J Incl Phenom Macrocycl Chem (2012) 73:295–303
DOI 10.1007/s10847-011-0056-x

ORIGINAL ARTICLE

Evaluation and solubility improvement of Carvedilol: PSC[*n*]arene inclusion complexes with acute oral toxicity studies

Shobhana K. Menon · Bhoomika R. Mistry · Kuldeep V. Joshi · Nishith R. Modi · Divyesh Shashtri

Received: 23 July 2011 / Accepted: 25 September 2011 / Published online: 19 November 2011

Abstract The aqua phobic molecules that are practically insoluble in aqueous media demonstrate a staggeringly slow intrinsic dissolution rate. In this work, we exemplify the utility of calixarenes as a tool to form inclusion complexes with Carvedilol (CDL). It is poorly water soluble drug. CDL is a Biopharmaceutical Classification System (BCS) Class II drug and it is a nonselective β-adrenegenic blocking agent with α1-blocking activity. It is mainly used in the management of hypertension. The maximum complexation of the drug was accomplished after 48 h of stirring with *para* sulphonato calix[4]arene (PSC[4]arene) and *para* sulphonato calix[6]arene (PSC[6]arene) in water and evaporation of water to acquire solid complexes. The study includes characterisation of both the complexes—physical mixtures of drug and PSC[4]arene and PSC[6]arenes by different methods like Fourier-transform infra red spectroscopy, differential scanning calorimetry and powder X-ray diffraction, proton nuclear magnetic resonance. This studies shows that there is electrostatic interaction between drug and PSC[*n*]arenes. The complexation was determined by phase solubility study. The prepared complexes exhibited improved in vitro dissolution profile and decreased in vivo acute oral toxicity compared to the pure drug.

Keywords Carvedilol · PSC[*n*]arene · Inclusion complex · Acute oral toxicity · Phase solubility study · Dissolution study

S. K. Menon (✉) · B. R. Mistry · K. V. Joshi · N. R. Modi
Department of Chemistry, School of Sciences,
Gujarat University, Ahmedabad 380009, India
e-mail: shobhanamenon07@gmail.com

D. Shashtri
KBIPER College of Pharmacy, Gujarat University,
Gandhinagar 382023, India

Abbreviations

PSC[4]arene	*Para* sulphonato calix[4]arene
PSC[6]arene	*Para* sulphonato calix[6]arene
DSC	Differential scanning calorimetry
FTIR	Fourier-transform infra red
PXRD	Powder X-ray diffraction
CDs	Cyclodextrins
CDL	Carvedilol
PM-1	Physical mixture-1
PM-2	Physical mixture-2
OECD	Organisation for Economic Co-operation and Development
EPA	Environmental Protection Agency
CNS	Central nervous system
AOT	Acute oral toxicity
BCS	Biopharmaceutical Classification System
^{1}H NMR	Proton nuclear magnetic resonance

Introduction

Cyclodextrins (CDs), the cyclic oligosaccharides discovered in 1891 are still esteemed novel excipients of unexplored potential. The drug formulations concentrating CDs have been approved for marketing in Japan, Europe and US [1]. The supramolecular structures imprinted between CDs and polymers have inspired interesting exploitations of novel supramolecular biomaterials [2].

Calixarenes are cyclic oligomers which are synthesized by condensation of *p-tert* butyl phenol with formaldehyde [3]. In calixarenes, *para* sulphonato calixarenes (*para* sulphonato calix[4]arene, PSC[4]arene and *para* sulphonato calix[6]arene, PSC[6]arene) are widely used as

complexing agents for organic molecules. These molecules possess the most eminent known aqueous solubility, >0.1 mol/L [4, 5]. The biochemistry of the *para*-sulfonatocalix[*n*]arenes has demonstrated rapid development during the past 10 years. The highly various biomedical applications of these molecules now include antiviral, anti-thrombotic activities, enzyme blocking and protein complexation. Its future is bright as the possibility of its use in the diagnosis of prion-based diseases has shown better results. Their innocuous quality, so far as it is known at present, may open up their succeeding use in medications [6].

Carvedilol (CDL) is chemically (±)-1-(carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino]-2-propanol. It is used for the treatment of mild to moderate high blood pressure. It is a non selective beta-adrenoreceptor antagonist and α-1 adrenoreceptor antagonist and a vasodilator [7]. CDL is easily soluble in dimethylsulfoxide, methylene chloride and methanol but sparingly soluble in 95% ethanol and isopropanol and practically insoluble in water. Inclusion of CDL in the hydrophobic cavity of PSC[*n*]arene is one of the appropriate approaches to solve solubility troubles. There have been several methods to prepare inclusion complexes of cyclodextrin and different class of drugs [8–15]. CDL with *β*-cyclodextrin inclusion complex was reported by Wen et al. [16–18]. Liu et al. conclude that the complexation ability of the calixarenes is largely driven by electrostatic interaction, rather than the hydrophobic interaction in the CDs complexation. Furthermore, Shinkai also stated that the calixarene cavity is more hydrophobic than that of beta-cyclodextrin [19, 20]. The molecular structures of pure CDL, PSC[4]arene, PSC[6]arene and PSC[4]arene: CDL and PSC[6]arene: CDL are shown in Fig. 1.

Till date there is no report in the literature survey where PSC[*n*]arenes inclusion complexes with CDL have been reported and this is the first report of such inclusion complexes. There are methods reported from our research group, for carbamazepine and mycophenolate mofetil (MMF) with PSC[4]arene, PSC[6]arene and calix[4]resorcenarene [21, 22]. The encouraging results received from the above studies motivated us to of investigated inclusion complex of CDL with PSC[*n*]arene and hence in the present investigation we have prepared novel formulation of CDL by way of an inclusion complex with PSC[*n*]arene to increase the solubility and dissolution rate of the drug. The inclusion complex of CDL was characterized by different analytical techniques like Fourier-transform infra red (FT-IR), powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC), proton nuclear magnetic resonance (^{1}H NMR) and phase solubility study. The work included phase solubility study, in vitro release study and the in vivo study of drug.

Materials and methods

Materials

CDL was a sample gifted from Cadila Pharmaceuticals Ltd., Ahmedabad, India. Both calixarenes PSC[4]arene (MW = 744.74) and PSC[6]arene (MW = 1,117.11) were synthesised by reported procedures [23]. PSC[4]arene and PSC[6]arene were characterised by Elemental analysis and various spectroscopic methods like FT-IR, ^{1}H NMR, ^{13}C NMR and compared with the reported values. Purified water was prepared by Millipore (Synergy) system. All

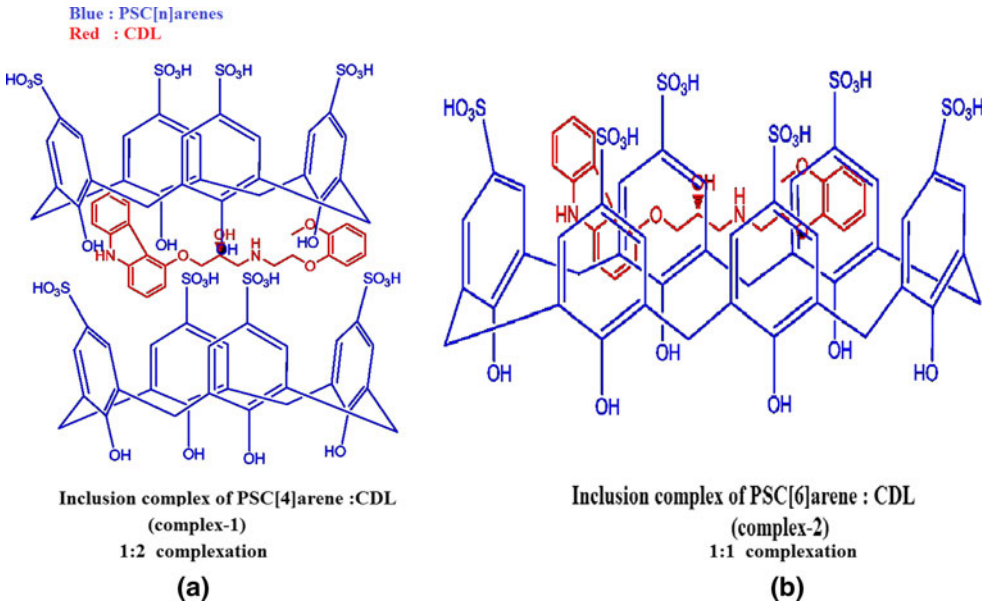

Fig. 1 Schematic proposal of the chemical structures of inclusion complex of: **a** PSC[4]arene: CDL and **b** PSC[6]arene : CDL

reagents and solvents used were of analytical grade. The 0.45 μm and 0.8 μm nylon filters (Millipore, Bedford, MA, USA) were used for filtration.

Preparation of solid inclusion complexes

The solid complexes CDL: PSC[4]arene (complex 1) and CDL:PSC[6]arene (complex 2) were prepared by rotary shaking. A molar ratio mixture of 1:1 CDL: PSC [6]arene (81:223 mg) and 1:2 CDL:PSC[4]arene (81: 297 mg) were taken into 250 mL stoppered conical flasks containing 150 mL distilled water. The solutions were shaken in a rotary shaker for 48 h at ambient temperature (27 °C) and then solution mixtures were filtered through 0.8 μm filter. The solutions were dried at 40 °C under vacuum (2–5 torr) to get solid complexes.

Preparation of physical mixture

To make the physical mixture, taken pure CDL:PSC[4] arene in 1:2 ratio which is physical mixture-1 (PM-1) and for physical mixture-2 (PM-2) we have taken pure CDL:PSC[6]arene in 1:1 ratio. Crushed both the mixtures well and characterized using various spectroscopic methods.

Phase solubility diagram

The phase solubility study of CDL with PSC[4]arene and PSC[6]arene were executed according to Higuchi and Connors [24]. Phase solubility studies were accomplished at 27 °C temperature, in triplicate. Excess quantity of CDL (250 mg) was added into different conical flasks containing 150 mL aqueous solutions of PSC[4]arene and PSC[6]arene at different concentrations. Flasks were then sonicated for 1/2 h and shaken at a rate of 100 strokes per min in orbital shaker incubator (Newtronic, India) for 48 h. The suspensions were then filtered out through 0.45 μm filter and assayed for CDL by using HPLC. The solubility of CDL was also determined in water at 27 °C by the same method. The association constant (Kc) for both the complexes formed was calculated from the slope of the phase solubility diagram and the solubility of CDL in water at 27 °C. The apparent stability constants of the complex 1 and complex 2 were respectively counted on from the slope and intercept of the straight lines of the phase-solubility diagrams, harmonizing to the following equation;

$$K_C = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (1)$$

where K_C is the stability constant and S_0 (intercept) is the intrinsic solubility of the chemical compound in the absence of complexing agent.

DSC

DSC analysis of samples was carried out employing a Shimadzu DSC 60. The samples (2.5–5.0 mg) were accurately weighed and sealed in aluminium pans and scanned at a heating rate of 10.0 °C min^{-1}. The aluminium sample pan having 50 μm thickness, was sealed and the samples were scanned from 25 °C up to 400 °C. Differential scanning calorimeter was adjusted with a heating rate of 10.0 °C and was calibrated with nitrogen purging at 30 mL min^{-1}. All samples CDL, PSC[4]arene, PSC[6]arene, complex 1, complex 2, PM-1 and PM-2 were examined.

PXRD

The PXRD analysis of both the complexes was executed by powder X-Ray diffractometer SEIFERT, FPM XRD-7 with Cu Kα radiation in transmission system mode. Each sample was set in the cavity of an aluminium sample holder flattened with a glass slide to present a proper surface texture and stuck in into the sample holder. The PXRD patterns of pure drug and both the complexes were recorded between $2\theta = 10$ and 45^{o} at tube power of 40 kV and 30 mA.

FT-IR spectroscopy

FTIR analysis of pure CDL, PSC[4]arene, PSC[6]arene, their complexes 1 and 2, PM-1 and PM-2 were recorded to assure the complexation. Samples were analysed in an FT-IR spectrometer Bruker, Tensor-27, USA using SCOPUS software. FT-IR spectra of all the samples were recorded by potassium bromide (KBr) disk method and scanned at the wave number region 4000–400 cm^{-1}.

^{1}H NMR study

^{1}H NMR spectra of PSC[4]arene, PSC[6]arene, CDL, complex 1 and for complex 2 were scanned on 400 MHz FT-NMR Bruker-Avance-400. The analysis range for the analysis is from 0.5 to 15 ppm. All the samples were dissolved in Dimethyl Sulphoxide (DMSO).

RP-HPLC analysis

HPLC system consisting CROMPACK ISOS isocratic pump with UV detector. It is manually injector (20 μL loop, RHEODYNE 7125, USA) with SPINCHROM software (ver. 2.4.1.93). The column was used for the separation was wakosil C_{18} (250 mm × 4.6 mm I.D., 5 μm particle size). The flow rate was 1.5 mL min^{-1}. The mobile phase consisting of water: MeOH (50:50% v/v) was filtered through 0.45 μm membrane filter. All the

measurements were carried out at ambient temperature (27 °C) and the wavelength of detector was set at 241 nm.

In vitro drug release from the complexes

In vitro dissolution profile was executed using USP dissolution apparatus type-II (USP-II). The complete dissolution of CDL, PSC[4]arene, PSC[6]arene, complex 1, complex 2, PM-1 and PM-2 were studied in 0.1 N of HCl (pH = 1.2) containing 0.1% w/v sodium lauryl sulphate in 900 mL at 37 ± 5 °C for both the complexes. The study was carried out at 37 ± 5 °C with a rotary motion of 50 rpm for 200 min [25]. A sample of physical mixture equivalent weight to 12.5 mg of CDL was employed in each test. At pre-set time intervals, 5 mL of the samples were accumulated by means of a syringe fitted with a pre-filter and the same volume of dissolution medium was added to maintain the sink condition. These samples were analyzed for drug content by evaluating the absorbance at 241 nm using UV Spectrophotometer (JASCO V-570). All the samples were analysed in triplicate.

In vivo studies

Acute oral toxicity (AOT) studies, for both the complexes, were carried out.

AOT studies (LD_{50})

AOT studies (average lethal dose) of both the complexes were determined using Swiss albino mice. The procedure followed is essentially the same as reported earlier by Menon et al. [22]. All the animals were abstained for 3 h anterior to the experimentation and were allotted with single dose of both the complexes. In this the dosage range of both the inclusion complexes are from 2500–12,000 mg/Kg dissolved in double distilled water and noticed for short condition toxicity for 48 h. The dose of following animal was determined as per Organisation for Economic Co-operation and Development (OECD) guideline 425. For both the complexes all the animals were also noticed for long-term toxicity for 14 days. The data of AOT study were analysed using "AOT425" software, which is provided by Environmental Protection Agency (EPA), USA.

Result and discussion

Phase solubility diagram

In the present work, complexation of CDL with PSC[4]arene and PSC[6]arene were carried out in an effort to improve its solubility and dissolution rate. The determination of CDL in phase solubility experiments was performed by HPLC. The phase solubility studies exposed a linear relationship (correlation coefficient > 0.994) amongst the aqueous drug solubility with enhancement in PSC[4]arene and PSC[6]arene concentration (Fig. 2) with the formation of soluble complexes. The slopes of the curves found in the regression analysis would suggest the formation of soluble complex of CDL: PSC[6]arene (slope 0.95) with 1:1 stoichiometry and CDL:PSC[4]arene (slope 0.47) with 1:2 stoichiometry (Fig. 3). The level of complexation was calculated based on the solubility plot. The stability constant Kc values were found to be $3.07 \times 10^4\ M^{-1}$ and $6.0 \times 10^5\ M^{-1}$ for complex 1 and complex 2 respectively. The values of the complexes were according to Higuchi and Connors phase solubility diagram [24]. It shows that the complex formed was effectively in stable form. This suggests a 1:1 and 1:2 stoichiometry of both the complexes. The solubility of CDL enhanced as a function of PSC[*n*]arene concentration.

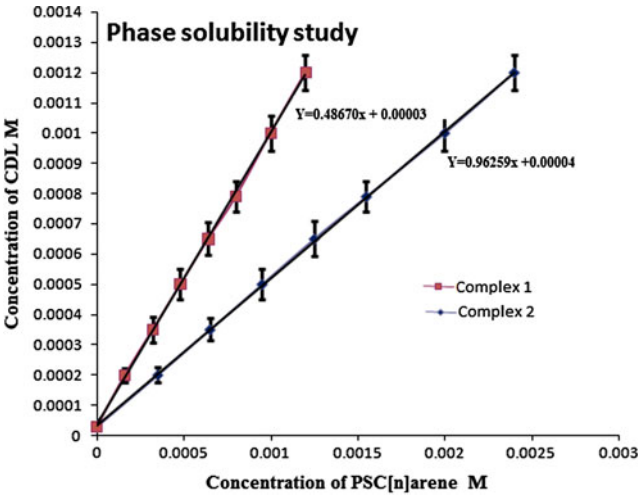

Fig. 2 Phase solubility diagram of PSC[4]arene, PSC[6]arene and CDL system in water at 27 °C. Each data point is the mean of three determinations

Differential Scanning Calorimetry (DSC)

DSC thermograms of pure CDL, PSC[4]arene, PSC[6]arene, complex 1, complex 2, PM-1 and PM-2 (Fig. 4) were taken. Out of these, the DSC thermogram of pure drug was characterized by a single, sharp melting endothermal peak at 114 °C throughout the analysis. Further, both the complexes accomplished vanishing of the all endothermic peaks which was available in CDL. It suggests that the complexation process really occurred and not the physical mixture [26].

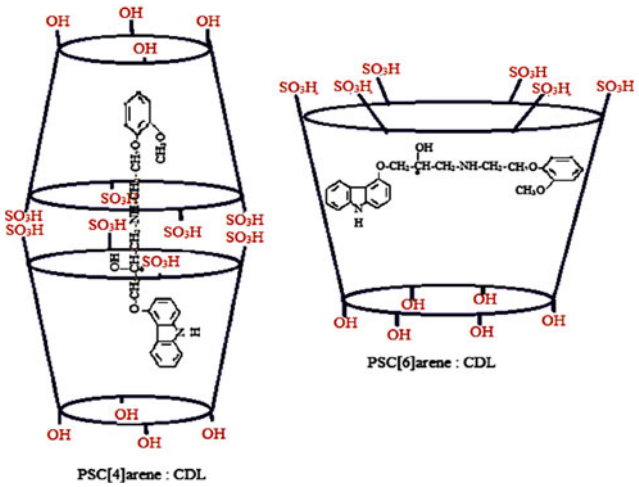

Fig. 3 Inclusion complex of PSC[4]arene and PSC[6]arene with CDL

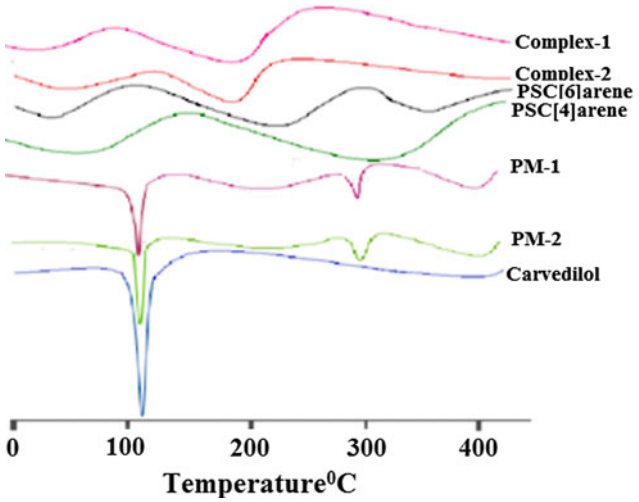

Fig. 4 DSC analysis of Complex-1, Complex-2, PSC[6]arene, PSC[4]arene, PM-1, PM-2 and CDL

FTIR spectral analysis

The FTIR data of the pure CDL, PSC[4]arene, PSC[6]arene, PM-1, PM-2, complex 1 and complex 2 are shown in Fig. 5. Pure CDL showed a peak at 3449.06 cm^{-1} for –NH stretching, 3273 cm^{-1} for –O–H bending of alcohols, 2940 cm^{-1} for C–H stretching of alkane, 1453 cm^{-1} for C=C aromatic stretching and 1020 cm^{-1} for C–H stretching.

PSC[4]arene, PSC[6]arene, PM-1 and PM-2 showed –O–H peaks at 3450, 3243 and 3230 cm^{-1}. The ν-SO_3 peaks were observed at 1185, 1210 and 1050 cm^{-1}. In the IR spectra of complexes the PSC[*n*]arene, main bands were found to overlap with the characteristic drug peaks because of low content of drug substance in both the complexes and

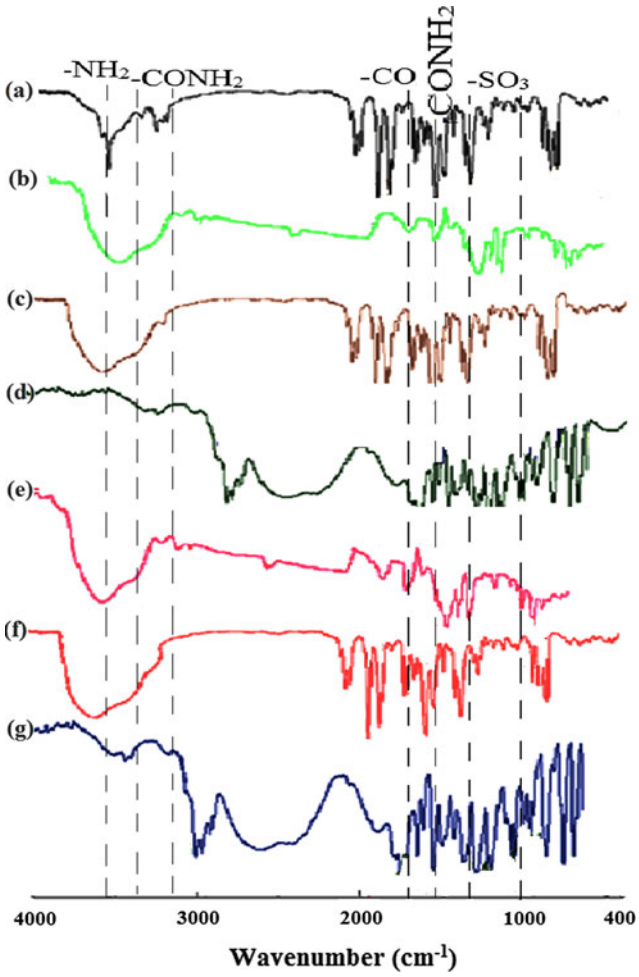

Fig. 5 FT-IR spectral analysis of (**a**) CDL (**b**) PSC[4]arene (**c**) PM-1 (**d**) Complex-1 (**e**) PSC[6]arene (**f**) PM-2 (**g**) Complex-2

also perhaps due to the inclusion of this part of the molecule inside the PSC structure.

The IR spectra of complex 1 and complex 2 did not show any new peak, demonstrating that no new chemical bonds were formed in both the complexes. However, the CDL characteristic peak at 3449 cm^{-1} for –NH stretching vanished because of overlapping of –OH peak of PSC[*n*]arene. Hence it could not be detected in the IR spectra of both the complexes 1 and 2. The peaks of νSO_3 at 1185, 1210 and 1050 cm^{-1} were changed over to lower wavelength to 1180, 1204 and 1040 cm^{-1}.

PXRD studies

The diffraction studies of CDL, PSC[4]arene and PSC[6]arene, PM-1, PM-2 and the solid complexes were carried out by PXRD (Fig. 6). The diffraction pattern of all the compounds showed sharp peaks. Pure CDL was in crystalline in nature which was clearly demonstrated by its characteristic PXRD pattern. The PXRD patterns of CDL

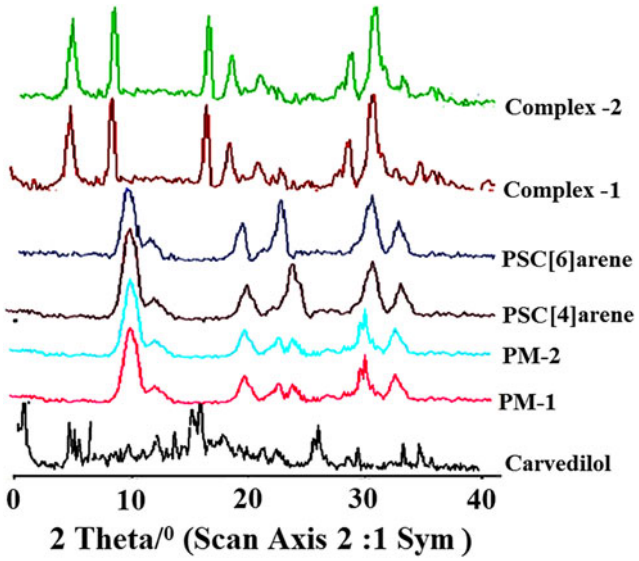

Fig. 6 PXRD data of Complex-2, Complex-1, PSC[6]arene, PSC[4]arene, PM-2, PM-1

show peaks at 2θ values 11.04, 13.26, 17.80, 18.38, 20.06, 23.16, 27.40, 33.20 and 36.04. The PXRD patterns of both PSC[4]arene and PSC[6]arene show the peaks at 12.03, 13.55, 19.19, 24.06, 30.16 and 34.10. PXRD diagram of physical mixtures demonstrate the superposition of both component, although the intensity of CDL pattern remarkably reduced. It indicates lower crystallinity of CDL compared to the pure CDL. Both the inclusion complexes did not show CDL peaks in XRD pattern, which could suggest that this drug is in complexed form. The decreasing in CDL crystallinity was responsible for the increase in solubility as previously reported [27]. Results of XRD support DSC results which show that inclusion complex was formed.

NMR analysis

Both the inclusion complexes were confirmed by nuclear magnetic resonance (NMR) also. ^{1}H NMR chemical shifts corresponding to carvediol in the absence and presence of PSC[4] and PSC[6]arene. In PSC[4]arene and PSC[6]arene there is an aromatic –OH peak and sulphonic acid peak appeared at 9.8 (S,8H, Ar–OH), 9.8 (S, 6H, Ar–OH) and 8.2 (s,8H, Ar–SO_3H), 8.30 (s, 6H, Ar–SO_3H) respectively. The study complex 1 and complex 2 showed a minor change in chemical shift, δ value, of the Ar–OH in the range of δ 10.1–10.3 and –SO_3H protons in the range of δ 8.4–8.6. The downfield shift in the protons of –OH & –SO_3H group can be assigned to the interaction of hydroxyl and sulphonic acid groups with the drug molecule. Thus NMR studies confirmed the results of the phase solubility studies that a 1:2 complex is formed between PSC[4]arene (complex-1) and 1:1 between drug and PSC[6]arene (complex-2) [28].

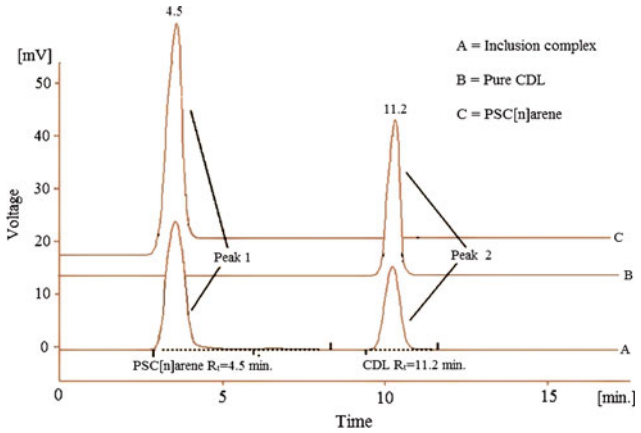

Fig. 7 RP-HPLC Chromatogram of PSC[*n*]arene (Peak 1) and CDL (Peak 2)

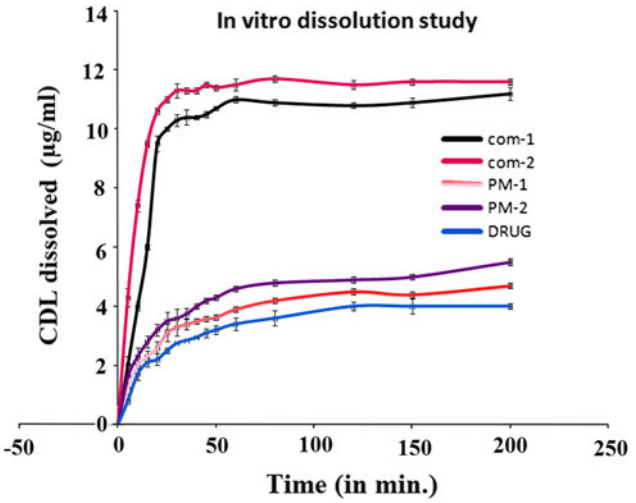

Fig. 8 In vitro release profile of CDL, PM-1, PM-2, Complex 1 and Complex 2

HPLC analysis

HPLC analyses of both the complexes were carried out by Isocratic reverse-phase HPLC method.

HPLC analysis of Inclusion complex (A), Pure CDL (B) and PSC[*n*]arene (C) showed in the Fig. 7. From the analysis the retention time of PSC[*n*]arene and for pure CDL appeared at 4.5 min and 11.2 min respectively. This same retention time appears in the Inclusion complex. Hence, from the RP-HPLC analysis it can be concluded that CDL was present in inclusion complex.

In vitro release study

The results of in vitro study for PM-1, PM-2, Complex 1, Complex 2 and pure CDL of drug dissolved (µg/mL) versus time was shown in Fig. 8. This figure shows a burst effect in the releasing profile especially for complex 2.

In vitro drug release study indicates 11.6 CDL dissolved (μg/mL) at the end of 200 min with complete release by the end of 4 h. From the figure both the complexes presented higher dissolution profile than pure CDL (Fig. 8). The increase in CDL dissolved from the PSC[*n*]arene comprising systems can be attributed to formation of a readily soluble complex in the dissolution medium [29]. Enhanced dissolution of CDL when physically mixed with PSC[*n*]arene may be due to an improved wettability of the drug molecules at the early phases of the dissolution process [30]. The dissolution study shows that the dissolution rate of CDL-PSC[4]arene and PSC[6]arene complexes was found to be higher than pure CDL.

The enhancement of rate of dissolution of the inclusion complex could be explicated from raise in solubility, and x-ray diffraction study and the amended wet ability of the drug by the inclusion complexation [31–37].

In vivo acute toxicity study

The in vivo toxicity study in mice was acquired using AOT425 software. The AOT of both the complexes, CDL, PM-1 and PM-2 were checked in Swiss albino mice. Animals were observed separately during first 30 min after dosing and thereafter for 48 h. There are no signals of toxicity in the animals treated with both the complexes. No death rate occurred during the limit test. No significant gross internal determinations were noticed at post mortem on study day 14. All the animals appeared normal by day 2 or earlier and throughout the remainder of the study. There was no change found in skin, fur, eyes, and mucous membranes and also respiratory, circulatory, autonomic and central nervous system (CNS). At the end of the test living animals were weighed. There was no crucial change in the body weight as compared to the initial weight on first day. CDL is reported to have an oral LD_{50} doses in male and female mice and male and female rats are over 8000 mg/Kg, [36] while the both complexes showed AOT value is 12000 mg/Kg.

Conclusion

In conclusion, the results of phase solubility, DSC, XRD confirmed the formation of inclusion complex. Reduction of crystallinity of drug was confirmed by DSC and XRD. X-Ray differactogram of complexes showed no change in the characteristic pattern of XRD; however, the peak intensity reduced in both the complexes. In DSC thermogram, no corresponding endotherms were present in complexes compared to physical mixture and pure drug. The FT-IR and ^{1}H NMR results show no chemical interaction (no new bond formation) between drug and PSC[*n*]arene. Because of reduced crystallinity dissolution of drug increases as compared to the physical mixture and pure drug which show an enhancement of aqueous solubility of CDL.

Acknowledgments Financial assistance from UGC, New Delhi is kindly acknowledged.

Appendix

See the Tables 1 and 2.

Table 1 AOT study of mice in both the complexes

Animal	Time (h)	Complex 1 dose (mg kg^{-1})	Complex 2 dose (mg kg^{-1})	Survival
1	12	2500		√
	24			√
	36			√
	48			√
2	12	5000		√
	24			√
	36			√
	48			√
3	12	7500		√
	24			√
	36			√
	48			√
4	12	10,000		√
	24			√
	36			√
	48			√
5	12	12,500		×
	24			–
	36			–
	48			–
6	12	12,000		√
	24			√
	36			√
	48			√

√ Animal survive, × animal not survive

Table 2 In vitro release data of pure CDL, PM-1, PM-2, complex-1 and for complex-2

Time (min)	CDL (μg/mL)	PM-1 (μg/mL)	PM-2 (μg/mL)	Complex-1 (μg/mL)	Complex-2 (μg/mL)
0	0	0	0	0	0
5	0.8	1.2	1.6	2	4.3
10	1.7	2	2.3	4	7.4
15	2.1	2.3	2.8	6	9.5

Table 2 continued

Time (min)	CDL (μg/mL)	PM-1 (μg/mL)	PM-2 (μg/mL)	Complex-1 (μg/mL)	Complex-2 (μg/mL)
20	2.2	2.6	3.2	9.5	10.6
25	2.5	3.1	3.5	10	11
30	2.75	3.3	3.6	10.3	11.3
35	2.85	3.4	3.75	10.4	11.3
40	2.95	3.5	4	10.4	11.3
45	3.1	3.58	4.2	10.5	11.5
50	3.2	3.63	4.3	10.7	11.4
60	3.4	3.9	4.6	11	11.5
80	3.6	4.2	4.8	10.9	11.7
120	4	4.5	4.9	10.8	11.5
150	4	4.4	5	10.9	11.6
200	4	4.7	5.5	11.2	11.6

References

1. Loftsson, T., Jarho, P., Masoon, M., Jarvinen, T.: Cyclodextrins in drug delivery. Expert Opin. Drug Deliv. **2**, 335–351 (2005)
2. Xian, J., Loh, J.: Cyclodextrin-based supramolecular architectures: syntheses, structures, and applications for drug and gene delivery. Adv. Drug Deliv. Rev. **60**, 1000–1017 (2008)
3. Gutsche, C., Dhawan, B., Kwang, H., Muthukrishnan, R.: Calixarenes. 4: the synthesis, characterisation and properties of the calixarenes from *p-tert* butylphenol. J. Am. Chem. Soc. **103**(13), 3782–3792 (1981)
4. Gutsche, C., Stoddart, C.: Calixarenes Revisited Monographs in Supramolecular Chemistry. The Royal Society of Chemistry, Cambridge (1998)
5. Vicens, J., Bohmer, V.: Calixarenes: A Versatile Class of Macrocyclic Compounds. Kluwer, Dordrecht (1991)
6. Perret, F., Lazar, A., Coleman, A.: Biochemistry of the *para*-sulfonato-calix[*n*]arenes. Chem. Commun. 2425–2438 (2006)
7. Antman, E.: Cardiovascular therapeutics: a companion to Braunwald's heart disease, pp. 99, 100 (2009)
8. Zhao, M., Wang, H., Yang, B., Tao, H.: Identification of cyclodextrin inclusion complex of chlorogenic acid and its antimicrobial activity. Food Chem. **120**, 1138–1142 (2010)
9. Ficarra, R., Ficarra, P., Di Bella, M., Raneri, D., Tommasini, S., Calabro, M., Gamberini, M.: Study of b-blockers: b-cyclodextrins inclusion complex by NMR, DSC, X-ray and SEM investigation. J. Pharm. Biomed. Anal. **23**, 33–40 (2000)
10. Jun, S., Kim, M., Kim, J., Park, H., Lee, S., Woo, J., Hwang, S.: Preparation and characterization of simvastatin/hydroxypropyl-b-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process. Eur. J. Pharm. Biopharm. **66**, 413–421 (2007)
11. Sapkal, N., Kilor, V., Bhusari, K., Daud, A.: Evaluation of some methods for preparing gliclazide-β-cyclodextrin inclusion complexes. Trop. J. Pharm. Res. **6**(4), 833–840 (2007)
12. Ganapathy, H., Lee, M., Park, C., Lim, K.: Sustained release applications of a fluoroalkyl ester-functionalized amphiphilic cyclodextrin by inclusion complex formation with water-soluble drugs in supercritical carbon dioxide. J. Fluor. Chem. **129**, 1162–1166 (2008)
13. Lu, Y., Zhang, X., Lai, J., Yin, Z., Wua, W.: Physical characterization of meloxicam-cyclodextrin inclusion complex pellets prepared by a fluid-bed coating method. Particuology **7**, 1–8 (2009)
14. Leea, C., Kima, S., Youna, Y., Widjojokusumoa, E., Leea, E., Kimb, J., Leea, Y., Tjandrawinatac, R.: Preparation of bitter taste masked cetirizine dihydrochloride/cyclodextrin inclusion complex by supercritical antisolvent (SAS) process. J. Supercrit. Fluids **55**, 348–357 (2010)
15. Dóris de Castroa, A., Silva Jr, N., Curya, B., Evangelistaa, R., Freitas, L., Gremiãoa, M.: A new approach to the granulation of cyclodextrin inclusion complexes. Chem. Eng. J. **164**, 316–321 (2010)
16. Wen, X., Tan, F., Jing, Z., Liu, Z.: Preparation and study the 1:2 inclusion complex of CDL with cyclodextrin. J. Pharm. Biomed. Anal. **34**, 517–523 (2004)
17. Chaulmaot, G., Yao, C., Pino, V., Anderson, J.: Determining the stoichiometry and binding constants of inclusion complexes formed between aromatic compounds and β-cyclodextrin by solid-phase microextraction couple to high-performance liquid chromatography. J. Chromatogr. A **1216**, 5242–5248 (2009)
18. Shewale, B., Sapkal, N., Raut, N., Gaikwad, N., Fursule, R.: Effect of hydroxypropyl-β-cyclodextrin on solubility of carvedilol. Indian J. Pharm. Sci. **70**(2), 255–257 (2008)
19. Liu, Y., Han, B., Chen, Y.: Inclusion complexation of acridine red dye by calixarenesulphonates and cyclodextrins: opposite fluorescent behaviour. J.Org. Chem. **65**, 6227–6230 (2000)
20. Arimura, T., Nagasaki, T., Shinkai, S., Matsuda, T.: Thermodynamic study of *p*-sulfonated calixarene complexes in aqueous solution. J. Org. Chem. **54**, 3766–3768 (1989)
21. Panchal, J., Patel, R., Menon, S.: Preparation and physicochemical characterization of carbamazepine (CBMZ): *para*-sulfonated calix[*n*]arene inclusion complexes. J. Incl. Phenom. Macrocycl. Chem. **67**(8), 201–208 (2010)
22. Menon, S., Modi, N., Mistry, B., Joshi, K.: Improvement of some pharmaceutical properties of mycophenolate mofetil (MMF) by *para* sulphonatocalix[4]resorcinarene inclusion complex. J. Incl. Phenom. Macrocycl. Chem. **70**, 121–128 (2011)
23. Shinkai, S., Mori, S., Koreishi, H., Tsubaki, T., Manabe, O.: Hexasulfonated calix[6]arene derivatives: a new class of catalysts, surfactants, and host molecules. J. Am. Chem. Soc. **108**(9), 2409–2416 (1986)
24. Higuchi, T., Connors, K.: Phase-solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
25. Swamy, P., Shilpa, H., Shirsand, S., Gada, S., Kinagi, M.: Role of cogrinding in enhancing the *in vitro* dissolution characteristics of carvedilol. Int. J. Pharma Sci. Res. **1**(5), 232–237 (2010)
26. Bhutani, S., Hiremath, S., Swamy, P., Raju, S.: Preparation and evaluation of inclusion complexes of carvedilol. J. Sci. Ind. Res. **66**, 830–834 (2007)
27. Eugene, F.F., Timothy, A.H.: In: Lachmann, L., Liberman, H.A., Kanig, J.L. (eds.) The Theory and Practice of Industrial Pharmacy, 3rd edn, p. 171. Varghese Publishing House, Mumbai (1987)
28. García-Sosa, I., María Ramírez, F.: Synthesis, solid and solution studies of paraquat dichloride calixarene complexes. molecular modelling. J. Mex. Chem. Soc. **54**(3), 143–152 (2010)
29. Vogel, H., Vogel, W.: Drug discovery and evaluation: pharmacological analysis. Springer, Berlin (1997)
30. Mohamed, A., Hussein, A., Bhathena, S., Hafez, Y.: The effect of dietary menhaden olive and coconut oil fed with three levels of vitamin E on plasma and liver lipids and plasma fatty acid composition in rats. J. Nutr. Biochem. **13**, 435–441 (2002)
31. Chadha, R., Saini, A., Gupta, S., Arora, P., Thakur, D., Jain, D.: Encapsulation of rifampicin by natural and modified b-cyclodextrins: characterization and thermodynamic parameters., J.

Incl. Phenom. Macrocycl. Chem. 67:1–2, 109–116. doi: 10.1007/s10847-009-9682-y
32. Araujo, D., Tsuneda, S., Cereda, M., Gel, F., Carvaiho, G., Prete, P., Fernandes, S., Yokaichiya, F., Franco, M., Mazzaro, I., Fraceto, L., De, A., Braga, F., Paula, E.: Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-beta-cyclodextrin inclusion complex. Eur. J. Pharm. Sci. **33**, 60–71 (2008)
33. Catarina, M., Rui, A., Pereira, D., Francisco, J.: Multimodal molecular encapsulation of nicardipine hydrochloride by b-cyclodextrin, hydroxypropyl-b-cyclodextrin and triacetyl b-cyclodextrin in solution. Structural studies by ^{1}H NMR and ROSEY experiments. Eur. J. Pharm. Sci. **18**, 285–296 (2003)
34. Nalawade, P., Aware, B., Dand, N., Kadam, V., Hirlekar, R.: Solid state characterization of the inclusion complex of valsartan with methyl b-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. **65**, 377–383 (2009)
35. Kadam, V., Gattani, S.: Dissolution enhancement of carvedilol by using surfactant as a coating material. Asian J. Pharm. Clin. Res. **2**, 3 (2009)
36. Murthy, T., Sowjanya, G.: Development of discriminatory method for dissolution of carvedilol marketed formulations. Int. J. ChemTech Res. **2**, 1047–1050 (2010)
37. http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/020297s013lbl.pdf. NDA 20-297/S-013 (2005)