Pharmaceutical Research, Vol. 22, No. 6, June 2005 (© 2005)
DOI: 10.1007/s11095-005-4594-z

Research Paper

Spray-Dried Amorphous Solid Dispersions of Simvastatin, a Low T_g Drug: *In Vitro* and *in Vivo* Evaluations

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Received January 20, 2005; accepted March 9, 2005

Purpose. To obtain free flowing, stable, amorphous solid dispersions (SDs) of simvastatin (SIM), a drug with relatively lower glass transition temperature (T_g) by spray drying technique, and to perform comparative *in vivo* study in rats, which could justify the improvement in rate and extent of *in vitro* drug release.

Methods. Dichloromethane suspensions of SIM either alone or in combination with PVP (1:1 or 1:2 parts by weight) were spray dried with proposed quantity of Aerosil 200 (1:1, 1:1:1, 1:2:2 parts by weight of SIM, Aerosil 200 and PVP, respectively). SDs were characterized initially in comparison with pure drug and corresponding physical mixtures in same ratios by drug content, saturation solubility, SEM, DSC, XRPD, IR, and *in vitro* drug release. SD 1:2:2 was further subjected to accelerated stability testing and checked for *in vitro* drug release and presence of crystallinity using DSC and XRPD. In addition, improvement in rate and extent of *in vitro* drug release from SD 1:2:2 was justified by *in vivo* study in rats.

Results. Combination of SD and surface adsorption techniques has been attempted to overcome the limitations of spray drying technique for amorphization of low T_g drugs. Based on powder characteristics, drug content, saturation solubility, and feasibility of processing into tablets; SD 1:2:2 was selected as the optimized formulation. During initial characterization, SEM, DSC, and XRPD analyses confirmed the presence of amorphous form in SD 1:2:2. IR spectroscopy revealed possibility of hydrogen bonding interaction between SIM and PVP in SDs. Also, there was dramatical improvement in rate and extent of *in vitro* drug release of SD 1:2:2. Insignificant decrease in dissolution was observed with no evidence of crystallinity during accelerated stability studies of SD 1:2:2. Moreover *in vivo* study in rats also justified the improvement in therapeutic efficacy of SD 1:2:2 over pure SIM.

Conclusions. Thus, present study demonstrates high potential of spray drying technique for obtaining stable amorphous SDs of low T_g drugs.

KEY WORDS: amorphous; *in vitro* drug release; *in vivo* study; low T_g; solid dispersions.

INTRODUCTION

Amorphous substances form a separate class of solids, distinct from the more common and well-known crystalline solids. The three-dimensional long-range order that normally exists in a crystalline material does not exist in the amorphous state and the position of the molecules relative to one another is more random. Pharmaceutical materials that are processed by high-energy processes such as freeze drying, spray drying, jet milling, melt extrusion, and so forth, are often rendered at least partially amorphous (1,2). This occurs by the virtue of the fact that these processes create conditions that can prevent crystallization or mechanically disrupt the structure of an existing crystalline material. The high internal energy and specific volume of the amorphous state relative to the crystalline state can lead to enhanced dissolution and bioavailability, but can also create a possibility that it may spontaneously convert back to the more stable crystalline state during processing or storage.

As stated earlier, the application of spray drying technique to obtain amorphous form of the drug substance, either alone or in combination with a hydrophilic polymer is now well known (1–4). The technique has desirable characteristics that the resultant particles are spherical and free flowing. The method also offers advantage that granulation and drying are completed in one step. In the previous paper (5), we demonstrated that solid dispersions (SDs) of a poorly water-soluble drug valdecoxib could be prepared by spray drying. It was also confirmed that the resultant SDs were stable and remarkably improved the dissolution property of the drug. However, in case of drugs having relatively low glass transition temperature (T_g), it is very difficult to obtain stable amorphous product in the form of a free flowing powder by spray drying. As the outlet temperature rises above the T_g, there is always a possibility that the final product is present in the supercooled rubbery state. Also such product is often sticky or tacky, which causes decrease in product recovery and hampers handling in subsequent

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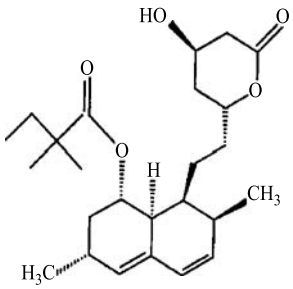

Fig. 1. Chemical structure of SIM.

processes (6). Under such circumstances, an adsorbent can be incorporated in the system to facilitates the transport of spray dried product upto the collection vessel. Colloidal silicon dioxide (Aerosil 200) is non-porous, hydrophilic adsorbent with a high specific surface area (200 m^2/g) (7). SDs containing Aerosil 200 have shown good drug dissolution properties and physical stability under dry conditions (8) and the hydrogen bonding potential of silanols in the local environment on silica surfaces is also well documented (9–11).

Simvastatin (SIM) is a cholesterol-lowering agent; widely used to treat hypercholesterolemia. SIM is a potent inhibitor of 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 (12,13). The chemical structure of SIM is shown in Fig. 1. It is a white to off-white, non-hygroscopic, crystalline powder that is practically insoluble in water. Its T_g is relatively low (around 35°C). Such drugs after oral administration often show dissolution as the rate-limiting step for their *in vivo* absorption and the appearance of the pharmacological effect. Therefore improvements in solubility and/or dissolution rate of poorly water-soluble drugs may lead to enhancement in their bioavailability (14–16).

In the current study, spray drying of a low T_g model drug SIM, either alone or in combination with a hydrophilic polymer polyvinylpyrrolidone K30 (PVP) was attempted with the aid of Aerosil 200 in different proportions. The SDs were characterized initially in comparison with pure drug and corresponding physical mixtures (PMs) in the same ratios. SD in the ratio of 1:2:2 parts by weight of SIM, Aerosil 200 and PVP, respectively was further subjected to accelerated stability testing as per ICH guidelines at 40°C/75% RH up to 3 months and checked for *in vitro* drug release along with presence of crystallinity using DSC and XRPD. In addition, the improvement in rate and extent of *in vitro* drug release from SD 1:2:2 was justified by *in vivo* study in rats.

MATERIALS AND METHODS

Materials

SIM was a generous gift from IVAX India Pvt. Ltd. (Mumbai, India). PVP (BASF), Aerosil 200 (Degussa), Pharmatose DCL 21 (DMV International), Avicel PH 102 and Ac-Di-Sol (FMC Corporation) were supplied by Get-Rid Pharmaceuticals Ltd. (Pune, India). All other chemicals and solvents were of reagent grade.

Preparation of SDs and PMs

SIM either alone or in combination with PVP (1:1 or 1:2 parts by weight) was dissolved in sufficient amount of dichloromethane. To these clear solutions proposed quantity of Aerosil 200 (Table I) was slowly added to obtain uniform suspensions. Spray drying was carried out using laboratory scale spray dryer (Jay Instruments & Systems Pvt. Ltd., Mumbai, India) under following set of conditions: Inlet temperature, 35°C; outlet temperature, 26–28°C; feed rate, 4–6 ml/min; atomization air pressure, 2 kg/cm^2 and aspiration, −250 mmWC. PMs in the same ratios were also prepared by physically mixing drug and excipients thoroughly for 10 min in a mortar until a homogeneous mixture was obtained. All the samples were passed through fine mesh (150 μm) and stored in desiccated environment until further study.

Drug Content

SDs equivalent to 10 mg of SIM were weighed accurately and dissolved in suitable quantity of methanol. The drug content was determined at 238 nm by UV-spectrophotometer (V-530, JASCO, Japan).

Saturation Solubility

To evaluate increase in solubility of SIM after spray drying (as in SDs) or only by the presence of hydrophilic polymer (as in PMs), saturation solubility measurements were carried out as follows: known excess of different formulations of SIM was added to 10 ml of phosphate buffer (pH 6.8). Samples were rotated at 20 rpm in a water bath (25°C) for 48 h. Samples were then filtered, suitably diluted and analyzed spectrophotometrically at 238 nm.

Scanning Electron Microscopy (SEM)

Samples were mounted on a double faced adhesive tape and sputtered with thin gold-palladium layer by sputter coater unit (VG-Microtech, UK) and surface topography was analyzed with a scanning electron microscope (Stereoscan S120, Cambridge, UK) operated at an acceleration voltage of 10 kV.

Differential Scanning Calorimetry (DSC)

DSC studies were carried out using differential scanning calorimeter equipped with an intracooler (DSC 821^e,

Table I. Composition of SDs and PMs of SIM

Type of formulation	Composition (parts by weight)		
	SIM	Aerosil 200	PVP
SD/PM 1:1	1	1	—
SD/PM 1:1:1	1	1	1
SD/PM 1:2:2	1	2	2

Mettler-Toledo, Switzerland). Indium/Zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 20°C/min over a temperature range of 20–150°C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50 ml/min.

X-Ray Powder Diffraction (XRPD)

The XRPD patterns were recorded on X-ray diffractometer (PW 1729, Philips, The Netherlands). The samples were irradiated with monochromatized CuKα radiation (1.542 Å) and analyzed between 2 to 40 °2θ. The voltage and current were used 30 kV and 30 mA, respectively. The range and the chart speed were 2×10^3 CPS and 10 mm/°2θ, respectively.

Infrared (IR) Spectroscopy

IR spectroscopy was performed on fourier transformed-infrared spectrophotometer (V 5300, JASCO, Japan). The pellets of drug and KBr were prepared on KBr-press (Spectra Lab., India). The spectra were scanned over wave number range of 4000 to 400 cm^{-1}.

Tablet Formulation and *in Vitro* Drug Release

All the formulations of SIM (equivalent to 10 mg of SIM) were compressed into tablets using 10-station, single rotary, B-tooling tablet machine (Rimek, MINI PRESS-I, Karnavati Engineering Ltd., Gujarat, India) using FFBE 8 mm die and punch set. Tabletting excipients like Pharmatose DCL 21 and Avicel PH 102 (diluents), Ac-Di-Sol (disintegrant) and magnesium stearate (lubricant) were also added as per the composition given in Table II. The average weight of tablet was maintained at 200 mg by accordingly adjusting the quantity of Pharmatose DCL 21. The tablets were evaluated for thickness and hardness using diametrial hardness tester (PTB, Pharmatest, India). The dissolution studies were performed using USP 24 type II dissolution test apparatus (TDT-06P, Electrolab, India). The tablets of different formulations equivalent to 10 mg SIM were 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 SIM was determined spectrophotometrically at 238 nm. Analysis of data was done using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India).

Stability Study

The accelerated stability of SD 1:2:2 was checked as per ICH guidelines at 40°C/75% RH upto 3 months. Periodically (initial, 15 days, 1 month, and 3 months) samples were removed and checked for *in vitro* drug release and presence of crystallinity using DSC and XRPD studies.

In Vivo Study in Rats

The Hypolipidemic activity of SD 1:2:2 was determined in comparison with pure SIM in healthy albino rats (Wistar strain) of either sex and weighing between 150 and 200 g. The animals were procured from National Toxicology Centre (Pune, India) and housed in the animal house of Poona College of Pharmacy (Pune, India). General and environmental conditions were strictly monitored. Animal handling routines were performed according to Good Laboratory Practice. Animals had free access to food and water was made available *ad libitum*. The research protocol of the animal experimentation was approved by the Institutional Animal Ethics Committee of Poona College of Pharmacy, Pune, India.

The animals were divided into 3 groups of 4 animals each. The treatment was given for 14 days. Each group daily received 2 ml of coconut oil orally. Reference and Test groups additionally received aqueous suspensions of pure SIM and SD 1:2:2 (equivalent to 10 mg/kg body weight) respectively, prepared using 2% w/v gum acacia as a suspending agent. 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) and high density lipoprotein (HDL) cholesterol levels by the *in vitro* diagnostic kit (ACCUREX BIOMEDICAL PVT. LTD., Mumbai, India). The statistical analysis for the determination of differences in lipid profiles of treatment and control groups was done by unpaired *t*-test and $p < 0.01$ was taken as significant.

Table II. Tablet Composition for Different Formulations of SIM

	Tablet composition (average weight 200 mg)				
Type of formulation	Amount of formulation equivalent to 10 mg of SIM (mg)	Pharmatose DCL-21 (mg)	Avicel PH-102 (mg)	AC-DI-SOL (mg)	Magnesium stearate (mg)
Pure SIM	10	130			
PM 1:1	20	120			
PM 1:1:1	30	110			
PM 1:2:2	50	90	50	8	2
SD 1:1	27.03	112.97			
SD 1:1:1	37.5	102.5			
SD 1:2:2	51.02	88.98			

RESULTS AND DISCUSSION

Preparation of SDs

Combination of SD and surface adsorption techniques has been attempted to overcome the limitations of spray drying technique for amorphization of a low T_g drug. As there was restriction on maintenance of outlet temperature below 35°C, dichloromethane was the only solvent of choice. Initially SIM and Aerosil 200 in various proportions were tried for feasibility of spray drying (data not shown). Based on powder characteristics, drug content, saturation solubility and feasibility of processing into tablets; minimum ratio of 1:1 parts by weight of SIM and Aerosil 200 (SD 1:1) was finalized. SD 1:1 was a free flowing powder with drug content of 74 ± 2%w/w and saturation solubility of 32.7 ± 1.6 μg/ml (Table III). This low drug content could be possibly due to the losses of a low T_g drug during spray drying. This indicated need for incorporation of PVP in different proportions (Table I) as a SD carrier, which would increase T_g and subsequently drug content and saturation solubility. SD 1:1:1 presented significant improvement in saturation solubility (56 ± 2.4 μg/ml). However, the drug content was still in the range of 80 ± 2% w/w. Finally, SD 1:2:2 was selected as the optimized formulation with drug content of 98 ± 2% w/w and approximately fivefold increase in saturation solubility (69 ± 3.1 μg/ml) in comparison with pure SIM (15 ± 1.04 μg/ml). Further increase in the amount of PVP could have resulted in improved solubility, but at the same time would have hampered the powder characteristics favourable for tablet formulation.

SEM

The microphotographs of pure SIM and its SDs are shown in Fig. 2 (A–D). Pure drug consisted of a mixture of some large crystals (8 to 10 μm) with microparticles, which might have been generated due to micronization or any other size reduction process at the time of manufacturing. SDs in different ratios revealed significant changes in particle shape and surface topography due to impact of spray drying process. SD 1:1 and 1:1:1 appeared as irregular shaped agglomerates with presence of few microcrystals, suggesting possibility of residual crystallinity. Slight surface smoothness observed in SD 1:1:1, in comparison to SD 1:1, could be attributed to the incorporation of PVP. SD 1:2:2 on the other hand looked like porous, spherical agglomerates with particle size in the range of 5–15 μm, suggesting presence of amorphous state.

Table III. Saturation Solubilities of Different Formulations of SIM

Type of formulation	Saturation solubility (μg/ml)[a]
Pure SIM	15 ± 1.04
PM 1:1	16.4 ± 2.1
PM 1:1:1	18.2 ± 1.1
PM 1:2:2	19.7 ± 0.7
SD 1:1	32.7 ± 1.6
SD 1:1:1	56 ± 2.4
SD 1:2:2	69 ± 3.1

[a] Mean ± SD, n = 3.

Thermal Analysis and XRPD Studies

Pure crystalline SIM was characterized by a single, sharp melting endotherm at 139.5°C (ΔH 77.39 J/g) during DSC (Fig. 3a) and prominent diffraction peaks in the range of 8–32°2θ during XRPD (Fig. 4a). Thermograms of PMs revealed broadening of melting endotherm of the drug along with significant decrease in enthalpy of fusion (142.9°C and 29.35 J/g, 141.72°C and 19.65 J/g, and 141.78°C and 12.84 J/g for PM 1:1, 1:1:1, and 1:2:2, respectively) (Fig. 3b–d). Consequently, there was significant decrease in intensity of some major SIM crystalline peaks (28.8, 22.8, 19.6, 18, 9.6°2θ) in diffractogram of PM 1:1 (Fig. 4b) and significant elevation of diffractograms in PM 1:1:1 and PM 1:2:2 (Fig. 4c and d). In general this partial loss of crystallinity may be observed due to physical presence of amorphous excipients (17).

The thermograms of SDs (1:1 and 1:1:1) (Fig. 3e and f) showed a very shallow endotherm at around 130°C (ΔH 5.13 J/g and 0.68 J/g, respectively), indicating presence of residual crystallinity. Also, the diffractogram of SD 1:1 (Fig. 4e) revealed presence of very low intensity peaks (28.8, 22.8, 18°2θ), which supported the observations of DSC. However, diffractogram of SD 1:1:1 (Fig. 4f) was unable to reveal the traces of crystallinity, might be due to influence of amorphous nature of Aerosil 200. The presence of residual crystallinity could be correlated with the amount of SD carrier, because as the amount of PVP was increased, the thermogram of SD 1:2:2 (Fig. 3g) presented a straight line with absence of any thermodynamic transitions. This clearly indicated the existence of amorphous state of drug, which was also confirmed by XRPD (Fig. 4g) showing a halo, characteristic to amorphous form. These results of DSC and XRPD were strongly supported by the SEM observations.

Ideally, as the thermogram of SD 1:2:2 presented no incidence of crystallinity, it should indicate the presence of T_g. There is a possibility that the presence of high amount of Aerosil 200 could have masked the thermodynamic transitions. Hence, in separate set of experiments (data not shown), the increase in T_g of SIM due to formation of SD with PVP (in the ratio 1:2 parts by weight) was determined by DSC. The observed T_g value was also compared with that of the predicted value obtained by fitting the data in the Couchman–Karasz (C-K) equation [Eq. (1)] (18).

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (1)$$

where w_1 and w_2 are weight fractions of each component and T_{g1} and T_{g2} are their corresponding T_g values. K in the C-K equation; a thermodynamic model, is defined as follows:

$$k = \frac{\Delta C_{p^2}}{\Delta C_{p^1}} \quad (2)$$

where ΔC_p is the difference in heat capacity at T_g.

The observed T_g of 114.82°C perfectly matched with the predicted value. Also, there was only a single T_g observed over the entire temperature range of DSC measurements.

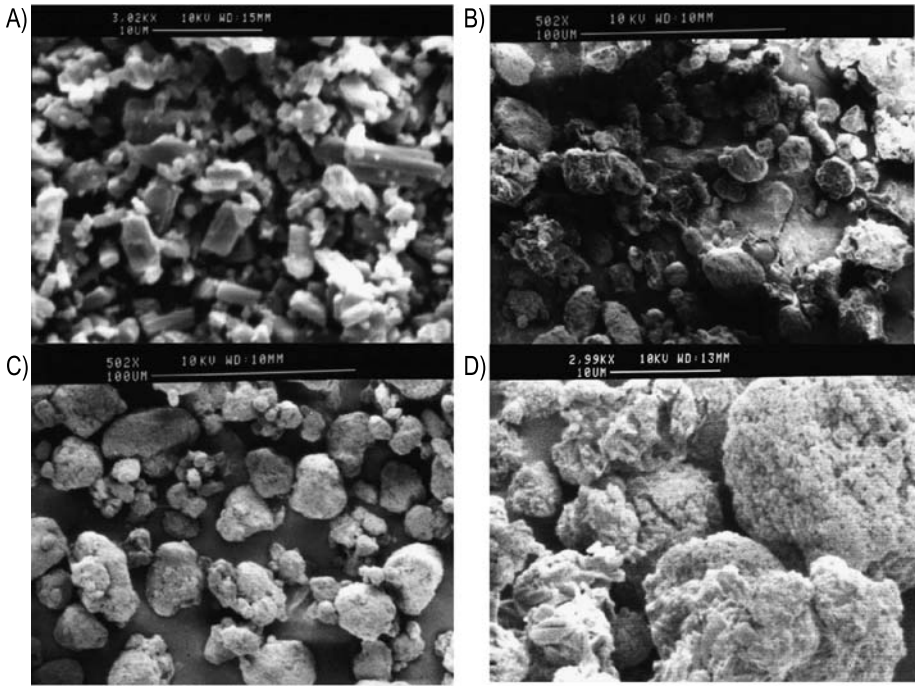

Fig. 2. SEM microphotographs of different formulations of SIM. Key: pure SIM (A); SD 1:1 (B); SD 1:1:1 (C); SD 1:2:2 (D).

This indicated perfect miscibility of drug and polymer to form uniform SD.

IR Spectroscopy

IR spectrum of pure SIM (Fig. 5a) presented characteristic peaks at 3553 cm^{-1} (free O-H stretching vibrations), 3011, 2959, and 2872 cm^{-1} (C-H stretching vibrations) and 1714 cm^{-1} (stretching vibrations of ester and lactone carbonyl functional group). The spectra of PMs should ideally be equivalent to the addition spectrum of drug, polymer and adsorbent. But in this case PMs in different proportions presented no significant difference in individual IR spectrum. Hence, only representative spectrum is shown (Fig. 5b). The overall spectrum appeared to be influenced by the incorporation of Aerosil 200. The presence of a broad prominent peak at 1107 cm^{-1} (strong Si-O linkage) is characteristic to Aerosil 200. As stated earlier, the hydrogen bonding potential of silanol groups in the local environment

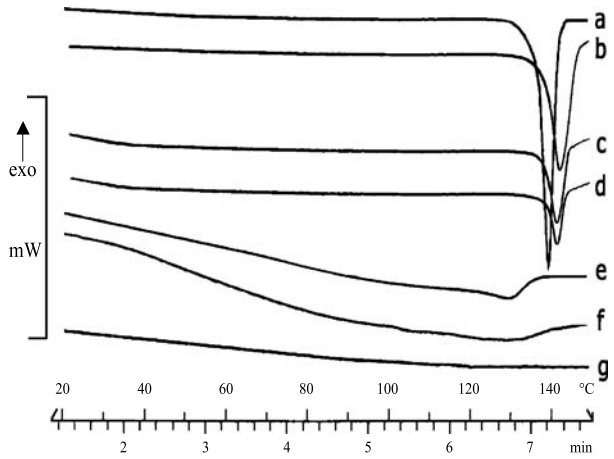

Fig. 3. DSC thermograms during initial characterization of different formulations of SIM. Key: pure SIM (a); PM 1:1 (b); PM 1:1:1 (c); PM 1:2:2 (d); SD 1:1 (e); SD 1:1:1 (f); SD 1:2:2 (g).

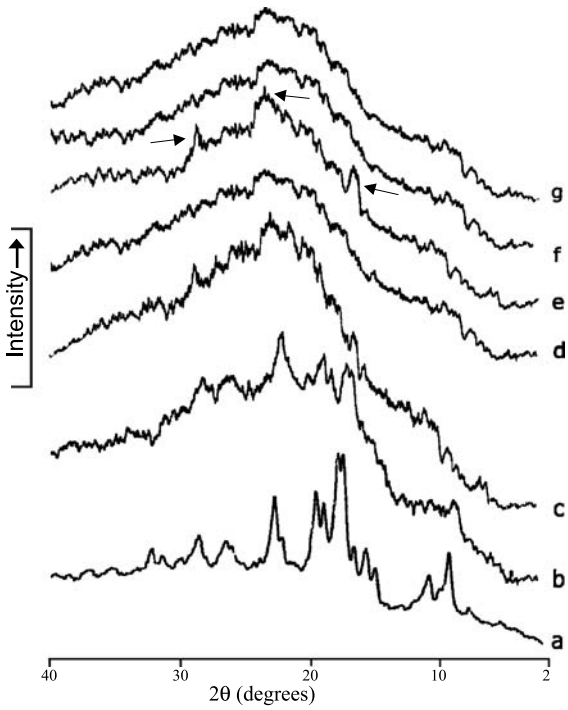

Fig. 4. XRPD patterns during initial characterization of different formulations of SIM. Key: pure SIM (a); PM 1:1 (b); PM 1:1:1 (c); PM 1:2:2 (d); SD 1:1 (e); SD 1:1:1 (f); SD 1:2:2 (g).

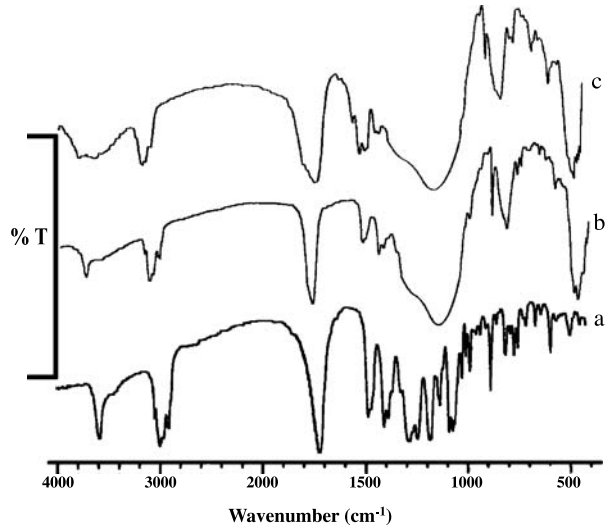

Fig. 5. IR spectra of different formulations of SIM. Key: pure SIM (a); representative PMs (b); representative SDs (c).

of silica is well documented, there is always a possibility that during preparation of PMs (trituration of drug and excipients for 10 min and passing through fine mesh) the carbonyl functional group of SIM can form very weak hydrogen bonds with silanol groups of Aerosil 200. This was clearly indicated by slight shift and broadening of 3549 cm^{-1} (O-H) and 1699 cm^{-1} (carbonyl) stretching vibration peaks of SIM in all spectra of PMs. This observation was in accordance with the data generated from DSC and XRPD studies. Similarly for SDs, only representative spectrum is shown due to the lack of significant difference in individual spectrum (Fig. 5c). In contrast to PMs, SDs presented possibility of hydrogen bonding between SIM and PVP. Each pyrrolidone moiety of PVP has two groups (=N– and C=O) that can potentially form hydrogen bond with the drug at molecular level in SD

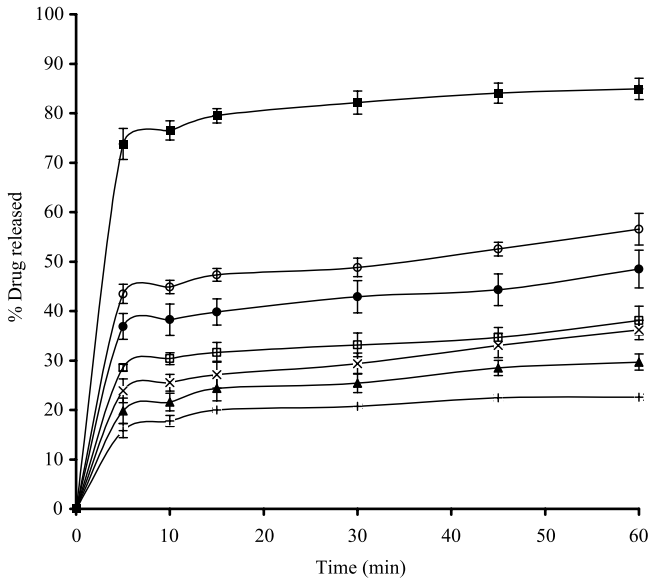

Fig. 6. Comparative dissolution profiles of different formulations of SIM. Key: pure SIM (+); PM 1:1 (▲); PM 1:1:1 (×); PM 1:2:2 (□); SD 1:1 (●); SD 1:1:1 (○); SD 1:2:2 (■).

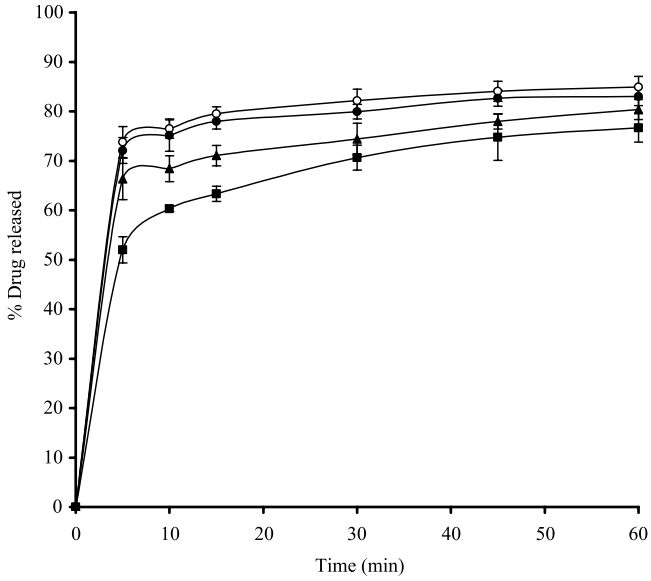

Fig. 7. Dissolution profile of SD 1:2:2 during stability study at different time intervals. Key: initial (○); 15 days (●); 1 month (▲); 3 months (■).

formulation. However, steric hindrance precludes the involvement of nitrogen atom in intermolecular interactions, thus making the carbonyl group more favorable for hydrogen bonding (19). Significantly broad peaks at 3553 cm^{-1} and 1678 cm^{-1} suggested hydrogen bonding interaction between free O-H group of SIM and carbonyl group of PVP.

Tablet Formulation and *in Vitro* Drug Release

Different formulations of SIM were compressed into tablets as per the compositions given in Table II. The tablet thickness and hardness were in the range of 3.6–3.8 mm and 6–8 Kp, respectively. Figure 6 shows *in vitro* drug release profiles of different formulations of SIM in tablet form. Pure SIM was characterized by only 22.57% drug release within 60-min in phosphate buffer (pH 6.8). PMs presented slight improvement in drug release and saturation solubility (Table III). This could be attributed to the improved wettability of drug particles by the physical presence of hydrophilic amorphous excipients. Also as indicated by IR spectroscopy, the possibility of weak hydrogen bonding interaction between drug and Aerosil 200 would increase

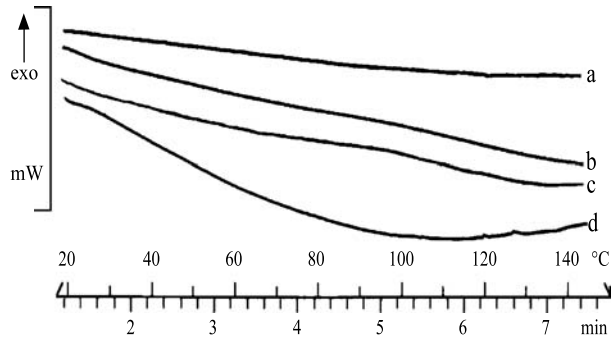

Fig. 8. DSC profiles of SD 1:2:2 during stability study at different time intervals. Key: initial (a); 15 days (b); 1 month (c); 3-months (d).

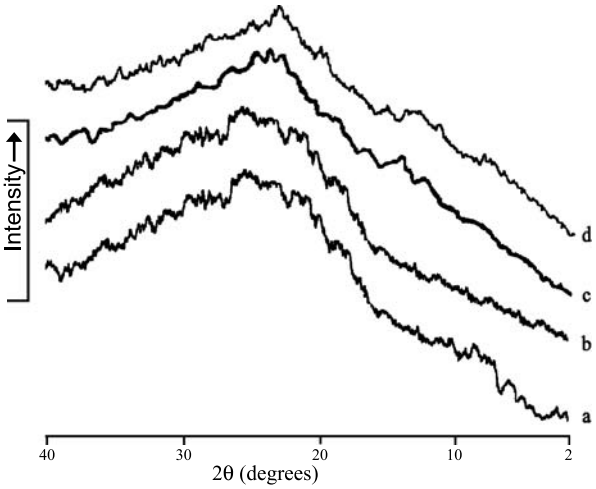

Fig. 9. XRPD profiles of SD 1:2:2 during stability study at different time intervals. Key: initial (a); 15 days (b); 1 month (c); 3 months (d).

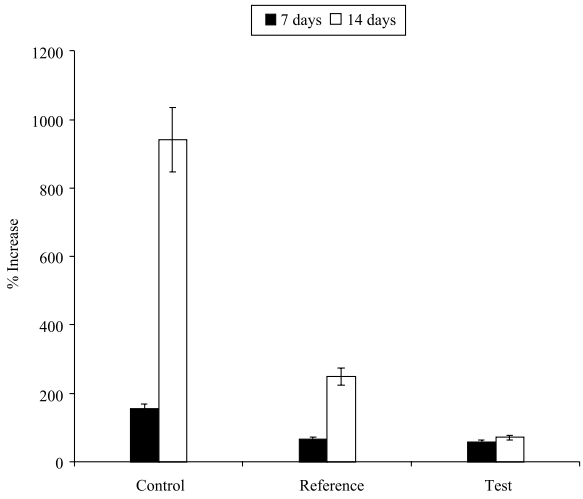

Fig. 11. Percent increase in serum TG levels of experimental groups at different time intervals.

the drug dissolution to some extent. SDs on the other hand, exhibited dramatical improvement in rate as well as extent of *in vitro* drug release with increase in the amount of PVP. SD 1:2:2 presented highest drug release (85% drug released in 60-min). During initial 5 min, there was approximately fivefold increase in the amount of drug released from SD 1:2:2 compared with pure SIM. This improved drug release could be attributed to presence of amorphous form of SIM, as confirmed by SEM, DSC, XRPD and IR studies.

Stability Study

It is well-known that amorphous drugs formulated in the form of SDs tend to recrystallize on storage at high temperature and humidity. Hence in the present study, accelerated stability studies were performed at 40°C/75% RH as per the ICH guidelines. Based on the results of initial characterization the SD 1:2:2 was thought to be the superior formulation and hence was further subjected to accelerated stability. There was insignificant decrease in *in vitro* drug release (Fig. 7) over the period of 3 months. Also the DSC and XRPD observations (Figs. 8 and 9, respectively) indicated presence of amorphous form of SIM. A characteristic halo, concave nature of the thermogram at the end of 3 months of storage, could be attributed to the loss of absorbed moisture by the extremely hydrophilic excipients during storage at higher relative humidity. Thus, the improved stability of SD 1:2:2 could be explained on the basis of combination of several effects: (i) increased T_g (114.82°C), which was well above the storage temperature; (ii) hydrogen bonding between the drug and the polymer; (iii) antiplasticizing effect of the polymer; (iv) entrapment of the drug molecules in the polymer matrix during solvent evaporation. [As the solvent is removed during the preparation of SD, viscosity of the system increases very rapidly leading to a decrease in drug mobility. When the solvent is evaporated completely, drug molecules are frozen in the polymer matrix. A crystal lattice is not formed, but the drug molecules are randomly ordered comparable to the liquid state and exhibit short-range order over only a few molecular dimensions,

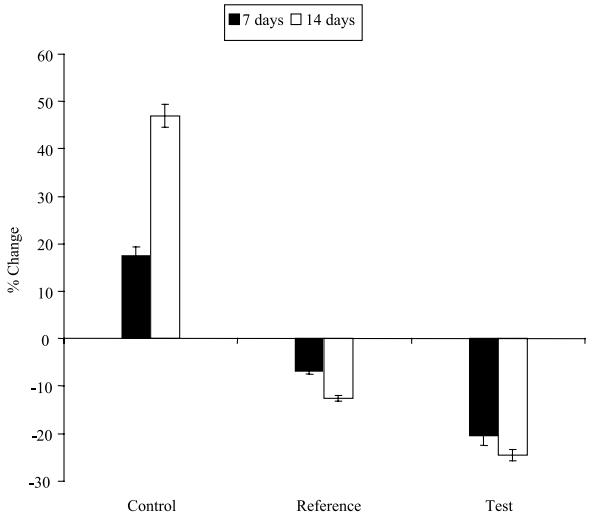

Fig. 10. Percent changes in serum total cholesterol levels of experimental groups at different time intervals.

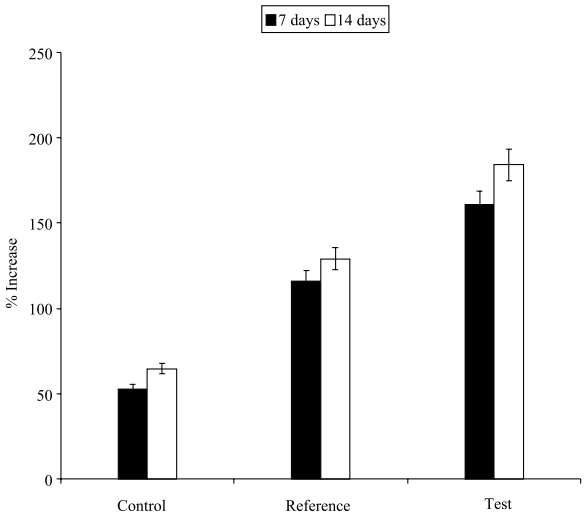

Fig. 12. Percent increase in serum HDL-cholesterol levels of experimental groups at different time intervals.

Table IV. Serum Lipid Profiles of Various Experimental Groups at Different Time Intervals

Experimental groups	Time intervals	Total cholesterol (mg/dl)*	TG (mg/dl)*	HDL- cholesterol (mg/dl)*
Control	Initial	52.5 ± 2.54	64.2 ± 16.80	24.2 ± 1.75
	7 days	61.7 ± 3.04	163.3 ± 8.42	37.1 ± 1.79
	14 days	77.2 ± 3.21	668.3 ± 78.16	40.3 ± 1.96
Reference	Initial	45.9 ± 2.94	67.5 ± 9.67	21.2 ± 2.68
	7 days	42.8 ± 4.04	111.4 ± 11.10	45.9 ± 1.95**
	14 days	40.2 ± 3.98	235.5 ± 22.16**	48.5 ± 1.50**
Test	Initial	56.5 ± 3.46	72.4 ± 10.26	20.5 ± 2.86
	7 days	44.9 ± 3.91	113.6 ± 18.14	53.5 ± 1.17
	14 days	42.7 ± 3.61	123.5 ± 22.31	58.4 ± 1.61

*Mean ± SD, n = 4, $p < 0.001$, **$p < 0.01$.

which is characteristic of amorphous form (20)]; (v) adsorption on the surface of amorphous Aerosil 200. Being hydrophilic in nature it enables preferential adsorption of moisture on its surface thereby acting as a buffer for SD system of drug and polymer. The possibility of interaction of Aerosil 200 with SIM and PVP to form ternary SD system was very low. It is well documented that for formation of such system incipient mechanochemical stress is the prerequisite and such stress can only be induced by cogrinding or melt quenching (21,22). Also, in case of solvent evaporation technique such as spray drying, which is employed in the present study, the use of polar solvents like methanol will favour hydrogen bonding between silanol groups of Aerosil 200 and carbonyl group of SIM. However, due to limited choice of solvents for spray drying of low T_g drugs, samples were prepared in dichloromethane, which is relatively non-polar in nature. It is reported that (23) fumed silica forms gelled structure in non-polar solvents, where silica particles are forced to interact with each other through hydrogen bonding interactions between the adjacent surface silanol groups to form a colloidal gel.

In Vivo Study in Rats

Hypolipidemic drugs like SIM (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 (24,25). This pharmacodynamic effect is reported to be dose dependent (25) hence, was used as a basis for the comparison of *in vivo* performance of pure SIM and SD 1:2:2. 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 different time intervals are presented in Table IV and the corresponding % changes in lipid profiles are plotted in Figs. 10–12. As expected, after 7 days of treatment with excess coconut oil, control group showed significant increase in total cholesterol, TG and HDL-cholesterol. Whereas, reference group showed around 7% decrease in total cholesterol, 65% increase in TG and 46% increase in HDL-cholesterol. Interestingly, test group in comparison to reference presented threefold decrease in total cholesterol, almost similar increase in TG and 1.4-fold increase in HDL-cholesterol. After 14 days of similar treatment, control group showed further increase in all the lipid levels. The reference group showed slight further decrease in total cholesterol, significant increase in TG and negligible increase in HDL-cholesterol. Test group on the other hand, presented further twofold decrease in total cholesterol, negligible increase in TG and further 1.4-fold increase in HDL-cholesterol in comparison with the reference group. Thus, at the end of 14 days study, SD 1:2:2 performed better than pure SIM in reducing total cholesterol and TG levels and increasing HDL-cholesterol levels. This could be primarily attributed to the improved solubility and dissolution associated with amorphization of the drug.

CONCLUSIONS

Amorphous SDs of SIM; a low T_g drug and PVP were successfully prepared by spray drying with the aid of Aerosil 200 as adsorbent. Initial characterization confirmed the presence of amorphous form in SD 1:2:2. IR spectroscopy revealed possibility of H-bonding interactions in both PMs and SDs, which was also supported by DSC and XRPD observations. SD 1:2:2 exhibited dramatical improvement in rate as well as extent of *in vitro* drug release. Very slight decrease in dissolution was observed with no evidence of crystallinity during accelerated stability studies of SD 1:2:2. Moreover *in vivo* study in rats also justified the improvement in therapeutic efficacy of SD 1:2:2 over pure SIM. Thus, present study demonstrates high potential of spray drying technique for obtaining stable amorphous SDs of low T_g drugs.

ACKNOWLEDGMENTS

The authors acknowledge the support of IVAX India Pvt. Ltd. (Mumbai, India) and Get-Rid Pharmaceuticals (Pune, India) for providing gift samples of SIM and other excipients, respectively. One of the authors (Anshuman Ambike) is thankful to CSIR (New Delhi, India) for providing financial support in terms of senior research fellowship. The authors wish to thank UGC (New Delhi, India) for support under special assistance program. The authors are thankful to Prof. S. L. Bodhankar for his support during the *in vivo* study.

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