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PhD thesis

THE STUDY OF PHYSICO-CHEMICAL PROPERTIES OF CRYSTALLINE AND AMORPHOUS MATERIALS

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1. Introduction

A major part of modern pharmaceutical research is constituted by formulating a base material of high purity with reproducible physical, chemical and biological properties. The majority of solid organic and inorganic materials occur in a crystalline form in nature, and the same is true for materials produced synthetically. A crystalline material is characterised by a regular, well-definable crystalline structure in which the molecules form a three-dimensional structure.

However, under certain circumstances materials can appear in an amorphous form, too. One possibility for this is when a partially or entirely amorphous form arises during the formulation of materials. The most common such technological procedures can be classified into four groups:

1. condensation from a vapour state
2. supercooling of the melt
3. mechanical activation of a crystalline material. e.g. milling, pressing of a crystalline material
4. rapid precipitation from solution, e.g. freeze drying or spray drying

In pharmacy, amorphous character is general in the case of polymer molecules used as auxiliary materials or in the case of peptides and proteins of a great molecular mass and with a therapeutic effect, but is may also occur with smaller organic and inorganic molecules.

The regular three-dimensional lattice typical of crystalline materials is absent from amorphous materials, only short-range order can be seen in them. Among others, this explains why the amorphous form differs from the crystalline form in terms of certain thermodynamic properties, such as melting point, vapour pressure or solubility; therefore it is essential to know these properties in order to understand and to quantify spontaneous changes occurring during the storage or handling of materials.

2. Aims

The aims of my research can be summarized as follows:

⇒ The aim of my thesis was to investigate the crystalline and amorphous nature of materials, and as part of this to review particularly the properties of amorphous character, its features which are important from the pharmaceutical aspect, its occurrence and importance in

certain fields of pharmacy such as pharmaceutical technology, pharmacology and biopharmacy.

⇒ Another aim was to summarize the methods suitable for studying crystalline and amorphous structures, to review their possibilities of application, their advantages and disadvantages.

⇒ One of the aims of my experimental work was to work out a reliable method which can be used for the quantitative measurement of the degree of crystallinity of materials. α-lactose monohydrate was chosen as the model material for this.

- The task was to prepare an amorphous form from this material and also to work out the procedure for this. This was realized with crystallization from a solution, with spray drying.
- Crystalline-amorphous material mixtures of different proportions were needed for the examinations, so another task was to work out how to produce them. Physical mixtures were prepared for this purpose.
- Finally, an analytical method had to be chosen and the investigation parameters had to be set for the quantitative measurement of the degree of crystallinity. Our aim was to perform this measurement with an instrument which is used in a relatively wide range. Moreover, the procedure had to satisfy "modern" requirements such as environmental protection and cost effectiveness, too.

⇒ A further aim of the experimental work was to study melt technology and its pharmaceutical applicability. This technology includes procedures which are advantageous in several respects, yet their use is not widespread enough.

- We had to model thermal processes occurring in melt technology and by means of this to study the thermal behaviour of the materials chosen. This is all the more important because the thermal treatment of materials is performed not only in melt technology but they are exposed to heat effect in the course of other technological procedures as well, e.g. during spray drying, the preparation of solid solutions or even tablet pressing. Therefore it is very important to know the thermal behaviour of materials as it can help us to predict what changes base materials may go through during various technological processes.

– Sugar alcohols (mannitol, sorbitol) were chosen as model materials for the work. The aim of the examinations was to determine in what form these materials can be used in melt technology. The thermal properties of pure mannitol, sorbitol and their mixtures were investigated. Furthermore, it was examined what effect prior melting and solidification had on these materials and on their processibility.

3. Materials and methods

Lactose

Alpha-lactose monohydrate was chosen as the model material because it is well known that its crystalline and amorphous forms, as auxiliary materials, influence the production and stability of solid-state dosage forms.

A sample of alpha-lactose monohydrate (Pharmatose DCL 15, DMV International, the Netherlands) was used as the reference material (corresponding to 100% crystalline lactose). The particle size was in the range of 50–280 μm.

Amorphous sample

In order to prepare totally amorphous lactose crystalline alpha-lactose monohydrate was dissolved in water at a ratio 1:10 to obtain a solution for spray drying. Spray dried (*SD*) lactose was prepared using an A/S NIRO Atomizer (Copenhagen, Denmark). The processing conditions were as follows: feed rate: 20 ml/min, inlet and outlet temperature: 175°C and 80°C. The crystallinity of the SD lactose is considered as 0%. The average particle diameter of SD lactose was 5–30 μm. The resulting amorphous particles were kept in a glass vial and stored in a desiccator at 30% relative humidity (*RH*) and room temperature (50-60% RH is the critical RH for crystallization of amorphous lactose).

Physical mixtures

Physical mixtures of amorphous and crystalline lactose were prepared to achieve 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 100% crystalline content by weight. The components were weighed to a total amount of 25 g and were mixed in a Turbula mixer (Turbula WAB, System Schatz, Switzerland) with 10 g of 2 mm glass beads. The powder mixture was stored at 30% RH and room temperature up to the analysis.

Sugar alcohols

Sugar alcohols are widely used in the pharmaceutical and food industry. They have the sweetness of sugars but they have a lower caloric value, they are not cariogenic and they are suitable for diabetics.

β-*D*-mannitol (Ph.Eur.4) and *D*-sorbitol (Ph.Eur.4) were used for the study. The particle size of both materials was in the range of 0.16-0.32 mm.

Mannitol has a strong tendency to crystallize, nonetheless exists in fully or partially amorphous state in certain formulations. Because of its low chemical reactivity and low hygroscopicity, it is used in tablet formulation as filling and binding material in a direct compression of suckable, chewable and effervescent tablets. In food industry they are used for the production of sweets and chewing gums. The modern use of spray-dried mannitol is the stabilization of proteins in aerosols. It is also frequently used as auxiliary material in spray- and freeze drying.

Sorbitol is the stereoisomer of mannitol, it is easily compressible but its disadvantage is its high hygroscopicity.

Both materials can be used for the preparation of solid dispersions, in order to increase the dissolution rate of poorly water soluble drugs and to amorphize materials with a propensity for polymorphism. In the preparation of solid dispersion systems the drugs can be mixed in melted excipients and after solidification this is suitable for further processing. Sorbitol and mannitol are suitable for this purpose because they are very stable to heat and melt without decomposition.

Physical mixtures

Physical mixtures were prepared to achieve 0, 10, 30, 50, 70, 90 and 100% sorbitol content by mass. The components were weighed and then mixed in a Turbula mixer (Turbula WAB, System Schatz, Switzerland) for 10 min. The prepared mixtures were divided into two parts. The first portion was processed hereafter as a physical mixture, the second portion was melted and then the solidified melt was used for further studies. During melting, the mixtures were heated in a furnace up to 170ºC and then cooled at room temperature. The solidified by cooling materials were pulverized and sieved (0.16-0.32 mm). During the preparation and storage of the mixtures <55% relative humidity was ensured because of the strong plasticizing effect of water.

Tablet forming

A Korsch EKO eccentric tablet machine (Emil Korsch Mashinenfabrik, Berlin, Germany) was applied for tablet forming. The compression tools were single, flat punches 10 mm in diameter, furnished with strain gauges. The rate of compression was 30 tablets/min with a pressure forces 2, 4 and 6 kN, at room temperature of 25±2ºC and relative humidity of 45±2%. The mass of tablets was 0.20 g. The tablets were stored at temperature of 20±2ºC and at 45±5% RH.

Examination parameters

X-ray powder diffraction (XRPD)

XRPD profiles were taken with an X-ray powder diffractometer (Philips PW 1050/70 PW 1710). The measurement conditions were as follows: radiation source: Cu Kα, scan speed (2θ): 0.035, step size (2θ): 0.035, time per step (s): 1.0.

Near Infrared Spectroscopy (NIRS)

The diffusion reflectance was measured by a Hitachi U-3501 UV/VIS/NIR spectrophotometer (Hitachi Ltd., Japan) equipped with integrating sphere (d=60 mm) and PbS detector. Solid samples were placed into the 5-mm layered sample holder of the instrument and the diffuse reflectance spectra were recorded in the 200–2600 nm wavelength range.

Differential Scanning Calorimetry (DSC)

DSC studies were performed using a DSC 821^e(Mettler-Toledo GmbH, Switzerland). The instrument was calibrated using indium.

In the case of lactose samples, samples of approximately 4 to 4.5 mg were weighed into non-hermetically sealed aluminium pans. The samples were heated from 20 to 240°C at a heating rate of 5°C min^{-1}.

In the case of sugar alcohols, samples of 15-20 mg were heated in a hermetically sealed aluminium pan. At first the samples were heated from 25ºC to 200ºC at a heating rate of 3.5°C min^{-1}. Then the samples were cooled in liquid nitrogen, subsequently the mixtures were reheated from -20ºC to 200ºC at the rate of 3.5°C min^{-1}.

Study of tablets

The tablets were stored in sealed glass containers at room temperature of 20±2ºC and 45±5% relative humidity. The crushing strength was investigated with Heberlein apparatus (Flisa, Le Locle, Switzerland). The crushing strength was determined one day (24 h) after formulation because of the texture change (elastic recovery).

4. Results

Lactose

Quantification of the crystallinity by XRPD

It was confirmed with X-ray powder diffraction that the initial α-lactose monohydrate sample was a 100% crystalline material. X-ray diffractograms of the samples with different amorphous content were recorded.

Analysis by Multiple Linear Regression (*MLR*) showed close correlation between intensity values at chosen four different 2θ values of X-ray diffractograms and crystallinity of mixtures. Therefore, XRPD is indeed suitable for determining the crystallinity of α-lactose monohydrate (**Figure 1**). The results of other methods were compared to these results, and proved their suitability.

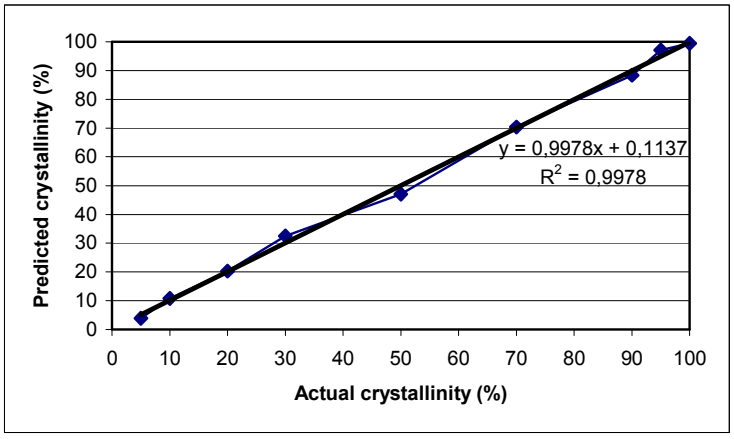

Figure 1 Relation between predicted and actual crystallinity of physical mixtures of crystalline and amorphous lactose, determined by conventional X-ray powder diffraction

Quantification of the crystallinity by NIRS

Suitability of NIRS was studied for the quantification of crystallinity.

Three spectra of each sample were collected and subsequently averaged to produce a single spectrum used for further analysis. Quantification of crystallinity was performed using second-derivative spectra in order to minimize the well-known effects of particle size and variable scattering of NIR radiation. Using the second-derivative values, MLR was performed at chosen characteristic wavelengths.

In the case of an unknown sample, after taking the spectrum, the degree of crystallinity (%) of the sample can be obtained if the second derivatives of the absorbance values are substituted into the calibration equation. **Figure 2** shows the relation between the actual and predicted crystallinity determined by NIRS.

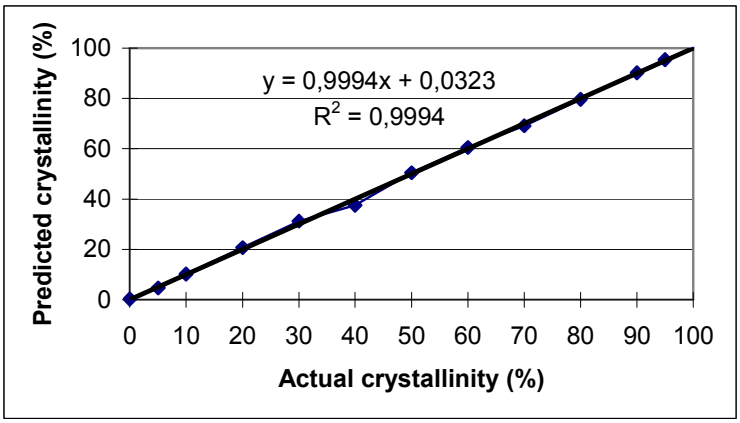

Figure 2 Relation between predicted and actual crystallinity of physical mixtures of crystalline and amorphous lactose, determined by Near Infrared Spectroscopy

The value of the correlation coefficient of the determination (R^2=*0.9994*) shows a close correlation. Thus, NIR spectroscopy is an appropriate method for the quantitative evaluation of crystallinity of pharmaceutical products, proven by the comparison of NIRS and XRPD methods **(Figure 3)**.

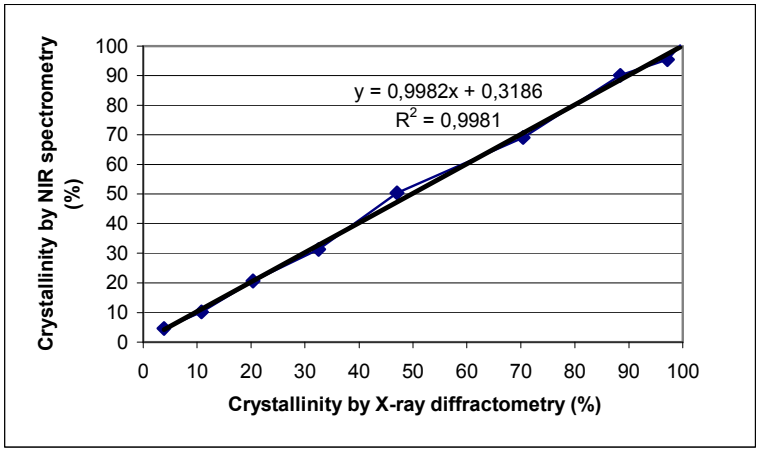

Figure 3 Relationship between the values of the degree of crystallinity determined with the NIRS and XRPD methods

Quantification of the crystallinity by DSC

DSC measurements were performed for the investigation of thermal behaviours of samples.

The amorphous form of lactose was identified by the presence of an exothermic peak at 167°C, which represented the transformation of amorphous to crystalline form. It is followed by two endothermic peaks, one at 210°C and the other at 216°C. These melting peaks belong to alpha- and beta-lactose, respectively. It confirmed the transformation of the amorphous form of lactose to the two types of crystalline form by heating.

In case of the 100% crystalline sample the DSC diagram has an endothermic peak at 144°C, which represents the loss of crystalline water. This is proven by thermogravimetric analysis, where the sample loses 4.34% water in the range of 130-160°C. The endothermic peak is followed by two melts of α and β forms at 213°C and 224°C.

In different mixtures, the ratio of the height and areas under curves of these two peaks varied, but no relation could be proven between crystallinity and these peaks. With the increase of amorphous component in the mixtures, the height of the endothermic peak (typical for crystalline form) decreases and the height of the exothermic peak (typical for amorphous form) increases on the DSC diagrams.

Both parameters typical for endothermic and for exothermic processes were studied as a function of degree of crystallinity. On the basis of this, the transition energy values of the amorphous component were used for quantitative evaluation.

20-100% amorphous content (0 to 80% crystallinity) was possible to be determined by DSC during the measurements. In cases of lower amorphous content, the exothermic peaks were characterless, unsuited for quantitative evaluation. **Table I** shows the energy values of the exothermic peak typical for crystallization in the mixtures with different proportions.

Table I Transition energy values of the mixtures with different crystallinity

Degree of crystallinity (%)	Transition energy (J/g)
0	112.15
5	99.31
10	95.64
20	78.3
30	55.93
50	39.45
60	33.73
70	28.97
80	14.01

The regression analysis of these values generates the calibration equation, with this equation the crystallinity of an unknown lactose sample can be easily calculated when the transition energy value is known. **Figure 4** shows the predicted crystallinity (determined by regression analysis) as a function of actual crystallinity.

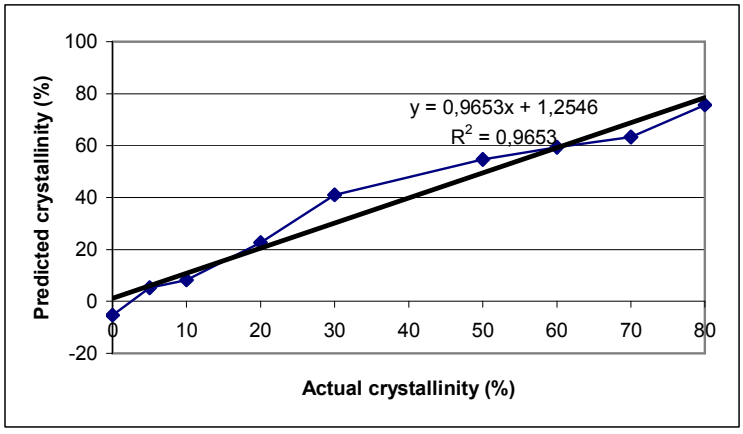

Figure 4 Relation between predicted and actual crystallinity of physical mixtures of crystalline and amorphous lactose, determined by DSC

The correlation (*0.9653*) is less close than in case of X-ray diffraction studies (*0.9978*), nevertheless DSC can be applied safely, especially for the quantitative evaluation of lactose samples with high amorphous content (0 to 80% crystallinity). However, when the lactose sample contains 80 to 100% crystalline part, X-ray diffraction is recommendable for exact quantitative determination.

Sugar alcohols

With the help of the DSC methode thermal properties of mannitol and sorbitol was studied and characteristic thermal parameters were recorded (**Table II).**

Table II Characteristic thermal parameters of sorbitol and mannitol

	T_m (ºC)	ΔH_m(J/g)	T_g (ºC)
sorbitol	96.8	217	-2.3
mannitol	165	338	-

On the first DSC scan pure sorbitol melted at 96.8ºC, (ΔH_m=217 J/g), and by cooling it cannot be recrystallized from its melt. If vitrified systematically, this leads to an amorphous state, which can be proved by X-ray powder diffraction. On the second DSC scan at -2.3ºC glass transition (*T*g) was observed.

The starting mannitol was β-*D*-mannitol, observed by XRPD, which melted at 165ºC on the first heating. The melt crystallized during cooling as α-*D*-mannitol, therefore polymorph transition took place. In case of mannitol no glass transition was observed on the second DSC scan.

It can be stated that neither pure mannitol nor pure sorbitol is suitable for hot melt technology. Because of mannitol's high melting temperature, it cannot be used in case of materials which decompose at this temperature. Sorbitol's vitrified state after melting is difficult to handle during further processing. Our additional aim was to eliminate these problems with mixing the two sugar alcohols.

During the examination of the mixtures, the decrease in the melting temperature of mannitol was observed when the ratio of sorbitol was increased in the mixture, because mannitol dissolves in sorbitol melt (**Figure 5**). On the other hand, the melting point of sorbitol was only slightly affected by the presence of mannitol with a higher melting point.

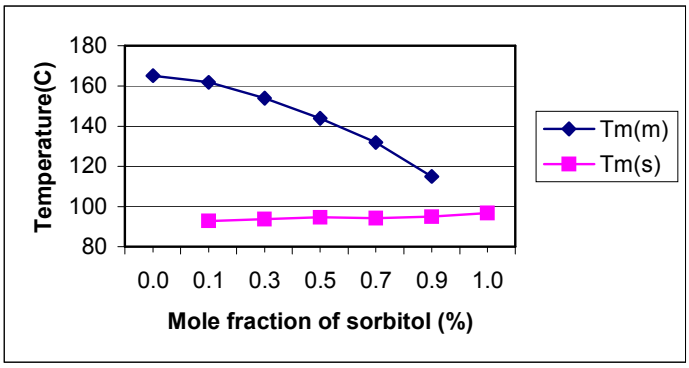

Figure 5 The melting temperature (*Tm*) of sorbitol (*s*) and mannitol (*m*) in relation to mole fraction of sorbitol in the mixtures

When the ratio of sorbitol was lower than 30%, no glass transition was detected on the second DSC scan. When the sorbitol content was above 90%, only glass transition was detected during the second heating (**Table III**).

Table III Thermal parameters of sorbitol and mannitol mixtures during the second heating

Sorbitol content (%)	T_g (ºC)	T_{cr} (ºC)	T_m (ºC)
0	-	-	165
10	-	-	161.5
30	-	52.2	154
50	4.91	59.8	142.8
70	1.96	81.2	131.8
90	-1.39	-	-
100	-2.3	-	-

It can be stated that the vitrification of sorbitol cannot be avoided with the addition of mannitol. However much mannitol is mixed with sorbitol, the crystallization of sorbitol cannot be achieved. When the high melting point of mannitol is the problem during processing, this can be improved with the addition of sorbitol. The melting point of mannitol decreases when mixed with sorbitol as seen in **Figure 5**. A further question is whether these mixtures are technologically suitable for further processing.

In the case of tablets prepared from physical mixtures, the crushing strength increased with the increase of the sorbitol ratio at all three pressure forces. The melted and solidified mixtures were pressed with the same method as the simple physical mixtures. In the case of melted mannitol, tablets could not be pressed with pressure forces 2 and 4 kN because the tablets broke easily and disintegrated. With the other mixtures all three pressure forces could be used. The crushing strength of the tablets prepared from melted mixtures increased with the increase of pressure forces and also with the increase of the sorbitol ratio. The tablets formulated from the solidified melt of pure sorbitol were the strongest.

The comparison of physical mixtures and melted/solidified samples with the same component ratio shows that, when the sorbitol content was lower than 50%, tablets of physical mixtures were stronger, and when the sorbitol content was above or equal to 50%, the tablets of melted mixtures had higher crushing strength values.

5. Summary

The results of my research could be summarized as follows:

⇒ In the case of lactose, it was proved with X-ray diffraction that the initial material was really 100% crystalline α-lactose monohydrate, and also that a 100% amorphous product could really be formulated by its spray drying, with the parameters developed by us. The examination of the mixture samples confirmed that the samples obtained with the proper physical mixture of the initial crystalline and amorphous materials represented the various degrees of crystallinity well.

The X-ray diffraction examinations confirmed the fact already known from literature that this method is very reliable in the quantitative determination of the degree of crystallinity. For this reason, it was expedient to choose this method as a starting method and to compare the other two procedures with it.

The results of our NIRS examinations revealed that this method is also suitable for determining the degree of crystallinity with proper accuracy, in particular in the case of crystallinity lower than 5%.

It was confirmed with DSC examinations that the procedure can be used excellently for studying the crystalline-amorphous transition, important thermodynamic parameters of the sample can be given with its use.

In the quantitative determination it can actually be considered to be a semi-quantitative method as only minimum 20% of amorphous content could be detected with certainty. However, the advantage of DSC is that great amorphous content not detectable with X-ray diffraction can also be identified quantitatively.

In the case of lactose, the presence of the amorphous form in the sample influences compressibility. This parameter is important as lactose is widely used as an auxiliary material in tablet formulation. With respect to compressibility, it is ideal if the product contains more than 30% of amorphous part. DSC can be used to determine this, and there are several other cases where its accuracy suffices. As the DSC method is commonly used for other examinations in pharmaceutical industry, the determination of the degree of crystallinity can add to its range of application.

$\Rightarrow$ The examinations with the chosen sugar alcohols, sorbitol and mannitol, were mainly aimed at determining in what form these materials can be used in melt technology and also at studying their thermal behaviour.

It was proved during the investigations that the use of D-mannitol or D-sorbitol alone is disadvantageous in melt technology. It is true for both materials that they do not decompose at their melting point, which in itself would make them suitable for the preparation of solid dispersions. However, the use of mannitol is limited by its high melting point and also by its polymorhic transition after melting (β-D-mannitol$\rightarrow$ α-D-mannitol). Moreover, after sorbitol is melted, it is vitrified in a gel-like form (T_g= -2.3°C) so its handling is very difficult or even impossible during further processing.

We tried to eliminate these problems by mixing the two materials. It can be concluded from the research that the glass transition of sorbitol after melting cannot be avoided and the crystallization of the material cannot be achieved with the addition of mannitol. The high melting point of mannitol can be decreased by adding sorbitol. Thus its original melting point

of 165°C can even be lowered to 115°C. However, in this case the mix shows glass transition characteristic of pure sorbitol, so it is not advantageous in respect of further processing. A similar situation arises in the case of a eutectic mixture (melting point: 93.6°C) containing 1.8% mannitol and 98.2% sorbitol.

When the thermoanalytical data were compared with the crushing strength studies performed, the mixture of 30% mannitol and 70% sorbitol was found to be the best. The melting point of this sample was 131.8°C. After melting and solidification, we could press tablets with the greatest crushing strength from this sample.

The sample preparation we used was a good model for the material changes occurring during melt technology, and it could be monitored and described well with the chosen method (DSC).

A good possibility in processing base materials is offered by the preparation of solid solutions or dispersions, when the active ingredient is dispersed in the melt of the excipients, and after solidification it becomes suitable for further processing. Thus, for example, the solubility of poorly soluble active ingredients can be enhanced or materials with a propensity for polymorphism can be amorphized.

However, neither mannitol nor sorbitol is suitable for this in itself, the desired thermal properties could be achieved and the requirements expected during further processing could be met only by combining the two materials in proper proportions.

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