Study of Differential Scanning Calorimetry of Atorvastatin in Solid Solution

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Abstract

In this experiment, lipid matrices were prepared by mixing and dissolving atorvastatin in lipidic excipients in presence of surfactants. The solid solutions were analyzed by differential scanning calorimetry (DSC) to predict whether or not the crystalline structure of atorvastatin can be converted into solid solution. DSC thermogram of atorvastatin and drug loaded self-emulsifying drug delivery systems (SEDDs) showed sharp melting endotherm at 161.19^0C $(161.19 - 168.76)^0$C which corresponded to its melting with normalized energy of 6.687 J/g. The thermogram of atorvastatin present in the preparations showed the endotherm within the range of $(145 - 155)^0$C which indicated the presence of atorvastatin crystals in preparations. The melting endotherm at $(56.54 – 62.49)^0$C was represented by glyceryl monostearate (GMS) which was present in the formulations. No drug crystal was observed when atorvastatin was mixed and dissolved in GMS-Tween 80 combinations and Cremophore RH 40 – soyabean oil combination. But the presence of drug crystal was remarkable in case of Atorvatatin- GMS-Cremophore RH 40 combinations. So the DSC thermogram represented the identity of each of the components and indicated the absence of interaction or complexation throughout the process of SEDDs preparation and on storage.

Keywords: SEDDS, DSC, atorvastatin

Introduction

Oral delivery systems designed for poorly water-soluble drugs include micelles with surfactants, microemulsions, self-emulsifying / microemulsifying drug delivery systems (SEDDS / SMEDDS), solid dispersions, microspheres and cyclodextrin inclusion complexes. These delivery systems have been shown to enhance oral bioavailability and therapeutic effects of poorly water-soluble drugs mainly by improving the poor solubility.

For the therapeutic delivery of lipophilic active moieties (Class II drugs), lipid based formulations are inviting increasing attention. Amongst many such delivery options, like incorporation of drug in oils, surfactant dispersions, emulsions and liposomes, one of the most popular approaches are the self-emulsifying drug delivery systems (SEDDS). SEDDS are mixtures of oils and surfactants, ideally isotropic, and sometimes containing cosolvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation. Many researchers have reported various rational applications of SEDDS for delivering and targeting lipophilic drugs, *e.g.,* coenzyme Q10, vitamin E, halofantrine and cyclosporin A (Burcham and Maurin, 1997; Serajuddin *et al.*, 1988; Myers and Stella, 1992; Schwendener and Schott, 1996; Charman *et al.*, 1992; Pouton, 1985; Shah *et al.*, 1994; Kommuru *et al.*, 2001; Julianto *et al.*, 2000). Potential advantages of these systems include enhanced oral bioavailability (enabling dose reduction), more consistent temporal profiles of drug absorption, selective drug targeting toward a specific absorption window in the GI tract, and drug protection from the hostile environment in the gut. For selecting a suitable self-emulsifying vehicle, drug solubility in various components, identification of emulsifying regions and resultant droplet size distribution need careful monitoring, since these are drug-specific systems. Atorvastatin, a crystalline compound, is practically insoluble in water and hence poorly absorbed from the GI tract (Khoo *et al.*, 2000; Gao *et al.*, 1998; Pouton and Charman, 1997). Atorvastatin is a selective, competitive inhibitor of Hydroxy-3-Methyl-Glutaryl Coenzyme (HMG-CoA) reductase, the rate-limiting

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enzyme that converts 3-hydroxyl-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including chelosterol.

Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes. Clinical and pathologic studies show that elevated plasma levels of total cholesterol (total-C), low density lipoprotein-cholesterol (LDL-C), and apolipoprotein B (apo B) promote human atherosclerosis and are risk factors for developing cardiovascular disease, while increased levels of high density lipoprotein-cholesterol (HDL-C) are associated with a decreased cardiovascular risk. In animal models, atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL. In the present study, SEDDS formulations containing atorvastatin were developed using different proportions of oils and surfactant systems for oral administration (Kang *et al.*, 2005; McClelland *et al.*, 1991; Cheng *et al.*, 1993; Ambike *et al.*, 2005; *USP 24/NF 19,* USP Convention, Rockville 2000).

Differential scanning calorimetry can be used to measure a number of characteristic properties of a sample. Using this technique it is possible to observe fusion and crystallization events as well as glass transition temperatures (T_g). DSC can also be used to study oxidation, as well as other chemical reactions. Glass transitions may occur as the temperature of an amorphous solid is increased. These transitions appear as a step in the baseline of the recorded DSC signal. This is due to the sample undergoing a change in heat capacity; no formal phase change occurs. As the temperature increases, an amorphous solid will become less viscous. At some point the molecules may obtain enough freedom of motion to spontaneously arrange themselves into a crystalline form. This is known as the crystallization temperature (T_c). This transition from amorphous solid to crystalline solid is an exothermic process, and results in a peak in the DSC signal. As the temperature increases the sample eventually reaches its melting temperature (T_m). The melting process results in an endothermic peak in the DSC curve. The ability to determine transition temperatures and enthalpies makes DSC an invaluable tool in producing phase diagrams for various chemical systems. In addition, the amount of crystalline structure (% crystallinity) can be quantified directly from the DSC melting endotherm by comparing the measured heat of fusion with that for a standard of known crystallinity. Alternatively, in polymer blends it is often possible to quantify blend composition based on the relative size of the crystalline melting peaks provided thermal history effects are constant. (Godkar, 1996; Patil *et al.*, 2004; Kim *et al.*, 2000; Vogel and Vogel, 1997; Elson, 1992; Hunter and Hirst, 1997).

Materials and Methods

Atorvastatin (Ronbaxy, India), Methanol (Merk, Germany), Dimethyl sulfoxide (DMSO)-Merk, Germany, Glyceryl monosterate (GMS)-Gatefosse, France, Glyceryl distearate (GDS)- Gatefosse, France, Glyceryl behenate (GBH)- Gatefosse, France, Poly ethylene glycol (PEG 6000)- Gatefosse, France, Tween 80-Merk, Germany, Soyabean oil–Gatefosse, France, Chremophore RH 40 (BASF), Chremophore EL (BASF), Avicel (Merk, Germany), Medium chain triglyceride (Mijtai, Taiwan), Liquid paraffin (Bangladesh).

Preparation of lipid matrix (SEDDS): Drug matrix was prepared by using drug (atorvastatin) and different excipients. First, atorvastatin & excipients were weighed accurately and taken in a vial. The mixture was then melted in a liquid paraffin bath at (160-162)^{0}C. The prepared solution was allowed to cool. Several matrices were prepared by this manner by using different excipients in different drug-polymer ratio (Table 1).

Preparation of microscope slide of prepared lipid matrix: Here, small quantity of prepared matrix from each vial was spreaded over a microscope slide and observed under electronic microscope to see whether any crystal is present or not.

Quantitative Analysis for Drug Matrix: Quantitative analysis of 15 formulations (matrices) was done for drug content determination. For analysis, small portion (approximately 10 mg) of each matrix was accurately weighed and dissolved in 100 ml of methanol. Then it was thoroughly shaken by using vortex mixer and 1 ml of this solution was taken in another 50 ml volumetric flask & diluted up to the mark. The solution was then filtered and absorbance was taken at 246 nm. By using the absorbance and standard curve equation, the drug content for each

matrix was calculated which is shown in Table 2 and Figure 1.

Thermal analysis of lipid matrix: Thermal analysis is a branch of materials science where the properties of materials are studied as they change with temperature. Differential scanning calorimetry is a thermoanalytical technique whereby the difference in heat flow between a sample and reference are measured as a function of temperature. Here, the sample and reference were maintained at the same temperature throughout the experiment. The temperature programs for DSC analysis were designed such that the temperature increases linearly as a function of time. The sample and an inert reference material are held in two different chambers. Symmetrical heating of the cell, and therefore of two containers, was achieved by constructing the furnace from a metal of high thermal conductivity. The provision of a gas flow through the cell, to sweep away volatiles, provided the required atmosphere, and to assist in heat transfer.

Table 1. Formulation of lipid matrices

Ingredients	Formulation code (mg)														
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F10	F11	F12	F13	F14	F15
Atorvastatine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
GMS	100	100	100	100	100	100	100	100	100	-	-	-	-	-	100
Tween-80	20	60	100	-	-	-	-	100	-	-	-	-	100	100	100
RH-40	-	-	-	20	60	80	-	-	100		150	60	-	-	-
Soyabean oil	-	-	-	-	-	-	-	200	200	-	-	-	-	-	-
Medium chain triglyceride	-	-	-	-	-	-	-	-	-	135	100	-	-	-	-
Dimethyl sulfoxide	-	-	-	-	-	-	-	-	-	65	50	-	-	-	-
Cremophore-EL	-	-	-	-	-	-	-	-	-	200		-	-	-	-
Poly ethylene glycol 6000	-	-	-	-	-	-	-	-	-			40		
Glyceryl behenate	-	-	-	-	-	-	-	-	-	-	-	-	100		-
Glyceryl distearate	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-
Avecil 101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200

Table 2. Quantitative analysis of lipid drug matrix (% potency)

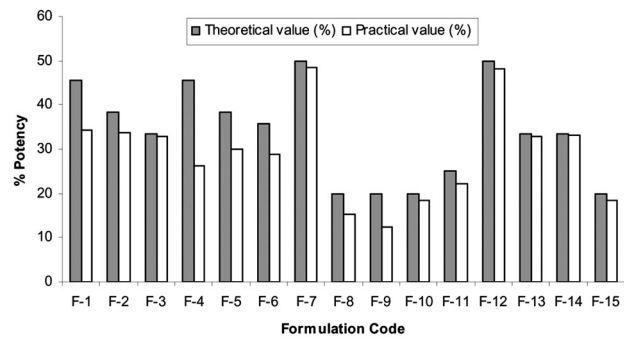| Parameter	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11	F-12	F-13	F-14	F-15
Theoretical value (%)	45.45	38.46	33.33	45.5	38.46	35.71	50.00	20.00	20.00	20.00	25.00	50.00	33.33	33.33	20.00
Practical value (%)	34.22	33.84	33.02	26.3	30.1	28.89	48.5	15.3	12.5	18.5	22.2	48.25	33	33.12	18.6

Figure 1. Quantitative analysis of lipid drug matrix.

Results and Discussion

Microscopic analysis of prepared matrices: The vials containing matrices were observed physically to see the physical state of matrices. Then small amount of matrix from each vial was spreaded on microscopic slide and placed under electronic microscope and presence or absence of crystals was observed. The results are given in Table 3.

Thermal analysis of matrices: DSC thermogram of drug (atorvastatin) and drug loaded matrices are shown in Figures 2-7. Atorvastatin showed sharp melting endotherm at 161.19^{0}C $(161.19 \ -168.76)^{0}$C (Table 4). The

thermogram of atorvastatin present in the preparations showed the endotherm within the range of $(145\ {-155})^0$C which indicated the presence of atorvastatin crystals in there preparations. The melting endotherm at $(56.54 - 62.49)^0$C was represented by GMS which was present in the formulations. No drug crystal was observed when atorvastatin was mixed and dissolved in GMS-Tween 80 combinations (Figure 4) and Cremophore RH 40 – soya oil combination (Figure 6). But remarkable presence of drug crystal was observed in case of atorvatatin- GMS-Cremophore RH 40 combinations (Figure 6).

Table 3. Physical state and microscopic observation of matrices.

Formu-lation code	Lipid matrix	Physical state	Presence of crystals
F-1	PEG 6000	Solid	+
F-2	Tween 80	Solid	+
F-3	GMS & Tween 80	Solid	-
F-4	GMS & Crem. RH 40	Solid	+
F-5	GMS & Avecil PH 101	Solid	-
F-6	Soybean Oil	Solid	+
F-7	Cremophore RH 40	Solid	-
F-8	Soybean Oil & Cremophore RH 40	Semi Solid	-
F-9	MCTG & Crem. RH 40	Solid	+
F-10	MCTG & Crem.-EL	Liquid	-
F-11	PEG 6000 & Crem.-EL	Solid	-
F-12	PEG 6000 & Tween 80	Solid	-
F-13	Tween 80 and GBH	Solid	-
F-14	Tween 80 and GDS	Solid	-
F-15	GMS, Tween 80 and Avecil 101	Solid	-

'+' = Presence of Crystal;   '-' = Absence of Crystal

Table 4. Melting point table

Compound	Melting point range	Exact melting point
Atorvastatin (as standard)	161.19-168.76 ℃	161.19 ℃
GMS	56.54 - 68.49 ℃	64.03 ℃
Atorvastatin (in combination with excipients)	150-155 ℃	150-155 ℃

So the DSC thermogram represented the identity of each of the components and indicated the absence of interaction or complexation throughout the process of SEDDs preparation and on storage.
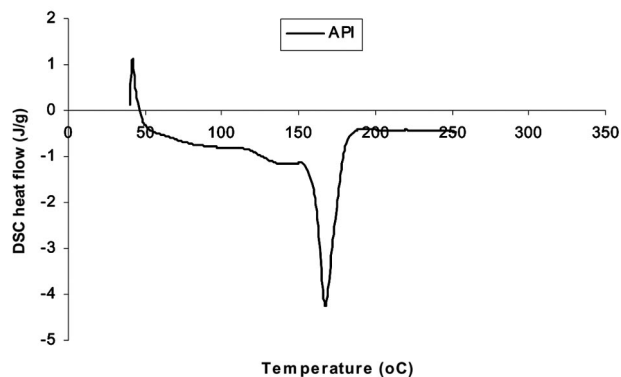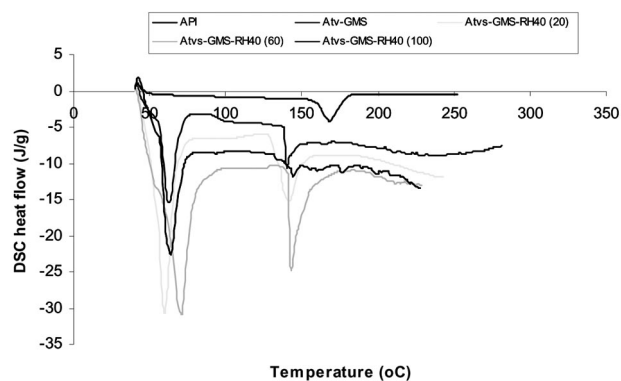

Figure 2. DSC curve for atorvastatin.

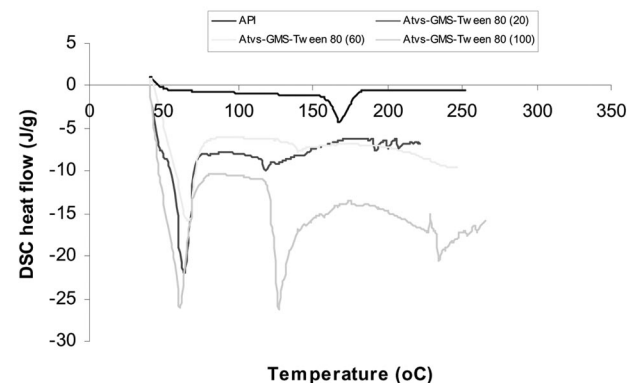Figure 3. DSC curve for atorvastatin-glyceryl monostearate-chremophore RH40 combination.

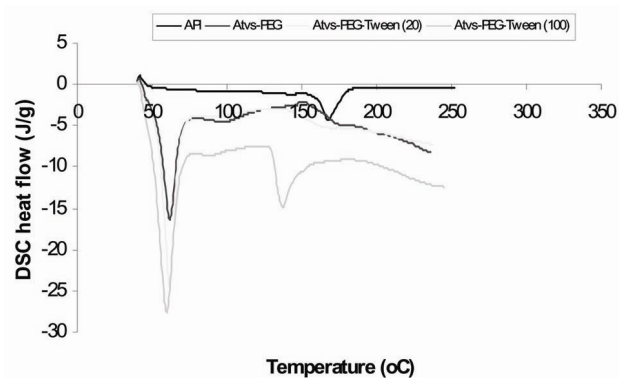Figure 4. DSC curve for atorvastatin-glyceryl monostearate-tween 80 combination.

Figure 5. DSC curve for atorvastatin-poly ethylene glycol-tween 80 combination.
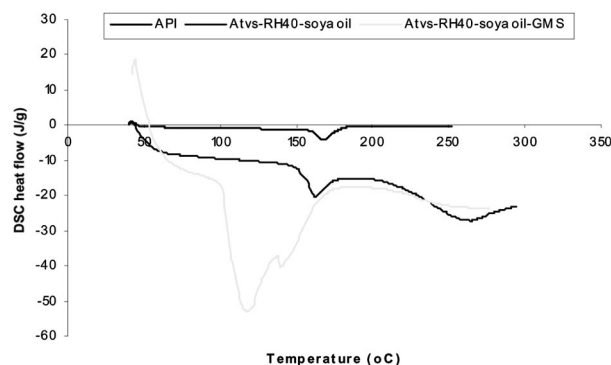

Figure 6. DSC curve for atorvastatin-glyceryl monostearate-chremophore RH 40-soyabean oil combination.
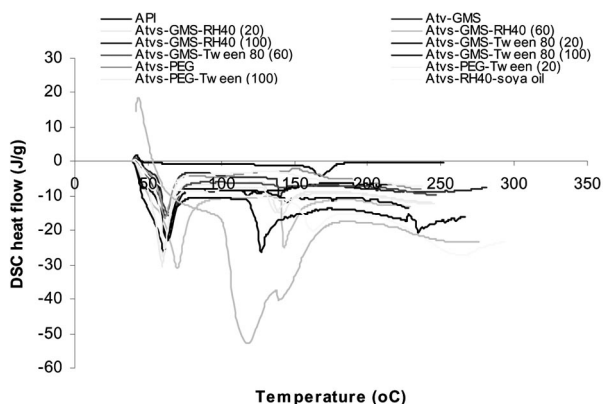

Figure 7. DSC curve for atorvastatin-glyceryl monostearate-tween 80-RH40-cremophor RH 40-poply ethylene glycol-soyabean oil combination.

Conclusion

This experiment indicated that crystalline structure of atorvastatin can be converted into solid solution with lipidic excipients in presence of surfactants. Dissolution studies of this matrix will be needed to ensure the faster bioavailability of atorvastatin from solid solution. This study illustrated the potential of atorvastatin SEDDS for oral administration. Further studies are required to establish a correlation between pharmacokinetics and pharmacodynamic responses of atorvastatin when administered in the form of SEDDS.

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