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ORIGINAL ARTICLE

Solid state characterization of the inclusion complex of valsartan with methyl β-cyclodextrin

Nalawade Pravin · Aware Babasaheb · Dand Neha · Kadam Vilasrao · Hirlekar Rajashree

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Abstract In this work, we illustrate the usefulness of cyclodextrins, namely, methyl-β-cyclodextrin (MβCD), an amorphous, methylated derivative of the natural β-cyclodextrin, as a tool to form an inclusion complex with Valsartan (VAL), a poorly water soluble drug. The phase solubility study showed A_L type of curve with slope less than one indicating the formation of complexes in 1:1 molar ratio of drug and CD. The stability constant was found to be 538.14 ± 5.4 $Mole^{-1}$. Solid binary systems between VAL and MβCD were prepared experimentally in a stoichiometry 1:1 by different techniques (physical mixing, kneading, co-evaporation). Afterward these products were characterized by Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM) and ^{1}H Nuclear magnetic resonance study (^{1}H NMR). The results obtained suggested that co-evaporation methods yield a higher degree of amorphous entities suggesting the formation of inclusion complex between VAL and MβCD. The dissolution of VAL from the binary systems was studied to select the most appropriate system for the formulation development. It was concluded that the preparation technique played an important role in the dissolution behavior of the drug and the inclusion complex between VAL and MβCD obtained by co-evaporation method allowed better performance.

Keywords Methyl β-cyclodextrin · Valsartan · Phase solubility studies · Inclusion complexes · Kneading · Co-evaporation · Release rate

N. Pravin · A. Babasaheb · D. Neha · K. Vilasrao · H. Rajashree (✉)
Department of Pharmaceutics, Bharati Vidyapeeth's College of Pharmacy, CBD Belapur, Sector-8, Navi Mumbai 400614, India
e-mail: rshirlekar@redifmail.com

Introduction

Drug solubility and stability are two very important factors with respect to drug administration and drug delivery. In recent years, to overcome the drugs stability and solubility limitations, several approaches have been investigated. Cyclodextrins (CDs) have been extensively used as complexing agents to improve solubility and stability of a variety of poorly soluble and labile drugs. Cyclodextrins are cyclic organic compounds obtained by enzymatic transformation of starch. Among this class of "host" molecules, the β-cyclodextrin (βCD) is one of the most abundant natural oligomers and corresponds to the association of seven glucose units exhibiting a cavity with a hydrophobic character whereas the exterior is strongly hydrophilic. This peculiar structure allows guest molecules to be included into the cavity via non-covalent bonds to form inclusion complexes [1].

Natural CDs have limited water solubility that negatively influences water solubility and the stability of the formed complex. To overcome this problem alkyl moiety, such as hydroxyalkyl or methyl, on free hydroxyl groups of βCD were introduced. The complexing ability of CD derivatives was significantly modified in respect to the parents. The potential use of natural cyclodextrins and their synthetic derivatives for improvement of the solubility, stability and/or bioavailability by formation of inclusion complexes with drugs has been extensively studied [2, 3].

Methyl-β-cyclodextrin (MβCD) offers a significant advantage, as a host molecule, over βCD since its solubility in aqueous solution at room temperature (>2,000 mg/mL) is significantly higher than the latter one (18.5 mg/mL). It is expected that the higher solubility of MβCD in aqueous solution will contribute to a higher solubility of the drug when in the complexed state [4].

Valsartan (VAL) is a potent, highly selective antagonist of angiotensin II AT1 receptor [5, 6] and lowers the blood pressure in hypertensive patients. According to the Biopharmaceutical Classification Scheme, VAL can be considered as a class II drug, a water insoluble, lipophilic and highly permeable compound. Cappello et al. have already demonstrated the ability of hydroxypropyl β-cyclodextrin (HP-βCD) to complex VAL to increase drug solubility and to overcome stability problems of VAL [7]. The purpose of this work was to investigate the ability of MβCD to include VAL which may result in better solubility.

The most widely used approach to study inclusion complexation is the phase solubility method described by Higuchi and Connors [8], which examines the effect of a solubilizer or ligand (cyclodextrin) on the drug being solubilized (the substrate). Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggest the formation of inclusion complexes with poor solubility [9].

Inclusion complexes between VAL and MβCD in solid state were prepared by kneading (KN) and coevaporation (CE) methods. Binary systems in solid state were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and ^{1}H nuclear magnetic resonance (^{1}H NMR).

Materials and methods

Materials

Valsartan (VAL) was kindly supplied by Torrent (India); CAVASOL® W7 M PHARMA (Methyl -β-cyclodextrin M.W. = 1,310 and D.S. = 1.8) was supplied by Wacker Fine Chemical Corporation (Germany). These chemicals were used as received without further purification.

All other chemicals and solvents were of analytical reagent grade. Double distilled water was used throughout the study.

Phase solubility studies

Phase solubility studies were carried out in deionised water at 25 °C according to the method reported by Higuchi and Connors [9]. An excess of VAL (50 mg) was added to 10 mL of distilled water containing increasing amounts of MβCD. The resulting suspensions were shaken for 48 h and then filtered through 0.45 μm Millipore filter, appropriately diluted and analyzed by UV–Vis spectrophotometer (SHIMADZU UV 1601) at 250 nm. The apparent stability constant K_s was calculated from the phase solubility diagram according to the Eq. 1.

$$Ks = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where, S_0 is the solubility of drug in absence of CDs.

Preparation of binary mixtures

All binary mixtures were prepared in 1:1 molar ratio between drug and MβCD on the basis of the results obtained from the preliminary phase solubility studies.

Physical mixtures (PM) were prepared by simple mixing, in a mortar with pestle for 10 min, the powders of both VAL and MβCD.

Kneaded (KN) product was obtained by adding small amounts of water to MβCD placed in mortar and mixing to obtain a homogenous paste. Then the saturated ethanolic solution of drug was added and the mixture was kneaded for 3–4 h. During the kneading process few drops of water were introduced to maintain a suitable consistency. The resulting mass was dried in an oven at 45 °C for 48 h and the solid was finally ground.

Co-evaporated (CE) product was obtained by adding alcoholic solution of VAL to aqueous solution of MβCD under sonication. The final solution was subjected to co-evaporation at 45 °C and the solid obtained was ground.

Characterization of VAL-CD complexes

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) analysis was performed on METTLER TOLLEDO—DSC-822^{e} (Japan) using 5 mg samples in covered aluminum pans under dry nitrogen purge (50 mL/min) at a heating rate of 10 °C/min over a temperature range of 30–200 °C.

Fourier transform infrared spectroscopy

Fourier Transform Infrared (FT-IR) spectra of VAL, PM and CE systems of VAL and MβCD were taken with Jasco-700 FTIR spectrophotometer (Shimadzu, Japan) using discs of each sample containing 0.01 g of sample in 0.1 g of potassium bromide between the wavelengths of 4,000–400 cm^{-1} at resolution of 2 cm^{-1}.

Scanning electron microscopy (SEM)

The surface morphology of VAL, MβCD and CE system of VAL with MβCD were visualized using Scanning Electron Microscope (JSM-5510, JEOL, USA). The samples were mounted on a brass stub using double sided tape and then

sputtered with a thin layer of gold. The photographs were taken at an acceleration voltage of 20 kV.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra of VAL, MβCD and CE systems were taken at 25 °C by a Bruker DPX Digital Nuclear Magnetic Resonance Model (USA) operating at a proton frequency 400 MHz using a 5 mm sample tubes. DMSO [2.5 ppm from tetramethylsilane (TMS)] was used as a solvent. Chemical shifts were expressed in ppm downfield from the signal (0 ppm) of TMS. The magnetic field remained stable with the deuterium field lock, being confirmed by negligible change in the signal frequency before and after each experiment.

In vitro dissolution studies

In vitro dissolution studies of VAL and VAL-MβCD solid systems were performed in 900 mL of dissolution medium, phosphate buffer pH 6.8 (USP) using a USP XXIII type 2 dissolution rate test apparatus (Model VDA-6DR, Veego Scientific, Mumbai, India). Amount contained 40 mg equivalent of VAL and a speed of 100 rpm at a temperature of 37 ± 0.5 °C were used in each test. A 5 mL aliquot was withdrawn at different time intervals and filtered using a 0.45 µm nylon disc filter; each sample was replaced with 5 mL of fresh dissolution medium. The filtered samples were suitably diluted, if necessary, and assayed by measuring the absorbance at 250 nm. The dissolution experiments were conducted in triplicate.

Results and discussion

Phase solubility studies

The phase solubility diagram of VAL with MβCD is shown in Fig. 1. Solubility of plain VAL was found to be 0.178 mg/mL as compared to 0.17 mg/mL reported by Marti et al. in United States Patent application publication with publication no. US 2003/0207930A1 dated 6th November 2003 and 0.085 mg/mL stated by Cappello et al. [7]. VAL solubility increased linearly with cyclodextrin concentration and the slope was smaller than unity, over the entire concentration range studied, indicating an A_L type diagram with the formation of a complex with 1:1 stoichiometry according to Higuchi and Connors. The estimated K_S of the inclusion complex was 538 ± 5.4 as compared to 296 ± 7 for the inclusion complex of VAL with HP-βCD as reported by Cappello et al. [7]. Thus it can be concluded that VAL formed more stable complexes

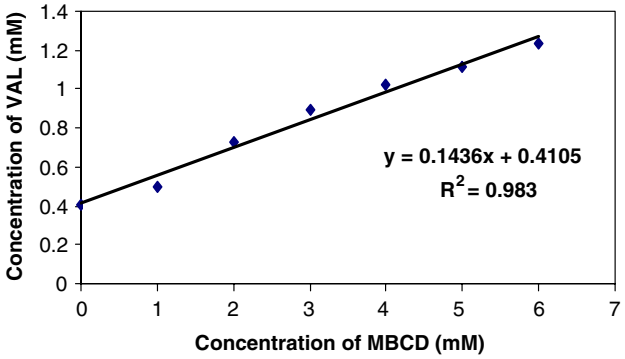

Fig. 1 Phase solubility curve for VAL:MβCD in distilled water at 37 ± 0.5 °C (n = 3)

with MβCD which may be due to the extension of the hydrophobic cavity without steric hindrance and provision of greater inclusion ability.

Differential scanning calorimetry

DSC analysis has been shown to be a very powerful analytical tool in the characterization of solid-state interactions between drugs and CDs and is a rapid analytical technique commonly used for evaluating drug–excipient interactions through the appearance, shift, or disappearance of endothermal or exothermal effects and/or variations in the relevant enthalpy values [10]. The thermal curves of pure components and binary systems are shown in Fig. 2. VAL was characterized by a single endothermic peak at 115 °C. The DSC curve of MβCD showed a broad endothermic

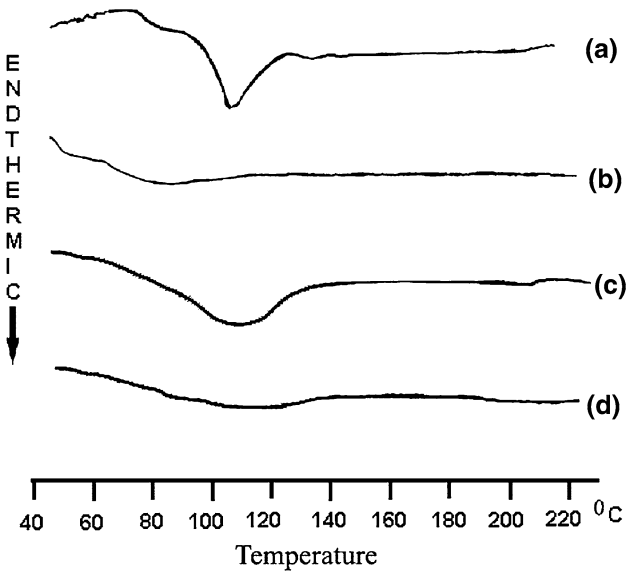

Fig. 2 Differential Scanning Images of (a) VAL, (b) MβCD, (c) physical mixture of VAL and MβCD, (d) inclusion complex of VAL with MβCD

effect around 40 °C and 100 °C associated with water loss [11]. In the PM system, the peak characteristic of the drug showed reduced intensity which disappeared in case of DSC curve of inclusion complex. However, the absence of this peak in the CE inclusion complex systems suggested the formation of an amorphous inclusion complex through molecular encapsulation of the VAL inside the MβCD cavity. The marked broadening and reduction in intensity of VAL endotherm when passing from physical mixture up to its disappearance in the co-evaporated complex, was indicative of complete drug amorphization and/or inclusion complexation [12].

Fourier transform infrared spectroscopy

The FTIR spectra of the inclusion complexes exhibited some significant differences (shifts, broadening or attenuation) in the characteristic bands revealing a modification of the drug environment. The characteristic bands of VAL were two carbonyl absorption bands at 1,733.89 and 1,602.74 cm^{-1} assigned to the carboxyl carbonyl and amide carbonyl stretching, respectively as seen in Fig. 3.

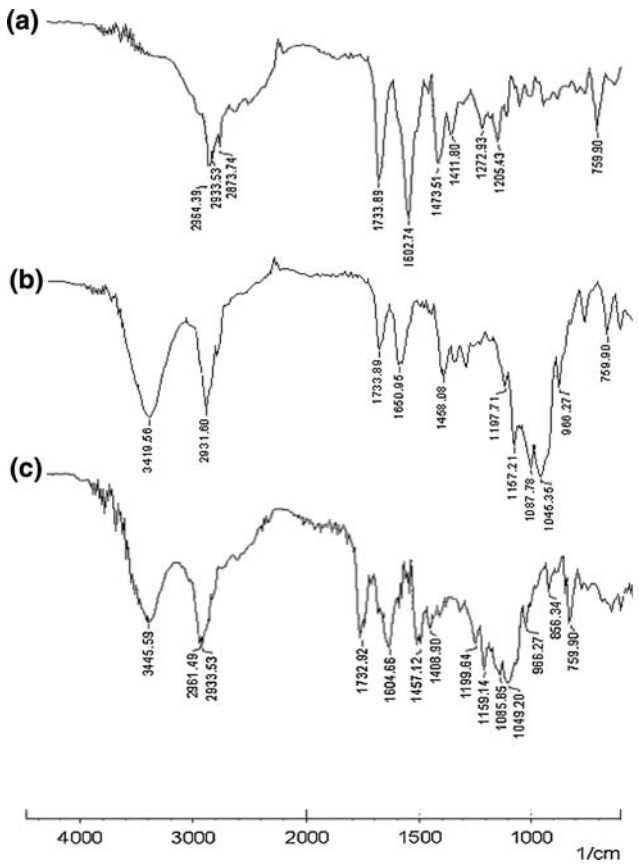

Fig. 3 FT-IR spectra of (a) VAL, (b) physical mixture of VAL and MβCD, (c) inclusion complex of VAL with MβCD

In case MβCD systems, the absorption band for carboxyl carbonyl group of pure drug was shifted to 1,732.92 cm^{-1} in the PM with MβCD, which was not changed in case of CE inclusion complex. The absorption band for amide carbonyl group was shifted to 1,604.66 cm^{-1} in case of physical mixture and to 1,650.95 cm^{-1} in case of CE inclusion complex. The shift in amide carbonyl band from 1,602.63 to 1,650.95 cm^{-1} in case of inclusion complexes with MβCD was within the range for amide group (1,695–1,600 cm^{-1}). It indicated that there were no chemical changes involved. This peak shifting towards lower frequency with change in intensity suggested change in the environment of the carbonyl group associated with amide moiety. The slight shifting of absorption band for the carbonyl group of amide to a lower frequency can be attributed to the breakdown of the intermolecular hydrogen bonds associated with crystalline drug molecule and the formation of hydrogen bonding of drug with MβCD [13].

Scanning electron microscopy

The morphology and particle size of VAL, MβCD, and CE binary systems were analyzed by SEM (Fig. 4). VAL appeared as irregular shape crystals. SEM of MβCD showed spherical particles, an observation similar to that made by Figueiras et al. [12]. Following inclusion complexation, MβCD showed loss of sphericity, smooth surface and reduced size of the particles. A drastic change in the morphology and shape of the drug particles was observed in the inclusion complex; it was no longer possible to differentiate the two components, drug and cyclodextrin. Hence changes in the particle shape and size, suggested an apparent interaction between drug and MβCD [14].

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

In the CD molecule, the hydrogen atoms H_3 and H_5 are located in the interior of the cavity and hydrogen atoms H_1, H_2, H_4 and H_6 are located on the outer surface of the cavity. When any guest molecule gets incorporated in the CD cavity, the hydrogen atoms located inside the cavity experience significant changes in the δ ppm values. But in case of association of guest molecule with CD, the hydrogens on the exterior surface show shifts in δ ppm values. In this convection, a positive sign of Δ ppm shows a downfield displacement and a negative sign shows an up field displacement. In case of MβCD CE complexes (Table 1), the shift in δ ppm values for H_1, H_2, H_3, and H_5 were 0.007, 0.052, 0.197 and −0.026 respectively. In the presence of the drug, an appreciably downfield shift for H_3 and upfield shift for H_5 was observed in the MβCD spectra,

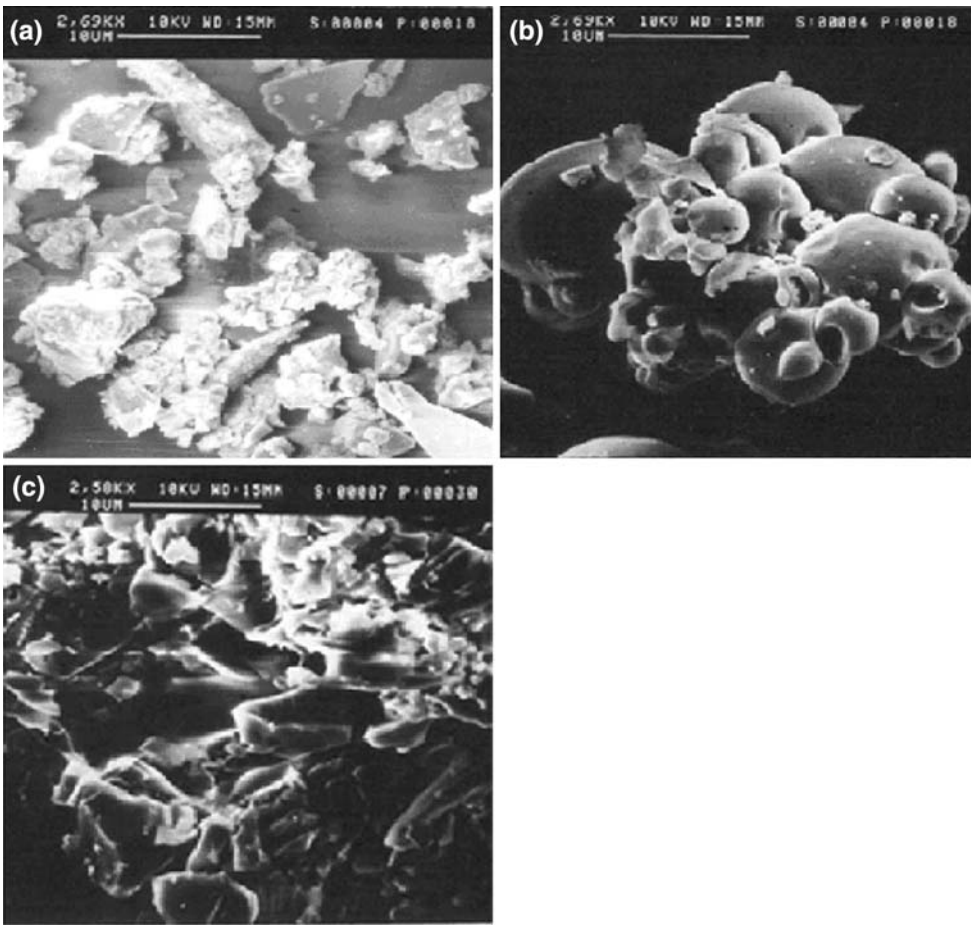

Fig. 4 Scanning electron images of **a** VAL, **b** MβCD, **c** inclusion complex of VAL with MβCD

Table 1 ^{1}H chemical shifts corresponding to MβCD in the absence and presence of VAL

Assignments	MβCD δ (ppm)	VAL:MβCD δ (ppm)	$\Delta\ \delta$ (ppm)	Shift
H1	5.069	5.076	0.007	Down field
H2	3.194	3.246	0.052	Down field
H3	3.491	3.688	0.197	Down field
H5	3.552	3.526	−0.026	Up field

$\Delta\delta = \delta_{(\text{complexed})} - \delta_{(\text{free})}$

which demonstrated a clear involvement of the protons in host–guest interaction. Since both H_3 and H_5 protons are located in the interior of the MβCD cavity, and H_3 protons are near the wide side while H_5 protons are near the narrow side, the shifts in H_3 and H_5 values suggested that either the entrance of the drug was made by the narrow side of the cavity or wide side of the cavity. In the ^{1}H NMR spectra of the CE inclusion complex of VAL with MβCD, the magnitude of peak was lowered as compared to the magnitude of peak for free drug, which also indicated that there were changes in the environment of hydrogen of drug molecule. This suggested formation of complex between VAL and MβCD, and this interaction may be due to hydrogen bonding between drug and CDs [15].

In vitro dissolution study

In dissolution study, plain VAL showed 17.50% release at the end of 5 min with complete release by the end of 4 h. As seen from Fig. 5, PM demonstrated higher dissolution profile than plain VAL. The KN and CE systems showed still higher amount of VAL dissolved than PM. The CE system of VAL-MβCD showed better VAL release than that reported for VAL-HPβCD by Cappello et al. [7]. Kang et al. in their study of complexation of campothesin with modified CDs found that randomly substituted MβCD had a significantly higher solubilising effect compared to other CDs [16]. Similar results have also been reported by Castillo et al. [17] for Albendazole and by Loftsson et al. [18] for ETH-615.

The relative parameters of the dissolution process, i.e., the percentage of VAL dissolved in 5 min (DP) in the absence or in the presence of MβCD (PM, KN and CE systems), and the relative dissolution rate (RDR) of the PM, KN and CE samples calculated at $t = 5$ min are

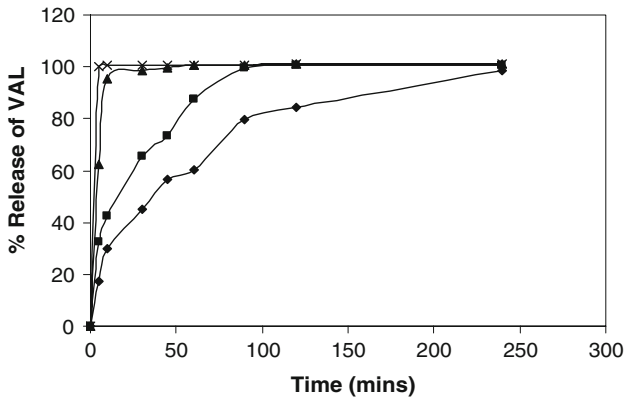

Fig. 5 Dissolution curves of VAL (*filled diamond*), physical mixture of VAL:MβCD (*filled square*), kneading mixture of VAL:MβCD (*filled triangle*), co-evaporation mixture of VAL:MβCD (×) in phosphate buffer pH 6.8 at 37 ± 0.5 °C ($n = 6$)

Table 2 Percentage drug release (DP) and relative release rate (RDR) at $t = 5$ of VAL and its solid systems with MβCD (± indicates SD for the respective values)

Sample	Percent release (DP) at $t = 5$ min	Relative release rate (RDR) at $t = 5$ min
VAL	17.50 ± 1.6	1.4
PM	32.25 ± 0.7	2.58
KN	62.58 ± 0.4	5.00
CE	99.89 ± 0.9	7.99

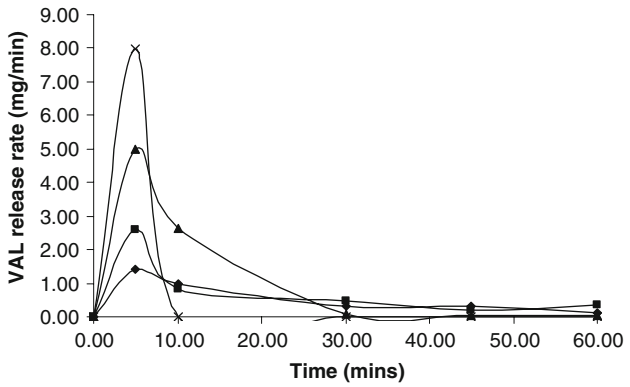

Fig. 6 Relative release rate of VAL (*filled diamond*), physical mixture of VAL:MβCD (*filled square*), kneading mixture of VAL:MβCD (*filled triangle*), co-evaporation mixture of VAL:MβCD (×) in phosphate buffer pH 6.8 at 37 ± 0.5 °C ($n = 6$)

shown in Table 2. The PM produced approximately a 2-fold increase of VAL dissolution rate (RDR 2.58). The kneaded complex (KN) produced 3.5 fold increase of VAL dissolution rate (RDR 5.00). The highest amount of solubilized drug was obtained with the CE sample (DP 99.89 ± 0.9%), that exhibited a 5.7 fold increase in dissolution rate (RDR 7.99). The graph of release rate against time is shown in Fig. 6.

The increase in VAL dissolved from the MβCD containing systems can be ascribed to formation of a readily soluble complex in the dissolution medium [7] and to their different drug–CD interaction degree occurring at solid state. Increased dissolution of VAL when physically mixed with MβCD may be due to an improved wettability of the drug particles at the early stages of the dissolution process [19]. The superior performances in dissolution testing exhibited by CE system may be attributed to several factors such as formation of soluble complexes, superior wettability, amorphisation, reduction of particle size [20] and presence of highly hydrophilic carriers such as MβCD [21].

Conclusion

Phase solubility profiles indicated that the solubility of VAL is significantly increased in the presence of MβCD. Information obtained from the DSC, FTIR, SEM, and ^{1}H NMR studies showed that VAL-MβCD inclusion complex can be prepared in 1:1 molar ratio by CE method. CE method enhanced the dissolution of VAL by 5.7 fold. Therefore a suitable dosage form incorporating this complex might show improvement in oral bioavailability of VAL.

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References

1. Dupuy, N., Barbry, D., Bria, M.S., Vrielynck, M.L., Kister, J.: ^{1}H NMR study of inclusion compounds of phenylurea derivatives in β-cyclodextrin. Spectrochim. Acta. [A] **61**, 1051–1057 (2005). doi:10.1016/j.saa.2004.04.031
2. Davis, M.E., Brewster, M.E.: Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. Drug Discov. **3**, 1023–1035 (2004). doi:10.1038/nrd1576
3. Martin Del Valle, E.M.: Cyclodextrins and their uses: a review. Process Biochem. **39**, 1033–1046 (2004). doi:10.1016/S0032-9592(03)00258-9
4. Hedges, A.R.: Industrial applications of cyclodextrins. Chem. Rev. **98**, 2035–2044 (1998). doi:10.1021/cr970014w
5. Criscione, L., De Gasparo, M., Buehlmayer, P., Whitebread, S., Ramjoue, H.P., Wood, J.M.: Pharmacological profile of valsartan: a potent orally active, nonpeptide antagonist of the angiotensin II AT1 receptor subtype. Br. J. Pharmacol. **110**, 761–766 (1993)
6. Dina, R., Jafari, M.: Angiotensin II—receptor antagonist: an overview. Am. J. Health Syst. Pharm. **57**, 1231–1240 (2000)
7. Cappello, B., Clelia, D.M., Iervolino, M., Ageness, M.: Improvement of solubility and stability of valsartan by hydroxypropyl beta cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. **54**, 289–294 (2005). doi:10.1007/s10847-005-9004-y

8. Higuchi, T., Connors, K.A.: Advances in Analytical Chemistry and Instrumentation, vol. 4, p. 117. Willey-Interscience, New York (1965)
9. Challa, R., Ahuja Ali, A.J., Khar, R.K.: Cyclodextrins in drug delivery: an updated review. AAPS. PharmSciTech. **6**, 329–357 (2005). doi:10.1208/pt060243
10. Verma, R.K., Garg, S.: Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extended release formulations. J. Pharm. Biomed. Anal. **35**, 449–458 (2004). doi:10.1016/j.jpba.2004.01.012
11. Figueiras, A., Carvalho, R.A., Ribeiro, L., Torres-Labandeira, J.J., Veiga, F.J.B.: Solid-state characterization and dissolution profiles of the inclusion complexes of omeprazole with native and chemically modified beta-cyclodextrin. Eur. J. Pharm. Biopharm. **67**, 531–539 (2007). doi:10.1016/j.ejpb.2007.03.005
12. Mura, P., Maestrelli, F., Cirri, M.: Ternary systems of naproxen with hydroxypropyl-betacyclodextrin and aminoacids. Int. J. Pharm. **260**, 293–302 (2003). doi:10.1016/S0378-5173(03)00265-5
13. Loftsson, T., Masson, M.: Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. **225**, 15–30 (2001). doi:10.1016/S0378-5173(01)00761-X
14. Ribeiro, A., Figueiras, A., Santos, D., Veiga, F.: Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-β-cyclodextrin. AAPS PharmSciTech. **9**(4), 1102–1109 (2008). doi:10.1208/s12249-008-9143-8
15. Catarina, M.F., Rui, A.C., Saul Pereira, D.C., Francisco, J.B.: Multimodal molecular encapsulation of nicardipine hydrochloride by β-cyclodextrin, hydroxypropyl-β-cyclodextrin and triacetyl β-cyclodextrin in solution. Structural studies by ^{1}H NMR and ROSEY experiments. Eur. J. Pharm. Sci. **18**, 285–296 (2003). doi:10.1016/S0928-0987(03)00025-3
16. Kang, J., Kumar, V., Yang, D., Chowdhury, P., Hohl, R.: Cyclodextrin complexation: influence on the solubility, stability, and cytotoxicity of camptothecin, an antineoplastic agent. Eur. J. Pharm. Sci. **15**, 163–170 (2002). doi:10.1016/S0928-0987(01)00214-7
17. Castillo, J., Palomo-Canales, J., Garcia, J., Lastres, J., Bolas, F., Torrado, J.: Preparation and characterization of albendazole–cyclodextrin complexes. Drug Dev. Ind. Pharm. **25**(12), 1241–1248 (1999). doi:10.1081/DDC-100102294
18. Loftsson, T., Petersen, P.: Cyclodextrin solubilization of ETH-615, a Zwitterionic drug. Drug Drug Dev. Ind. Pharm. **24**(4), 365–370 (1998). doi:10.3109/03639049809085632
19. Hassan, M.A., Suleiman, M.S., Najib, N.M.: Improvement of the in vitro dissolution characteristics of famotidine by inclusion in β-Cyclodextrins. Int. J. Pharm. **58**, 19–24 (1990). doi:10.1016/0378-5173(90)90282-9
20. Veiga, F., Fernandes, C., Maincent, P.: Influence of preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary system. Drug Dev. Ind. Pharm. **27**, 523–532 (2001). doi:10.1081/DDC-100105177
21. Zingone, G., Rubessa, F.: Preformulation study of the inclusion complex warfarin-β-cyclodextrin. Int. J. Pharm. **29**, 3–10 (2005). doi:10.1016/j.ijpharm.2004.11.013