ANOMERIC COMPOSITION AND SOLID STATE PROPERTIES OF LACTOSE

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ABSTRACT

Lactose is a widely used excipient in capsules and tablets. It has two anomeric forms, α (usually a monohydrate) and β (anhydrous). Lactose NF XVI is usually α-lactose monohydrate. Physical properties, such as thermal behavior, x-ray diffraction characteristics, and true density of the anomers are different and not clearly understood. Pure samples of each anomer are difficult to prepare and all commercial lactose samples, especially the directly compressible grades, contain a certain amount of each anomer. It is not clearly established in what physical form the two anomers are present in a commercial sample. The physical form, and also certain differences in the physical properties, may depend upon the anomeric composition.

An accurate and rapid gas chromatographic (GC) method for the determination of anomeric composition was developed. It involved derivatization of the lactose samples using trimethylsilylimidazole (TSIM). A mixture of TSIM in dimethylsulfoxide (DMSO) and pyridine (PYR) was used. DMSO dissolved the samples and PYR stabilized the solutions by preventing a phase separation which occurred if only TSIM and DMSO were used. Alpha-rich samples were dissolved directly into the mixture. Beta-rich samples were first dissolved in DMSO and then derivatized using a mixture of TSIM and PYR. An OV-225 column with helium as carrier gas was used for separating the anomers. The relative response of the anomers at a flame ionization detector was equal. Thus, the relative anomeric peak areas could be used as relative anomeric

amounts. This avoided the use of an internal standard. The anomeric composition of a number of lactose samples was determined and was found to vary from 1.9 to 98.4% α.

A study of the thermal behavior of commercial lactose samples using differential scanning calorimetry and thermal microscopy showed that all α-lactose monohydrate rich samples exhibited a dehydration peak followed by a melting peak when heated in an open pan. In sealed pans, the dehydration peak split into two components because of an overlap of an exotherm (due to dissolution of anhydrous lactose in the liquid water formed in the sealed pan, and recrystallization of β-lactose from the solution) with the endothermic dehydration peak. The extent of the split varied with the heating rate (which controls the extent of dissolution). Two new peaks, an endotherm and an exotherm, also appeared after the dehydration peak. The endotherm is due to anomeric conversion (determined using the GC method) rather than melting, and the exotherm is due to recrystallization into a new crystal lattice as the sample became β-rich. Since β-rich samples normally have a higher melting point than α-rich samples, the melting peak shifted to a higher temperature when sealed pans were used. An unstable anhydrous α-lactose sample also showed the endotherm (anomeric conversion) and the exotherm (recrystallization of the β-rich form).

On the basis of their powder x-ray diffraction patterns, the lactose samples can be classified into three types: 1. α-lactose monohydrate rich, 2. β-rich, and, 3. samples showing peaks of both α-

lactose monohydrate and β-lactose. It was shown using quantitative x-ray diffraction that samples did not contain their anomeric impurity as a simple physical mixture.

The true density of the lactose samples also varied with their anomeric composition. Beta-rich samples had greater true density than α-rich samples. This can be attributed to: 1. a simple physical mixture of α-lactose monohydrate and β-lactose crystals, 2. a continuous substitutional solid solution, 3. an interstitial solid solution, or, 4. a mixture of two solid solutions. The first possibility was ruled out using quantitative x-ray diffraction because the relative anomeric x-ray peak intensities did not match the anomeric composition determined by GC. The second possibility was ruled out because there was no gradual shift of peaks in the x-ray diffraction patterns with the anomeric composition. The formation of an interstitial solid solution was not possible because this occurs only if the solute and solvent have very different molecular sizes. The quantitative x-ray diffraction experiments suggest that most samples contain a mixture of two solid solutions.

Sorbed-moisture and surface area are important factors in tabletting. Various commercial lactose samples had specific surface areas ranging from 0.108 to 0.574 m^2/g. Moisture-desorption and sorption were found to depend more on the relative crystallinities of the samples than on their surface areas.

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Abbreviations for lactose samples:

Name	Abbreviation
α-Lactose Monohydrate	α-L or A-L
NF Lactose Hydrous grade 60S	L60S
NF Lactose Hydrous grade 80S	L80S
NF Lactose Hydrous grade 80M	L80M
NF Lactose Hydrous Capsulating grade	LCAPS
NF Lactose Hydrous Impalpable	IPL-S
NF Lactose Anhydrous	ANL
Impalpable Lactose	IPL-F
Spray Process Lactose	SPL
Fast Flo Lactose	FFL
DCLactose 11	DCL-11
DCLactose 21	DCL-21
DCLactose 30	DCL-30
Crystalline β-Lactose	β-DMV or B-DMV
β-Lactose 'Baker'	β-BAK or B-BAK
β-Lactose	β-CCF or B-CCF
Stable Anhydrous Lactose	SANL
Unstable Anhydrous Lactose	UANL

Other abbreviations:

ACN	Acetonitrile
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
FID	Flame ionization detector
GC	Gas chromatography
h	Hour(s)
HMDS	Hexamethyldisilazane
kV	Kilovolts
LiF	Lithium fluoride
mA	Miliamperes
P_2O_5	Phosphorus pentoxide
PYR	Pyridine
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TSIM	Trimethylsilylimidazole

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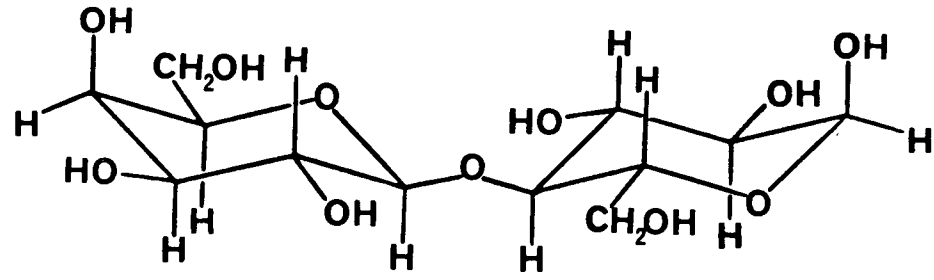
1. INTRODUCTION

Lactose is a disaccharide [4-(β-D-galactopyranosyl)-D-glucose]. It exists in two anomeric forms, designated as α-lactose and β-lactose (Scheme I). Molecular structures of the lactose anomers are closely analogous to the isomeric α and β-glucose. The anomeric designations refer to the configuration of substituents on the carbon-1 of the glucose moiety.

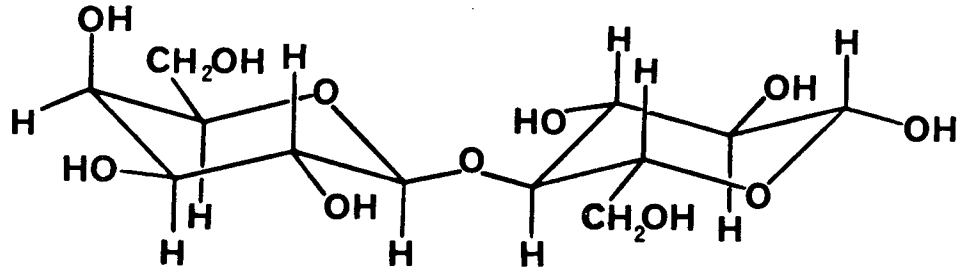
Types of lactose

A number of distinct types of lactose can be produced by various crystallization and drying processes. These types can vary in their content of α and β-lactose, crystalline and amorphous lactose, and in their chemical state, i.e., hydrated or anhydrous lactose (Handbook of Pharmaceutical Excipients, 1986). Lerk *et al.* (1984) describe lactose as a solid which occurs in one of four crystalline forms or in an amorphous state. These different types are as follows.

i. Crystalline α-lactose monohydrate: the most common form.
ii. Crystalline β-lactose.
iii. Unstable anhydrous α-lactose: a very hygroscopic form.
iv. Stable anhydrous α-lactose: a nonhygroscopic form.
v. Amorphous lactose: also called amorphous lactose glass.



α - LACTOSE



β - LACTOSE

SCHEME I

Preparation of different types of lactose

The crystalline forms of lactose are prepared by recrystallization from aqueous solutions. In aqueous solutions, the lactose anomers exist in a mutarotational equilibrium. The aqueous solubility of the anomers is temperature dependent. Below 93.5°C, the α-anomer is the less soluble form. Therefore, it recrystallizes from the aqueous solutions below this temperature. Above 93.5°C, it becomes the more soluble form, allowing the β-anomer to recrystallize (Whittier, 1944; Visser, 1982; Olano *et al.*, 1983). Due to the mutarotational equilibrium, it is difficult to obtain one anomer completely free from the other. However, α-rich or β-rich samples can be prepared.

The hygroscopic unstable anhydrous α-lactose is prepared by heating α-lactose monohydrate, *in vacuo*, at temperatures of 110-130°C. The non-hygroscopic stable anhydrous α-lactose is prepared by heating α-lactose monohydrate in a moist atmosphere at temperatures above 110°C (Sharp, 1943) or by desiccation with suitable liquids, such as dry methanol (Lim and Nickerson, 1973). Depending on the conditions of the thermal dehydration, products with anomeric composition different from the initial α-lactose monohydrate can be obtained. For example, Olano *et al.* (1983) prepared up to 95% β-lactose by thermal treatment of α-lactose monohydrate in the presence of water vapor.

The amorphous lactose is produced by rapid drying of a lactose solution. Due to the rapid drying, the viscosity of the solution

increases quickly such that the formation of crystals does not occur (Nickerson, 1974). This form is also called amorphous (non-crystalline) lactose glass, and is a constituent of spray dried, freeze dried or roller dried lactose samples.

Other types of lactose

Apart from the types mentioned above, some novel types of lactose, so called "molecular complexes" of anhydrous α-lactose and β-lactose, have also been reported. Reports by Olano *et al.* (1977), Parrish and Brown (1982) and Simpson *et al.* (1982) describe α/β molecular complexes with α/β anomer ratios of 5/3, 3/2 and 4/1. Lerk *et al.* (1984) reported the formation of a "β/α-lactose compound" with 1:1 α:β ratio during a DSC analysis of α-lactose monohydrate.

Pharmaceutical utilization of lactose

Various types of lactose have found applications in pharmaceutical formulation. The Handbook of Pharmaceutical Excipients (1986) gives an exhaustive listing of the pharmaceutically important lactose types called Pharma-Lactose-Types. These are useful in solid dosage form manufacture, for example, as diluents for compressed and molded tablets and capsules.

With the increased use of the direct compression method of tablet preparation, various direct compression types of lactose have been

prepared and used under such names as anhydrous lactose, impalpable lactose, spray process lactose, spray dried lactose, fast-flo lactose, or lactose beadlets (Batuyios, 1966; Fell and Newton, 1970; Henderson and Bruno, 1970; Vromans *et al.*, 1985).

Presence of α and β-anomers together in a given type of lactose

It is clear from the description of different types of lactose, and their methods of preparation, that all samples of lactose can be expected to contain both anomers together. This immediately raises a question. How is one anomer incorporated in a product consisting mainly of the other anomer, or in what physical state are the lactose anomers incorporated into a product containing both of them? Such questions are yet to be answered completely.

In order to describe a lactose product which contains both anomers, terms like "complex", "compound" or "mixture" have been frequently used (Simpson *et al.*, 1982; Olano *et al.*, 1983; Lerk *et al.*, 1984). Although some evidences have been presented by these authors to substantiate the existence of such types, the problem still lacks a complete answer. It is necessary to clearly establish whether the two anomers exist as a simple physical mixture, solid solution, eutectic mixture, or an altogether new compound (or complex). Formation of new compounds between the two anomers under certain specific experimental conditions has been mentioned above. To confirm their existence as truely new phases, these compounds would have to be characterized fully

and it would be necessary to prove that they are not merely artifacts of a given specific set of experimental conditions. The best solution to this problem would be to establish a two component phase diagram of the lactose anomers. This would be complicated by the fact that α-lactose is a monohydrate while β is anhydrous. Nevertheless, important information in this connection can be obtained if physical properties, such as thermal behavior, powder x-ray diffraction characteristics, and the true densities, of a number of lactose samples with different anomeric compositions are studied and interpreted.

Physical properties of lactose anomers

A survey of the extensive literature available on lactose points to a lack of agreement between various reports on its physical properties. These properties include thermal behavior, x-ray diffraction characteristics, and true density of the anomers. An overview of these disagreements is presented below.

A. Differences in thermal behavior of lactose

A summary of the differences in the melting points of different types of lactose is presented in Table I. This stresses the observation mentioned above that the literature lacks agreement regarding the thermal behavior of lactose. Besides those mentioned in Table I, other important reports on the thermal behavior of lactose include those by Berlin *et al.* (1971), Itoh *et al.* (1977), Ross (1978), Krycer and Hersey

Table I. Melting points of different types of lactose samples.

Lactose type	m.p.(°C)	Reference
α-hydrate	201.6	Olano *et al.* (1983)
	217	Simpson *et al.* (1982)
	210	Lerk *et al.* (1984)
Unstable α-anhydrous	222.8	Olano *et al.* (1983)
	210	Lerk *et al.* (1984)
Stable α-anhydrous	216	Simpson *et al.* (1982)
	210	Lerk *et al.* (1984)
β-lactose	229.5	Itoh *et al.* (1978)
	252	Olano *et al.* (1983)
	232	Simpson *et al.* (1982)
	237	Lerk *et al.* (1984)

(1981), and Lerk *et al.* (1980). Berlin *et al.* (1971) and Lerk *et al.* (1980) both reported changes in the thermograms of lactose when the samples were heated in sealed pans. The dehydration peak was reported to split into two parts and some new peaks were reported to arise. These were left unexplained.

Fernandez-martin *et al.* (1980) and Lerk *et al.* (1984) reported anomeric conversion during the thermal treatment of α-lactose monohydrate on a DSC. The two reports disagree in explaining the thermal transitions and attribute similar peaks to different causes. For example, the peak that Fernandez-martin *et al.* (1980) believed to occur due to anomerization was reported by Lerk *et al.* (1984) to occur due to melting.

It is clear from these disagreements that the thermal behavior of lactose lacks a complete interpretation and requires an extensive investigation before it can be fully understood.

B. Differences in x-ray diffraction characteristics of lactose

X-ray diffraction characteristics and crystal structure of various crystalline types of lactose have been studied by Seifert and Labrot (1961), Knoop and Samhammer (1962), Buma and Weigers (1967), Beevers and Hansen (1971), Fries *et al.* (1971) and Hirotsu and Shimada (1974). While all of these reports agree that the lactose crystals (both α and β) belong to the monoclinic crystal system, there is a disagreement

between the reported lattice parameter values (Table II). Hence, the calculated x-ray densities of the different lactose samples also disagreed between these reports. Buma and Weigers (1967) found the x-ray density of β-lactose to be 1.63 g/cm^3, while Hirotsu and Shimada (1974) reported it to be 1.586 g/cm^3. Similarly, the x-ray density of α-lactose monohydrate was found to be 1.52 (Beevers and Hansen, 1971) and 1.540 g/cm^3 (Fries *et al.*, 1971). It is possible that these authors used lactose samples of different anomeric compositions. Depending on the physical form in which the two anomers were actually present in these samples, their x-ray diffraction behavior would be expected to differ.

C. Differences in true density of lactose

Besides the disagreements between their x-ray densities, the lactose samples differed in their experimentally determined true densities as well. The true density of β-lactose was found to be 1.59 by Buma and Weigers (1967), 1.570 by Hirotsu and Shimada (1974), and 1.62 g/cm^3 by Buma (1978). Similarly the true densities of α-lactose samples were found to be 1.497 (Seifert and Labrot, 1961), 1.545 (Buma, 1965), 1.53 (Beevers and Hansen, 1971), and 1.537 g/cm^3 (Fries *et al.*, 1971). This again suggests that the lactose samples were probably different in their anomeric composition and contained the two anomers in different physical forms. Thus, it is necessary to understand how the true density would change as the anomeric composition changes. Conversely, the true density, in combination with other physical

Table II. Differences between the lattice parameters of different types of lactose.

Lactose Type	Lattice Parameters				Reference
	a(Å)	b(Å)	c(Å)	β°	
α-hydrate	7.864	21.894	4.897	105.97	Seifert and Labrot (1961)
	7.96	21.80	4.81	109.11	Knoop and Samhammer (1962)
	7.98	21.68	4.836	109.78	Buma and Weigers (1967)
	7.815	21.567	4.844	106.2	Beevers and Hansen (1971)
	7.982	21.652	4.824	109.57	Fries *et al.* (1971)
β-lactose	10.81	13.34	4.84	91.25	Buma and Weigers (1967)
	10.839	13.349	4.954	91.31	Hirotsu and Shimada (1974)

properties can be expected to provide an insight into the physical state of the two anomers if they are present together in a given sample of lactose.

D. Other properties of lactose

As stated earlier, lactose is a commonly used excipient in tabletting. Like many other excipients, lactose samples are also expected to possess sorbed moisture which can affect their tabletting behavior (Umprayn and Mendes, 1987), and processing characteristics prior to tabletting (Tabibi and Hollenbeck, 1984). The sorbed moisture also affects the determination of the heat of solution (Pikal *et al.*, 1978; Suryanarayanan and Mitchell, 1985), an important physical property in studying the physical state of pharmaceutical solids. It is, therefore, necessary to determine the amount of sorbed moisture present in a given sample of lactose. Since surface area is an important factor in determining moisture sorption (Zografi *et al.*, 1984) and tabletting behavior, it is important to determine the surface area of the various samples of lactose.

Quantitative determination of anomeric composition of lactose

It is clear from the information presented above that all lactose types can be expected to contain both anomers, and that the anomeric composition can be expected to affect their physical properties. Therefore, a prerequisite for any study involving the various lactose

types is an analytical method that can be used to accurately and speedily determine their anomeric composition. A brief account of the literature concerning the quantitative determination of lactose anomers is presented in the following section.

Methods of quantitative analysis of lactose anomers

The lactose anomeric composition can be determined quantitatively by four main types of methods. These methods are based on the following techniques:

i. Solubility determination,
ii. Polarimetry,
iii. Differential scanning calorimetry, and
iv. Chromatography.

The solubility method (Choi *et al.*, 1949) utilizes the difference in solubility behavior of the lactose anomers. The polarimetric method (Troy and Sharp, 1930; Sharp and Doob, 1941) involves determination of the optical rotation of aqueous lactose solutions before and after the mutarotational equilibrium has been attained. Both, the solubility and polarimetric, methods are unsuitable for rapid analysis of lactose samples, because these involve prolonged equilibration of lactose solutions.

The DSC method (Ross, 1978) is based on the difference in the melting points of the lactose anomers. It carries little reliability

considering the fact that lactose undergoes anomeric conversion during the thermal analysis by DSC (Fernandez-martin *et al.*, 1980).

The most useful are the chromatographic methods. Both, LC and GC, have been applied to lactose anomer determination. A number of LC methods for the separation and determination of lactose from other carbohydrates have been reported in the literature, but there is only one (Beebe and Gilpin, 1983) that deals specifically with the separation and quantitation of the lactose anomers. It involves the separation of the anomers from a totally aqueous mobile phase using two columns connected in series. The columns have to be maintained at ≈4°C in order to reduce the mutarotation, which makes the method somewhat inconvenient for lactose anomer analysis.

The GC methods of lactose anomer analysis are based on the method of Sweely *et al.* (1963) which involves separation of the carbohydrates, and related polyhydroxy compounds, after conversion to their TMS derivatives. The trimethylsilylation was carried out in PYR using HMDS and TMCS. Reineccius *et al.* (1970), Newstead and Gray (1972) and Roetman (1982) used this procedure for the derivatization of lactose anomers in PYR solutions. Newstead and Gray (1972) reported mutarotation of lactose in PYR, and, therefore, proposed the use of rigorously dried PYR below room temperature and derivatization immediately after dissolution. This made the method very tedious. To avoid this problem, Roetman (1982) replaced a part of PYR with DMSO, in which mutarotation does not occur, but retained the same derivatizing

reagents. These reagents cause the formation of a precipitate which has to be removed by centrifuging, or the derivatized lactose anomers have to be extracted using a separate solvent like hexane (De Neef, 1969). These additional steps make the method lengthy. More recently, Gianetto *et al.* (1986) used a new TMS reagent, namely, TSIM, which provides a single step derivatization of lactose. These authors were interested only in the determination of total amount of lactose. Nevertheless, they mentioned that the chromatograms were complicated by the presence of multiple peaks resulting from anomeric separation.

Gas chromatography offers the most rapid method of lactose anomeric composition analysis. The total analysis times required are roughly of the order of 30 minutes. The detection (usually by flame ionization) is simple. The signals from the detector can be easily processed using the sophisticated electronic integrators available with most of the modern gas chromatographs. These advantages make the GC method the method of choice for lactose anomer analysis.

Goal of the present research

It is clear from the brief account of the vast literature on lactose presented in the last few pages that there is a serious lack of agreement between various reports on its physical properties. This lack of agreement gains importance considering the fact that lactose, in its various types, is a commonly employed excipient in tabletting. Since the tabletting behavior of a solid is intimately related to its physical properties, it is important to establish a better understanding of the various physical properties of lactose. The goal of the present research is to provide such an understanding. In the process of understanding the physical properties, an attempt to explain the form in which the two anomers are incorporated in a given product is another important part of the proposed research. A number of commercially available, and some specially prepared, lactose samples were analyzed for their anomeric composition and some physical properties.

Specific objectives of the present research

1. To develop a gas chromatographic method of analysis of the anomeric composition of different samples of lactose.
2. To establish in what physical form one anomer is incorporated in a product consisting mainly of the other anomer.
3. To study and explain the complex and not clearly understood thermal behavior of the lactose anomers.
4. To study such physical properties of the different commercial grades of lactose as true density and powder X-ray diffraction patterns and correlate them with the anomeric composition;
5. To characterize various commercial samples of lactose on the basis of their surface area and moisture-desorption and sorption properties.

2. EXPERIMENTAL

2.1 APPARATUS

Cahn Gram electrobalance, Cahn Division, Ventron Instruments Corporation.

Differential scanning calorimeter (model 910) with a thermal analyzer (series 99), Du Pont Instruments.

Gas chromatograph (model 5830A) with a flame ionization detector, and a GC terminal (model 18850A), Hewlett Packard.

Gas chromatographic syringe, Hamilton Co.

Gastight syringe, Hamilton Co.

Hot stage, model FP2, Mettler.

Incubator, Isotemp, Fisher Scientific Co.

Microscope, model S-Kt, Nikon.

Pycnometer, Multipycnometer, Quantachrome Corporation.

Refrigerated circulator bath, model 2376, Forma Scientific.

Surface area analyzer, Quantasorb, Quantachrome Corporation.

Vacuum oven, model 5831, National Appliance Co.

Vacuum pump, General Electric.

Vials (polytetrafluoroethylene-lined, screw-capped), Reacti-Vials, Pierce Chemical Co.

X-ray diffraction setup consisting of an x-ray generator (model 1710), x-ray diffractometer (model 1729), and an x-ray detector (model PW 1711/10), Philips.

2.2 MATERIALS

Acetonitrile, BDH Chemicals.

Alpha-lactose monohydrate, BDH Chemicals.

Beta-lactose 'Baker', J.T. Baker Chemical Co. (through Electron Microscopy Sciences).

Beta-lactose, Cooperative Condensfabriek Friesland.

Beta-lactose, crystalline, De Melkindustrie Veghel.

Carbon tetrachloride, BDH assured, BDH Chemicals.

Chromosorb W(HP) 80-100 mesh, Applied Science Laboratories Inc.

Cyanopropylphenylmethyl silicone (OV-225), Applied Science Laboratories Inc.

DCLactose 11, DCLactose 21, and DCLactose 30, De Melkindustrie Veghel.

Dimethylformamide, silylation grade, Pierce Chemical Co.

Dimethylsulfoxide, silylation grade, Pierce Chemical Co.

Ethyl bromide, Baker analyzed, J.T. Baker Chemical Co.

Fast flo lactose, product # 316, Foremost Whey Products.

Gas Chrom P, 100-120 mesh, Applied Science Laboratories Inc.

Impalpable lactose, product # 312, Foremost Whey Products.

Indium, Calibration sample kit, Perkin Elmer Corporation.

Lithium fluoride, extra pure, BDH Chemical Co.

Methyl silicone (OV-101), Applied Science Laboratories Inc.

NF lactose anhydrous, Sheffield Products.

NF lactose hydrous, grades 60S, 80S, 80M, capsulating, and impalpable, Sheffield Products.

3% phenylmethyl silicone (OV-17) on Chromosorb W(HP) 80-100 mesh, Western Chromatography Supplies.

Pyridine, silylation grade, Pierce Chemical Co.

Spray process lactose, product # 315, Foremost Whey Products.

Tin, Calibration sample kit, Perkin Elmer Corporation.

Trimethylsilylimidazole, Sigma Chemical Co.

Trimethylsilylimidazole in pyridine (TRI-SIL Z), Pierce Chemical Co.

2.3 DEVELOPMENT OF A GAS CHROMATOGRAPHIC PROCEDURE FOR QUANTITATIVE DETERMINATION OF LACTOSE-ANOMERS

Required features of the procedure

Carbohydrates are non-volatile compounds which cannot be analyzed by GC unless their volatile derivatives are first formed (Laker, 1980). The first target was thus to select a suitable derivative for the lactose anomers. Secondly, it was important to establish a derivatization method which would not affect the anomeric composition of the lactose samples. Like most derivatization methods, this method would also require a solvent for initial dissolution of the solid samples. The solvent should not induce mutarotation of the lactose anomers. For the method to be suitable for routine quantitative analysis, the dissolution and derivatization steps would have to be rapid and reproducible. Conditions, such as the presence of water and application of heat, which favour mutarotation, would have to be avoided.

Selection of a derivative

Carbohydrates can be made volatile by conversion to their methyl, acetyl, trifluoroacetyl or TMS derivatives (Robards and Whitelaw, 1986). The first three derivatives are prepared by methods which do not meet one or more of the requirements in the previous section. These involve either lengthy sample preparation, application of heat or conversion of

the carbohydrates to forms like oximes which do not differentiate between isomers (Bishop, 1962; Bourne *et al.*, 1950; Gunner *et al.*, 1961; Sawardeker *et al.*, 1965). The TMS derivatives, on the other hand, can be prepared easily and under conditions that would not affect the initial composition of the isomeric carbohydrates. Therefore, these were the derivatives of choice for quantitative determination of lactose anomers.

Selection of a TMS reagent

Earlier trimethylsilylation methods for the analysis of lactose involved the use of multiple silylating reagents, namely, HMDS and TMCS (Sweely *et al.*., 1963; Newstead and Gray, 1972; Olling, 1972). The reaction caused the formation of a precipitate which had to be removed by centrifugation, or, the derivatives had to be extracted using a different solvent, such as hexane, leading to time-consuming and tedious sample processing (De Neef, 1969). This difficulty was overcome by using a trimethylsilylating reagent, TSIM, which offered a rapid and single step derivatization at room temperature for most carbohydrate samples (Brittain, 1971). A formulation of this reagent, TRI-SIL Z, is commercially available. TRI-SIL Z was successfully used for the determination of calcium glucoheptonate epimers (Suryanarayanan and Mitchell, 1984). Aside from one report (Gianetto *et al.*, 1986), which was concerned only with the determination of the total amount of lactose, this formulation has received little attention in the determination of lactose anomers. Thus, it was decided to use TRI-SIL Z

for the derivatization of lactose samples.

Selection of a solvent

Preliminary experiments showed that, at room temperature, the lactose samples did not dissolve equally rapidly in TRI-SIL Z. Upon vortex-mixing, the α-lactose rich samples dissolved within 1-2 minutes, while the β-rich samples required more than 30 minutes. TRI-SIL Z contains PYR which is known to cause mutarotation (Hine, 1956). Although derivatization is a much faster reaction than mutarotation (Oates and Schrager, 1967; Cowie and Hedges, 1984), the slow dissolution of β-rich samples in TRI-SIL Z presented a situation where the possibility of the occurrence of sufficient mutarotation to induce errors in the relative anomeric compositions of lactose samples could not be ruled out.

To overcome this problem, ACN, DMF, and DMSO were tried as other solvents. There was no improvement in the dissolution using ACN and DMF. In DMSO, on the other hand, the dissolution of both, α and β-rich, samples was sufficiently rapid; α-rich samples dissolved in less than 1 minute, while the β-rich samples took 5-10 minutes of vortex-mixing to dissolve completely. The solutions, when stored in a desiccator for up to a week, remained stable (no phase separation). DMSO has been used before to improve the dissolution of lactose samples prior to the derivatization by HMDS and TMCS (Roetman, 1982; Visser, 1988). To avoid mutarotation due to traces of water, it was necessary to use rigorously

dried or specially prepared silylation grade of the solvent. A commercially available silylation grade DMSO (Pierce) was thus selected as the initial solvent. The next step was to formulate a mixture of DMSO and TSIM which would dissolve and derivatize lactose samples simultaneously.

Formulation of a derivatization mixture

TRI-SIL Z is a 21% w/v (≈ 22% v/v) mixture of TSIM in PYR. Therefore, the first derivatization mixture tried was a 22% v/v mixture of TSIM in DMSO. While the α-rich samples dissolved in this mixture as rapidly as in pure DMSO, the dissolution of the β-rich samples was considerably retarded (more than 30 minutes). Although the dissolution of α-rich samples was quick, the solutions separated into two phases when kept at the room temperature.

Such a phase separation would be acceptable if it did not affect the relative anomeric composition. To test this, the two phases were injected into the GC separately. The relative peak areas corresponding to the two phases were different from each other and also from the relative peak area of the solution before the phase separation. This indicated that the initial relative peak areas changed after the phase separation, apparently due to mutarotation. The mutarotation probably occurred in the lactose rich polar phase which was also expected to contain the water of crystallization from the α-lactose monohydrate crystals. It should be recalled at this stage that water causes

mutarotation of lactose anomers.

In order to test the effect of the amount of TSIM on the phase separation, mixtures of TSIM in DMSO ranging from 10 to 22% v/v were prepared and used. The solutions were stable at low concentrations of TSIM, but yielded multiple chromatographic peaks implying incomplete derivatization. Therefore, a mixture consisting of 22% v/v TSIM in DMSO was retained and it was decided to establish an alternative method to eliminate the phase separation.

A phase separation was reported also when HMDS and TMCS were used to derivatize lactose dissolved in DMSO (Roetman, 1982). PYR was added to keep the two phases together. The addition of PYR to the solution of lactose in TSIM-DMSO mixture also yielded stable solutions. Since PYR favours mutarotation, the minimum amount of PYR that would prevent the phase separation was selected by varying its relative amount with DMSO, keeping the TSIM concentration at 22% v/v. This amount was found to be 58.5%. Thus, the final formulation for derivatization consisted of 19.5% DMSO, 22% TSIM and 58.5% PYR (v/v).

Optimization of the gas chromatographic conditions

A. Stationary phase

Three stationary phases of increasing polarities were selected for use in GC experiments:

i. 3% OV-101 on 100-120 mesh Gas Chrom P,

ii. 3% OV-17 on 80-100 mesh Chromosorb W, and

iii. 3% OV-225 on 80-100 mesh Chromosorb W.

These were packed into silanized glass columns (4mm i.d. X 0.9m length). Helium (30 mL/min) was used as the carrier gas for all three columns. The detector used was an FID. The temperatures of the injection port and FID were set at 250°C. The column temperatures were typically about 225°C, and were varied according to the experiment under consideration. For example, when monosaccharides were tried as internal standards, the initial column temperatures had to be decreased in order to separate their peaks from the solvent peak. This increased the retention times of lactose peaks. So, to keep the lactose anomer peaks approximately at their earlier retention times, the temperatures were increased at programmed rates to typically about 250°C following the elution of the monosaccharides.

Of the three stationary phases tried, OV-225 provided the best separation of the lactose anomers. Therefore, this stationary phase was used for further optimization of the GC conditions.

B. Column temperature and carrier gas flow

The column temperature was varied from 175 to 225°C. Also, the helium flow rate was varied from 20 to 50 mL/min. The most satisfactory separation was obtained at a column temperature of 215°C and a helium

flow rate of 40 mL/min. The peaks were sharp and symmetric. Total run time was approximately 10 minutes with a separation factor of 1.51 between the two anomeric peaks (Fig. 1).

C. Injection port temperature

A variation of injection port temperature from 225 to 290°C did not cause a significant difference in the anomeric peaks and their total area counts (Fig. 2). The injection port temperature was thus set at 275°C.

D. Detector temperature

A variation in the FID temperature from 250 to 325°C did not significantly affect the anomeric peaks and their total area counts (Fig. 2). The FID temperature used was 275°C.

Sample processing

Once the constituents of the derivatization formulation were selected it was necessary to determine the order in which these were to be added to obtain the anomeric composition in a short time of analysis keeping a minimum sample processing time. The following were three possible ways of processing the lactose samples.

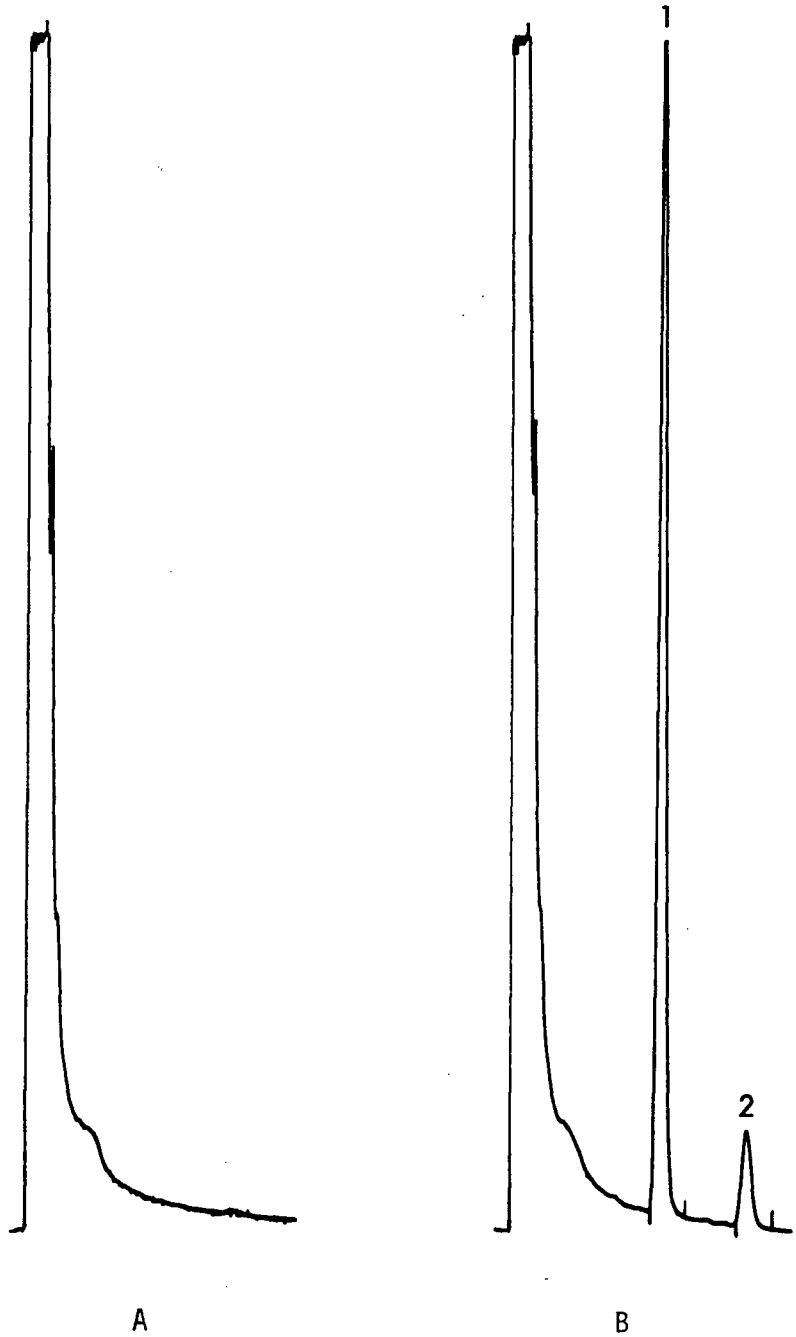


Fig. 1. Representative gas chromatogram of lactose anomers (sample used: DCL-11). A: a blank chromatogram; B: chromatogram showing lactose peaks at retention times of 5.52 min (α-lactose, peak 1) and 8.54 min (β-lactose, peak 2)

Chromatographic conditions: stationary phase, 3% OV-225 on Chromosorb W (HP) in a coiled glass column (4mm i.d. x 0.9m length); injection port temperature, 275°C; column temperature, 215°C; FID temperature, 275°C; carrier gas (helium) flow rate 40mL/min.

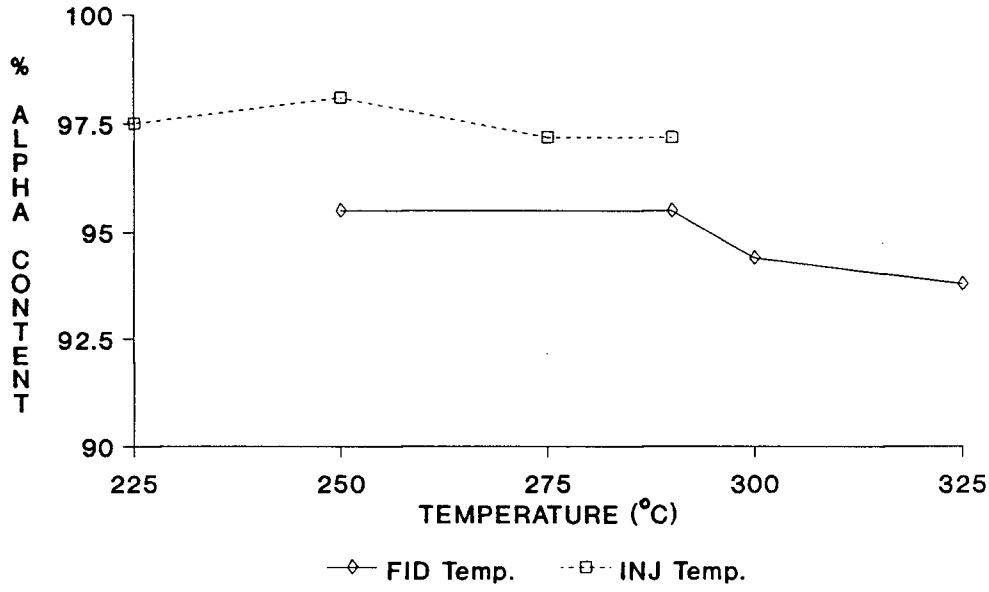


Fig. 2. Variation of anomeric composition as a function of FID and injection port temperatures (the plots correspond with two different solutions of lactose).

1. Sample + DMSO + TSIM-PYR mixture,
2. Sample + DMSO-TSIM mixture + PYR, and
3. Sample + TSIM-DMSO-PYR mixture.

To test whether the three methods lead to the same anomeric composition, two samples, α-L (α-rich) and DCL-21 (β-rich), were processed by each method. The solutions were left under ambient conditions and injected into the GC intermittently. Table III shows the anomeric compositions obtained using the three methods and their statistical evaluation. ANOVA and Tukey's multiple comparison tests showed that the methods 2 and 3 produced average anomeric composition not significantly different from each other (p=0.05). On the other hand, method 1 produced anomeric composition significantly different from those produced by both methods 2 and 3. For both of the samples used, the α-content determined by method 1 was significantly less than that determined by the methods 2 and 3. This could be due to mutarotation. Mutarotation would result in the conversion of α-anomer to β in the case of α-L, and of β-anomer to α in the case of DCL-21. This indicated that, in the case of α-rich samples like α-L, concurrent derivatization and dissolution were important otherwise traces of water of crystallization released into the solution from the crystals would cause mutarotation before TSIM was added. Thus, the methods of choice for α-L, and other α-rich samples, were 2 and 3. Method 3, being more convenient, was selected for processing all α-rich samples. On the other hand, in the case of β-rich samples like DCL-21, it seemed

Table III. Determination of anomeric composition of α-L and DCL-21 by GC using three different methods of sample processing.

Method[a]	% α content (± 1 s.d.)[b]	
	α-L	DCL-21
1	96.5 (0.2)	23.1 (0.1)
2	98.0 (0.2)	23.9 (0.4)[c]
3	98.3 (0.2)	23.8 (0.1)

Statistical evaluation:

A. ANOVA

Total SS	10.0156	1.184
Group SS	9.7266	1.458
Error SS	0.2891	0.356
D.F.:		
Total	11	10
Group	2	2
Error	9	8
F	151.4189	16.3841

Conclusion: The mean α-contents obtained from the three methods for both samples were significantly different from each other (p=0.05).

B. Tukey's multiple comparison test for difference between the different average alpha-contents (p=0.05)

Comparisons:		
Method 3 Vs 1	Significant	Significant
Method 2 Vs 1	Significant	Significant
Method 3 Vs 2	Not Significant	Not Significant

a. See page 29 for details.
b. Average of four injections of one sample each.
c. Average of three injections of one sample.

important to obtain a rapid dissolution prior to derivatization. As mentioned before, the presence of TSIM in DMSO (as in methods 2 and 3) significantly retards the dissolution, allowing the anomers enough time to mutarotate. Therefore, method 1 was the method of choice for samples which were known to be β-rich.

Use of an internal standard

An ideal internal standard for quantitative analysis of the lactose anomers would be a structurally similar compound (e.g., a carbohydrate or a polyhydric alcohol) which could be derivatized with TSIM and would elute at a retention time preferably shorter than lactose. Initial trials with these compounds did not prove successful. Under the conditions which produced the most satisfactory separation of lactose anomers, monosaccharides (glucose, mannose) and polyhydric alcohols (sorbitol, mannitol) had short retention times and almost co-eluted with the solvent. Disaccharides (sucrose, maltose, cellobiose, melibiose) produced peaks overlapping with the lactose peaks. Trisaccharides (raffinose, maltotriose) had long retention times which resulted in broad peaks unsuitable for quantitative work. An added complication was the presence of multiple peaks in the chromatograms of isomeric carbohydrates like glucose and maltose. This would necessitate the prevention of the mutarotation of not only the lactose anomers, but also the isomers of the internal standard.

Relative FID response of lactose anomers

If it could be shown that FID responses of the two anomers were equal, then the relative peak areas could be used as their relative amounts in a given lactose sample. This would avoid the problem of including an internal standard. The equality of FID responses could be easily proved by injecting equal amounts of 100% pure α and β anomers. However, such pure forms were not available. In such a situation the relative response was determined in the following way.

- it was assumed initially that the relative responses were equal,
- based on this assumption, the anomeric ratios of an α and a β-rich sample were determined using the peak area ratios,
- mixtures of these two samples in different ratios were prepared and the resultant anomer ratios were calculated, correcting the amount of α-lactose for the water of crystallization,
- the mixtures were derivatized and injected into the GC,
- peak area ratios of these mixtures were determined,
- the area ratios were plotted against the calculated anomer ratios,

If the initial assumption was correct, then the plot would be a straight line with a slope of 1. This was found to be true (Table IV, Fig. 3). Thus the relative area counts can be used as relative anomer amounts for each lactose sample.

Table IV. Determination of the relative response of the lactose anomers using mixtures of an α-rich sample (α-L) with β-rich samples (β-BAK and β-CCF).

β/α Concentration Ratio	β/α Peak Area Ratio Using	
	β-BAK	β-CCF
0.018		0.018±0.001
0.280		0.280±0.001
0.465	0.499±0.002	
0.539		0.513±0.003
0.684	0.685±0.005	
0.786	0.782±0.014	
0.962	0.903±0.015	
1.049		1.039±0.003

Regression analysis:

r^2	0.9936	1.0
Slope	0.9501	0.9908
95% C.I. for the slope	0.8091 to 1.0911	0.9754 to 1.0061
Intercept	0.0233	0.002

Conclusion: The two slopes were not significantly different from 1 when tested by the Student's t-statistic (p=0.05) using a two-tailed null hypothesis [H_0: slope=1] in conjunction with ANOVA.

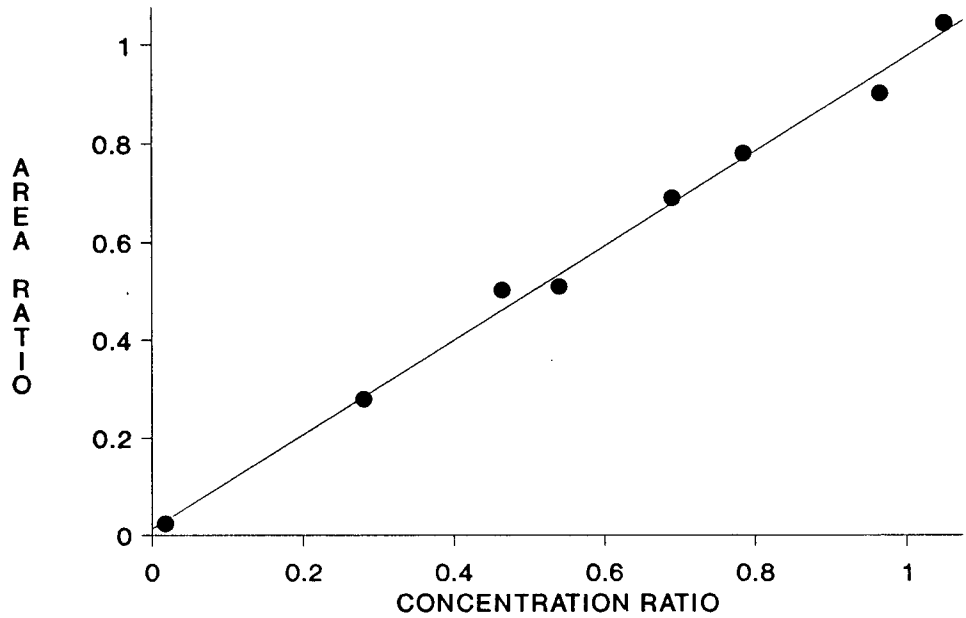


Fig. 3. Plot of relative anomeric response (β/α area ratio) at FID against β/α concentration ratio.

Optimization of derivatization time

A solution of α-L in DMSO was divided into two portions. One portion was derivatized using TSIM-PYR mixture, and injected into the GC at 5, 15, 25 and 35 minutes after the addition of the mixture. The second portion was similarly derivatized, and injected 10, 20, and 30 minutes after addition of the TSIM-PYR mixture to it. There was no significant increase in the total area count of the anomer peaks after approximately 20 minutes indicating complete derivatization within this time (Fig. 4). Thus, the derivatization time was fixed at 20 minutes.

Mutarotation in solution and derivatized state

A solution of DCL-21 in DMSO was kept in a P_2O_5 desiccator at room temperature. Also, a sample each of solutions of α-L and ANL was derivatized and the solutions were stored at 4°C. Intermittently, aliquots of the DCL-21 solution were removed and derivatized using a TSIM-PYR mixture. Portions (3 μL) of this, and the derivatized α-L and ANL solutions, were injected into the GC over a prolonged period of time. The anomeric composition following each injection was determined. The results of this experiment (Table V) indicate that the amount of mutarotation that occurs in approximately 1 h is insignificant. One hour is approximately the amount of time required to carry out three GC runs and, thus, obtain a statistical estimate of the anomeric composition.

Table V. Mutarotation of lactose in DMSO solution and derivatized state.

Solution and Storage	Composition		% Change
	Before	After	
DCL-21, room temp, 96 h, in DMSO	23.3% α	24.0% α	0.7%
α-L, 4°C, 28 h, derivatized	98.2% α	97.8% α	0.4%
ANL, 4°C, 67 h, derivatized	26.1% α	26.6% α	0.5%

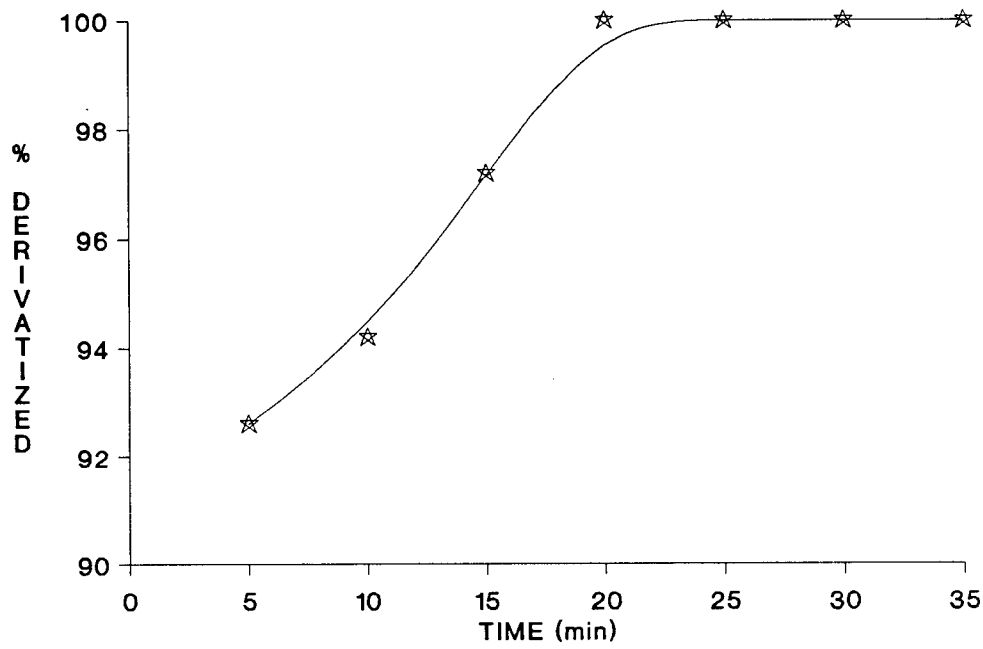


Fig. 4. Extent of derivatization (% of total lactose, based on total area of the anomeric peaks) as a function of time.

Determination of anomeric composition of lactose samples

Approximately 100 μg of α-rich lactose samples were dissolved in 225 μL of the derivatization mixture by vortex-mixing in a Reacti-Vial. The solutions were left at room temperature in a P_2O_5 desiccator until the completion of the derivatization reaction. Using a GC syringe, 3 μL aliquots of the solutions were injected into the GC.

The β-rich samples were processed by a slightly different procedure (method 1, page 29). The derivatization mixture was divided into two components, namely, DMSO and TSIM-PYR mixture. Approximately 100 μg of the samples were dissolved in DMSO by vortex-mixing. The TSIM-PYR mixture was then added to derivatize the dissolved lactose. The total amount of DMSO and TSIM-PYR mixture was maintained at 225 μL. The derivatization reaction was allowed to proceed to completion in a P_2O_5 desiccator, following which 3 μL aliquots of the solutions were injected into the GC.

In order to obtain a statistical estimate, the relative anomeric composition of each lactose sample was obtained by determining the average relative peak area counts of three injections each of three different solutions of each sample (total nine injections of each sample).

2.4 STUDY OF THERMAL BEHAVIOR OF LACTOSE

Differential scanning calorimetry

A DSC coupled with a thermal analyzer was used to study the thermal behavior of the different samples of lactose. Most DSC scans were carried out with 1 to 5 mg samples under a flowing stream of nitrogen at 20 psi. The samples were weighed out directly in different types of sample-pans using an electrobalance. The pans are designated as follows:

A - Open pan (standard pan with crimped edges).

B - Sealed pan (volatile pan).

C - Sealed pan with a small pinhole.

The heating rate employed in most scans was 10°C/min. At times, it was changed to 2.5 or 20°C/min to obtain better insight into the thermal transitions.

Calibration of DSC

The DSC was calibrated using standard samples of Indium (m.p. 156°C) and Tin (m.p. 231°C).

Thermal microscopy

A microscope with a hot stage was used for direct observation of the thermal transitions of lactose.

Preparation of UANL and SANL

The unstable anhydrous α-lactose was prepared by heating an accurately weighed L80S sample *in vacuo* at 110-112°C in a petridish and concurrently removing the water vapor by P_2O_5 kept inside the vacuum oven. The dehydration was complete after about 5 h as indicated by a weight loss of more than 5%. No further weight loss was recorded after this time.

The stable anhydrous α-lactose was prepared according to the method of Sharp (1943). An accurately weighed sample was heated at ≈140°C in a covered petridish in a hot air oven at atmospheric pressure. The sample was, thus, heated in a more or less moist atmosphere inside the covered petridish. It lost the water of crystallization in approximately 8 h (more than 5% weight loss) to yield SANL. A sample of this product was exposed to ambient conditions for about 1 week to verify its stability. The exposed sample did not show any dehydration peak when a DSC run was carried out after this time, which affirmed the stability of the anhydrous product.

Changes in anomeric composition during DSC runs

Fernandez-Martin *et al.* (1980) and Lerk *et al.* (1984) reported changes in the anomeric composition of lactose during DSC runs. In order to investigate the dependence of the changes in the anomeric composition on DSC conditions, and the effect these changes would have

on lactose thermograms, the samples pans were removed from the DSC cell at different points on the thermograms and stored in a P_2O_5 desiccator. When cooled down to the room temperature, small amounts of the samples were removed from the pans, and their anomeric compositions were determined using the GC method described in Section 2.3.

Determination of weight loss during DSC runs

In order to determine the weight-loss of lactose samples due to moisture-desorption and/or dehydration, during the DSC runs, the sample pans were removed from the DSC cell, allowed to cool down to room temperature in a P_2O_5 desiccator, and weighed on an electrobalance. The difference between the weights before and after heating was expressed as % weight loss due to the thermal changes mentioned above.

2.5 STUDY OF POWDER X-RAY DIFFRACTION PROPERTIES OF LACTOSE

Powder x-ray diffraction patterns of lactose samples

Different samples of lactose were mixed with an internal standard, LiF, in 1:1 ratio. The mixtures were packed into sample-holders of an x-ray diffractometer and the diffraction patterns were recorded from 9 to 50°2θ at a scan speed of 0.02°2θ/sec. The x-ray source was maintained at 40 kV and 20 mA. Ni-filtered CuKα x-rays (wavelength 1.54178 Å) were used throughout. Parameters of the diffraction patterns such as peak positions and the corresponding interplanar spacings, and

peak intensities were recorded on a computer terminal interfaced with the x-ray detector.

Quantitative use of the powder x-ray diffraction

The purest available crystalline grades of lactose anomers, namely, L80S (98.4% α) and β-DMV (94.9% β), were selected for quantitative x-ray diffraction work. Mixtures of the two in known weight ratios were made to obtain anomeric compositions from about 14 to 89% α. The mixtures were then mixed with LiF in 1:1 ratio. The resultant mixtures were ground just enough to pass them completely through sieve #200 (particle size 75 μm). Powder x-ray diffraction patterns of these different mixtures were recorded. Ratios of intensities of the most characteristic peak of the α-lactose monohydrate (20.216 ± 0.019°2θ, n=5) to that of the most characteristic and non-interfering peak of LiF (45.224 ± 0.043°2θ, n=42) were plotted against the percent α-content for each mixture. Each mixture prepared above was scanned at least three times to obtain statistical average at each point on the plot. The surface of the packing in the sample cells was disturbed and relevelled each time to test the effect of preferred orientation on peak intensities.

Relationship between anomeric composition and peak intensities

Mixtures of the commercial grades of lactose as received from the suppliers were prepared with LiF in 1:1 ratio. X-ray diffraction

patterns of each mixture were recorded and the ratios of the peak intensities of the most characteristic peaks of α-lactose monohydrate (about 20.1°2θ) and β-anomer (about 21.1°2θ) -if both peaks were present in the diffraction patterns- to that of LiF were obtained. The relative amounts of the two anomers could be determined by taking the ratios of these two ratios. Since the peak intensity of LiF would be cancelled out by this procedure, the relative amounts were actually determined by taking the ratios of the peak intensities corresponding to the two anomers only. Nevertheless, the internal standard, LiF, was retained in order to negate any instrumental variations.

2.6 DETERMINATION OF TRUE DENSITY OF LACTOSE SAMPLES

Determination by pycnometry

A multipycnometer was used for the determination of true density of lactose samples. Prepurified helium gas was used as the displacement fluid. Lactose samples were accurately weighed directly into the sample cell of the pycnometer. All samples were outgassed prior to the true density determination. The sample cell size and the outgassing procedure were selected as follows.

A. Selection of sample cell size

The multipycnometer allows the use of three sample cell sizes. These are, large, small and micro (volumes: 149.59, 28.25 and 12.29 cm^3,

respectively). The three cells yielded true density values which were significantly different from each other (ANOVA, p=0.05). For example, the true density values of a representative sample, namely, α-L, as obtained from the large, the small and the micro cell, were 1.538, 1.524 and 1.502 g/cm^3 respectively.

In order to establish which sample cell yielded the most reliable value, the true density of α-L was determined using a previously developed suspension density method (Suryanarayanan and Mitchell, 1985). The true density obtained from this method (1.5385 ± 0.0006, n=3) was close to the value obtained using the large cell (1.538 ± 0.001, n=6). Thus, this cell was used for all further density determinations. Since β-CCF is a specially prepared sample and is unavailable in the amount (≈90g) required by the large cell, its true density was determined only by the suspension density method.

B. Outgassing of lactose samples

The samples have to be properly equilibrated with helium before the true density can be determined using the multipycnometer. Three methods of outgassing were initially tested for this purpose:

i. purging of the samples with a continuous flow of helium for approximately 45 minutes.

ii. applying vacuum to the samples to remove the adsorbed gases from the sample, and relieving the vacuum by introducing helium into the sample cell; repeating the procedure until

equilibration.

iii. pressurizing the samples with helium and subsequently depressurizing it; repeating the procedure until equilibration.

The three methods yielded true density values not different from each other. The last method was essentially a repetition of the density determination procedure itself. It was a useful method because the true densities of the samples could be calculated after each pressurization and depressurization cycle, and whether the samples were equilibrated or not could be verified by testing whether the density values were constant or not after repetitive outgassing cycles. This method was quicker than the other two methods and was used for further density determinations.

Determination by suspension density method

The suspension fluid for α-rich samples consisted of a mixture of ethyl bromide and carbon tetrachloride. For the β-CCF sample, only carbon tetrachloride was used as the suspension fluid. The method was improved over that used by Suryanarayanan and Mitchell (1985) by using a refrigerated circulating bath which afforded a precise ($\pm$ 0.05°C) control and a rapid change and reequilibration of the temperature of the suspension fluids.

2.7 DETERMINATION OF MOISTURE-DESORPTION AND SORPTION CHARACTERISTICS OF LACTOSE SAMPLES

Moisture-desorption of lactose samples

Initial experiments, involving heating at 80 and 100°C *in vacuo*, led to a loss of water of crystallization as indicated by weight loss of 5% or more. In a previously published method (Tabibi and Hollenbeck, 1984) on the moisture-desorption of a direct compression form of sucrose, a disaccharide like lactose, a temperature of 70°C was used *in vacuo*. In order to avoid dehydration, accurately weighed samples of lactose were kept in a vacuum oven at a lower temperature (60°C) for a prolonged period of time. Intermittently, the samples were taken out of the oven and weighed. It was difficult to obtain a stable weight because the samples would start sorbing moisture from the atmosphere and gaining weight. Therefore, the weight gain was followed over a short period of time (2-3 minutes) and plotted against time. The curve obtained was fitted using a polynomial fitting program. The initial parts of the curves were linear and were extrapolated to time zero to obtain the weight at the moment the samples were taken out of the oven. Difference between the initial weight and the weight after drying was expressed as % weight loss due to drying.

Moisture-sorption of lactose samples

Lactose samples dried as above were transferred to chambers at relative humidities of 15, 32 and 52% (maintained using different saturated salt solutions) at a temperature of 25°C in an incubator. Relative humidities greater than 52% are not expected to be encountered in a tablet manufacturing area. The samples were removed from the constant humidity chambers intermittently and weighed. The weights were fairly constant, therefore, the extrapolation method was not required. Difference between the initial weights and the weights after the exposure to moisture was expressed as % weight gain due to moisture-sorption.

2.8 CHARACTERIZATION OF LACTOSE SAMPLES BASED ON THEIR SURFACE AREA

Specific surface areas of different lactose samples were determined by the multipoint BET method using a surface area analyzer (Lowell, 1973). Three different concentrations (0.0347, 0.0720 and 0.1093%) of krypton (adsorbate) in helium (carrier) were used. Nitrogen was used as the calibration gas.

Calibration curves for determination of surface area

Volumes of nitrogen ranging from 2 to 35 μL were injected into the flowing stream of Kr/He mixture using a gastight syringe. The detector signals so obtained were recorded as peaks on a strip chart recorder.

The peak heights were measured, and the peak areas were determined using triangular approximation. The peak heights and peak areas were plotted against the volume of nitrogen injected. The plots were used as calibration curves for the determination of surface area of lactose samples. The precision of the calibration curves was tested periodically by injecting known volumes of nitrogen.

Determination of surface area of lactose samples

Lactose samples (about 100-250mg) were weighed out accurately in the sample cell of the surface area analyzer. The heights and areas of the desorption peaks of lactose were determined. Volumes of nitrogen that would produce peaks of the same heights and areas were determined from the calibration curves. The peak heights, the corresponding volumes of nitrogen, and the ambient temperature and atmospheric pressure were fed into a computer program (Orr, 1983), based on the BET equation, which calculated the surface area of each lactose sample.

3. RESULTS AND DISCUSSION

3.1 ANOMERIC COMPOSITION OF LACTOSE SAMPLES

Quantitative analysis of different samples of lactose by the GC method described in Section 2.3 revealed that all samples contain both anomers in different relative amounts. The anomeric composition of each sample of lactose is shown in Table VI.

3.2 SOME COMMENTS ON THE GC METHOD OF LACTOSE ANOMER ANALYSIS

The results obtained by the GC method have a high degree of precision as is evident by the low coefficient of variation associated with the anomeric composition (Table VI). The precision and short sample processing and GC run times make the method suitable for routine analysis of lactose samples. The sample handling involves a single-step derivatization using only one derivatizing reagent. Earlier methods used multiple reagents (Sweely *et al.*, 1963; Newstead and Gray, 1972; Olling, 1972; Roetman, 1982).

The experiments conducted during the development of the present GC method indicate that the lactose anomers have equal FID responses. Thus, a simple determination of the relative areas of the anomeric peaks gives the relative anomeric amounts.

Table VI. Anomeric composition of different samples of lactose.

Lactose Samples	α		% β
	% ± 1s.d.[a]	% Coeff. of Var.[a]	
α-L	98.2 ± 0.1	0.12	1.8
L60S	98.0 ± 0.1	0.10	2.0
L80S	98.4 ± 0.1	0.10	1.6
L80M	98.1 ± 0.2	0.20	1.9
LCAPS	98.2 ± 0.1	0.14	1.8
IPL-S	98.2 ± 0.1	0.14	1.8
ANL	26.1 ± 0.4	1.56	73.9
IPL-F	96.9 ± 0.1	0.10	3.1
SPL	96.8 ± 0.1	0.14	3.2
FFL	94.6 ± 0.3	0.34	5.4
DCL-11	89.8 ± 0.3	0.25	10.2
DCL-21	23.3 ± 0.4	1.74	76.7
DCL-30	85.2 ± 0.6	0.67	14.8
β-DMV	5.1 ± 0.2	4.07	94.9
β-BAK	30.6 ± 0.2	0.78	69.4
β-CCF	1.9 ± 0.1	4.40	98.1
SANL[b]	92.0 ± 0.3	0.31	8.0
UANL[b]	92.9 ± 0.1	0.10	7.1

a. Based on 3 injections each of 3 samples.
b. Based on 3 injections each of 1 sample.

3.3 THERMAL BEHAVIOR OF LACTOSE

Observations on lactose thermograms

All α-rich lactose samples exhibit a dehydration peak between 140-145°C, followed by a melting peak between 210-220°C, when heated in open pans (Fig. 5, Table VII). In sealed pans, the dehydration peak shifts to a slightly higher temperature. The melting peak is shifted similarly but more significantly (by ≈10°C; Fig. 5). Also, two new peaks appear in the thermograms and the dehydration peak splits into two components when sealed pans are used.

All β-rich samples melt typically around 235°C (Fig. 5). Some β-rich samples (β-BAK, ANL and DCL-21) also show a dehydration peak revealing that the α-lactose present in these samples is hydrated.

Two samples, UANL and β-DMV, have melting points lower than expected. UANL was prepared by a rapid dehydration of L80S (Section 2.4). Apparently, the dehydration left the crystals in a poorly crystalline form which have a lower melting point . The unexpectedly low melting peak temperature of β-DMV can be explained on similar lines. This sample contains 5.1% α-lactose as an impurity. As suggested later (Section 3.4), this impurity is probably incorporated inside the β-lactose crystals. This incorporation may lead to a significant disruption of the crystal lattice causing it to melt at a lower temperature than expected.

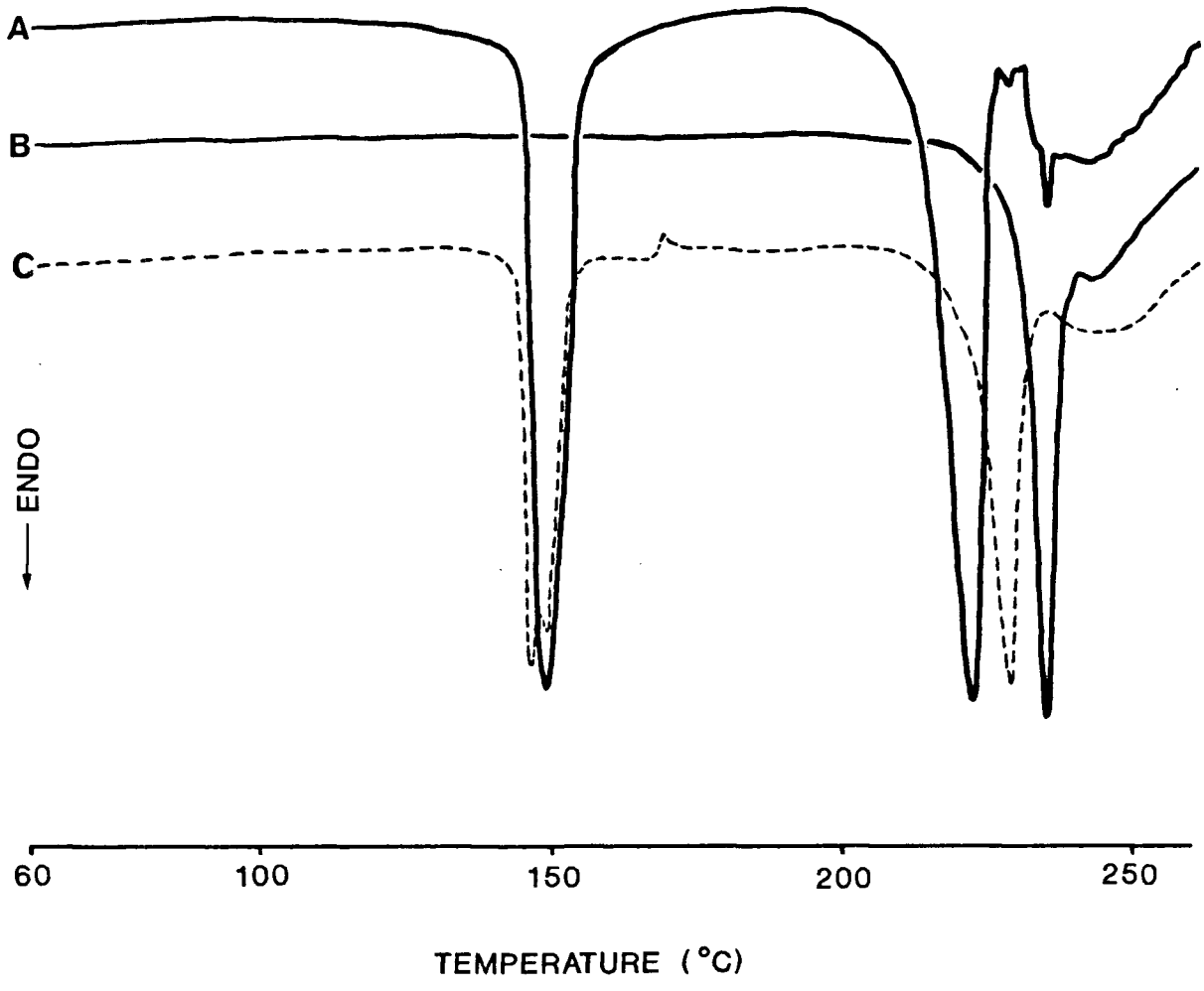


Fig. 5. DSC thermograms of representative samples of lactose (heating rate 10°C/min). A: an α-rich sample (L80S, open pan); B. a β-rich sample (β-CCF, open pan); C. an α-rich sample (L80M, sealed pan).

Table VII. Temperatures of dehydration and melting peaks of different grades of lactose (heating rate 10°C/min).

Lactose Grade	Temperature (°C) at dehydration peaks			Temperature (°C) at melting peaks		
PANS[a]:	A	B	C	A	B	C
α-L	144	148[d]	146[c]	215	224	216
L60S	145[b]	149[b]	148	218	222	220
L80S	145[c]	150	148	218	227	211[b]
L80M	145	148[d]	145	217	228	220[b]
LCAPS	145	148[d]	145	217	230	221[b]
IPL-S	144	146[d]	144	216	226	218
IPL-F	144	147[d]	145	217	228	219
SPL	144[c]	144[c]	145[c]	216[e]	229	219[b]
FFL	144	147[d]	144	215	226	218[b]
DCL-11	140[c]	145[d]	146[d]	217	226	219
DCL-30	145[d]	146	146[d]	219	215	220
DCL-21	127[e]	133[e]	128[e]	236	234	237
ANL	133[e]	135[e]	134[e]	235	234	236
β-BAK	129[e]	131[e]	132[e]	235	232	236
β-DMV	-	-	-	213[b]	214[b]	214[b]
β-CCF	-	-	-	235	236	236
SANL	-	-	-	-	-	219
UANL	-	-	-	211	207	-

a. A- open pans; B- sealed pans; and C- sealed pans with a small pinhole.
b. Shoulder at the leading edge of the peak.
c. Shoulder at the lagging edge of the peak.
d. Splits into two peaks.
e. Very broad peak.

Presence of new peaks when sealed pans are used

A feature not referred to in Table VII is the presence of a second endothermic peak immediately followed by an occasional exothermic peak between the dehydration and the melting peaks when sealed pans are used. In an earlier report, Lerk *et al.* (1984) thought that the peaks were due to melting (endothermic) of unstable anhydrous α-lactose (formed due to dehydration) and its subsequent recrystallization (exothermic) into a new form that melted at a higher temperature. This was supported by an observation that the two new peaks were invariably present in the case of an unstable anhydrous lactose sample irrespective of the pan type employed. However, in the present study, thermal microscopy of α-lactose monohydrate did not reveal such a melting. Thus, the endotherm must be due to some other reaction.

It is known that lactose undergoes anomeric conversion during the DSC runs (Fernandez-Martin *et al.*, 1980; Lerk *et al.*, 1984). It is also known that the mutarotation of lactose in aqueous solutions involves a bond breaking in the glucose moiety of the lactose molecule and rearrangement of the hydroxyl group at carbon-1 to a new anomeric configuration (Hine, 1956; Morrison and Boyd, 1987). Such a break up of a bond requires absorption of energy, and if it occurred during the DSC runs, then it would appear as an endotherm on the thermograms. The second endothermic peak can be attributed to such an anomeric conversion, rather than melting, based on the following evidences:

i. Analysis of an α-rich sample (L80S, particle size 106-149 μm, heating rate 10°C/min) before and after the endothermic peak disclosed a considerable degree of anomeric conversion (from the initial 98.4% α to 8.9% α after the peak).

ii. Lactose undergoes degradation to monosaccharides when it melts. These monosaccharides have been reported to appear as separate chromatographic peaks if a GC analysis of the molten samples is carried out (Fernandez-Martin *et al.*, 1980). In the present study, no such peaks were seen during the GC analysis of the lactose samples after the second endothermic peak.

iii. As mentioned earlier, the thermal microscopy did not reveal any melting at the temperatures of the second endotherm.

The fact that the exotherm is only occasionally observed can be explained on the basis of the erratic nature of the process of recrystallization. Recrystallization requires the initial formation of nuclei. Nucleation is dependent on experimental conditions. In the case of lactose, one such condition would be the formation of sufficient β-anomer before the initial sample (α-lactose monohydrate) can recrystallize into the new crystals of β-lactose. Even if enough anomeric conversion has occurred, the nuclei formation and subsequent crystal growth may not occur, because this involves rearrangement of the lactose molecules into a new crystal lattice. In the solid state, such a rearrangement would be restricted, and the recrystallization would be only a matter of chance leading to only an occasional exothermic peak.

Shifting of melting peak to a higher temperature in sealed pans

Although there is a lack of agreement between the literature values of the melting points of individual lactose anomers, it is clearly established that the melting point of β-lactose is higher than that of α-lactose (Olano *et al.*, 1983). This fact is documented by the data in Table VII (except for β-DMV).

The previously reported observation (Fernandez-martin *et al.*, 1980; Lerk *et al.*, 1984) that lactose undergoes anomeric conversion during the DSC runs has now been confirmed by the present experiments. The data in Table VIII show that the conversion occurs to a greater degree in a sealed pan (where water vapor cannot escape from the pan) than in an open pan (where the water formed due to dehydration is lost by vaporization). Thus, the conversion appears to require the presence of water vapor formed upon dehydration of the α-lactose monohydrate. As the sample in a sealed pan becomes richer in β-content, it melts at a higher temperature giving rise to a shift of the melting point of the starting α-rich lactose sample (as determined in an open pan) to a higher temperature (Table VII).

This is further supported by the fact that one of the specially prepared samples of lactose, β-CCF (98.1% β), melts at a higher temperature than the α-rich samples. The sample was prepared by heating an α-lactose monohydrate sample in a steel-bomb in an oven for a prolonged period (Visser, 1988). The bomb prevented escape of water due

Table VIII. Change in anomeric composition during DSC runs; initial sample: L80S (98.4% α).

Heating Rate (°C/min)	Particle Size (μm)	Pan Type	Temperature[a] (°C)	% α Content[b]
2.5	< 75	B	157	6.6 ± 0.2
	75-106	B	162	6.6 ± 0.3
	106-149	B	160	6.6 ± 0.3
	149-180	B	160	7.5 ± 0.3
	180-250	B	160	7.2 ± 0.1
	106-149	A	165	82.9 ± 0.1
10	106-149	B	156	46.0 ± 0.1
20	106-149	B	159	77.0 ± 0.1

a. The temperature at which samples were removed from the DSC cell for GC analysis.
b. Determined by GC method; average ± 1 s.d., 3 injections of one sample each.

to vaporization and provided an analogous situation to that of the sealed pans.

Dependence of anomeric conversion on other experimental conditions

Apart from the type of pan used, the conversion of α to β-form is expected to depend on other experimental conditions as well. These conditions include the following.

i. Initial particle size of the sample: since the anomeric conversion requires the presence of water vapor, it is expected to depend on the exposed surface area, and hence the particle size, of the lactose crystals.

ii. Heating rate: since the heating rate controls the time allowed for the crystals to stay in contact with the water vapor, it is also expected to affect the extent of the anomeric conversion.

The results (Table VIII) suggest that the presence (as in a sealed pan) and the duration of the presence (dependent on the heating rate) of water vapor are more important factors than the initial particle size of the samples.

Formation of a β/α-lactose compound during DSC runs

Lerk *et al.* (1984) reported the formation of a β/α-lactose compound crystal at approximately 200°C when an α-lactose monohydrate

sample was heated in a DSC pan at 2°C/min. The "compound crystal" was shown to have an α/β ratio of 1:1. The pan type used was not specified. Thermograms similar to those reported by these authors were obtained in the present study only in the sealed pans. Therefore, it could be assumed that Lerk *et al.* (1984) used sealed pans in their study. The results presented in Table VIII show that, at a similar heating rate of 2.5°C/min in a sealed pan, the conversion to β-lactose is almost complete (only ≈7% α remaining) at a lower temperature (≈160°C) than 200°C reported by Lerk *et al.* (1984). The conversion is not as complete (as much as 82.9% α remaining) when an open pan is used at 2.5°C/min. An approximately 1:1 β/α ratio is obtained at a heating rate of 10°C/min in a sealed pan, again at a lower temperature (156°C). The conversion of the α to β-lactose is even lower (77% α remaining at 159°C) at 20°C/min. These results indicate that the formation of a β/α-lactose compound crystal as reported by Lerk *et al.* (1984) is merely an artifact of the experimental conditions, since other anomer ratios can be obtained by changing the pan type and heating rate.

Lerk *et al.* (1984) described the lactose sample with 1:1 α/β ratio as a "β/α-lactose compound" based on the x-ray diffraction patterns of this sample and of α-lactose monohydrate (4% β), β-lactose, stable anhydrous α-lactose (20% β) and unstable anhydrous α-lactose (18% β). An examination of the x-ray diffraction patterns published by these authors indicates that β/α lactose compound crystals have some matching peaks with each of the above samples. The relative intensities of the peaks are, however, different. Simpson *et al.* (1982) indicated a

similar match up between the x-ray diffraction peaks of two anhydrous lactose samples, 5:3 and 4:1 α:β compounds (prepared by methanol dehydration), while the relative peak intensities differed. They attributed the match up of the peaks to essentially identical unit cells of the two samples, and the intensity differences to differences in the spatial compositions of the unit cells. Therefore, it is possible that the samples studied by Lerk *et al.* (1984) had the same unit cell, and the intensity differences were due to differences in the spatial composition of the crystal lattice. It is also possible that the partial conversion of the anhydrous α-lactose sample to the β-form, as found in the present work, may give rise to more than one anhydrous phase in the 1:1 α/β lactose sample. This also would explain why the peaks in the different samples matched up and why the peak intensity differences occurred. This possibility cannot be ruled out on the basis of the information provided by Lerk *et al.* (1984). In such a situation, the sample produced by the thermal treatment on the DSC should not be called a new "compound" as a new compound would be expected to possess a completely new crystal structure and, hence, a completely different x-ray diffraction pattern.

On the other hand, Simpson *et al.* (1982) showed, using a density gradient column, that the 5:3 or 4:1 compounds formed only one band in the column indicating that these samples consisted of only one homogeneous crystalline phase each. Nevertheless, the two samples had matching peaks in their diffraction patterns suggesting that they were not two different compounds.

Splitting of dehydration peak in a sealed pan

In a sealed pan the dehydration peak splits into two distinct components. This observation was reported earlier (Lerk *et al.*, 1980; Berlin *et al.*, 1981), but was left unexplained. In our view, this was an important feature of the lactose thermograms.

In a sealed pan the water lost by the sample due to dehydration is retained within the pan. The pan being a hermetically sealed system, the water cannot completely vaporize and, an equilibrium will exist between liquid water and water vapor. When lactose dehydrates, it leaves an anhydrous solid. This would dissolve in the liquid water. It is known that the dissolution of an anhydrous solid is exothermic. Therefore, the dissolution of anhydrous lactose would give rise to an exothermic peak. Since this would happen at the same time as the dehydration, the overall result would be an overlapping of an exothermic peak and an endothermic peak. Thus, the exothermic peak of dissolution appears as a split in the endothermic peak of dehydration. It is also to be expected that the part of the sample, which is still a monohydrate, would also dissolve and contribute to the endothermic peak, because the dissolution of α-lactose monohydrate is endothermic.

The extent of splitting is expected to increase as the extent of dissolution increases. The extent of dissolution can be controlled by varying the heating rates. This was indeed the case as demonstrated by Fig. 6. The peak splitting became more significant at slower heating

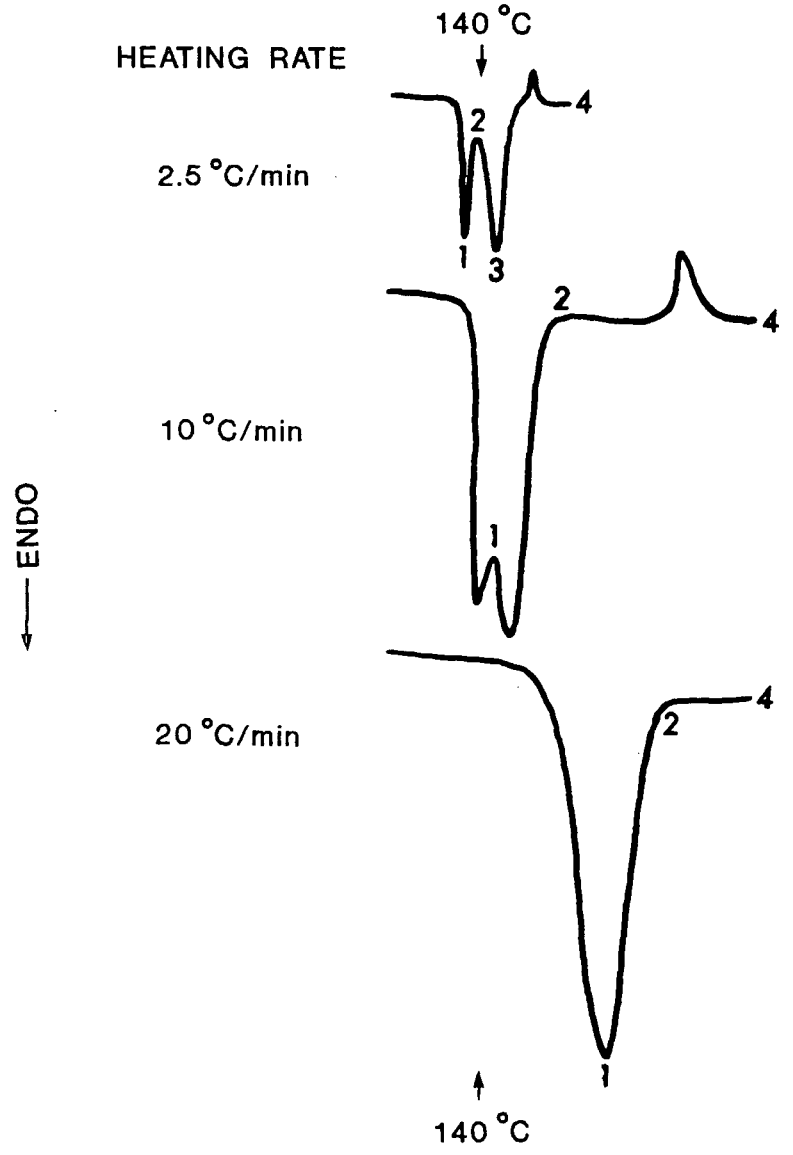


Fig. 6. Dependence of splitting of lactose dehydration peak on heating rate (sample: L80S; particle size: 106-149μm; sealed pan). Numbers on the thermograms refer to Table IX. These numbers are placed to identify those points on the thermograms where the DSC runs were stopped for removing samples for GC analysis and do not necessarily show the location of similar points on the different thermograms.

rates. At 20°C/min, there occurred only a shoulder; at 10°C/min the shoulder became prominent or, in some cases, even developed into a split (Table VII), and at 2.5°C/min the split was almost up to the baseline.

Other causes of splitting of dehydration peak

Dissolution at the temperatures of the dehydration peak of α-lactose monohydrate would favour the formation of the β-anomer. This anomer is anhydrous, therefore, its dissolution also will be exothermic. If the temperature of a saturated solution of a solid with an exothermic heat of solution is raised, the solid recrystallizes from the solution in accordance with the Le Chatelier's principle (Mullin, 1972). During the DSC scan, the temperature of the sample is increased at a constant rate, which would result in the recrystallization of the β-anomer from its solution due to the above reason. Since recrystallization is also an exothermic process, it would also contribute to the exothermic split in the dehydration peak.

Dissolution of lactose in sealed pans during the DSC runs

The dissolution of lactose during the dehydration peak was confirmed by a visual observation of the lactose crystals taken out immediately after the exothermic split. The crystals were bonded together by an apparently continuous and glass-like phase. When the sample was removed from the DSC sample pan and processed for its GC analysis, this phase, like β-rich products, did not dissolve in DMSO as

rapidly as the bulk of the crystals which suggested that it was a β-rich phase. Since melting at this stage of the DSC runs was ruled out based on the GC and thermal microscopic evidences as before, the possibility that the phase was formed due to rapid cooling of a melt when the pan was removed from the DSC cell did not exist. Thus, this phase could only be formed from a solution. The solution recrystallized very quickly due to the rapid cooling giving a glass-like appearance to the product. Dissolution of lactose at the temperature of the dehydration peak would cause accelerated mutarotation of α to the β-anomer, hence the glass-like phase would be rich in the β-anomer and would dissolve slowly in DMSO during the GC analysis.

It is important to realize at this stage that the amount of lactose dissolved and the extent of recrystallization over the short range ($\approx$5°C) of temperatures encountered during the exothermic split are expected to be very small. The relative importance of these processes in affecting the appearance of the lactose thermograms can be established by determining the energy changes involved in each process. This would be complicated by the fact that the dehydration, the dissolution and the recrystallization, all occur at the same time. Hence, it would be difficult to isolate the relative contributions of each process to the total energy change.

Components of the split peak

The split peak has three components, an initial endotherm corresponding to dehydration, an exothermic component, and a third endothermic component. The reasons that give rise to the first two components have been mentioned before. The last endotherm could be due to a still continuing dehydration and/or anomeric conversion in the solid state as explained earlier. Figure 6 and the data in Table IX indicate that the samples lost weight throughout the split peak. Moreover, most of the conversion occurred at this last endotherm, especially when the heating rate was 2.5°C/min. Thus, this last endotherm was due to both dehydration of the part of the sample that was still hydrated, and anomeric conversion.

The endotherm corresponding to the anomeric conversion does not appear as a sharp peak. The conversion occurs over a range of temperature. Thus, it appears only as a shift in the baseline. The shift is most apparent at the heating rate of 10°C/min. At the heating rates of 2.5 and 20°C/min, the shift is absent although the anomeric conversion does occur.

Thermal behavior of UANL

The unstable anhydrous α-lactose was prepared by rapid dehydration of L80S by heating *in vacuo*, and concurrent removal of water vapor from the vacuum oven by P_2O_5 (Section 2.4). This sample produced an

Table IX. Anomeric conversion and weight loss of L80S (106-149 μm, initial α-content, 98.4%).

Heating Rate (°C/min)	Pan Type	Observation Point[a] (Temperature[b], °C) 1	2	3	4
			% α-anomer content[c]		
2.5	B	--	80.7(140)	64.8(142)	6.6(160)
10	B	93.3(145)	74.8(154) 46.0(156)	--	8.9(180)
20	B	78.2(152)	77.3(159)	--	63.2(180)
			% weight loss		
2.5	A	--	2.98	3.85	5.37
	C	--	2.90	3.47	--

a. Refer to Fig. 6.
b. The temperature at which samples were removed from the DSC cell for GC analysis.
c. As determined by the GC method.

interesting thermogram (Fig. 7) in which an endothermic peak (160-170°C) was immediately followed by an exotherm (≈177°C). The endotherm was reported (Lerk *et al.*, 1984) to be due to melting of the unstable anhydrous lactose, followed by its recrystallization (the exotherm) into another form which melted at a higher temperature (211°C). In the present study, no melting was observed when thermal microscopy of UANL was performed. Moreover, GC analysis of this sample at various points on the first endotherm (Table X) indicated a significant degree of anomeric conversion from the initial α to the β-anomeric form. Absence of new GC peaks due to the products of degradation (monosaccharides) also ruled out melting. The endotherm should thus be attributed to anomeric conversion (see page 55) rather than to melting.

Since there was a major change in the initial anomeric composition, recrystallization of lactose into a new form was expected. This appeared as the exotherm. However, the degree of anomeric conversion was not as marked as in the case of crystalline α-lactose monohydrate and the melting point did not shift to a higher temperature (Table VII).

A short summary of various reactions that occur during the thermal transitions of lactose is presented in Scheme II.

Weight loss of lactose samples during DSC runs

The total expected weight loss for an α-lactose monohydrate sample

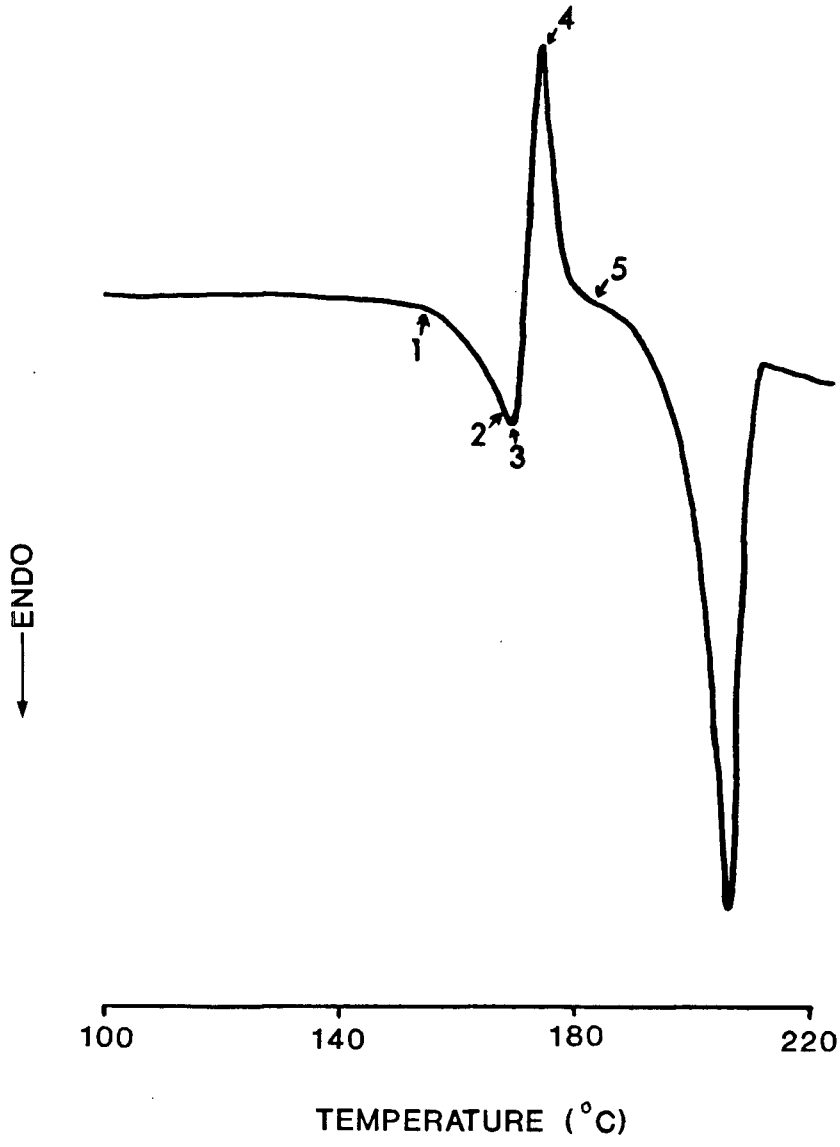


Fig. 7. DSC thermogram of UANL (open pan, 10°C/min). Numbers on the thermogram refer to Table X.

Table X. Anomeric conversion during the DSC runs of UANL (initial α-content, 92.9%).

Pan Type	Observation Point[a]	Temperature[b] (°C)	%α[c]
A	1	159	90.5
A	2	173	72.4
A	3	174	63.7
A	4	177	64.1
A	5	183	63.2

a. Refer to Fig. 7.
b. The temperature at which samples were removed from the DSC cell for GC analysis.
c. As determined by the GC method.

Scheme II

Thermal transitions of lactose samples on a DSC

Pan Type[a]	Reactions[b]	Peak Maxima (°C)
A[1]	α-L.M ⟶ α-L + H_2O(l) (98.4%α) ⟶ α-L + β-L + H_2O(v) (82.9%α)	140-145
	α-L + β-L ⟶ melt	213-219
B[1]	α-L.M ⟶ α-L + H_2O(l) (98.4%α) (dissolution) ⟶ α-L + β-L + H_2O(v)	144-150
	α-L ⟶ α-L + β-L (anomerization; 7%α)	≈170
	α-L + β-L ⟶ α-L + α-L.β-L (recrystallization)	≈180
	α-L + α-L.β-L ⟶ melt	222-230
A,B[2]	β-L + α-L.M ⟶ β-L + α-L + H_2O(l) ⟶ β-L + α-L + H_2O(v)	127-135
	β-L + α-L ⟶ melt	232-234
A,B[3]	β-L ⟶ melt	235-236

a. A = open pan; B = sealed pan.
b. α-L.M = α-lactose monohydrate; l = liquid; v = vapor.
1. Representative samples: L80S, α-L, L80M, L60S.
2. Representative samples: DCL-21, ANL, β-BAK.
3. Representative sample: β-CCF.

upon complete removal of the water of crystallization is about 5%. If sorbed moisture is also present then the total weight loss would be more than 5%. Most α-rich samples showed a weight loss corresponding to their content of α-lactose monohydrate (Table XI).

In the case of ANL, β-BAK, DCL-11, DCL-21 and DCL-30, which contain significant amounts of both of the anomers, the loss in weight should correspond with the amount of α-lactose present if all of it was assumed to be a monohydrate. While the assumption was found to be approximately true in the case of DCL-11, DCL-21 and DCL-30, the ANL and β-BAK showed weight losses less than expected (Table XII). Since the latter two did show the dehydration peak (Fig. 8), it can be concluded that the α-lactose present in them was only partially hydrated. The results show clearly that DCL-30, DCL-21 and ANL are not anhydrous products as claimed by Vromans *et al.* (1985) and Sheffield Product Information (1986). Part of the weight loss shown in Tables XI and XII is presumably due to the loss of sorbed moisture. The amount of sorbed-moisture was determined separately and is dealt with in Section 3.6.

3.4 POWDER X-RAY DIFFRACTION PROPERTIES OF LACTOSE SAMPLES

General Observations

The diffraction patterns and the peak parameters of an α-rich sample (L80S, 98.4% α), a sample containing significant amounts of each

Table XI. Weight lost by lactose samples after the peak of dehydration (determined by weighing the samples in pan C).

Lactose Grade	Weight Lost %
α-L	5.72
L60S	4.73
L80S	4.44
L80M	5.46
LCAPS	5.15
IPL-S	6.11
IPL-F	5.80
SPL	6.77
FFL	5.68
DCL-11	5.77
DCL-30	2.60
DCL-21	1.56
ANL	1.16
β-BAK	1.19
β-DMV	0.40
β-CCF	0.90
SANL[a]	--
UANL[a]	--

a. Samples prepared and stored in a P_2O_5 desiccator.

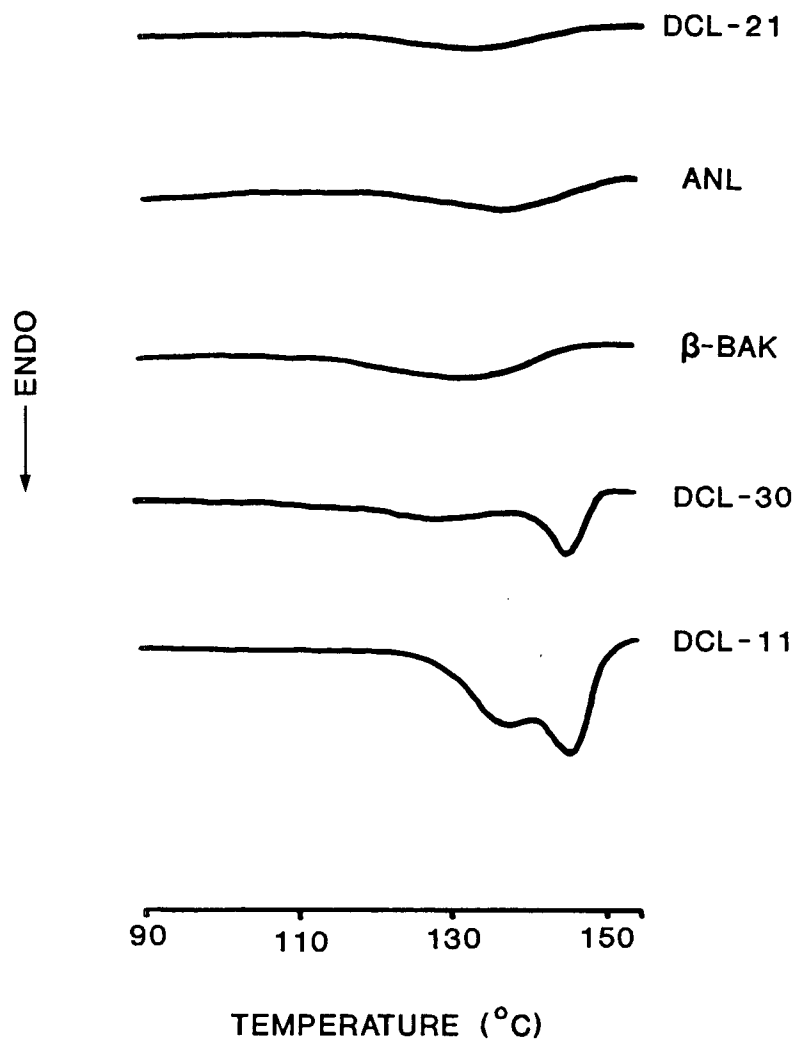


Fig. 8. DSC thermograms of lactose samples showing dependence of dehydration peaks on α-content (sealed pans, 10°C/min). Refer also to Table XII.

Table XII. Comparison of expected and actual weight loss after the peak of dehydration.

Lactose Grade	% α	Weight Loss (%)	
		Expected[a]	Actual[b]
DCL-21	23.3	1.17	1.56
ANL	26.1	1.31	1.16
β-BAK	30.6	1.53	1.17
DCL-30	85.2	4.26	5.60
DCL-11	89.8	4.47	5.77

a. Determined assuming that all of the α-lactose is in monohydrate form.
b. Determined by weighing the samples in pan C after the peak of dehydration.

anomer (β-BAK, 69.4% α), and a β-rich sample (β-CCF, 98.1% β) are given in Figs. 9-11 and the accompanying Tables XIII to XV. All α-rich samples produced diffraction patterns resembling that of L80S. The β-rich samples showed patterns similar to that of β-CCF. Samples containing significant proportions of both anomers (DCL-21 and ANL) had diffraction patterns similar to that of β-BAK.

Samples SANL and UANL have different crystal structures from L80S (from which these were prepared) and other samples as indicated by the presence of some new peaks in their diffraction patterns (Tables XVI and XVII and Fig. 12 and 13 respectively). The major peak in the powder x-ray diffraction pattern of SANL is at 19.6°2θ, while the major peak of UANL is at a much lower angle of 11.9°2θ. However, there is some match up of peak positions with other samples of lactose. This indicates that the thermal dehydration changes the crystal structure of α-lactose monohydrate to some extent, but apparently the unit cell remains the same. Also, the change in the crystal structure is dependent on the conditions of dehydration (see the methods of preparation of UANL and SANL, page 40).

Change in powder x-ray diffraction patterns with anomeric composition

Figure 14 shows the changes in the diffraction patterns of some selected samples of lactose with their anomeric composition. An examination of the figure suggests that the diffraction patterns can be classified into at least three types:

Table XIII. Major peaks in the powder x-ray diffraction pattern of L80S.

2θ	d(Å)	I/I_{100} (%)
12.70	6.97	26.9
16.65	5.32	30.9
19.28	4.60	11.2
19.75	4.49	17.8
20.14	4.41	100.0
21.39	4.15	22.4
36.41	2.47	17.2
37.11	2.42	20.0
37.67	2.39	10.1
38.40	2.34	17.8

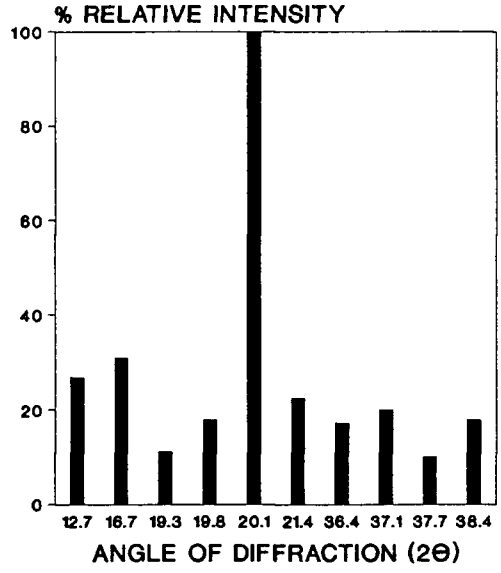


Fig. 9. Powder x-ray diffraction pattern showing major peaks of an α-rich sample (L80S).

Table XIV. Major peaks in the powder x-ray diffraction pattern of β-BAK.

2θ	d(Å)	I/I_{100} (%)
10.69	8.27	27.8
19.25	4.61	54.8
19.71	4.50	23.9
20.09	4.42	19.2
20.81	4.27	36.5
21.13	4.20	100.0
23.83	3.73	18.7
25.68	3.47	21.2
31.84	2.81	19.8
36.67	2.45	19.0

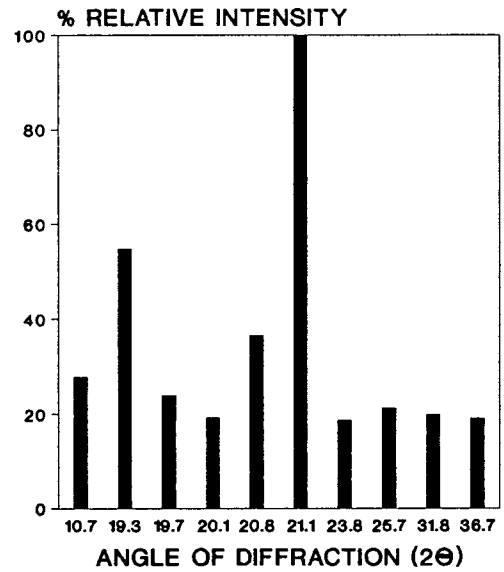


Fig. 10. Powder x-ray diffraction pattern showing major peaks of a sample containing significant amounts of both anomers (β-BAK).

Table XV. Major peaks in the powder x-ray diffraction pattern of β-CCF.

2θ	d(Å)	I/I_{100} (%)
10.70	8.27	30.6
19.29	4.60	52.1
20.84	4.26	39.7
21.14	4.20	100.0
23.86	3.73	19.5
24.14	3.69	15.7
25.69	3.16	25.5
28.22	3.16	14.8
31.72	2.82	15.1
36.72	2.45	18.8

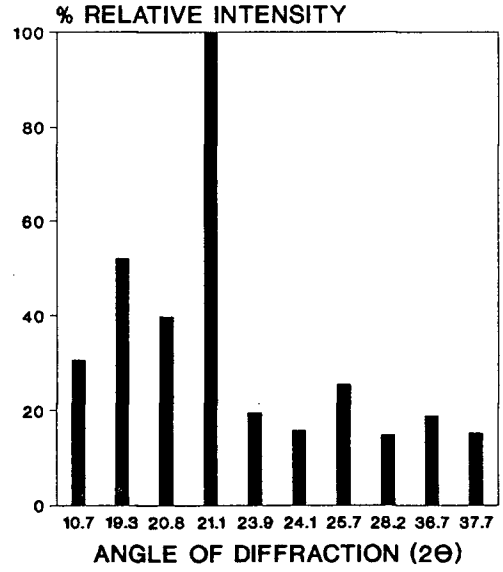


Fig. 11. Powder x-ray diffraction pattern showing major peaks of a β-rich sample (β-CCF).

Table XVI. Major peaks in the powder x-ray diffraction pattern of SANL.

2θ	d(Å)	I/I_{100} (%)
12.55	7.05	17.8
18.22	4.87	19.6
18.73	4.74	21.4
19.24	4.61	60.9
19.61	4.53	100.0
20.15	4.20	39.4
21.38	4.16	21.6
23.39	3.80	34.5
27.25	3.27	20.5
36.07	2.49	25.5

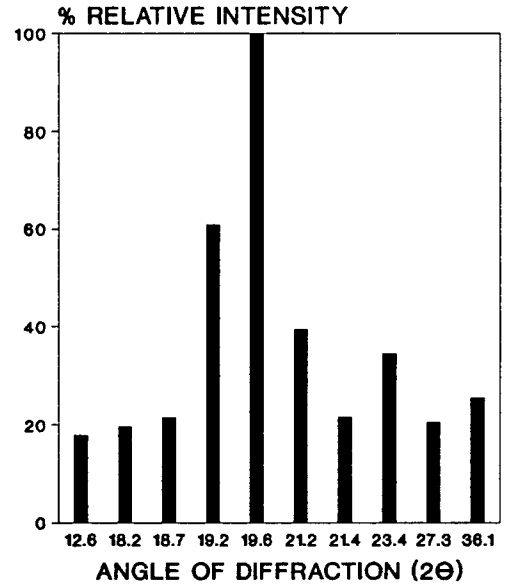


Fig. 12. Powder x-ray diffraction pattern showing major peaks of stable anhydrous α-lactose (SANL).

Table XVII. Major peaks in the powder x-ray diffraction pattern of UANL.

2θ	d(Å)	I/I_{100} (%)
11.86	7.46	100.0
12.69	6.98	80.4
18.14	4.89	46.0
19.28	4.60	62.2
20.10	4.42	17.9
20.81	4.27	30.8
21.61	4.11	35.6
24.08	3.69	16.6
25.58	3.48	17.2
36.13	2.49	18.0

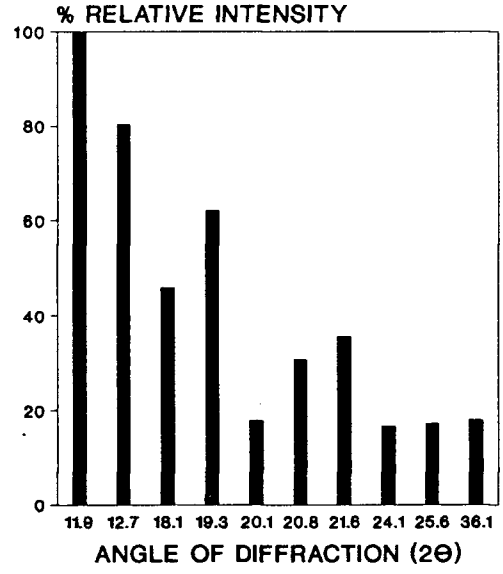


Fig. 13. Powder x-ray diffraction pattern showing major peaks of unstable anhydrous α-lactose (UANL).

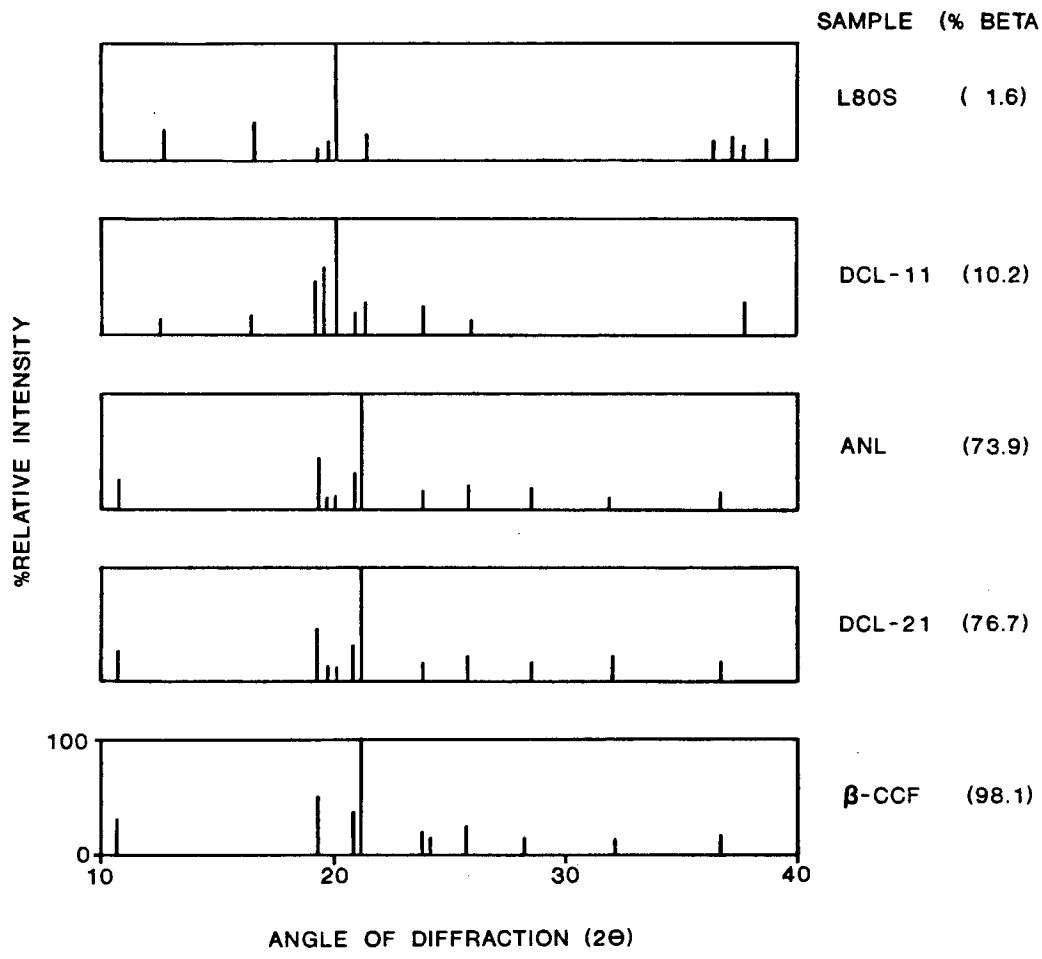


Fig. 14. Changes in powder x-ray diffraction patterns with anomeric composition (determined by GC) of selected lactose samples.

i. patterns corresponding to the α-rich samples (L80S) with the major peak at around 20.1°2θ;

ii. patterns corresponding to samples containing significant proportions of both anomers (β-BAK, ANL, and DCL-21) showing the major peak around 21.1°2θ with less intense peaks at 19.3°2θ and 20.1°2θ which correspond to α-rich samples;

iii. patterns corresponding to β-rich samples with the major peak at 21.1°2θ (β-CCF).

The shift of the major peaks from lower to higher 2θ values (i.e., to lower interplanar spacings) for lactoses of higher β-content than α-rich samples indicates that the crystal lattice of β-lactose is more constricted than α-lactose monohydrate. This is because the size of the β-lactose molecules is smaller than that of the α-lactose monohydrate molecules. This suggests that the true density of β-lactose samples should be greater than α-lactose monohydrate. This is supported by the true density data presented in Table XX (Section 3.5).

As mentioned above, β-BAK, ANL and DCL-21 show peaks in their diffraction patterns that are characteristic of α and β anomers. It is possible, therefore, that these samples contain the two anomers as a simple mixture. If this were true, then the relative peak ratio should correspond with the relative anomeric amounts present in the samples. This quantitative relationship will be discussed in the remaining part of this section.

Quantitative use of powder x-ray diffraction

In order to try to determine the state in which one anomer is incorporated in a product consisting mainly of the other anomer, it was decided to use the powder x-ray diffraction of lactose quantitatively.

It is known that in the case of a mixture of two crystalline substances, the powder x-ray diffraction pattern is a superimposition of the diffraction patterns of the two substances. A quantitative relationship exists between the relative peak-intensities and the relative amounts of the two components (Cullity, 1978a). If, in a given lactose sample, the two anomers are present as a simple physical mixture, then the powder x-ray diffraction pattern of the sample would show peaks characteristic of the two anomers. The relative intensities of these peaks would correspond with the relative amounts of the two anomers. To test this hypothesis, however, it would be necessary to first obtain the diffraction patterns of 100% pure anomeric samples. Such samples are not available. The quantitative relationship between peak-intensities and anomeric compositions was tested by a procedure described in Section 2.5. This procedure involved certain precautions which are discussed as follows.

A. Selection of particle size

In a situation where a given product is suspected to consist of different phases in the solid state, as was the case for lactose (the

suspected phases being α-lactose monohydrate and β-lactose), the particle size selected for quantitative powder x-ray diffraction is of importance. Grinding and subsequent sieving of the samples to obtain just enough material of a given particle size for recording an x-ray scan is prone to errors. This is because a particular phase may be more brittle than the other(s), and, therefore, would be present in a greater quantity in the undersize sieve-fraction than in the oversize fraction. This would lead to the determination of incorrect relative peak-intensity ratios. To avoid such an error, the lactose samples were first weighed, mixed with LiF, and then ground just enough to completely pass them through the sieve #200 (particle size 75 μm). This ensured that the whole sample, not just a part, was used.

B. Avoiding microabsorption effect

The microabsorption effect arises when the linear absorption coefficients of the components of a mixture, or their particle sizes, are considerably different. The difference in the linear absorption coefficient leads to errors in the quantitative results due to incorrect intensity calculations (Cullity, 1978b). The linear absorption coefficients (μ) of the lactose anomers are very close to each other ($\mu_{\alpha H2O}$=11.6664 cm^{-1} and μ_{β}=11.8579 cm^{-1}) (Appendix A). Thus, this factor is not expected to cause errors in the intensity ratio calculations. Since the particle size of the different mixtures was the same, the microabsorption effect could be considered to have been avoided.

C. Use of LiF as an internal standard

In order to eliminate the effect of amorphous scattering and instrumental variations such as fluctuations in the x-ray source, an internal standard was employed. Lithium fluoride is a suitable internal standard for lactose as it belongs to the highly symmetrical cubic crystal system which has only a few intense and non-interfering peaks. Lactose belongs to the monoclinic crystal system (Beevers and Hansen, 1971; Hirotsu and Shimada, 1974). Also, LiF is believed to dilute the samples under study and, thus, avoid the problem of their preferred orientation (Otsuka and Kaneniwa, 1983). In the concentration (50% w/w) of LiF used in this study, the preferred orientation of lactose samples can be assumed to be nominal. This is also evident from the data presented in Table XVIII (see below for discussion).

The intensity ratio vs α-content plot for the mixtures

The plot of the maximum peak-intensity ratio (lactose/LiF) against the calculated α-content was a straight line (Table XVIII, Fig. 15) with an intercept on the x-axis. This proved that there would indeed exist a quantitative relationship between the anomeric composition and the x-ray peak intensities if a given lactose sample contained the two anomers as a simple physical mixture. It is necessary to point it out at this stage that, in quantitative powder x-ray diffraction studies, a plot of integrated peak-intensities is more reliable than a plot of maximum peak-intensities. However, if the particle size of the samples is not

Table XVIII. Relationship between peak-intensity ratio and the anomeric composition of lactose.

% α-content[a]	Lactose/LiF Intensity Ratio (± 1 s.d., n=3)
14.48	0.104 ± 0.016
23.74	0.166 ± 0.010
33.03	0.331 ± 0.027
42.41	0.445 ± 0.010
51.74	0.493 ± 0.021
61.18	0.590 ± 0.014
70.47	0.683 ± 0.029
79.72	0.871 ± 0.026
89.03	0.969 ± 0.038

Regression analysis:

r^2: 0.987

Slope: 0.0115 Standard Error : 0.0005
95% C.I.: 0.0103 to 0.0126

Intercept:-0.077 Standard Error : 0.0284
95% C.I.:-0.144 to -0.0099

a. The amount of LiF was maintained at 49.97 ± 0.04%; thus % α could be plotted instead of Lactose/LiF amount ratio. Samples used to prepare the mixtures: L80S and β-DMV.

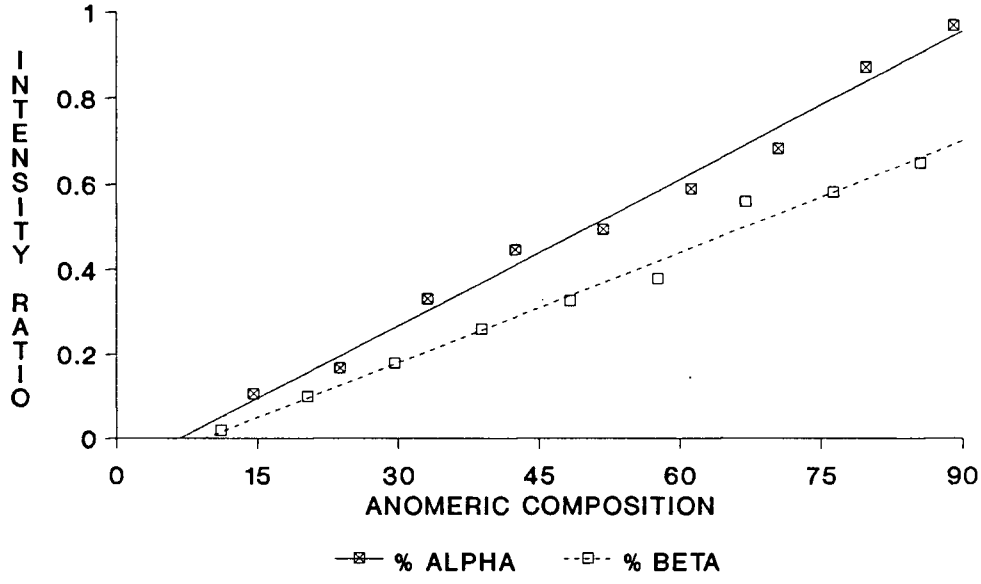


Fig. 15. Plot of lactose/LiF x-ray peak intensity ratios with anomeric composition (determined by GC).

variable, as in the present case, the plot of maximum peak-intensities can be reliably used (Cullity, 1978c).

Figure 15 shows the plots of the peak intensity ratios (anomeric peak/ LiF peak) with respect to the % α and % β-content. The x-axis intercepts of the plots were significantly different ($p=0.05$) from 0%. The intercept of 6% α for the plot of α-L/LiF peak intensity ratios against % α indicates that the 5.1% α (GC determination; Table VI, page 50) in β-DMV is probably incorporated inside the β-crystals. Similarly, the intercept of 9% β for the plot of β-L/LiF peak intensity ratios against % β indicates that the 1.6% β in L80S is probably incorporated inside the α-lactose monohydrate crystals. Although the powder x-ray diffraction does not reveal the exact nature of this incorporation, it certainly suggests that the anomeric impurities in the lactose samples (L80S and β-DMV) are not present as a simple physical mixture.

To test the quantitative relationship between the anomeric composition and the x-ray peak-intensities in the commercial samples of lactose, mixtures of these samples as received from the suppliers were prepared with LiF in 1:1 ratio. Their diffraction patterns were recorded and the relative amount of the anomers in each sample was obtained by determining the relative peak-intensities of the most characteristic peak of each anomer (Section 2.5). The diffraction patterns of some samples (L60S, SPL, FFL, and DCL-30) did not show one or other of the two characteristic anomeric peaks; therefore, their relative anomer amounts could not be determined by this method.

Although only the lactose peak-intensities were used for the relative anomer content determination, LiF was retained in each mixture in order to test for any instrumental variation. The LiF peak intensities showed a coefficient of variability of 6.7% (n=18), which is a good degree of precision considering the large number of factors that can affect the peak intensities. Such factors include preferred orientation, powder packing uniformity between different x-ray scans, homogeneity of the mixtures prepared and instrumental stability.

The lactose amounts determined by using the x-ray peak intensities as above were compared with the anomeric compositions determined earlier by the GC method. The data are presented in Table XIXA and XIXB. Since ANL, DCL-21 and β-BAK contain significant amounts of each anomer, their x-ray patterns are expected to provide better information on the physical state of the two anomers. It appears that although their diffraction patterns do contain peaks that can be attributed separately to the two anomers, the peak-intensity ratios do not reflect the true anomer ratio (GC determination). On an average, the amount of β-anomer estimated by the x-ray method is 12.8% less than that expected according to the GC results. The underestimation of the amount of β-lactose by the x-ray method indicates that the difference in β-content is possibly due to a partial incorporation of the β-anomer inside the crystal lattice of α-lactose.

Comparison of the anomeric compositions obtained by the x-ray and

Table XIXA. Comparison of the anomeric composition as obtained from powder x-ray diffraction and GC methods.

Lactose Samples	Anomeric Composition (% α)	
	GC method	x-ray method
ANL	26.1	12.7
DCL-21	23.3	12.8
β-BAK	30.6	16.1

Table XIXB. Comparison of the amount of the minor anomeric component as obtained from powder x-ray diffraction and GC methods.

Lactose Samples[a]	Minor Component (% β or α)	
	GC method	x-ray method
α-L	1.8	5.9
L80S	1.6	8.8
L80M	1.9	7.7
IPL-S	1.8	7.7
IPL-F	3.1	7.5
LCAPS	1.8	5.5
DCL-11	10.2	14.7
β-CCF	1.9	10.9
β-DMV	5.1	11.0

GC methods for the remaining samples shows that the x-ray method overestimates the amount of the minor component (Table XIXB). In such a situation, the overestimation may occur due to the presence (and overlapping) of a peak from the major component in the region of the diffraction pattern where the most characteristic peak of the minor component is expected to be present.

As far as the sensitivity of the x-ray diffraction technique in estimating the amounts of a given phase in a physical mixture is concerned, it has been used to detect as low as 1% of a given phase incorporated into another phase as a simple physical mixture (Cullity, 1978d). However, this required very careful preparation of samples. The fact that the lactose samples in the present study have been obtained from different manufacturers should be borne in mind when the comparisons as in Table XIX are drawn. Different conditions used in the preparation of these samples will result in differences in crystal properties. Such differences are important when the amount of a given anomer to be quantitatively determined by the x-ray method is small.

The results obtained in the present case, nevertheless, indicate that the lactose anomers in a given sample are not present as a simple physical mixture. It appears that while, at this stage, it is difficult to draw any final conclusion regarding the actual physical state of the anomers in commercial lactoses, it is theoretically possible to obtain such information by a careful combination of the x-ray and GC methods and other physical properties, such as true density. A study of changes

in crystal lattice parameters with the anomeric composition could establish the nature of incorporation of the anomers in each other. This would, however, require careful recrystallization of a series of lactose samples with different anomeric compositions. Factors such as differences in their particle sizes would have to be avoided. The problem of overlap of the anomeric peaks would have to be eliminated by a proper correlation of the individual anomeric contributions to the intensities of peaks in the regions of interest in the diffraction patterns. X-ray diffraction has been used to interpret different phases in a given sample (Cullity, 1978d), and seems to be a potential tool for this purpose in the case of lactose.

3.5 DETERMINATION OF TRUE DENSITY OF LACTOSE SAMPLES

Determination by pycnometry

A multipycnometer was used for determination of the true density of the lactose samples. The instrument uses an air-displacement technique (Franklin, 1949) which is based on the Archimedes' principle of fluid displacement, the fluid in this case being prepurified helium gas. The instrument has a sample cell of accurately known volume. The cell is filled with a known weight of the powder sample under study. Any adsorbed gases are removed from the powder by an outgassing procedure. A known quantity of helium under pressure is allowed to flow into the cell from a precisely known reference volume. This causes the gas to expand and the pressure to drop to a lower value. By applying

the gas laws to these pressures, the volume of helium surrounding the powder particles and penetrating into the pores and cracks is calculated (Martin *et al.*, 1969). The difference between this volume and the volume of the empty cell is the volume of the powder. Knowing the powder volume, the true density of the sample can be determined, since the weight of the powder is known.

Helium is the gas of choice because its small atomic size assures its easy penetration into small cracks and pores. Its behavior as an ideal gas is also desirable since ideal gas laws are used in the process of determination of the powder volume.

True density and anomeric composition of lactose

True densities of the lactose samples are presented in Table XX. An examination of these data indicates that the true density of lactose varies with the anomeric composition of the samples. The true density of α-rich lactose samples is lower than that of the β-rich samples and appears to increase with the β-content of the samples. This would be true in each of the four following situations:

i. the two anomers are present as a simple physical mixture of their individual crystals;

ii. one anomer forms a continuous substitutional solid solution in the other;

iii. the β-anomer forms an interstitial solid solution in α-lactose monohydrate;

Table XX. True density of lactose samples[a] determined by a multipycnometer.

Lactose Grade	%β	True Density ± 1s.d.	n
α-rich samples:			
L80S	1.6	1.538 ± 0.0005	8
α-L	1.8	1.538 ± 0.0007	6
LCAPS	1.8	1.540 ± 0.0003	8
IPL-S	1.8	1.541 ± 0.0011	8
L80M	1.9	1.540 ± 0.0005	8
L60S	2.0	1.536 ± 0.0003	8
IPL-F	3.1	1.539 ± 0.0010	6
SPL	3.2	1.538 ± 0.0003	8
FFL	5.4	1.533 ± 0.0004	8
DCL-11	10.2	1.535 ± 0.0005	8
DCL-30	14.8	1.492 ± 0.0007	8
Samples containing significant amounts of both anomers:			
β-BAK	69.4	1.561 ± 0.0003	5
ANL	73.9	1.564 ± 0.0013	8
DCL-21	76.7	1.561 ± 0.0008	8
β-rich samples:			
β-DMV	94.9	1.589 ± 0.0004	8
β-CCF	98.1	1.589	

a. True densities of SANL and UANL were not determined by this method.
b. Determined by suspension density method.

iv. for the samples which contain more than 9% β in α-lactose monohydrate or more than 6% α-lactose monohydrate in β-lactose, they may be a mixture of two random solid solutions, one α-rich and the other β-rich.

Each of the above four possibilities is discussed below.

i. Formation of a simple physical mixture

This possibility has been investigated by a quantitative x-ray method described in Section 3.4. The results indicate that a given sample of lactose does not contain crystals of each anomer as a simple physical mixture, because the anomeric composition obtained from the x-ray method does not correspond with the actual anomeric composition determined using the GC method. This is supported by an observation by Buma and Van der Veen (1974) that the β-anomeric impurity in an α-lactose sample was incorporated inside the α-crystals. The exact nature of this incorporation was not investigated. However, it has been reported that β-lactose molecules are specifically taken up by certain faces of the α-lactose monohydrate crystals when the latter are grown from a mother liquor containing both anomers (Visser and Bennema, 1983). The present work indicates that up to 9% β-lactose can be incorporated into the α-lactose monohydrate crystals. This value is in good agreement with a value of 7% β-lactose in α-lactose monohydrate determined by Fries *et al.* (1971) in a previous study.

The incorporation of small amount of α-impurity in the β-rich samples can be explained in a similar manner. The β-lactose crystals can be grown from aqueous solutions of lactose above 93.5°C (Itoh *et al.*, 1978). At the end of the crystallization process, when the β-lactose crystals are separated by filtration, the temperature drops below 93.5°C, which favours the recrystallization of α-lactose monohydrate from the traces of the mother liquor adhering to the β-lactose crystals. Thus small amounts of α-lactose monohydrate are present at the surface of the β-lactose crystals, and not as separate distinct crystals.

In the case of samples like β-CCF, prepared by thermal dehydration of an α-lactose monohydrate sample in a steel bomb, the dehydrated crystal lattice rearranges itself into a more compact form. This explains why the true density of β-rich samples is greater than α-rich samples and why the major peaks in their x-ray diffraction patterns are present at higher 2θ values. The presence of 1.9% α-lactose as an impurity in β-CCF can be accounted for by assuming that the conversion of α to the β-anomer was incomplete. This amount of impurity is probably incorporated into the new crystal lattice where it is unlikely to be detected by the x-ray method.

The density of DCL-30, a partially anhydrous lactose sample (Section 3.3) is, however, anomalous. This sample has been apparently prepared by thermal dehydration of an α-lactose monohydrate sample. Comparison of the x-ray diffraction patterns of DCL-30 with other α-rich

samples shows a marked reduction in the peak intensities indicating that the sample is less crystalline. It is known that the true density of a solid decreases as its crystallinity decreases (Hüttenrauch, 1978).

ii. Formation of a continuous substitutional solid solution

If one anomer forms a continuous substitutional solid solution in the other anomer, it would be expected to affect the melting point of the other anomer in a continuous manner, giving rise to a continuous relationship between the anomeric composition and the melting point. That is, if the melting points of the lactose samples are plotted as a function of their anomeric composition, a smooth curve should result (Findlay, 1951). Such a relationship between α-lactose monohydrate and β-lactose cannot be obtained, because, as mentioned in Section 3.3, the α-anomer undergoes conversion to the β-anomer when heated, for example, during the DSC runs. Since the identity of the initial sample is lost during heating, it is not possible to obtain the temperature-composition relationship in the case of lactose samples.

Another property that can be made use of in this context is the diffraction of x-rays by different lactose samples. If the lactose samples formed a continuous solid solution, the x-ray diffraction pattern would be expected to change gradually with anomeric composition. This is not the case as is evident from the powder x-ray diffraction patterns of lactose samples presented in Section 3.4. The diffraction patterns can be categorized into three distinct types: (i) those due to

the α-rich samples, (ii) those due to the samples containing significant proportions of both anomers, and (iii) those due to the β-rich samples. Thus, the possibility of the formation of continuous solid solution between α-lactose monohydrate and β-lactose can be ruled out. It is possible that a certain amount of one anomer can be substitutionally incorporated into the crystal lattice of the other without changing its lattice, and, hence, its x-ray diffraction pattern. In the present study, a 6% incorporation of α-lactose into β-lactose and 9% β-lactose in α-lactose monohydrate (7% according to Fries *et al.*, 1971) was observed by quantitative x-ray technique (Section 3.4).

The possibility of formation of a continuous solid solution if the samples consist of anhydrous α-lactose and β-lactose can be discussed similarly. As mentioned earlier, the 1:1 α/β "compound," as described by Lerk *et al.* (1984), may not be one homogeneous phase (Section 3.3). The 1:1 α/β compound is more likely to be a mixture of anhydrous phases than one of the series of continuous solid solutions. Simpson *et al.* (1982), on the other hand, described each of the 5:3 and 4:1 α/β lactoses as being one homogeneous phase (Section 3.3). It is possible that these samples belong to a continuous solid solution system. If this were true, a gradual shift in the peak positions in their x-ray diffraction patterns would be observed with the change in their anomeric composition. As shown by the data in Table XXI, this does not appear to be the case. Samples rich in anhydrous α-lactose (SANL and UANL, both 92-95% α), the samples of intermediate compositions (5:3 and 4:1 α/β samples), and the β-rich samples (β-CCF, 98.4% β), all show matching

Table XXI. Interplanar spacings corresponding to some common peaks in the diffraction patterns of some anhydrous lactose samples.

Sample	Interplanar Spacings[a] (Å)	Reference
UANL	3.19, 3.41, 3.48, 4.11, 4.27, 4.42, 4.60, 4.89, 6.97, 7.46	This study.
	3.20, 3.82, 4.13, 4.42, 4.63, 4.93, 7.0	Bushill *et al.*, 1965.
	3.41, 3.48, 4.13, 4.31, 4.67, 4.93, 7.63	Buma and Weigers, 1967.
SANL	3.13, 3.80, 4.01, 4.20, 4.53, 4.61, 4.87, 7.05	This study.
	3.41, 3.74, 3.83, 4.02, 4.25, 4.58, 4.65, 4.93, 7.14	Buma and Weigers, 1967.
	3.2, 3.82, 4.21, 4.56, 4.65, 4.9, 7.2	Bushill *et al.*, 1965.
4.1 α/β Sample	3.19, 3.79, 4.00, 4.18, 4.54, 4.88, 7.13	Simpson *et al.*, 1982.
5:3 α/β Samples	3.2, 3.82, 4.04, 4.42, 4.65, 4.93, 7.1	Bushill *et al.*, 1965.
	3.2, 3.79, 4.01, 4.41, 4.65, 4.86, 7.13	Simpson *et al.*, 1982.
β-lactose	3.16, 3.47, 3.69, 3.73, 4.20, 4.26, 4.60, 8.27	This study. (β-CCF)
	3.23, 3.48, 3.74, 3.97, 4.23, 4.29, 4.53, 4.62, 8.43	Buma and Weigers, 1967.

a. Underlined interplanar spacings indicate the three most intense peaks in the diffraction patterns.

peaks. The peaks at approximately 3.8, 4.6 and 4.9Å remain almost unchanged throughout. This suggests that these samples have essentially the same unit cell and it is unlikely that they belong to a continuous solid solution system. It is difficult to draw conclusions in this context, though, because the different samples were prepared under different conditions. A series of carefully prepared anhydrous samples with a range of anomeric compositions would have to be studied for their x-ray diffraction patterns and true densities. A study of changes in the lattice parameters with the anomeric composition might be more fruitful. It should be noted, however, that most of the pharmaceutical types of lactose consist of α-lactose monohydrate and β-lactose, and that anhydrous lactose samples are only a special case.

iii. Formation of an interstitial solid solution by β-lactose

Another possibility is the formation of an interstitial solid solution. This happens only when the solute molecule is very small compared to the solvent molecule, so that it can enter the interstices of the solvent lattice without causing much distortion (Cullity, 1978d). Since the molecular size of β-lactose is not considerably smaller than that of α-lactose monohydrate, the formation of an interstitial solid solution between the two anomers would not be expected.

iv. The presence of two (α-rich and β-rich) random solid solutions

On the basis of the quantitative x-ray diffraction results, it has been suggested that samples containing significant amounts of both anomers, such as β-BAK, ANL, and DCL-21, are not simply mixtures of the crystals of α-lactose monohydrate and β-lactose (Section 3.4). The true density values of these samples are intermediate between those of the α and β-rich samples. This can be explained on the basis of the existence of a mixture of the following two phases in these samples: (1) a solid solution of α in β, and (2) a solid solution of β in α. The possibility of the existence of these two phases seems logical, as this would also explain why the peaks characteristic of the two anomers are present in the x-ray diffraction patterns of these samples. Moreover, the partial incorporation of the two anomers in each other would also explain the observation that the anomeric compositions obtained from the x-ray and the GC methods do not agree (Section 3.4).

3.6. MOISTURE-DESORPTION AND SORPTION CHARACTERISTICS OF LACTOSE

The moisture content of some lactose samples was determined by drying the samples at 60°C *in vacuo*. The dried samples were then exposed to three different humidities at 25°C. The weight changes in each case were followed for prolonged periods of time. The results are presented in Tables XXII and XXIII, and Figs. 16 and 17.

Table XXII. Moisture-desorption of lactose samples (at 60°C, *in vacuo*).

Time of Drying (Days)	% Weight Loss of				
	IPL-F	α-L	β-BAK	FFL	SPL
0.25	0.32	0.37	0.38	0.43	0.81
1	0.35	0.35	0.42	0.45	0.90
2	0.37	0.33	0.44	0.48	0.96
3	0.38	0.36	0.41	0.46	0.95
4	0.36	0.37	0.39	0.46	0.99
5	0.42	0.38	0.43	0.44	1.03
6	0.41	0.40	0.42	0.50	1.13
7	0.41	0.39	0.41	0.54	1.13
139	0.52	0.50	0.59	0.60	1.18
Surface Areas (m^2/g):	0.511	0.426	0.428	0.137	0.142

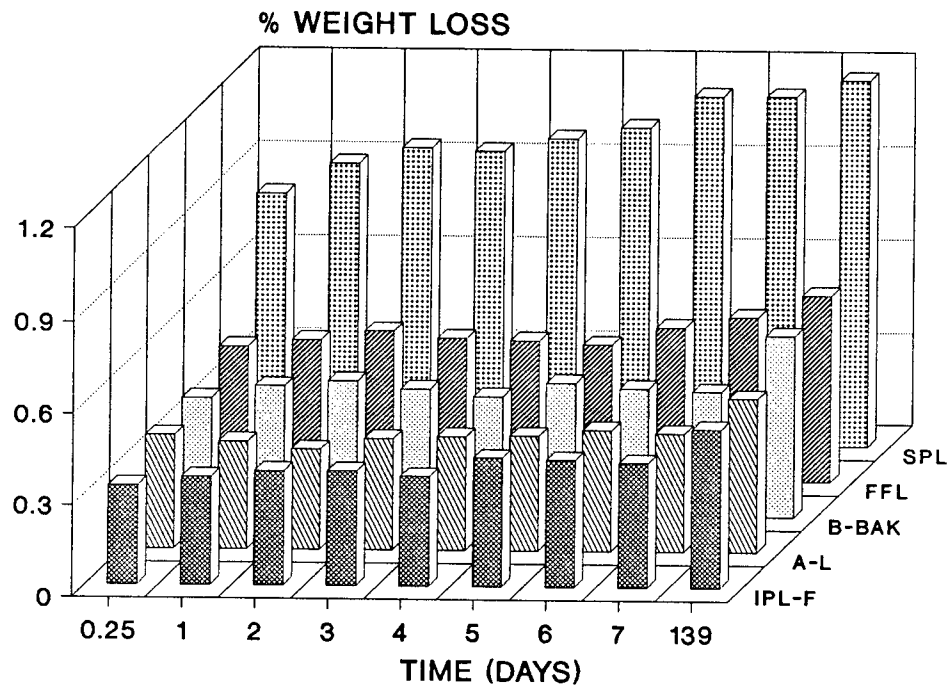


Fig. 16. Weight loss of some lactose samples as a function of time upon drying *in vacuo* at 60°C.

Table XXIII. Moisture-sorption of lactose samples after 76 h (25°C) at different relative humidities.

% R.H.	% Weight Gain of			
	IPL-F	β-BAK	FFL	SPL
15	0.35	0.28	0.38	0.57
32	0.40	0.45	0.47	0.69
52	0.49	0.45	0.58	0.92

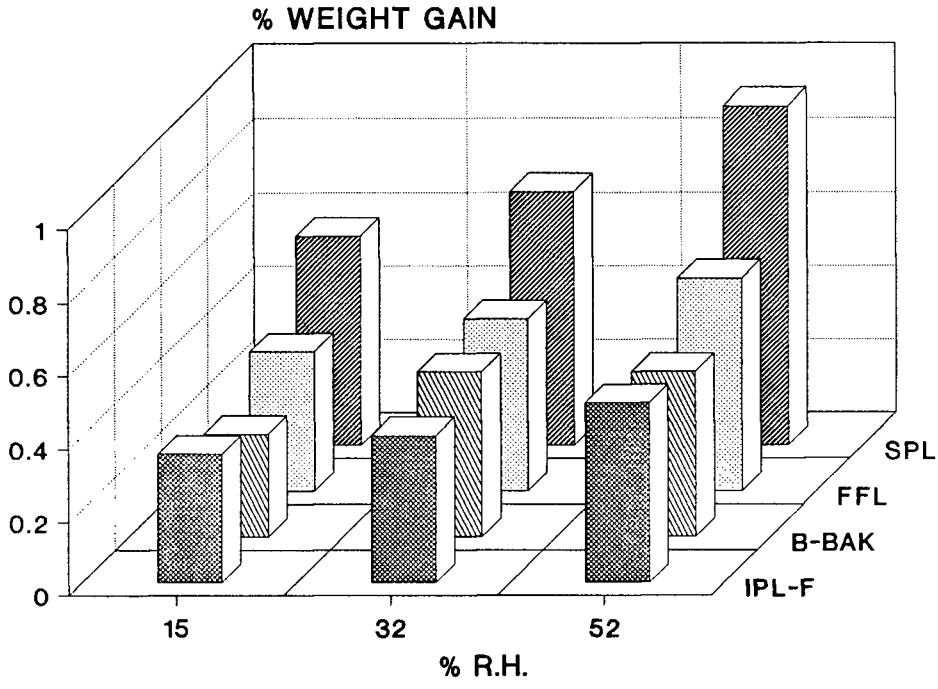


Fig. 17. Weight gain of some lactose samples after moisture-sorption for 76 h at 25°C as a function of % relative humidity.

Observations on the moisture-desorption and sorption of lactose

Lactose samples are expected to contain sorbed moisture along with the water of crystallization. Lactose is an excipient in tabletting. The moisture content of excipients is an important factor in deciding their flow and compression characteristics, and the hardness of their tablets (Umprayn and Mendes, 1987). Also, moisture is a critical factor that affects physical properties like the heat of solution and heat of wetting of a solid sample (Pikal *et al.*, 1978; Hollenbeck *et al.*, 1978; Suryanarayanan and Mitchell, 1985). For this purpose, it is necessary to obtain samples that are dry, i.e., devoid of sorbed moisture. It was, therefore, important to determine the amount of sorbed moisture in lactose and differentiate it from the water of crystallization. The moisture-desorption method described in Section 2.7 appears to achieve this effectively. This is because the weight loss due to drying the lactose samples at 60°C *in vacuo* (even for an extended period of over 4.5 months) does not correspond with their weight loss due to dehydration during DSC runs (which includes both moisture-desorption and dehydration). This can be easily seen by comparing Tables XI and XXI.

Irrespective of their anomeric composition, the lactose samples lose most of their sorbed-moisture within one day of drying at 60°C *in vacuo* with further gradual weight loss occurring over a prolonged drying period (Fig. 16). The spray dried product, SPL, contains almost two times as much moisture as compared with the other forms of lactose. It also sorbs a larger amount of moisture at the relative humidities used

in this experiment. It is known that the spray dried samples are poorly crystalline due to rapid crystallization during the spray-drying process, and, thus, are in a higher energy, or activated, state. Such products are expected to possess greater reactivity. This explains why SPL tends to sorb more moisture than the other lactose samples and, correspondingly, lose greater weight due to moisture-desorption. These observations are in accordance with the reported enhanced moisture-sorption by poorly crystalline β-lactam antibiotics prepared by spray drying (Pikal *et al.*, 1978).

Specific surface area is another important factor that decides the extent of moisture-sorption by a given solid sample (Umprayn and Mendes, 1987). It is clear from the observations in Tables XXII and XXIII that there is no correlation between the surface area and moisture-sorption of the lactose samples studied. In fact the relatively non-crystalline sample, SPL, despite its lower surface area, shows considerably greater degree of moisture-sorption than the crystalline lactose samples (α-L, IPL-F and β-BAK). Thus, it is apparent that the crystallinity of lactose samples is a more important factor than surface area in determining their moisture-sorption characteristics.

3.7 CHARACTERIZATION OF THE LACTOSE SAMPLES ON THE BASIS OF THEIR SURFACE AREA

Calibration curve for the determination of the surface area

The calibration curves of the volume of nitrogen injected into the flowing stream of Kr/He mixture versus the peak heights and peak areas are shown in Fig. 18. Both curves have good correlation (r^2=0.999). The curves were intermittently checked by injecting known volumes of nitrogen. The peak heights and areas were essentially invariable at different times indicating a good degree of precision associated with the two curves. The intercept (2.48 mm^2) of the peak area curve was not significantly different (p=0.05) from zero. The peak height curve had an intercept (2.7 mm) significantly different (p=0.05) from zero. Despite this, the curve could be used for surface area determination as the error would be common in both the sample and the calibration peak heights.

Surface area of the lactose samples

The surface area values of the different lactose samples are shown in Table XXIV. The surface areas of some selected samples were calculated by both the peak height and the peak area methods. The values are within acceptable limits of each other suggesting that any one of the two peak parameters, namely, height and area, could be reliably used for surface area calculation.

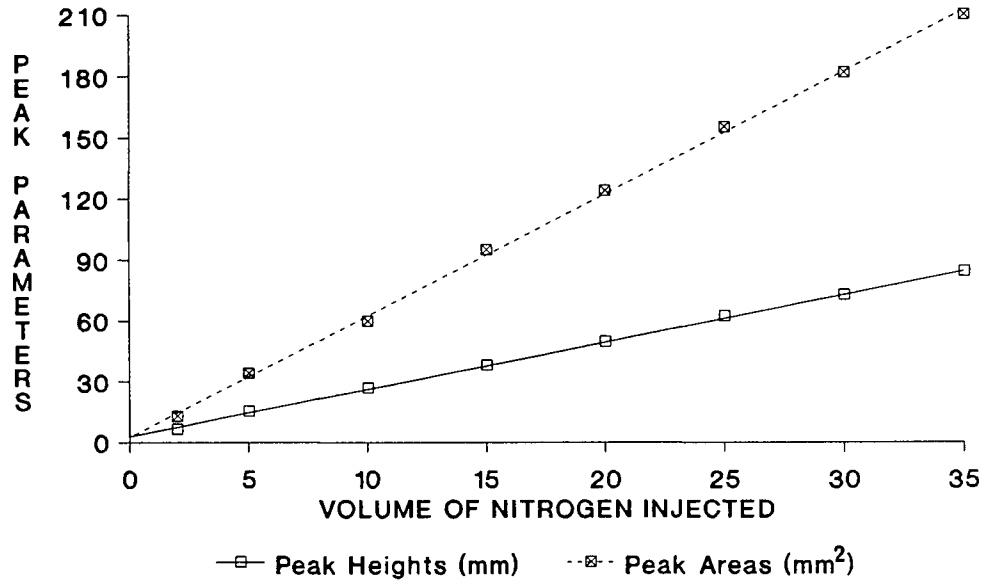


Fig. 18. Calibration curves for surface area analysis of lactose samples using Quantasorb. Both the peak heights and peak areas are shown.

Table XXIV. Specific surface area of lactose samples.

Lactose Sample	Specific Surface Area (m^2/g) Peak height method	n
α-L	0.426	2
L60S	0.108	2
L80S	0.090	2
L80M	0.293	2
IPL-S	0.574	1
ANL	0.397	1
IPL-F	0.511	1
SPL	0.142	1
FFL	0.137	1
DCL-11	0.185 (0.179)[a]	2
DCL-21	0.307 (0.300)[a]	2
DCL-30	0.168 (0.166)[a]	2
β-BAK	0.428	2
SANL	0.254	1
UANL	1.673	1

a. Values in parentheses show the specific surface area determined by peak area method.

4. SUMMARY

1. Fourteen commercially available and four specially prepared samples of lactose were analyzed for their anomeric composition and physical properties.

2. An accurate and rapid GC method for separation and subsequent quantitative determination of lactose anomers was developed. It was shown that the lactose anomers have equal responses on a FID detector. This enabled the relative peak areas to be used as relative anomeric composition of the lactose samples without the necessity of an internal standard.

3. The thermal behavior of various samples of lactose was studied using a DSC. The following conclusions were drawn.
   i. In a sealed pan, the dehydration peak of α-lactose monohydrate splits into two parts. This is because an exothermic peak (due to dissolution of the dehydrated sample into liquid water and recrystallization of β-lactose from the solution) overlaps the endothermic peak of dehydration.
   ii. Alpha-lactose undergoes an endothermic anomeric conversion to the β-anomer during the dehydration peak and also immediately after it; the anomeric conversion is dependent on the heating rate and pan type used.
   iii. The β-rich material formed in (ii) recrystallizes into a new crystal form, especially in a sealed pan, giving rise to a new

exotherm in the thermograms.

iv. The melting peak in a sealed pan shifts to a higher temperature due to the anomeric conversion.

v. A previously reported formation of a 1:1 β/α-lactose compound is an artifact of the experimental conditions, since other anomeric compositions can be obtained by varying the DSC conditions.

vi. Certain commercial samples, claimed to be anhydrous, are not truely so because they show the dehydration peak.

4. The powder x-ray diffraction patterns of the various samples of lactose could be classified into three categories corresponding to:

   i. α-lactose monohydrate rich samples with a characteristic peak at 20.1°2θ;

   ii. β-rich samples with a characteristic peak at 21.2°2θ;

   iii. samples showing characteristic peaks of both the α-lactose monohydrate and β-lactose.

5. Quantitative powder x-ray diffraction studies indicated the following.

   i. In a lactose sample containing a simple physical mixture of α-lactose monohydrate and β-lactose, the relative anomeric amounts show a linear relationship with the relative peak intensities.

   ii. A small amount of β or α anomeric impurity can be incorporated

into the α-lactose monohydrate or β-rich samples without being detected by the x-rays.

iii. Samples showing characteristic peaks of both anomers are probably made up of two phases, one α-rich and the other β-rich, each containing a certain amount of the other anomer as impurity.

6. The true density of lactose samples determined by a Quantachrome multipycnometer is reliable only if the largest cell is used.

7. An examination of the true density values in conjunction with the powder x-ray diffraction patterns revealed that, in a given sample, α-lactose monohydrate and β-lactose do not exist as a continuous solid solution. Formation of an interstitial solid solution is also not possible. It is possible that the lactose samples contain two solid solutions, one α-rich and the other β-rich.

8. Along with the water of crystallization, the lactose samples also contained sorbed moisture. The moisture-desorption and sorption appears to depend on the relative crystallinity of the samples rather than on their surface area.

9. Various samples of lactose obtained from different commercial sources had different surface areas, suggesting that their compaction behavior during a tabletting process may be different.

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APPENDIX A

Calculation of linear absorption coefficients of lactose anomers

The linear absorption coefficient, μ, of a compound is given by:

$$\mu = \rho \Sigma p_e . \mu_e/\rho_e$$

where,

ρ = density of compound,

p_e = proportion by weight of each constituent element with mass absorption coefficient μ_e/ρ_e.

μ_e/ρ_e for CuKα radiation for C=4.22, H=0.391 and O = 11.0 cm^2/g (Cullity, 1978e).

Therefore, the linear absorption coefficient for lactose can be calculated as follows:

i. α-lactose monohydrate:

p_e for C=0.40002, H=0.06714 and O=0.53284.

Thus, $\mu_{\alpha H2O}$ = 1.54{(0.40002x4.22)+(0.06714x0.391)+(0.53284x11)}

= 11.6664 cm^{-1}.

ii. β-lactose:

p_e for C=0.42108, H=0.06478 and O=0.51414.

Thus, μ_β = 1.59 {(0.42108x4.22)+(0.06478x0.391)+(0.51414x11)}

= 11.8579 cm^{-1}.