Dissolution improvement of albendazole by reconstitutable dry nanosuspension formulation

Ph.D. thesis

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1. Introduction

One of the major obstacles of the development of highly potent new drug candidates is the poor water solubility of these compounds, which hinders their therapeutic application. Nanonization is one of many formulation strategies capable of improving the low bioavailability of these active ingredients.

Pharmaceutical nanosuspension is defined as colloidal, biphasic systems, where solid drug particles are very finely dispersed in an aqueous vehicle, without any matrix material, stabilized by surfactants and/or polymers, prepared by suitable methods for drug delivery applications. The particle size of the solid particles in nanosuspensions is usually less than 1 μm with a mean particle size ranging between 200 and 600 nm. The potential benefits of the nanosuspension technology are increased drug dissolution rate, increased bioavailability of a drug, reduced variability and fed/fasted effects. They have low incidence of side effects caused by the excipients, increased resistance to hydrolysis, oxidation and increased physical stability to settling. By surface modifications they can also provide passive targeting for specific tissues and altering biotransformation.

Drug nanocrystals have another outstanding feature because they can distinctly increase adhesiveness to surface/cell membranes, which lead to an improvement of oral absorption and penetration capability in case of topical routes of administrations. The most common disadvantages of the utilization of nanosuspensions are high energy investment during manufacturing, immunotoxicity and non-specific uptake in reticuloendothelial system (RES) organs. There are two converse methods available for manufacturing nanosuspensions: the ‘bottom-up’ and the ‘top-down’ approach technologies. The ‘bottom-up’ technology is an assembling method from molecules and the ‘top-down’ variant is a disintegration approach from large particles, microparticles. There are also newly developed combination techniques available, merging the advantages of already employed production methods.

The ‘top-down’ dry milling (e. g. jet milling) is not efficient to obtain crystal sizes in the nanometer range; therefore, wet-milling is applied. Wet-milling means that the drug particles are dispersed in a surfactant/stabilizer solution; the obtained macro suspension is then subjected to milling energy.

In pearl milling, the macro suspension of the active is filled into a milling bowl, containing smaller or larger coated milling pearls as milling media (typically in the size of 0.2 mm or 0.4–0.6 mm) made of ceramics (cerium or yttrium stabilized zirconium-dioxide), stainless steel or glass. The pearls are moved by a stirrer, the drug is ground to nanocrystals in between the pearls.

Albendazole (ABZ) is a benzimidazole carbamate-type, broad-spectrum anthelmintic for the treatment of intestinal helminth infections. In addition, demonstrated systemic anti-hydatid activity and is now recognized to have important application in treatment of human cystic and alveolar echinococcosis. This compound has a pH dependent, poor water solubility and high membrane permeability (log P = 3.83) Thus, it is classified as a BCS class II. compound. There are two polymorphs available for ABZ, Form I (commercially available form) and From II (recrystallized from N,N - dimethylformamide). Temperature dependent solubility studies revealed, that on 25 ^{0}C solubility of Form I is better, than Form II. Application of a metastable Form I may be advantageous for exploiting higher solubility in the gastrointestinal tract.

2. Aims and objectives

This Ph.D. thesis summarizes the development of a redispersable solidified nanosuspension, containing ABZ nanocrystals. It is divided into four major sections.

The first section emphasizes the potential of the optimization of loading composition and formulation factors of the top down wet planetary bead milling method. Process parameter optimization was performed by design of experiments (DOE) approach. Long-term physical stability determination of the optimized nanosuspension formula as a liquid dosage form was not investigated in this study, only a short 56 day long demonstration was included.

The second section focuses on the stabilization principal of the obtained milled albendazole nanosuspension, as well as the starting macro suspension by the post-processing solidification wet granulation method, applying microcrystalline cellulose (MCC) as carrier (**Figure 1**). The particle size distribution after redispergation was studied in various dissolution media and zeta-potential in demineralized water.

The third part summarizes the in vitro solubility and dissolution studies, which describes the impact of the particle size reduction and the solubilization on the water solubility of albendazole along with the dissolution rate values in various aqueous-based pH buffer solutions.

Finally, the last chapter summarizes the solid state characterization and morphological studies. These experiments involved the investigation and comparison of raw material albendazole, this active processed in milled suspensions after drying and in granules absorbed by solid carrier, the solid carrier MCC and physical mixtures of these components.

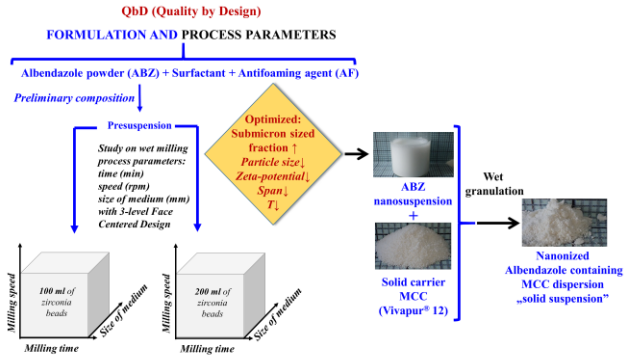

Figure 1. *Graphical abstract of the optimization of wet-milling for ABZ nanosuspension development and post processing solidification by wet granulation*

3. Methods

Ideal loading composition determination for wet-milling

Loading composition optimization evaluations were carried out in the smallest laboratory sized container (12 ml), at high milling speed (500 rpm), also with high volume of applied milling beads (4.8 ml), while various milling programs were compared. Total process time was 60 minutes. For determination of albendazole loadings 1.00% (w/w), 3.00% (w/w) and 4.00% (w/w) were investigated. The influence of the surface-active agents and polymers on wet milling of albendazole were compared in concentrations of 0.40% (w/w). Best performed emulsifier concentration was screened between the ranges of 0.30% (w/w) to 1.00% (w/w), application of antifoaming agent dimethylpolysiloxane at higher concentrations (> 0.50% (w/w)) was also involved.

Process parameter optimization of wet-milling by factorial design and desirability approach

In order to characterize the relationship between formulation factors and their impact on the output variables response surface methodology based on face centered central composite design was utilized.

In this study submicron sized fraction (%) (Y_1), volume-weighted mean particle size (D [4,3]) (µm) (Y_2), span of particle size distributions (Y_3), zeta-potential (mV) (Y_4) and milling temperature (^{0}C) (Y_5) values were selected as responses (dependent variables). X_1, X_2, X_3 values were the independent variables, where X_1 represented the milling time (20, 40 and 60 minute long programs were screened). X_2 represented the milling speed (effect of 200, 400 and 600 rpm on wet milling were investigated). X_3 represented the sizes of milling beads (effect of d = 0.1, d = 0.3 and d = 1.0 mm sized zirconia beads on wet milling were compared). Milling experiments were carried out in 500 ml containers, with two different volumes of milling beads (100 ml and 200 ml) as category factors. A three-level (+1, 0, -1) factorial design for the optimization of the independent variables with 32-32 runs (5-5 center points) was applied to each category.

Nano- and macro suspension solidifications by wet granulation processes

Freshly prepared ABZ nano- and macrosuspensions were transformed to solid forms by wet granulation processes, applying microcrystalline cellulose (MCC) (Vivapur® 12)

as solid carrier. ABZ nanosuspensions produced in milling container of 50 ml capacity with 20 ml of d = 0.1 mm sized zirconia beads at 400 rpm rotation speeds and 120 minutes long 5:5 cyclic milling programs. After every milling process the obtained ABZ nanosuspension was added to the carrier and mixed manually by kneading for 3 minutes, then sieved through a stainless steel sieve with mesh size of d = 180 μm and dried in a drying chamber, on 40 ^{0}C for 2 days. This process was repeated 8 times to achieve a desirably low dose. Solid suspension of unmilled albendazole was prepared the same way, but instead of milling, a magnetic stirrer was employed for the dispergation of albendazole at 400 rpm mixing speed, for 120 minutes on 24 ^{0}C.

Thermodynamic solubility studies

Thermodynamic solubility studies were determined using a slightly modified version of the classical saturation shake-flask method in dissolution media at pH = 1.2 (0.1 N hydrochloric acid), pH = 4.5 (phosphate-buffer) and pH = 6.8 (phosphate-buffer) for optimized albendazole nanosuspension. For albendazole containing solid suspensions investigations were carried out in media

at pH = 1.20 (0.1 N hydrochloric acid), pH = 6.50 artificial rumen fluid (ARF) and pH=6.80 (phosphate-buffer). Mean thermodynamic solubility values were determined by UV-Vis spectrophotometry from the linear calibrations of ABZ in the relevant media.

In vitro dissolution studies

Dissolution tests were performed employing USP apparatus 2 (paddles) methods at 75 rpm rotation speed for suspensions and 100 rpm for solidified suspensions at 37 ± 0.2 ^{0}C bath temperatures, in 900 ml aqueous-based buffer solutions at pH = 1.2 (Ph. Eur. 9) (in doses of 200 mg), pH = 4.5 phosphate buffer solution (Ph. Eur. 9) (in doses 100 mg) and pH = 6.8 phosphate buffer solution (Ph. Eur. 9) (in doses 100 mg). Dissolution profiles of unmilled and milled Vivapur® 12 dispersions containing 200 mg of albendazole, compared to 200 mg of albendazole powder were studied in 900 ml of pH = 1.2 (Ph. Eur. 9), pH = 6.50 Artificial Rumen Fluid (ARF) and pH = 6.8 phosphate buffer solution (Ph. Eur. 9).

Solid state characterization investigations

Comparison of the diffraction patterns of starting material ABZ powder, dried milled and unmilled suspensions were evaluated by powder X-Ray diffractometry (PXRD).

Comparison of the phase transitions and thermoanalytical behaviors of albendazole, MCC, granules containing milled and unmilled albendazole were investigated by differential scanning calorimetry (DSC). Physicochemical properties of MCC carrier, albendazole powder, granules of unmilled solid suspension and milled solid suspension were examined and compared with fourier transform infrared spectroscopy (FT-IR).

Morphological investigations of solid particulate systems

In order to get an actual understanding of particle morphology microscopic techniques are preferred. In search for milled albendazole nanocrystals on the surface of the milled dispersion, the surfaces of the albendazole powder, MCC carrier (Vivapur® 12), their physical mixture and the albendazole milled dispersion have been scanned and compared with atomic force microscopy (AFM) and scanning electron microscopy (SEM).

4. Results and discussions

Surfactant assisted media milling process

Best emulsifier for ABZ containing nanosuspension development was Tween 80 in minimal concentration of 0.50% (w/w). Additional antifoaming agent (AF) dimethylpolysiloxane in concentration of 0.01% (v/v) slightly increased submicron sized fraction from 86.84% to 92.89%. Ideal drug loading investigations involved the screening of 1.00%, 3.00% and 4.00% (w/w) ABZ at predetermined best performed conditions. Best-case scenario was the application of 3.00% (w/w) ABZ, which yielded maximal submicron sized fraction, with minimal polydispersity values (**Figure 2**).

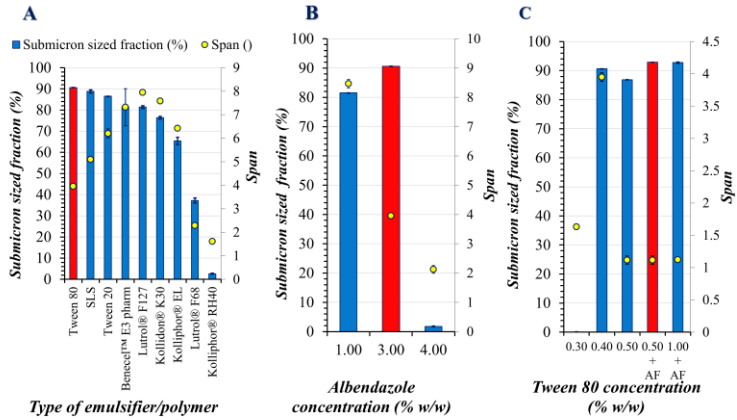

Figure 2*. Loading composition optimization for wet planetary bead milling of ABZ*

When influence of zirconia beads with different sizes were compared on milled ABZ particle size distribution parameters and suspension zeta-potential values at 100 ml of applied zirconia beads result showed improving performance on size reduction with the utilization of smaller beads, consequently showed inverse proportionality with the size of milling medium. Nanosuspension criteria were easily fulfilled with the application of d = 0.1 mm sized beads at low to mediocre milling speed intervals (200-434 rpm), even at shorter operations (25.6-59.4 mins) (**Figure 3**).

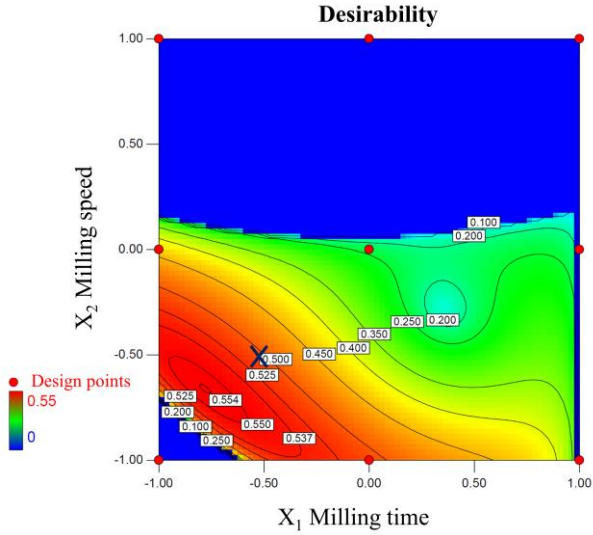

Figure 3*. Contour plot of the nanosuspension criteria by numerical optimization of desirability function, where x marks the chosen process parameters*

For preparation of optimized composition chosen settings were 300 rpm milling speed with 30 minutes long 5:5 cyclic, milling operation. Milling temperature at the end of the process was 24.0 ^{0}C, showed a minimal + 6.0 ^{0}C elevation compared to the temperature of starting surfactant solution. Washing the beads after milling with an additional 33.33% (w/w) of total mass loading with surface active agent solutions and mixed with ABZ nanosuspensions have demonstrated reasonable ABZ yield (83.05% (w/w)).

Thermodynamic solubility studies

For optimized nanosuspension formulation, maximal gains in solubility were observed in medium at pH = 6.80, where milling boosted initial solubility by 1.45-folds compared to surfactant dispersion. Studies with solidified ABZ dispersions indicated, that maximal gains were registered in artificial rumen fluid (ARF) at pH = 6.50, where particle size reduction boosted initial solubility by 1.98 times compared to surfactant dispersion (**Figure 4**).

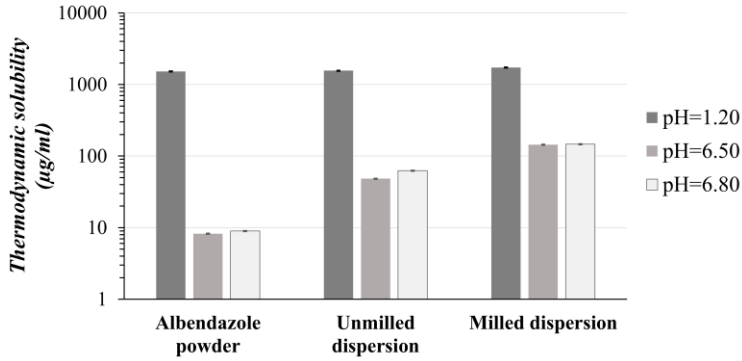

***Figure** 4. Mean thermodynamic solubility values of albendazole powder substance, milled and unmilled dispersions in various pH buffer solutions (n = 3)*

In vitro dissolution studies

For optimized nanosuspension formulation, maximal gains in mean dissolution rate constants were calculated in medium at pH = 1.20, where particle size reduction boosted initial value by 13.50-folds compared to surfactant dispersion. Studies with solidified ABZ dispersions indicated, that maximal gains were calculated in artificial rumen fluid (ARF), where particle size reduction improved performance by 43.88% compared to the dispersion containing unmilled ABZ particles (**Figure 5**).

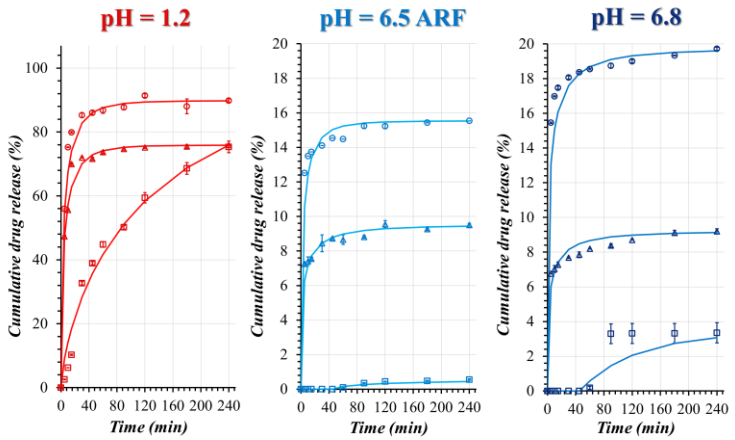

Figure 5*. Comparison of the fitted in vitro dissolution profiles of ABZ solid suspensions containing nanocrystals (○), solid suspensions containing unmilled ABZ (Δ) and raw ABZ powder (□), n = 3, mean values ± SDs*

Solid state characterization investigations

PXRD, DSC and FT-IR studies identified both forms of albendazole present in starting powder liquid and solid formulations. DSC and FT-IR investigations indicated partial crystalline-amorphous transition of ABZ when subjected to milling operation. PXRD analysis revealed a polymorphic form change of ABZ from Form I to Form II., when coarse milling programs were applied (d = 0.3 mm sized beads loaded in container capacity of 50 ml at high speed of 600 rpm).

Morphological investigations of solid particulate systems

Careful investigations of the surfaces of microparticulate carrier systems revealed, that no ABZ nanocrystals were detected on the surfaces of carrier particles, which ensured the incorporation of nanocrystals inside the pores of the MCC. Particle size distribution parameters after reconstitution were slightly altered, probably due to the slower tray-drying process (**Figure 6**).

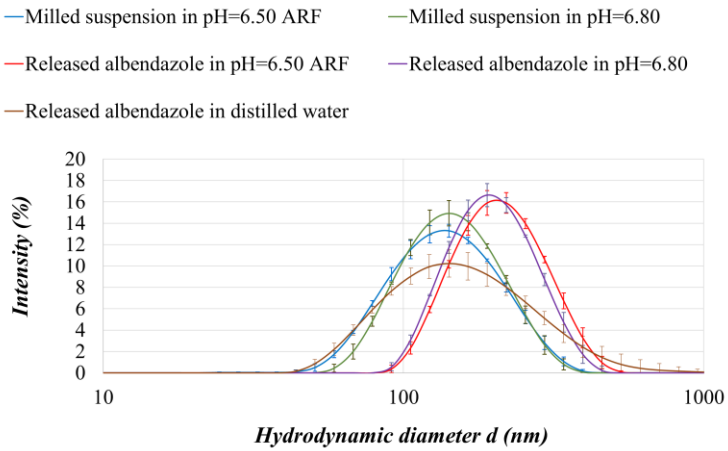

Figure 6. *Comparison of the PSDs by intensities (%) of milled albendazole suspensions and milled albendazole crystals released from Vivapur® 12 dispersions in various pH buffer solutions (n = 5), mean values ± SDs*

5. Conclusions

The main objective of this work was to develop dry nanosuspension drug delivery system containing BCS class II. ABZ active pharmaceutical ingredient to offset the undesirable physicochemical and biopharmaceutical properties of this model drug.

Optimized dispersion containing milled ABZ has improved the thermodynamic solubility by 1.98 times in artificial rumen fluid (ARF) and by 1.33 times in phosphate buffer at pH = 6.8 (Ph. Eur. 9) compared to the unmilled dispersion supporting the principals of the Ostwald-Freundlich equation.

Maximal gains in mean dissolution rate values were calculated in artificial rumen fluid (ARF), where particle size reduction improved performance by 43.88% compared to the dispersion containing unmilled ABZ.

Quality control studies have demonstrated the successful reconstitution of ABZ nanocrystals from the solid drug delivery system.

ABZ crystals displayed partial crystalline amorphous transition due to milling by DSC investigations and the crystalline fraction contained both polymorphs confirmed by PXRD examinations.

Although crystallinity, polymorphs and structures of ABZ have been thoroughly studied, along with polymorphy inducing factors separately, there are no publications investigating the effect of pearl/bead milling on ABZ polymorphy. This work as a novelty offers the identification of extreme milling conditions, where disadvantageous ABZ Form I → Form II conversion is realized, therefore lowering initial ABZ solubility. This research can give a hint to formulation scientists to avoid these critical conditions during ABZ nanosuspension development by wet planetary bead milling techniques. The influence of process parameters (capacity of the milling container, size of the milling medium, milling speed, milling time) and drying on ABZ polymorphism have been studied. Results have concluded, that there was a full polymorphic conversion of ABZ from Form I to Form II, which was more pronounced with the application of d = 0.3 mm sized zirconia beads loaded in smaller milling container (50 ml) and with the utilization of coarse milling programs involving 600 rpm rotation speeds, during 30-180 minute long operations.

6. List of publications related to topic of the Ph.D. thesis

1. Fülöp Viktor, Balogh Emese, Jakab Géza, Antal István, A nanogyógyszerek és nanotechnológia formulálási vonatkozásai I. Bevezetés, biofarmáciai szempontok, 2016 Acta Pharmaceutica Hungarica 86:2 43-52.

2. Fulop V, Jakab G, Bozo T, Toth B, Endresik D, Balogh E, Kellermayer M, Antal I, Study on the dissolution improvement of albendazole using reconstitutable dry nanosuspension formulation, 2018 European Journal of Pharmaceutical Sciences 123, 70-78. https://doi.org/10.1016/j.ejps.2018.07.027

3. Fülöp, V., Jakab, G., Tóth, B., Balogh, E., Antal, I., Study on Optimization of Wet Milling Process for the Development of Albendazole Containing Nanosuspension with Improved Dissolution, 2020 Periodica Polytechnica Chemical Engineering 64(4), 401-420, https://doi.org/10.3311/PPch.15569