Carbohydrate Polymers 98 (2013) 122–131

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Spray-dried voriconazole–cyclodextrin complexes: Solubility, dissolution rate and chemical stability

Tijana Miletic[a], Kachrimanis Kyriakos[c], Adrijana Graovac[a], Svetlana Ibric[b,*]

[a] Research and Development, Hemofarm a.d., Vrsac, Serbia
[b] Department of Pharmaceutical Technology, Faculty of Pharmacy, Belgrade, Serbia
[c] Department of Pharmaceutical Technology, Aristotle University of Thessaloniki, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 23 February 2013
Received in revised form 28 May 2013
Accepted 29 May 2013
Available online 7 June 2013

Keywords:
Voriconazole
Cyclodextrins
Solubility
Dissolution
Stability
Molecular docking

ABSTRACT

The present work investigates the effect of complexation with hydroxypropyl-beta-cyclodextrin (HPBCD) and 2-O-methyl-beta-cyclodextrin (2-O-MBCD), on voriconazole solubility, dissolution rate and chemical stability. Drug–cyclodextrin complexes were prepared as aqueous solutions, which were spray-dried, and their properties were compared to wet ground samples and physical mixtures. DSC analysis revealed absence of crystalline voriconazole from spray-dried complexes. FTIR spectroscopy indicated changes in the H-bonding network of the hydroxyl groups of cyclodextrin following drug inclusion. Dissolution rate of voriconazole was significantly higher from spray-dried complexes with either cyclodextrin in comparison with free drug, physical mixtures, or wet ground mixtures. However, two degradation impurities were found in aged samples, with slightly higher impurity level with HPBCD. Performed solubility studies suggested that 2-O-MBCD is more efficient solubilizer. Molecular docking simulations showed a difference in the 1:1 binding affinities and sites, with HPBCD surprisingly forming complexes of much lower energy, thus suggesting a multiple rather than a 1:1 complexation.

1. Introduction

As the number of new poorly soluble drugs is increasing (Dubin, 2006), exploring ways of improving their solubility and bioavailability is becoming an essential part of formulation development. Complexation of the drugs with cyclodextrins is one of the possible strategies for enhancing the drug's solubility (Brewster & Loftsson, 2007). Some of the additional advantages of cyclodextrins application include the potential for improvement of the drug's stability, safety, organoleptic properties (Challa, Ahuja, Ali, & Khar, 2005; Freitas et al., 2012; Hu, Zhang, Song, Gu, & Hu, 2012). Among many factors that can influence the character of drug–cyclodextrin interaction, the most important is the nature of the cyclodextrin used (Redenti, Szente, & Szejtli, 2000; Szejtli, 2004). Many chemically modified beta cyclodextrins, such as methyl- or hydroxypropyl-substituted are currently under testing or have already been approved for use in pharmaceutical products. Hydroxypropyl-beta-cyclodextrin (HPBCD) is one such marketed product, known for its good aqueous solubility and low toxicity (Brewster & Loftsson, 2007), while 2-O-methyl-beta-cyclodextrin (2-O-MBCD) has been presented by its manufacturer as a novel, easy-to-use, solubilizing aid for pharmaceutical formulations with potentially more beneficial properties than the HPBCD (oral dosage forms and injectable ones) (*Kleptose® Crysmeb exp* e-brochure).

Voriconazole, or (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, has properties suitable for complexation with cyclodextrins. Complexation of antifungal azoles and beta-derivatives of cyclodextrins has attracted the attention of researchers (Buchanan, Buchanan, Edgar, & Ramsey, 2007; Ribeiro, Figueiras, Santos, & Veiga, 2008), and there is already a product containing an voriconazole–beta-cyclodextrin derivative complex, marketed by Pfizer (New York, USA) under the brand name Vfend® in the form of powder for solution for injection. However, voriconazole is a single diastereomer, which in addition to having low aqueous solubility, suffers from stability problems due to retro-aldol reactions in solution (Buchanan et al., 2007). This fact can potentially add a level of complexity when preparing voriconazole–cyclodextrin complexes.

Therefore the main focus of this study was to examine the influence of type of cyclodextrin on the properties of a poorly soluble model drug, voriconazole. For that purpose, two cyclodextrins, HPBCD and 2-O-MBCD were used. The potential for using HPBCD and 2-O-MBCD for voriconazole solubility improvement, and comparing their relative effectiveness in solubilizing voriconazole, was assessed by phase-solubility studies and a 2^3 factorial design, performed similarly as in some previous studies (Borghetti, Lula,

* Corresponding author at: Department of Pharmaceutical Technology, Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia. Tel.: +381 693343500; fax: +381 113071049.
E-mail address: svetlana.ibric@pharmacy.bg.ac.rs (S. Ibric).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.05.084

Sinisterra, & Bassani, 2009; Brewster & Loftsson, 2007; Loftsson, Hreinsdottir, & Masson, 2005). Drug–cyclodextrin complexes in powder form were prepared by spray-drying and compared with wet ground samples as well as simple physical mixtures applying thermo-analytical methods, attenuated total reflectance FTIR spectroscopy (ATR-FTIR) and dissolution rate testing. A computational method, molecular docking simulation was used as an attempt to gain insight into the binding of drug into the cavity of CDs at the molecular level (Miro et al., 2012; Wang, Ouyang, Liu, Yuan, & Liu, 2013; Yuan, Jin, & Xu, 2012).

2. Materials and methods

2.1. Materials

Voriconazole (Medichem Manufacturing Limited, Malta) was used as the active ingredient; (2-hydroxy)propyl-beta-cyclodextrin, MS value (degree of molar substitution) ~0.99 (Kleptose HP, Roquette, France) and 2-O-methyl-beta-cyclodextrin, MS value ~0.57 (Kleptose Crysmeb, Roquette, France), were used for the inclusion of voriconazole.

All other reagents were of analytical grade.

2.2. Solubility studies

2.2.1. Phase-solubility studies

Effect of complexation with cyclodextrins on the drug solubility was evaluated by solubility studies. The amount of voriconazole was kept in excess as the concentration of 2-O-MBCD (0–160 mM) or HPBCD (0–320 mM) was increased. The resultant dispersions were stirred on a magnetic stirrer at room temperature for 48 h. All dispersions were filtered through a 0.45-μm membrane filter, suitably diluted and assayed spectrophotometrically for voriconazole at 255 nm, on a Camspec M330 UV spectrophotometer (Camspec Inc., Cambridge, UK).

2.2.2. Factorial design

A 2^3 factorial design was applied in order to assess the statistical significance of cyclodextrin type (HPBCD, 2-O-MBCD), excess amount of drug (500 mg and 1000 mg), and mixing time (30 and 60 min), on voriconazole solubilization. For all experiments 5% aqueous solutions of cyclodextrins were prepared (HPBCD or 2-O-MBCD), and 500 mg or 1000 mg of voriconazole was added. Dispersions were then stirred on a magnetic stirrer at room temperature during 30 or 60 min. After mixing, dispersions were centrifuged, the obtained supernatants were suitably diluted and analyzed spectrophotometrically at 255 nm (8453 UV–vis spectrophotometer, Agilent, Santa Clara, USA). Results for solubility of voriconazole were then statistically analyzed as a response to different combinations of examined factors, with the assistance of Design-Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA).

2.3. Molecular docking simulations

Docking simulations were performed using the AutoDock Vina software program that uses a sophisticated gradient local optimization algorithm, and automates grid map generation and result clustering procedures (Trott & Olson, 2010). The starting coordinates of voriconazole were taken from the crystal structure published in the Cambridge Structural Database (Ravikumar, Sridhar, Prasad, & Rao, 2007; CSD Ref code CEXMAU). Since it is impractical to simulate all possible isomers of substituted cyclodextrins, one representative isomer of HPBCD was chosen for the simulation, on the basis of the declared molar substitution (MS) of the commercial product (Kleptose HP, MS = 0.99), with a total of seven (2-hydroxy)propyl substituents, four on the O6 oxygens, which is the most reactive site, and the remaining three placed on the next most reactive site (Gramera & Caimi, 1969), the O2 oxygen position (Fig. 1a). For 2-O-MBCD (Fig. 1a), starting coordinates were taken from the crystal structure published in the Cambridge Structural Database (CSD Ref code IQOZIX), suitably adapted to match the declared MS of 0.57 by placing four methyl groups on the O2 oxygens.

Initial geometries of all structures were optimized prior to the docking runs, using the AMMP force field program as implemented in the VegaZZ molecular modeling software (Pedretti, Villa, & Vistoli, 2002). Subsequently, non-polar hydrogen atoms were merged, Kollman united-atom type charges, and solvation parameters were added with the aid of the AutoDock Tools program (Morris et al., 1998).

The geometry of the resultant bound conformations with the highest scores was further optimized using the AMMP force field program, and the lowest energy complexes were subsequently submitted to a full geometry optimization at the semi-empirical level of theory, using the PM6 Hamiltonian with the localized molecular orbital method, and simulating the effects of solvation with the COSMO continuum model (Klamt & Schumann, 1993) with a dielectric constant of 78.4. A single point energy run was performed using the PM6-DH2 method (Korth, Pitonak, Rezac, & Hobza, 2010), in order to account for the effects of non-covalent interactions (dispersion and hydrogen bonding). The binding energies, ΔE, were calculated from the difference in the heat of formation of the individual components and their complex, according to the Eq. (1):

$$\Delta E = E(complex) - E(bCD) - E(Vor) \quad (1)$$

This combined docking – semi empirical geometry optimization procedure is considered to increase the accuracy of docking simulations (Stigliani, Bernardes-Genisson, Bernadou, & Pratviel, 2012). All calculations were performed using MOPAC2012 (Stewart, 1990) and the results were evaluated using the WinMostar (TENCUBE Institute, Ltd.) graphical user interface to MOPAC.

2.4. Preparation of solid systems

2.4.1. Spray-dried samples

Solid drug–cyclodextrin systems were prepared by spray-drying the final clear solution obtained after filtration of suspensions from the phase-solubility studies. Spray-drying was performed on a Büchi B-191 mini spray-drier (Büchi Laboratoriums-Technik AG, Flawil, Switzerland) under the following conditions: inlet air temperature 120 °C, outlet air temperature 70 °C, pump set to 15%, aspirator 100% and atomization 550 l/h.

2.4.2. Wet ground samples

Powder samples were also prepared by the wet grinding technique, with CD:drug molar ratio 1:1. Cyclodextrin was wetted with few droplets of methanol in a ceramic mortar. The required amount of voriconazole was then slowly added and the powders were mixed with the help of a ceramic pestle for about 15 min. During this process, an appropriate quantity of methanol was added in order to maintain a suitable consistency of mixture. The final product was subsequently dried in an oven at 40 °C for 2 h and then allowed to equilibrate at room temperature during 24 h.

2.4.3. Physical mixtures

Simple physical mixtures of voriconazole and HPBCD or 2-O-MBCD (CD:drug molar ratio 1:1) were prepared by blending the powders in a polyethylene bag.

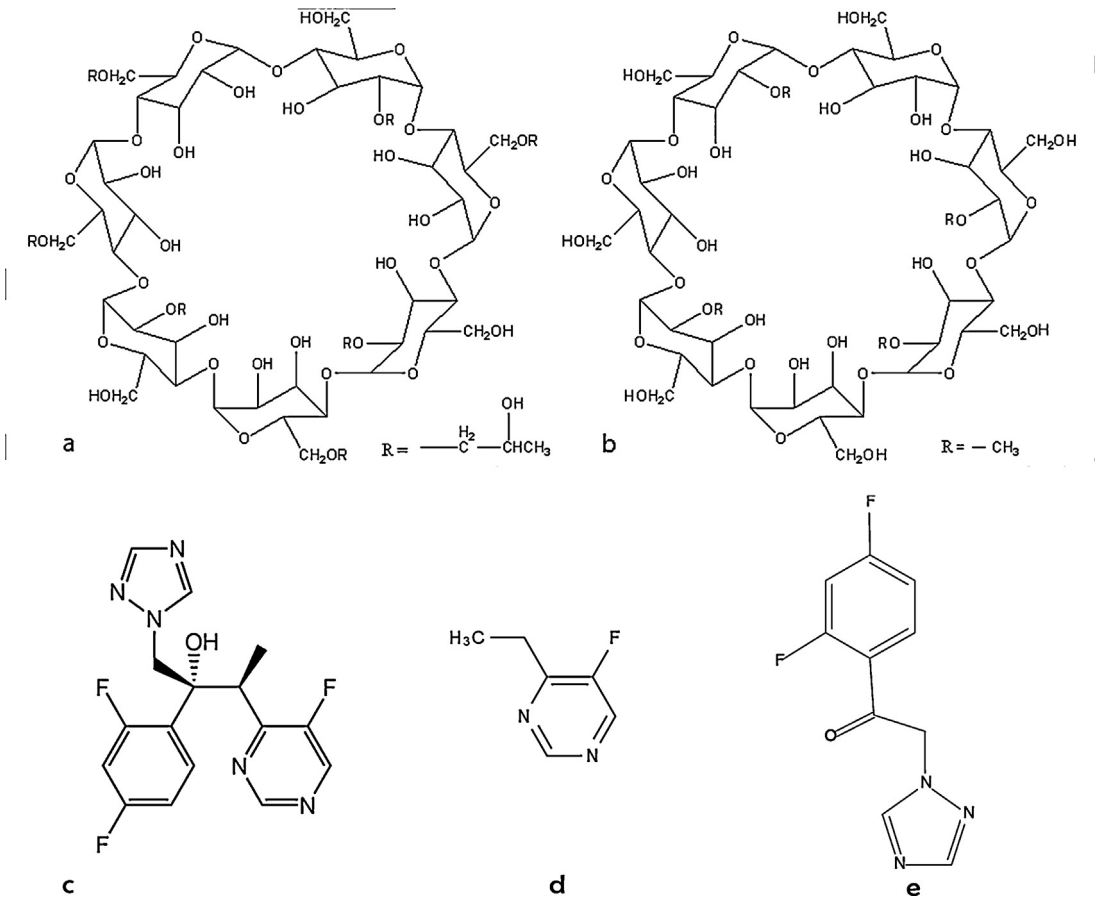

Fig. 1. Molecular structure of HPBCD (a), 2-O-MBCD (b), voriconazole (c), Impurity 1 (d), and Impurity 2 (e).

2.5. Characterization of powder samples

2.5.1. Determination of water content in cyclodextrins

For the initial characterization of cyclodextrins used in the study, semi-micro determination of water was conducted by Karl–Fischer titrimetry. It is based upon the quantitative reaction of water with sulphur dioxide and iodine in a suitable anhydrous medium in the presence of a base with sufficient buffering capacity (Ph.Eur. 2.5.12 Method A), by using the Karl–Fischer titrator (835 Titrando, Metrohm, Switzerland).

For comparison purposes, loss on drying was also determined for cyclodextrin by a gravimetric method for samples of 2.5 g, using a Halogen moisture analyzer (Mettler-Toledo HR 83, Greifensee, Switzerland) at a temperature of 105 °C, and measurement time until the mean weight loss drops below 1 mg/50 s.

2.5.2. Differential scanning calorimetry (DSC)

DSC measurements of powder samples were carried out using a Shimadzu DSC50 Differential Scanning Calorimeter (Shimadzu Corporation, Tokyo, Japan). Thermal behavior was studied after one scan performed by heating the samples (2–5 mg) in a sealed aluminum pan from 30 °C to 300 °C, at a rate of 10 °C/min, using nitrogen as purge gas (flow rate 25 cm^3/min). An empty pan was used as a reference.

2.5.3. Thermogravimetric analysis (TGA)

TGA measurements of powder samples were carried out using a Shimadzu TGA50 Thermogravimetric Analyzer (Shimadzu Corporation, Tokyo, Japan). The weight loss was monitored during heating the samples (2–5 mg) in an aluminum pan from 30 °C to 120 °C, at a rate of 10 °C/min, and under a nitrogen flow of 25 cm^3/min. Results were expressed as percent weight loss at the end of heating, which is assumed to represent water loss (percent water).

2.5.4. Fourier transform infrared spectroscopy (FTIR)

Attenuated total reflectance (ATR) FTIR spectra were obtained on a Shimadzu IR Prestige 21 spectrometer (Shimadzu Europa GmbH, Duisburg, Germany), with a horizontal Golden Gate MkII single-reflection ATR system (Specac, Kent, UK) equipped with a heated diamond top plate, ZnSe lenses and a 4000 series temperature controller. Spectra were recorded between 4000 and 500 cm^{-1} at a resolution of 4 cm^{-1}. A number of 32 scans were added for each spectrum.

2.5.5. Dissolution study

Dissolution study was conducted on a DT800 Dissolution Tester (Erweka, Heusenstamm, Germany) at 37 ± 0.5 °C, using USP Apparatus II (paddle rotating at 50 rpm) with 900 ml phosphate buffer pH 6.8, acetate buffer pH 4.5, and 0.1 M hydrochloric acid, used as dissolution media. Selection of dissolution media for dissolution profile analysis was made in accordance to relevant FDA guidance (FDA guidance for industry on Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System, 2000).

The solid formulations (pure drug, spray-dried complex and physical mixture with HPBCD, spray-dried complex and physical

mixture with 2-O-MBCD) were filled into hard gelatin capsules (Size #0). Fill weight of each capsule corresponded to approximately 10–11 mg of drug. At pre-set time intervals, aliquots were withdrawn from the dissolution medium, filtered through a 0.45-μm membrane filter, transferred to the spectrophotometer (8453 UV–vis spectrophotometer, Agilent, Santa Clara, USA) and assayed for voriconazole at 255 nm, by comparison with a calibration curve. Reference solutions that were used for calibration were prepared using voriconazole reference standard supplied by the manufacturer, methanol and dissolution media, to obtain solutions with concentration of 16 μg/ml, 80 μg/ml and 160 μg/ml of voriconazole, which were used to measure absorbance at wavelength of 255 nm comparing to the methanol as the blank solution. Obtained calibration curve had a coefficient of determination $R^2 > 0.990$. The test was repeated three times, and mean values and relative standard deviation were calculated.

2.5.6. Visualization of particles

Scanning electron microscopy analysis was performed using scanning electron microscope (Phenom G2 Pro, Phenom-World, The Netherlands). For sample preparation, double sided carbon adhesive pad was placed to a sample stub, then sample was placed on top of the exposed adhesive making sure it was firmly attached to the stub before placing the sample into the SEM. The samples were photographed at a voltage of 5 KV.

2.5.7. Determination of impurities in spray-dried complexes

Several impurities of voriconazole were monitored in spray-dried complexes: Impurity 1 (4-ethyl-5-fluoropyrimidine) and Impurity 2 (1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone) which are degradation products, and Impurity 3, epi-voriconazole, which is the 2S, 3R isomer of voriconazole (Fig. 1). Impurities were determined using appropriate high-performance liquid chromatography (HPLC) methods.

The analysis of Impurity 1 and Impurity 2 was performed using an Agilent HPLC system, 1200 Series (Agilent, Santa Clara, CA, USA). The chromatographic procedure was carried out using a C-18, 100 mm × 4.0 mm column packed with particles of silica (3 μm) (Hypersil, Thermo, Thermo Fisher Scientific Inc., MA, USA). The mobile phase consisted of 0.0125 M solution of sodium dihydrogen phosphate dihydrate (buffer pH 4.0) and acetonitrile in 90:10 ratio. The flow rate was 1.0 ml/min, the injection volume was 50 μl, column temperature was 35 °C and the UV detector was set at 254 nm.

The analysis of chiral Impurity 3 was performed using an Agilent HPLC system, 1200 Series (Agilent, Santa Clara, CA, USA). The chromatographic procedure was carried out using a C-18, 150 mm × 4.6 mm column packed with particles of silica (5 μm) (Zorbax Eclipse, Agilent, Santa Clara, CA, USA). The mobile phase consisted of 0.01 M solution of ammonium acetate and 0.02 g/ml 2-hydroxypropyl-beta-cyclodextrin (buffer pH 5.0) and acetonitrile in 78:22 ratio. The flow rate was 2.0 ml/min, the injection volume was 50 μl, column temperature was 25 °C and the UV detector was set at 254 nm.

Standard solutions were adequately prepared using voriconazole and impurities reference standards (supplied by the voriconazole manufacturer), acetonitrile and diluent and they were used to check System suitability criteria.

Test solutions were adequately prepared from spray-dried powder samples. The responses (peak area) for the major peaks (voriconazole and each impurity) were measured and the their quantity was calculated using the equation $Cs \times (Au/As)$, where *Au* is the area of peak of interest in the test solution, *As* is the area of peak of interest in the standard solution, and *Cs* is the concentration of substance of interest in the standard solution.

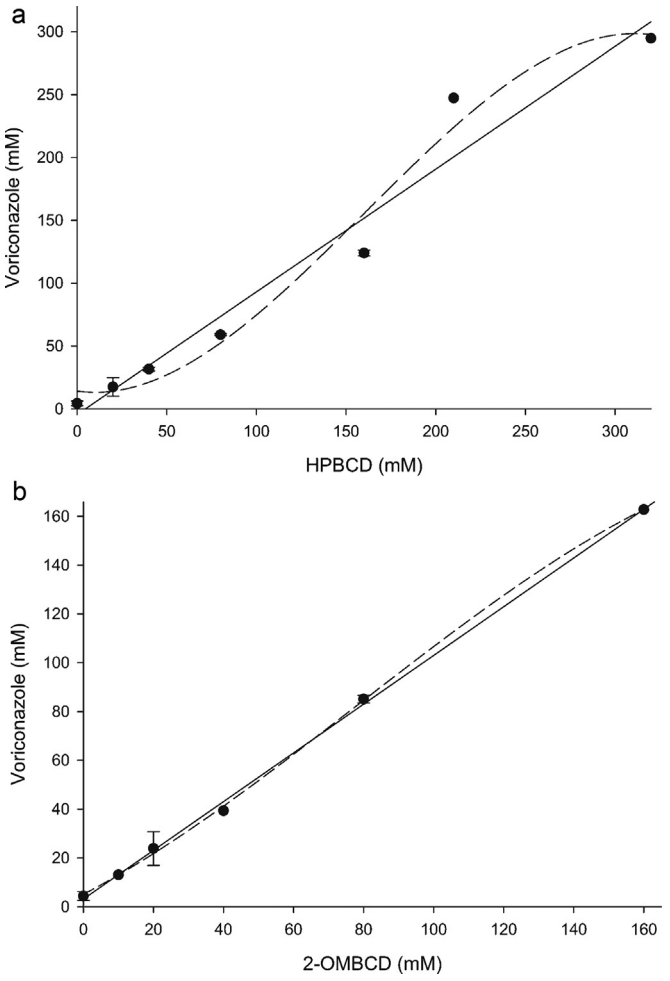

Fig. 2. Phase-solubility diagrams for voriconazole in aqueous solutions of HPBCD (linear function curve fit: straight line, cubic function curve fit: dashed line) (a) and 2-O-MBCD (linear function curve fit: straight line, cubic function curve fit: dashed line) (b).

3. Results and discussion

3.1. Solubility studies

3.1.1. Phase-solubility studies

Fig. 2 sums up the results of the phase-solubility studies performed and analyzed using the approach described by Brewster and Loftsson (2007) and Higuchi and Connors (1965). For this study, a range of CD concentrations were selected which cover the maximum solubility of CDs in water at room temperature: 0–160 mM for 2-O-MBCD and 0–320 mM for HPBCD. For both 2-O-MBCD and HPBCD, the solubility of voriconazole increased linearly with the concentration of cyclodextrins over the CD concentration range up to 160 mM, corresponding to the A_L type profile (Brewster & Loftsson, 2007). For HPBCD after 160 mM, drug concentration began to plateau which was indicative of A_N type profile, and that CD is less effective at higher concentrations (Brewster & Loftsson, 2007). For voriconazole–HPBCD solutions Buchanan et al. (2007) also reported that over the lower HPBCD concentration range A_L type profile could be recognized, as well as existence of A_N profile at higher concentrations. A_N profiles were explained by bulk changes imparted to the solvent by the solubilizer at various concentrations and/or self-association of the solubilizer (HPBCD) at high concentrations.

A fit to the initial linear portion of the curve could be used for calculation of the apparent binding constant, which provides

information about the relative strength of the drug–cyclodextrin interaction. However, intrinsic solubilities for poorly water-soluble drugs are typically very low and difficult to measure accurately and this can significantly impact the accuracy of the reported binding constant (Brewster & Loftsson, 2007; Miro et al., 2012). Therefore, when comparing cyclodextrins in a given medium, observing the difference in achieved solubility results for the same concentrations of the different cyclodextrins could be more meaningful (Buchanan et al., 2007). With 2-O-MBCD somewhat higher solubility was achieved than with HPBCD solutions of the same molar concentration. As the slope obtained for 2-O-MBCD is greater than for HPBCD, it appears that 2-O-MBCD is a more efficient solubilizer for voriconazole. By simple phase-solubility diagram analysis it is difficult to further distinguish whether simple (1:1) or higher order complexes are being formed (Brewster & Loftsson, 2007; Loftsson et al., 2005).

The stoichiometry of the system can be probed by curve fitting of the diagram with linear, quadratic or a cubic model (Brewster & Loftsson, 2007). In this study the solubility curve for complexation with HPBCD was better fitted by a cubic function ($R^2 = 0.978$) than by a linear and quadratic function ($R^2 = 0.958$ and $R^2 = 0.958$, respectively), suggesting a 1:3 drug:CD complex formation rather than 1:1 or 1:2 complex (Fig. 2a). Similar stoichiometry was suggested in a patent application for a formulation of voriconazole with sulfo-butyl-ether-beta-cyclodextrin (Harding, 2003).

As for complexation with 2-O-MBCD fit was equally good for linear, quadratic and cubic function ($R^2 = 0.9990$, 0.9992 and 0.9994 respectively), therefore providing virtually no information about whether complexes are 1:1 or higher order (Fig. 2b). For 2-O-MBCD, which is a beta-cyclodextrin derivative still not in wide use, it has been pointed out by its manufacturer (*Kleptose® Crysmeb exp* e-brochure), that solubilization of drugs by aqueous solutions of 2-O-MBCD should be high and depending linearly on CD concentration. There should be no complication characteristic that leads to self-association and non-linear decrease of solubilization efficiency at high concentrations, which is a common problem for some other cyclodextrin derivatives such as HPBCD (Buchanan et al., 2007; Loftsson, Masson, & Brewster, 2004; Piel et al., 2006). This is explained by the size of the methyl substituents, which are small and do not function as guests at high concentration. Also, the cavity of beta-cyclodextrin is slightly extended by methyl substituents, which are equally stereo-rigid as the cavity itself (Mosher & Thompson, 2007). This should all increase the solubilization power of 2-O-MBCD, which seems to be in agreement with the results obtained in this study. Owens, Fell, Coleman, and Berridge (2000) studied the nature of interaction between the voriconazole stereoisomers and a number of derivatised CDs and concluded that the size of the CD toroid and the nature of the derivative side chain have significant effect on the nature of the complexation, but also that consideration of the three-dimensional spatial arrangement of each stereoisomer is important. Therefore, the application of molecular modelling techniques may be helpful in clarifying the nature of interaction of drug and CD by suggesting stable three-dimensional models of complexes.

3.1.2. Factorial design

Results of factorial analysis indicate that the most significant factor ($p < 0.05$) affecting voriconazole solubility was cyclodextrin type (Table 1 and Table A.1, supplementary data). The amount of drug was also a significant factor for the rate of the drug solubilization, since after the addition of higher amount of drug, solubility results were higher in the same time points in comparison with lower amount of drug. This is in agreement with the results of Borghetti et al. (2009), where it was suggested that in more concentrated conditions the interaction between the drug and cyclodextrin was easier and the complex formation seemed to occur faster.

Table 1
Design of experiments and responses for 2^3 factorial design.

Sample no.	Independent variables			Response
	A	*B*	*C*	*R*
1	+1	+1	+1	7.20
2	+1	−1	+1	6.95
3	+1	+1	−1	4.65
4	+1	−1	−1	4.55
5	−1	+1	+1	4.64
6	−1	−1	+1	4.28
7	−1	+1	−1	4.52
8	−1	−1	−1	4.17

Coding of factors and factor levels: *A* = cyclodextrin type: −1 corresponds to HPBCD, +1 to 2-O-MBCD; *B* = mixing time: −1 corresponds to 30 min, +1 to 60 min; *C* = voriconazole amount: −1 corresponds to 500 mg, +1 to 1000 mg; *R* = voriconazole solubility (mg/ml).

The only important interaction effect was the one between cyclodextrin type and mixing time. The longer mixing time helped to improve voriconazole solubility more with HPBCD, whereas with 2-O-MBCD solubilization occurred with shorter mixing time (Table 1). It was noticed that after addition of 1000 mg of voriconazole, larger amount of voriconazole was dissolved in the examined period of time in 2-O-MBCD than in HPBCD solutions (Table 1). That means that drug solubilization was easier and therefore faster with 2-O-MBCD, which was also assumed by simple observation of the visual appearance of dispersions (presence of visible undissolved drug particles) during mixing. Therefore, the results of factorial design statistical analysis suggest that 2-O-MBCD is a more efficient solubilizer than HPBCD, in agreement with phase-solubility studies.

3.2. Molecular docking simulations

The docking simulations followed by further geometry optimization of the highest score complexes, revealed that the most stable complex between voriconazole and 2-O-MBCD is formed when the fluoropyrimidine ring is included in the cavity (Fig. 3a1, $\Delta E = -14.6$ kcal/mol), while less stable inclusion compounds can be formed when the triazole or the difluorophenyl ring is included in the cavity (Fig. 3a2 and a3, $\Delta E = -9.1$ and -6.0 kcal/mol, respectively). The interactions responsible for the inclusion seem to be purely hydrophobic in nature, as no indications of hydrogen bonding can be found in the optimal complex geometries.

HPBCD on the other hand, shows a preference of forming stable inclusion compounds mostly when the difluorophenyl (Fig. 3b1 and b3, $\Delta E = -88.9$ and -57.3 kcal/mol, respectively) or the fluropyrimidine ring is included in the cavity (Fig. 3b2, $\Delta E = -84.8$ kcal/mol). Inclusion of the difluorophenyl group into the CD toroid while the CD side chains interacted with the triazole and pyrimidine groups of voriconazole stereoisomers, was also discussed in a study by Owens et al. (2000). Strong hydrophilic interactions including hydrogen bonds between the hydroxypropyl groups of HPBCD and several hydrophilic groups of voriconazole explain the calculated low energy of the complexes. It is remarkable that the calculated energy of voriconazole–HPBCD complexes is much lower than those of 2-O-MBCD complexes, which implies that it should be a much better solubilizer for voriconazole (Table 2). The discrepancy between computer simulations and experimental results may be attributed to the self-association and formation of multiple complexes of HPBCD molecules, in agreement with the phase-solubility studies that suggested a multiple complexation and not the formation of 1:1 complexes between voriconazole and HPBCD. Self-association

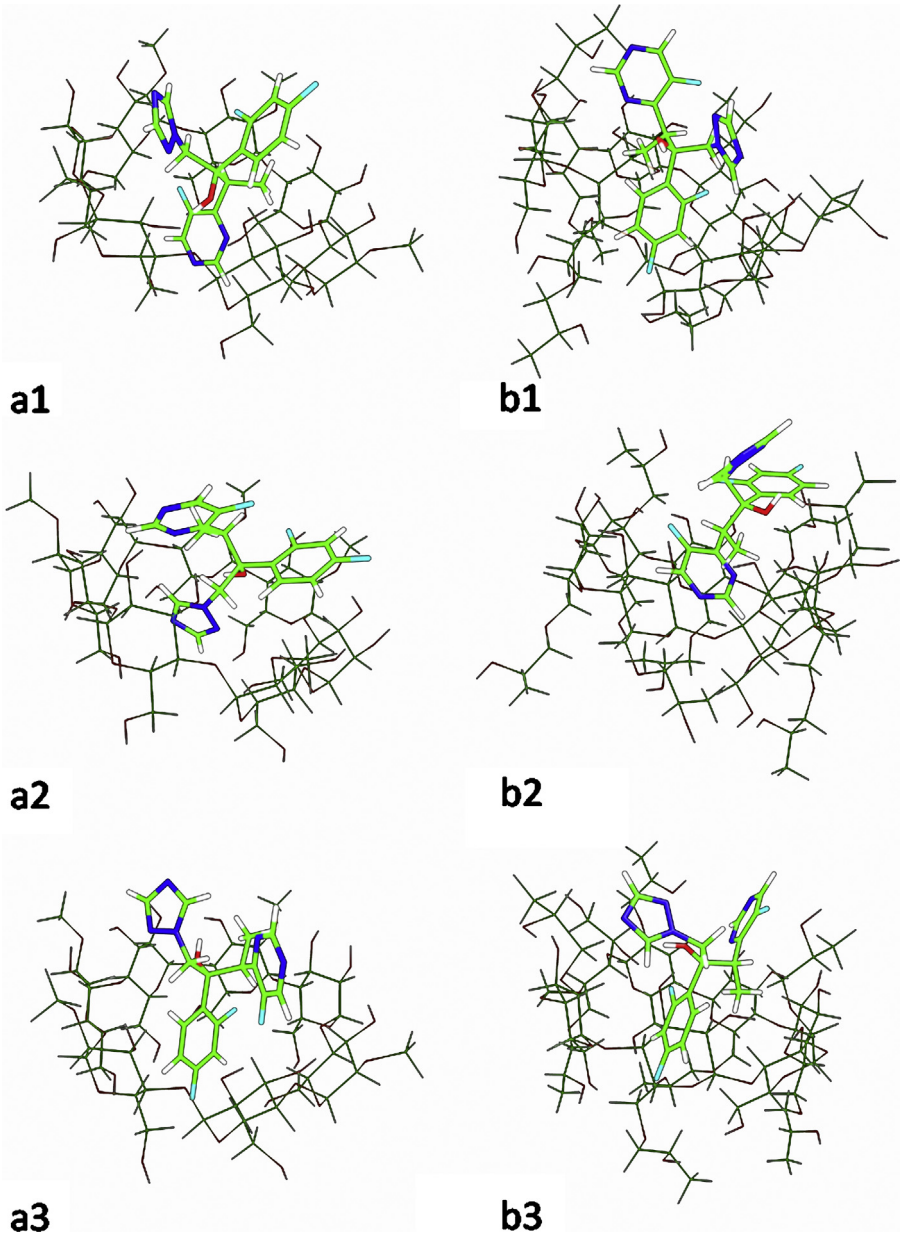

Fig. 3. Lowest energy voriconazole–2-O-MBCD (a1–3), and voriconazole–HPBCD (b1–3) complexes identified by PM6-DH2 energy minimization of the highest score results of docking simulations.

of HPBCD molecules is very likely due to the presence of hydrogen bond donor and acceptor groups at the rim of the cavity. Formation of HPBCD–HPBCD complexes would prevent voriconazole from entering the cavity and forming the stable complexes predicted by the 1:1 docking simulations.

Table 2
Semi-empirical level binding energies of voriconazole–cyclodextrin complexes, calculated using the PM6-DH2 Hamiltonian.

Complex no.[a]	PM6-DH2 binding energy, ΔE (kcal/mol)	
	2-O-MBCD	HPBCD
1	−14.6	−88.9
2	−9.1	−84.8
3	−6.0	−57.3

[a] Key as in Fig. 3.

3.3. Characterization of powder samples

3.3.1. Thermal analysis

From the DSC thermograms (Fig. 4), it is seen that pure voriconazole shows a melting endotherm with a maximum at 133 °C, in agreement with the reported melting point of 128–134 °C (Herbrecht, 2004), indicating that the raw material belongs to the polymorphic form I described in US patent US20090023922 (Benito et al., 2009). Regarding the spray-dried products, the total absence of a melting endotherm was observed while the physical mixtures and the co-ground products show clear indications of the presence of crystalline voriconazole (melting peak maximum at 132 °C with HPBCD and 133 °C with 2-O-MBCD for physical mixtures, and for co-ground products, 129 °C with HPBCD, and 131 °C with 2-O-MBCD). This slight melting point depression can indicate that some kind of interaction occurs with the cyclodextrins during co-grinding (Lin et al., 2012), although in previous studies a similar

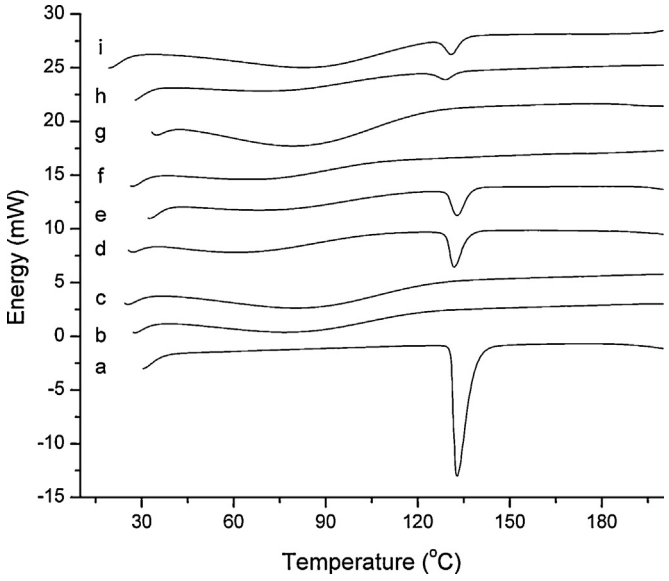

Fig. 4. Representative DSC thermograms: voriconazole (a), HPBCD (b), 2-O-MBCD (c), physical mixture of voriconazole and HPBCD (d), physical mixture of voriconazole and 2-O-MBCD (e), voriconazole–HPBCD inclusion compound produced by spray drying at 160 mM HPBCD concentration (f), voriconazole–2-O-MBCD inclusion compound produced by spray drying at 160 mM 2-O-MBCD concentration (g), voriconazole–HPBCD inclusion compound produced by solvent-drop co-grinding (h) and voriconazole–2-O-MBCD inclusion compound produced by solvent-drop co-grinding (i).

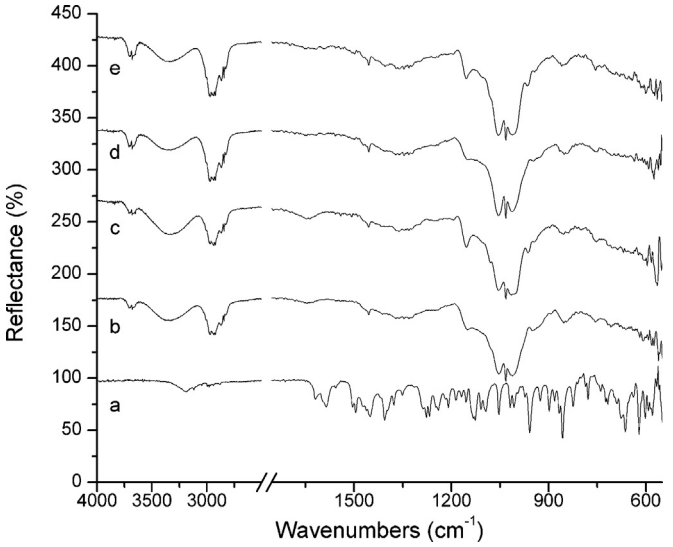

Fig. 5. Representative ATR-FTIR spectra: voriconazole (a), HPBCD spray-dried (b), 2-O-MBCD spray-dried (c), voriconazole–HPBCD inclusion compound produced by spray-drying at 160 mM HPBCD concentration (d), and voriconazole–2-O-MBCD inclusion compound produced by spray-drying at 160 mM 2-O-MBCD concentration (e).

melting point depression of voriconazole in the presence of polymers, was considered insignificant (Beinborn, Lirola, & Williams, 2012). Although the wet grinding method appears as an attractive solvent and energy saving alternative, more experimental work is required for the optimization of its parameters, which is beyond the scope of the present study.

The absence of a melting endotherm of the drug in the spray-dried product indicates that the total voriconazole amount exists in complexed form and is amorphous (Fig. 4f and g). Another feature of the thermograms is the broad endotherm spanning from room temperature to 100 °C, with a maximum in the range of 79–80 °C that probably corresponds to the loss of strongly bound water from the cyclodextrin hydration shell. No effect of the cyclodextrin molar concentration in the spray-drying solution was observed in the thermograms, indicating that the amounts of cyclodextrins used in the present study were suitable and sufficient to fully include the drug in a molecular state.

Results of thermogravimetric analysis, performed for the purpose of moisture content determination in powder samples prepared by different methods, were obtained by heating up to 120 °C where the water loss is expected to occur, and are presented in Table A.2 (supplementary data). Results for physical mixtures and wet ground samples of drug and CDs are similar to the results for CDs alone (Tables A.2 and A.3, supplementary data). For samples with two different cyclodextrins prepared by spray-drying, it was observed that products with 2-O-MBCD had much higher water content than products with HPBCD, when using the same process parameters. It seems that it is more difficult to remove the water from 2-O-MBCD than from HPBCD complex during the spray-drying process, suggesting that the formulation with 2-O-MBCD might be more hygroscopic than with HPBCD, which could affect the cost-effectiveness of spray-drying process, the next steps in potential production of solid oral dosage forms, and potentially the extent of physical and chemical stability of resulting powders over a prolonged time period.

3.3.2. FTIR spectroscopy

Fig. 5 illustrates ATR-FTIR spectra of voriconazole, cyclodextrins and spray-dried products. Voriconazole (Fig. 5a) shows characteristic bands of polymorph I (patent US20090023922), in agreement with the melting range observed in the DSC thermograms. Specifically, the CN, CF and CC stretching bands appear at 3190–3046 cm^{-1}, 1495–1451 cm^{-1} and 1585–1451 cm^{-1}, respectively, in agreement with Xiang-Gen, Li-Na, Meng, and Hao-Ran (2011). The FTIR spectra of spray-dried pure cyclodextrins (Fig. 5b and c) show a broad reflectance band in the range of 3100–3600 cm^{-1} with a maximum ~3340 cm^{-1}, which is attributed to the OH stretching vibrations, indicating strong association of the hydroxyl groups via hydrogen bonds. However, the existence of lower intensity sharp peaks in the range of 3600–3700 cm^{-1}, indicates the presence of free OH groups that possibly belong to adsorbed water that is not fully involved in hydrogen bonds.

The FTIR spectra obtained for voriconazole–CD spray-dried complexes (Fig. 5d and e) displayed only the typical bands of CD, indicating that free voriconazole cannot be detected in the samples. This might actually indicate that voriconazole is completely included in the cyclodextrin cavity, as discussed by Yang et al. (2010), where the observed close agreement of the FTIR band disappearance with the reported sites of complexation of itraconazole and HPBCD was considered to be credible evidence for the presence of true inclusion complexes (Peeters, Neeskens, Tollenaere, Van Remoortere, & Brewster, 2002). The spectra for the physical mixtures of CD and voriconazole (Fig. A.1d and A.1e, supplementary data) showed the typical bands of the CD and voriconazole, indicating weak or no interaction between the drug and the cyclodextrin when physically mixed.

3.3.3. Dissolution study

Dissolution profile was tested in three different media (phosphate buffer pH 6.8, acetate buffer pH 4.5 and 0.1 M hydrochloric acid pH 1.2) for the assessment of drug solubility and dissolution rate improvement. Fig. 6 shows the percent of voriconazole dissolved vs time for a voriconazole–HPBCD and voriconazole–2-O-MBCD complex, in comparison to corresponding physical mixture

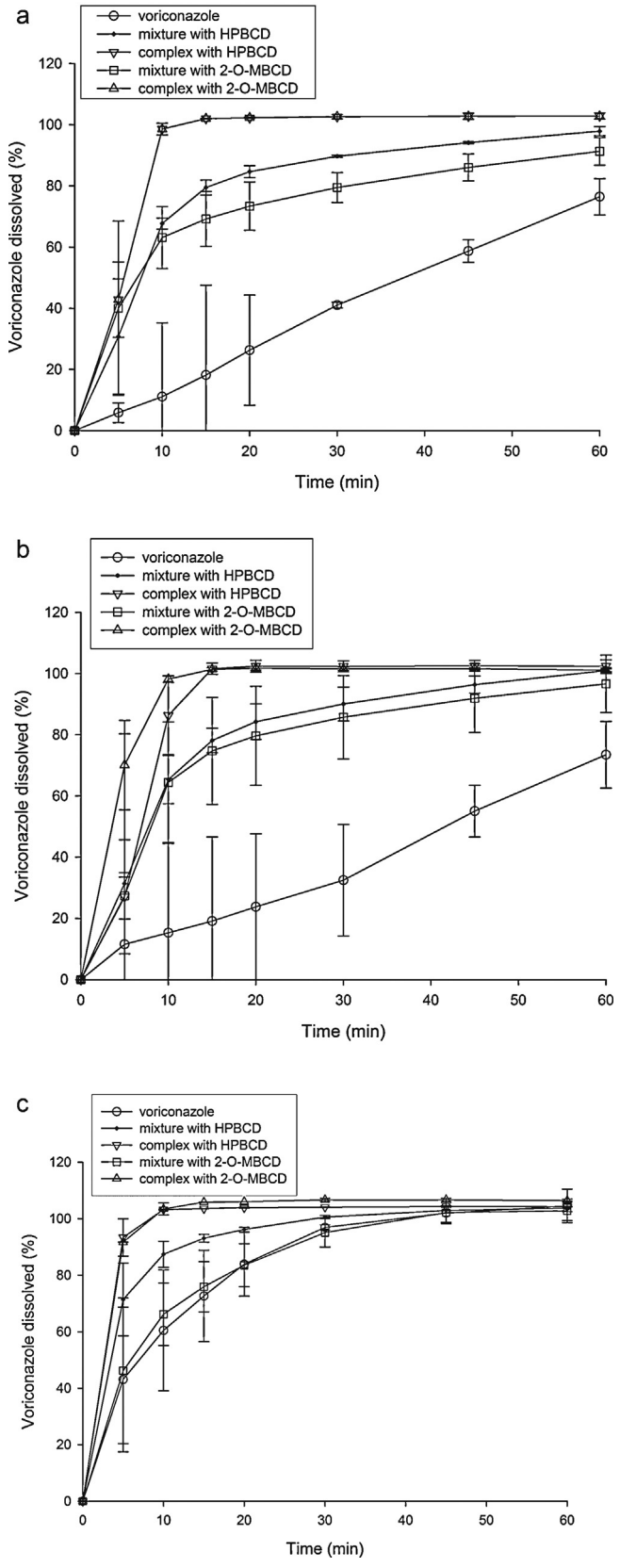

Fig. 6. Dissolution profiles for voriconazole from various solid samples – dissolution method conditions: Apparatus II (Paddle), agitation speed 50 rpm, 900 ml of dissolution media: phosphate buffer pH 6.8 (a), acetate buffer pH 4.5 (b) and 0.1 M hydrochloric acid pH 1.2 (c).

and pure voriconazole. There was an obvious difference in dissolution rate between these samples.

In physical mixtures with drug, the presence of cyclodextrin contributed to some in vitro dissolution rate improvement. Voriconazole was dissolved faster from the physical mixture with HPBCD than from the capsules with the pure drug, or from the physical mixture with 2-O-MBCD. The reason for difference in dissolution rate from physical mixture with 2-O-MBCD and HPBCD could be attributed to different solubility of CD derivatives and/or to smaller particle size of 2-O-MBCD and the adsorbed air on their surface, which made the wetting somewhat slower, which was not the issue with larger particles of HPBCD. Also, during the cyclodextrin solution preparation it was observed that the 2-O-MBCD particles were floating and more intensive mixing was needed in order to wet the powder and dissolve it.

From spray-dried complexes more than 80% of voriconazole was dissolved in all three media after 10 min, regardless of type of cyclodextrin. Considering the pH-dependent voriconazole solubility, interestingly it was observed that for the spray-dried complexes with cyclodextrins dissolution profile of drug was independent of pH. The possibility that the solubility of a drug can become independent of the pH through the application of CD for drug complexation was also reported by Nacsa, Berkesi, Szabo-Revesz, and Aigner (2009), as well as Buchanan et al. (2007). By comparing the relative standard deviation values (represented by error bars in Fig. 6), it was concluded that the dissolution was very rapid and uniform only from the spray-dried drug–CD complex, which is a significant desirable characteristic of the dissolution profile.

Overall, results obtained in this study suggest that the complexation of voriconazole with both cyclodextrins can be used as a formulation strategy for solubility and dissolution rate enhancement. There was not much difference in maximum solubility between complex and mixture of drug and CD, probably because in case of mixture, complex formation and solubilization took place in the dissolution medium. This however, may not happen in the gastro-intestinal tract, where absorption takes place following dissolution. Therefore, prior formation of complexes is expected to be beneficial.

3.3.4. *Visualization of particles*

The visualization of the particles was performed using scanning electron microscopy. With both CDs similar particles were produced by spray-drying the solutions of complexes. A representative photomicrograph is presented in Fig. 7. It was observed that the particles were of spherical shape, most of which were with wrinkled and some with smooth surface. The presence of apparently hollow particles was also observed. Spherical shape is typical for spray drying of solutions. An empty interior can result from gas expansion in the droplets with a vapour-impervious film or from air entrained in the liquid feed. Since the particle formation kinetics is controlled by the evaporation rate, the rising droplet viscosity, and the components' receding as the liquid droplet dries, it imposes stresses on the forming surface which control the morphology of the spray dried particles (Snyder & Lechuga-Ballesteros, 2008). Probably particles became wrinkled and hollow because they were subjected to high drying rates (total residence time of the droplets/particles in the Büchi mini spray dryer is approximately 1 s (Lee & Brandenberger, 2002)). The detailed physics of the entire droplet-to-particle formation process is highly complex and dependent upon both process variables and formulation physicochemical properties (Snyder & Lechuga-Ballesteros, 2008).

3.3.5. *Long term stability of voriconazole–cyclodextrin inclusion complexes*

The long term stability of voriconazole–cyclodextrin inclusion complexes upon storage in ambient conditions was examined

Table 3
Results for impurities determined in spray-dried samples initially and after 12 months of storage.

Impurity	Complex with HPBCD		Complex with 2-O-MBCD	
	Initially (%)	After 12 months (%)	Initially (%)	After 12 months (%)
Impurity 1[a]	<0.05	0.24	<0.05	0.16
Impurity 2[b]	<0.05	0.46	<0.05	0.31
Impurity 3[c]	<0.05	0.05	<0.05	0.06
Total impurities	<0.05	0.75	<0.05	0.53

[a] Impurity 1: 4-ethyl-5-fluoropyrimidine.
[b] Impurity 2: 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone.
[c] Impurity 3: (2S,3R)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; (epi-voriconazole).

after 12 months by differential scanning calorimetry and thermogravimetric analysis (results not shown). No sign of voriconazole crystallization could be identified in the thermograms (complete absence of endotherm peaks corresponding to the melting of voriconazole). Additionally, examination of the water content by TGA showed no significant change. These results could indicate good physical stability over the 1 year time frame examined to date.

Although initially the level of impurities in powders was below 0.05%, determination of impurities in aged spray-dried complexes with both cyclodextrins showed that there might be some chemical stability issues. Results for voriconazole impurities determined in spray-dried samples after 12 months of storage are presented in Table 3.

Only small amounts of Impurity 3 or epi-voriconazole were found in aged spray-dried powders, which might indicate that voriconazole epimerization is not very likely to take place. Impurity 1 and Impurity 2 are degradation products that are the result of splitting the voriconazole molecule at the butanol backbone (Fig. 1). With both CDs, some chemical degradation of voriconazole was observed. The difference in impurity levels between HPBCD and 2-O-MBCD complexes is not particularly big, but it might be the result of the difference in binding affinity and the orientation of voriconazole molecule in the inclusion complex with different CD derivatives, as it was observed in phase-solubility study and molecular docking simulations. In case of 2-O-MBCD, the somewhat extended cavity might additionally contribute to somewhat better drug stability. However, complexation with either CD did not prevent degradation of voriconazole that was present in the complex as the less stable, amorphous form. As it was shown in some other studies, higher mobility of amorphous drug:CD complexes can be the reason for chemical instability irrespective of the chemical nature of the side chain or cavity dimensions of CD (Hong, Shah, & McGonagle, 2011). The other reason for instability might be the specific bound conformations of drug:CD complexes, which could not stabilize the drug (Mosher & Thompson, 2007). Molecular modeling results revealed that the parts of the voriconazole molecule that are most probably included in the CD cavity are its rings (difluorophenyl, fluoropyrimidine, or triazole). Therefore, the most sensitive part of the voriconazole molecule, butanol backbone, would probably stay outside the CD cavity and therefore remain unprotected against the degradation and formation of Impurities 1 and 2. This might indicate that, in order to prevent the degradation of voriconazole in complex, CD derivative with larger substituents might be necessary to provide a steric barrier which might stabilize the drug (Mosher & Thompson, 2007).

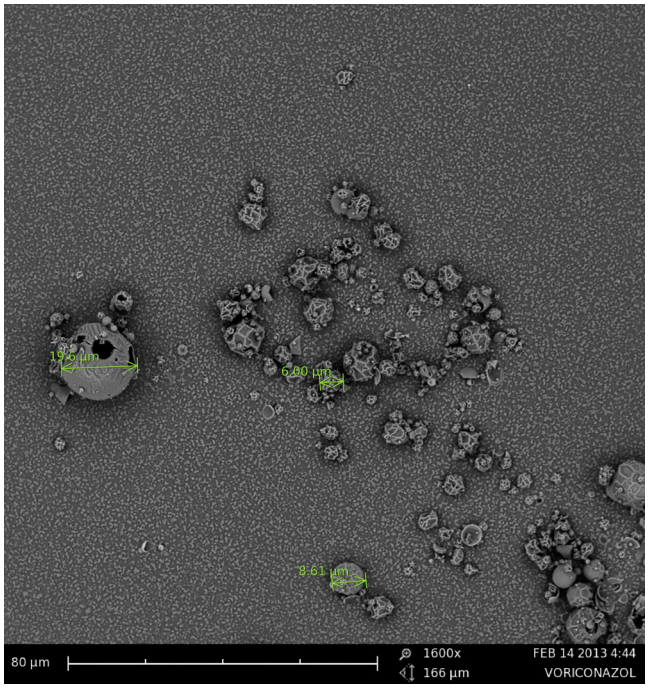

Fig. 7. Representative SEM photomicrograph of voriconazole:CD spray-dried particles.

4. Conclusions

From phase-solubility studies, the 2-O-MBCD derivative seemed to be more efficient in complexation than HPBCD as indicated by the greater slope of the solubility diagram. This was additionally confirmed by performed factorial design study, which indicated that CD type was the most significant factor for voriconazole solubilization, and that complexation occurred easier and faster with 2-O-MBCD. Molecular docking simulations showed a marked difference in the 1:1 binding affinities, with HPBCD forming complexes of much lower energy. This discrepancy between the predicted and experimentally observed solubilizing ability of HPBCD suggested a multiple rather than a 1:1 complexation. Spray-drying of cyclodextrin solutions proved to be an efficient technique for the preparation of highly soluble inclusion complexes of voriconazole with both HPBCD and 2-O-MBCD. The spray-dried products were free of crystalline voriconazole, and provided higher solubility and dissolution rate. Although physically stable enough over a prolonged period of storage, the observed chemical instability of powders that was slightly more pronounced with HPBCD, may lead to a more detailed study and further formulation development. Combining of applied methods helped in better understanding the nature of complexation of voriconazole with two CD derivatives and how it can affect drug properties such as solubility, dissolution rate and stability.

Acknowledgment

This work was supported by the Project TR 34007, Ministry of Education and Science, Republic of Serbia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.05.084.

References

Beinborn, N. A., Lirola, H. L., & Williams, R. O., 3rd. (2012). Effect of process variables on morphology and aerodynamic properties of voriconazole formulations produced by thin film freezing. *International Journal of Pharmaceutics*, *429*, 46–57.

Benito, M., Molins, E., & Contreras, J. (2009). *Process for preparing voriconazole, new polymorphic form of intermediate thereof, and uses thereof*. US Patent application number:20090023922

Borghetti, G. S., Lula, I. S., Sinisterra, R. D., & Bassani, V. L. (2009). Quercetin/β-cyclodextrin solid complexes prepared in aqueous solution followed by spray-drying or by physical mixture. *AAPS Pharmaceutical Science Technology*, *10*(1), 235–242.

Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews*, *59*, 645–666.

Buchanan, C. M., Buchanan, N. L., Edgar, K. J., & Ramsey, M. G. (2007). Solubility and dissolution studies of antifungal drug:hydroxybutenyl-β-cyclodextrin complexes. *Cellulose*, *14*, 35–47.

Challa, R., Ahuja, A., Ali, J., & Khar, R. K. (2005). Cyclodextrins in drug delivery: An updated review. *AAPS Pharmaceutical Science Technology*, *6*(2). Article 43.

Dubin, C. H. (2006). Formulation strategies for poorly soluble drugs. *Drug Delivery Technology*, *6*(6), 34–38.

Freitas, M. R. d, Rolimb, L. A., Soares M.F.d.L.R., Rolim-Neto, P. J., Albuquerque, M. M. d., & Soares-Sobrinho, J. L. (2012). Inclusion complex of methyl-beta-cyclodextrin and olanzapine as potential drug delivery system for schizophrenia. *Carbohydrate Polymers*, *89*, 1095–1100.

FDA guidance for industry on Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. 2000. www.fda.gov

Gramera, R. E., & Caimi, R. J. (1969). *Cyclodextrin polyethers and their production*. US Patent 3,459,731.

Harding, V. D. (2003). *Pharmaceutical formulations containing voriconazole*. US patent 006632803B1.

Herbrecht, R. (2004). Voriconazole: therapeutic review of a new azole antifungal. *Expert Review of Anti-infective Therapy*, *2*, 485–497.

Higuchi, T., & Connors, K. A. (1965). Phase solubility techniques. *Advances in Analytical Chemistry and Instrumentation*, *4*, 117–212.

Hong, J., Shah, J. C., & McGonagle, M. D. (2011). Effect of cyclodextrin derivation and amorphous state of complex on accelerated degradation of ziprasidone. *Journal of Pharmaceutical Sciences*, *100*, 2703–2716.

Hu, L., Zhang, H., Song, W., Gu, D., & Hu, Q. (2012). Investigation of inclusion complex of cilnidipine with hydroxypropyl-beta-cyclodextrin. *Carbohydrate Polymers*, *90*, 1719–1724.

Klamt, A., & Schumann, G. (1993). COSMO: A new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *Journal of the Chemical Society Perkin Transactions*, *2*, 799–805.

Kleptose® Crysmeb Exp methyl beta-cyclodextrin from Roquette, e-brochure. Accessed 04.10.12. http://www.roquette-pharma.com/brochures/03/visio.html

Korth, M., Pitonak, M., Rezac, J., & Hobza, P. (2010). A transferable H-bonding correction for semiempirical quantum-chemical methods. *Journal of Chemical Theory and Computation*, *6*, 344–352.

Lee, G., & Brandenberger, H. (2002). Spay-drying of macromolecules for inhalation therapy. *Evaporation & Life Science*, *25*, accessed April 2013. http://www.buchi.com/uploads/media/BestBuchi25_BUE.pdf

Lin, H.-L., Lin, S.-Y., Lin, C.-C., Hsu, C.-H., Wu, T.-K., & Huang, Y.-T. (2012). Mechanical grinding effect on thermodynamics and inclusion efficiency of loratadine–cyclodextrin inclusion complex formation. *Carbohydrate Polymers*, *87*, 512–517.

Loftsson, T., Hreinsdottir, D., & Masson, M. (2005). Evaluation of cyclodextrin solubilization of drugs. *International Journal of Pharmaceutics*, *302*, 18–28.

Loftsson, T., Masson, M., & Brewster, M. E. (2004). Self-association of cyclodextrins and cyclodextrin complexes. *Journal of Pharmaceutical Sciences*, *93*, 1091–1099.

Miro, A., Ungaro, F., Balzano, F., Masi, S., Musto, P., Manna, P. L., et al. (2012). Triamcinolone solubilization by (2-hydroxypropyl)-beta-cyclodextrin: A spectroscopic and computational approach. *Carbohydrate Polymers*, *90*, 1288–1298.

Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., et al. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, *19*, 1639–1662.

Mosher, G., & Thompson, D. O. (2007). Complexation: cyclodextrins. In J. Swarbrick (Ed.), *Encyclopedia of Pharmaceutical Technology* (third edition, pp. 671–696). New York, USA: Informa Healthcare USA.

Nacsa, A., Berkesi, O., Szabo-Revesz, P., & Aigner, Z. (2009). Achievement of pH-independence of poorly-soluble ionizable loratadine by inclusion complex formation with dimethyl-β-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *64*, 249–254.

Owens, P. K., Fell, A. F., Coleman, M. W., & Berridge, J. C. (2000). Complexation of voriconazole stereoisomers with neutral and anionic derivatised cyclodextrins. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *38*, 133–151.

Pedretti, A., Villa, L., & Vistoli, G. (2002). VEGA: A versatile program to convert handle and visualize molecular structure on Windows-based PCs. *Journal of Molecular Graphics*, *21*, 47–49.

Peeters, J., Neeskens, P., Tollenaere, J. P., Van Remoortere, P., & Brewster, M. E. (2002). Characterization of the interaction of 2-hydroxypropyl-β-cyclodextrin with itraconazole at pH 2, 4 and 7. *Journal of Pharmaceutical Sciences*, *91*, 1414–1422.

Piel, G., Piette, M., Barillaro, V., Castagne, D., Evrard, B., & Delattre, L. (2006). Betamethasone-in-cyclodextrin-in-liposome: The effect of cyclodextrins on encapsulation efficiency and release kinetics. *International Journal of Pharmaceutics*, *312*, 75–82.

Ravikumar, K., Sridhar, B., Prasad, D. K., & Rao, A. K. S. B. (2007). Voriconazole, an antifungal drug. *Acta Crystallographica*, *E63*, 565–567.

Redenti, E., Szente, L., & Szejtli, J. (2000). Mini-review drug/cyclodextrin/hydroxy acid multicomponent systems. Properties and pharmaceutical applications,. *Journal of Pharmaceutical Sciences*, *89*(1), 1–8.

Ribeiro, A., Figueiras, A., Santos, D., & Veiga, F. (2008). Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-β-cyclodextrin. *AAPS Pharmaceutical Science Technology*, *9*(4), 1102–1109.

Snyder, H. E., & Lechuga-Ballesteros, D. (2008). Spray drying: theory and pharmaceutical applications. In L. L. Augsburger, & S. W. Hoag (Eds.), *Pharmaceutical Dosage Forms: Tablets, Volume 1, Unit Operations and Mechanical Properties* (pp. 227–256). New York, USA: Informa Healthcare USA Inc.

Stewart, J. (1990). MOPAC: A semiempirical molecular orbital program. *Journal of Computer-Aided Molecular Design*, *4*, 1–105.

Stigliani, J.-L., Bernardes-Genisson, V., Bernadou, J., & Pratviel, G. (2012). Cross-docking study of InhA inhibitors: A combination of Autodock Vina and PM6-DH2 simulations to retrieve bio-active conformations. *Organic and Biomolecular Chemistry*, *10*, 6341–6349.

Szejtli, J. (2004). Past, present, and future of cyclodextrin research. *Pure and Applied Chemistry*, *76*(10), 1825–1845.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function efficient optimization and multithreading. *Journal of Computational Chemistry*, *31*, 455–461.

Wang, D.-W., Ouyang, C.-B., Liu, Q., Yuan, H.-L., & Liu, X.-H. (2013). Inclusion of quinestrol and 2,6-di-O-methyl-beta-cyclodextrin: Preparation, characterization, and inclusion mode. *Carbohydrate Polymers*, *93*(2), 753–760.

Xiang-Gen, W., Li-Na, Y., Meng, X., & Hao-Ran, J. (2011). Anti-infectious activity of intravitreal injectable voriconazole microspheres on experimental rabbit fungal endophthalmitis caused by *Aspergillus fumigatus*. *Journal of Pharmaceutical Sciences*, *100*(5), 1745–1759.

Yang, W., Chow, K. T., Lang, B., Wiederhold, N. P., Johnston, K. P., & Williams, R. O., 3rd. (2010). In vitro characterization and pharmacokinetics in mice following pulmonary delivery of itraconazole as cyclodextrin solubilized solution. *European Journal of Pharmaceutical Sciences*, *39*, 336–347.

Yuan, C., Jin, Z., & Xu, X. (2012). Inclusion complex of astaxanthin with hydroxypropyl-beta-cyclodextrin: UV, FTIR 1H NMR and molecular modeling studies. *Carbohydrate Polymers*, *89*, 492–496.