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Changes in Anomeric Composition of Different Crystalline Forms of Lactose During Thermal Treatment

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ABSTRACT

The effect of heat on lactose crystals is investigated at different temperatures and humidities. Changes may be explained in terms of dissolution, after loss of water of crystallisation, and mutarotational equilibria.

INTRODUCTION

During the last few years, several thermal analysis procedures such as thermogravimetry (Liskowitz *et al.*, 1980), differential scanning calorimetry (DSC) (Berlin *et al.*, 1971; Ross, 1978) and differential thermal analysis (DTA) (Itoh *et al.*, 1977) have been applied to the study of the thermal behaviour of crystalline forms of lactose. The results show that thermograms are affected by heating rate, container type, sample size

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and purity of the starting sample. As a consequence, the interpretation of results by different authors is often contradictory and the changes shown by lactose during thermal treatment are not easily explained. Moreover, little is known about the possible chemical changes undergone by the various forms of lactose at temperatures below their melting points. The subject has been studied by Fernández-Martín *et al.* (1980) by combining the use of DSC with gas–liquid chromatography (GLC) of samples. These authors indicated that experimental parameters and anomeric purity of sample were a very important factor in the thermal behaviour of α-lactose hydrate.

In this laboratory, interest has centred on the early stages of the thermal treatment of lactose (mainly mutarotation) as part of a study of the chemical reactions (in the solid state) at relatively high temperatures. In the present work, the effect of thermal treatment of lactose at different relative humidities using various crystalline forms has been studied.

MATERIAL AND METHODS

Several crystalline forms of lactose were prepared according to previously described methods (Lim & Nickerson, 1973; Olano *et al.*, 1977; Olano & Rios, 1978). Purity was checked by polarimetry and GLC. All samples were >99% lactose. Anomeric compositions were: α-hydrate, 95%; unstable anhydrous α-lactose, 92%; stable anhydrous α-lactose, 97%; β-lactose, 88·5%; molecular complex, 64·5% α and 35·3% β.

Thermal treatments

A few milligrams of a finely divided crystalline sample of lactose were placed in the bottom of a capillary tube (0·71 mm inside diameter × 1 mm outside diameter) sealed at one end. This tube was placed into a wider one (1·31 mm inside diameter × 1·80 mm outside diameter) according to the scheme shown in Fig. 1. Tubes of different volumes were obtained by varying the length, so that differences in heat transfer were avoided. Water was added to the external tube when 100% relative humidity was required; the external tube was sealed and held in a vertical position to avoid any direct contact between liquid water and solid sample. Isothermal and programmed-temperature treatments were undertaken in the oven of a Sigma 3B gas chromatograph (Perkin-Elmer).

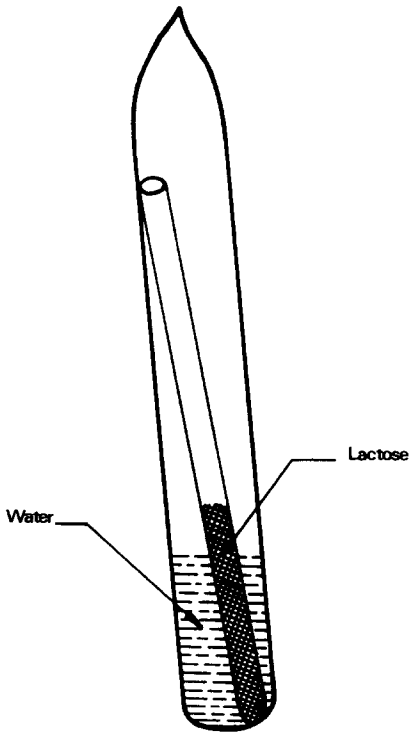

Fig. 1. Scheme of double capillary used for heat treatment of samples in water vapour atmosphere.

Temperatures were calibrated with an external chromel/alumel thermocouple. Capillaries containing samples were introduced into the oven and the temperature was maintained at 100 °C for 10 min. Thereafter, it was raised at several heating rates to the final desired temperature. After thermal treatment the tubes were broken and the sugar samples dissolved in dimethyl sulfoxide (DMSO) to impede further mutarotation.

Analyses

GLC analyses of lactose were carried out on the trimethylsilyl (TMS) ethers, prepared by reaction with trimethylsilylimidazole in DMSO. In some cases phenyl-β-D-glucoside was used as an internal standard. TMS derivatives were injected in a Sigma 3B chromatograph equipped with dual s/s columns of 3 m × 3·17 mm packed with 3% OV-17 on Chromosorb W-HP 80–100 mesh. Carrier gas was N_2 at 25 ml/min. Peak

areas were measured as the product of height and the mean base and corrected with previously determined response factors.

RESULTS

Heating under dynamic conditions

This type of treatment, usual in thermal analysis, was carried out not only to compare chemical results with thermal data from the literature, but also because the dynamic nature of heating makes the study of possible changes undergone by a substance before reaching a specified temperature of interest. Thus, samples of α-lactose hydrate were heated to different final temperatures at rates ranging from 1 to 8 °C/min. Because, at rates higher than 4 °C/min, transformations were not reproducible, all programmed temperature treatments were carried out at 4 °C/min.

Since this investigation was undertaken to study the changes occurring during the early stages of heating before degradation started, it was necessary to know the maximum temperature to which lactose could be heated without undergoing degradation.

GLC analysis (with an internal standard) showed that, when lactose was heated to final temperatures below 165 °C, mutarotation to β-lactose was the only change observed. Heating to temperatures above 175 °C caused lactose degradation. Chromatograms of TMS derivatives of heated samples showed the formation of monosaccharides (galactose and glucose), lactulose and other disaccharides, as well as shifts in the anomeric composition of the remaining lactose. These events were also observed by Fernández-Martín *et al.* (1980). In our samples the total material accounted for by the GLC analysis was low because many degradation products (e.g. caramel polymers) cannot be analyzed by GLC. Table 1 shows that degradation of lactose started above 165 °C and increased with temperature, reaching 92% at 215 °C; simultaneously, a decrease of the previously formed β-anomer was observed, and the percentage of α-anomer in the remaining lactose increased until a distribution similar to equilibrium in solution was reached. Anomeric mixtures of this type have been found previously (Pincok & Kiovsky, 1966; Broido *et al.*, 1966) when sugars were melting.

As a consequence of these results, the subsequent experiments were carried out below 170 °C. Several experiments were performed by heating

TABLE 1
Degradation of α-Lactose Hydrate (9 mg) in Sealed Capillaries (58 mm^3) During Heat Treatment (4 °C/min) from 100 °C Up to Different Final Temperatures

Final temperature (°C)	*Anomeric composition* %α	%β	*% remaining lactose*
100	95·0	5·0	100
165	9·5	90·5	100
175	9·6	90·4	98·4
185	11·7	88·3	85·9
195	21·0	79·0	63·7
205	46·4	53·6	32·9
215	50·3	49·7	7·6

the α-hydrate in open capillaries in order to allow water vapour to escape, thus favouring crystal dehydration. Under these conditions less than 30 % of the initial alpha form mutarotated to the beta form. In the absence of water vapour, anhydrous forms of lactose remained unaltered when heated in either open or sealed capillaries. However, when α-hydrate was heated in sealed capillaries up to 90–95 % of the initial sample was converted to β-lactose. The ratio of the sample weight/tube volume

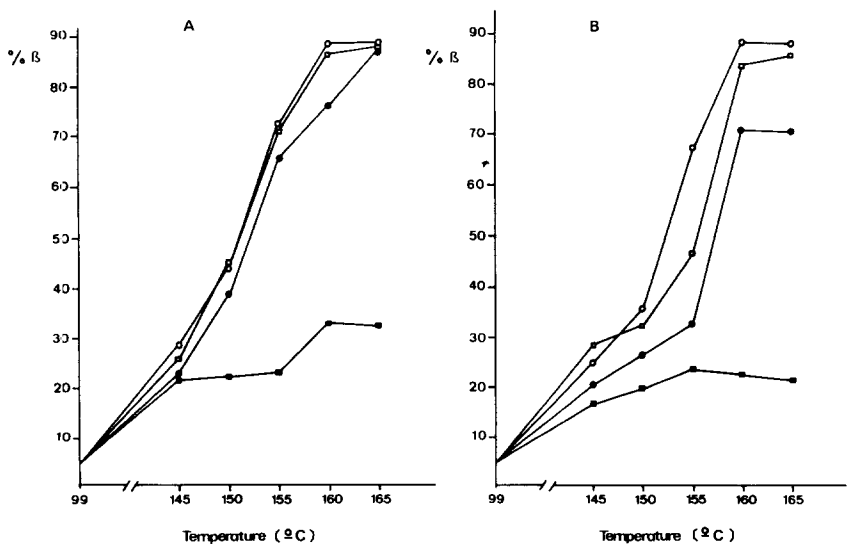

Fig. 2. Mutarotation of α-lactose hydrate during heat treatment at 4 °C/min in (A) 58 mm^3 and (B) 116 mm^3 capillaries (■) 3 mg; (●) 6 mg; (□) 9 mg; (○) 12 mg sample.

affected the extent of transformation; the higher the ratio, the higher was the percentage of lactose formed. This can be clearly related to the increased relative humidity created by the hydration water released during heating (Gillis, 1920). Maximum transformation to β-lactose was achieved at 100% relative humidity. The results are shown in Fig. 2.

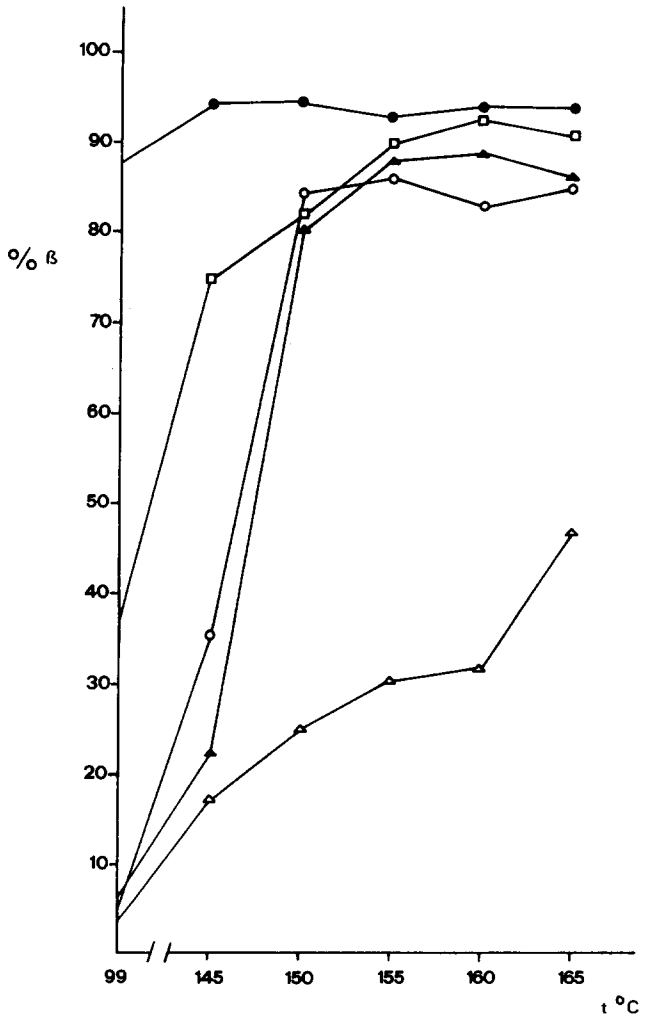

Fig. 3. Mutarotation during heat treatment at 4 °C/min of crystalline forms of lactose (6 mg in 116 mm^3 capillaries) at 100% relative humidity. (△) stable anhydrous α-lactose; (▲) unstable anhydrous α-lactose; (○) α-lactose hydrate; (□) molecular complex α/β; (●) β-lactose.

In several reported DSC and DTA studies of α-lactose hydrate a first endotherm occurring at 110 °C is unanimously attributed to dehydration (Berlin *et al.*, 1971; Ross, 1978; Fernández-Martín *et al.*, 1980; Lerk *et al.*, 1980; Itoh *et al.*, 1981). When α-lactose is heated in an open container, another endotherm corresponding to the melting of α-lactose is found, beginning at about 200 °C with a maximum at 212 °C. Berlin *et al.* (1971) showed that, during heating of α-hydrate in sealed pans, two new peaks (an exo- and an endotherm) appeared at about 165 and 185 °C. These authors interpreted these peaks as sorption of the water released accompanied by mutarotation (the exotherm) and the melting of α- and β-lactose (the second and third endotherms). Other authors (Lerk *et al.*, 1980; Itoh *et al.*, 1981) reported similar results but without giving an interpretation. Fernández-Martín *et al.* (1980) found that results similar to these were dependent on the purity of the initial sample, but did not

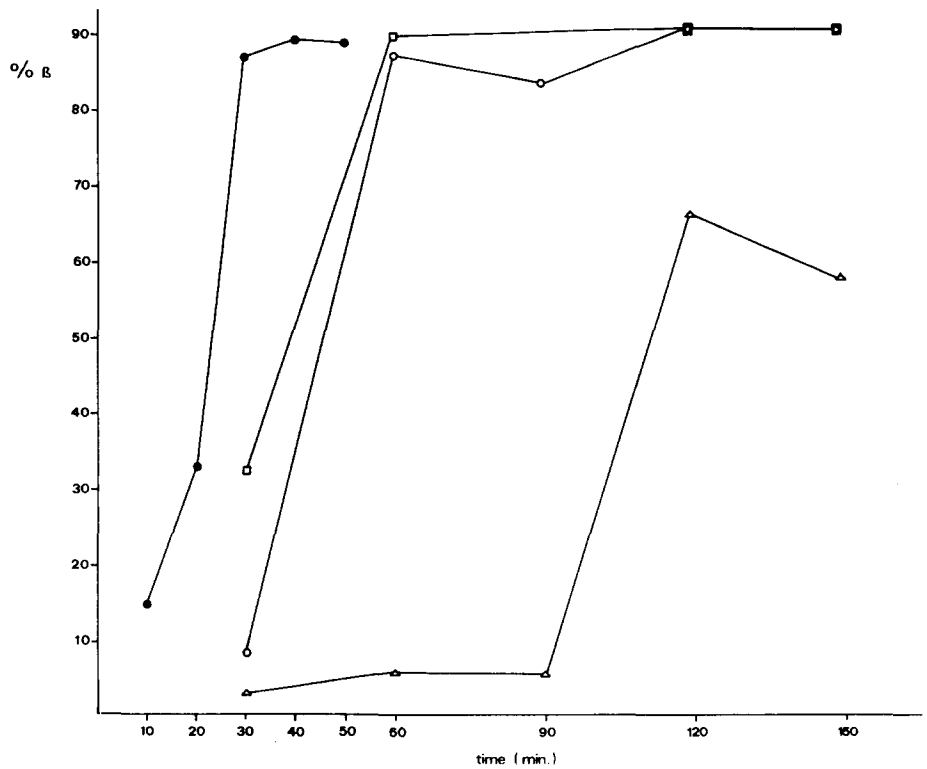

Fig. 4. Mutarotation of α-lactose hydrate (6 mg, 58 mm^3 capillaries) at several temperatures: (△) 129 °C; (○) 132 °C; (□) 134 °C; (●) 136 °C.

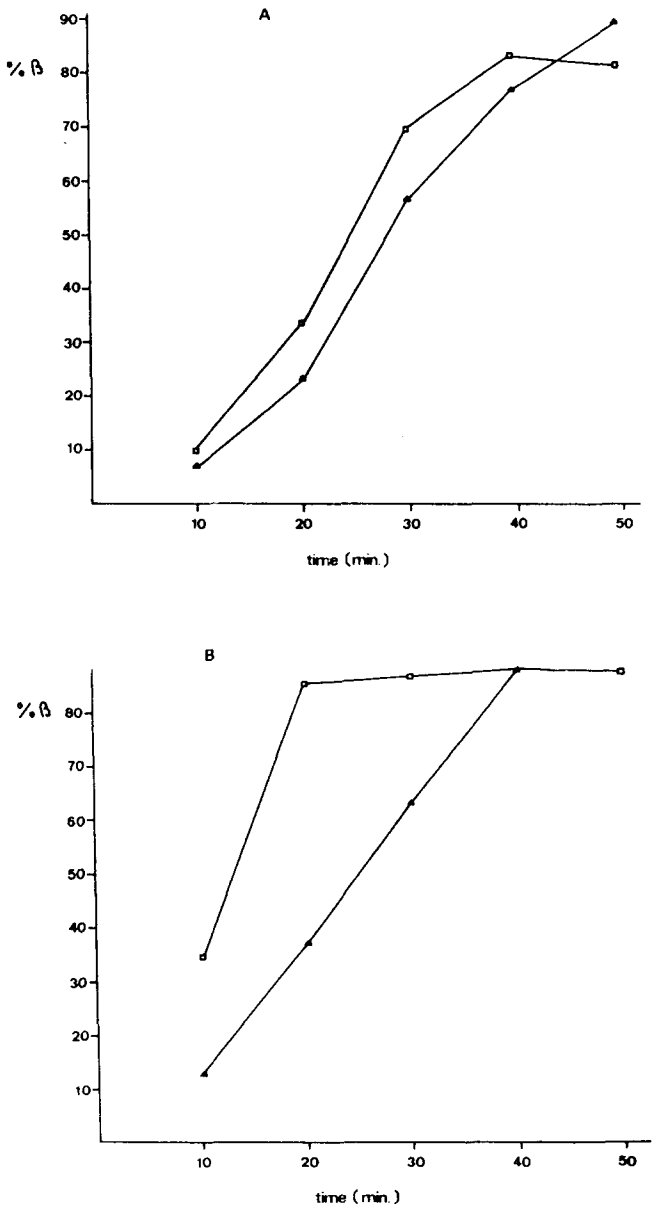

Fig. 5. Murarotation of α-lactose hydrate (□) and stable anhydrous α-lactose (△) (6 mg, 116 mm^3 capillaries at 100% relative humidity at 132 °C (A) and 136 °C (B)).

attempt to correlate mutarotation with the quantity of water present. The amount of β-lactose formed in this case was less than 70%.

In order to confirm the rôle of water in the transformation of α- to β-lactose, a series of experiments were carried out by adding water to the bottom of the external tubes. The amount added was calculated to be just sufficient to maintain 100% relative humidity during the heat treatment. All anhydrous forms were heated in this manner and the α-hydrate was also heated in this way for comparative purposes. The results obtained are shown in Fig. 3. In all cases β-lactose formed in high yield; the initial β-lactose sample, which contained 15% of the α-anomer, yielded 95% β-lactose after treatment. α-Hydrate, unstable anhydrous α-lactose and the molecular complex mutarotated analogously. However, stable anhydrous α-lactose yielded only a maximum of 50% β-lactose.

Heat treaments under isothermal conditions

All crystalline forms of α-lactose were heated at 129, 132, 134 and 136 °C for different times in sealed capillaries. The anhydrous forms (stable and unstable) remained unaltered whereas α-hydrate was transformed to the β form. Transformation rate increased with temperature, as is shown in Fig. 4. At 129 °C no changes were observed during the first 90 min; thereafter transformation began, reaching 67% after 120 minutes' heating. At 136 °C, maximum transformation (90% β) was reached in 30 min.

Heat treatments at 100% relative humidity caused mutarotation of both anhydrous forms of α-lactose. The results obtained during heat treatment of α-hydrate and stable anhydrous α-lactose are compared in Fig. 5. At 132 °C transformation was similar for both crystalline forms whereas, at 136 °C, the mutarotation rate was slower for stable anhydrous α-lactose. The slower increase of mutarotation rate with temperature observed for the α-anhydrous stable form can explain the low percentage of β-lactose formed during its dynamic heating (see Fig. 3).

DISCUSSION

From these results, the effect of relative humidity in thermally induced mutarotation of crystalline forms of α-lactose is well established but the detailed mechanism of interaction between lactose and water molecules

during this type of mutarotation is difficult to explain. When heating α-hydrate, the first step is the release of the hydration water forming the unstable anhydrous α-lactose, a porous form without sufficient rearrangement to form a stable crystalline lattice structure. This unstable form did not mutarotate in the absence of water vapour; therefore, it is possible that these hygroscopic crystals are able to sorb water vapour which forms microsolutions on the surface. Dissolution of the anhydrous unstable form in water is an exothermic process and possibly corresponds to the exothermic peak found in DSC analysis by Berlin *et al.* (1971). The β-lactose formed on the surface of α-lactose crystals could induce the rearrangement of the remaining lattice to the stable, dense β form. The influence of β-lactose on the crystalline habits of the other forms is well known (Nickerson, 1974). According to this hypothesis, α-lactose could mutarotate without disappearance of the solid form, as observed using a hot-plate microscope. Samples submitted to heat treatment showed a partial sinterization on the surface of crystals. This was attributed to a solubilization effect; partial melting was not considered since sinterization took place several tenths of a degree below the melting point and also because β-anomer equilibrates very rapidly with α on melting (Broido *et al.*, 1966; Pincok & Kiovsky, 1966).

The case of the stable anhydrous α form seems slightly different. Although some thermal properties of this form have been studied in the absence of water vapour (Berlin *et al.*, 1971; Lerk *et al.*, 1980), no calorimetric and vapour sorption data are available at very high relative humidity. Nevertheless, Berlin *et al.* (1971) showed that β-lactose, also a stable anhydrous form, sorbs water rapidly at 97% relative humidity, forming a concentrated solution whereby lactose is capable of mutarotation. It is feasible that stable anhydrous α-lactose sorbed water at high relative humidity and temperature and dissolved to form β-lactose by a similar mechanism.

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