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ORIGINAL ARTICLE

Preparation, characterization and pharmacodynamic activity of supramolecular and colloidal systems of rosuvastatin–cyclodextrin complexes

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Abstract In the present study influence of nature of selected cyclodextrins (CDs) and of methods of preparation of drug–CD complexes on the oral bioavailability, in vitro dissolution studies and pharmacodynamic activity of a sparingly water soluble drug rosuvastatin (RVS) was investigated. Phase solubility studies were conducted to find the interaction of RVS with β-CD and its derivatives, which indicated the formation of 1:1 stoichiometric inclusion complex. The apparent stability constant ($K_{1:1}$) calculated from phase solubility diagram were in the rank order of β-CD < hydroxypropyl-β-cyclodextrin (HP-β-CD) < randomly methylated-β-cyclodextrin (RM-β-CD). Equimolar drug–CD solid complexes prepared by different methods were characterized by the Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). FTIR study demonstrated the presence of intermolecular hydrogen bonds and ordering of the molecule between RVS and CDs in inclusion complexes. DSC and XRD analysis confirmed formation of inclusion complex by freeze dried method with HP-β-CD and RM-β-CD. Aqueous solubility and dissolution studies indicated improved dissolution rates of prepared complexes in comparison with drug alone. Moreover, CD complexes demonstrated of significant improvement in reducing total cholesterol and triglycerides levels as compared to pure drug. However the in vivo results only partially agreed with those obtained from phase solubility studies.

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Keywords Antihyperlipidemic · β-CD · Complexation · HP-β-CD · RM-β-CD · Supramolecular

Abbreviations

CDs	Cyclodextrins
RVS	Rosuvastatin
β-CD	β-cyclodextrin
HP-β-CD	Hydroxypropyl-β-cyclodextrin
RM-β-CD	Randomly methylated-β-cyclodextrin
KND	Kneading
PM	Physical mixtures
COEVP	Coevaporation
FZD	Freeze dried

Introduction

Rosuvastatin (RVS) is a lipid-lowering statin [1, 2]. It is a selective, potent and competitive inhibitor of enzyme 3-hydroxy-3 methyl glutaryl Coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMG-CoA to mevalonate a precursor of cholesterol and thereby checks the synthesis of cholesterol [3]. RVS is sparingly soluble in water and has low absolute bioavailability [4]. However, it has a partition coefficient (octanol/water) of 0.13 at pH of 7.0. Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability.

The versatile pharmaceutical material cyclodextrin's (CDs) are classified into hydrophilic, hydrophobic, and ionic derivatives, belonging to family of family of the macrocyclic oligosaccharides [5]. The CD structure provides a molecule shaped like a segment of a hollow cone with an exterior hydrophilic surface and interior electron-

rich hydrophobic cavity [6]. This molecule is capable of forming stable, supramolecular structures with various molecules, fitting partially or completely in the host molecular cavity [7, 8].

Complexation of pharmaceutical compounds with CDs leads to alteration of physical, chemical and biological properties of guest molecules. The main advantages in the pharmaceutical use of CDs are the increase in solubility, improved stability and bioavailability of drugs [9]. Both the nature of the CD (native or chemically modified, crystalline or amorphous) and the method of complexation may play a role in drug solubilization [10]. Therefore, it seemed of interest to extend our investigation to prepare supramolecular and colloidal complexes from different methods for RVS with crystalline native β-CD (β-Cd) and its amorphous and highly soluble derivatives, hydroxypropyl-β-CD (HP-β-Cd) and randomly methylated-β-CD (RM-β-Cd).

Materials and methods

Materials

RVS was obtained as a kind gift; β-CD, HP-β-CD and RM-β-CD were gifted by Roquette (Lestrem, France). All other reagents and solvents were of analytical grade (with purity >99.5 %) and thus were used without any further purification. HPLC grade methanol (Merck, Mumbai, India) was used as received.

Methods

Phase solubility studies

Phase solubility studies of RVS with selected CD derivatives (β-CD, HP-β-CD and RM-β-CD) were carried out according to Higuchi and Connors [11]. RVS in excess of its solubility was weighed into a series of screw-capped vials containing aqueous solutions of CDs of concentrations ranging from 0 to 10 mM/l. The sealed vials were agitated and equilibrated on a rotary shaker for 48 h at room temperature (30 ± 1 °C). The clear supernatant was passed through 0.45 μm Millipore filter. The drug content of samples after suitable dilution with methanol was determined spectrophotometrically (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan) at λ_{max} of 244 nm. Methanol was used as blank solution for analysis of samples. The presence of CD derivatives did not interfere with the assay. The apparent stability constant ($K_{1:1}$) was calculated from the initial straight portion of the phase solubility diagram using the following equation:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{slope})}$$

where, S_0 is the intrinsic solubility of drug in the absence of CD derivatives at 30 ± 1 °C.

Preparation of RVS–CD supramolecular and colloidal complexes

The supramolecular and colloidal complexes of RVS with β-CD, HP-β-CD and RM-β-CD were prepared in 1:1 molar ratio of respective simple components (drug and CDs) by physical mixture (PM), kneading (KND), coevaporation (COEVP) and freeze drying (FZD) methods [12, 13].

Characterization of RVS–CD supramolecular and colloidal complexes

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure RVS, β-CD, HP-β-CD, RM-β-CD, RVS–CDs PMs and complexes (in 1:1 molar ratio) were monitored as KBr disc using a Shimadzu FTIR (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan) for comparison. The scans were executed at a resolution of 8 cm^{-1}, from 4,500 to 500 cm^{-1}.

Differential scanning calorimetry (DSC)

The stability and thermal behavior of RVS and its prepared supramolecular and colloidal complexes with β CD, HP-β-CD and RM-β-CD were traced by DSC. DSC was carried out in the temperature range of 50–180 °C in a stream of nitrogen atmosphere on Shimadzu-Thermal Analyzer DT 40. The accurately weighed sample was placed in an aluminum pan, and an empty aluminum pan was used as reference. The scanning rate was 10 °C/min and the nitrogen flow was 50 ml/min.

X-ray diffraction (XRD)

X-ray powder diffraction patterns were taken at ambient temperature and were obtained with a Philips diffractometer (PW 1729, Eindhoven, Netherlands). The samples were irradiated with monochromatized Cu K_α radiation and were analyzed in the 2θ angle range of 3–40° and the process parameters were set as: scan step size of 0.02° scan step time of 1.54 s. The voltage and current used were 40 kV and 36 mA, respectively.

Drug content

The RVS–CDs supramolecular and colloidal complexes prepared by various methods viz. PM, KND, COEVP and FZD were assayed for RVS content by dissolving a specific amount of complex in suitable quantity of methanol and analyzing spectrophotometrically at 244 nm by UV–Vis spectrophotometer (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan).

Aqueous solubility

An excess amount of RVS and RVS–CDs supramolecular and colloidal complexes was added to 5 ml of the 0.05 M citrate buffer (pH 6.6) in test tubes sealed with stoppers. The test tubes were vortex-mixed for 5 min and then sonicated for 30 min. They were kept in a constant-temperature shaking bath maintained at 30 ± 1 °C until reaching equilibrium. A portion of solution was withdrawn and then filtered using a 0.45 μm Millipore filter and adequately diluted with methanol. The drug concentration was determined at 244 nm by UV–Vis spectrophotometer (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan).

In vitro dissolution studies

Dissolution studies of RVS and supramolecular colloidal complexes of RVS–CDs were performed using USP paddle type II dissolution test apparatus at 37 ± 0.5 °C using 900 ml dissolution medium (0.05 M citrate buffer pH 6.6 as per U.S. Food and Drug Administration) and 50 rpm for 180 min as dissolution medium. Aliquots, each of 5 ml, from the dissolution medium were withdrawn at suitable time intervals and replenished by an equal volume of fresh dissolution medium. The withdrawn samples were filtered, suitably diluted and analyzed for RVS content by measuring its absorbance at λ 244 nm. The results are presented as mean ± SD of triplicate experiments. The resulting dissolution curves were analyzed in terms of the dissolved drug percentage (DP_t) and the dissolution efficiency (DE_t) according to the equation:

$$DE_t = \frac{\int_0^t D\,dt}{D100\,t} \times 100$$

where D is the percentage of the dissolved drug at time t and $D100t$ is the area of the rectangle corresponding to a total dissolution (100 %) at the same time [14, 15].

Pharmacodynamic activity

Experimental protocol

In order to measure the antihyperlipidemic effect, male Sprague–Dawley rats of 16–19 weeks age, weighing 180–200 g was selected for the study. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28 ± 0.2 °C, humidity 60–70 %, 12 h light/dark cycle). The rats were allowed to acclimatize environment for 48 h and supplied with a standard pellet diet and water ad libitum. After acclimatization the animals were randomly distributed into four groups of six animals each. The treatment was given for 15 days. Each group daily received cholesterol diet and butter (1 ml twice a day) using gavage-feeding needles. After feeding, standard and test groups were administrated orally 3 ml of 2 % w/v gum acacia aqueous suspensions containing RVS, RVS–HP-β-CD FZD complex [RVS–HP-β-CD (FZD)] and RVS–RM-β-CD FZD complex [RVS–RM-β-CD (FZD)] equivalent to 5 mg/kg body weight respectively. Also, control group received daily 3 ml of 2 % w/v gum acacia solution containing equivalent amount of CD as in formulation via. oral administration. All treatments were continued for 15 day. Blood samples were collected at 0th day, after 7 days and after 15 days following an overnight fast. Blood samples were collected under light ether anesthesia by retro-orbital puncture into heparinized tubes and plasma was obtained by immediate centrifugation of blood samples using cooling centrifuge at 3,000 rpm (Remi, India) for 5 min at room temperature. Serum samples were analyzed for total cholesterol (TC) and triglyceride (TG) using commercial diagnostics kits.

Group I	Control group of hyperlipidemic rats received a dose of 3 ml of 2 % w/v gum acacia solution containing equivalent amount of CD as in formulation.
Group II	Standard group of hyperlipidemic rats received powder RVS in suspension form (5 mg/kg).
Group III	Hyperlipidemic rats treated with RVS–HP-β-CD (FZD) (5 mg/kg).
Group IV	Hyperlipidemic rats treated with RVS–RM-β-CD (FZD) (5 mg/kg).

Statistical analysis

The results were expressed as mean ± SD. Statistical comparisons Groups I, II, III and IV were carried out using

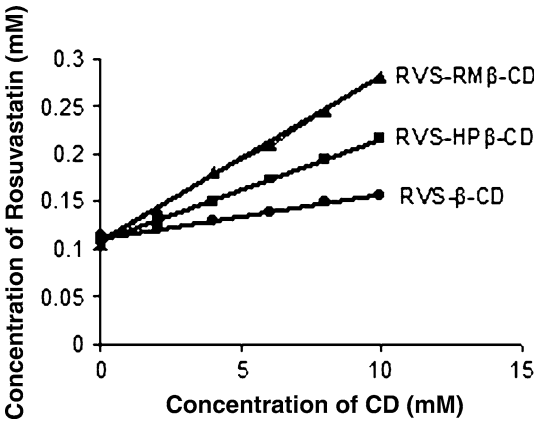

Fig. 1 Phase solubility diagram of RVS with various CDs

one-way ANOVA. Differences below $P < 0.05$ implied statistically significance [16].

Results and discussion

Phase solubility studies

This study allowed us to follow the inclusion phenomena and evaluate the apparent stability of the complexes. The phase solubility diagram of RVS as a function of β-CD and its selected modified derivatives (HP-β-CD and RM-β-CD) concentration at room temperature (30 ± 1 °C) is shown in Fig. 1.

The aqueous solubility of RVS was increased linearly as a function of the concentration range studied for parent molecule β-CD and its selected modified derivatives (HP-β-CD and RM-β-CD). The phase solubility diagrams of RVS with β-CD, HP-β-CD and RM–β-CD complexes can be classified as type A_L according to Higuchi and Connors [11]. The slope of phase solubility diagrams was less than 1 in all the cases, indicating that the inclusion complexes could be of the first order with respect to the CDs (1:1 stoichiometry). The increase in solubility was may be due to the formation of a 1:1 supramolecular and colloidal complex in solution with selected CDs. However increase in solubility with β-CD was very minute or less. Stability constant ($K_{1:1}$) values obtained for RVS–CDs complexes were in the rank order of β-CD (354 M^{-1}), HP-β-CD (884 M^{-1}) and RM-β-CD (1,429 M^{-1}). The values of ($K_{1:1}$) indicated that the supramolecular and colloidal complexes formed between RVS and selected CDs are quite stable. In fact, values of stability constants within the range of 100–1,000 M^{-1} are believed to indicate an ideal value [17]. Actually, smaller values of ($K_{1:1}$) indicate a too weak interaction between drug and CD, while larger values are symptomatic of an incomplete drug release from the inclusion complex.

Characterization of RVS–CD complexes

Fourier transform infrared spectroscopy

The FTIR spectra of RVS reveals the presence of characteristic peaks at 3,400 cm^{-1} due to (–OH) stretching, at 1,700 cm^{-1} due to (–C=O) bond stretching, at 1,380–1,650 cm^{-1} corresponding to (C–N) bond stretching and aliphatic (–CH) stretching shows peak at 2,967 cm^{-1}. The FTIR spectra of the all the PMs (RVS–β-CD, RVS–HP-β-CD and RVS–RM-β-CD) corresponds simply to superposition of FTIR spectra of pure RVS and respective CD. However the FTIR spectra's of RVS–CDs prepared by KND, COEVP and FZD method shows considerable differences with those of their corresponding constitutes. The inclusion complexes of RVS–β-CD shows decrease broadness of (–OH) group stretching, because a decrease in frequency of a specific peak is generally seen on complexation, which indicates on ordering of the molecule [18, 19]. The peak broadening may also be due to utilization of hydrogen atom for intermolecular bond formation.

In case of RVS–HP-β-CD complexes (KND, COEVP and FZD) FTIR spectra demonstrate increase in broadband at the region of 3,300–3,400 cm^{-1} due to hydrogen bonding as compared to plain HP-β-CD spectra. FTIR spectra's also reveals decrease peak intensity of aliphatic (–CH) stretching below 3,000 cm^{-1} along with decrease in peak intensity of (–C–O–C) bond.

However in case of RM–β-CD complexes (KND, COEVP and FZD) FTIR spectra shows peaks at 3,300 and 3,400 cm^{-1} region along with peak broadening due to hydrogen bonding. The two bands at the region of 1,415 and 1,000 cm^{-1} show the presence of SO_2 group of the drug though the peak intensity is decreased due to complexation. FTIR spectra's of RM-β-CD also shows a shift of peak of –C–O–C group. These modifications clearly indicate the presence of host–guest interactions and suggest the formation of stable hydrogen bonds between RVS and selected CDs.

Differential scanning calorimetry (DSC)

When guest molecules were embedded into CDs cavities, their melting, boiling or sublimating points generally shifted to different temperatures or disappears [20]. The thermal behavior of pure components (RVS, β-CD, HP-β-CD and RM-β-CD) and of the different equimolar (1:1 molar ratio) prepared complexes PM, KND, COEVP and FZD was studied using DSC are presented in Figs. 2, 3, 4.

DSC scan of RVS shows a characteristic endothermic peak at 129 °C, which represents its melting point. The DSC scans of pure CDs showed endothermic peaks associated with loss of water at 100–120 °C for β-CD, 70 and

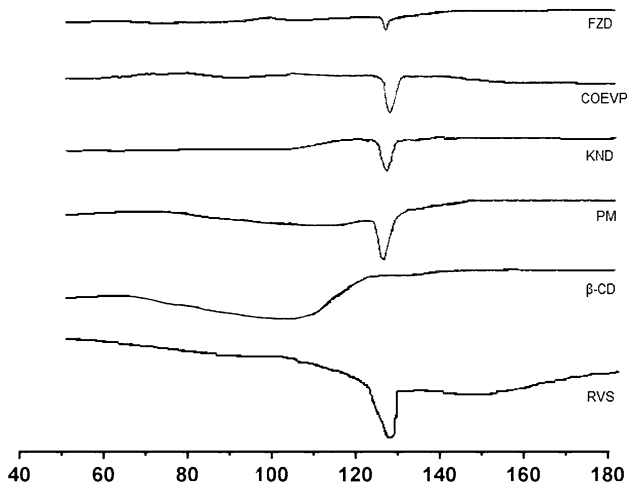

Fig. 2 DSC scans of RVS and RVS–β-CD complexes prepared by PM, KND, COEVP and FZD method

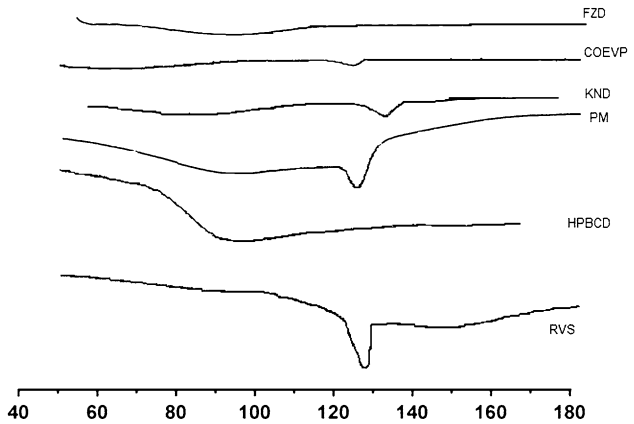

Fig. 3 DSC scans of RVS and RVS–HP-β-CD complexes prepared by PM, KND, COEVP and FZD method

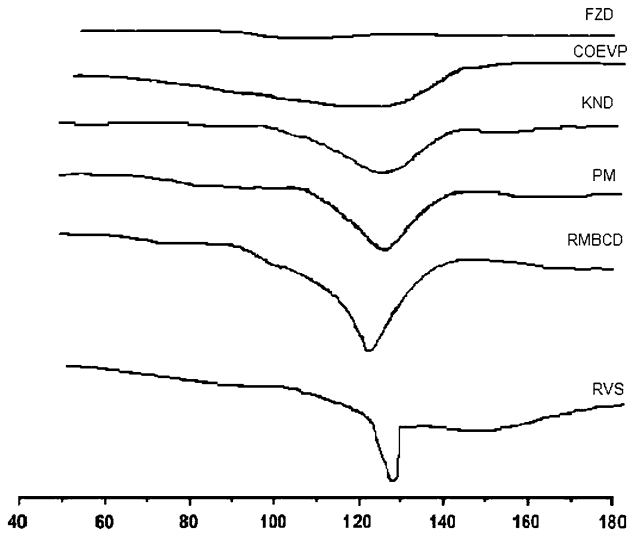

Fig. 4 DSC scans of RVS and RVS–RM-β-CD complexes prepared by PM, KND, COEVP and FZD method

100 °C for HP-β-CD, 90–140 °C for RM-β-CD. The DSC scans of PMs of RVS with all the selected CDs showed endothermic peak at 129 °C, which is characteristic RVS or peak nearly identical to that of pure RVS along with a broad peak of respective CDs. This indicates that no complexation. In kneaded, co-evaporated and FZD complexes of RVS–β-CD (Fig. 2) the endothermic peak of the drug was still recognizable. However, peak intensity is strongly reduced in FZD complexes, which may be attributed as a consequence of interaction between the components (RVS and β-CD) [21]. Total disappearance of RVS endothermic peak was not observed in any of the complexes with β-CD. This phenomenon is generally considered as indicative weak interaction in the solid state between RVS and β-CD.

The DSC scans of RVS–HP-β-CD inclusion complexes prepared by (KND and COEVP, Fig. 3) also showed the presence of peaks of both pure compounds with the difference that the peak intensity (or height) was strongly reduced in case of COEVP and the drug melting endothermic peak had slightly shifted from its original position of 129–134 °C in case of KND complex. Thus the results indicate that certain fraction of RVS molecule was included into the HP-β-CD cavity**.** However, no endothermic peak other that indicating the water loss from sample was noted in the FZD complex suggesting, the molecular encapsulation of the drug inside the HP-β-CD cavity [22, 23].

The DSC scans of RVS–RM-β-CD complex prepared by KND (Fig. 4) showed a broad peak while in complex prepared by COEVP (RVS–RM-β-CD (COEVP)), Fig. 4) the drug endothermic peak shifted from 129 to 138 °C with significant decrease in peak intensity. This shift of peaks indicated that there was an interaction between the two compounds. In RVS–RM-β-CD complex prepared by FZD (RVS–RM-β-CD (FZD), Fig. 4) disappearance of the endothermic peak assigned to the RVS at 129 °C indicates that the maximal/complete inclusion complex have been formed during processing.

Total disappearance of drug thermal profile was observed in the FZD complexes with HP-β-CD and RM-β-CD. This phenomenon is generally considered as indicative of complex formation/drug amorphization and/or stronger interaction in the solid state between RVS–HP-β-CD and RM-β-CD. The negative values of enthalpy changes (data not shown) indicate that the interaction processes of RVS with selected CDs are exothermic.

X-ray diffractometry

The XRD patterns of pure components and of the different equimolar (1:1 molar ratio) prepared complexes are shown in Figs. 5, 6, 7. The XRD pattern of RVS shows intense

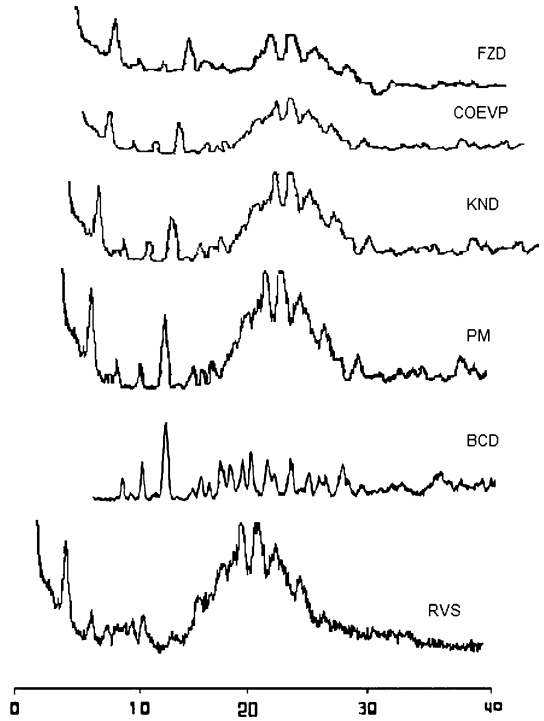

Fig. 5 X-ray *diffractogram* of RVS and RVS–β-CD complexes prepared by PM, KND, COEVP and FZD method

peaks at 4.6, 19.8, and 22.3. X-ray diffractogram of the PMs of RVS with all the selected CDs consisted of the superimposed figures of each of the pure components with the peaks of RVS being attenuated due to dilution and particle size reduction during mixture. Also, diffraction peaks relevant to RVS were detectable in all the complexes with β-CD (Fig. 5) while in case of FZD complexes RVS–β-CD and KND complexes of RVS with HP-β-CD and RM-β-CD peak intensity was reduced to certain extent, indicating no or little complexation which may be due to weak ionic interactions.

However, coevaporated complex of RVS with HP-β-CD (Fig. 6) showed undefined, broad, diffused peaks with low intensities. Though this signifies an amorphous nature of prepared complex, a few sharp peaks having less intensity were also observed. Instead the coevaporated complex of RVS with RM-β-CD showed peaks of diminished intensity suggesting almost complete amorphization of the drug (Fig. 7), indicating the existence of molecular interactions between the two species.

Complete drug amorphization was observed in both FZD complexes of RVS with HP-β-CD and RM-β-CD Figs. 6, 7. The diffraction pattern of the FZD complexes showed a single very broad band in which the diffraction peaks of the drug and CDs disappeared. This phenomenon confirmed that an inclusion complex between drug and CDs (HP-β-CD and RM-β-CD) was formed.

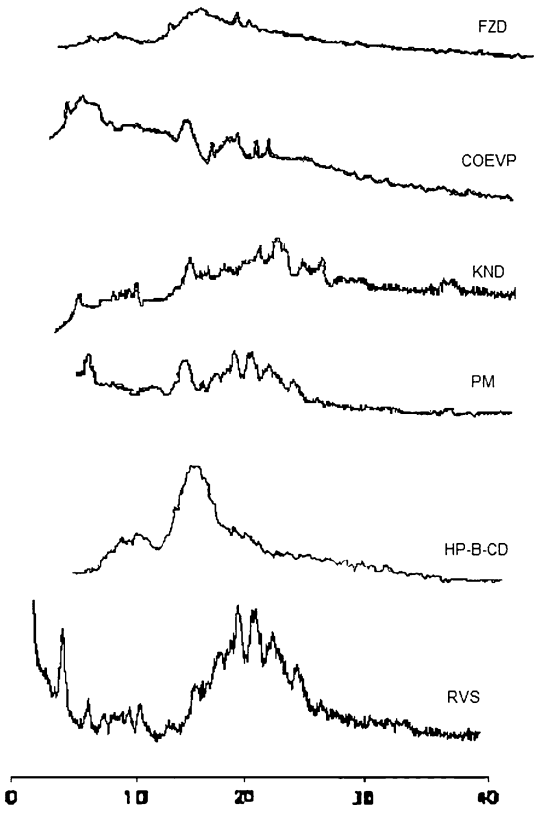

Fig. 6 X-ray *diffractogram* of RVS and RVS–HP-β-CD complexes prepared by PM, KND, COEVP and FZD method

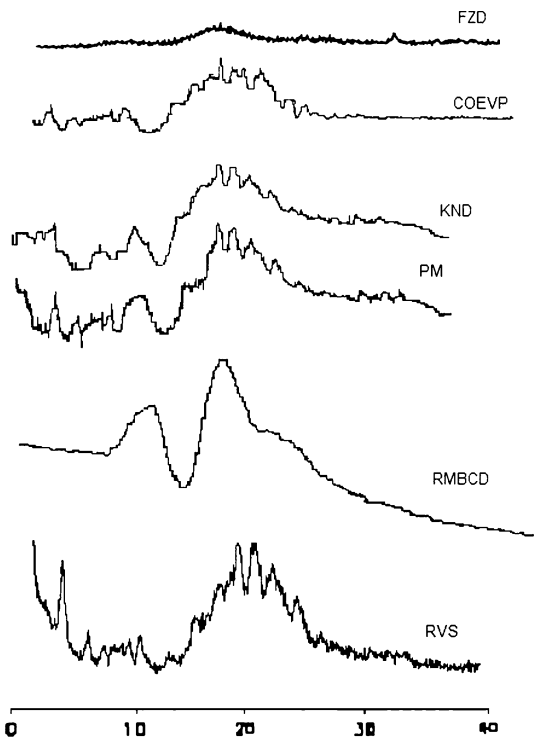

Fig. 7 X-ray *diffractogram* of RVS and RVS–RM-β-CD complexes prepared by PM, KND, COEVP and FZD method

Drug content and aqueous solubility

The actual drug content of each prepared complexes are reported in Table 1. The RVS–β-CD complex prepared by physical method showed least (15 ± 2 %) while RVS–RM-β-CD complex prepared by FZD method showed highest (94 ± 2 %) percent drug content. It is obvious from results that there is no significant increase in percent drug content for complexes prepared by using β-CD by PM, KND and COEVP method except slight increase (approx 6 %) in case of FZD complex (RVS–β-CD (FZD), Table 1). This slight increase may be due to weak ionic interaction between components in solution.

As can be seen in case of HP-β-CD and RM-β-CD FZD complexes (drug content 88 ± 2 %, 94 ± 2 % respectively) shows a good agreement between theoretical and actual drug content (Table 1). While coevaporated complexes showed mediocre (RVS–HP-β-CD (COEVP), 55 ± 2 %) and high percent drug content (RVS–RM-β-CD (COEVP), 78 ± 2 %) respectively, this can be attributed to partial inclusion of drug in CD cavity. However in the case of complexes prepared by physical and kneaded method, due to poor solubility, the drug was not completely dissolved and the actual drug content was consequently lower than theoretical one.

Table 1 Drug content and aqueous solubility of complexes of RVS prepared by different methods at 37 ± 0.5 °C

Type of complex	% Theoretical drug content	% Actual drug content (n = 3)	Solubility (mg/ml)
RVS	100	100	5.0 ± 0.2
RVS + β-CD (PM)	100	15.0 ± 2	5.3 ± 0.32
RVS + β-CD (KND)	100	15.4 ± 2	5.5 ± 0.31
RVS + β-CD (COEVP)	100	15.6 ± 2	5.5 ± 0.11
RVS + β-CD (FZD)	100	21 ± 2	6.6 ± 0.36
RVS + HP-β-CD (PM)	100	18.3 ± 2	6.1 ± 0.52
RVS + HP-β-CD (KND)	100	22.7 ± 2	12.2 ± 0.42
RVS + HP-β-CD (COEVP)	100	55.3 ± 2	16.56 ± 0.22
RVS + HP-β-CD (FZD)	100	88.1 ± 2	36.65 ± 1.02
RVS + RM-β-CD (PM)	100	32.3 ± 2	7.1 ± 0.54
RVS + RM-β-CD (KND)	100	40.1 ± 2	17.75 ± 0.17
RVS + RM-β-CD (COEVP)	100	78.5 ± 2	24.25 ± .008
RVS + RM-β-CD (FZD)	100	94.2 ± 2	46.07 ± 0.9

The Aqueous solubility of the drug alone and prepared complexes is reported in Table 1. RVS indicated an aqueous solubility of 5.0 ± 0.2 mg/ml. It can be seen that, the RVS–β-CD complexes prepared by different methods did not show any significant increase in drug solubility except FZD complex, which showed very small increase i.e. 1.3 fold in comparison to drug alone. Complexes of RVS with HP-β-CD and RM-β-CD prepared by PM method also showed very little increase in drug aqueous solubility. While complexes prepared by KND and COEVP methods [RVS–HP-β-CD (KND), RVS–HP-β-CD (COEVP) and (RVS–RM-β-CD (KND), (RVS–RM-β-CD (COEVP)] exhibited approximately a 2.24, 3.31 fold and 3.55, 4.85 fold increase in aqueous solubility, compared to drug alone. However, FZD complexes of both the CD derivatives [RVS–HP-β-CD-β-CD (FZD) and RVS–RM-β-CD (FZD)] significantly increased drug solubility to 7.33 and 9.34 fold. This significant increase of the solubility of FZD complexes can be attributable to the formation of amorphous, highly soluble and stable inclusion complex. This interesting finding is coherent with the well-admitted principle claiming that the less the aqueous solubility of the pure drug, the greater the relative solubility enhancement by the CD complexation [24].

In vitro dissolution studies

Along with the effect of type of CD derivatives used on solubility of RVS the effect on solubility as a function of the method used for the preparation of the complexes was also studied.

The results in terms of DE and percent of active ingredient dissolved (DP_{10}, DP_{20}) after 10 and 20 min are presented in Table 2. Results shows that inclusion complexation of RVS with selected CD derivatives increased the dissolution rate of the drug, except in case of β-CD which demonstrated only a slight increase of 4.17 in DP_{20} of complex prepared by FZD method (RSV–HP-β-CD (FZD)). This increase follows the order: RM-β-CD (FZD) > HP-β-CD (FZD) > RM-β-CD (COEVP) > HP-β-CD (COEVP) > RM-β-CD (KND) > HP-β-CD (KND) > RM-β-CD (PM) > β-CD (FZD) > HP-β-CD (PM) > β-CD (COEVP) ≈ β-CD (KND) > β-CD (PM) > RVS, suggesting that dissolution rate was influenced by the preparation method of the binary mixtures (Table 2). Results demonstrate that in case of complexes of HP-β-CD and RM-β-CD prepared by using PM method showed a very slight increase of the dissolution rate. The slight improvement in dissolution rate obtained with PMs can be attributed to both, improved drug wettability and in situ formation of readily soluble complexes was also

possible [25]. The RVS dissolution rate from kneaded complexes of HP-β-CD and RM-β-CD was almost similar to dissolution rate from coevaporated complexes respectively (nearly 50 % of drug dissolved at 20 min), as expected from physicochemical characterizations.

A enhanced dissolution rate of the drug to a marked extent was found in the case of FZD complexes of HP-β-CD and RM-β-CD. The DE was increased up to 1.78 and 1.91 fold probably due to several reasons: formation of soluble inclusion complex, amorphization of the drug, better wettability and reduction of particle size leading to the intermolecular hydrogen bonds [26, 27]. The high dissolution rate of the FZD complex with RM-β-CD (RVS–RM-β-CD (FZD)) compared to that of the HP-β-CD (RVS–HP-β-CD (FZD)) might be attributed to the higher inclusion ability of RM-β-CD and increase in hydrophobicity [28]. However, as reported above, the RVS solubility increased by CD complexation indicating that CDs are useful tools for promoting RVS solubility and, therefore, its bioavailability.

Table 2 Dissolution parameters of complexes of RVS prepared by different methods at 37 ± 0.5 °C (mean ± SD, n = 3)

Drug/complexes	Solubility parameters		
	DP_{10}	DP_{20}	DE_{30}
RVS	29.12 ± 0.88	39 ± 1.06	39.95 ± 0.08
RVS + β-CD (PM)	29.86 ± 0.38	39.43 ± 1.37	40.12 ± 1.22
RVS + β-CD (KND)	30.24 ± 0.04	40.52 ± 1.28	41.32 ± 1.18
RVS + β-CD (COEVP)	30.38 ± 1.35	40.98 ± 1.15	41.18 ± 1.14
RVS + β-CD (FZD)	32.06 ± 1.05	43.17 ± 1.49	43.98 ± 1.33
RVS + HP-β-CD (PM)	30.78 ± 1.21	41 ± 1.06	41.22 ± 1.23
RVS + HP-β-CD (KND)	36.23 ± 1.37	50 ± 0.08	46.29 ± 1.34
RVS + HP-β-CD (COEVP)	38.65 ± 1.05	52 ± 1.32	48.92 ± 1.01
RVS + HP-β-CD (FZD)	60.49 ± 0.78	70 ± 1.05	71.35 ± 1.12
RVS + RM-β-CD (PM)	25.27 ± 1.71	41 ± 0.06	39.93 ± 1.06
RVS + RM-β-CD (KND)	40.50 ± 0.78	51 ± 0.02	47.63 ± 0.04
RVS + RM-β-CD (COEVP)	42.08 ± 1.06	53 ± 2.01	49.25 ± 1.13
RVS + RM-β-CD (FZD)	69.26 ± 1.10	76 ± 1.78	76.54 ± 1.09

Each value is the average of three determinations (coefficient of variation CV < 2.5 %)

DP_{10}, *DP_{20}* percent drug dissolved at 10 and 20 min, *DE_{30}* dissolution efficiency at t = 30 min (calculated from the area under the dissolution curve at t = 30 min and expressed as % of the area of the rectangle described by 100 % dissolution in the same time)

Pharmacodynamic activity

To examine whether the notable increase in dissolution rate observed with CDs complexation may lead to differences in pharmacological effects, we explored the antilipidemic profiles of RVS and frieze dried complexes (RVS–HP-β-CD (FZD), RVS–RM-β-CD (FZD)).

The reported dose dependent pharmacodynamic effect of RVS i.e. lowering of TC and increment in high-density lipoprotein levels was used as a basis for the comparison of in vivo performance. The effect of RVS and selected FZD complexes of HP-β-CD and RM-β-CD on serum lipid profiles of hyperlipidemic rats are shown in Table 3.

After 7 days of treatment standard group showed approximately 13 % decrease in TC, 87 % increases in TG. In contrast, RVS–HP-β-CD (FZD) and RVS–RM-β-CD (FZD) showed 2.2 and 1.5 fold decrease in TC and 2.47 (0.53 times more) and 1.3 fold (0.25 times more) increases in TG level of test group III and IV. After 15 days of similar treatment, control group showed further increase in all the lipid levels. The standard group showed approximately 1.85 fold decrease in TC and 1.76 fold increase in TG. On the other hand, test groups III and IV presented further 2.4 and 1.8 fold decrease in TC and less increase in TG in comparison with the standard group. It is interesting to note that the decrease in TC level is more and increase in TG level is less in second week i.e. from day 7 to day 15 than values obtained in first week i.e. from day 1 to day 7. This results of change in levels of TC and TG values in second week as compared to first week indicate sustain release of drug from both the complexes. These results only partially agreed with those obtained from phase solubility studies. In fact, the significant difference observed for the aqueous solubility and the stability constant of the RVS–HP-β-CD (FZD) complex in comparison to the

Table 3 Pharmacodynamic parameters of complexes of RVS prepared by different methods at day 0th, 7th and 15th (n = 4)

Groups	Day	TC (mg/dl)	TG (mg/dl)
Group I: control group	0th	78 ± 0.12	61 ± 0.23
	7th	92 ± 1.2	178 ± 0.68
	15th	137 ± 1.01	605 ± 0.95
Group II: standard group	0th	76 ± 0.15	60 ± 0.35
	7th	67 ± 0.18	112 ± 0.15
	15th	56.5 ± 0.02	153 ± 0.27
Group III: RVS + HP-β-CD (FZD)	0th	80 ± 0.55	63 ± 1.02
	7th	60.2 ± 1.15	84 ± 1.58
	15th	34.7 ± 1.04	92 ± 1.21
Group IV: RVS + RM-β-CD (FZD)	0th	81 ± 1.11	65 ± 1.07
	7th	67.5 ± 1.18	105 ± 0.25
	15th	48.5 ± 0.22	118 ± 0.18

RVS–RM-β-CD (FZD) complex was not reflected in pharmacological response of the respective complexes. In particular, no significant difference ($P > 0.1$) was found between the decrease in TC and TG levels by complexes with HP-β-CD and RM-β-CD and this may be attributed to large stability constant of RVS–RM-β-CD system which might have reduced drug in vivo absorption rate. Thus, at the end of 15 days study, both RVS–HP-β-CD (FZD) and RVS–RM-β-CD (FZD) inclusion complex performed better than pure RVS in reducing TC and TG levels. This could be primarily attributed to the improved solubility and dissolution associated with inclusion complex between RVS and selected CD derivatives.

Conclusion

Our results demonstrate that β-CD derivatives are able to improve RVS dissolution properties. It was observed that the properties of prepared complexes of RVS with β-CD and its derivatives (HP-β-CD and RM-β-CD) are influenced by the type of CD. Result also demonstrates that the preparation method strongly influenced the ability of CD to include the drug. The best results in vitro were obtained by RM-β-CD FZD complex, suggesting a true inclusion of RVS with RM-β-CD. Pharmacodynamic activity on hyperlipidemic rats demonstrated that the hypolipidemic efficacy of the drug is not modified by the complexation with any of the CDs derivative studied. However in contrast to in vitro results the FZD complex of RVS with HP-β-CD proved to be more efficacious as compared to FZD complex of RM-β-CD in vivo. The pharmacodynamic activity in rats indicates that the RVS–CDs complexes might be used in developing a new solid oral formulation with an in vivo performance much better than that of RVS alone. Thus the considerable increase in bioavailability of the drug CD complex can be used to reduce the dose of drug. At last the proposed complex (RVS–HP-β-CD (FZD)) can be an important new means which could offer new dosage regimen and may help to improve patient compliance by reducing pill burden which will further improve adherence of patient to the therapy.

Further work is in progress to study the encapsulation of prepared drug complex as an model drug complex for biopharmaceutical class II drugs into various polymeric system to assess the expected improvement in oral bioavailability along with desired release profiles with a view to develop improved generic medicines/formulation for existing poorly or sparingly soluble drugs.

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