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Research paper

Preparation and characterization of simvastatin/hydroxypropyl-β-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process

Seoung Wook Jun [a], Min-Soo Kim [a], Jeong-Soo Kim [a], Hee Jun Park [a], Sibeum Lee [a], Jong-Soo Woo [b], Sung-Joo Hwang [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon, Republic of Korea
[b] Hanmi Pharmaceutical Co. Ltd., Kyungkido, Republic of Korea

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Abstract

In the present study, the practically insoluble drug, simvastatin (SV), and its inclusion complex with hydroxypropyl β-cyclodextrin (HP-β-CD) prepared using supercritical antisolvent (SAS) process were investigated to improve the aqueous solubility and the dissolution rate of drug, thus enhancing its bioavailability. Inclusion complexation in aqueous solution and solid state was evaluated by the phase solubility diagram, differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). The phase solubility diagram with HP-β-CD was classified as A_L-type at all temperatures investigated, indicating the formation of 1:1 stoichiometric inclusion complex. The apparent complexation constants ($K_{1:1}$) calculated from phase solubility diagram were 774, 846 and 924 M^{-1} at 25, 37 and 45 ± 0.5 °C, respectively. No endothermic and characteristic diffraction peaks corresponding to SV was observed for the inclusion complex in DSC and PXRD. FT-IR study demonstrated the presence of intermolecular hydrogen bonds between SV and HP-β-CD in inclusion complex, resulting in the formation of amorphous form. Aqueous solubility and dissolution studies indicated that the dissolution rates were remarkably increased in inclusion complex, compared with the physical mixture and drug alone. Moreover, SV/HP-β-CD inclusion complex performed better than SV in reducing total cholesterol and triglyceride levels. This could be primarily attributed to the improved solubility and dissolution associated with inclusion complex between drug and HP-β-CD. In conclusion, SAS process could be a useful method for the preparation of the inclusion complex of drug with HP-β-CD and its solubility, dissolution rate and hypolipidemic activity were significantly increased by complexation between SV and HP-β-CD.

Keywords: Simvastatin; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Supercritical antisolvent (SAS) process

1. Introduction

Simvastatin (SV) is a cholesterol-lowering agent that is derived synthetically from a fermentation product of *Aspergills terreus* [1] and widely used to treat hypercholesterolemia. SV, an inactive lactone, is converted to corresponding β,δ-dihydroxy acid in liver by cytochrome P450 (CYP) 3A after oral administration. SV is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol [2,3]. However, it is practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract [4,5]. Therefore, it is

* Corresponding author. National Research Laboratory of Pharmaceutical Technology, College of Pharmacy, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Republic of Korea. Tel.: +82 42 821 5922; fax: +82 42 823 3078.
E-mail address: sjhwang@cnu.ac.kr (S.-J. Hwang).

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very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability.

Supercritical particle generation processes are new and efficient route for improving the bioavailability of pharmaceutically active compounds [6]. Carbon dioxide (CO_2) is currently the most commonly used supercritical fluid because of its low critical temperature and pressure ($T_c = 31.0$ °C, $P_c = 7.38$ MPa). Apart from being non-toxic, non-flammable and inexpensive, the low critical temperature of carbon dioxide makes it attractive for processing heat-labile pharmaceuticals. Our preliminary studies demonstrated that the supercritical antisolvent (SAS) process could improve the dissolution of SV by a 1.5-fold increase in the aqueous solubility (pH 6.8) [7]. However, a complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD) using SAS process has been further investigated because this improvement in dissolution has proven to be insufficient.

Previously, it was reported that SV formed inclusion complex with α-CD and β-CD using ESI-MS experiments and molecular modeling [8]. Cyclodextrins (CDs) are cyclic oligosaccharides, which are produced by enzymatic degradation of starch by a glucosyltransferase most commonly derived from *Bacillus macerans* and have been recognized as useful pharmaceutical excipients [9]. Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate and bioavailability of poorly water-soluble drugs. Especially, HP-β-CD is widely used in the pharmaceutical field owing to its high aqueous solubility and ability to stabilize drug molecules [10,11]. In addition, supercritical fluid processes were recently proposed as a new alternative method for the preparation of drug–cyclodextrin complexes. Supercritical carbon dioxide (SC-CO_2) is suggested as a new complexation medium due to its properties of improved mass transfer and increased solvating power [12–16].

In present study, SAS process was applied to prepare the inclusion complex of SV with HP-β-CD because SV as a model drug was poorly soluble in SC-CO_2. The type of complexation and complexation constant were established according to phase solubility study. The dissolution properties and hypolipidemic activity of inclusion complex were evaluated compared with those of SV alone and the corresponding physical mixture. Differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were used to characterize properties in solid state.

2. Materials and methods

2.1. Materials

Simvastatin (SV) as a model drug was purchased from Kyongbo Pharmaceutical Co., Ltd. (Korea) and HP-β-CD (Kleptose® HPB) was obtained from Roquette-Freres (Lestrem Cedex, France). Carbon dioxide (CO_2) with high purity of 99.99% was supplied from Myungsin General Gas Co., Ltd. (Korea). Male Sprague–Dawley rats were obtained from Samtaco Co., Ltd (Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Phase solubility diagram

The phase solubility diagram was performed by the method reported by Higuchi and Connors [17]. Excess amount of SV was added to aqueous solution (pH 6.8) containing various concentrations of HP-β-CD (0, 2, 4, 6, 8 and 10 mM) and then was shaken for 48 h at different temperatures, 25, 37 and 45 ± 0.5 °C. After equilibrium was reached, the samples were withdrawn and filtered through a 0.45 μm PVDF membrane filter and suitably diluted with methanol. Drug concentration was determined spectrophotometrically (UV-1240, Shimadzu, Japan) at 240 nm. The phase solubility diagram was constructed by plotting the total dissolved drug concentration against the total HP-β-CD concentration. The apparent complexation constant ($K_{1:1}$) of the complex was calculated as following equation (Eq. (1)) from phase solubility slope, where the intercept is the intrinsic solubility of drug in the absence of HP-β-CD at 25, 37 and 45 ± 0.5 °C, respectively;

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept } (1 - \text{Slope})} \tag{1}$$

Also the change in enthalpy (ΔH) on complexation was determined using Van't Hoff equation, Eq. (2)

$$\ln\left(\frac{K_2}{K_1}\right) = \Delta H \frac{T_2 - T_1}{RT_2T_1} \tag{2}$$

The change in Gibbs free energy (ΔG) and in entropy (ΔS) upon complexation was determined using the following equation (Eq. (3)) and Eq. (4), respectively.

$$\Delta G = -RT \ln K \tag{3}$$

$$\Delta S = \frac{(\Delta H - \Delta G)}{T} \tag{4}$$

2.3. Preparation of SV/HP-β-CD complex using SAS process

The SAS process is described in detail everywhere [18–20]. The drug/HP-β-CD solution was prepared by dissolving drug and HP-β-CD (1:1 molar ratio) in mixture solvents of dichloromethane and ethanol (1:2 v/v%). The SAS process was performed at 40 °C and 120 bars for temperature and pressure, respectively. The same experiment was carried out without HP-β-CD. After the spraying of drug/HP-β-CD solution into the particle formation vessel was completed, an additional SC-CO_2 continued to flow into the vessel for further 120 min to remove residual solvent from precipitated particles and then slowly depressur-

ized to atmospheric pressure. For comparisons, physical mixture (PM) in the same ratio was also prepared by physically mixing SV and HP-β-CD thoroughly for 3 min in a mortar until a homogeneous mixture was obtained.

2.4. Differential scanning calorimetry (DSC)

Prior to DSC analysis, a baseline was obtained which was used as a background. DSC analyses of samples were carried out on DSC S-650 (Sinco, Ltd., Korea). Temperature and enthalpy were calibrated with the standard materials indium (melting point = 156.6 °C) and zinc (melting point = 419.5 °C) at a heating rate of 5 °C/min. Samples (3–4 mg) were accurately weighed and sealed in aluminum pans and heated at a rate of 5 °C/min. The measurements were performed at a heating range of 40–200 °C under a nitrogen purge. A nitrogen flow rate of 20 ml/min was used for DSC run. All samples were analyzed in duplicate.

2.5. Powder X-ray diffractometry (PXRD)

Powder X-ray diffraction patterns of samples were obtained using powder X-ray diffractometer (Generator: Rigaku, D/max-IIIC, Goniometer: θ/θ goniometer), with Ni filtered Cu-Kα line as the source of radiation, which was operated at the voltage 40 kV and the current 45 mA. Each sample was placed in the cavity of an aluminum sample holder flattened with a glass slide to present a good surface texture and inserted into the sample holder. In order to measure the powder pattern, the sample holder and detector were moved in a circular path to determine the angles of scattered radiation and to reduce preferred sample orientation. All samples were measured in the 2θ angle range between 0° and 60° with a scan rate of 3°/min and a step size of 0.01°. All samples were analyzed in duplicate.

2.6. Fourier-transform infrared (FT-IR) spectroscopy

Fourier-transform infrared (FT-IR) spectra were obtained by ATR FT-IR spectrometer (Travel IR, USA) equipped with a DTGS detector. A resolution of 2 cm^{-1} was used and 64 scans were co-added for each spectrum over a frequency range of 4000–650 cm^{-1}. All samples were analyzed in duplicate.

2.7. Scanning electron microscopy (SEM)

The morphology of samples was determined using scanning electron microscope (SEM) (HITACHI S-3000N, Japan), operated at an accelerating voltage of 20 kV (filament current of 1.75 μA, beam current of 30–40 mA and probe current of 250 pA). Samples were prepared by mounting ≈0.5 mg of powder onto a 5 mm × 5 mm silicon wafer affixed via graphite tape to an aluminum stub. The powder was then sputter-coated for 40 s at beam current of 38–42 mA with a 200 Å layer of gold/palladium alloy.

2.8. Drug content

SAS processed SV/HP-β-CD complex equivalent to 10 mg of SV was accurately weighed and dissolved in suitable quantity of methanol (MeOH). The drug content was determined at 240 nm by UV-spectrophotometer (UV-1240, Shimadzu, Japan).

2.9. Aqueous solubility

An excess amount of sample was added to 5 ml of the phosphate buffer solution (pH 6.8) 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 37 ± 0.5 °C until reaching equilibrium (for 48 h). A portion of solution was withdrawn and then filtered with a PVDF syringe filter (0.45 μm) and adequately diluted with methanol. The drug concentration was determined at 240 nm by UV-spectrophotometer (UV-1240, Shimadzu, Japan).

2.10. Preparation of the tablets containing the SV/HP-β-CD complex

The SV, SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex were compressed into tablets, based on formulations [4]. Microcrystalline cellulose (Avicel® PH101, 50 mg/tablet) and Ac-Di-Sol (8 mg/tablet) were added as a tablet diluent and disintegrant, respectively. The average weight of tablet with different formulations equivalent to 10 mg of SV was maintained at 200 mg by accordingly adjusting the amount of lactose. All ingredients of the tablet formulation except magnesium stearate were mixed for 15 min in a plough shear blender at 400 rev./min. After the addition of magnesium stearate (1% of the total weight of the mixture), the blend was mixed again for 1 min at 400 rev./min and compressed into tablets with a total weight of 200 mg on an ERWEKA® EKO (Germany). The tablets were evaluated for thickness and hardness using diametrical hardness tester (PTB, Pharmatest, India).

2.11. Dissolution studies

The dissolution studies from tablets containing SV, SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex were carried out using USP XXIV type II dissolution test apparatus (VK 7000, Vankel, USA). The tablets of different formulations equivalent to 10 mg of SV was placed in the dissolution vessel containing 900 ml phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. Concentration of SV was determined spectrophotometrically at

240 nm. Dissolution efficiency (DE) was calculated from the area under the dissolution curves at given time and expressed as a percent of the area of the rectangle described by 100% dissolution in the same time [21].

2.12. In vivo studies in rats

The hypolipidemic activity of SAS processed SV/HP-β-CD complex was determined in comparison with SV in male Sprague–Dawley rats (Samtaco, Korea), weighing between 150 and 200 g. The rats were housed in a cage and maintained on a 12 h light/dark at room temperature (25 °C) and relative humidity of 55 ± 10%, with free access to water and *ad libitum*. General and environmental conditions were strictly monitored. All animal experiments were performed according to the "Guidelines for the Care and Use of Laboratory Animals" at Chungnam National University. The animals were divided into three groups of three animals each. The treatment was given for 14 days. Each group daily received 2 ml of coconut oil orally using gavage feeding needles. After the feeding of coconut oil, reference and test groups were administered orally 1 ml of 2% w/v gum acacia aqueous suspensions containing SV and SAS processed SV/HP-β-CD complex (equivalent to 10 mg/kg body weight), respectively. Also, control group received daily 1 ml of 2% w/v gum acacia solution via oral administration. Blood samples were collected under light ether anesthesia by retroorbital puncture; initially, after 7 days and after 14 days. The serum samples were analyzed for total cholesterol, triglycerides (TG) by the *in vitro* diagnostic kit (Stanbio Laboratory, USA).

2.13. Statistical analysis

The statistical significance of differences among formulations was estimated using one-way analysis of variance (ANOVA) followed by Tukey's test in solubility data and lipid profiles, and Student–Newman–Keuls test in terms of drug dissolution efficiency, percent of drug dissolved at a given time and relative dissolution rate (RDR) using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Phase solubility diagram

Fig. 1 shows the phase solubility diagram of drug in the presence of various HP-β-CD concentrations at 25, 37 and 45 ± 0.5 °C. The phase solubility diagram of SV in various HP-β-CD concentrations was linear A_L type, as classified by Higuchi and Connors [17]. This suggests a 1:1 stoichiometry of the complexes over the concentration range (0–10 mM) investigated. The solubility of SV increased as a function of HP-β-CD concentration. The apparent complexation constant ($K_{1:1}$) and various thermodynamic parameters such as changes in enthalpy

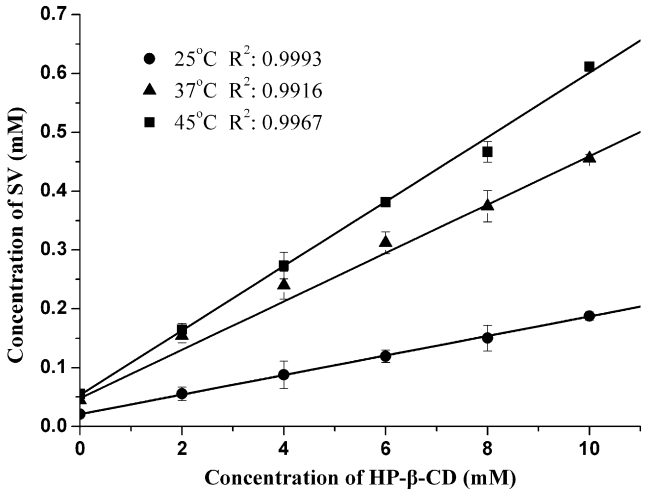

Fig. 1. Phase solubility diagram of SV as a function of HP-β-CD concentrations at different temperatures, 25, 37 and 45 ± 0.5 °C ($n = 3$, mean ± SD).

(ΔH), Gibb's free energy (ΔG) and entropy (ΔS) of complexation are listed in Table 1. The apparent complexation constants ($K_{1:1}$) calculated from phase solubility diagram were 774, 846 and 924 M^{-1} at 25, 37 and 45 ± 0.5 °C, respectively. It was observed that the apparent complexation constant increased with increasing temperature, probably due to the decrease in the interaction forces, such as van der Waals and hydrophobic forces. Changes in thermodynamic parameters during complexation are an intricate phenomenon and are a result of changes in van der Waals interaction energy, hydrogen bonding and hydrophobic interaction between the guest and cyclodextrin. The values of the Gibbs free energy ΔG were negative, indicating that the complexation with HP-β-CD is spontaneous.

3.2. Differential scanning calorimetry (DSC)

The DSC thermograms of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex are shown in Fig. 2. SV was characterized by a single, sharp melting endotherm at 139.5 °C (ΔH = 77.39 J/g) during DSC analysis and the thermogram of HP-β-CD showed a very broad endothermic effect, which attained a maximum around 70 and 100 °C, respectively, due to the release of water molecules [22]. The SAS processed SV was also characterized by a single, sharp melting endotherm like SV, indicating the presence of crystallinity after SAS process. The DSC curve of SV/HP-β-CD (PM) shows two peaks: a broad endotherm between 50 and 150 °C corresponding to the water loss of the HP-β-CD, followed by the endothermal melting peak at 139.5 °C characteristic of SV. However, the complete disappearance of endothermic peak corresponding to SV was observed for SAS processed SV/HP-β-CD complex, indicating the formation of an amorphous inclusion complex, the molecular

Table 1
Apparent complexation constants ($K_{1:1}$) and thermodynamic parameters for complexation of SV with HP-β-CD at different temperatures, 25, 37 and 45 ± 0.5 °C ($n = 3$)

Temperature (°C)	$K_{1:1}$ (M^{-1})	ΔH (kJ/mole)	ΔG (kJ/mole)	ΔS (J/mole/K)
25	774	6.82	−16.48	78.20
37	846	7.05	−17.26	78.96
45	923	6.94	−18.05	78.58

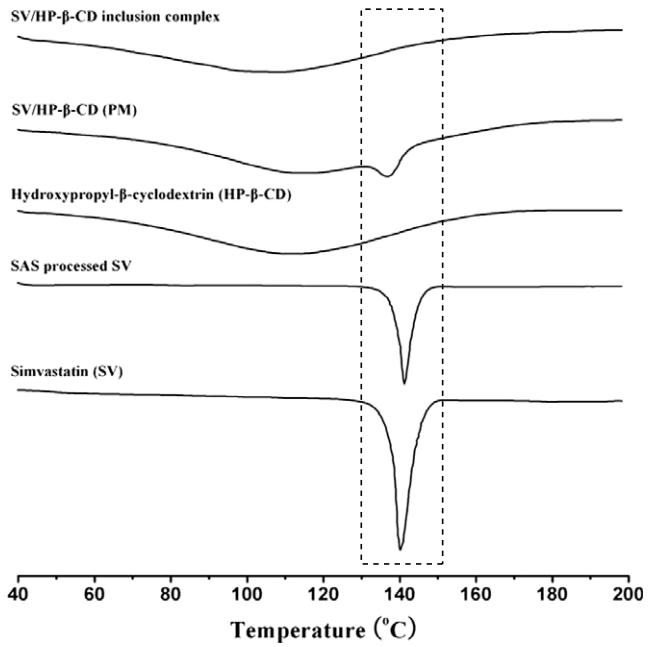

Fig. 2. DSC thermograms of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex.

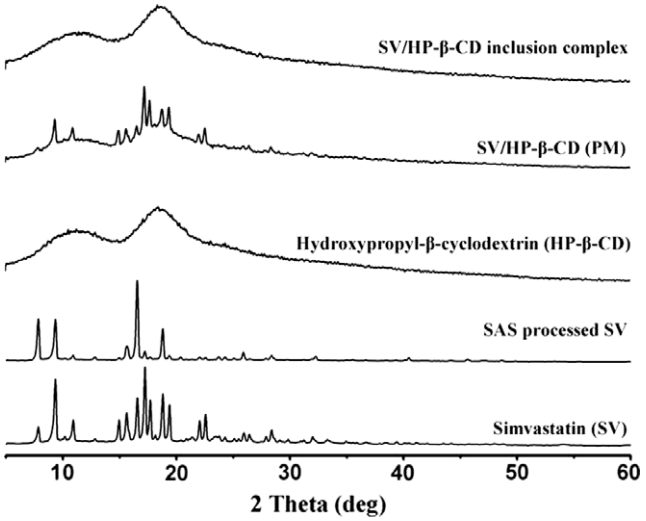

Fig. 3. Powder X-ray diffraction patterns of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex.

encapsulation of the drug inside the HP-β-CD cavity [23,24].

3.3. Powder X-ray diffractometry (PXRD)

A further supporting evidence for the formation of inclusion complex between SV and HP-β-CD was obtained from the powder X-ray diffraction patterns. Powder X-ray diffraction patterns of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex are represented in Fig. 3. The presence of several distinct peaks in the PXRD of SV at a diffraction angle of 2θ 7.85°, 9.35°, 10.92°, 12.85°, 15.62°, 16.48°, 17.24°, 18.81°, 22.58°, 25.95°, 28.38° and 31.99° revealed that the drug is present as a crystalline form. The several distinct peaks corresponding to SV were still observed in PXRD of SAS processed SV and SV/HP-β-CD (PM), indicating that drug maintained its crystallinity. On the other hand, the PXRD of SAS processed SV/HP-β-CD complex was characterized by the complete absence of any diffraction peak corresponding to SV. These results indicate that the drug is no longer present as a crystalline form when complexed with HP-β-CD during SAS process, but exists in the amorphous state. These results may be attributed to a possible complexation of SV inside the HP-β-CD during SAS process, suggesting the possible interaction such as hydrogen bonding between SV and HP-β-CD.

3.4. Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex are represented in Fig. 4. The FT-IR spectra of SV showed the presence of the following peaks: 3553 cm^{-1} (Free O–H stretching vibration), 3011, 2959, and 2872 cm^{-1} (C–H stretching vibrations), 1714 cm^{-1} (stretching vibration of ester and lactone carbonyl functional group) and the FT-IR spectra of HP-β-CD showed prominent absorption bands at 3414 cm^{-1} (for O–H stretching vibrations), 2933 cm^{-1} (for C–H stretching vibrations) and 1164 cm^{-1}, 1083 cm^{-1} (C–H, C–O stretching vibration). In addition, the FT-IR spectra of the SAS processed SV and SV/HP-β-CD (PM) did not show any significant differences from the respective spectra of the SV and HP-β-CD. However, the FT-IR spectrum of the SAS processed SV/HP-β-CD complex exhibited some significant differences. The characteristic absorption peaks of the carbonyl group of SV in the range 1600–1800 cm^{-1} have disappeared from the spectrum of the inclusion complex. This

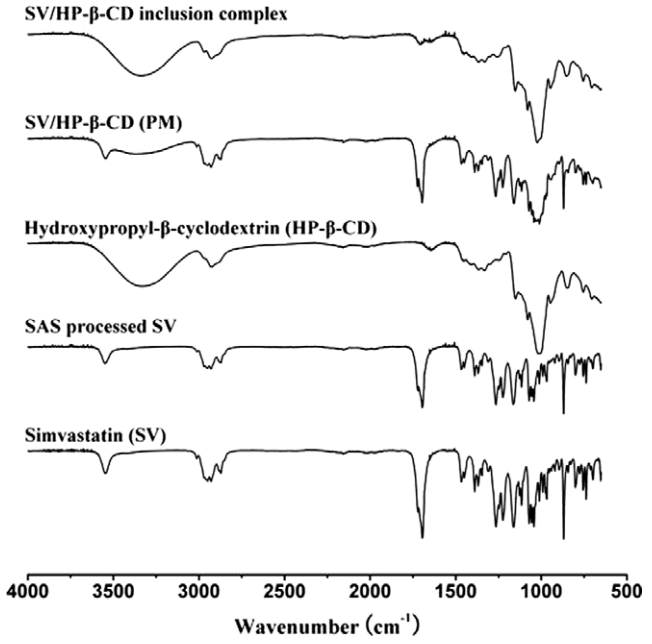

Fig. 4. FT-IR spectra of SV, SAS processed SV, HP-β-CD, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex.

can be probably due to the inclusion complexation of drug into the HP-β-CD cavity. Based on these results, the C=O group of lactone ring of SV might be involved in the inclusion complexation.

3.5. Scanning electron microscopy (SEM)

The SEM of SV, HP-β-CD, SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/ HP-β-CD complex is shown in Fig. 5. SV and SAS processed SV consisted of a mixture of large crystals. It seemed that the morphology of drug was not affected by SAS process. HP-β-CD consisted of hollow spherical particle. SV/HP-β-CD (PM) has appeared as irregular-shaped crystals. In contrast, a drastic change in the morphology and shape of drug was observed in the SAS processed SV/HP-β-CD complex, revealing an apparent interaction in the solid state.

3.6. Drug content and aqueous solubility

The drug content within tablets was 96 ± 2% for all formulations ($n = 3$). Aqueous solubility of SV at the end of 48 h in phosphate buffer solution (pH 6.8) indicated an aqueous solubility of 24.4 ± 1.5 μg/ml (Table 2). No further increase in solubility was observed at the end of 4 and 7 days. The observed drug solubility was in close agreement with that reported by Anshuman et al. [25]. As mentioned earlier, the SAS processed SV exhibited approximately a 1.5-fold increase (33.6 μg/ml) in aqueous solubility (pH 6.8), compared with SV. However, SV/HP-β-CD (PM) significantly increased drug solubility to 229.4 ± 6.3 μg/ml and SAS processed SV/HP-β-CD complex further enhanced drug solubility to 296.9 ± 13.0 μg/ml.

3.7. Dissolution studies

The tablet thickness and hardness were in the range of 3.6–3.8 mm and 6–8 Kp, respectively. Fig. 6 shows the dissolution profiles of different formulations of drug in tablet form. The percent drug dissolved after 10 and 30 min, dissolution efficiency at 60 (DE_{60}) and RDR at 5 min were calculated to compare dissolution profiles from tablets containing SV, SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex (Table 3). The results in Table 3 show that the dissolution of drug from tablet containing SAS processed SV/HP-β-CD complex was dramatically improved, as compared with SV. The SAS processed-SV/HP-β-CD complex exhibited faster dissolution rates than SV with approximately 6.6- and 34-fold increases in terms of both DE_{60} and RDR. The analysis of variance showed that there are significant differences among the formulations ($F_{3,20} = 10352.305$, $P < 0.001$), which in order of increasing DE_{60} were ranked by the Student–Newman–Keuls test as follows: SV < SAS processed SV < SV/HP-β-CD (PM) < SAS processed SV/HP-β-CD complex. Based on the results from our studies, the higher dissolution rate of drug from tablets containing SAS processed SV/HP-β-CD complex was probably attributed to the complexation with HP-β-CD during supercritical antisolvent (SAS) process, leading to the intermolecular hydrogen bonds.

3.8. In vivo studies in rats

Hypolipidemic drugs like SV (HMG-CoA reductase inhibitors) are known to reduce elevated total cholesterol and TG levels in blood. At the same time they cause elevation of the HDL-cholesterol levels, which promote the removal of cholesterol from peripheral cells and facilitate its delivery back to the liver. This pharmacodynamic effect is reported to be dose dependent hence, it was used as a basis for the comparison of *in vivo* performance of SV and its inclusion complex with HP-β-CD. Administration of excess coconut oil, which is a rich source of saturated fatty acids, promotes biosynthesis of cholesterol in liver and leads to hypercholesterolemia [26,27]. The serum lipid profiles of all the experimental groups at initial, 7- and 14-day time intervals are presented in Table 4 and the corresponding % changes in lipid profiles are plotted in Figs. 7 and 8. As expected, after 7 days of treatment with excess coconut oil, control group showed significant increase in total cholesterol, TG. However, reference group showed approximately 10% decrease in total cholesterol, 90% increases in TG. It is interestingly noted that test group presented 4-fold decrease in total cholesterol and similar increase in TG as compared with reference group. After 14-day of similar treatment, control group showed further increase in all the lipid levels. The reference group showed

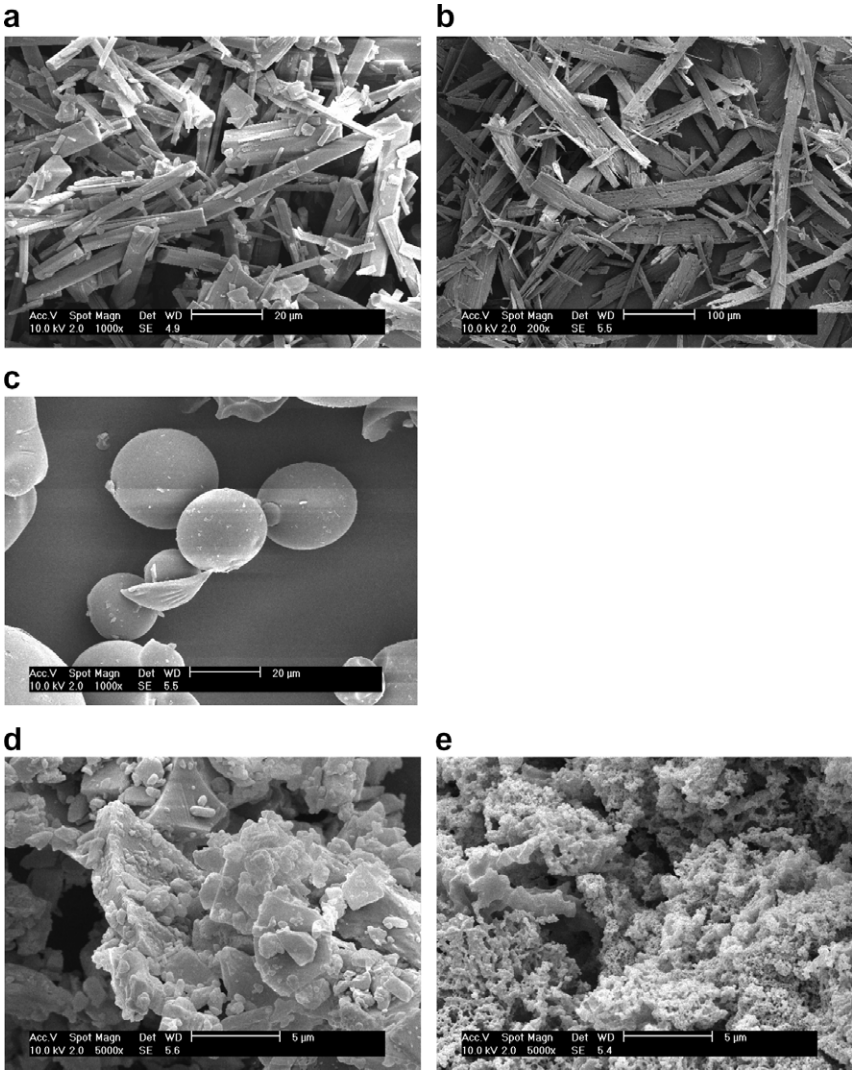

Fig. 5. Scanning electron microscopy (SEM) of SV (a), SAS processed SV (b), HP-β-CD (c), SV/HP-β-CD (PM) (d), and SAS processed SV/HP-β-CD complex (e).

Table 2
Aqueous solubility of SV in phosphate buffer solution (pH 6.8) at 37 ± 0.5 °C after 48 h

Formulations	Aqueous solubility[a] (μg/ml)
SV	24.4 ± 1.5
SAS processed SV	33.6 ± 9.0
SV/HP-β-CD (PM)	229.4 ± 6.3[b]
SAS processed SV/HP-β-CD complex	296.9 ± 13.0[c]

[a] Solubility data at the end of 48 h expressed as mean ± SD for $n = 3$.
[b] Indicates $P < 0.05$ between SV and SV/HP-β-CD (PM).
[c] Indicates $P < 0.05$ between SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex.

approximately 2-fold decrease in total cholesterol and significant increase in TG. On the other hand, test group presented further 2.5-fold decrease in total cholesterol and slight increase in TG in comparison with the reference group. Thus, at the end of 14-day study, SV/HP-β-CD inclusion complex performed better than pure SV in reduc-

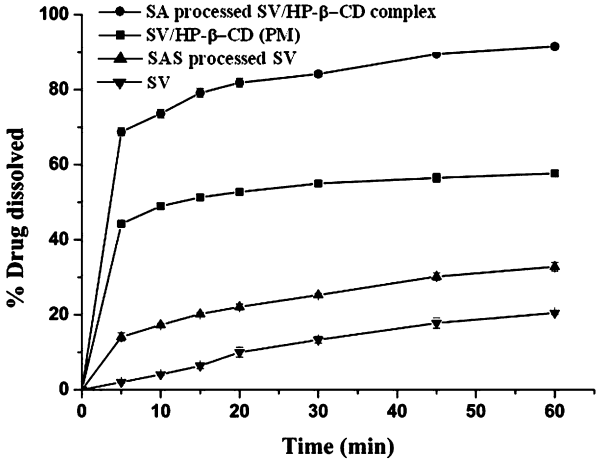

Fig. 6. Dissolution profiles of tablets containing SV, SAS processed SV, SV/HP-β-CD (PM), and SAS processed SV/HP-β-CD complex in phosphate buffer, pH 6.8 ($n = 6$, mean ± SD).

Table 3
Dissolution efficiency at 60 min (DE)[a], % drug dissolved[a] at 10, 30 min and relative dissolution rate (RDR)[a,b] from tablets containing SV, SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex ($n = 6$, mean ± SD)

Formulations	% drug dissolved[a]		DE[a] (60 min)	RDR[b] (5 min)
	10 min	30 min		
SV	4.1 ± 0.25	13.3 ± 0.88	12.1 ± 0.70	1.0
SAS processed SV	17.3 ± 0.36[c]	25.3 ± 0.45[c]	24.0 ± 0.40[c]	6.9
SV/HP-β-CD (PM)	49.0 ± 0.78[d]	55.0 ± 0.73[d]	51.4 ± 0.58[d]	21.9
SAS processed SV/HP-β-CD complex	73.6 ± 1.10[e]	84.2 ± 0.72[e]	80.1 ± 0.51[e]	34.1

[a] Calculated from the area under the dissolution curve at 60 min and expressed as % of the area of the rectangle described by 100% dissolution in the same time.
[b] Ratio between amount of drug dissolved from tablets containing SAS processed SV, SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex and that dissolved from SV at 5 min.
[c] Indicates $P < 0.05$ between SV and SAS processed SV.
[d] Indicates $P < 0.05$ between SAS processed SV and SV/HP-β-CD (PM).
[e] Indicates $P < 0.05$ between SV/HP-β-CD (PM) and SAS processed SV/HP-β-CD complex.

Table 4
Serum total cholesterol and TG of experimental groups at initial, 7- and 14-day time intervals, respectively

Experimental groups	Time intervals	Total cholesterol (mg/dl)[a]	TG (mg/dl)[a]
Control	Initial	63.0 ± 1.57	53.1 ± 0.36
	7 days	73.4 ± 0.85	142.4 ± 1.69
	14 days	98.1 ± 1.36	535.5 ± 4.51
Reference	Initial	60.4 ± 1.45	53.2 ± 1.27
	7 days	54.5 ± 0.98[b]	100.9 ± 1.35[b]
	14 days	48.2 ± 1.77[b]	163.5 ± 1.20[b]
Test	Initial	61.5 ± 0.85	56.3 ± 1.61
	7 days	34.2 ± 2.03[c]	92.2 ± 1.3[c]
	14 days	30.8 ± 0.40[c]	104.1 ± 2.80[c]

[a] Mean ± SD ($n = 3$).
[b] Indicates $P < 0.01$ between control and reference.
[c] Indicates $P < 0.01$ between reference and test.

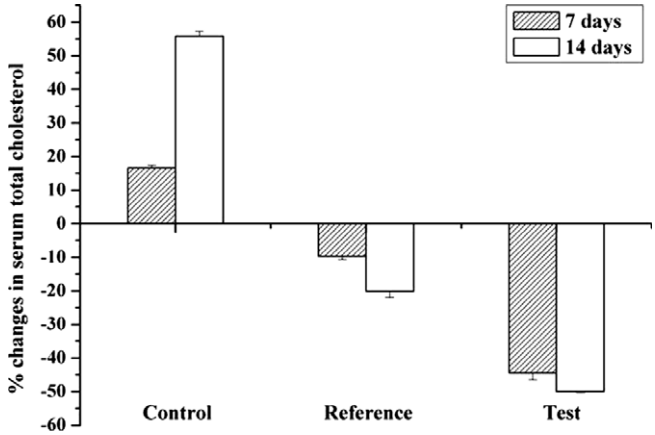

Fig. 7. Percent changes in serum total cholesterol of experimental groups at 7- and 14-day time intervals, respectively ($n = 3$, mean ± SD).

ing total cholesterol and TG levels. This could be primarily attributed to the improved solubility and dissolution associated with inclusion complex between SV and HP-β-CD.

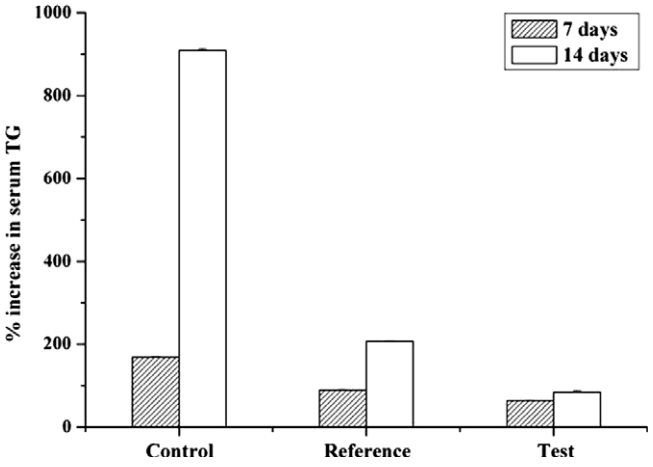

Fig. 8. Percent increase in serum TG of experimental groups at 7- and 14-day time intervals, respectively ($n = 3$, mean ± SD).

4. Conclusions

In this study, SAS process has been applied to prepare inclusion complex of SV with HP-β-CD. The phase solubility diagram with HP-β-CD was classified as A_L-type at all temperatures investigated, indicating the formation of 1:1 stoichiometric inclusion complex. The apparent complexation constants ($K_{1:1}$) calculated from phase solubility diagram were 774, 846 and 924 M^{-1} at 25, 37 and 45 ± 0.5 °C, respectively. No endothermic and characteristic diffraction peaks corresponding to SV was observed for the inclusion complex in DSC and PXRD. FT-IR study suggested the presence of intermolecular hydrogen bonds between SV and HP-β-CD in inclusion complex, resulting in the formation of amorphous form. Aqueous solubility and dissolution studies indicated that the dissolution rates were remarkably increased in SAS processed SV/HP-β-CD complex, compared with the physical mixture and drug alone. Moreover, SV/HP-β-CD inclusion complex performed better than SV in reducing total cholesterol and TG levels. This could be primarily attributed to the

improved solubility and dissolution associated with inclusion complex between SV and HP-β-CD. In conclusion, SAS process could be a useful method for the preparation of inclusion complex of drug with HP-β-CD and its solubility, dissolution rate and hypolipidemic activity were significantly increased by complexation between drug and HP-β-CD.

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