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Investigation of drug polymorphism: Case of artemisinin

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ABSTRACT

The polymorphism of the anti-malarial compound artemisinin was examined. The phase behavior of solid artemisinin has experimentally been investigated using differential scanning calorimetry and temperature-resolved X-Ray powder diffraction. In addition, complementary solution studies and suspension experiments were performed. The results clearly confirm the existence of two modifications of artemisinin, which are related enantiotropically. The orthorhombic modification is the thermodynamically stable form at low temperatures, while the triclinic form is the stable one at higher temperatures with a transition temperature of ~130 °C. Problems associated with analysis of the polymorphic phase behavior are comprehensively addressed.

1. Introduction

A solid substance which is able to form different crystalline lattices at same chemical composition shows polymorphic behavior [1]. Around one-third of the organic compounds appear in different crystalline forms [2]. Various polymorphs differ in their physical properties, with the solubility and dissolution rate being of major importance for an active pharmaceutical ingredient (API) [3].

To guarantee the quality and effective dosage of a drug it is necessary to identify the polymorphic behavior of an API during the pharmaceutical development as early as possible and, afterwards, to secure a robust control of the production of the desired polymorph [2].

The substance studied here is artemisinin (Fig. 1), chemically a sesquiterpen lactone with an endoperoxide function, isolated from *Artemisia annua* (sweet wormwood) growing in China and Vietnam. It was recommended from the World Health Organization as the currently most effective anti-malarial drug [4].

The existence of two crystalline forms of artemisinin, an orthorhombic and a triclinic modification, is already known. Their crystal structures, in addition to the absolute configuration, have been determined in 1988 and 1997 [6,7]. Chan et al. [7] reported very close melting temperatures of the two forms with 154.88 °C and 155 °C for the orthorhombic and the triclinic form, respectively. Other melting data given in the literature do not state the identity of the solid phase studied and vary between 153–154 °C [8], 156–157 °C [9], 158–159 °C [10] and 153–155 °C [11]. Chan et al. [7] describe the formation of the triclinic modification from cyclohexane solution.

Thus, the properties of the two polymorphs, their formation conditions and in particular their relative thermodynamic relationship are so far not clearly characterized, but very important in the industrial pharmaceutical practice for the design of crystallization and purification strategies. Different packing arrangements of molecules in the crystal lattice influence almost all pharmaceutical properties of the solid substance [12]. For this reason, it is of crucial relevance to have knowledge about the interconversion between pairs of polymorphs or solvates due temperature or solvent changes.

In the present study the polymorphism of artemisinin is examined in detail. X-Ray powder diffraction (XRPD) (also temperature-resolved) and differential scanning calorimetry (DSC) were used to elaborate and to understand the nature of the polymorphs present and the thermodynamics of the polymorphic transition both in solid and solution phase.

2. Experimental

2.1. Materials

The studies were carried out using several artemisinin sources: (a) synthesis product kindly provided from collaboration partners* (*Group of Peter H. Seeberger, Max Planck Institute of Colloids and Interfaces in Potsdam and Free University of Berlin, Institute of Chemistry and Biochemistry) (purity >98%), commercial product (CAS-Nr. 63968-64-9) purchased by (b) TCI Europe with a purity

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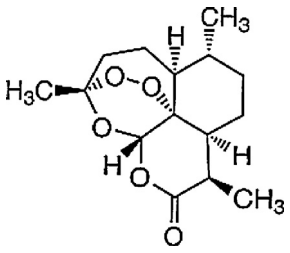

Fig. 1. Chemical structure of artemisinin [5].

of >97% and (c) Sigma-Aldrich with a purity >98% and (d) solid phase of pure artemisinin obtained from own evaporation crystallization experiments. All solid materials were checked for identity and crystallinity. Before further application they were crushed in a mortar to provide finely dispersed powder samples for DSC and XRPD measurements.

HPLC analytical grade solvents toluene and ethanol (each of >99.8% purity) were obtained from VWR International and Merck KGaA respectively.

2.2. X-Ray powder diffraction measurements

X-Ray powder diffraction was used to identify and characterize the solid phases obtained during the studies of artemisinin. X-Ray powder data were collected on an X'Pert Pro diffractometer (PANalytical GmbH, Germany) using Cu-*K*α radiation. Samples were scanned in a 2Theta range from 4° to 30° with a step size of 0.017° and a counting time of 50 s per step. In order to elaborate the phase behavior with respect to temperature, temperature-resolved XRPD measurements were performed. The samples were heated stepwise in a temperature range of 30–147 °C with a heating rate of 1 K/min. At certain temperatures samples were held isothermal for a period of 15 min to allow for establishment of equilibrium before the XRPD patterns were taken. Samples were subsequently cooled down to 30 °C with 10 K/min. The samples were prepared after carefully grinding in a mortar as fine powder on Si single crystal discs for XRPD measurements at ambient temperature. For temperature-resolved XRPD studies a plate-like sample holder, made from Cr-coated Cu with a 0.2 mm sample depth was used. The heating is provided directly via the sample holder bottom, which is closely contacted to the heater. A heat conductivity paste further allows for optimal heat transfer.

2.3. Differential scanning calorimetry studies

Melting temperatures and enthalpies of fusion were determined by differential scanning calorimetry using a Setaram DSC131 (Setaram, France). The DSC device was carefully calibrated against the temperatures and enthalpies of fusion of water, indium and tin. Samples prepared as described before were weighed into sealed Al-pans (usually 10–12 mg) and heated from 30 °C to 165 °C at a rate of 2 K/min, while purging with helium. The measurements were repeated several times to obtain averaged values with standard deviations.

2.4. Complementary solution studies and suspension experiments

To confirm the thermodynamic relationship of the polymorphs, complementary solution and suspension experiments were performed. First, equilibration studies of suspensions of artemisinin in various ethanol/toluene solvent mixtures were done at different temperatures between 5 °C and 40 °C. Fast evaporation experiments were carried out using various clear saturated solutions of artemisinin in ethanol/toluene solvent mixtures at ambient temperature. Therefore, solutions were saturated at different temperatures between 5 °C and 20 °C. Further, a slurry experiment was performed by stirring 200 mg of a 1:1 mixture of the artemisinin polymorphs in 1 ml ethanol at room temperature for 12 h. All solid phases obtained were analyzed with XRPD.

3. Results

3.1. Identification and analyses of solid phases in the artemisinin system

Fig. 2 shows the XRPD patterns of the solid artemisinin samples used in this work. Measured patterns are compared with references for the two artemisinin polymorphs derived from single crystal data given in the Cambridge Structural Database (CSD) [13].

Patterns taken from solid (a)–(c) clearly correspond to the crystal structure of the orthorhombic modification and those taken from solid (d) match the pattern of the triclinic modification, all with slight differences in crystal orientation in particular for samples of the orthorhombic form.

As indicated in Fig. 2, for reasons of simplification the orthorhombic modification will be labeled with Mod. I (for modification I) and the triclinic modification with Mod. II (for modification II) throughout the following part of the manuscript.

In order to elaborate the phase behavior with respect to temperature, temperature-resolved XRPD measurements for both artemisinin modifications were performed. Figs. 3 and 4 show the collected XRPD patterns of Mod. I and Mod. II in dependence on temperature, each compared to the reference structures.

For Mod. I subsequent heating and cooling cycles, with the last after complete melting of artemisinin, have been measured.

In the temperature range between 30 °C and 125 °C with increasing temperature no visible deviation from the reference of Mod. I can be noticed. Exclusively, peaks belonging to Mod. I are present here. Slight peak shifts to lower angles are related to the lattice expansion with increasing temperature. With further temperature increase up to 135 °C additional peaks appear, that clearly originate from the presence of Mod. II (e.g. peaks at ~9°,10 to 11 and 12 to 13 as well). At 147 °C, the conversion from Mod. I to Mod. II is completed. With decreasing temperature no backward transition from Mod. II to Mod. I occurred. Even after complete melting and recrystallization of the sample, Mod. II remained stable.

On the right hand side of Fig. 3 an interesting detail of the powder pattern at 135 °C is highlighted. The peaks have been indexed and the resulting *h k l*-values added to the figure. A closer look on the 2Theta range between 6° and 14° clearly verifies that a partial reorganization of the crystal lattice of Mod. I to Mod. II takes place. A splitting of the peaks indexed with [0 0 1] and [1 0 0] belonging to the Mod. II is observed. The increasing temperature causes changes of the unit cell parameters. The parameter *c* of the triclinic cell of Mod. II indexed with [0 0 1] is the most sensible one with greater temperature dependence than the parameter *a* indexed with [1 0 0]. As a result, the peak [0 0 1] splits from [1 0 0] and both slightly shift to lower angles.

However, contrary to the case described above, recrystallization of Mod. I occurred occasionally. This particularly happened in runs without sample melting where a small residual amount of Mod. I remained in presence of Mod. II acting as seed crystals for Mod. I to be formed back. Thus, Mod. I of artemisinin reversibly transformed into Mod. II with increase of temperature and vice versa with decrease of temperature, indicating an enantiotropic relationship of both forms.