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Original Article

SYNTHESIS AND EVALUATION OF OLMESARTAN MEDOXOMIL COMPLEX WITH SBE_7 β-CD FOR ENHANCED DISSOLUTION AND BIOAVAILABILITY

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ABSTRACT

Objective: Olmesartan is a BCS class II anti-hypertensive drug with limitations of low aqueous solubility and bioavailability. Present work was designed to use complexation as an approach to explore the ability of modified cyclodextrin such as sulphobutyl ether$_7$ β-cyclodextrin (SBE_7β-CD) to develop an inclusion complex with poorly soluble olmesartan medoxomil (OLM) for improving its dissolution rate and relative bioavailability.

Methods: Inclusion complexes were prepared by different techniques of physical mixing, kneading and lyophilisation. Prepared complexes were characterized by differential scanning calorimetry (DSC), powder x-ray diffractometry (X-RPD), proton nuclear magnetic spectroscopy (^{1}H NMR) and fourier transforms infrared spectroscopy (FT-IR). Molecular interaction and encapsulation in an inclusion complex at the molecular level were considered using docking chemistry.

Results: Phase solubility analysis revealed 13 fold increase in aqueous solubility with stability constant K_s=249 M^{-1}at 1:1 stoichiometry of complexation. DSC and XPRD confirmed the reduction of crystallinity in complexes with improved solubility. ^{1}H NMR and FT-IR studies depicted the interaction among functional groups with varied hydrogen shifts confirmed by molecular modelling. Dissolution studies of complexes have shown an improved dissolution rate when compared to plain OLM. Pharmacokinetic profile of OLM/SBE_7β-CD has shown a significant enhancement in the relative bioavailability.

Conclusion: SBE_7β-CD may be successfully used as a carrier for the oral administration of OLM with enhanced bioavailability subjected to future scale up.

Keywords: Complexation, Molecular modelling, Relative bioavailability, Olmesartan medoxomil, Sulphobutyl ether$_7$ β-cyclodextrin.

INTRODUCTION

Olmesartan medoxomil (OLM) chemically (5-methyl-(2-Oxo-1,3-dioxol-4-yl)methyl-4-(1-hydroxy-1-methyl ethyl)-2-propyl-1-[2′(1H) -tetrazole-5yl)1,1′biphenyl-(4-yl) methyl]-1H-imidazole-5-carboxylate) is an ester prodrug that is quickly and fully hydrolyzed to the active metabolite olmesartan by both arylesterase and albumin during gastrointestinal absorption [1] as shown in fig. 1a.

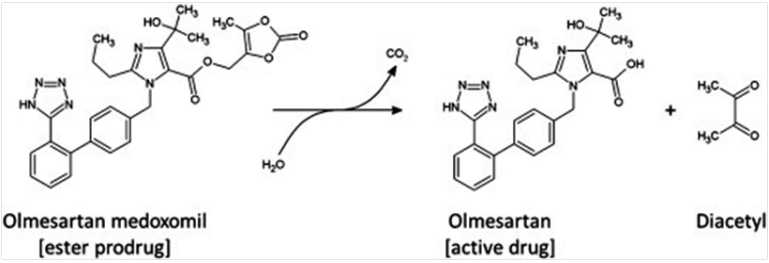


Fig. 1a: Conversion of OLM to its active metabolite OL

OLM is a weak base (pKa =4.3) with high molecular weight of 558.59 [1, 2] highly lipophilic and classified as class II drug according to the biopharmaceutical classification system (BCS) where its dissolution is the rate-limiting step for absorption. The drug has an oral bioavailability of about 29% only [3, 4]. It is an angiotensin II receptor blocker that selectively blocks the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. It was approved by the US Food and Drug Administration (FDA) in 2002 for the treatment of hypertension either singly or in combination [5].

Various approaches such as self-micro emulsifying drug delivery systems (SMEDDS) and lipid-based formulations have been investigated for enhancing the oral bioavailability of OLM [6]. Recent reports highlighted the cardioprotective and the nephro protective effects of OLM [7-9]. Considering the potential of protective effects shown in recent studies and dissolution related problems associated with OLM utility a strategy utilizing cyclodextrin complexation for improving the solubility and relative bioavailability of OLM has been explored for the first time. Of all the cyclodextrin derivatives SBE_7β-CD was proved to be a very good solubilizer for the drugs with high molecular weight [10]. Aqueous solubility of SBE_7β-CD (70 g/100 ml at 25°C) is appreciably higher than β-cyclodextrin (1.85 g/100 ml at 25°C) [11]. SBE_7β-CD is a chemically modified β-CD commercially available as Captisol® and is cyclic hydrophilic oligosaccharide with the negative charge in aqueous media as shown in fig. 2.

Furthermore, SBE_7β-CD does not exhibit the nephrotoxicity and cytotoxic effects on intestinal epithelial Caco-2 cells like β-

cyclodextrin [12]. Thus selected complexing agent was considered to be very safe for oral administration [3, 13] and also found to be superior solubilizer compared to β-CD because of its greater electronic interactions with drug [14, 15]. Considering the above favourable aspects in terms of solubility, electronic interactions and safety modified cyclodextrin was chosen for further studies.

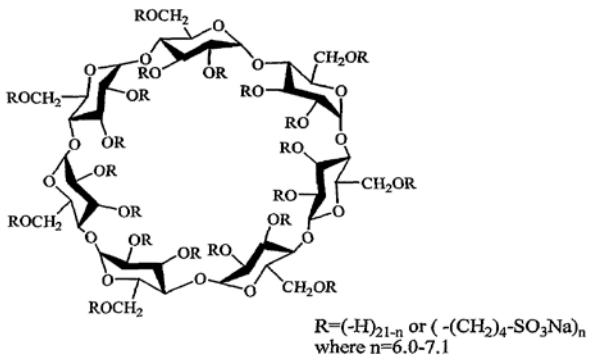


Fig. 2: Structure of sulfobutyl ether$_7$β-cyclodextrin

In the present investigation, we studied the ability of selected cyclodextrin to form an inclusion complex with OLM to enhance the solubility, dissolution rate and relative bioavailability of OLM. The OLM/SBE$_7$β-CD complex formation was confirmed and characterized by various techniques like Differential Scanning Calorimetry (DSC), X-Ray Powder Diffraction (X-RPD), Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) and Fourier Transform Infra Red Spectroscopy (FT-IR). Furthermore, we have also studied different modes of interactions. The superior efficacy of the OLM/SBE$_7$β-CD complexes with respect to *in vitro* dissolution rate and *in vivo* oral bioavailability has been successfully demonstrated in comparisons to pure OLM.

MATERIALS AND METHODS

Materials

OLM form B (Benicar) was kindly supplied by Daiichi Sankyo research Centre in India, Gurgaon. Captisol® (Sulphobutyl Ether$_7$β-Cyclodextrin) was purchased from CyDex pharmaceuticals (USA). Milli-Q water was used throughout the study and solvents methanol, acetonitrile (J. T. Baker, USA) used were of analytical grade.

Animals

Young adult male Wistar rats weighing 200-220 g were procured from the animal house Ranbaxy (Gurgaon, India). Rats were housed in properly ventilated cages at room temperature (24±2^{o}C) and 40-60% relative humidity while on a regular 12 h light-dark cycle. The animals were acclimatized for a period of three days prior to the start of experimentation. The animal protocol to carry out the *in vivo* study was reviewed and permitted by the Institutional Animal Ethics Committee, Ranbaxy-RCI (approval no: DS2012/011) and their guidelines were followed throughout the study.

Preliminary phase solubility studies

A phase solubility study was carried out in triplicates in distilled water at 25ºC to investigate the effect of SBE$_7$β-CD on the solubility of OLM [16]. An excess amount of drug was added to 2 ml aqueous solution containing different molar concentrations of SBE$_7$β-CD (average molecular weight 2,163) in increasing amounts from 0-80 mM and kept on shaker water bath (Gas-col® test tube shaker, USA) for 72 h at 25 °C. After equilibrium, the solutions were centrifuged (eppendorf centrifuge) at 3100 rpm for 15 min and filtered using 0.45 µm, 25 mm PTFE (poly tetra fluoro ethylene) filter and diluted suitably with distilled water to determine the concentration of OLM using waters acquity ultra-pressure liquid chromatography (UPLC) system with a photodiode array detector at 256 nm. Chromatographic analysis was done using sunniest C-18HT column (2 µm, 50×2.1 mm) with a gradient flow program of two mobile phases consisting of water: acetonitrile (95:5, v/v) with 0.05 % formic acid (A) and acetonitrile with 0.05 % formic acid (B) at a flow rate of 0.7 ml/min. The solubility phase profile of OLM was plotted for the concentration (mM) of drug and complexing agent and apparent stability constant K_s of the complex was determined from the graph using equation given by Higuchi and Connors (1965).

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

Where, the slope was obtained from the graph and S_o was the equilibrium solubility of OLM in water without cyclodextrin.

Jobs plot of continuous variation

Jobs plot of continuous variation method [17] was used to determine the stoichiometry of the binding event between drug and complexing agents. Stock solutions of 100 µM OLM and SBE$_7$β-CD in distilled water were prepared. The samples were prepared by mixing different volumes of OLM and complexing agent in such a way that a mole fraction of OLM and CD were in the range of 0-1. The peak area difference between the samples containing drug with and without SBE$_7$β-CD were measured using UPLC. Complex stoichiometry was determined from the plot of the peak area difference versus the mole fraction of SBE$_7$β-CD.

Preparation of OLM/SBE$_7$β-CD physical mixture and inclusion complexes

The inclusion complexes of OLM with SBE$_7$β-CD in 1:1 ratio were prepared by three different methods

Physical mixture (PM)

The physical mixture was prepared by simple mixing of equimolar quantities of OLM and SBE$_7$β-CD in a mortar pestle for 10 min. The prepared mixture was passed through BSS 120 sieve mesh for uniform size and kept in the desiccator until further analysis performed.

Kneading method

Equimolar quantities of OLM and SBE$_7$β-CD were added separately to 10 ml of methanol and mixed thoroughly. Prepared drug solution was slowly added to methanolic SBE$_7$β-CD under trituration and the mixture was further kneaded for 3–4 h until evaporation of methanol. During the process of kneading few drops of water was added to maintain appropriate consistency of the mass. The resultant mass was dried in an oven (Shel Lab Oven, USA) at 45^{o}C for 48 h. The dried kneaded product (KP) was finally grounded and stored as discussed for physical mixture [18].

Lyophilisation method

Equimolar quantities of OLM and SBE$_7$β-CD were added to 10 mlof methanol and water respectively. Both solutions were mixed, and the resultant solution was frozen in a deep freezer (New Brunswick Scientific, Germany) at-70 ^{o}C for about 1 h. The frozen mixture was further freeze-dried in the freeze dryer (Heto dry winner with rotary vane pump, Germany) at-110 °C to-120 ^{o}C under vacuum for 8 h. The resultant lyophilization product (LP) was sieved as discussed earlier and stored accordingly [19, 20].

Characterization of the inclusion complexes of OLM in solid state

Differential scanning calorimetry (DSC)

The thermal properties of OLM, SBE$_7$β-CD, PM and inclusion complexes (KP and LP) were investigated using a DSC (Shimadzu QA-100 Thermal Analyzer) calibrated with indium. 3 mg of respective sample (plain OLM, SBE$_7$β-CD, PM and inclusion complexes) were accurately weighed into aluminium pans, sealed and scanned at a rate of 10 ^{o}C/min between 40 - 250 ^{o}C in nitrogen atmosphere applied at 40 ml/min.

X-Ray powder diffraction analysis (X-RPD)

The crystalline or amorphous state of complexes was studied by X-RPD. The X-RPD patterns of plain OLM, SBE$_7$β-CD, PM and inclusion

complexes (KP and LP) were recorded on an analytical Xpert powder x-ray diffractometer (Philips P) using Ni-filtered, CuKα radiation wavelength of 1.5405 A° was used as x-ray source over the diffraction angle range (2θ) of 5–40 ° at 1 °/min with voltage of 40 kV and 25 mA current.

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

The ^{1}H-NMR spectra of plain OLM, PM, inclusion complexes (KP and LP)and SBE_7β-CDwere performed in DMSO and plain heavy water (D_2O) respectively on a Bruker ultra shield 400 MHz NMR [21]. The spectra were processed using topspin 2.0 software.

Fourier transforms infrared spectrophotometry (FT-IR)

Drug excipients interactions were analysed by FT-IR studies (Perkin-Elmer Model 1600). Spectra of OLM, SBE_7β-CD, PM and inclusion complexes (KP and LP) containing an equivalent amount of 2.0 mg OLM were recorded in the range of 4000–400 cm^{-1} using KBr discs.

Molecular modelling studies

Molecular docking studies of OLM with sulfobutyl ether7 β-cyclodextrin [SBE7β-CD] were carried using GLIDE module of Schrödinger suite 2013. Co-ordinates for sulfobutylether$_7$ β-cyclodextrin were generated by adding sulfobutyl ether group at 7thposition of β-cyclodextrin. Further, it has been prepared for docking by adding hydrogen and minimized. The 3D structure of OLM was sketched and minimized by OPLS-2005 molecular mechanics force field using Ligprep module of Schrödinger. A total of 10 conformations were generated. Grid was generated around sulfobutyl ether 7β-cyclodextrin with x: 19.7004; y: -8.0605; z: 0.2067 co-ordinates. Glide XP docking was performed. Molecular docking of OLM with β-cyclodextrin was also performed to compare the binding affinity with that of SBE_7β-CD [22].

***In vitro* dissolution profile of complexes**

The *in vitro* dissolution test of prepared complexes was performed in 0.1 M PBS buffer pH 6.8 (900 ml) using USP Type II apparatus (DISTEK dissolution system 4300) maintained at 37±0.5 °C, operated at the speed of 50±2 rpm. Plain OLM, PM and inclusion complexes (all equivalent to 20 mg of OLM) were weighed accurately and placed in the dissolution medium. Aliquots of 2 mlwere withdrawn at fixed time intervals from the dissolution medium and replaced with an equivalent amount of fresh media. The withdrawn samples were filtered through a 0.22 µm nylon filter and the amount of OLM released in the dissolution medium was determined by defining the peak area of the suitably diluted aliquot using UPLC.

Determination of dissolution parameters

Dissolution data was analyzed using Dissolution Efficiency (DE_{15}) at 15 min and Mean dissolution time (MDT) [23]. DE is the area under the dissolution curve up to a certain time 't' expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time. Representative equation is as follows:

$$DE = \frac{\int_0^t Y \times dt}{\int_0^t Y100\ t} (2)$$

It is preferable to choose a time interval corresponding to 70–90% dissolution unless one wishes to compare an early part of the dissolution curve. In the current study t_1=0 and t_2=60 min were chosen. Mean dissolution time is the arithmetic mean value of any dissolution data and is described as below:

$$MDT = \frac{\sum_{j=1}^{n} t\Delta M_j}{\sum_{j=1}^{n} \Delta M_j} (3)$$

Where j is the sample number, n is the number of dissolution sampling times, t is the time at the midpoint between t and t-1(calculated with $(t+t-1)/2$) and ΔM_j is the additional amount of drug dissolved between t and t-1.

***In vivo* relative bioavailability study**

In vivo relative bioavailability was carried out on 6-week-old male wistar rats weighing 200-220 gm. Plain OLM, PM and inclusion complexes were administered as a suspension using 0.5% methylcellulose via oral gavage at 5 mg/kg as OLM. Blood samples of about 0.3 mlwere collected using heparinized tubes at predetermined time intervals 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h and centrifuged at 12,000 rpm for 10 min. Plasma samples were stored at -20 °C until analysis.

Bioanalytical determination

The bioanalytical method was developed using UPLC to determine the concentrations of OLM in rat plasma. A 100 µl aliquot of plasma was transferred into a glass tube. A measured volume of 200 µl 1M HCl and 5 mlof extraction solvent tertiary butyl methyl ether (TBME) was added to this mixture. OLM was extracted from plasma after vortex mixing and centrifugation at 3000 rpm for 10 min. The supernatant was transferred into a glass tube and evaporated at 40 °C under a gentle stream of nitrogen gas. The residue was reconstituted with 100 µl of methanol and was injected into the UPLC system. The plasma concentrations were determined by using calibration equation obtained by least-squares linear regression over the range 50-2000 ng/ml in plasma. The limit of detection (LOD), the lower limit of quantification (LOQ), accuracy and precision were determined as per the limits according to FDA bio-analytical guidelines [24].

RESULTS AND DISCUSSION

Binary systems were prepared by different techniques such as PM, KM and LM.

Phase solubility studies

The effect of modified cyclodextrin on the aqueous solubility of a drug was evaluated using solubility phase method. The phase solubility diagram for OLM in sulphobutyl cyclodextrin complex is represented in Fig.3a. The aqueous solubility of the drug increased linearly as a function of SBE_7β-CD concentration with a correlation coefficient (r^2) of 0.998. Phase diagram can be assigned as A_L type according to Higuchi and Connors method.

Complex formation occurs at 1:1 stoichiometry ratio since slope was lower than 0.002. An increase in solubility of the drug from 0.0073±0.0003 mM (without cyclodextrin) to 0.0950±0.0022 (in 80 mM cyclodextrin) was obtained. The apparent stability constant (K_S) of OLM: SBE_7β-CD complex (1:1) was calculated as 249 $M^{-1}$from the linear plot of the phase-solubility diagram [25]. It was observed that SBE_7β-CD yielded a 13 fold increase in the aqueous solubility of OLM.

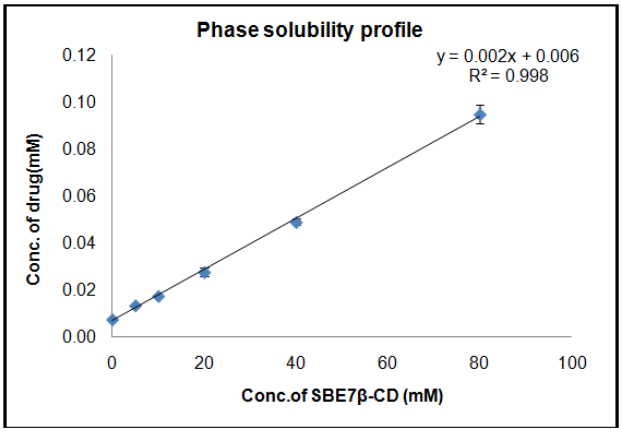


Fig. 3a: Phase solubility studies for olmesartan medoxomil (OLM) in the presence of sulphobutyl ether$_7$ β-cyclodextrin (SBE_7β-CD)

Jobs plot of continuous variation

The solubility of OLM with modified cyclodextrin was determined by continuous variation method. fig. 3b displays job plot of differences in peak area of the drug as a function of mole fraction of complexing agent. The jobs plot of OLM with SBE_7β-CD exhibit a max at R= 0.5 with high relative symmetry indicating the complex possess a peak corresponding to 1:1 stoichiometry (table 1).

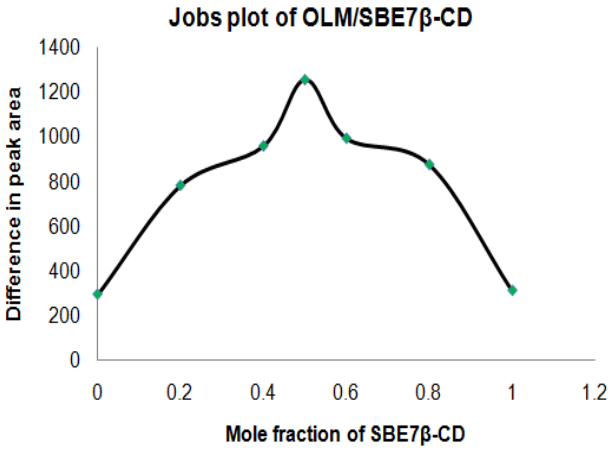


Fig. 3b: Jobs plot of OLM/SBE$_7$β-CD

Table 1: Jobs plot of continuous variation

S. No.	Mole fraction of SBE7 β-CD	Difference in the peak area of complexed and uncomplexed OLM
1	0	295
2	0.2	782
3	0.4	958
4	0.5	1254
5	0.6	992
6	0.8	875
7	1	312

Characterization of the inclusion complexes

Differential scanning calorimetry (DSC)

The DSC thermograms of OLM, PM, KP and LP are shown in fig. 4a-4e. The DSC of plain OLM and SBE$_7$β-CD has shown a sharp endothermic peak at 183.1 ^oC and 87.4^oC corresponding to the melting point of the drug.

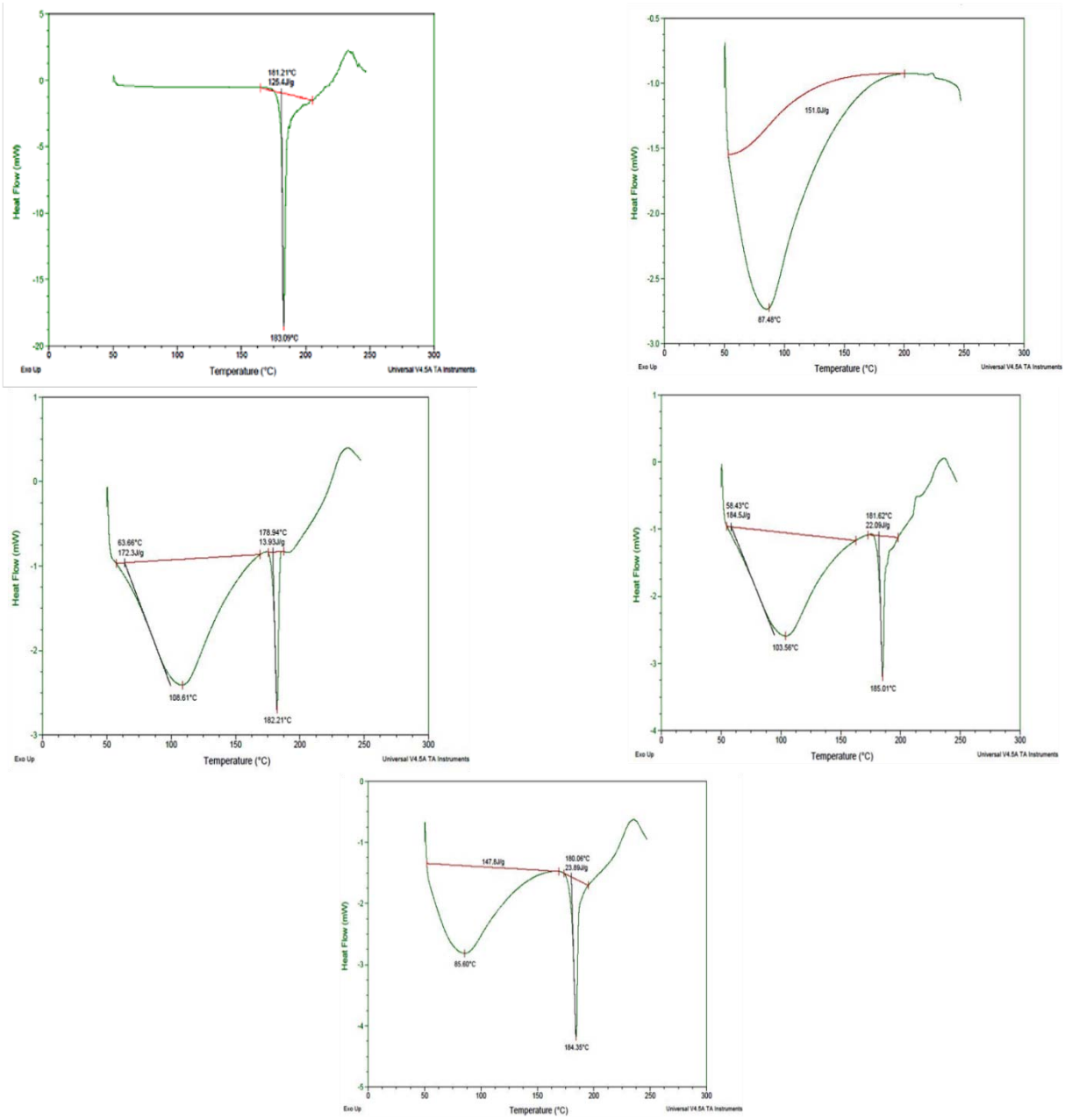


Fig. 4: DSC thermogram of a) olmesartan (OLM) b) sulphobutyl ether$_7$β-cyclodextrin c) physical mixture (PM), d) kneaded product (KP) and e) lyophilised product (LP)

The peak at SBE_7β-CD was due to the liberation of crystal water. A characteristic well recognizable peak of the drug corresponding to its melting point was slightly reduced in the physical mixtures of drug (endothermic peak at 182.2°C) but in other inclusion complexes, there was a slight increase in melting points of drug (185°C and 184.3°C) respectively.

The PM and inclusion complexes retained endothermic peaks of both SBE_7β-CD and OLM indicated that there was no chemical interaction, but the heat of fusion (J/gm), intensity and height of the endotherm was reduced re-presentated that complexation may result in reduced crystallization of OLM. Similarly, the endothermic peak of SBE_7β-CD at 87.5 °C was shifted to 108.6°C, 185.0°C and 85.6 °C for PM (partial complex), KP and LP respectively indicating the formation of stable complexes.

X-ray powder diffraction (X-RPD)

The physical state of the drug in inclusion complex was verified by another thermal technique X-RPD pattern along with DSC for confirmation. Diffraction patterns for drug and complexes LP are shown in fig. 5a-5e. The characteristic peaks of OLM were at 9 °, 12°, 14°, 16°, 18°, 19° and 21.2±0.2° on 2θ confirmed crystalline nature of drug while the absence of sharpness in peaks of cyclodextrins clearly revealed amorphous halo nature. Crystallinity was determined by comparing some representative peak height [OLM peak at 22.12° (2θ)] in the diffraction pattern of the binary systems with those of a reference. The relative degree of crystallinity (RDC) was defined according to the equation:

$$RDC = \frac{Isam}{Iref} \quad (4)$$

Where I_{sam} is the peak height of sample and I_{ref} is the peak height at the same angle for the reference with the highest intensity [26, 27].

The RDC values of corresponding binary systems of OLM/SBE_7β-CD were shown in table 2. All sharp peaks mainly at ~22 ° on 2θ were seen with a reduced intensity to a great extent of ~2000 in PM, ~1500 in KP and ~1000 in LP and were diffused which indicated decreased crystalline nature of OLM in all cases. Most importantly the significant peaks of OLM at 12 ° (~2200), 19 ° (~3500) and 24 °(~4,050) on 2θ were absent in the X-RPD of the KP and LP inclusion complexes. The results of the X-RPD studies indicated that drug had formed an inclusion complex with a simultaneous reduction in the crystallinity in order of OLM>PM>KP>LP.

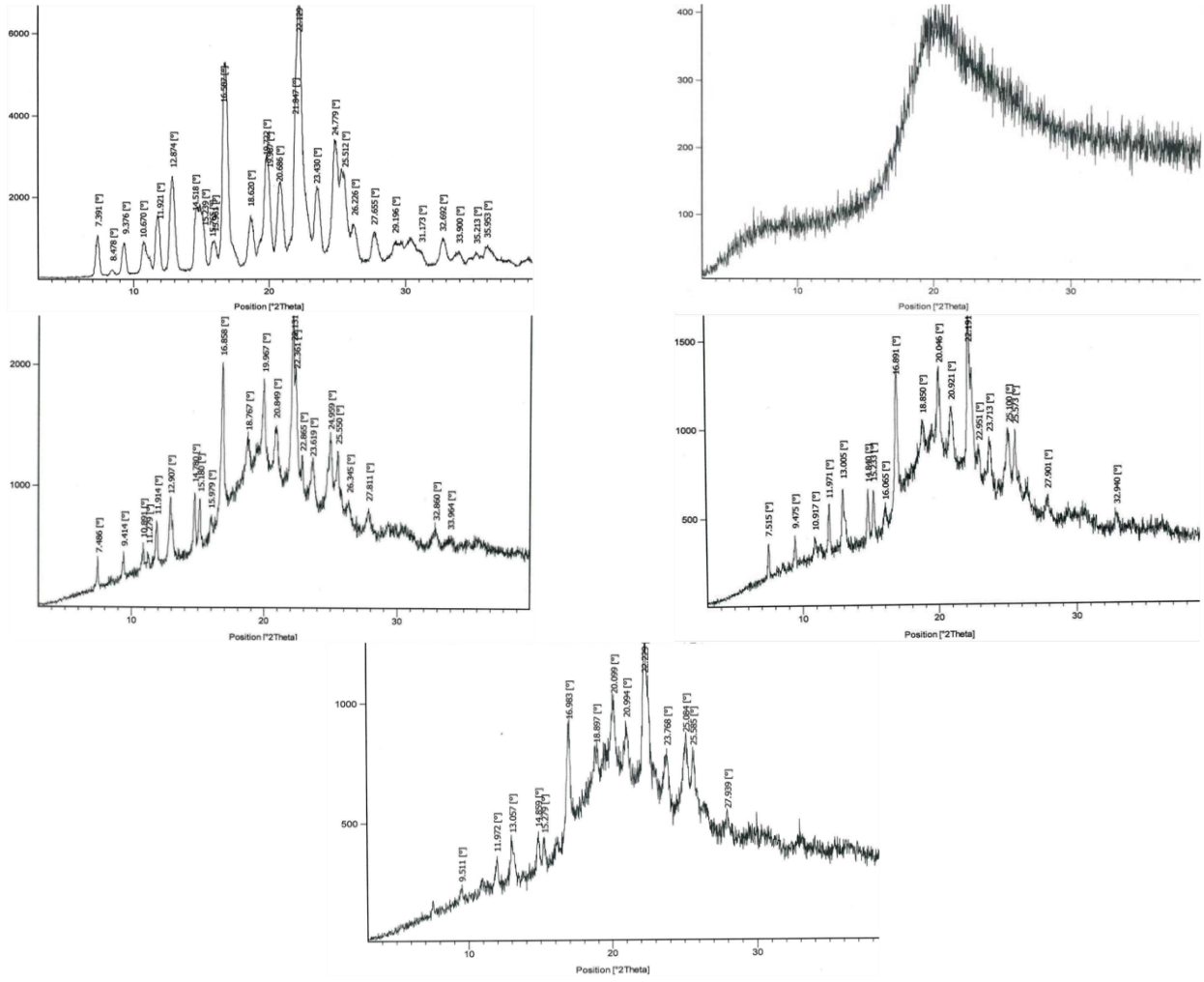


Fig. 5: XRD pattern of a) olmesartan (OLM) b) sulphobutyl Ether$_7$β-cyclodextrin c) physical mixture (PM), d) kneaded product (KP) and e) lyophilised product (LP)

Table 2: Peak intensities and RDC values for OLM: SBE_7 β-CD complexes

Parameters	OLM	PM	KP	LP
2θ	6658	2035	1243	903
RDC	1	0.305	0.186693	0.135626

Nuclear magnetic resonance studies (^{1}H-NMR)

^{1}H-NMR can provide evidence of the inclusion of drug molecule within the cavity of SBE$_7$β-CD. In general, if a guest or drug molecule is located within CD cavity inner hydrogen and OH groups are shielded by the drug while outer groups are unaffected by complex formation. The interaction among groups can be seen by chemical shifts in drug molecule. The ^{1}H-NMR spectra of OLM and its complexes were recorded to study closely the interaction of the drug with cyclodextrin. The ^{1}H-NMR spectra of OLM and SBE$_7$β-CD are shown in fig. 6a-6b. The chemical shifts observed for OLM and SBE$_7$β-CD were given below: ^{1}H-NMR ($CDCl_3$) δ: 7.81(dd, 1H), 7.52-7.6(m, 3H), 7.05(d, 2H), 6.85(d, 2H), 5.42(s, 1H),5.05(s, 1H), 2.58(t, 3H), 2.07(s, 3H), 1.57-1.59(m, 2H), 1.47(s, 6H), 0.89(t,3H).

SBE$_7$β-CD ^{1}H-NMR (D2O): δ ppm 3.82 (H5 proton), 3.75(H6 proton), 3.66 (H1′ proton), 3.54 (H2 proton), 2.89 (H4′ proton), and 1.72 (H3′ H2′ protons) SBE$_7$β-CD ^{1}H-NMR (CD3OD/D2O): δ ppm 5.04 (H1 proton), 4.03(H3 proton), and 3.57 (H4 proton). fig. 6c-6e: Shows the ^{1}H-NMR spectra of the PM, KP and LP inclusion complexes

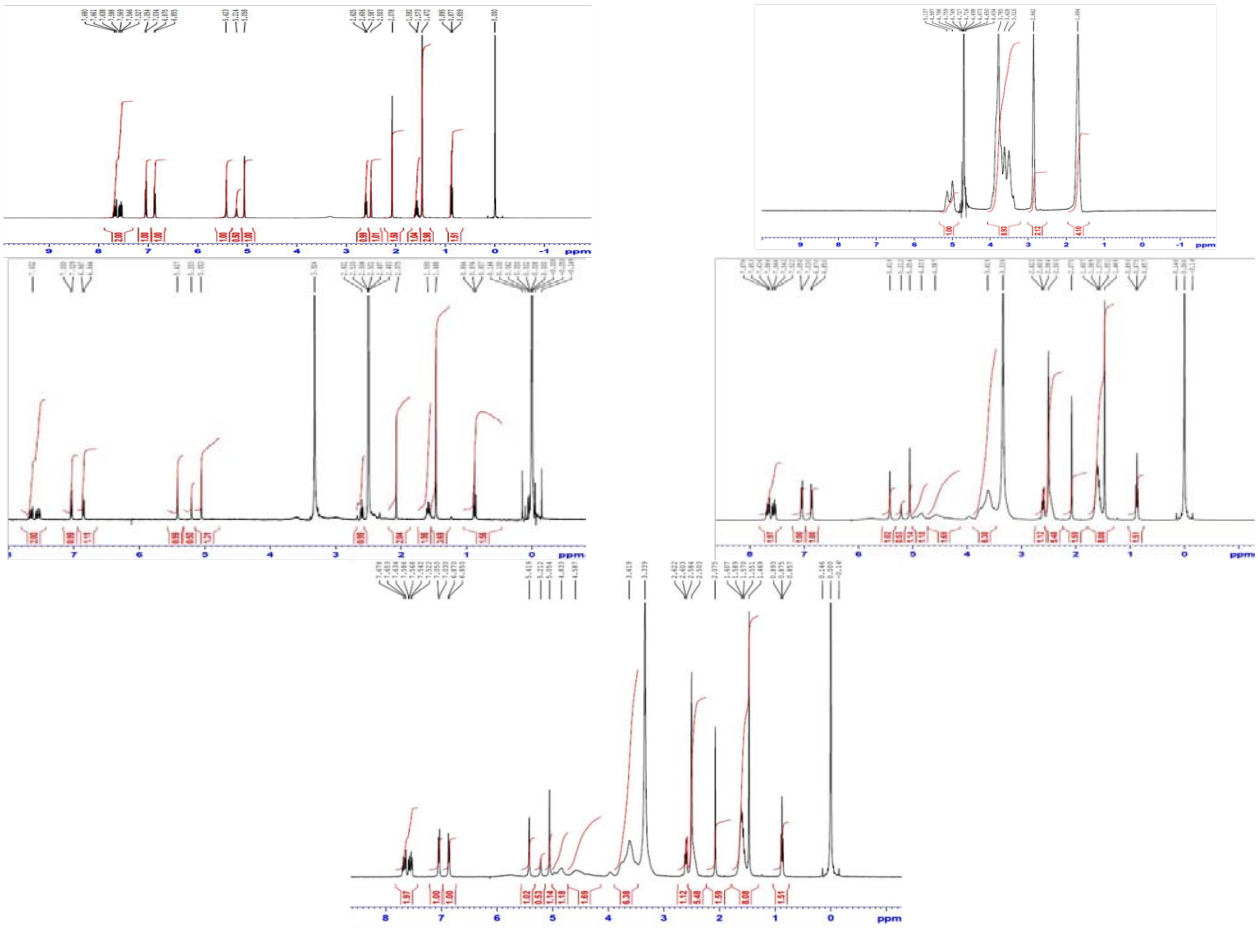


Fig. 6: NMR spectra of a) olmesartan medoxomil OLM, b) sulphobutyl Ether$_7$β-cyclodextrin (SBE$_7$β-CD), c) physical mixture (OLM/SBE$_7$β-CD), d) kneaded product and e) lyophilised product

The NMR spectra show characteristic protons of OLM and the protons in the cavity of SBE$_7$β-CD are distinct and can be used as probes to monitor the interaction between OLM and SBE$_7$β-CD. It is evident in fig. 6c-6e that there were eminent changes in the prototype of the NMR signals of the protons of aromatic benzyl groups of OLM for both the PM and inclusion complexes. In addition changes in the pattern of the NMR signals of the cavity protons of SBE$_7$β-CD hydroxyl groups were observed both for the PM and the inclusion complexes. These changes clearly indicated some form of interaction between SBE$_7$β-CD OH groups and OLM aromatic groups in both the PM as well as the inclusion complexes. However, due to difficulties in extracting accurate changes in the chemical shifts elaborate characteristics of the interaction cannot be inferred and the discussed interactions were further supported by the results from molecular modelling studies.

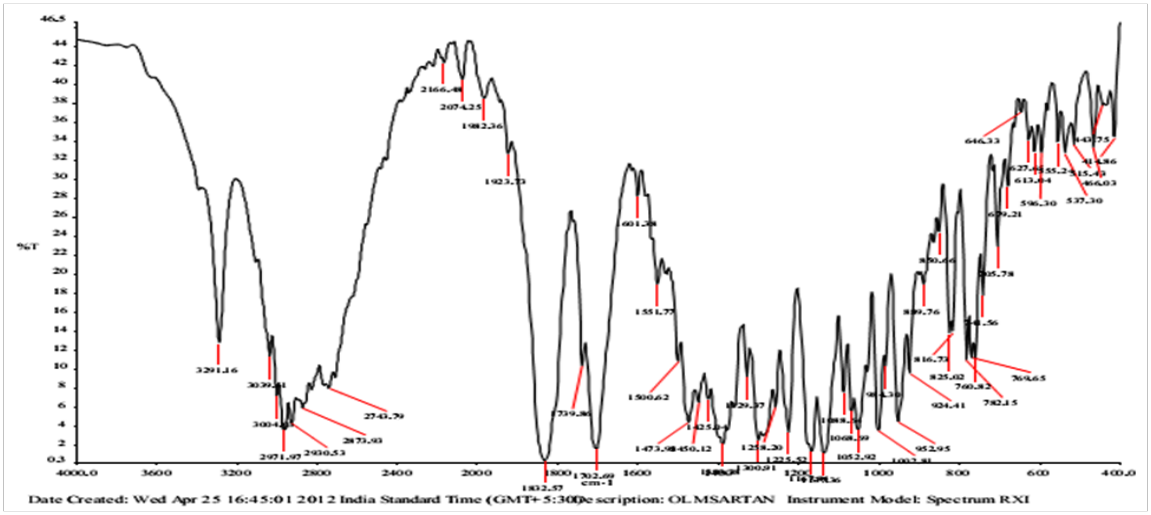
^{1}H-NMR results showed that the principal chemical shifts (δ) in plain OLM were at 7 ppm for aromatic hydrogens and for the plain SBE$_7$β-CD were at 4 ppm for either groups. These chemical shifts were absent in complexes indicating strong interactions.

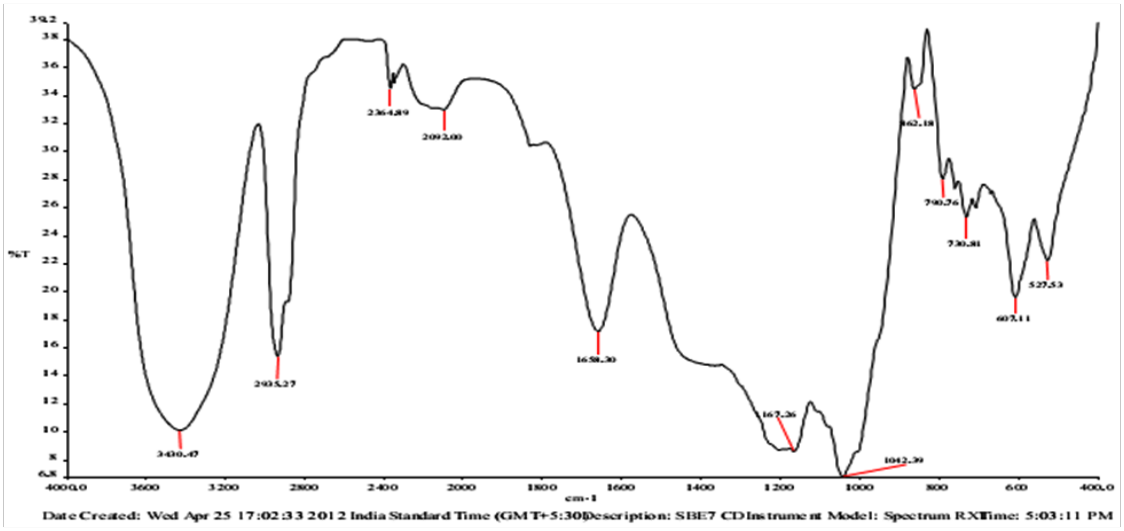
Fourier transforms infrared spectroscopy (FT-IR)

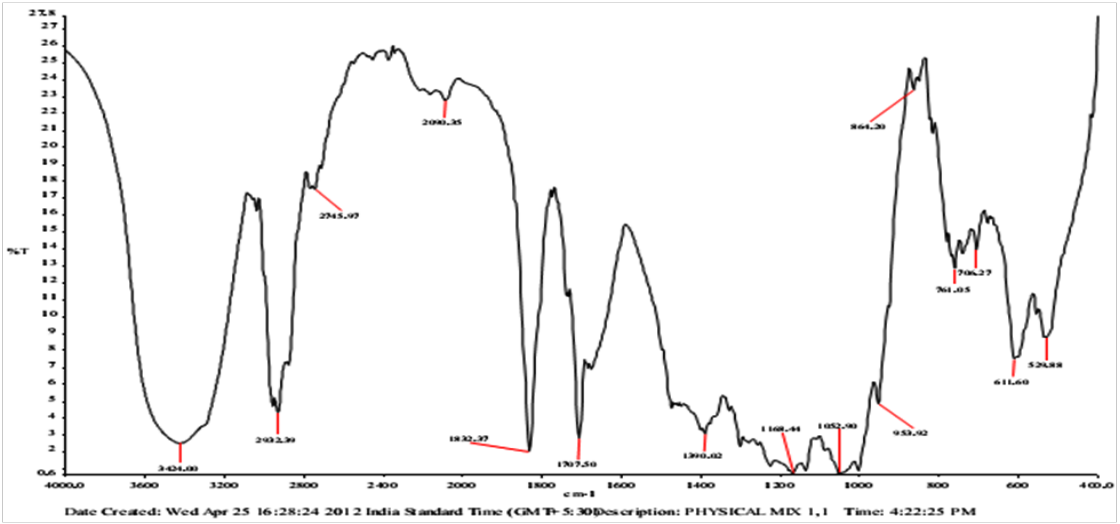
The FT-IR spectra of plain OLM, SBE$_7$β-CD, PM, KP and LP were shown in fig. 7a-7e.

The principal peak hydrogen stretching of the NH group, C-H of aromatic rings and aliphatic groups for plain OLM at 3291.16 cm^{-1}, 3000-3100 cm^{-1} and 2850-2960 cm^{-1} respectively got enclosed with the broad alcoholic OH stretch peak at 3430 cm^{-1} of SBE$_7$β-CD in the physical mixture and inclusion complexes. The principal peak for C=C bond stretching of aromatic groups at 1500-1600 cm^{-1} range of plain OLM was absent in PM and inclusion complexes indicating a strong interaction or bonding between OLM and SBE$_7$β-CD. The hydrogen of CH_3 group and aromatic C=O group stretching's in plain OLM, PM and inclusion complexes formed an umbrella deformation at 1380 cm^{-1} and 1832 cm^{-1}respectively were absent in plain SBE$_7$β-CD indicating that those groups were freely exposed. Along with this the specific C=O group stretching at 1707 cm^{-1} of plain OLM was seen in PM and other inclusion complexes indicating that part was not entrapped by SBE$_7$β-CD. But the C-O and C-N is stretching at 1260-1000 and 1340-1020 cm^{-1} range of plain OLM was not exposed

in PM and other inclusion complexes. These results suggested that both the complexes and the PM possess a strong interaction between SBE_7β-CD and OLM being more conspicuous in the complex. The enclosure of IR absorption bands of the hydrogen of OH, an aromatic ring and aliphatic groups, the C=C stretching of the aromatic group in OLM by the OH group of SBE_7β-CD were signposts of interaction between OLM and SBE_7β-CD. In the PM the interaction between OLM and SBE_7β-CD was of a random nature.







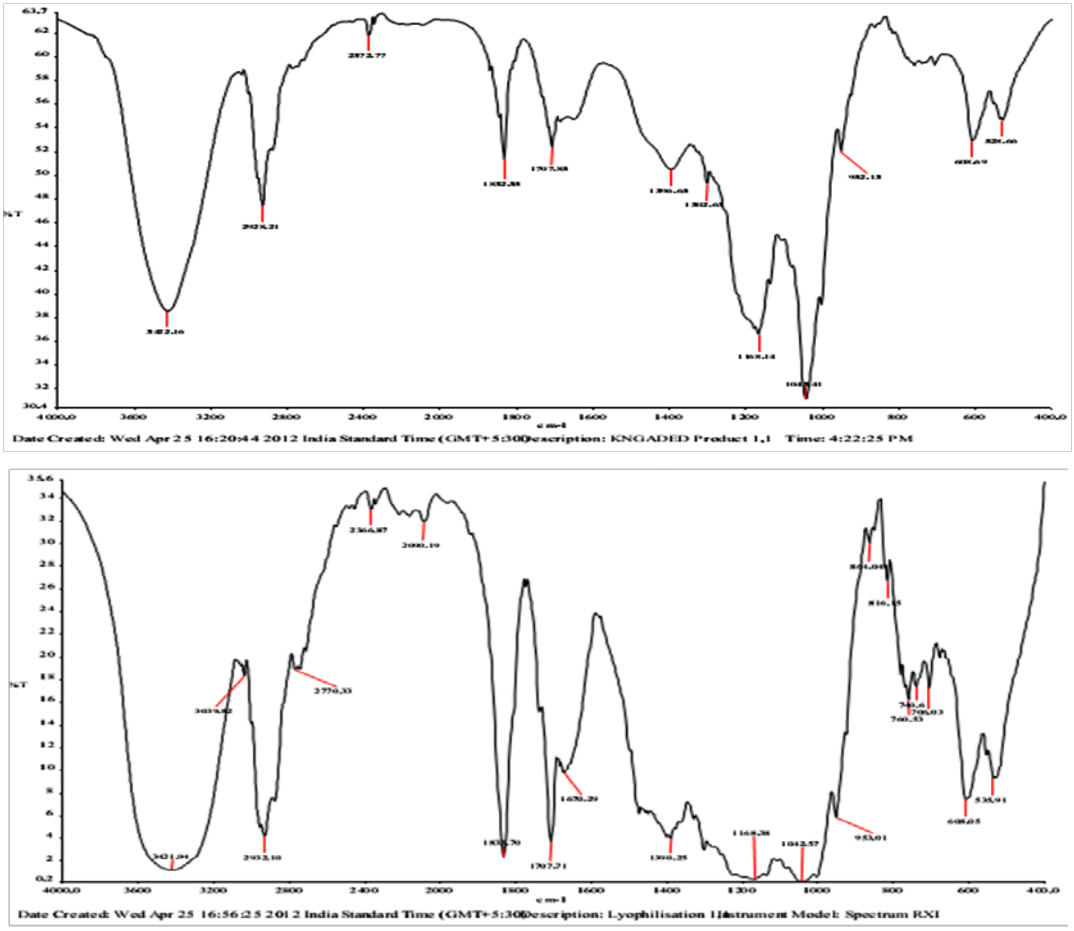


Fig. 7: FT-IR spectra of (a) olmesartan medoxomil OLM, (b) sulphobutyl ether$_7$β-cyclodextrin (SBE$_7$β-CD), (c) physical mixture (OLM/SBE$_7$β-CD), (d) kneaded product and (e) lyophilised product

Molecular modelling studies

In SBE$_7$β-CD randomly the OH groups in cyclodextrin were replaced with the sulphobutyl ether groups. As the actual substitution pattern and the configurations have not been revealed by the manufacturer in the present investigation three possible isomers of SBE$_7$β-CD were employed in the molecular modelling studies.

The results of the docking studies of OLM with the three different SBE$_7$β-CD isomers have been shown in fig. 8a-8c. It was evident that in three isomers that OLM was partially embedded in the SBE$_7$β-CD cavity ie. the aromatic rings lie within the SBE$_7$β-CD cavity. This clearly explained the change in the pattern of the IR and NMR signals of aromatic protons in the OLM/SBE$_7$β-CD complex. It was evident from fig. 8c that in third isomer OLM interacted with the sulphobutyl either arms of SBE$_7$β-CD.

In molecular docking, the intermolecular hydrogen bond between the tetrazole group of olmesartan and the OH of the glucose unit of SBE$_7$β-CD was shown in fig. 8a. In contrast, the complex with isomer 2 shows hydrogen bonding occurred between the NH of the tetrazole group of OLM and the 3-OH group of the glucose unit of SBE$_7$β-CD.

In isomer 1 hydrogen bond between carboxylic-OH groups and OH group of glucopyranose units of cyclodextrin was present, whereas in isomer 3 hydrogen bonds between imidazole ring of OLM and OH group of glucopyranose units of cyclodextrin was observed. After the molecular modelling studies of three isomers, we have noticed that the hydrophobic part of OLM was enclosed by the hydrophobic cavity of SBE$_7$β-CD which ultimately enhanced the solubility of OLM.

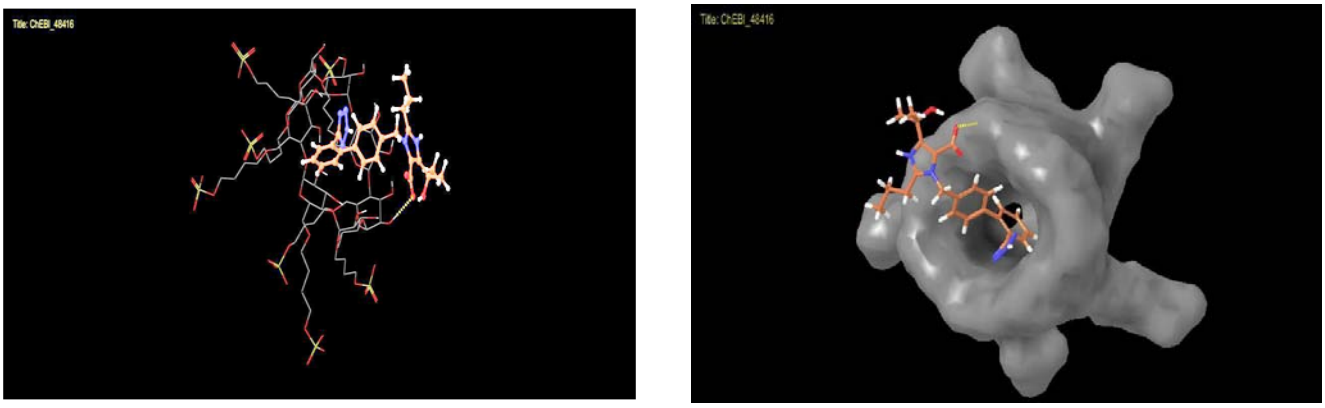


Fig. 8a: 3D structures of the complex between the olmesartan medoxomil and sulphobutyl ether$_7$β-cyclodextrin (SBE$_7$β-CD) isomer 1

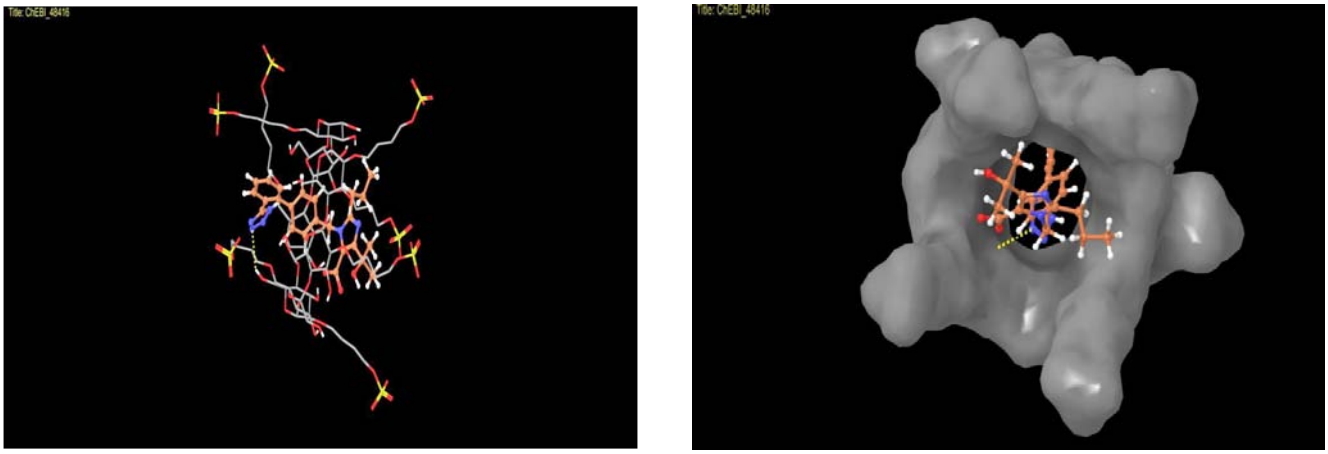


Fig. 8b: 3D structures of the complex between the olmesartan medoxomil and sulphobutyl ether$_7$β-cyclodextrin (SBE$_7$β-CD) isomer 2

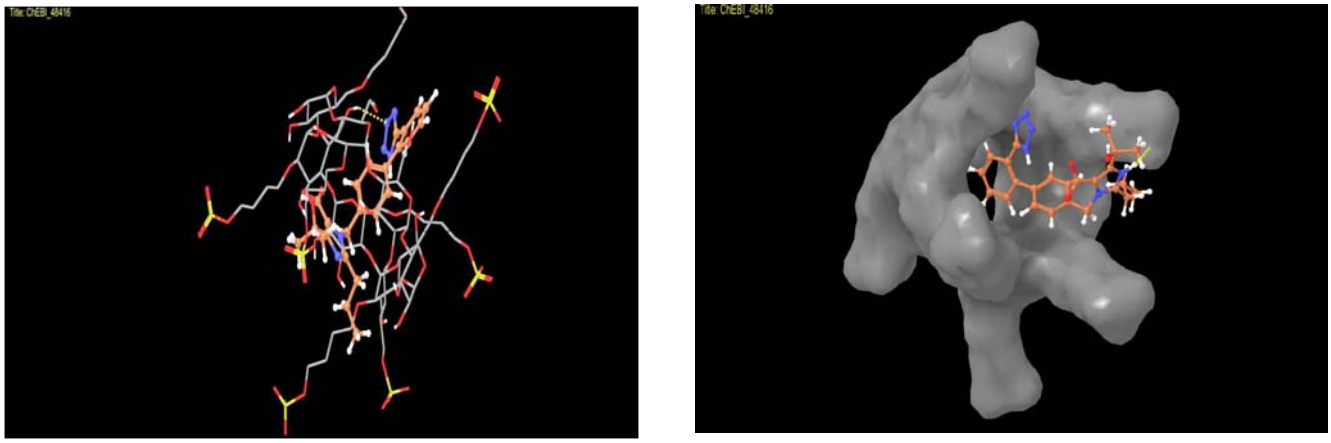


Fig. 8c: 3D structures of the complex between the olmesartan medoxomil and sulphobutyl ether$_7$β-cyclodextrin (SBE$_7$β-CD) isomer 3

***In vitro* dissolution study**

The dissolution profiles of the plain drug, physical mixture and inclusion complexes were presented as the mean percentage release of drug with respect to time as shown in fig. 9a. The percentage release after 120 min for both inclusion complexes KP and LP were shown 99.98 % and 99.78% respectively than PM and OLM with 66.36% and 57.63% respectively.

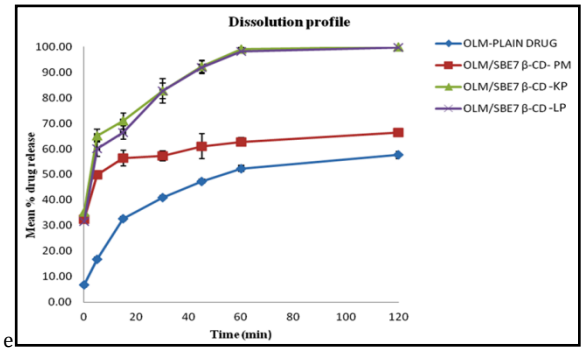


Fig. 9a: *In vitro* dissolution profiles of pure olmesartan medoxomil, PM and inclusion complexes

Dissolution profiles were compared with dissolution efficiency at 15 min (DE$_{15}$) and mean dissolution times (MDT) as given in table 3.

The significant enhancement of the dissolution efficiency that occurred with kneaded and lyophilized products has been attributed to an increase of OLM solubility upon complexation in the solid state due to the reduction of crystalline nature. MDT was considerably lower for the KP and LP indicating a faster dissolution rate for complexes compared to plain OLM. The *in vitro* results have shown that drug dissolution rates of complexes were evidently higher than that of the plain drug which can be attributed to enhanced drug solubility achieved by the complexation technique. This was also confirmed by X-RPD studies due to the decrease of crystalline nature in complexes.

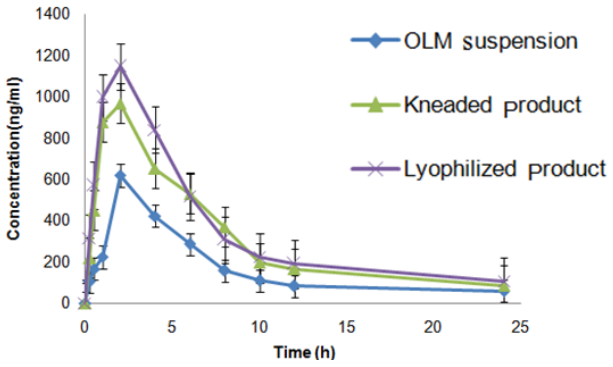


Fig. 9b: Mean plasma concentration-time profiles of olmesartan medoxomil, PM and inclusion complexes after oral administration

***In vivo* relative bioavailability in rats**

The relative bioavailability (BA) study in rats was conducted to evaluate absorption of inclusion complexes compared with the plain drug. Plasma OL levels (metabolite) were measured to assess the pharmacokinetic parameters because OL levels reflect OLM pharmacokinetics [28]. The mean plasma OL levels were higher for inclusion complexes than the plain OLM suspension as shown in fig. 9b. It has been reported that cyclodextrin/drug complexes yield improvement in oral bioavailability and therapeutic efficacy [29-32]. In view of this the OLM/SBE_7β-CD complex is expected to show higher therapeutic efficacy or enhanced relative oral bioavailability.

The pharmacokinetics parameters for OLM such as maximum plasma concentration (C_{max}), plasma half-life ($t_{1/2}$) and area under the concentration-time curve (AUC) were listed in table 4.

Table 3: Mean dissolution time and dissolution efficiency of plain OLM, PM, KP and LP

S. No.	Sample	Mean dissolution time (in min)	Dissolution Efficiency
1	OLM plain drug	31	50.56%
2	Physical mixture	27	55.43%
3	Kneaded product	18.4	63.96%
4	Lyophilized product	19.2	63.51%

Table 4: Pharmacokinetic parameters of pure OLM, KP and LP (5.0 mg/kg oral dose) in rats

Parameters	OLM	KP	LP
$AUC_{0\text{-}24h}$ (ng. h/ml)	4041.4±159	7480.9±292	8481.8±331
$AUC_{0\text{-}\infty}$ (ng. h/ml)	4293.1±311	7906.3±329	8950.7±372
Cmax (ng/ml)	617.3±43	997.5±217	1145.2±221
$T_{1/2}$ (h)	2.97±0.31	3.48±0.17	3.09±0.14
Relative % Bio-availability*	-	185.8	209.9

*% Relative Bio-availability = $AUC_{OLM}/AUC_{complex}$

The relative BA of OLM in KP and LP was 185.2, 185.8 and 209.9% respectively. The $AUC_{(0–24\ h)}$ and $AUC_{(0–\infty)}$ of OLM were only 4041 and 4293 ng. h/ml respectively because slower dissolution of OLM particles in suspension is the rate-limiting step for intestinal absorption. The AUC and C_{max} of inclusion complexes were increased which implied enhancement of solubilization of drug in inclusion complexes by improving oral bioavailability by overcoming the dissolution step. The relative bioavailability, C_{max} and AUC of the inclusion complexes were significantly higher than those of the plain drug suspension due to enhanced solubility.

CONCLUSION

The inclusion complexes of OLM were successfully prepared by employing modified sulphobeta cyclodextrin though different techniques physical mixing, kneading and freeze-drying. Phase solubility profile of inclusion complexes provides evidence of improved solubility with 13 fold increase as compare to a plain drug with complexation stoichiometry as 1:1 confirmed by jobs plot. DSC and XRD showed a reduction in crystallinity and no interaction among components. NMR predicted different hydrogen shift during complexation. Complexation was further supported through FTIR. The inclusion complex exhibited significantly higher *in vitro* dissolution profile as compared with plain OLM drug. The *in vivo* pharmacokinetic parameters showed enhancement of relative bioavailability of inclusion complexes with good correlation. Hence, the inclusion complexes of OLM with SBE_7β-CD have more feasibility for further development as an efficient oral delivery system with enhanced bioavailability at a low cost.

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CONFLICT OF INTERESTS

Declared None

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