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Comparative analysis of zaleplon complexation with cyclodextrins and hydrophilic polymers in solution and in solid state

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ABSTRACT

The aim of this work was to investigate the potential synergistic effect of water-soluble polymers (hypromellose, HPMC and polyvinylpyrrolidone, PVP) on zaleplon (ZAL) complexation with parent β-cyclodextrin (βCD) and its randomly methylated derivative (RAMEB) in solution and in solid state. The addition of HPMC to the complexation medium improved ZAL complexation and solubilization with RAMEB ($K_{ZAL/RAMEB} = 156 \pm 5\ M^{-1}$ and $K_{ZAL/RAMEB/HPMC} = 189 \pm 8\ M^{-1}$; $p < 0.01$), while such effect was not observed for βCD ($K_{ZAL/\beta CD} = 112 \pm 2\ M^{-1}$ and $K_{ZAL/\beta CD/HPMC} = 119 \pm 8\ M^{-1}$; $p > 0.05$). Although PVP increased the ZAL aqueous solubility from 0.22 to 0.27 mg/mL, it did not show any synergistic effects on ZAL solubilization with the cyclodextrins tested. Binary and ternary systems of ZAL with βCD, RAMEB and HPMC were prepared by spray-drying. Differential scanning calorimetry, X-ray powder diffraction and scanning electron microscopy demonstrated a partial ZAL amorphization in spray-dried binary and ternary systems with βCD, while the drug was completely amorphous in all samples with RAMEB. Furthermore, inclusion complex formation in all systems prepared was confirmed by solid-state NMR spectroscopy. The *in vitro* dissolution rate followed the rank order ZAL/RAMEB/HPMC > ZAL/RAMEB = ZAL/βCD/HPMC > ZAL/βCD ≫ ZAL, clearly demonstrating the superior performance of RAMEB on ZAL complexation in the solid state and its synergistic effect with HPMC on drug solubility. Surprisingly, when loaded into tablets made with insoluble microcrystalline cellulose, RAMEB complexes had no positive effect on drug dissolution, because HPMC and RAMEB acted as a binders inside the tablets, prolonging their disintegration. Oppositely, the formulation with mannitol, a soluble excipient, containing a ternary RAMEB system, released the complete drug-dose in only 5 min, clearly demonstrating its suitability for the development of immediate-release oral formulation of ZAL.

1. Introduction

Insomnia is one of the most common health-related problems that can affect several aspects of life quality. It is estimated that one third of adults in the general population display insomnia symptoms, while its prevalence is even higher in elderly populations [1]. Zaleplon (ZAL; N-[3-(3-cyanopyrazolo [1,5-a] pyriminidin-7-yl) phenyl]-N-ethylacetamide) is a non-benzodiazepine hypnotic agent which is indicated for the short-term treatment of insomnia in 10 mg doses. Unlike benzodiazepines, ZAL has a low potential for abuse and it does not cause rebound insomnia or other discontinuation effects [2]. After oral administration the drug is extensively metabolized into pharmacologically inactive metabolites, resulting in an absolute oral bioavailability of only 30%. Furthermore, ZAL absorption might be significantly impaired when administrated with a high fat meal [3]. Since hypnotics like ZAL are usually administered at bedtime, a formulation capable of overcoming such problems and thus increasing the therapeutic efficiency of hypnotic drugs is preferred. The development of such a formulation might be rather challenging, taking into account the poor aqueous solubility of ZAL [4].

In order to enhance ZAL solubility, one of our previous works studied its complexation with a series of natural and chemically modified cyclodextrins by means of phase solubility studies, spectrofluorimetry and ^{1}H NMR spectroscopy [5]. The results showed that parent β-cyclodextrin (βCD) and its randomly methylated derivative (RAMEB) were the best complexing agents for ZAL, in terms of increased aqueous drug solubility and stability of the complexes formed. However, the pharmaceutical use of cyclodextrins, particularly methylated derivatives, is limited mainly by formulation restrictions and a potential toxicity [6,7]. Therefore, it would be useful to increase their solubilizing and complexing effect by the addition of suitable auxiliary substances, in order to reduce the amount of cyclodextrin in use. Different hydrophilic polymers,

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such as carboxymethylcellulose, hypromellose, polyethylene glycol or polyvinylpyrrolidone, at low concentration of 0.1–0.5% (w/v), can drastically enhance cyclodextrin solubilization of the drug through ternary drug–cyclodextrin–polymer complex formation [8,9]. Taking this into account, it seemed of interest to extend our studies on the multicomponent technology as a strategy to further improve cyclodextrin efficiency in the solubilization and complexation of the poorly soluble ZAL. The effect of adding small amounts of hypromellose (HPMC) or polyvinylpyrrolidone (PVP) to the complexation media on the ZAL/cyclodextrin interaction was investigated by phase solubility studies in order to assess their synergistic effects with cyclodextrins tested on the ZAL aqueous solubility. The most efficient carrier/polymer systems were selected and prepared as spray-dried solid samples, monitoring the effect of added polymeric substance on the drug–cyclodextrin interaction in the solid state. With this aim, a comprehensive solid state analysis of prepared binary and ternary systems was performed, using differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD) and scanning electron microscopy (SEM). Although these methods are conventionally used to characterize solid drug/cyclodextrin interaction products [9–13], they cannot give a clear answer about the nature of drug–carrier solid state interaction. In this regard, we used a solid state NMR, a method known for its ability to reveal the structural and dynamic behavior of the individual components of the drug/carrier solid systems, as well as their interaction, despite the chemical and physical complexity of these systems [14]. Changes in dissolution properties of spray-dried samples were monitored using pharmacopeial methodology. Furthermore, the *in vitro* performance of selected spray-dried samples loaded into different tablet formulations was investigated in order to critically evaluate their potential for the development of an immediate-release oral formulation of the drug.

2. Materials and methods

Zaleplon (ZAL, 99.7% purity) was kindly donated by Belupo d. d. (Croatia). Natural β-cyclodextrin (βCD) and randomly methylated-β-cyclodextrin (RAMEB) with an average degree of substitution per anhydroglucose unit of 1.8 were obtained from Wacker Chemie GMBH (Germany). Hydrophilic polymers used were hypromellose (HPMC, Methocel® E5 Premium LV, Colorcon, England) and polyvinylpyrrolidone (PVP, Kollidon 30®, BASF The Chemical Company, Germany). Microcrystalline cellulose (Avicel® PH 101, Sigma–Aldrich GmbH, Germany), spray-dried mannitol (Mannogem™ EZ, SPI Pharma, Germany), lactose (Pharmatose 100 M, DFE Pharma, Germany) and magnesium stearate (Sigma–Aldrich, Germany) were used for tablet preparation. All chemicals and solvents used in this study were of analytical reagent grade.

2.1. Phase solubility studies

An excess amount of ZAL (50 mg) was added to 20 ml of an aqueous cyclodextrin solution, containing 0 to 12.5 mM of βCD or 0 to 48 mM of RAMEB, respectively (binary systems). To test the possible effect of hydrophilic polymers on the cyclodextrin solubilization of ZAL, ternary systems were prepared by adding 0.05% (w/v) of polymer (HPMC or PVP) to binary systems with βCD or RAMEB. All samples prepared in sealed glass containers were first sonicated for 30 min in an ultrasonic bath (Branson B1210E-DTH, Danbury, USA) thermostated at 70 °C and then magnetically stirred at a constant temperature of 25.0 ± 0.5 °C. After 24 h, when the complexation equilibrium was reached, the samples were centrifuged at 3500 rpm for 10 min, to separate the undissolved drug. Aliquots of the supernatant were filtered through a 0.45 μm Millipore membrane filter. Drug concentrations in the filtrate were determined spectrophotometrically at 335 nm (Ultrospec Plus, LKB, Pharmacia, Sweden), as described previously [5]. It was verified that ZAL did not absorb to the filter used for the separation of the undissolved solids.

The apparent stability constants (K_s) of complexes formed were calculated from the phase-solubility diagram [15], according to Eq. (1):

$$K_s = \frac{tg\alpha}{s_0 \times (1-\alpha)} \quad (1)$$

where $tg\alpha$ is the slope of the phase solubility diagram and s_0 is ZAL solubility in the absence of CD.

2.2. Preparation of spray-dried binary and ternary systems

For binary system preparation in the solid state, 0.5 g of the drug and an equimolar amount of each cyclodextrin (βCD and RAMEB, respectively) were separately dissolved by sonication in 100 mL of 96% (v/v) ethanol and 200 mL of water, respectively. The solutions of ZAL and CD were mixed together and subjected to spray-drying using a Büchi 190 Mini Spray Dryer (Flavil, Switzerland) equipped with a standard 0.5 mm nozzle. The drying conditions were as follows: compressed air flow rate of 700 NL h^{-1}, inlet temperature of 160 °C, outlet temperature of 90 °C, and sample flow rate of 10 mL min^{-1}.

For ternary system preparation, an amount of a hydrophilic polymer (HPMC) corresponding to the 10% (w/w) of the drug quantity was added to the cyclodextrin solution, while the rest of the procedure was the same as in case of binary system preparation. Furthermore, in order to evaluate the influence of spray-drying procedure on the solid-state properties of the drug, ethanol–water solution of ZAL without CDs was also prepared following the same procedure, and the solid product was isolated as described above.

2.3. Solid state characterization of spray-dried products

The DSC analysis was performed with a Perkin-Elmer DSC 7 instrument (PerkinElmer, Inc., USA). The instrument was calibrated with indium (99.98% purity; melting point of 156.61 °C and fusion enthalpy of 28.71 J g^{-1}) prior to the analysis of the samples under nitrogen purge. Accurately weighted samples (2–5 mg, Mettler M3 Microbalance) were placed in sealed aluminium pans with pierced lid and scanned at a heating rate of 10 °C min^{-1} over the temperature range from 30 to 300 °C. The relative degree of drug crystallinity (RDC) in the samples was calculated according to Eq. (2):

$$\mathrm{RDC} = \frac{\Delta H_{\mathrm{spd}}}{\Delta H_{\mathrm{drug}}} \times 100\% \quad (2)$$

where ΔH_{spd} and ΔH_{drug} are the fusion enthalpies of the ZAL in the spray-dried product (normalized to the drug content) and in the pure drug, respectively. Measurements were carried out in triplicate and the relative standard deviation of crystallinity data was <2.5%.

The XRPD analysis was performed at ambient temperature using a Philips PW1750 X-ray diffractometer (Philips Electronic Instruments, Netherlands), with CuKα_1 radiation ($\lambda_{CuK\alpha}$ = 0,15418 nm) over the 5–40° 2Θ range at a scan rate of 0.02°/s. The pattern was collected with 40 kV of tube voltage and 30 mA of current in step scan mode.

Solid-state NMR spectra were recorded on a Bruker Avance II NMR spectrometer operating at 9.4T, which means 100.16 MHz for ^{13}C. Samples (cca. 100 mg powder) were packed into 4 mm