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Thermochimica Acta 380 (2001) 55–65

thermochimica acta

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Comparison of two melting range analysis methods with lactitol monohydrate

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Received 20 February 2001; accepted 12 June 2001

Abstract

In pharmacopoeia, the melting point is determined by a standard method with a melting point instrument. The melting point can also be determined with differential scanning calorimetry (DSC). In this study, the standard method and DSC method are compared for determining the melting range of lactitol monohydrate.

The effect of initial temperature, grinding, and drying on the melting range of different lactitol monohydrate samples was studied by a melting point instrument. The melting point and melting enthalpy of the stable form of lactitol monohydrate was identified by DSC. The statistical analysis of the results is based on a *t*-test. All studied variables had a small effect on the melting range. Repeatability of these two methods was calculated. The intermediate precision was also determined for the measurements of the melting point instrument. The melting range of lactitol monohydrate taking into account the intermediate precision is 94–100°C determined with a melting point instrument. The melting range determined with the DSC is according to these measurements 93–99°C and the melting enthalpy is $58.5 \pm 1.0\,\mathrm{kJ\,mol^{-1}}$.

Keywords: Lactitol monohydrate; Melting point; Melting range; Melting enthalpy

1. Introduction

Lactitol, 4-*O*-β-D-galactopyranosyl-D-glucitol, is a sweetener which can substitute for sucrose in many food applications. Lactitol is a safe and valuable low-calorie food ingredient that is well tolerated by people with diabetes because it does not affect the blood glucose or insulin levels. Fermentation by oral bacteria is negligible, and is thus suitable for products that are safe for our teeth [1,2]. Lactitol is prepared by catalytic hydrogenation of the glucose moiety of lactose [2,3].

Lactitol exists in at least four crystallized crystalline forms: anhydrous lactitol (abbreviated as A2) [4], lactitol monohydrate (M1) [5,6], lactitol dihydrate (D) [7,8], and lactitol trihydrate (T) [9]. The melting point of lactitol monohydrate is according to Kanters et al. [5] 120–121°C and according to Kivikoski [6] 92–94°C. The melting point, 120–121°C, does not exist for the stable form of lactitol monohydrate according to this study.

In the present study, the melting point of the stable form of lactitol monohydrate was determined using a melting point instrument and differential scanning calorimetry (DSC). The melting range is determined in pharmacopoeia by a melting point instrument using a specific method [10]. On the other hand, the melting

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0040-6031/01/$ – see front matter
PII: S0040-6031(01)00637-2

point can also be determined by DSC. The aim of this study is to compare these measurement methods for determining the melting temperature under repeatability conditions. The melting range can be determined from the lower and upper limit of the melting range, taking into account the intermediate precision (r_D).

The upper limit of the melting range is the melting point of the sample. The lower limit of the melting range corresponds with the starting point of melting. The onset temperature in the DSC-measurement corresponds with the starting point of the melting, and the peak temperature in the DSC-measurement corresponds with the melting point measured by the pharmacopoeia method.

2. Experimental

The effect of the initial temperature, grinding, and drying on the melting range of four different monohydrate samples (one recrystallized sample and three different production lots) were studied with a Büchi 535 melting point instrument. The instrument was calibrated using the following standards: acetanilide, vanillin and USP reference materials. The capillary tubes (length 10 cm, internal diameter 0.8–1.2 mm and wall thickness 0.2–0.3 mm) were purchased from Hirchman Laborgeräte, Germany. The heating rate was set to $2^{\circ}C\,min^{-1}$ at 88°C (about 10°C below the presumed melting point) and the initial temperature was set at about 5°C below the presumed melting point. The initial temperature in this study was the temperature at which the sample capillary tube was inserted into the oil bath of the melting point instrument.

Three different initial temperatures (91, 92 and 93°C) were tested and the melting ranges were measured for both ground and unground samples. Six parallel measurements were made at each test point for determining the influence of the initial temperature on the melting point of lactitol monohydrate. Samples used were sample 1 (recrystallized reference sample, lactitol monohydrate, in-house reference standard, 12 March 1994, Cultor Ltd., Kantvik, Finland) and sample 2 (lactitol monohydrate, lot. L225 40158, 5 April 1994, Xyrofin Ltd., Kotka, Finland). Measurements were taken for sample 1, both for a dried and an undried material, and for sample 2, only for an undried material. The obtained results were tested by the *t*-test with the confidence level of 95%.

The drying in this study means that the sample was kept at room temperature in a vacuum over anhydrous silicagel for 24 h prior to measurement. Drying is done to remove free water from the sample. The undried sample means that a small sample was drawn from the sample container just before the measurement. The influence of drying was studied for samples 1–3 (lactitol monohydrate, lot. L125 0141, 27 October 1991, Xyrofin Ltd., Kotka, Finland), and sample 4 (lactitol monohydrate, lot. L125 20756, 7 September 1994, Xyrofin Ltd., Kotka, Finland). Six parallel measurements were taken for different production lots (samples 2–4) and for the in-house reference standard at each test point. The determinations were made for ground material and the test tubes of different production lots were put into the melting point instrument at 93°C and the tube of sample 1 at 91, 92, and 93°C. Grinding in this study means that the sample was gently ground by hand with a porcelain mortar. Grinding was done gently to avoid formation of amorphous structures.

The effect of grinding on the melting range was studied by measuring all the test points for ground and unground samples. The samples used were samples 1–4.

The overall method performance was studied during an intermediate precision test for sample 4. During this test, a sample was analyzed independently by different analysts. Six parallel measurements were taken by each analyst over 5 days according to the method which also includes sample drying and grinding before the measurement. The initial temperature was 93°C. Because all the studied variables had a small effect (drying negligible) on the melting range, the sample, which was used for the intermediate precision test was dried in a vacuum over anhydrous silicagel for 24 h and finally gently ground before packing into the capillary tube. The evaluation of these results is based on International Standard ISO 5725 referring to one laboratory [11].

Pure lactitol monohydrate (sample 5; lactitol monohydrate, lot. 20707, 3 November 1994, Xyrofin Ltd., Kotka, Finland) for DSC-measurements was obtained from Cultor Ltd. and its purity was over 98.5%

(determined by HPLC by Cultor Ltd.). The amount of water of lactitol monohydrate was determined by a TG and the structure of the sample was confirmed as the stable form of lactitol monohydrate by a DSC and a X-ray powder diffractometer.

The melting point and the melting enthalpy of the sample were identified by DSC with four different heating rates. Six parallel measurements were taken at each test point. The results were tested by a paired *t*-test by the SPSS-program [12] with the confidence level of 95%.

The DSC-measurements were carried out on PerkinElmer DSC-7 using 50 μl aluminum sample pans (PerkinElmer part No. B014-3019 and cover PerkinElmer part No. B014-3040) with capillary holes. The temperature calibration was carried out by indium and benzoic acid standards and the energy calibration by the indium standard. The calibration was done for all heating rates. The sample weight was about 5 mg. The heating rates were 5, 10, 15 and 20°C min^{-1} and the temperature range was 30–180°C.

3. Results and discussion

When the paired *t*-test is used to analyze the results, the degrees of freedom are almost half of independent cases. It means that the significant two-tail value which tells when to ignore the zero hypothesis must be higher than with independent cases. The 95% confidence interval of the mean (risk level, $\alpha = 0.05$) provides the interval believed to comprise the real value of the measurement at 95% probability. The CI_{95} value used is the interval from the mean value of the measurements to the lower (or upper) limit of the confidence interval [13].

The effect of the initial temperature and grinding of the sample on the melting ranges of the recrystallized reference standard and linear regression curves with R^2 (correlation coefficient) values for unground and ground sample 1 are illustrated in Fig. 1 and for sample 2 in Fig. 2. The *t*-test results for the lower limit of the melting range are presented in Table 1 and the *t*-test results for the melting point in Table 2, for unground and ground samples.

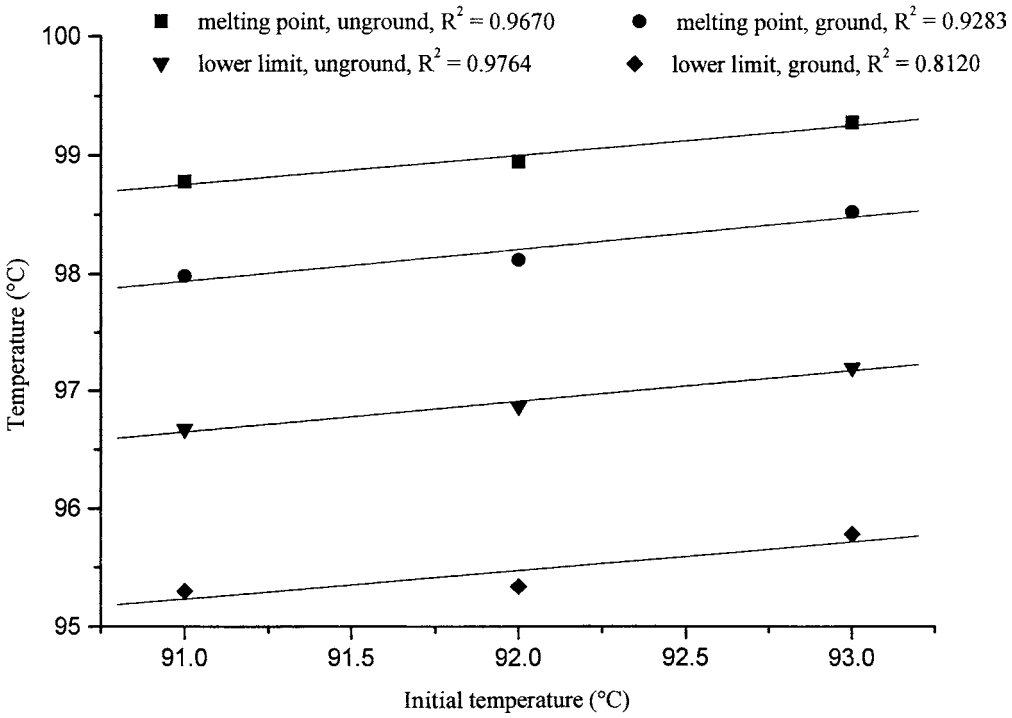

Fig. 1. The effect of initial temperature on the melting range of sample 1.

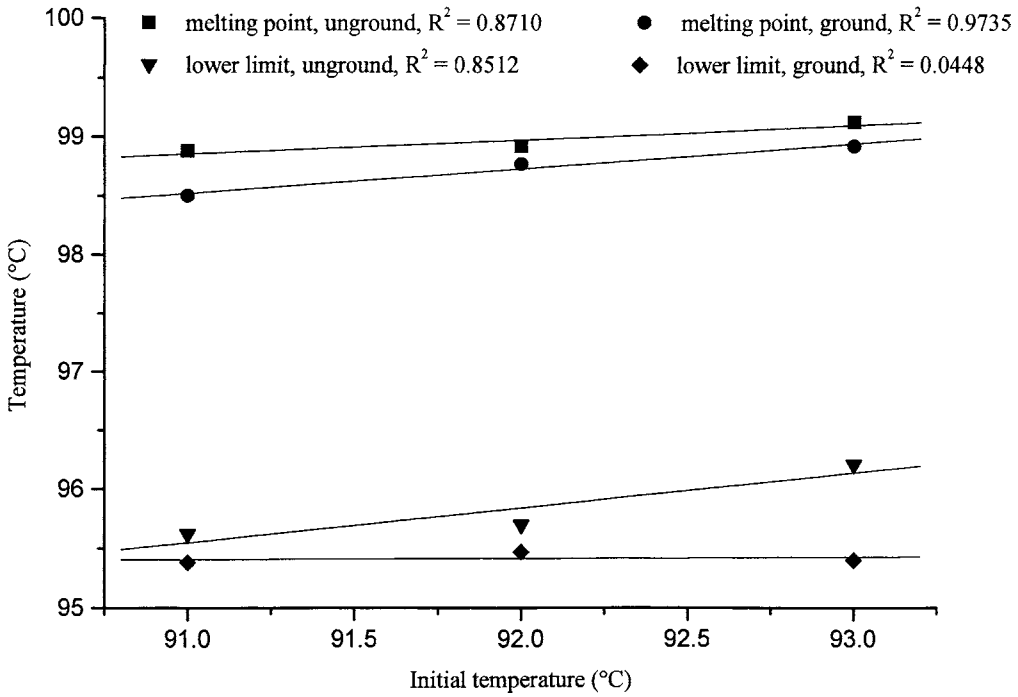

Fig. 2. The effect of initial temperature on the melting range of sample 2.

When analyzing the effect of the initial temperature with the paired *t*-test the results obtained clearly indicate that the melting range is dependent on the temperature at which the packed capillary tube is inserted into the instrument. In the case of a recrystallized sample (sample 1), the influence of the initial temperature on the melting range is obvious whereas it is not so clear with the production lot (sample 2). The melting range shows linear dependence on the initial temperature in both cases.

Drying in this study means that the sample was kept in a vacuum over anhydrous silicagel for 24 h before measurement. The undried sample means that a small sample was drawn from the sample container just before the measurement. The obtained results are shown in Table 3 and in Fig. 3 (sample 1) and Fig. 4 (samples 2–4). According to the obtained results, sample drying has statistically a significant effect only on the lower limit of the melting range of the in-house reference standard at the confidence level of 95%.

In order to get a clear picture about the effect of grinding on the melting range, all the points tested in this study always included both alternatives, i.e., ground and unground samples. Grinding in this study means that the sample was gently ground by hand with a porcelain mortar. Fig. 5 shows the results drawn in the same graph. The results of the recrystallized reference standard are located on the left side of the figure and the results obtained for the production lots are shown on the right side of the figure. From those data points, it is evident that the grinding of the sample has a clear effect on the melting range of lactitol monohydrate. In Table 4, the *t*-test results are shown of the in-house reference standard and industrially produced lactitol monohydrate samples. When result pairs obtained for ground and unground samples are analyzed with the paired *t*-test, the result is always the same: grinding statistically has a significant effect on the melting range at the confidence level of 95%.

The overall method performance was studied by analyzing a selected sample, drawn from sample 4 by different analysts over a 5-day period. The method used also included sample drying and grinding before the measurements. Six parallel measurements were

Table 1
t-Test results of lower limit of melting range for unground and ground samples: effect of the initial temperature

	Sample 1			
	Unground, initial T 92°C	Unground, initial T 93°C	Ground, initial T 92°C	Ground, initial T 93°C
Mean	96.86	97.19	95.34	95.78
Variance	0.095	0.034	0.150	0.055
Observations	12	12	12	12
d.f.	11		11	
t stat	−5.087		−4.776	
t critical two-tail	2.201		2.201	
	Sample 1			
	Unground, initial T 91°C	Unground, initial T 93°C	Ground, initial T 91°C	Ground, initial T 93°C
Mean	96.67	97.19	95.30	95.78
Variance	0.022	0.034	0.075	0.055
Observations	12	12	12	12
d.f.	11		11	
t stat	−8.042		−6.250	
t critical two-tail	2.201		2.201	
	Sample 2			
	Unground, initial T 92°C	Unground, initial T 93°C	Ground, initial T 92°C	Ground, initial T 93°C
Mean	95.70	96.20	95.47	95.40
Variance	0.008	0.080	0.007	0.012
Observations	6	6	6	6
d.f.	5		5	
t stat	−3.423		1.195	
t critical two-tail	2.571		2.571	
	Sample 2			
	Unground, initial T 91°C	Unground, initial T 93°C	Ground, initial T 91°C	Ground, initial T 93°C
Mean	95.62	96.20	95.38	95.40
Variance	0.046	0.080	0.014	0.012
Observations	6	6	6	6
d.f.	5		5	
t stat	−5.127		−0.277	
t critical two-tail	2.571		2.571	

performed by each analyst. The results are presented in Tables 5 and 6.

The repeatability standard deviation or the intra-assay precision standard deviation (s_r) is the value measured on different days and the intermediate precision standard deviation or the inter-day method precision standard deviation (s_D) is the value of all measured data points [14]. The evaluation of these standard deviations was continued by applying the International Standard ISO 5725 [11] referring to one laboratory (Table 7). Repeatability (r) can be calculated as 2.8 × repeatability standard deviation with the probability of 95% [15]. The intermediate precision (r_D) can be calculated as 2.8 × intermediate precision standard deviation with the probability of 95%. The melting range can be determined from the lower and upper limit of the melting range (Table 7) taking into account the intermediate precision (r_D). This range is according to these measurements 94–100°C.

The paired t-test was also chosen for DSC-measurement results. The zero hypothesis for DSC-measurements is that the scanning rate does not affect the melting point of lactitol monohydrate. Slow heat transfer slightly affects the enthalpy values measured with 5°C min^{-1}, but the peak and onset values can still be obtained confidently. For that reason, the results

Table 2
t-Test results of melting point for unground and ground samples: effect of the initial temperature

	Sample 1			
	Unground, initial T 92°C	Unground, initial T 93°C	Ground, initial T 92°C	Ground, initial T 93°C
Mean	98.95	99.28	98.12	98.52
Variance	0.132	0.067	0.060	0.129
Observations	12	12	12	12
d.f.	11		11	
t stat	−3.100		−2.818	
t critical two-tail	2.201		2.201	
	Sample 1			
	Unground, initial T 91°C	Unground, initial T 93°C	Ground, initial T 91°C	Ground, initial T 93°C
Mean	98.78	99.28	97.98	98.52
Variance	0.063	0.067	0.092	0.129
Observations	12	12	12	12
d.f.	11		11	
t stat	−3.983		−3.781	
t critical two-tail	2.201		2.201	
	Sample 2			
	Unground, initial T 92°C	Unground, initial T 93°C	Ground, initial T 92°C	Ground, initial T 93°C
Mean	98.92	99.12	98.77	98.92
Variance	0.050	0.074	0.031	0.038
Observations	6	6	6	6
d.f.	5		5	
t stat	−1.130		−1.307	
t critical two-tail	2.571		2.571	
	Sample 2			
	Unground, initial T 91°C	Unground, initial T 93°C	Ground, initial T 91°C	Ground, initial T 93°C
Mean	98.88	99.12	98.50	98.92
Variance	0.022	0.074	0.044	0.038
Observations	6	6	6	6
d.f.	5		5	
t stat	−1.784		−4.110	
t critical two-tail	2.571		2.571	

obtained with a heating rate of 5°C min^{-1} were ignored when determining the melting enthalpy.

The *t*-test results of the measured melting onsets and melting peaks are in Table 8. There, it can be seen that the scanning rate significantly affects the melting onset temperature of lactitol monohydrate. For that reason, the melting range of lactitol monohydrate is determined by linear regression in an isothermic situation. The software used was Microcal Origin [16]. According to linear regression, the onset temperature of the melting peak of lactitol monohydrate at an isothermal situation is 93.3 ± 0.1°C and the peak temperature of the melting peak is 97.1 ± 0.1°C. The correlation coefficient R^2 value was 0.9974 for the onset temperature and 0.9998 for the peak temperature. The onset temperature corresponds with the lower limit of the melting range measured with the melting point instrument. The onset temperature of the melting peak is lower at an isothermal situation than when measured by a melting point instrument with the heating rate of 2°C min^{-1}. Therefore, the onset and peak temperatures measured with DSC should be determined from the equations of linear regression. The equation for the onset temperature is

$$Y = 0.232X + 93.3$$

Table 3
t-Test results of lower limit of melting range and melting point for undried and dried samples

	Sample 1			
	Undried, lower limit	Dried, lower limit	Undried, melting point	Dried, melting point
Mean	95.68	95.26	98.21	98.20
Variance	0.110	0.074	0.093	0.198
Observations	18	18	18	18
d.f.	17		17	
t stat	5.680		0.102	
t critical two-tail	2.110		2.110	
	Other samples			
	Undried, lower limit	Dried, lower limit	Undried, melting point	Dried, melting point
Mean	95.37	95.38	98.87	98.76
Variance	0.022	0.012	0.080	0.030
Observations	18	18	18	18
d.f.	17		17	
t stat	−0.287		1.538	
t critical two-tail	2.110		2.110	

where Y is the temperature (°C) and X the heating rate (°C min^{-1}). The corresponding equation for the peak temperature is

$$Y = 0.499X + 97.1$$

According to those equations, the onset temperature of the melting peak is 93.8°C and the peak temperature of the melting peak is 98.1°C when determined with the heating rate of 2°C min^{-1}. The melting rate can be

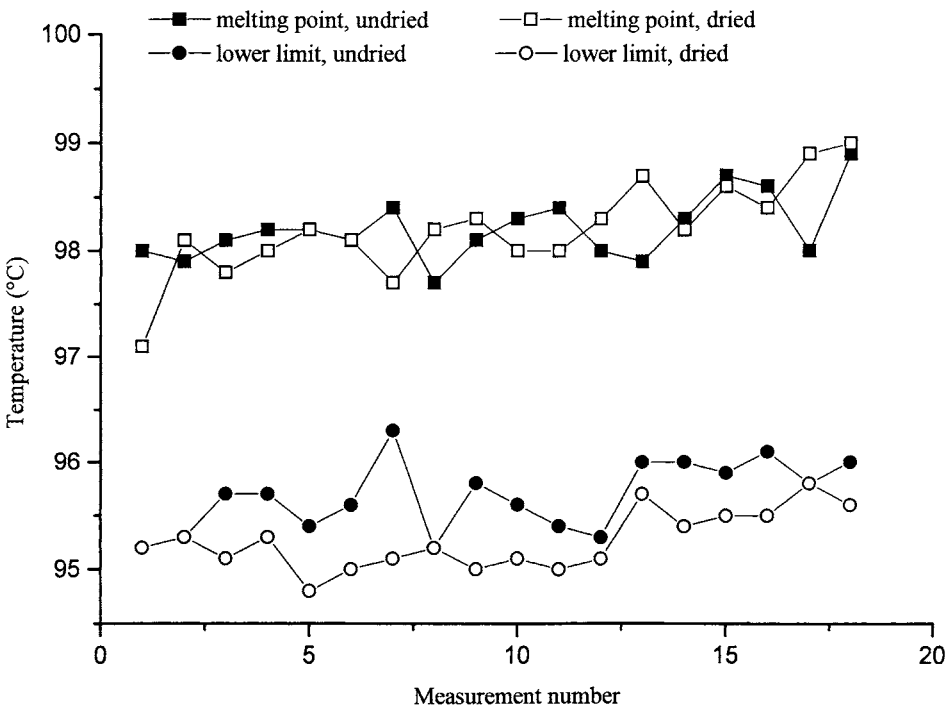

Fig. 3. The effect of drying on the melting range of sample 1.

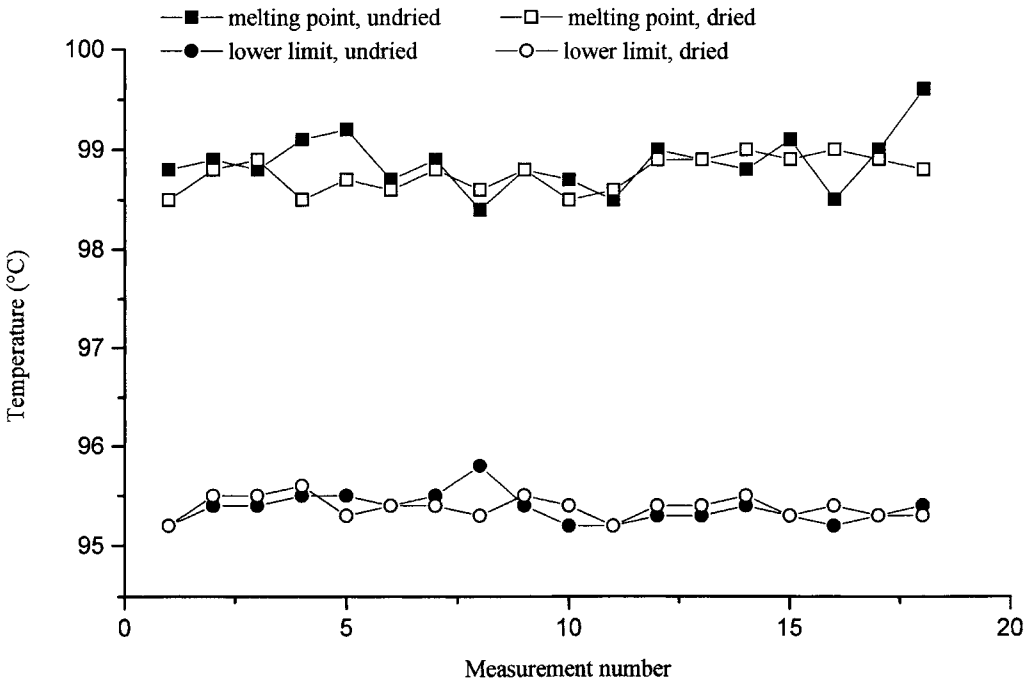

Fig. 4. The effect of drying on the melting range of samples 2–4.

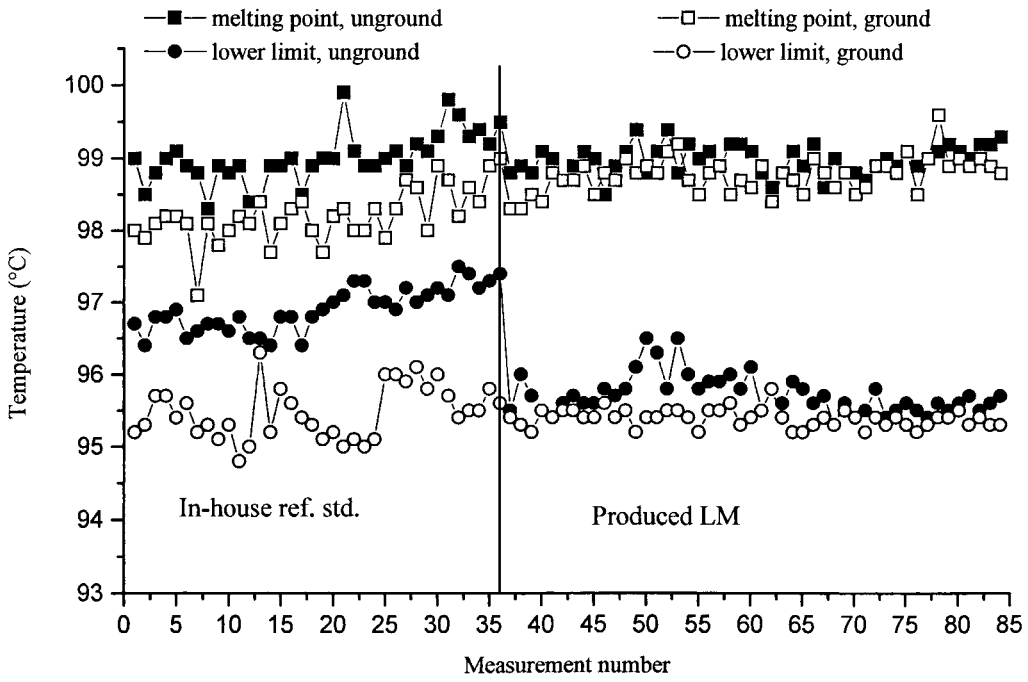

Fig. 5. The effect of grinding on the melting range of lactitol monohydrate (all test points).

Table 4
t-Test results comparison between unground and ground samples

	All data			
	Unground, lower limit	Ground, lower limit	Unground, melting point	Ground, melting point
Mean	96.23	95.43	98.99	98.53
Variance	0.427	0.067	0.077	0.173
Observations	84	84	84	84
d.f.	83		83	
t stat	11.272		9.664	
t critical two-tail	1.989		1.989	
	Sample 1			
	Unground, lower limit	Ground, lower limit	Unground, melting point	Ground, melting point
Mean	96.91	95.47	99.01	98.21
Variance	0.096	0.135	0.127	0.141
Observations	36	36	36	36
d.f.	35		35	
t stat	19.230		12.065	
t critical two-tail	2.030		2.030	
	Other samples			
	Unground, lower limit	Ground, lower limit	Unground, melting point	Ground, melting point
Mean	95.72	95.39	98.98	98.77
Variance	0.071	0.015	0.041	0.061
Observations	42	42	42	42
d.f.	41		41	
t stat	8.291		5.432	
t critical two-tail	2.012		2.012	

determined from the onset and peak temperatures determined with DSC, taking into account the repeatability values. This range is according to these measurements 93–99°C. The heating rate does not affect the melting enthalpy, therefore, it was determined as the mean of measurements. The melting enthalpy was $58.5 \pm 1.0\,\mathrm{kJ\,mol^{-1}}$. Repeatability of the onset and peak temperatures were also determined at each heating rate (Table 9).

A homogeneous crystal size of the pure monohydrate M1 sample could not be achieved by grinding, and the crystal size slightly affected the results of the DSC-measurement. Therefore, the DSC-measurements were taken only for unground samples.

Table 5
Results of intermediate precision test: lower limit of the melting range

Sample	Day 1 (°C)	Day 2 (°C)	Day 3 (°C)	Day 4 (°C)	Day 5 (°C)
1	95.4	95.2	95.4	95.1	94.8
2	95.5	95.1	95.7	95.2	94.8
3	95.3	95.1	95.8	95.2	94.6
4	95.4	95.1	95.4	95.0	94.6
5	95.3	95.0	95.6	95.1	94.6
6	95.3	95.1	95.6	95.2	94.5
Mean	95.37	95.10	95.58	95.13	94.65
S.D.	0.082	0.063	0.160	0.082	0.122
CI_{95}	0.086	0.066	0.168	0.086	0.129

Table 6
Results of intermediate precision test: melting point or upper limit of the melting range

Sample	Day 1 (°C)	Day 2 (°C)	Day 3 (°C)	Day 4 (°C)	Day 5 (°C)
1	98.9	98.3	98.7	97.7	98.7
2	99.0	98.8	99.0	97.7	98.6
3	98.9	97.8	98.9	97.7	98.6
4	99.0	98.4	99.1	97.4	98.7
5	98.9	98.2	98.9	97.7	98.7
6	98.8	98.3	99.1	98.0	98.7
Mean	98.92	98.30	98.95	97.70	98.67
S.D.	0.075	0.323	0.152	0.190	0.052
CI_{95}	0.079	0.339	0.159	0.199	0.054

Table 7
Repeatability and intermediate precision at laboratory of Xyrofin Ltd., Kotka, Finland

Melting range	Mean (°C)	Repeatability standard deviation, s_r (°C)	Intermediate precision standard deviation, s_D (°C)	Repeatability, r (°C)	Intermediate precision, r_D (°C)
Lower limit	95.17	0.11	0.36	0.30	1.02
Upper limit	98.51	0.19	0.55	0.52	1.55

Table 8
t-Test results for the melting onset and peak values of lactitol monohydrate measured with DSC: comparison of different heating rates

	Sample 5			
	Onset, 5°C min^{-1}	Onset, 10°C min^{-1}	Peak, 5°C min^{-1}	Peak, 10°C min^{-1}
Mean	94.39	95.64	99.62	102.07
Variance	0.031	0.008	0.052	0.028
Observations	6	6	6	6
d.f.	5		5	
t stat	−16.191		−17.386	
t critical two-tail	2.571		2.571	
	Sample 5			
	Onset, 10°C min^{-1}	Onset, 15°C min^{-1}	Peak, 10°C min^{-1}	Peak, 15°C min^{-1}
Mean	95.64	96.85	102.07	104.66
Variance	0.083	0.018	0.028	0.272
Observations	6	6	6	6
d.f.	5		5	
t stat	−14.639		−15.825	
t critical two-tail	2.571		2.571	
	Sample 5			
	Onset, 15°C min^{-1}	Onset, 20°C min^{-1}	Peak, 15°C min^{-1}	Peak, 20°C min^{-1}
Mean	96.85	97.85	104.66	107.07
Variance	0.018	0.030	0.272	0.409
Observations	6	6	6	6
d.f.	5		5	
t stat	−11.467		−7.525	
t critical two-tail	2.571		2.571	

Table 9
Repeatability for onset and peak temperature values of lactitol monohydrate measured with DSC

Melting range	Scanning rate (°C min^{-1})	Mean (°C)	Repeatability standard deviation, s_r (°C)	Repeatability, r (°C)
Onset	5	94.39	0.176	0.49
	10	95.64	0.091	0.25
	15	96.85	0.136	0.38
	20	97.85	0.173	0.49
Peak	5	99.62	0.228	0.64
	10	102.07	0.168	0.47
	15	104.66	0.522	1.46
	20	107.07	0.639	1.79

4. Conclusion

The determination of the melting point with a melting point instrument is a fast and cheap way for controlling the quality of product. From the shape of the DSC-curve, it can be deduced whether the product is pure.

The melting range of pure stable lactitol monohydrate is 94–100°C when determined by a melting point instrument. It was found that the initial temperature, grinding, and drying had only a small effect on the melting range of lactitol monohydrate when it is determined with a melting point instrument independently of different analysts and different measuring times.

To get comparable results by the pharmacopoeia and DSC methods, the melting range of lactitol monohydrate should be determined with the same heating rate. When determining the melting range by the DSC, several heating rates should be used for samples. The result is obtained by extrapolating the onset and the peak temperatures to an isothermal situation by a linear regression method. Therefore, the onset temperature of the melting peak is lower than when measured by a melting point instrument with the heating rate of 2°C min^{-1}. The extrapolated onset and peak temperatures of the melting peak are 93.8 and 98.1°C, respectively, with the heating rate of 2°C min^{-1}. The melting range determined with the DSC is according to these measurements 93–99°C, which is 1°C lower than determined with the melting point instrument.

The DSC-curves give information not only of melting, but also whether other lactitol phases are present. From the shape of the DSC-curve, it can be deduced whether the product has a high quality. The determination of the melting point with a melting point instrument is a fast and cheap way for controlling the quality of the product. However, in case the melting of a sample does not fit the specified melting range, the measurement indicates only poor quality of the product but not specify its impurity or phase.

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