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Enhancement of dissolution and bioavailability of poorly soluble Telmisartan: Designing modified cyclodextrin inclusion complexes

Thirupati Ajmeera[1], Sonia Gera[1], Sravanthi Reddy Pailla[1], Mallika A.[1], Sujatha Dodoala[2] and Sunitha Sampathi[1*]

[1]*Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER-Hyderabad) Balanagar, Telangana-500037, India.*

[2]*Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupathi (A.P.),India.*

ABSTRACT

Present work investigated the ability of modified Cyclodextrin such as sulphobutyl ether 7β-cyclodextrin (SBE7β-CD) to form an inclusion complex with telmisartan (TEL), a poorly soluble drug and its related effects on dissolution rate and relative bioavailability. The binary systems of drug with modified cyclodextrin were prepared by different techniques like simple mixing, kneading and lyophilization. Molecular modelling studies (docking) were carried out to understand and demonstrate significant interactions between SBE7β-CD and telmisartan. Resulting complexes were characterized by differential scanning calorimetry, x-ray powder diffractometry (XRD), proton nuclear magnetic spectroscopy and infrared spectroscopy. The analytical studies authenticated the formation of an inclusion complex between telmisartan and sulfobutyl ether7β-cyclodextrin whereas XRD studies revealed an amorphous nature of the inclusion complex. Inclusion complexes displayed improved dissolution rate when compared to pure drug. Pharmacokinetic profile of complexes had shown a significant enhancement in the relative bioavailability of selected drug. Therefore, SBE7β-CD can be successfully used as a carrier for the oral administration of telmisartan with enhanced bioavailability and industrial applicability in terms of scale up.

Keywords: Complexation, inclusion complexes, molecular modelling, sulphobutyl ether 7β-cyclodextrin, telmisartan.

INTRODUCTION

In the present scenario of drug development the upcoming active pharmaceutical ingredients (API) are facing the challenges in terms of formulation development due to poor aqueous solubility [1]. To overcome above shortcomings, techniques like solid dispersion [2] and hot melt extrusion [3], surfactants [4] and cyclodextrin inclusion complexation [5] are commonly used. Recently most of the formulations have been reported with inclusion complex to enhance drug activity in terms of improving solubility, dissolution and bioavailability [6-9]. Cyclodextrins are among group of molecules containing cyclic oligosaccharides of six, seven or eight linked D-glucopyranose units [α, β and γ CDs] containing relatively lipophilic central cavity and a hydrophilic outer surface. The dual nature of the cylcodextrins helps in the incorporation, entrapment and solubility of the lipophilic molecule [10-12]. Literature studies clearly shows that CDs not only improve the drug properties of hydrophobic drugs but also helps in avoiding the use of harmful organic solvents, surfactant or lipids to enhance aqueous solubility and bioavailability [13-14].

Among the commonly used modified anionic CD derivatives, sulfobutylether7β-CD [SBE7β-CD] is a capable candidate with water-solubility [>50% w/v] in a broad pH range [15, 16]. SBE7β-CD is approved by FDA as a safer

complexing agent as compared to β–CD [17, 18]. Research work reported and suggested that complex with sulfobutyl ether-β-cyclodextrin improved the solubility of the formulations [19-21].
Telmisartan a potent, nonpeptide antagonist of the angiotensin II type-1 [AT1] receptor used in the treatment of essential hypertension [22]. It is chemically 2-[4-[[4-methyl-6-[1-methylbenzimidazol-2-yl]-2-propylbenzimidazol-1-yl] methyl] phenyl] benzoic acid that belongs to BCS class II drug which is practically insoluble in water [0.09 µg/ml] with absolute bioavailability of 42-58%. The solubility of telmisartan is strongly pH dependent, with maximum solubility observed at high and low pH. Under fed and fasted conditions TEL has shown significant variation in pharmacokinetic parameters (C_{max} and t_{max}) in preclinical studies [23-24]. Thus, there is a need of improving the solubility of the drug to have an optimum therapeutic effect. Literature studies revealed the make use of *β*-cyclodextrin and hydroxy propyl-*β*-cyclodextrin to learn the outcome of complexation lying on aqueous solubility and rate of dissolution in dissolution media but has not reported the effect of pH on complexation and molecular interaction among the complex [25-26] but certain setbacks in previous studies provides challenges to the formulator for successful use of cyclodextrin inclusion complexes of telmisartan for oral delivery.

In the present study telmisartan inclusion complexes with SBE7β-CD were prepared by various methods and characterized by different techniques. Molecular interaction of TEL and SBE7β-CD were explored by molecular docking studies and analyzed the factors responsible for the binding of drug with SBE7β-CD. *In vitro* dissolution studies and *in vivo* bioavailability studies of complex were compared with the pure drug as well as with marketed formulation.

EXPERIMENTAL SECTION

Chemical

Telmisartan was gifted by Eurobond Pharmaceuticals (Hyderabad, India). SBE7 β-CD was a gift sample from Daichisankyo (Gurgaon, India). Captisol® (Sulphobutyl Ether7β-Cyclodextrin) was purchased from Cydex pharmaceuticals (USA). Milli-Q water was used throughout the study and solvents methanol, acetonitrile (AR grade, Qualigens Chemicals, Mumbai, India) used were of analytical grade. Marketed tablets TELMA-40 (Glenmark Pharmaceutical, Ltd., Himachal Pradesh, India) was purchased from local pharmacy store.

Phase solubility studies

A phase solubility study was carried out in triplicates to investigate the effect of SBE7β-CD on the solubility of TEL. 2 mL of double distilled water containing different molar concentrations of SBE7β-CD (0, 2.5, 5, 10, 20, 40 and 80 mM) were taken in 5 mL screw vials to which excess of TEL was added and shaken (orbital shaker,JEIOTECH, Korea) at 25 °C for 72 h. After equilibrium, the solutions were centrifuged at 10,000 rpm Multifuge™ X3 Centrifuge, Thermo scientific) 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 drug using UV spectrophotometer [V-650, JASCO] at 294 nm. The phase solubility profile was plotted for the concentration [mM] of TEL and SBE7β-CD, the stability constant of the complex was resolute from the graph by the following equation:

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

Where, the slope was obtained from the graph and S_o was the equilibrium solubility of TEL in water.

Preparation of inclusion complexes

Inclusion complexes of telmisartan with SBE7β-CD were prepared in 1:1 ratio by following methods.

1. Kneading method [KM]: Equimolar quantities of TEL and SBE7β-CD were added separately to 10 mL of methanol and mixed thoroughly. Methanolic solution of SBE7β-CD was transferred into a mortar and to this saturated methanolic solution of the drug was added slowly. The mixture was further kneaded for 3–4 h until evaporation of methanol. During the kneading process few drops of water was added to maintain a suitable consistency. The resulting mass was dried in an oven [Osworld laboratory oven] at 45^{o}C for 48 h. The dried mass, kneaded product [KP] was finally grounded and sieved as discussed earlier [Gil et al., 2004].

2. Lyophilization method [LM]: Equimolar quantities of TEL and SBE7β-CD were added to 10 mL of methanol and water respectively and mixed thoroughly. Both solutions were mixed and the resultant solution was frozen in a deep freezer [Thermo scientific 5812, Germany] at -70°C for about 1 h. This mixture was further freeze dried in the freeze dryer [Skadi FD 5508, Europe] at -110°C to -120°C under vacuum for 8 h. The resultant lyophilization product [LP] was sieved as discussed earlier [27-28].

3. Physical mixture [PM]: In this method the physical mixture was prepared by simple mixing of equimolar quantities of TEL and SBE7β-CD in a mortar with pestle for 10 min and then the mixture was passed through BSS 120 sieve.

Characterization of drug inclusion complexes in solid state

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of TEL, SBE7β-CD, PM, inclusion complexes [KP and LP] were recorded on Perkin Elmer IR spectrophotometer [Inc., MA, US] with pellets prepared using potassium bromide.

Differential Scanning Calorimetry (DSC)

The thermal properties of TEL, SBE7β-CD, PM and inclusion complexes [KP and LP] were investigated using a Mettler DSC [Mettler Toledo, Germany] calibrated with indium. Accurately 5 mg each of pure TEL, SBE7β-CD, PM and inclusion complexes were weighed into aluminium pans, sealed and scanned at a rate of 10°C/min between 40°C and 250°C in nitrogen atmosphere applied at 40 mL/min.

X-Ray diffraction analysis (XRD)

X-ray diffraction (XRD) patterns of pure TEL, SBE7β-CD and lyophilized inclusion complex were recorded at room temperature on a Philips P analytical xpert powder X-ray diffractometer [Philips P] using Ni-filtered, Cu Kα radiation, with a voltage of 40 kV and 25 mA current. The scanning speed was 1°/min over the diffraction angle range [2θ] of 5 - 40 °C.

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

The ^{1}H-NMR spectra of pure TEL, SBE7 β-CD and lyophilized inclusion complex were taken in DMSO and for pure SBE7β-CD it was done in duterium water [D_2O] on a Bruker Ultra shield 400 MHz nuclear magnetic resonance [29] and the spectra were processed using topspin 2.0 software.

Molecular modelling studies

Molecular docking studies of telmisartan withSBE7β-CD were carried using GLIDE module of Schrödinger suite 2013. Co-ordinates for sulfobutylether7 β-cyclodextrin were generated by adding sulfobutyl ether group at 7th position of β-cyclodextrin. Further, it has been prepared for docking by adding hydrogen and minimized. 3D structure of telmisartan 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 sulfobutylether 7β-cyclodextrin with x: 19.7004; y: -8.0605; z: 0.2067 co-ordinates. Glide XP docking was performed. Molecular docking of telmisartan with β-cyclodextrin was also performed to compare the binding affinity with that of SBE7β-CD.

***In vitro* dissolution studies**

In vitro dissolution studies of pure drug, PM, drug inclusion complexes and marketed product [TELMA-40] were carried out using USP paddle method. Dissolution studies were carried out in 900 mL of 0.1N HCl [pH 1.2], 0.001N HCl [pH 3], acetate buffer [pH 4.5] and phosphate buffer [pH 6.8 and pH 7.4] at 75 rpm and temperature maintained at 37 ± 0.5 °C. At suitable time intervals [5, 10, 15, 30, 45, 60, 90, and 120 min] 5 mL of the sample were collected and filtered through 0.45µm membrane filter. The samples were analyzed at 294 nm by UV-visible spectrophotometer to calculate the drug release from each formulation in triplicates.

Mathematical Modelling of Drug Release

Comparison of dissolution data was analyzed using percentage of drug dissolved at 5 and 10 minutes [Q_5 and Q_{10}], dissolution efficiency [DE] at 15 min and mean dissolution time [MDT] [29]. DE is defined as the area under the dissolution curve up to a certain time 't' expressed as percentage of the area of the rectangle described by 100% dissolution in the same time. The equation is as follows:

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

Where "Y" is the percent of drug released as a function of time, t is the total time of drug release, and "Y100" is 100% drug release.

Mean dissolution time is the arithmetic mean value of any dissolution data and the equation is as below

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

Where j is the sample number, n is the number of dissolution sampling times, t is the time by the side of 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 male wistar rats**

In vivo pharmacokinetic studies were performed in male wistar rats of 220–250 g [8 weeks old] were supplied by M/S Tina Labs, India. All the experimental protocol were approved by the IAEC bearing CPCSEA number [1548/PO/1/11 CPCSEA dated 15.02.2012]. All rats were housed in individual with temperature controlled at 20 ± 2°C and a relative humidity of 50 ± 5% for one week. Twelve rats were randomly divided into two groups with six rats in each group. The rats were fasted for 10–12 h before the experiments with water ad libitum. The first group of animals received pure drug suspension while the second group of animals received optimized telmisartan/SBE7β-CD inclusion complexes [160 mg/kg, body weight, p.o]. Post dosing, 250 µL of the blood was collected from retro-orbital plexus at regular time intervals of 0, 2, 4, 6, 10, 15, 30, 60 and 120 min. The collected blood samples were centrifuged at 5000 rpm for 10 min and the obtained plasma [100 µL] was immediately stored at -20 °C until further analysis.

All animal care and experimental procedures were conducted according to the guiding principles in the use of animals in toxicology, as adopted by the CPCSEA guidelines. To 100 µL of plasma, 300 µL of acetonitrile containing 300 ng of internal standard [omeprazole] was added to precipitate the proteins. The samples were vortexed and centrifuged at 5,000 rpm for 10 min. The supernatants were separated and analyzed for drug content by HPLC RP Hypersil BDS C-18 column at a flow rate of 0.8 mL per minute using a mixture double distilled water and acetonitrile in the relative amount of 60:40 as mobile phase with pH adjusted to 3.2. The intra and inter day accuracy and precision for the bio-analytical method were validated and were found to be within the acceptable range as per the USFDA guidelines [32].

The calibration curve for telmisartan concentration in plasma was linear [R^2 =0.999] over the range of 100–1600 ng/mL. Standard non-compartmental analysis were performed to determine all the pharmacokinetic parameters including maximum plasma concentration [C_{max}], time taken for its occurrence [T_{max}], half-life [$t_{1/2}$], and area under the curve [AUC] for each rat by using the standard edition of kinetica software 5.0 trial version. The AUC was calculated from zero to infinity via the linear trapezoidal rule. The relative bioavailability was calculated using the following equation:

Student's *t*-test was performed to evaluate significant differences between the optimized formulation and the control. All values were considered statistically noteworthy at $p<0.05$.

RESULTS AND DISCUSSION

Phase Solubility Studies

The effect of SBE7 β-CD on the aqueous solubility of telmisartan was evaluated using the phase solubility curve as presented in [Fig.1]. Higuchi and cannons method was used and the solubility curve was classified as AL type. Inclusion complex between telmisartan and SBE7 β-CD molecules was formed in the ratio of 1:1.

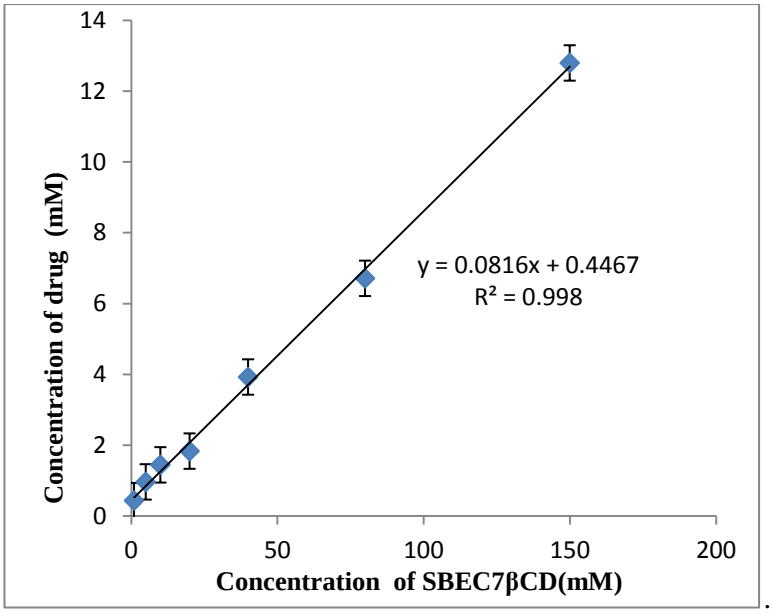

Figure1. Phase solubility analysis of telmisartan in SBE7β-CD

Telmisartan solubility increased by 13 folds at a concentration of 150 mM of SBE7 β-CD and apparent stability constant [KS] of telmisartan: SBE7 β-CD complex [1:1] was calculated as 239 M^{-1} from the linear plot of the phase-solubility illustration.

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR analyses mainly provide information on physicochemical compatibility of substances with respect to used excipients [33].The FT-IR spectra of pure TEL, pure SBE7β-CD, PM, KP and LP was shown in [Fig.2]. TEL exhibits characteristic peaks at 3418 cm^{-1} [N–H stretch], 3053 cm^{-1} [aromatic C–H stretch], 2957cm^{-1} [aliphatic C–H stretch], 1697 cm^{-1} [carbonyl group], and the peak at 1448 cm^{-1} indicated the presence of C=C aromatic group.

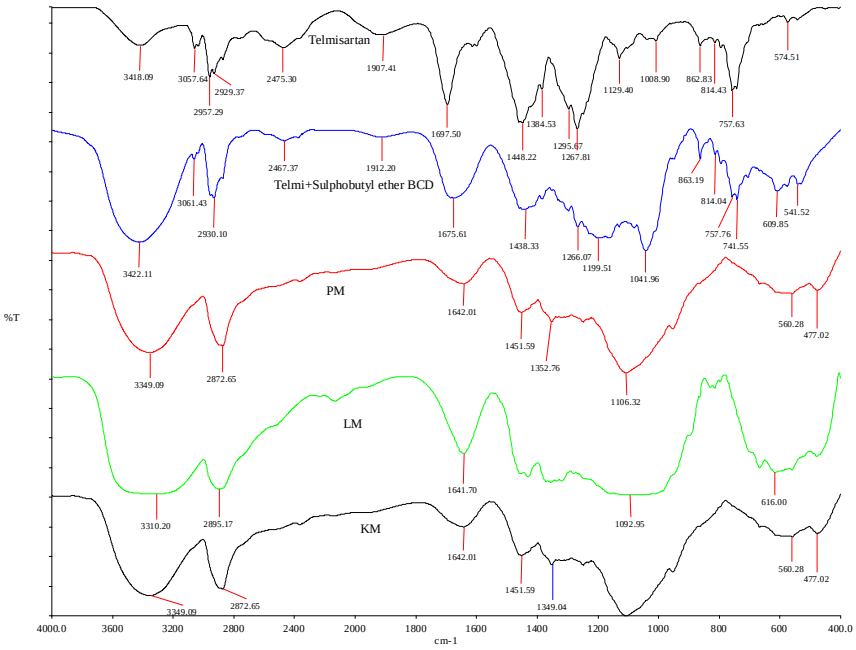

Figure2. FTIR spectra of pure drug, SBE7β-CD, and telmisartan / SBE7β-CD lyophilized inclusion complex

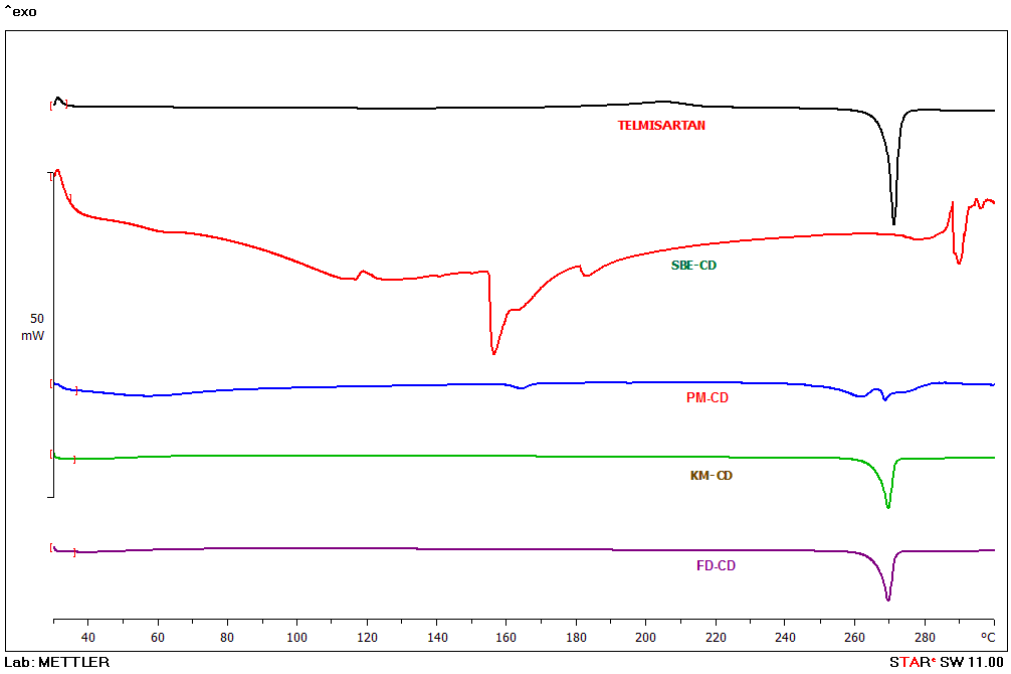

Figure3. DSC thermogram of pure drug, SBE7β-CD, telmisartan/ SBE7β-CD inclusion complex and lyophilized mixture

The main absorption of TEL carbonyl group appeared almost unchanged in the formulated inclusion complexes and physical mixture pointing out the absence of the interaction between the drug and the complexing agent.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of TEL, SBE7β-CD and inclusion complexes were shown in [Fig.3]. The thermal curve of pure telmisartan showed crystalline nature with sharp endothermic peak at 272°C corresponding to the melting point of the drug. In the inclusion complexes and physical mixtures of drug with SBE7β-CD, a characteristic and well recognizable endothermic peak of drug appeared at a temperature corresponding to its melting point which confirmed the absence of interaction between drug and complexing agent.

X-Ray diffraction analysis (XRD)

XRD patterns of pure TEL, SBE7β-CD and lyophilized inclusion complex were carried out. Prepared lyophilized inclusion complexes were selected in preliminary studies as it showed better solubility profile for drug than respective PM and KM. The solid-state form such as crystalline, polymorphs, solvates or amorphous solids of a drug substance can have a significant impact on drug's solubility, dissolution rate, bioavailability and stability in a pharmaceutical formulation [34]. Crystallinity was resolute by match up to some representative peak height in the diffraction pattern of the binary systems with those of a reference. The XRD patterns of pure drug (Fig. 4) was showing sharp individual peaks mainly at 2θ diffraction angles of 6°, 12°, 15° and 22° stipulating that telmisartan was in crystalline state.

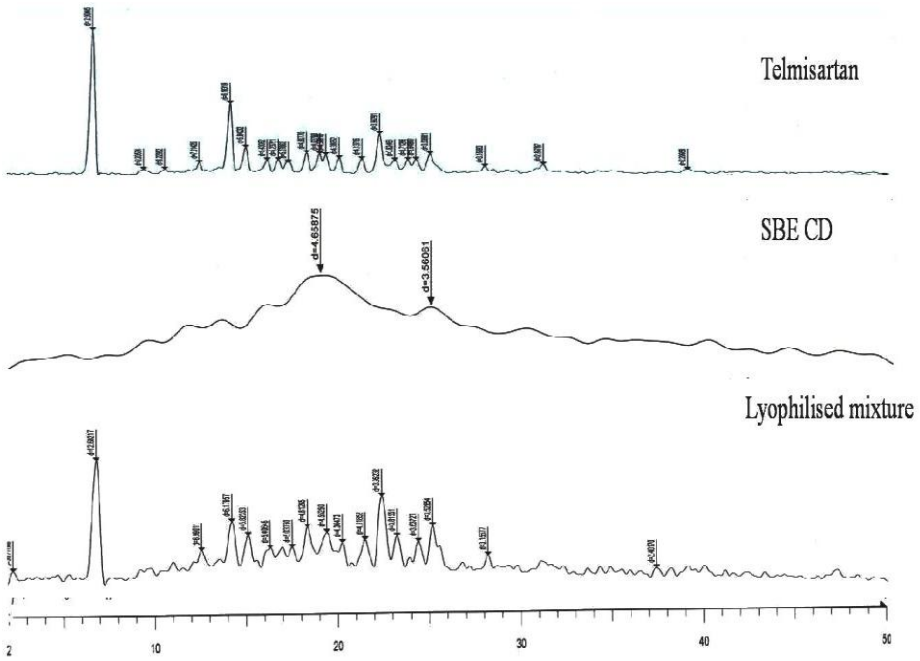

Figure4. X-Ray Diffraction spectra of the telmisartan (D), SBE7β-CD(C), and lyophilized mixture of telmisartan/ SBE7β-CD

Telmisartan showed sharp peak at 12.90405 (2θ) with highest peak intensity of 400 while in test at 12.69217 (2θ) with peak intensity of 63. The relative degree of crystallinity (RDC) was calculated according to the equation:

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

Where I_{sam} is the peak height of sample and I_{ref} is the peak height at the identical angle intended for the reference with the maximum intensity [35]. The peak height at 12.904050 was used for calculating the RDC of lyophilized binary system and drug. The RDC value of corresponding binary systems of telmisartan-SBE7β-CD was 0.1575. The characteristic crystalline peak of telmisartan in lyophilized mixtures with lesser peak size was observed due to the possibility of drug to undergo solid state transition from crystalline to amorphous form.

^{1}Nuclear Magnetic Resonance Studies [^{1}H-NMR]

^{1}H-NMR of pure TEL, SBE7β-CD and lyophilized inclusion complex was carried out. Prepared lyophilized inclusion complexes were selected for ^{1}H-NMR as in preliminary studies it showed better solubility profile for drug than respective PM and KM. The ^{1}H-NMR spectra of telmisartan and its complexes were recorded to gain deeper insight into the interaction of the drug with SBE7β-CD. Protons showed an upfield chemical shift due to a variation of local polarity and to the weedy contact with CD cavity hydrogen atoms. ^{1}H-NMR spectra of telmisartan, SBE7β-CD and complex are shown in Fig. 5. The chemical shifts for telmisartan and SBE7β-CD before and after complexation is shown in Table 1.

Table1. Chemical shifts, δ (ppm) and chemical shifts variations for telmisartan proton

TELMESARTAN δ (ppm)	TELMESARTAN/SBE7β-CD δ (ppm) LP	Δδ
0.895	0.894	0.001
0.877	0.875	0.002
0.859	0.857	0.002
1.592	1.59	0.002
1.573	1.572	0.001
1.472	1.468	0.004
2.625	2.601	0.024
2.606	0.583	2.023
2.587	2.51	0.077
5.423	5.416	0.007
5.214	5.203	0.011
7.68	7.651	0.029
7.661	7.63	0.031
7.638	7.535	0.103
7.588	7.586	0.002
7.569	7.568	0.001
7.546	7.542	0.004

7.527	7.522	0.005
7.054	7.05	0.004
7.034	7.029	0.005
6.875	6.866	0.009
6.855	6.846	0.009

It is evident from NMR spectra that the characteristic protons of TEL and the protons in the cavity of SBE7β-CD are different and used to explore as well as to check the interaction by the prominent changes in the pattern of NMR signals of the protons of aromatic benzyl groups of TEL in both PM and inclusion complexes. In addition, alterations in the pattern of the NMR signals of the cavity protons of hydroxyl groups in SBE7β-CD were sensible in the inclusion complexes. However, intricate aspects of the interaction could not be deduced due to difficulties in extracting accurate changes in the chemical shifts and the discussed interactions were further supported by the results from molecular modelling studies.

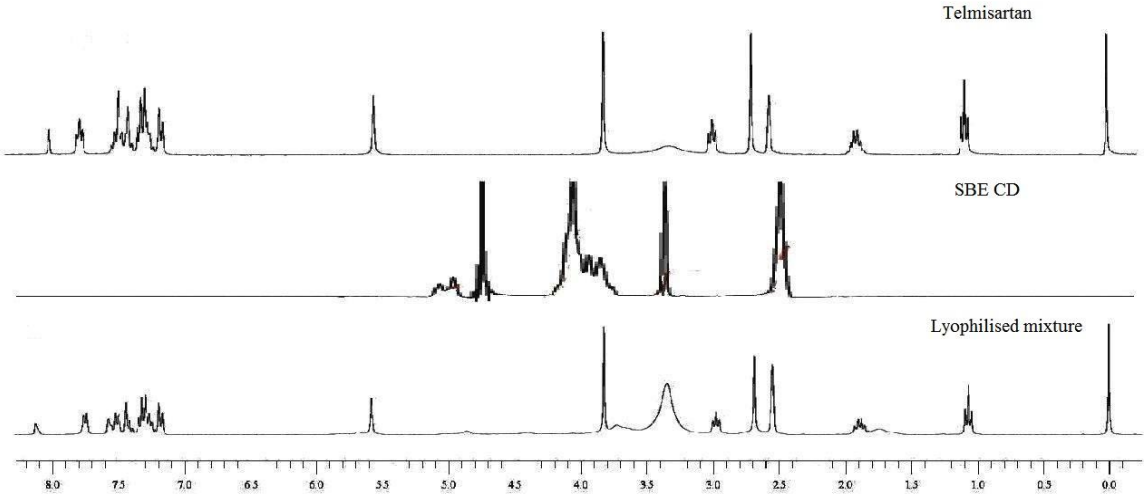

Figure 5. NMR spectra of the telmisartan(D), SBE7β-CD (C), and lyophilized mixture of telmisartan/ SBE7β-CD

Molecular modelling studies:

The molecular docking study of telmisartan with the SBEC 7β-CD has revealed formation of two hydrogen bonds with 3-OH and 4–OH group of dextrin ring with a distance of 1.8 and 1.93 ^{0}A respectively. Whereas docking of telmisartan with simple β-cyclodextrin showed one hydrogen bond formation with 2-OH group of dextrin ring with a distance of 2.36 ^{0}A. Telmisartan has showed docking score of -3.694 with SBEC7 β-CD and -3.065 with simple β–cyclodextrin. From Fig.6 it was evident that telmisartan was partially embedded in the SBEC7 β-CD cavity and formed 2 hydrogen bonds. Upon superposition of telmisartan binding pose in β–cyclodextrin and SBEC 7β-CD revealed that, in SBEC7 β-CD the hydrophobic core of drug molecule embedded into the cavity whereas in simple β–cyclodextrin, it is on periphery of the cavity which contributes for the formation of better inclusion complex between SBEC7 β-CD and telmisartan.

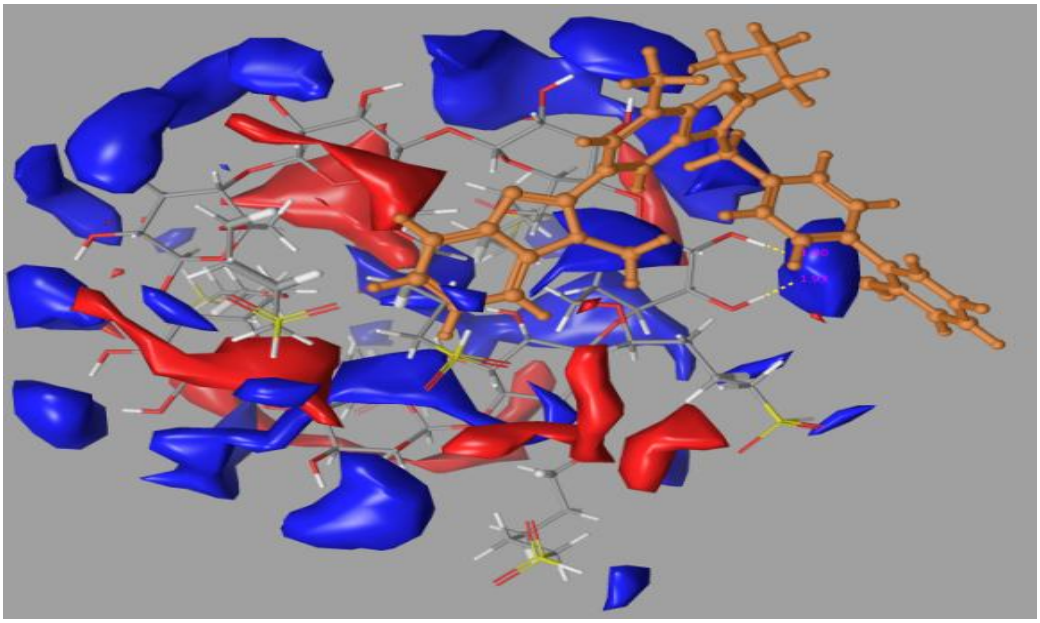

Figure 6. CADD analysis depicting 3D structures of the complex between the telmisartan with Sulphobutyl Ether7β-Cyclodextrin (SBE_7β-CD) and telmisartan with cyclodextrin

In vitro dissolution study

The *in vitro* [%] drug release profiles of LM, KM and PM were examined at different pH media [2, 3, 4.5, 6.8 and 7.4] in comparison to pure drug and marketed formulation at different time intervals and were shown in Fig. 7. Q_5, Q_{10}, DE_{15} and MDT were summarized in Table 3.

Table3. Dissolution parameters for PM, KM and LM inclusion complex and marketed formulation in different media's

Dissolution Parameters(in different pH medias)		*Q*5 min (%)	*Q*10 min (%)	DE15 (%)	MDT
pH 2	LM	60.22±2.54	100.9±1.62	98.24±1.47	4.66±0.61
	KM	55.0±3.42	99.8±2.48	96.64±4.38	4.9±0.68
	PM	45.22±4.38	99.7±1.82	88.21±2.66	5.81±1.20
	MF	25.46±2.10	54.8±4.27	42.86±4.34	26.5±5.07
	PD	4.78±1.12	16.3±2.26	9.24±0.46	67.48±2.68
pH 3	LM	40.51±4.28	94.9±2.67	70.94±0.99	7.42±1.18
	KM	16.78±1.49	66.0±4.65	41.45±4.36	15.28±2.25
	PM	14.25±1.08	59.6±6.02	35.18±4.18	18.8±1.47
	MF	26±2.66	57.8±4.98	46.87±4.20	28.5±2.46
	PD	2.34±0.74	6.5±1.62	3.92±0.78	139.45±6.89
pH 4.5	LM	20.21±2.35	51.51±5.64	42.64±3.87	17.23±1.24
	KM	13.35±2.32	36.08±4.36	20.29±1.84	20.85±2.88
	PM	21.45±1.68	38.76±4.52	33.16±3.68	21.56±2.64
	MF	9.5±2.36	15.69±2.99	14.1±3.42	88.89±4.68
	PD	8.02±1.02	19.40±2.64	15.35±2.44	118.2±6.44
pH 6.8	LM	75.65±2.69	92.25±5.07	85.45±4.69	4.096±0.54
	KM	65.75±3.69	78.25±6.47	70.98±1.62	6.272±0.18
	PM	16.32±0.20	52.32±4.46	24.12±2.14	17.95±5.67
	MF	21.45±2.65	57.11±1.17	39.42±2.42	27.05±5.02
	PD	1.32±0.2	4.47±0.89	2.94±0.44	126.23±1.14
pH 7.4	LM	19.96±2.10	54.88±1.28	42.82±2.63	16.28±4.10
	KM	13.53±1.16	38.13±2.98	25.27±0.99	28.11±5.21
	PM	12.2±1.96	35.85±5.41	18.28±0.02	24.56±3.66
	MF	20.89±0.20	50.98±4.85	39.72±2.10	32.94±0.83
	PD	5.81±0.098	7.5±0.17	6.24±0.06	140.12±4.38

LM, KM and PM showed 98, 96 and 88% drug release respectively in 0.1 N HCl at 15 min while marketed formulation and pure drug gave 42 and 9 % release. The significant enhancement of the dissolution efficiency was seen in kneaded and lyophilized products which may be attributed to an increase of TEL solubility upon complexation in the solid state due to reduction of crystalline nature and also owing to high energetic amorphous state of drug in inclusion complex. MDT was considerably lower for the KP and LP indicating a faster is solution rate for complexes as compared to pure TEL [36]. In acetate buffer [pH 4.5], percentage drug release was decreased

to 42, 20, 33 % from LM, KM and PM respectively. However, at pH 6.8 DE_{15} has increased to 85.45, 70, 24% of LM, KM and PM while marketed formulation and pure drug showed 39.42 and 2.9%. From the results it can be credited that telmisartan has low solubility as pH increases.

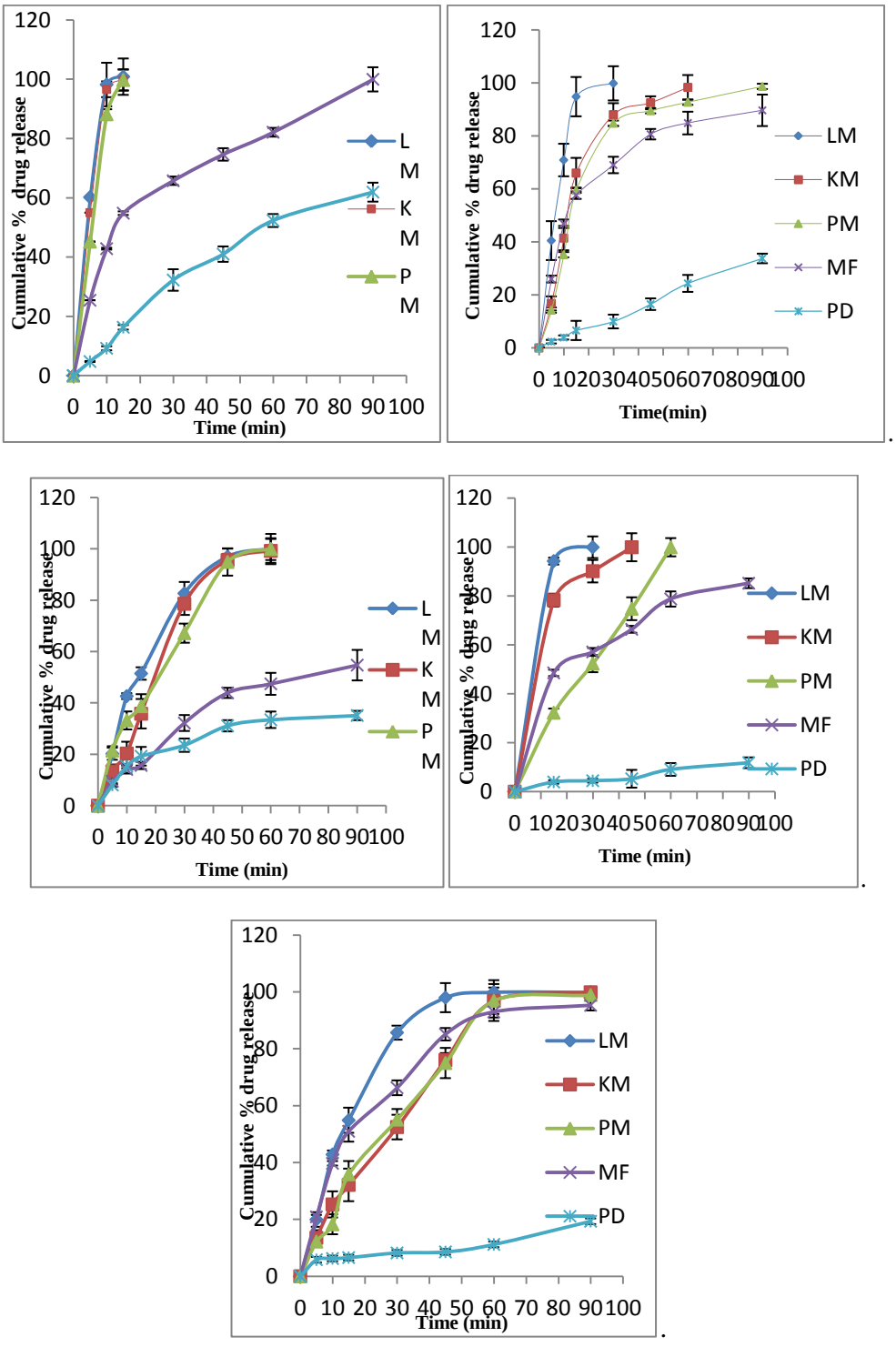

Figure7. Dissolution profile of the pure telmisartan, inclusion complex (PM, KM, LM) and marketed formulation in (a) 0.1N HCL, (b) 0.001N HCL, (c) 4.5pH acetate buffer, (d) 6.8pH phosphate buffer and (e) 7.4pH phosphate buffer

***In vivo* relative bioavailability study**

The incorporation of TEL into inclusion complexes was explored as a method to enhance the solubility and release of the drug. Plasma concentration profiles of pure drug solution and inclusion complex [LM] were shown in [Fig.8]. Pure telmisartan has given the C_{max} of 3568.27ng/ml at t_{max} 60 minutes while in optimized formulation an enhanced

C_{max} of 12042.7 ng/ml at t_{max} of 10 min was achieved. *In vivo* studies showed that inclusion complex released the drug immediately as compared to pure drug suspension (Table 4).

Table4: Pharmacokinetic parameters for pure drug and optimized formulation

Formulation	C_{max}(ng/ml)	T_{max}(min)	$AUC_{0-\infty}$(ng/min/ml^{-1})	$T_{1/2}$ (min)
Pure drug	3568.27	60	165872	5.534
Optimized formulation	12042.71	10	678521	4.923

The AUC $_{[0-\infty]}$ for pure drug and optimized formulation was 1, 65,872 and 6, 78,521 ng.hr/mL. The differences in AUC were attributed to the slower dissolution of pure TEL particles in suspension form which is the rate-limiting step for intestinal absorption and relative BA of TEL was increased by 4 folds as compared to pure drug.

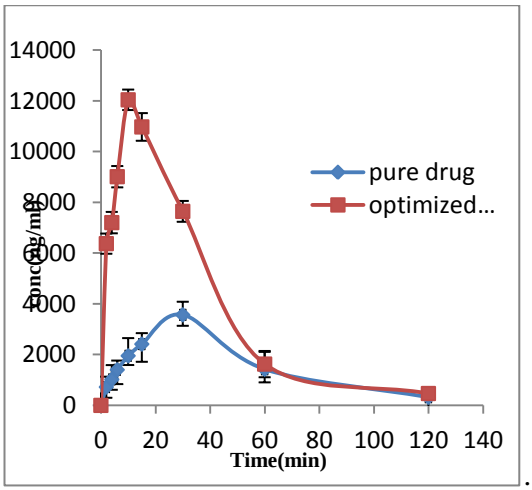

Figure 8 Plasma concentration *vs.* time profile after oral administration of the optimized formulation of telmisartan compared with the pure drug

CONCLUSION

The stability constants A_L [*Ks*] in phase solubility profile of inclusion complexes provides evidence of improved solubility. The results of various experimental techniques and molecular modelling studies are in compliance with each other confirming the formation of complex between TEL and SBE7β-CD.Theinclusioncomplexexhibitedsignificantly higher *in vitro* dissolution profile as compared with pure drug. The *in vivo* pharmacokinetic parameters showed enhancement of relative bioavailability of inclusion complexes. The *in vitro* and *in vivo* study data were correlating each other. So, the inclusion complexes of TEL with SBE7β-CD have more feasibility for further development as an efficient oral delivery system with enhanced bioavailability.

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