identity of the peaks was supported by measuring the changes in relative size as lactose mutarotated in aqueous solution during a 360 min experiment [27]. While mutarotation makes it difficult to obtain β-lactose completely free of α and vice versa, it is relatively slow (24 h is required to reach mutarotation equilibrium under our conditions [27, 37]) (see Sect. 2.2.5). The problem is minimized by injecting the sample into the instrument within 1 min and separating peaks within 5 min [27]. The areas under the peaks were used to calculate the proportions of the anomers in the lactose sample.

2.2.8. *Microscopic analysis of powder using a microscope with polarized light*

Samples of freeze-dried amorphous lactose were monitored for the absence of

crystalline material by examination under a microscope with polarized light (Model: Wetzlar, Leica Mikroskopie & Systeme GmbH, Germany) according to the method by Roetman and Van Schaik [38].

3. RESULTS AND DISCUSSION

3.1. TGA/SDTA for the determination of surface water, water of crystallization and amorphous lactose

The TGA/SDTA curves of various crystalline lactose and amorphous lactose are shown in Figures 2 to 6. The TGA/SDTA shows simultaneous data on the change in weight and thermal profile of the lactose samples in an analysis for sequential and separate description of the surface water, water of crystallization and amorphous lactose. The total water of the sample can be obtained by addition. Table I lists the weight loss and peaks on the TGA and SDTA thermograms of the various crystalline lactose and amorphous lactose.

The TGA curve of crystalline α-lactose monohydrate shows three distinctive weight losses (Fig. 2). The first weight loss (ca. 0.1 g·100 g^{-1}, marked between the first two flags on the TGA curve) occurred between 40 and 130 °C (SDTA thermogram, total treatment time was 18 min), was presumably the loss of surface water. The second weight loss (ca. 4.7 g·100 g^{-1}, marked between the second and third flags on the TGA curve) occurred between 130 and 170 °C and peaked at 153 °C (total treatment time was 8 min), was the loss of water of crystallization. This is in agreement with the literature [8] that α-lactose monohydrate released its water of crystallization when heated above 150 °C.

Before the third loss in weight (still within the second and third flags on the TGA thermogram, Fig. 2), there was a peak at 177 °C (SDTA thermogram), not accompanied by an appreciable loss in weight, which was presumably the peak of the anomerization of α-lactose to β-lactose (shown on DSC curves in the literature [7]). It is unlikely that all water would have been removed in the first endotherm. A small amount of water vapor was sufficient to cause anomerization of α- to β-lactos at temperatures above 150 °C [27, 45]. This is consistent with the top curve being not completely horizontal.

Heating of α-lactose monohydrate to 300 °C resulted in a continuous loss in weight (above 20 g·100 g^{-1}, the region beyond the third flag on the TGA curve, Fig. 2). There was an endothermic peak at 222 °C (SDTA thermogram) which was the melting of lactose crystal, followed by the decomposition of lactose. The sample was charred when taken out of the TGA/SDTA instrument. The SDTA thermogram in Figure 2 did not show the twin melting peaks of α- and β-lactose as mentioned in an earlier paper [14] for α-lactose monohydrate analyzed using DSC. The temperature of melting found in this experiment was different from the temperature of melting of α-lactose monohydrate reported in the literature (210 to 213 °C [14]; 202 °C [19]). It was similar to the temperature of melting of β-lactose at 224 °C reported by Gombas et al. [14] but different from that (252 °C) stated by Holsinger [19]. It was similar to the temperature of melting of α-anhydrous stable at 223 °C reported by Figura and Epple [9] and by Holsinger [19]. There are disagreements in the literature [9, 14, 19] on the temperatures of melting of various crystals of lactose which might be caused by the different methods of analysis used. Heating of the sample above 700 °C may allow the potentially new application of TGA/SDTA for the analysis of ash content (residue on ignition).

Figures 3 and 4 show that the SDTA curves of α-lactose anhydrous unstable and α-lactose anhydrous stable had a similar

Table I. Summary of the TGA/SDTA thermograms of crystalline and amorphous lactose.

Crystalline lactose	Surface water		Water of crystallization		Anomerization of lactose		Crystallization of amorphous lactose		Lactose melting and decomposition		Lactose anomer proportion (HPLC)	
	Temperature range	Weight loss ($g \cdot 100\ g^{-1}$)	Peak	Weight loss ($g \cdot 100\ g^{-1}$)	Peak	Weight loss ($g \cdot 100\ g^{-1}$)	Peak	Weight loss ($g \cdot 100\ g^{-1}$)	Peak	Weight loss ($g \cdot 100\ g^{-1}$)	α	β
α-Monohydrate	40 to 130 °C	0.12	153 °C	4.64	177 °C	–	–	–	222 °C	> 20	94.8	5.2
α-Anhydrous unstable	40 to 130 °C	3.03	153 °C	1.92	177 °C	–	–	–	211 °C	> 20	91.2	8.8
α-Anhydrous stable	40 to 130 °C	1.76	153 °C	0.39	177 °C	–	–	–	217 °C	> 20	89.4	10.6
β-Anhydrous (DCL21)	40 to 130 °C	0.36	–	–	–	–	–	–	234 °C	> 20	16.2	83.8
Amorphous lactose	40 to 130 °C	3.70	–	–	–	–	174 °C	–	222 °C	> 20	40.8	59.2

Note: "–" means data not available.

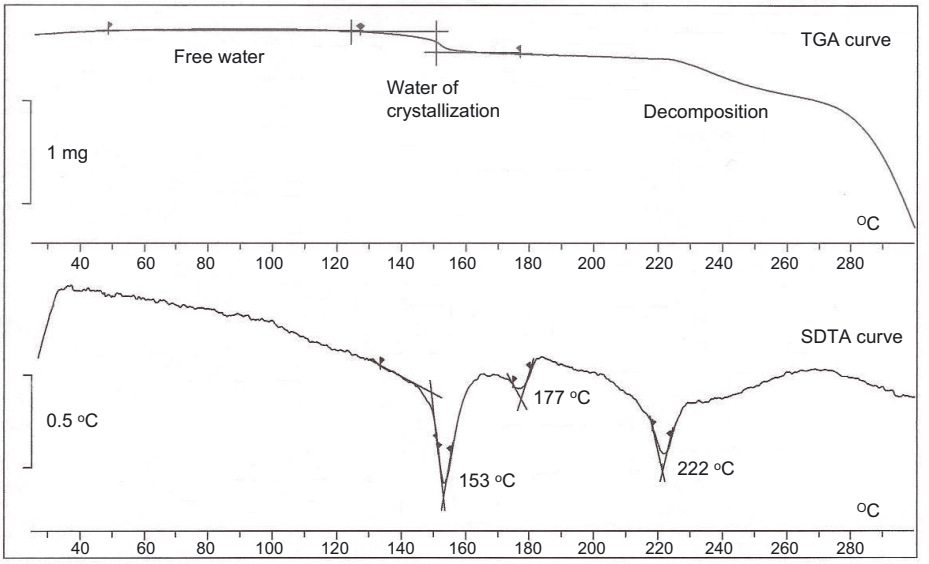

Figure 2. TGA and SDTA curves of α-lactose monohydrate measured from 25 to 300 °C with a heating rate of 5 °C·min^{-1}. The SDTA curve has a °C scale on the *x*-axis (instrument readout).

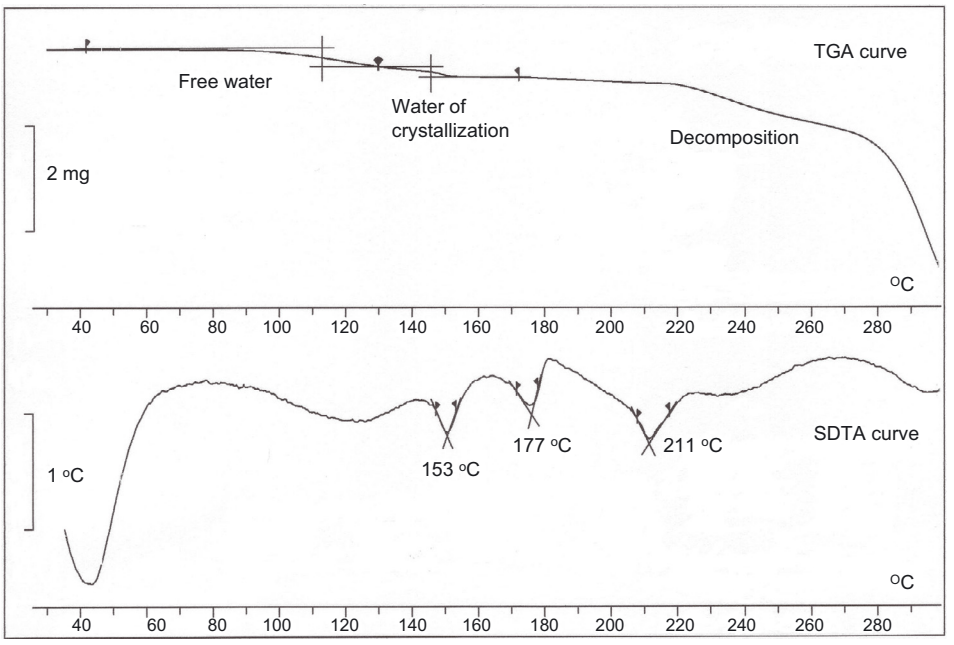

Figure 3. TGA and SDTA curves of α-lactose anhydrous unstable measured from 25 to 300 °C with a heating rate of 5 °C·min^{-1}. The SDTA curve has a °C scale on the *x*-axis (instrument readout).

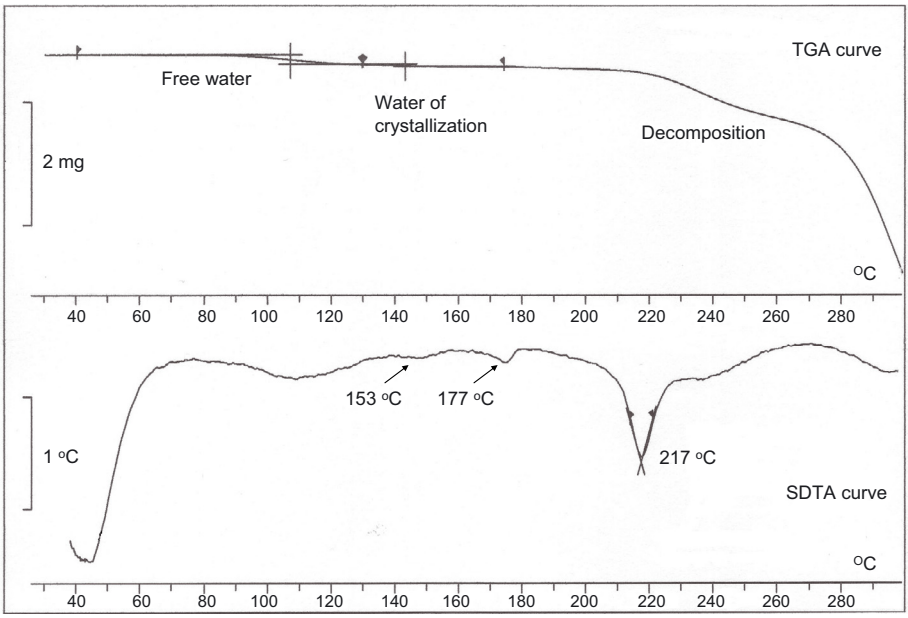

Figure 4. TGA and SDTA curves of α-lactose anhydrous stable measured from 25 to 300 °C with a heating rate of 5 °C·min^{-1}. The SDTA curve has a °C scale on the *x*-axis (instrument readout).

profile to that of α-lactose monohydrate. However, there was a larger reduction in weight between 40 and 130 °C indicating that the samples of anhydrous α-lactose contained initially more surface water than α-lactose monohydrate. It is reported in the literature that the anhydrous forms of α-lactose are more hygroscopic than α-lactose monohydrate with α-lactose anhydrous unstable being the most hygroscopic [8, 9, 19]. Figures 3 and 4 also show that the samples of anhydrous α-lactose contained some α-lactose monohydrate as indicated by the small weight loss at 153 °C corresponding to the presence of water of crystallization. Many researchers have reported difficulty in producing pure α-lactose anhydrous unstable and α-lactose anhydrous stable from the starting material α-lactose monohydrate [42].

The SDTA curves of α-lactose anhydrous unstable and α-lactose anhydrous stable show the peak of anomerization of lactose at 177 °C (Figs. 3 and 4 respectively). This peak was larger for α-lactose anhydrous unstable than for α-lactose anhydrous stable and the previous α-lactose monohydrate (Figs. 2–4). It seems that the size of this peak was affected by the surface water and total water content of the sample. The crystals in the samples of α-lactose anhydrous unstable and α-lactose anhydrous stable melted at 211 °C and 217 °C respectively.

Figure 5 shows the TGA/SDTA thermograms of anhydrous β-lactose Pharmatose DCL21. The SDTA curve of anhydrous β-lactose did not show the peaks for the loss of water of crystallization and the anomerization of lactose. There was a small reduction in weight between 40 and 130 °C for the loss of surface water followed by the melting of the crystals in the anhydrous β-lactose DCL21 sample at 234 °C (TGA and SDTA curves,

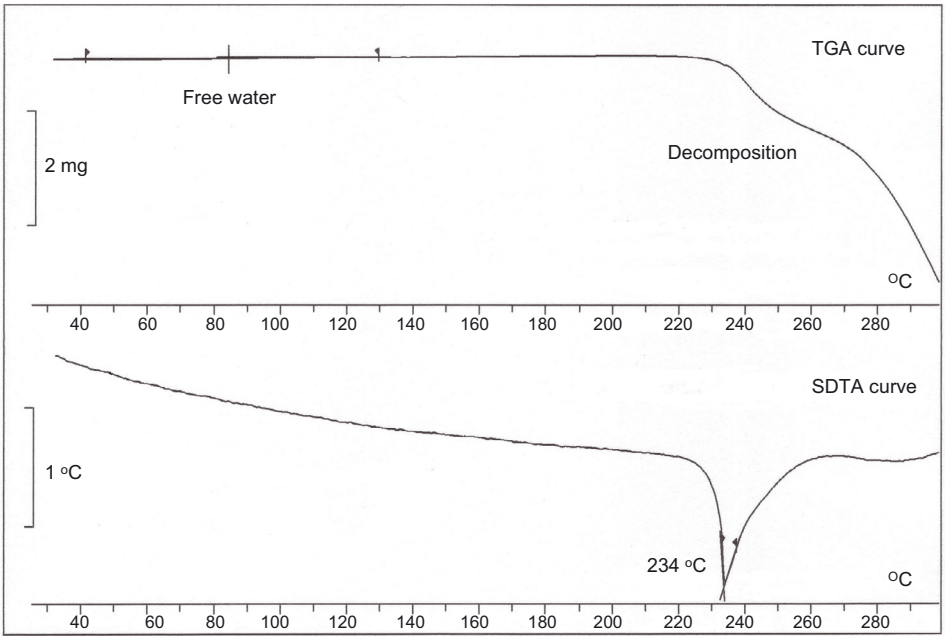

Figure 5. TGA and SDTA curves of anhydrous β-lactose Pharmatose DCL21™ measured from 25 to 300 °C with a heating rate of 5 °C·min^{-1}. The SDTA curve has a °C scale on the *x*-axis (instrument readout).

Fig. 5). The curves were as expected for a sample that was predominantly anhydrous β-lactose with a lesser amount of anhydrous α-lactose stable. The levels of the latter were too low for its characteristic peaks to appear in Figure 5.

These studies show that the TGA/SDTA allows the simultaneous monitoring of both change in mass and temperature profile of the same sample and is a more descriptive technique for the analysis of water content than the TGA method alone as described by some authors [3]. The TGA/SDTA may also be used as a reference to determine the best temperature required for water content analysis by conventional oven drying. The use of the TGA/SDTA for studying the transformation of crystals of lactose at high temperatures in non-hermetically sealed pans requires further study on the characterization of the crystals after heating (e.g. using X-ray diffraction [12,25]) and on the melting points.

Figure 6A shows the TGA/SDTA curves of amorphous lactose. The SDTA curve of amorphous lactose shows a distinctive endothermic peak and an exothermic peak. There was a weight loss between 40 and 130 °C corresponding to the loss of surface water (3.7 g·100 g^{-1}, TGA curve). An exothermic peak appeared at 174 °C (onset at 165 °C), which represented the conversion of amorphous lactose to crystals as reported in the literature [8, 14] on DSC thermograms. There was no change in weight observed in the TGA curve when crystals of lactose were formed from the amorphous lactose. The SDTA thermogram did not clearly show the curve indicating the glass transition as would appear on DSC analysis before

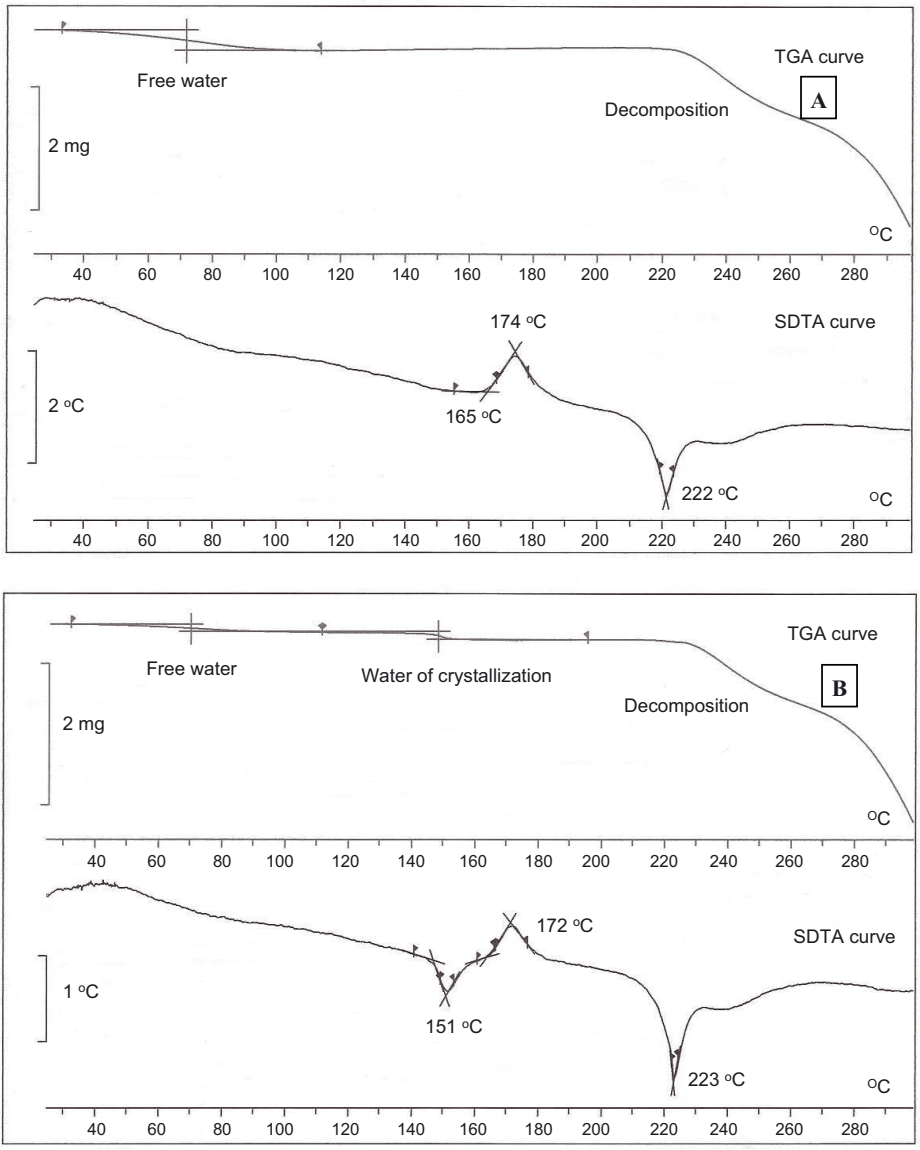

Figure 6. TGA and SDTA curves of a sample of freeze-dried lactose which contained 100 g·100 g^{-1} amorphous lactose (A) and a lactose sample which contained a physical mixture (1:1 ratio, anhydrous weight basis) of crystalline α-lactose monohydrate and freeze-dried amorphous lactose (B) The SDTA curve has a °C scale on the *x*-axis (instrument readout).

crystallization occurred. Heating of the samples in an open crucible at low relative humidity in the TGA/SDTA furnace and not hermetically sealed as normally done in a DSC analysis, constantly reduced the water content of the sample. This constant reduction in water might cause the glass transition to occur within a broad temperature range since the crystallization occurs more slowly than in hermetically sealed containers, where crystallization occurs at a constant moisture (T – Tg is constant) [40]. Thus, the glass transition temperature (Tg) was not clearly shown on the SDTA thermogram. The glass transition is a property of a non-equilibrium system and it is time dependent [4, 11]. The Tg of dry amorphous lactose is 101 °C [30].

Following crystallization, lactose melted at 222 °C (endothermic peak on SDTA curve) then decomposed (indicated by a large reduction in weight on the TGA curve). The melting profile of the amorphous lactose was similar to that shown in Figure 2 for the sample of α-lactose monohydrate. The amorphous lactose did not show the twin melting peaks of α- and β-lactose as mentioned earlier in the literature on DSC curve [14].

Figure 6B shows the SDTA thermogram of a sample which contained a mixture of amorphous lactose and α-lactose monohydrate. The thermogram consisted of a combination of peaks typical of amorphous lactose (Fig. 6B) and crystalline α-lactose monohydrate (Fig. 2), except for the peak of anomerization. There was a broad peak signifying the loss of surface water, followed by one for the loss of water of crystallization, then the exothermic peak of crystallization of amorphous lactose before the lactose finally melted and decomposed.

When varying known masses (up to 10 mg) of amorphous lactose were heated alone (as in Fig. 6A), the area under the exothermic peak (ca. 174 °C) was proportional to the mass of amorphous lactose ($y = 8.995x$, $r = 0.996$).

It was also found that the area under the peak of the exothermic crystallization was linearly related to the proportion of amorphous lactose for the samples that contained mixtures of crystalline α-lactose monohydrate and amorphous lactose ($y = 0.779x$, $r = 0.989$). This indicates that the area under the exothermic crystallization peak can be used for directly determining the content of amorphous lactose. The linear relationship suggests that any effect of the possible overlapping of the exothermic peak of amorphous lactose at 174 °C (Fig. 6A) with the endothermic peak of α-lactose monohydrate at 177 °C (Fig. 2) is negligible.

3.2. Thermochemical analysis for the detection of crystalline and amorphous lactose

The calorimetric data, contrasted with published values, are shown in Table II. The data for α-lactose monohydrate and amorphous lactose provide the clearest basis for comparisons with the published data. The published data are from pioneering studies and fail to distinguish between the stable and unstable variants (e.g. anhydrous α-lactose). The anhydrous β-lactose (DCL21) samples used here contained α-lactose (β:α = 83.8:16.2) while the published values are from old papers which contain little information about the purity nor the method of preparation of the β-lactose samples.

The experimental data (thermistor and 0.2 mm thermocouple) for α-lactose monohydrate and amorphous lactose agree with the published values in Table II. The negative heat of solution (also shown as a reduction of the temperature of water) indicates that the α-lactose monohydrate absorbed energy (endothermic reaction) when dissolved. This contrasted the positive heat of solution (exothermic reaction, shown as an increase in the temperature

Table II. Temperature change and the initial heat of solution of some forms of crystalline lactose and amorphous lactose.

Sample		Mass (g)		Temperature change (°C)	Initial heat of solution ($J \cdot g^{-1}$)
		H_2O	Lactose		
Thermocouple (0.3 mm)					
α-Lactose monohydrate		100	1.0011	–0.05	–20.5
Amorphous lactose		100	1.0000	0.09	37.7
Thermocouple (0.2 mm)					
α-Lactose monohydrate		100	1.0008	–0.120	–50.2
Anhydrous β-lactose DCL21		100	1.0025	–0.008	–3.3
α-Lactose anhydrous (stable)		100	1.0013	–0.032	–13.4
α-Lactose anhydrous (unstable)		100	1.0011	–0.031	–13.0
Amorphous lactose		100	1.0010	0.126	52.7
Thermistor					
α-Lactose monohydrate		100	1.0005	–0.1182	–49.4
Anhydrous β-lactose DCL21		100	1.0000	–0.0154	–6.3
α-Lactose anhydrous (stable)		100	1.0005	–0.0409	–17.2
α-Lactose anhydrous (unstable)[a]	First temperature change	100	1.0007	0.0244	10.0
	Stable system			–0.0078	–3.3
Amorphous lactose		100	1.0011	0.1369	56.9
Values in the literature[b]					
α-Lactose monohydrate	Hudson and Brown [20] at 20 °C	995	26.58	–0.304	–50.2
	Brown and Pickering [2] at 16 °C	–	–	–	–48.1
	Magie [31] at 21.6 °C	–	–	–	–48.1
	Hogan and Buckton [17] at 25 °C	100	0.200	–	–56.2
	Harjunen et al. [16] at 25 °C	100	0.400	–	–54.2
β-Lactose	Hudson and Brown [20] at 20 °C	700	15.98	–0.048	–9.6
	Brown and Pickering [2] at 16 °C	–	–	–	–22.6
	Magie and Hudson [32]	–	–	–	–15.1
α-Lactose anhydrous	Jorissen and Van de Stadt [24]	–	–	–	+30.5
	Magie [31] at 19.2 °C	–	–	–	–31.0
Amorphous lactose	Hogan and Buckton [17] at 25 °C	100	0.200	–	56.5
	Harjunen et al. [16] at 25 °C	100	0.400	–	53.3

Note: Heat of solution was calculated using the formula: $Q_{lactose} = Q_{water}$ and $Lm = c_p m\Delta T$; Q is heat generated or absorbed, L is heat of solution of lactose, m is mass of sample, c_p is heat capacity of water ($4.177\ J \cdot g^{-1} \cdot °C^{-1}$ at 25 °C [26]), ΔT is temperature difference of the water after dissolution [10]. Unless otherwise indicated, data of this study (not literature values) were the means of four replicates. The experiments were conducted at 25 °C.

[a] Temperature increased when sample was introduced (the calculated latent heat was $10.2\ J \cdot g^{-1}$), then reduced. The temperature change used for the calculation of heat of solution was derived after the system was stable.

[b] Data were published as calories/gram (1 calorie/gram = $4.184\ J \cdot g^{-1}$ [26]) and were calculated to $J \cdot g^{-1}$ in this table.

"–" means data not available.

of water) of the non-crystalline amorphous lactose. The measurements with the 0.3 mm thermocouple were not sufficiently sensitive. The thermistor is likely to provide a better basis for future development.

α-Lactose anhydrous unstable had a unique thermochemical pattern when dissolved in water. The thermistor data (Tab. II) show that α-lactose anhydrous unstable initially released a considerable amount of heat to the water then absorbed heat. This pattern could not be observed using the 0.2 mm thermocouple. Perhaps this explains the theory [19] that in the presence of water, α-lactose anhydrous unstable apparently forms the hydrate without first dissolving. Therefore, the first temperature change of the water might be the heat of transition of α-lactose anhydrous unstable to α-lactose monohydrate. The values for the initial heat of solution of α-lactose anhydrous unstable (after reaching stable condition) were negative (–3.3 $J \cdot g^{-1}$, Tab. II).

For known mixtures of amorphous lactose and α-lactose monohydrate, the temperature difference, y (°C), on dissolution was linearly related to the proportion, x, of amorphous lactose in the mixture ($y = 0.0026x - 0.121$, $r = 0.996$, $n = 4$). This simple laboratory-constructed calorimeter has potential for quickly and inexpensively estimating the proportion of amorphous lactose in lactose samples.

3.3. FT-IR technique for the identification of crystalline and amorphous lactose

Figure 7 shows that all spectra of the crystalline and amorphous lactose contained the bands at 3600–3200 cm^{-1} (stretching vibration of the hydroxyl group [33]), the weak band at 1650 cm^{-1} (bending vibration of the hydroxyl groups of crystal water [33]) and the band at 1200–1070 cm^{-1} (asymmetric stretching vibration of C-O-C in the glucose and galactose [33]). The distinguishing peaks in the spectra of the various forms of crystalline lactose are indicated with arrows in Figure 7. The band specific to α-anomer (920 cm^{-1}) found in this study was in agreement with that described by Nakanishi [33].

The FT-IR spectrum of α-lactose monohydrate (Fig. 7A) was in agreement with the IR spectra in the literature [1, 25, 35]. The FT-IR spectrum of α-lactose anhydrous unstable (Fig. 7B) was similar to the IR spectrum illustrated by Itoh et al. [22] but different from that of Kirk et al. [25]. The number of peaks on the FT-IR spectrum of α-lactose anhydrous unstable found in this study was the same as those on the spectrum of α-lactose monohydrate. The poor reproducibility of FT-IR for characterizing α-lactose anhydrous unstable was strongly affected by the hygroscopic nature of this anhydrous form of lactose and has been reported in the literature [25]. It is possible that the samples in this study and in the work of Itoh et al. [22] had partly re-hydrated to α-lactose monohydrate during the time of analysis, therefore giving the spectrum of α-lactose monohydrate.

The FT-IR spectrum of α-lactose anhydrous stable (Fig. 7C) was similar to the IR spectrum reported by Kirk et al. [25]. The FT-IR spectrum of Pharmatose DCL21 β-lactose (Fig. 7D) was in agreement with the IR spectra of β-lactose in the literature [1, 25, 35].

The FT-IR spectrum of amorphous lactose (Fig. 7E) found in this study was similar to that illustrated by Norris and Greenstreet [35]. In general, the spectrum of amorphous lactose could be distinguished from those of crystalline lactose by the number of peaks and the less defined peaks of the amorphous lactose spectrum (Fig. 7C). Crystals have an ordered three-dimensional network of molecules, which is lacking in the amorphous particles.

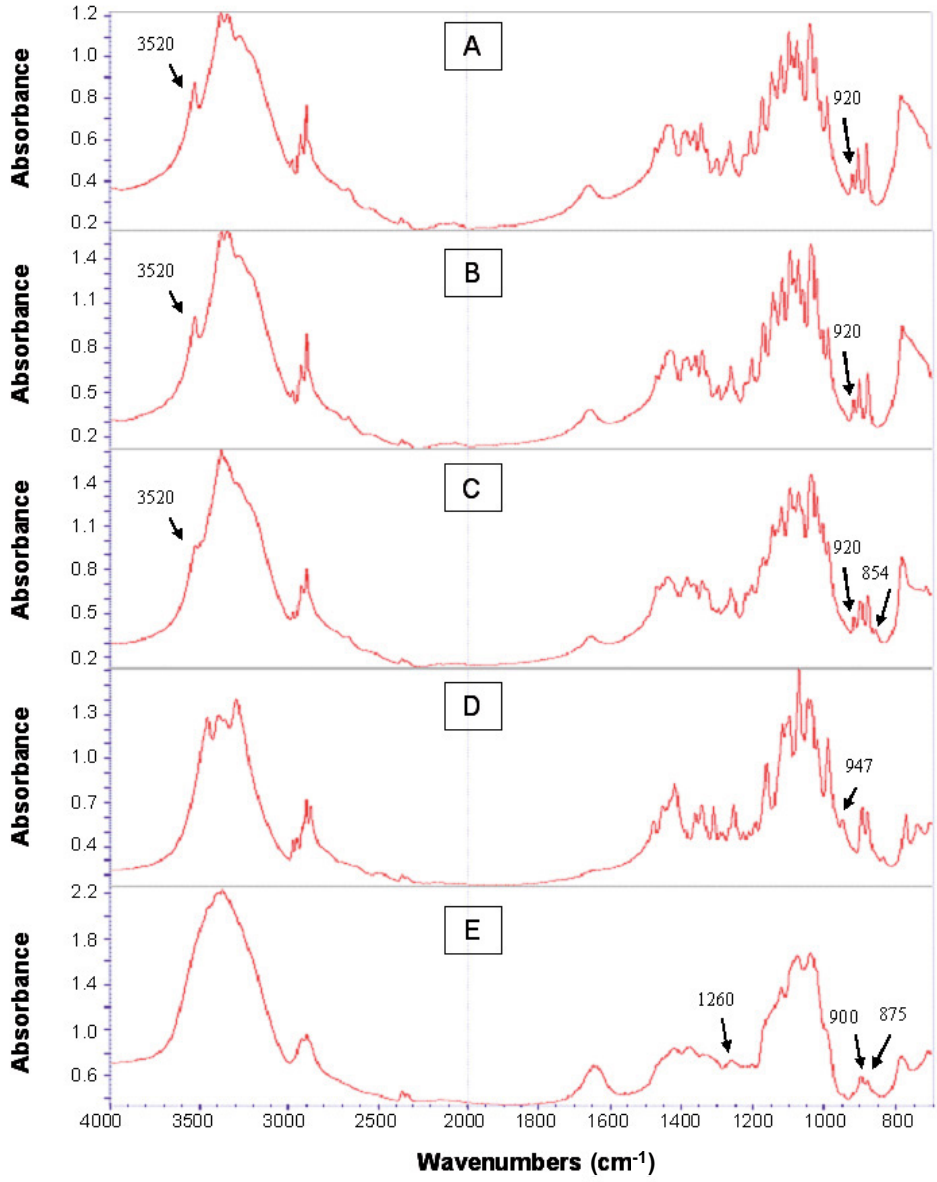

Figure 7. FT-IR spectra of (A) α-lactose monohydrate, (B) α-lactose anhydrous unstable, (C) α-lactose anhydrous stable, (D) anhydrous lactose DCL21 and (E) amorphous lactose.

The sharp peaks at 1260 cm^{-1}, 900 cm^{-1} and 875 cm^{-1} seem to allow the differentiation of crystalline from amorphous lactose (Fig. 7) and have not been reported in published papers.

The disadvantage of using FT-IR technique for the analysis of the degree of crystallization of lactose is the process of sample pre-treatment, which includes grinding of the sample with potassium bromide. Grinding of the sample prior to analysis might change the property of the crystals, such as transforming α- to β-anomer and reducing the degree of crystallization [42]. It was important to ensure that dry KBr was used to form the sample disc and the analysis was conducted relatively quickly to avoid moisture absorption by the hygroscopic types of lactose and premature crystallization of any amorphous lactose prior to analysis.

3.4. Water content of lactose analyzed by TGA/SDTA, Karl Fischer, Toluene distillation and the conventional oven methods

TGA/SDTA gave independent data on the surface water and water of crystallization of lactose in a single analysis. The total water content of the α-lactose monohydrate determined by TGA/SDTA (mean ± standard deviation: 4.76 (± 0.06) g·100 g^{-1} by addition from surface water 0.12 (± 0.02) g·100 g^{-1} and water of crystallization 4.64 (± 0.04) g·100 g^{-1}) was similar to that analyzed using the toluene distillation method (5.0 (± 0.0) g·100 g^{-1}). The TGA/SDTA gave lower total water content for the α-lactose monohydrate than Karl Fischer method (5.42 (± 0.34) g·100 g^{-1}) but much higher than the loss on drying in the oven at 120 °C for 16 h (3.74 (± 0.01) g·100 g^{-1}). The theoretical water content of α-lactose monohydrate is 5 g·100 g^{-1}.

The surface water content of the α-lactose monohydrate sample determined by the TGA/SDTA was lower than that determined by oven drying at 80 °C for 2 h (0.28 (± 0.04) g·100 g^{-1}).

It is difficult to justify the reliability of the individual values of water content determined by the different methods. For this purpose, a comparison was done on the water contents for estimating α-lactose monohydrate in the sample (Tab. III, calculated using the formula by Schuck and Dolivet [43]).

TGA/SDTA gave a reasonable estimate of the content of α-lactose monohydrate in the lactose powder sample (92.6 g·100 g^{-1}, Tab. III). The combination of Karl Fischer and loss on drying at 80 °C for 2 h, the reference methods in the United States Pharmacopeia [44], slightly overestimated the content of α-lactose monohydrate (103.2 g·100 g^{-1}, Tab. III). In contrast, the reference method in the Codex Alimentarius [6], the loss on drying in the oven at 120 °C (16 h) and 80 °C (2 h) underestimated the content of α-lactose monohydrate (68.3 g·100 g^{-1}, Tab. III). A combination of toluene distillation for total water and loss on drying in an oven (80 °C for 2 h) for surface water resulted in a reasonable estimate of α-lactose monohydrate (94.4 g·100 g^{-1}).

Calculation of the water of crystallization by difference between the total water and the surface water may serve as a potential source of errors when estimating the content of α-lactose monohydrate and thus, it may result in a less reliable presentation of the degree of crystallization as currently accepted in the literature and the industries [43]. Of the four methods examined, TGA/SDTA is the only one which allows a direct and specific measurement of the water of crystallization, thus avoiding the errors associated with calculation by difference and errors from the analysis of separate samples using different methods. Comparison of the methods of analysis for water content examined in this study is listed in Table IV.

Table III. The content of α-lactose monohydrate in a refined edible grade α-lactose monohydrate powder calculated from its water of crystallization.

Methods		National objectives	Water content ($g \cdot 100\ g^{-1}$)	α-Monohydrate ($g \cdot 100\ g^{-1}$ anhydrous basis)
TGA/SDTA	Temperature range 40 to 130 °C	Surface water	0.12	
	Peak temperature at 153 °C	Water of crystallization	4.64	92.6
Standard methods in USP [44]	Karl Fischer	Total water	5.42	
	80 °C/2 h	Surface water	0.28	
	By difference	Water of crystallization	5.14	103.2
Oven methods	120 °C/16 h	Total water	3.74	
	80 °C/2 h	Surface water	0.28	
	By difference	Water of crystallization	3.46	68.3
Toluene distillation & Oven method 80 °C/2 h	Toluene distillation	Total water	5.0	
	Oven method 80 °C/2 h	Surface water	0.28	
	By difference	Water of crystallization	4.72	94.4

Note: The content of α-lactose monohydrate (anhydrous basis) was calculated using the formula by Schuck and Dolivet [43] for the degree of crystallization of lactose. The theoretical maximum content of α-lactose monohydrate is 95 $g \cdot 100\ g^{-1}$ (anhydrous basis).

Table IV. Comparison of the methods for the analysis of water content of lactose.

Criteria		TGA/SDTA	Toluene distillation	Karl Fischer	Oven method (80 °C)	Oven method (120 °C)
Minimum detection limit for total water (as % of sample weight)		0.001%	> 0.2%	0.2%	0.01%	0.01%
Able to determine:	Total water	Yes (by addition)	Yes	Yes	No	Yes
	Surface water	Yes	No	No	Yes	No
	Water of crystallization	Yes	No	No	No	No
Time for analysis (h)		< 1	5	< 0.5	2	16
Provide other information		Yes	No	No	No	No
Sample weight (g)		0.0100	50–100	0.250	1.000–2.000	1.000–2.000
Reproducibility		Good	Good	Poor	Good	Good

The Karl Fischer method had been reported to show experimental difficulties and poor reproducibility between laboratories and operators for the determination of the water content of lactose [36]. It involved a tedious sample dissolution and/or water extraction from the sample in the system's solvent (e.g. methanol or mixtures of methanol and formamide). The toluene distillation is more reproducible than the Karl Fischer method but the toluene distillation involves a lengthy procedure (> 5 h) and requires large amounts of sample (minimum 50 g [23]).

There are many published criticisms (see [29] for a compilation) of the analysis of water content by the oven methods and they are supported by the results of this study. Another method for the loss on drying in an oven for the estimation of total water available in the literature is 105 °C for 5 to 7 h (40 Pa vacuum over dried zeolite powder [43]). Other methods for the estimation of surface water are 87 °C for 6 h [43] and 102 °C for 3 h [21]. Although the conventional oven methods for the loss on drying are simple and inexpensive, the methods determine the "loss of weight" which does not necessarily express the true water content [44]. The method may determine surface water, some part of the water of crystallization of lactose and possibly, other volatile matters [6, 44]. Other possible errors might also be inadvertent moisture loses or gains during weighing due to exposure to open atmosphere.

The TGA/SDTA method is sensitive (able to measure as low as 0.001 g·100 g^{-1} weight loss) and this is an important requirement for detecting a small amount of moisture. It is simple, relatively rapid and has good reproducibility in comparison to Karl Fischer, toluene distillation and the 16 h oven method at 120 °C. The smaller sample size of TGA/SDTA may result in a more uniform temperature distribution within the sample than that of the oven methods but it may also be a potential source of sampling errors of non-homogeneous samples. The TGA/SDTA is robust and may be applicable to other samples of dairy powders such as milk and whey.

3.5. The amorphous content of lactose analyzed by TGA/SDTA and the crystalline and amorphous lactose proportion of commercial spray-dried lactose

The amorphous content of commercial spray-dried lactose (Pharmatose DCL11™) determined by TGA/SDTA was 4.9 (± 0.4) g·100 g^{-1} (mean ± standard deviation). These values were consistent with those obtained by Nuclear magnetic resonance (NMR) evaluation (4.9 g·100 g^{-1}, data taken from [28]). A comparison of the TGA/SDTA method and some methods available in the literature for the analysis of amorphous lactose is listed in Table V. The ability of the TGA/SDTA to generate data for surface water, water of crystallization and amorphous lactose on a single run is noteworthy.

To illustrate the potential of the methods explored here, the possible proportions of some crystalline forms of lactose and amorphous lactose in commercial spray-dried lactose were calculated and presented in Table VI. The detailed composition can be calculated if sufficient information on the water content (i.e. surface water and water of crystallization), amorphous content and isomeric proportions is available. For dairy powders containing lactose (e.g. milk and dairy powders), an estimation of the total lactose content will also be required to calculate the composition of the lactose as in Table VI. A clear and detailed presentation of lactose composition is important when designing recommendations for better product handling, application, storage and processing. Follow-up studies are required to confirm and extend

Table V. Comparison of the methods for the quantitative analysis of amorphous lactose.

Criteria	TGA/ SDTA	Literature claims								
		^{13}C NMR [15]	XRPD [12, 14]	TAM [8]	DSC [12, 14]	Hyper-DSC [13, 41]	IGC [34]	NIRS [12, 18]	DVS [18]	Solution calorimetry [17]
Approximate minimum detection limit for amorphous	1%	< 0.5%	5–10% [14] 0.5% [12]	< 0.5%	Qualitative	< 1.5% [13] < 1% [41]	< 1%	< 1% [18] 0.5% [12]	0.7%	1%
Time for analysis (h)	< 1	0.5–10	< 1	0.5–4	< 1	< 0.5	5–13	< 0.5	5	< 1
Destroys sample	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Sample weight (mg)	5–15	500–700	400 [12]	20–300	4.0–4.5 [14] 3.5 [12]	1–3 [41]	500–1000	50 [18] 2000 [12]	150	200
Reproducibility	Good	Good	Good	Good	Poor [14]	Good	Good	Good	Good	Good
Calibration model required	Yes	Yes	Yes	Yes	Yes Linear [14] Non-linear [12]	Yes Linear	Yes	Yes	Yes	Yes
Provide structural information	No	Yes	Yes	No	No	No	No	Possible	No	No
Other information										
Surface H_2O	Yes	No	No	No	No	No	No	No	No	No
H_2O crystallization	Yes	No	No	No	Yes	Yes	No	No	No	No
Total H_2O	Yes	No	No	No	No	No	No	No	No	No
α-Monohydrate	Yes	Possible	Yes	No	Possible	Possible	Possible	No	No	No

Note: TGA/SDTA (thermal gravimetric analysis/single differential thermal analysis), NMR (nuclear magnetic resonance), XRPD (X-ray powder diffraction), TAM (thermal activity monitor – isothermal calorimetry), DSC (differential scanning calorimetry), IGC (inverse phase gas chromatography), NIRS (near infrared spectroscopy), DVS (dynamic vapor sorption).

Table VI. The proportion of crystalline and amorphous lactose (anhydrous basis) in a commercial sample of spray-dried lactose (Pharmatose DCL11™).

Data by analysis[a] (total sample = 105.0 g "as is basis")			Methods of analysis
Surface H_2O	0.72 (g·100 g^{-1})	0.8 g	TGA/SDTA (%H_2O × total sample)
H_2O (crystallization)	4.08 (g·100 g^{-1})	4.3 g	TGA/SDTA (%H_2O × total sample)
Total H_2O	4.80 (g·100 g^{-1})	5.0 g	By addition (Surface & H_2O crystallization)
Amorphous (anhydrous basis)	4.90 (g·100 g^{-1})	4.9 g	TGA/SDTA (%amorphous × total lactose content)
α/β-Lactose proportion	86.5:13.5	–	HPLC
Total lactose content ("anhydrous" basis)	95.2 (g·100 g^{-1})	100.0 g	Total solid by subtraction (total sample – total water)

α-Monohydrate (anhydrous basis) calculated using the formula by Schuck and Dolivet [43], eq. ((H_2O crystal × 19)/total lactose) × 100	81.7 g	
Other lactose anhydrous (where 0.8 g surface H_2O is attached, by subtraction of α-monohydrate content from total lactose content)	18.3 g	Proportion of lactose anhydrous

Assumed α:β in amorphous was 1:1.25 [37][c]	Crystalline anhydrous (Other lactose – amorphous)	Amorphous[b]
β-amorphous = amorph content × (1.25/(1+1.25))		
α-amorphous = amorph content × (1/(1+1.25))	13.4 g	4.9 g
β-anhydrous crystal = total β-anomer from HPLC – β-amorph	α 2.6	α 2.2
α-anhydrous crystal = total α-anomer from HPLC – (α-amorph + α-mono)	β 10.8	β 2.7

Summary

Therefore, the proportion of some crystalline and amorphous lactose in the 100 g total lactose (g·100 g^{-1}, anhydrous basis) is:	
α-monohydrate	81.7
α-anhydrous[d]	2.6
β-anhydrous	10.8
amorphous α-lactose	2.2
amorphous β-lactose	2.7
Total	100.0

Note: [a] Surface water and water of crystallization of lactose (analyzed using TGA/SDTA, n = 4, mean ± standard deviation) = 0.73 (± 0.02) g·100 g^{-1} and 4.09 (± 0.01) g·100 g^{-1} respectively. Proportion of β-anomer and α-anomer (analyzed using HPLC, n = 4, mean ± standard deviation) = 13.5 (± 0.1) : 86.5 (± 0.1).

[b] Proportion of amorphous lactose (analyzed using TGA/SDTA, n = 4, mean ± standard deviation) = 4.9 (± 0.4) g·100 g^{-1}.

[c] It was assumed that the isomeric proportion of amorphous lactose was the same as that in equilibrated solution at 25 °C (α:β was 1:1.25 [37]).

[d] The composition of the stable and unstable form of α-lactose anhydrous may be further determined by methods of analysis such as X-ray powder diffraction.

these findings, to examine the sensitivity of the TGA/SDTA method and to establish its application to lactose-containing products such as whey and milk powders.

4. CONCLUSIONS

When thermal gravimetric analysis integrated with single differential thermal analysis (TGA/SDTA) was applied to α-lactose monohydrate, anhydrous β-lactose DCL21, α-lactose anhydrous stable, α-lactose anhydrous unstable and amorphous lactose, changes in mass and SDTA temperature peaks characteristics of surface water, water of crystallization and amorphous lactose were identified and used to develop a new approach to the sequential, separate and direct determination of the surface water, water of crystallization and amorphous lactose. The TGA/SDTA showed the loss of surface water of lactose at 40 to 130 °C and the loss of water of crystallization at 130 to 170 °C (peak at 153 °C). The content of α-lactose monohydrate can be reliably determined by calculation from the proportion of the water of crystallization of the lactose samples. The amorphous lactose was detected by the peak of crystallization at 174 °C. The area under the crystallization peak was linearly correlated with the content of amorphous lactose. The thermochemical method shows that the proportion of amorphous lactose in a sample mixture with α-lactose monohydrate, was linearly correlated with the temperature change of the water and the heat of solution of the dissolved lactose powder. The heat of solution of α-lactose monohydrate was –49.4 $J \cdot g^{-1}$ and that of amorphous lactose was +56.9 $J \cdot g^{-1}$ (at 25 °C, measured using a thermistor). The FT-IR differentiated crystalline from amorphous lactose by the peaks at 1260 cm^{-1}, 900 cm^{-1} and 875 cm^{-1}, specific to crystalline lactose. The peaks of crystalline lactose were generally more defined and sharper than those of amorphous lactose. The proportion of some crystalline forms of lactose and amorphous lactose in commercial products can be calculated if sufficient information on the surface water, water of crystallization, amorphous content and isomeric proportions is available.

Acknowledgements: Dr Y. Listiohadi acknowledges the support of an Australian Postgraduate Award from the University of Western Sydney. This work was supported by a grant from Dairy Australia (formerly known as the Dairy Research and Development Corporation). The authors would also like to thank Fernz Specialty Chemicals (NSW, Australia) for providing the spray-dried lactose Pharmatose DCL11™ and anhydrous lactose Pharmatose DCL21™. An acknowledgment is extended to the late Dr B. Johnson (Principal Research Scientist of Food Science Australia), who provided generous guidance but passed away before the study was completed.

REFERENCES

[1] British Pharmacopoeia (BP), Medicinal and pharmaceutical substances, The Stationery Office, Great Britain, 1, 2001, pp. 23–20 to 23–23.

[2] Brown H.T., Pickering S.U., Thermochemistry of chemistry hydrolysis, J. Chem. Soc. 71 (1897) 783–795.

[3] Buckton G., Yonemochi E., Hammond J., Moffat A., The use of near infra-red spectroscopy to detect changes in the form of amorphous and crystalline lactose, Int. J. Pharm. 168 (1998) 231–241.

[4] Elmonsef Omar A.M., Roos Y.H., Glass transition and crystallization behaviour of freeze-dried lactose-salt mixtures, Lebensm. Wiss. Technol. 40 (2007) 536–543.

[5] FAO/WHO, Methods of analysis for sugars, in: Codex Alimentarius, CAC/RM 1/8-1969, FAO/WHO, Rome, 1969, p. 9.

[6] FAO/WHO, Codex standard for sugars, in: Codex Alimentarius, Codex STAN 212-1999 (Amd. 1-2001), FAO/WHO, Rome, 2001, pp. 1–5.

[7] Fernandez-Martin F., Morais F., Olano A., Thermal behaviour of lactose, in: Linko P., Malkki Y., Olkku J., Larinkari J. (Eds.), Food Process Engineering, volume 1: Food Processing Systems, Applied Science Publishers, Barking, Essex, UK, 1980, pp. 523–529.

[8] Figura L.O., The physical modification of lactose and its thermoanalytical identification, Thermochim. Acta 222 (1993) 187–194.

[9] Figura L.O., Epple M., Anhydrous α-lactose: a study with DSC and TXRD, J. Therm. Anal. 44 (1995) 45–53.

[10] Findlay A., Thermochemistry, in: Findlay A. (Ed.), Physical Chemistry, Longman, London, UK, 1954, pp. 186–198.

[11] Fitzpatrick J.J., Hodnett M., Twomey M., Cerqueira P.S.M., O'Flynn J., Roos Y.H., Glass transition and the flowability and caking of powders containing amorphous lactose, Powder Tech. 178 (2007) 119–128.

[12] Fix I., Steffens K.J., Quantifying low amorphous or crystalline amounts of alpha-lactose-monohydrate using X-ray powder diffraction, near-infrared spectroscopy, and differential scanning calorimetry, Drug Dev. Ind. Pharm. 30 (2004) 513–523.

[13] Gabbott P., Clarke P., Mann T., Royall P., Shergill S., A high-sensitivity, high-speed DSC technique: Measurement of amorphous lactose, Am. Lab. 35 (2003) 17–18, 20, 22.

[14] Gombas A., Szabo-Revesz P., Kata M., Regdon Jr G., Eros I., Quantitative determination of crystallinity of α-lactose monohydrate by DSC, J. Therm. Anal. Calorim. 68 (2002) 503–510.

[15] Gustafsson C., Lennholm H., Iversen T., Nystrom C., Comparison of solid-state NMR and isothermal microcalorimetry in the assessment of the amorphous component of lactose, Int. J. Pharm. 174 (1998) 243–252.

[16] Harjunen P., Lehton V.P., Koivisto M., Levonen E., Paronen P., Jarvinen K., Determination of amorphous content of lactose samples by solution calorimetry, Drug Dev. Ind. Pharm. 30 (2004) 809–815.

[17] Hogan S.E., Buckton G., The quantification of small degrees of disorder in lactose using solution calorimetry, Int. J. Pharm. 207 (2000) 57–64.

[18] Hogan S.E., Buckton G., The application of near infrared spectroscopy and dynamic vapor sorption to quantify low amorphous contents of crystalline lactose, Pharm. Res. 18 (2001) 112–116.

[19] Holsinger V.H., Lactose, in: Wong N.P., Jenness R., Keeney M., Marth E.H. (Eds.), Fundamentals of Dairy Chemistry, Van Nostrand Reinhold, New York, USA, 1988, pp. 279–342.

[20] Hudson C.S., Brown F.C., The heats of solution of the three forms of milk-sugar, J. Am. Chem. Soc. 30 (1908) 960–971.

[21] International Dairy Federation (IDF), Dried milk and dried cream: determination of water content: Standard 26A, International Dairy Federation, Belgium, 1993.

[22] Itoh T., Satoh M., Adachi S., Differential thermal analysis of α-lactose hydrate, J. Dairy Sci. 60 (1977) 1230–1235.

[23] Jones J.M., McLachlan T., The determination of moisture by the volatile solvent method, Analyst 52 (1927) 383–387.

[24] Jorissen W.J., Van de Stadt E., Ueber die Bindungswärme des Krystallwassers von organischen Verbindungen, J. Prakt. Chem. 51 (1894) 102–106.

[25] Kirk J.H., Dann S.E., Blatchford C.G., Lactose: A definitive guide to polymorph determination, Int. J. Pharm. 334 (2007) 103–114.

[26] Lide D.R., CRC Handbook of Chemistry and Physics, CRC Press, USA, 2004.

[27] Listiohadi Y.D., Modification of sugars by extrusion, MSc Honours Thesis, University of Western Sydney Hawkesbury, Richmond, NSW, Australia, 2000.

[28] Listiohadi Y.D., The caking of lactose, Ph.D. Thesis, University of Western Sydney, Richmond, NSW, Australia, 2004.

[29] Listiohadi Y.D., Hourigan J.A., Sleigh R.W., Steele R.J., Properties of lactose and its caking behaviour, Aust. J. Dairy Technol. 60 (2005) 33–52.

[30] Lloyd R.J., Chen X.D., Hargreaves J.B., Glass transition and caking of spray-dried lactose, Int. J. Food Sci. Technol. 31 (1996) 305–311.

[31] Magie W.F., The specific heats of certain organic solids, Phys. Rev. 16 (1903) 381–382.

[32] Magie and Hudson, Princeton Univ. Bull. April (1902). As cited by Hudson and Brown (1908), see above [20].

[33] Nakanishi K., Infrared Absorption Spectroscopy, Nankoudou Press, Tokyo, 1960.

[34] Newell H.E., Buckton G., Butler D.A., Thielmann F., Williams D.R., The use of inverse phase gas chromatography to measure

the surface energy of crystalline, amorphous, and recently milled lactose, Pharm. Res. 18 (2001) 662–666.

[35] Norris K.P., Greenstreet J.E.S., Infra-red absorption spectra of casein and lactose, Nature 181 (1958) 265–266.

[36] Roetman K., Methods for the quantitative determination of crystalline lactose in milk products, Neth. Milk Dairy J. 35 (1981) 1–52.

[37] Roetman K., Buma T.J., Temperature dependence of the equilibrium β/α ratio of lactose in aqueous solution, Neth. Milk Dairy J. 28 (1974) 155–165.

[38] Roetman K., Van Schaik M., The β/α ratio of lactose in the amorphous state, Neth. Milk Dairy J. 29 (1975) 225–237.

[39] Roos Y., Karel M., Crystallization of amorphous lactose, J. Food Sci. 57 (1992) 775–777.

[40] Roos Y., Karel M., Effects of glass transitions on dynamic phenomena in sugar containing food systems, in: Blanshard J.M.V., Lillford P.J. (Eds.), The Glassy State in Foods, Nottingham University Press, Leicestershire, UK, 1993, pp. 207–222.

[41] Saunders M., Podluii K., Shergill S., Buckton G., Royall P., The potential of high speed DSC (Hyper-DSC) for the detection and quantification of small amounts of amorphous content in predominantly crystalline samples, Int. J. Pharm. 274 (2004) 35–40.

[42] Savolainen M., Jouppila K., Pajamo O., Christiansen L., Strachan C., Karjalainen M., Rantanen J., Determination of amorphous content in the pharmaceutical process environment, J. Pharm. Pharmacol. 59 (2007) 161–170.

[43] Schuck P., Dolivet A., Lactose crystallization: Determination of α-lactose monohydrate in spray-dried dairy products, Lait 82 (2002) 413–421.

[44] United States Pharmacopeia (USP), The National Formulary: USP 23, NF 18 United States Pharmacopeial Convention, Washington, 1995, pp. 2257–2258.

[45] Van Leverink J., Extrusion process for the preparation of anhydrous stable lactose, U.S. Patent No. 4 280 997, 1981.