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Research paper

Preparation of amorphous indomethacin nanoparticles by aqueous wet bead milling and *in situ* measurement of their increased saturation solubility

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ABSTRACT

The aim of this study was to prepare amorphous indomethacin nanoparticles in aqueous media and to determine *in situ* their increased saturation solubility and dissolution rate. Drug nanosuspensions with a Z-average of ~300 nm were prepared by wet media milling and afterwards freeze-dried. The drug solid state was analyzed by DSC, XRD and FTIR before and after the milling process. Milling of amorphous indomethacin with polyvinylpyrrolidone (PVP) as stabilizer resulted in an amorphous nanosuspension which could not be redispersed in the nanosize range after freeze-drying. The combination of PVP and poloxamer 407 resulted in crystalline nanoparticles: poloxamer 407, a polymer with high molecular weight, competed with PVP for surface coverage, and hindered the interaction between PVP and indomethacin. This indicated the importance of sufficient drug-PVP interactions on the drug particle surface for amorphous state stabilization. Redispersable amorphous indomethacin nanoparticles were obtained by combining the anti-recrystallization effect of PVP with the particle size stabilization provided by sodium dodecyl sulfate. Solubility studies were performed *in situ*. The solubility of crystalline micronized indomethacin of 6.7 ± 1.3 μg/mL was increased up to 17.3 ± 2.8 μg/mL by its amorphization, with a factor of increase of 2.6. Indomethacin amorphization increased its dissolution rate by a factor of 30. Indomethacin nanocrystals resulted in an increased solubility of 2.6 times, with a solubility of 17.2 ± 0.4 μg/mL. The highest increase was obtained with amorphous indomethacin nanoparticles with a solubility of 35 ± 1.6 μg/mL and 5.2 times higher than the solubility of the original indomethacin. Amorphous indomethacin nanoparticles resulted in the highest dissolution rate, which increased from 0.003 μg/(mL s) to 2.328 μg/(mL s). The synergistic effect obtained by the combination of nanosize and amorphous solid state was demonstrated.

1. Introduction

Most new drug candidates are characterized by a poor aqueous solubility, potentially resulting in low bioavailability and failure of the therapy [1]. Different approaches have thus been developed aiming to increase the rate of dissolution or solubility of poorly soluble drugs. They can be divided into three main categories: physical modifications (e.g. amorphous form), chemical modifications (e.g. complexation) or miscellaneous methods (e.g. use of solubilizers) [2]. Among the physical modifications, nanosuspensions (nanocrystals) and amorphous formation are promising approaches.

In comparison to microcrystals, nanocrystals are characterized by a faster dissolution rate due to their increased surface area [3], and by a higher saturation solubility [4]. Their increased saturation solubility is described in Ostwald-Freundlich and Kelvin equations [5,6]. The enhanced solubility of nanocrystals results in a higher gradient between donor and acceptor compartments, therefore higher flux, and potentially increased drug bioavailability. The solubility increase reported for nanocrystals is, however, not remarkably high [7,8]. In addition, the solubility of nanocrystals is affected by their degree of crystallinity: preparation processes involving high shear forces and mechanical stress, as is the case in wet bead milling, which is one of the most used methods for preparation of nanosuspensions, reduce the crystallinity of drug particles and/or increase the presence of amorphous regions, thereby enhancing the saturation solubility [9]. One major drawback of nanocrystals is their physical instability due to high tendency of small particles to aggregate/agglomerate. This challenge is tackled by using the proper type and amount of stabilizer and by drying the nanosuspensions to obtain redispersable powders with a long-term stability [10,11].

Amorphous drugs do not possess the three-dimensional long-range order typical for crystalline compounds, but exhibit only a short-range

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molecular order. Their mechanical properties, such as elastic modulus, also differ from those of their crystalline counterparts, resulting in novel properties which could be favorable for formulation scientists. The amorphous form represents the most energetic solid state of the material and should hence provide enhanced thermodynamic properties in comparison to crystalline drugs, as a remarkable increase in solubility and therefore improved bioavailability [12,13]. The amorphous solid state is, however, characterized by higher molecular motion which results in spontaneous conversion back to the crystalline state, energetically more favorable. Thus, although remarkable increases in saturation solubility are reported for amorphous compounds [14–17], their tendency to crystallize has limited their commercial potential, and thus different studies have focused on how to stabilize amorphous pharmaceuticals [13,18,19].

Combining nanosize and amorphous solid state is a quite recent approach which, if the two techniques together provide synergistic effect, would result in the highest increase in saturation solubility. In addition to the remarkably increased solubility, this system would benefit from the high dissolution rate obtained by the particle size reduction to the nanometer range, and could hence represent a very promising approach for poorly soluble drugs [20,21]. Amorphous nanoparticles of an active compound were prepared by an emulsification process which is currently used for product development in the food sector [22,23]. Amorphous nanoparticles of a dye were prepared by precipitation of the dye from a water-miscible organic solution [24]. Furthermore, amorphous drug nanoparticles were prepared by the Nanomorph® technology, which consists in a controlled precipitation process [21]. So far, however, no pharmaceutical products based on amorphous nanoparticles have reached the market. The main concern is indeed the preservation of the amorphous solid state in addition to the particle size stabilization.

Although wet bead milling is the most used method for nanocrystal preparation, especially in the industry, no attempts for preparation of amorphous nanoparticles with this technique are reported, probably because of the presence of water, promoting recrystallization.

The aim of this study was to prepare amorphous drug nanoparticles by wet bead milling in aqueous media and to determine *in situ* their increased saturation solubility. The main challenge by using wet media milling as preparation method was the recrystallization tendency of the drug in the amorphous state once in contact with water. Indomethacin was selected as model drug due to its poor aqueous solubility [25] and its well-described solid state transformation and interaction with polymers [26–28]. The solid state of the drug nanoparticles was thoroughly investigated, with focus on understanding the amorphous state stabilization throughout the milling process. The synergistic effect of nanosize and amorphous solid state with regard to increased saturation solubility was tested and proofed by solubility studies which were performed *in situ*, which is a more accurate method than non *in situ* ones [8].

2. Materials and methods

2.1. Materials

Indomethacin (Fluka Chemie AG, Buchs, Switzerland), ibuprofen (Ibuprofen 70, BASF SE, Ludwigshafen, Germany), poloxamer 407 (Kolliphor® P407, BASF SE, Ludwigshafen, Germany), polyvinylpyrrolidone (Kollidon® 30, BASF SE, Ludwigshafen, Germany), sodium dodecyl sulfate (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), ultrapurified water purified by a Milli-Q-apparatus (Millipore GmbH, Darmstadt, Germany), 0.25–0.35 mm zirkonium beads (SiLibeads®, Sigmund Lindner GmbH, Warmensteinach, Germany).

2.2. Preparation of amorphous indomethacin

Amorphous indomethacin was prepared by quench cooling with liquid nitrogen of the crystalline γ-form, previously molten at 165 °C for ~3 min in a stainless steel beaker [29]. The amorphous drug was gently ground in a mortar before wet bead milling.

2.3. Preparation of nanoparticles

Nanosuspensions of 1% (w/w) indomethacin were prepared by aqueous wet bead milling. 1% (w/v) poloxamer 407 or 1% (w/v) polyvinylpyrrolidone or combinations of 1% (w/v) polyvinylpyrrolidone either with 0.5%/0.2% (w/v) poloxamer 407 or with 0.1% (w/v) sodium dodecyl sulfate (SDS) were used as stabilizers. The amorphous or crystalline drug was added to the surfactant solution and milled for 1 h at 3200 rpm with a Dyno®-Mill KDL-A (Willy A. Bachofen AG – Maschinenfabrik, Muttenz, Switzerland) in the discontinuous batch mode. Zirkonium beads (0.25–0.35 mm) were used as grinding agent in a ratio suspension:beads 1:1.6 (w/v). The suspensions were separated from the beads by filtration through a paper filter with a pore size of ~40 µm. Directly after preparation, the suspensions were exposed to shock-freezing with liquid nitrogen and afterwards freeze-dried (Alfa® 2–4 LD Plus freeze-dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The lyophilization process was performed at −47 °C and 0.055 mbar for 19 h. Pictures of the batches were taken with a photo camera (iPhone SE, Apple Inc., Cupertino, US).

2.4. Preparation of physical mixtures

Physical mixtures 1:1 (w/w) of crystalline (γ-form) or amorphous indomethacin with PVP were prepared by either shaking the two powders together for 1 min in a glass vial or by mixing them gently with a pestle in a mortar.

2.5. Characterization of nanoparticles and micronized powders

2.5.1. Particle size analysis

The particle size of original indomethacin was measured in triplicate by powder laser diffraction (Sympatec® Helos Rodos, Clausthal-Zellerfeld, Germany), while the particle size of amorphous indomethacin was determined by laser diffraction (Mastersizer® 2000, Malvern Instruments Ltd., Malvern, UK) (n = 5) after preparation of an aqueous suspension of the compound. The particle size of the nanosuspensions was measured in triplicate by photon correlation spectroscopy (PCS) using a Zetasizer® Nano ZS (Malvern Instruments Ltd., Malvern, UK). The particle sizes of the nanocrystals and amorphous nanoparticles after redispersion of the freeze-dried powders in the surfactant solutions were measured by PCS (n = 3).

2.5.2. Light microscopy

Optical microscopy pictures of the nanosuspensions before drying were taken (Zeiss Axioskop, Carl Zeiss Microscopy GmbH, Jena, Germany) in order to determine if large agglomerates and/or aggregates were present.

2.5.3. Drug content

The real drug content of the nanosuspensions was measured before freeze-drying by UV spectrophotometry (Agilent HP 8453, Agilent Technologies Inc., Santa Clara, US). The samples were diluted with a methanol:water mixture (1:1 v/v) as solvent.

2.5.4. Differential scanning calorimetry (DSC)

Thermograms of original (crystalline) indomethacin and amorphous indomethacin powders were recorded using a DSC 6000 (PerkinElmer Inc., Waltham, MA, USA). Samples of ~10 mg were accurately weighed