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Development of oral lyophilisates containing meloxicam nanocrystals using QbD approach

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ABSTRACT

The aim of this study was to develop oral lyophilisates with improved meloxicam (MEL) dissolution, optimizing each step of the preparation by design of experiments. First, meloxicam nanosuspensions were prepared by high-pressure homogenization (HPH), using PVP, Poloxamer or PEG as stabilizers and were subjected to freeze-drying using mannitol as cryoprotectant. The effects of the stabilizers and cryoprotectant were assessed and an optimal formulation was generated within the Design Space where the particle sizes and the PDIs are at their lowest values. The optimal formulation was used at the preparation of oral lyophilisates. Sodium alginate (SA) and croscarmellose sodium (CCS) were tested as matrix forming agents and three different freezing regimes were applied. The formulation was optimized, choosing the polymer that yielded both high mechanical strength and fast MEL dissolution. Poloxamer led to particle size reduction down to 10.27% of the initial size, meaning 477.6 ± 7.5 nm, with a slight increase during freeze-drying process. PEG showed lower nanonizing capacity during HPH, but freeze-drying produced further diminution of the particle size. Since Poloxamer provided advanced size reduction while preserving MEL crystallinity, it was used for the optimized formulation containing 1% Poloxamer and 5% mannitol added before freeze-drying. SA showed good structural properties when compared to CCS and allowed fast MEL dissolution at low ratios. The optimal formulation contained 1.157% of SA was subjected to thermal treatment during freeze-drying. It disintegrated in 3.33 s and released 77.14% of the MEL after 2 min. The quality by design (QbD) approach for the development of pharmaceutical products ensured high quality of the dosage form and good understanding of the preparation process.

1. Introduction

Oral solid dosage forms are preferred by patients for the accurate dosing, their stability, easy of administration. Still, for the special groups of patients: pediatric, geriatric, patients with disphagia, their intake imposes serious limitations. The orally disintegrating dosage forms gained attention from the pharmaceutical industry and academia for their numerous advantages: easy swallowing without water, pleasant taste, enhancement of patent life cycle and increase of bioavailability of poorly water soluble drugs (AlHusban et al., 2010, 2011).

In the case of orodispersible tablets (ODTs), bioavailability increases due to the quick disintegration, followed by dissolution of the active pharmaceutical ingredient (API) in the saliva, the direct absorption through the oral mucosa to the systemic circulation, bypassing the liver first-pass metabolism (AlHusban et al., 2011). Fast disintegration occurs with highly porous products obtained either by compressing at low compression forces, molding or by freeze-drying (Chandraseckhar et al., 2009). Among these methods, freeze-drying provides light, porous structures that disintegrate in a matter of seconds, "officially" known as oral lyophilisates.

When fast disintegration of the dosage form is granted, bioavailability can be further limited by the API solubility and dissolution rate (Ghosh et al., 2012; Sarnes et al., 2014). To overcome this issue, researchers developed solid dispersions, drug-cyclodextrin inclusion complexes and nanosized particles (Samprasit et al., 2015; Blagden et al., 2007). Particle size reduction to the nano range with the effective

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surface area increase showed promising results in terms of dissolution rate and bioavailability improvement.

From the plethora of APIs being the subject of nanonization, meloxicam is a substance whose action consists in the selective inhibition of cyclooxigenase-2 isoenzyme and therefore it has effective anti-inflammatory and analgesic properties (Ochi et al., 2014) that recommend it for both human and veterinary use (Monteiro et al., 2016). Besides that, it is also emerging as a promising drug for the treatment of Alzheimer's disease and cancer. It was categorized into class II of the Biopharmaceutics Classification System (BCS), meaning it exhibits low water solubility (4.4 μg/ml) and good membrane permeability (Ambrus et al., 2009). Following the intake of classical tablets, the peak plasma concentration is reached in 5–6 h (Dellgado et al., 2014), far too long for a quick onset of the effect, which motivates the development of a fast dispersible dosage form with highly soluble meloxicam nanocrystals.

Nanocrystal technologies usually provide sub-micron colloidal dispersions of the drug crystals in a solvent, which has to be eliminated to obtain the dry powder for the further preparation of a solid dosage form (Kumar et al., 2015; Lai et al., 2015). One interesting approach is the combination of nanosuspensions with the production of freeze-dried orodispersible tablets (Lai et al., 2011, 2014). It involves the nanosuspension preparation and mixing with the matrix forming and cryoprotectant excipients, followed by freeze-drying, thus obtaining the freeze-dried ODTs. However, the main issues generated by the preparation of this new dosage form relate to the crystals' stability before, after freeze-drying, and to the balance between the disintegration and structure resistance of the freeze-dried products. The nanosuspensions are thermodynamically unstable systems, which can be stabilized for a pharmaceutically relevant time by adding surfactants or polymers that act as stabilizers. A high number of reports acknowledged the stability dependence on the type and amount of stabilizer, but when freeze-drying process is involved, data about crystal aggregation tendency is still controversial (Chung et al., 2012).

The design of a new formulation requires complete information about the process parameters and the way they control the quality attributes. Optimization via empirical screening approach is time consuming and does not reveal the collective effects of process and formulation factors. Design of Experiments (DoE) method has been used to overcome these issues by offering a broad understanding on the relationship between independent and dependent variables. Previous studies reporting the development of oral lyophilisates containing nanocrystals (Lai et al., 2011, 2014) used traditional screening approach and focused on API dissolution. They pointed out a complex preparation process with numerous variables, each one having a potential impact on product characteristics. Therefore, we believe that a research study conducted by a method that allows their simultaneous study could add to the knowledge base valuable data.

In this study, a DoE approach was applied to understand and optimize the two important steps in the preparation of oral lyophilisates (OLs) containing API nanocrystals: nanosuspension preparation and oral lyophilisates preparation. MEL was used as a model drug due to its emerging wide clinical applications and to the fact that such formulations of OLs containing MEL nanoparticles have never been used. In the first step, we established the optimal stabilizer and the mannitol ratios, after the evaluation of crystal behavior before and after freeze-drying. The second step focused on the matrix forming agents' functions and on the freezing regime. We studied their influence on the mechanical structure and the further on MEL dissolution.

The nanosuspensions were obtained by pre-sonication and high-pressure homogenization, followed by freeze-drying that led to oral lyophilisates. The particle size, polydispersity index (PDI) and Zeta potential were investigated as responses in the first step, while in the second preparation step we evaluated the disintegration time, the texture analysis and the in vitro drug release.

2. Materials and Methods

2.1. Materials

The active pharmaceutical ingredient (API) - meloxicam (MEL) was purchased from Unichem Laboratoires Ltd., India. Mannitol (M) (Pearlitol 200M) and polyethylene glycol 4000 (PEG 4000) were purchased from Merck, Germany. PVP K25 (Kollidon 25) and Poloxamer 188 (Polox) (Kolliphor P188) were kindly donated by BASF, Germany. Croscarmellose sodium (CCS) (Ac-Di-Sol) was obtained from FMC BioPolymer, Belgium and the alginic acid sodium salt (SA) from Sigma-Aldrich, United Kingdom.

2.2. Methods

2.2.1. Nanosuspension Optimization

2.2.1.1. Design of Experiment. Previous research in nanosuspension preparation revealed the importance of the stabilizer type and concentration for the API dissolution behavior. The stabilizers should assure wetting of the hydrophobic surfaces and increase of the activation energy of the agglomeration process, therefore be a barrier to agglomeration (Van Eerdenbrugh et al., 2008). The type of stabilizer was set as qualitative variable (X_1). We chose two polymers: PVP, PEG and one nonionic surfactant, Polox. Their weight concentrations in volume were varied on three levels: 0.25 - 1 - 1.75% (w/V) (X_2). During the freeze-drying, a cryoprotectant was added to avoid freeze damage due to ice formation and particle aggregation (Wang et al., 2013). We chose mannitol as cryoprotectant, at concentrations comprised between 0 and 5% (X_3). The effects of the aforementioned parameters on the crystal size, polydispersity (PDI) and Zeta potential were investigated using a three-factor, three-level DoE.

The critical quality attributes (CQAs) of the nanosuspensions were the average particle size and the MEL crystallinity.

As responses, we chose the particle size and PDI after the size reduction (Y_1, Y_2) and after the freeze-drying (Y_3, Y_4). In order to assess the size and PDI variations produced by freeze-drying process only and test if they have any statistical significance within the DoE, we calculated the size and PDI changes from the following equations:

$$\text{Size variation } (Y_5) = (\text{initial size} - \text{final size}) * 100/\text{initial size}$$

$$\text{PDI variation } (Y_6) = (\text{initial PDI} - \text{final PDI}) * 100/\text{initial PDI}$$

The DoE modeling was performed using Modde 10.0 (Umetrics, Sweden) software and was used to provide a surface model for the six mentioned responses and an optimized formulation to take forward to the second step of the study.

2.2.1.2. Preparation of Nanosuspensions. The micronized MEL (with 4.51 ± 0.57 μm average size and polydispersity index equal to 1) was suspended in the aqueous stabilizer (PVP, Polox or PEG) solution, using a magnetic stirrer to a concentration of 0.75% (w/V). The suspensions were stirred for 10 min at 1000 rpm. Each of them was then sonicated for 10 min at 70% amplitude using a high power ultrasound device (Hielscher UP 200S Ultrasonic processor, Germany) to wet the drug. Further size reduction to nanorange was achieved by applying high-pressure homogenization (HPH) with an Emulsiflex C5 apparatus (Avestin, Ottawa, Canada). 2 cycles at 500 bar were applied, followed by 20 cycles at 1000 bar.

2.2.1.3. Freeze-drying of Nanosuspensions. MEL nanosuspensions were freeze-dried using a lab scale VirTis Advantage Plus freeze-drier (SP Scientific, Gardiner, USA). Briefly, four 0.5 ml samples were taken from each of the nanosuspensions and poured into blister sockets. The blisters were placed on the freeze-dryer shelf and cooled to − 50 °C at a rate of 1 °C/min, thus we applied a fast freezing regime. The temperature was kept constant for 6 h for complete product solidification. The primary drying was performed at − 20 °C for 20 h and vacuum of 0.2 mbar, followed by secondary drying at 5 °C for 6 h at