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Polymorphic transition of lipid particles obtained with the PGSS process for pharmaceutical applications

Lena Wollenweber*, Sabine Kareth, Marcus Petermann

Chair of Particle Technology, Ruhr-University Bochum, Universitätsstr. 150, DE-44801, Bochum, Germany

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ABSTRACT

The bioavailability of a pharmaceutical depends on the crystal structure of a polymorphic active ingredient or excipient. By changing the process parameters of the PGSS (Particles from Gas Saturated Solutions) process i.e. thermodynamic conditions of the solidification process it is possible to vary the crystal modification of polymorphic products. In a series of PGSS experiments microparticles of the polymorphic excipient tristearin as well as of mixtures of tristearin and the emulsifier sorbitan monolaurate were successfully produced. Pre-expansion pressure was varied between 6 and 22 MPa. The effects of process dependent parameters on the crystallization were investigated via scanning electron microscopy, differential scanning calorimetry and X-ray powder diffraction.

Metastable α-forms of tristearin were found immediately after the processing for all experiments. Further it was found that it is possible to shift the conversion rate of polymorphic transitions in the product either by varying the process parameters or by adding the emulsifier.

1. Introduction

The PGSS (Particles from Gas Saturated Solutions) process [1,2] has been successfully tested in terms of thermally gentle micronization of low-melting substances. Further the feasibility of producing composites was shown. Depending on the design of the PGSS process and selection of the substances to be processed, the composite materials may be single or multiphase. Two-phase composites were first manufactured and tested by Brandin [3]. The production of single as well as multiphase composite powders has already been investigated and has been proven to be feasible for applications in food and pharmaceutical industries [4–11]. Thus the PGSS process is feasible to produce drug delivery systems in order to gently process the containing bioactive ingredients. For example, a liquid antibiotic or pain reliever could be encapsulated in a fat in the PGSS process with regard to controlled release. In this case the bioavailability of the pharmaceutical system depends on the crystal structure of the excipient, since the crystallinity affects its dissolution behavior. The PGSS process is able to vary the crystal modification of polymorphic substances by changing the process parameters and therefore the thermodynamic conditions of the solidification process [12,13]. For other high pressure processes some authors showed already that an increasing pre-expansion pressure leads to an increase of the transformation tendency of polymorphic substances [14–17].

It is known that triglycerides like tristearin can crystallize in three polymorphs α, β' and β [18]. The three forms exhibit different physical properties like melting point and heat of fusion which can have an influence on the bioavailability of a pharmaceutical system where tristearin is an excipient. Knez et al. investigated the influence of pre-expansion pressure and temperature in a batch PGSS process on the crystal form and the degree of crystallinity of monostearate and tristearate. Products of monostearate were analyzed as stable β-form immediately after micronization. In case of tristearate the metastable β'-form was found for all process settings. A re-crystallization of all products was found which resulted in the stable β-form also for tristearate after three months of storage [19,20].

2. Materials and methods

The objective of this work was to find out whether the continuous PGSS process can create functionalized pharmaceuticals from polymorphic substances by setting the polymorphic type in order to create controlled delivery systems. Therefor it was figured out whether the polymorphs of a triglyceride which can function as a pharmaceutical excipient can be effected by the PGSS process conditions. This was done by PGSS processing and analyzing tristearin ($C_{57}H_{110}O_6$, M = 891.5 g/mol) as a model substance as excipient for pharmaceutical systems. Additionally, the influence caused by the presence of the emulsifier

* Corresponding author.
E-mail address: lena.wollenweber@gmail.com (L. Wollenweber).

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Table 1
Melting temperatures T_{fus} and enthalpies of fusion Δh_{fus} of the three polymorphic phases of tristearin. [21].

		T_{fus} [°C]	Δh_{fus} [J g^{-1}]
tristearin	α	54.2	128.0
	β'	63.6	154.2
	β	72.9	219.6

sorbitan monolaurate ($C_{18}H_{34}O_6$, M = 346.5 g/mol) in the gas saturated solution being processed was investigated. Melting temperatures and heats of fusion of the unprocessed polymorphic forms of tristearin are given in Table 1.

Aronhime et al. found that the polymorphic transformation from metastable α-tristearin in the case of conventional crystallization is significantly accelerated by adding the liquid surfactant Span 20 [22]. Their results are compared to a PGSS product consisting of tristearin and Span 20 within this work. In a series of experiments micro particles of the triglyceride tristearin, as well as of mixtures consisting of the fat and the co-surfactant sorbitan monolaurate (Span® 20) were processed with PGSS using CO_2 as sub- and supercritical fluid. CO_2 (99.9%) was purchased from YARA (Bad Hoenningen, Germany) and Span 20 from Sigma-Aldrich Co. LLC. (St. Louis, USA). Tristearin (Softenol 3118) was supplied by Cremer Oleo GmbH & Co. KG (Witten, Germany). Table 2 shows process conditions and processed substances of selected experiments that are exemplarily presented here. In case of an emulsion being processed by PGSS the preparation of the emulsion was as follows: Substances were weighed according to the desired composition and melted. The mixture was stirred on a heating plate for 15 min at 800 rpm to obtain a homogenous emulsion.

A flow sheet of the PGSS plant is presented in Fig. 1. CO_2 is supplied from a storage tank (A). It is pressurized (B) and heated (C) to the required set points. The melt comes from a heated vessel (I) and is fed to the mixing-block (D) via a second pump (J). The mass flows of the CO_2 and the melt are recorded when carrying out the experiment. The gas-saturated solution passes the nozzle-block (E). It expands through a nozzle of diameter 0.5 mm into a cylindrical spray tower (F). Here the powder can be collected. After filtering of the particles (G) the expanded gaseous carbon dioxide is exhausted to the environment.

Pre-expansion pressure was varied between 6 and 22 MPa which resulted in a temperature difference between pre- and post-expansion conditions (ΔT) from 65 to 85 °C. The effects of temperature and other process-dependent parameters on the crystallization process and in particular the influence on the polymorphic transformations during the micronization were studied. For this purpose, the polymorphic characteristics and thermal stability of the different polymorphic forms of the produced powders were analyzed using Differential Scanning Calorimetry (DSC) and as a non-affecting method for the sample X-Ray Powder Diffraction (XRPD, exposure time 60 min, 10 scan loops). The melting temperature and heat of transformation of the samples were determined by measuring the heat flow difference between the sample and a reference material while varying the temperature using a DSC131 EVO by Setaram Instrumentation (Caluire, France). DSC measurements were performed right after the production of the powders and repeated after certain storage times at 20 °C to investigate the polymorphic transition. Temperature was varied from 30 to 120 °C with a heating rate of 5 °C/min. XRPD measurements were conducted using a Guinier Camera G670 by HUBER Diffraktionstechnik GmbH & Co. KG (Rimsting, Germany) as a fully-fledged diffractometer. Powder diffractograms were generated by using a molybdenum anode (λ = 0.71 Å) as X-ray source. The step size was set at $\Delta 2\theta$ = 0.005°. Furthermore, morphological properties were figured out by Scanning Electron Microscopy (SEM). SEM delivers information about topography and composition of samples that are scanned by a focused electron beam. In this study, a high resolution thermally aided Field Emission Scanning Electron Microscope (FE-SEM) type LEO Gemini 1530 by Carl Zeiss AG (Oberkochen, Germany) was used. FE-SEMs with a field emission electron source deliver sample's surface information with a high resolution at low voltage.

Table 2
Process conditions and substances in the series of PGSS experiments with pre-expansion pressure p and temperature difference between pre- and post-expansion ΔT.

process conditions	p_0 = 6 MPa, ΔT = 65 °C	
	p_0 = 22 MPa, ΔT = 85 °C	
substances	tristearin	
	tristearin + 10 wt.-% Span 20	

3. Results

This work summarizes the results of a series of PGSS experiments. The pure substance tristearin as well as mixtures of tristearin and Span 20 have been successfully processed. For all experiments stable, solid free flowing powders were obtained.

Fig. 2 shows an SEM image of the raw material tristearin in an unprocessed state in a ten times larger scale. As common for crystalline structures the surface has polygonal and angular shapes. Needle and flake shaped surfaces and particles can be distinguished.

In Fig. 3 SEM pictures of four PGSS products are shown. On the left side pictures of powders produced at 6 MPa pre-expansion pressure which results in a temperature difference between pre- and post-expansion of ΔT = 65 °C can be seen while powders processed at 22 MPa pre-expansion pressure which results in a temperature drop before and after expansion of approximately ΔT = 85 °C can be found on the right side. First row of Fig. 3 illustrates SEM results for the processing of pure tristearin. Second row presents particles that were produced out of a mixture containing tristearin and the liquid surfactant Span 20.

First, it can be noticed that in contrast to the unprocessed substances all PGSS products show spherical shapes. Only the surfaces exhibit different states of scaled surfaces. Particles that were produced under higher pre-expansion pressure have a larger surface because they are significantly smaller and in addition to the flakes they have some needles on their surfaces. Particles that were micronized out of pure tristearin under low pressure consist of wide areas where there are now needles or flakes growing. Instead they possess some flat surface parts. The main conclusion drawn from these results is that there are some signs for crystallinity in all the products. DSC and XRPD experiments are performed to gain further information on the degree of crystallinity and the crystal forms of the products.

DSC analysis of tristearin shows a typical thermogram for each crystal state which is defined by literature and shown in Fig. 4 [24].

Values for the melting onset temperatures and the heat of fusion for the three polymorphs of tristearin are reported in literature, e.g. [24–26] α-crystals show an early melting peak (at approximately 54 °C). After that a polymorphic transition into β'-form takes place due to the constant heating of the sample. Further the β'-form melts and recrystallizes into the stable β-form within the analysis. Those three phenomena result in an exothermic peak while heating the sample. At the highest temperature, the thermogram of α-crystals shows the typical β melting peak at about 72 °C.

The DSC results shown here only consider the phase transitions during the first heating cycle since only that part of the analysis shows exactly the calorimetrical behavior of the actual PGSS powders. DSC thermograms are therefor cut before a sample temperature of 40 °C and after 100 °C respectively and slope corrected. Subsequently the baseline integration is made to figure out the heat of the correspondent phase transitions. The typical melting behavior of α-crystals is figured out for all the PGSS products directly after being produced. Independent on the pre-expansion pressure being high or low all the powders show α-melting behavior which can be seen in Fig. 5 for the upper continuous lines.