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Studies on lipid nanoparticle formulation of antihyperlipidemic drug

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ABSTRACT

Simvastatin is a biopharmaceutical classification system Class II drug which has less solubility and high permeability and therefore is slowly absorbed, which results in insufficient and uneven oral bioavailability. To overcome this limitation associated with the simvastatin, present work focuses on the formulation and evaluation of nanostructured lipid carriers of simvastatin which results in enhanced drug solubility and ultimately increased bioavailability. Simvastatin loaded nanostructured lipid carriers were produced by high shear homogenization coupled ultrasound method using Compritol 888ATO as solid lipid, Oleic acid as liquid lipid and surface tuned with non-ionic surfactant Tween 80. SIM loaded nanostructured lipid carriers were further characterised for mean particle size, polydispersity index, entrapment efficiency and drug release. The optimized formulation was evaluated for pharmacokinetics parameters such as C_{max}, t_{max} and AUC using suitable animal model. The study showed the potential of simvastatin loaded nanostructured lipid carriers as a carrier for enhancing the bioavailability of the drug as compared to the plain drug. This strategy can be used successfully in treatment of hypercholesterolemia.

KEYWORDS: Simvastatin, nanostructured lipid carriers, bioavailability, entrapment efficiency.

INTRODUCTION

Colloidal carrier system is receiving, growing interest in the field of drug delivery because they can offer several advantages in this area like, increased solubility and hence the increased bioavailability of poorly water soluble actives, which belong to the classes II and IV in the biopharmaceutical classification system (BCS), protection of drugs against degradation or alteration of their distribution after intravenous (i.v.) administration, increased drug loading capacity and the possibility of drug targeting and controlled release characteristics [1-3]. At the beginning of the 1990s, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been introduced as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. SLNs are composed of 0.1% w/w to 30% w/w solid lipid dispersed in an aqueous medium and if necessary, stabilized with preferably 0.5% w/w to 5% w/w surfactant. Composition of NLCs differs slightly as some part of solid lipid is substituted by liquid lipid. The incorporation of many cosmetic and pharmaceutical actives is feasible. The mean particle size of SLNs and NLCs is in the submicron range, ranging from about 40 nm to 1000 nm. SLNs and NLCs have the combine advantages of polymeric nanoparticles and o/w fat emulsions for drug administration such as good tolerability, lower cytotoxicity and not only increased bioavailability by oral administration but also increase in the drug stability. Another advantage of using lipid as excipient is biodegradability, cost effectiveness, suitable for the incorporation of lipophilic as well as hydrophilic drugs. Lipids have been used as carrier systems via the lymphatic transport to decrease the hepatic first pass metabolism causing an enhancement in bioavailability of many drugs [4, 5].

Simvastatin (SIM), a BCS Class II drug, is used as cholesterol-lowering agent in the treatment of hypercholesterolemia, dyslipidemia and coronary heart disease. It is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and it lowers the lipid concentration in the body [6]. SIM is practically insoluble in water, resulting in poor and variable oral bioavailability (around 5%). It has a short half life of about 2-3 h. It is rapidly absorbed from the gastrointestinal (GI) tract following oral administration, but undergoes

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extensive first-pass metabolism in the liver and/or GI mucosa by Cytochrome P3A [7].

This research work aimed at developing a lipid nanoparticulate system for a SIM which will lead to enhancement in its bioavailability and controlled release of drug. SIM NLCs formulations were developed by using Compritol 888ATO, Oleic acid and Tween 80 as formulation components using high shear homogenization (HSH) coupled ultrasound (US) method. This nanostructure improves drug loading and firmly retains the drug during storage. NLCs system minimizes some problems associated with SLNs such as low payload, drug expulsion on storage and high water content of SLNs dispersions. The prepared systems were characterized for particle size, entrapment efficiency (EE), drug release and pharmacokinetics study to prove the improvement in bioavailability. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies also carried out to check for crystallinity, polymorphic changes in SIM NLCs.

MATERIALS AND METHODS

Materials: SIM was kindly provided by Ranbaxy Laboratories, India. Solid lipids such as COMPRITOL ATO888 (Glyceryl behenate), PRECIROL ATO5 (Glyceryl palmitostearate) were obtained as gift sample from Gattefosse India Pvt. Ltd. IMWITOR 900 K (Glyceryl monostearate) was obtained as gift samples from Sasol GmbH, Germany. Glyceryl tristerate, Glyceryl tripalmitate, Oleic acid and TWEEN 80 were purchased from Sigma-Aldrich, Mumbai, India. Dialysis bag (molecular weight cut off 12‑14 kDa; pore size 2.4 nm) was supplied by Hi Media, Mumbai, India. All solvents and reagents used were of analytical reagent grade.

Methods:

Screening of lipids and oils: The solubility of SIM in different solid lipids was determined by a semi quantitative method. Lipid screening procedure used by Pardeike *et al.* was slightly modified [8]. A fixed quantity of drug was accurately weighed in a series of test tubes and different lipids were added in increasing order until drug is completely solubilised. The temperature of test tubes was maintained 10°C above the melting point of lipid used. The test tubes were intermittently shaken on cyclone mixer and observed visually for any drug residue. The amount of lipid required for solubilising fixed amount of drug was determined [9, 10]. For determining solubility in different oils, an excess amount of SIM was added to 2 ml of oil in a vial. After tightly capping, these vials were then kept on mechanical water bath shaker in which temperature was maintained at 37 ± 1 °C for equilibration. After equilibrium, each sample was centrifuged at 6500 rpm for 30 min using a centrifuge (Remi instruments, India) to separate the undissolved drug. Supernatant oil phase was removed, filtered through 0.45 µ membrane filter. The filtrate was diluted appropriately with methanol and absorbance was recorded by ultraviolet (UV) spectroscopy at λ_{max} of 238 nm. The concentration of SIM dissolved in oil was determined [11].

Preparation of NLCs: The preparation of NLCs was based on the principle of 'HSH coupled US' method as reported by Fang *et al.*[12]. Drug was solubilised in molten lipid phase along with liquid lipid (kept at 5-10 °C above the melting point of the lipid) in a water bath. The resulting drug-lipid mixture was poured into aqueous Tween 80 solution which was maintained at same temperature as lipid melt. The mixture was high shear homogenised using Ultra Turrax® T25 digital (IKA, Germany) at 8000 rpm for 2 min. The pre-emulsion thus formed was then sonicated for 5 min using Sonapros PR-250 M (Oscar Ultrasonics, Andheri). The resulting hot dispersion was cooled under magnetic stirring at 4-8°C to give NLCs dispersion. The composition of NLCs batches is as shown in Table 1.

Evaluation and characterization of SIM NLCs:

Particle size analysis: The particle size analysis of formulations was performed using NANOPHOX® (NX0073) particle size analyzer (Sympatec GmbH, Germany). An aliquot of NLC was diluted in deionised water prior to measurements. All the measurements were carried out in triplicate at a temperature of 25±2°C and at a fixed angle of 90° to the incident laser beam. Data was analysed by Windox software (Version 5.0) and values of mean particle size, polydispersity index (PI) and particle size distribution curve were recorded.

Entrapment efficiency: EE corresponds to the percentage of drug encapsulated within and adsorbed onto the nanoparticles. Nanoparticle dispersion was centrifuged at 14,000 rpm (Remi instruments, India) for 20 min to separate the nanoparticles. NLCs dispersion was aggregated by addition of electrolyte such as NaCl to facilitate the separation of the nanoparticles. After centrifugation the supernatant was analyzed for amount of free drug by using UV-spectrophotometric method after suitable dilution with methanol at λ_{max} of 238 nm [13]. The EE was calculated by the following equation:

$$\% \text{ Entrapment efficiency} = \frac{W_{\text{Initial drug}} - W_{\text{Free drug}}}{W_{\text{Initial drug}}} \times 100$$

Where, '$W_{Initial\ drug}$' is the weight of total drug added in the dispersion and '$W_{Free\ drug}$' is the weight of free drug found in the supernatant after centrifugation.

In vitro drug release: *In vitro* drug release was evaluated by using dialysis bag diffusion method [14]. Membrane was soaked in double-distilled water for 12 h before using for hydration. The pre-soaked dialysis bag was filled with NLCs dispersion equivalent to (5 mg) of SIM, tied at both ends and then immersed in the dissolution medium pH 7.0 buffer containing 0.5% SLS (500 ml) at 100 rpm and temperature was maintained at 37°C ± 0.5°C. Aliquots were withdrawn at different time intervals till 24 h from dissolution medium and replaced with 5 ml of fresh buffer maintained at same temperature in order to maintain perfect sink conditions. The withdrawn samples were filtered through 0.45 µ filter and analyzed at λ_{max} of 238 nm by using validated HPLC method. The % cumulative drug released versus time graphs were plotted.

X-ray diffraction (XRD) study: X-ray scattering measurements were carried out with Pan-analytical Xpert PRO MPD X-ray diffractometer. Anode material used was copper having $K\alpha_1$ and $K\alpha_2$ radiation wavelength of 1.5405 and 1.5444 respectively with generator voltage of 45 KV and tube current of 40 mA and detected using Xcelerator diffracted beam monochromator. XRD was carried out on Pure SIM, Pure Compritol 888ATO5 and SIM loaded NLCs.

Differential scanning calorimetric (DSC) study: DSC analysis was performed to check the drug‑lipid interaction in nanoparticulate formulations and crystallinity of drug. Samples were analyzed on SII Nanotechnology EXSTAR DSC 6220 in scanning range of 30-300°C at a heating rate of 10°C/min. DSC scans of plain drug, lipid, Drug-lipid physical mixture and NLCs formulation were recorded and compared.

Pharmacokinetic study: All animal procedures were performed according to the protocol approved by the Institutional Animal Ethics Committee of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai. Sprague–Dawley rats (Either sex, 200-250g) were procured from Glenmark Research Center, Mumbai. Animals were supplied with standard diet and free access to water under a 12 h light/dark cycle at the Animal Care Facility. Prior to dosing, the rats were fasted overnight (12–18 h) with free access to water. The SIM suspension and NLCs were administrated orally at a dose of 20 mg/kg. Blood samples were withdrawn from retro orbital plexus into tubes containing suitable anticoagulant at specified times intervals. Plasma was separated immediately by centrifugation (5000 rpm for 10 min at 4°C) and stored below -20 °C until further analysis.

Plasma samples were extracted for SIM by Liquid-Liquid extraction procedure. 25 µl of internal standard (Nicardipine hydrochloride, 20 µg/ml) was vortex mixed with 200 µl of plasma. Then 1000 µl of ethyl acetate as extracting solvent was added and vortex mixed for 1 min and centrifuged at 5000 rpm for 10 min. The organic supernatant layer was removed and evaporated to dryness under nitrogen at 40-50 °C. The residue thus obtained was reconstituted with 250 µl of methanol and an aliquot of 20 µl was injected into the HPLC for analysis. The concentration of SIM in rat plasma was determined by HPLC–UV detection.

The HPLC analysis was carried out using an Agilent Technologies 1200 series system, based on quaternary pump plus autosampler, UV detector and EZ-Chrome software. The column HiQSil® C_{18}HS (250 X 4.6 mm, 5 µm) was maintained at 25°C. An isocratic mobile phase used was Acetonitrile: 0.01 M Ammonium acetate (80:20 v/v) with the flow rate of 1ml/min and detection was carried out at λ_{max} of 238 nm. Pharmacokinetic parameters such as $C_{max,}$ T_{max} were calculated directly from the mean drug concentration-time profile curve of drug in plasma and area under the curve (AUC_{0-t}) by the method of residuals.

RESULTS AND DISCUSSION

Screening of lipids and oils: Lipid nanoparticles have been reported as useful tools in development of oral, injectable, topical dosage forms for poorly water soluble drugs [15]. The components selected to produce biodegradable and biocompatible lipid nanoparticles are of Generally Recognized as Safe (GRAS) status. Lipid solubility study carried out to determine the maximum solubility of drug in particular lipid with the aim to select a lipid based on the affinity of drug for that lipid matrix type. This also helps to choose lipid which can show high drug loading capacity as well as entrapment efficiency. Highest drug solubility in lipid also reduces chances of drug expulsion from lipid particles. It was found that Compritol 888ATO shows highest SIM solubilising capacity (114.3±3.512 mg/500 mg) over other lipids followed by Imwitor 900K (63.18 ± 3.294 mg/500 mg) and Precirol ATO5 (58.34 ± 2.450 mg/500 mg). Fig.1 shows the comparative solubility of drug in different lipids. Compritol 888 ATO and Precirol ATO5 showed higher solubilisation capacity as they are made up of mixture of fatty acid esters as compared to Imwitor 900 K as it is a

monoglyceride. These lipids also show lesser recrystallization and polymorphic changes as compared to other lipids.

To develop SIM loaded NLCs, oil with high drug solubility needs to be selected. Different oils such as oleic acid, Ethyl oleate, Miglyol 812, isopropyl palmitate (IPP) and isopropyl myristate (IPM). Amongst these oils, oleic acid had highest drug solubilising capacity about 38.67 ± 0.4136 mg/ml. Fig. 2 shows the drug solubility in different oils. Main role of oleic acid in NLCs formation will be to disorganize the structure of lipid matrix but it additionally improves drug incorporation ability and also increased drug loading.

Preparation of NLCs: High shear homogenization (HSH) followed by Ultrasonication (US) is a very simple and common method for preparation of SLNs and NLCs [16]. It is a method of choice for SLNs and NLCs preparation for drugs showing high solubility in molten lipids. The role of surfactant is not only to control the particle size and stability of dispersion but also to control the crystallization behaviour of the lipid particles including polymorphs [17]. Tween 80 was selected as model surfactant based on the preliminary surfactant screening from a range of surfactants reported in literature such as Tween 80, Tween 20, Solutol HS 15 and Poloxamer 188. Evaluations were based on particle size obtained and batch stability. Agrawal *et al.* have developed acitretin loaded NLCs using Tween 80 as model surfactant [18]. Oleic acid was used in concentrations of 30, 50 and 70 mg in optimized formula for SIM SLNs (optimization data not shown). Effect of concentration of oleic acid on particle size and EE is as shown in Table 2. Concentration of 30 mg oleic acid was used in further studies as it showed desired particle size and EE.

Evaluation of NLCs:

Particle size: Particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems [19-21]. SIM SLNs formulation (B1) showed particle size of 153.53 ± 64.50 nm. Incorporation of increasing amounts of oleic acid caused increase in the particle size of the batch as the total lipid content of the formulation was increased without any changes in the concentration of surfactant. Batches 'B2- B4' showed particle size in range of 165.52 ± 27.66 to 175.14 ± 24.15 nm. Batch 'B5' was taken after optimization of the lipid surfactant ratio with fixed concentration 30 mg of oleic acid and the particle size was on a slightly higher side around 200.19 ± 15.83 nm (Table 2).

Entrapment efficiency (EE): The % EE increased with increase in concentration of total lipid content of formulations. This is probably due to increase in viscosity of medium resulting in faster solidification of nanoparticles. This would further prevent the drug diffusion to external phase of medium [16]. This could also be attributed to the effect of liquid lipid i.e. oleic acid. This oleic acid can lead to imperfections in the solid lipid structure by incorporating itself inside the matrix of the solid lipid. Batch B1 showed EE of 79.20 ± 2.00 %. The optimum concentration of oil is important; as the concentration of liquid lipid amount plays a crucial role with respect to EE. Batch B2 showed maximum EE of 83.42 ± 1.66 % having oleic acid concentration of 30 mg (Table 2). As we further go on increasing the concentration of oil (B3 and B4) EE decreased. One probable reason for this could be the increased liquid lipid amount was not accommodated by solid lipid during solidification and was dispersed in the external aqueous phase. As drug has maximum solubility in this liquid lipid it got partitioned in to the liquid lipid. Post optimization batch 'B5' showed EE of 89.79 ± 2.25% as the amount of lipid and surfactant were optimized with respect to oleic acid concentration.

In vitro drug release: Based on the results of EE and particle size batch 'B5' was chosen for carrying out the *in vitro* drug release. For evaluating the *in vitro* performance of nanoparticles, drug diffusion studies using the dialysis technique are well documented in literature [22-24]. Hence, the dialysis bag diffusion technique was used to assess the drug release from the NLCs formulations. The dissolution medium used was pH 7.0 buffer with 0.5% SLS [25]. Sodium Lauryl Sulphate (SLS) was used to act as a wetting agent in low concentrations as plain SIM has low aqueous solubility [26]. The % drug release of optimized NLCs formulation and suspension of SIM are shown in fig. 3. Initially there was burst release about 10 % of drug within 1 h. This may be attributed to increased aqueous solubility of SIM leading to rapid dissolution of drug molecules present in the surface layer of particles. After that the release of the drug was at very slow rate. The % drug diffused at the end of the 24 h was 20 % only but in a sustained manner. This slowed down release may be because of the more imperfect matrix of NLCs which helps in entrapment of more drug. Diffusion of drug from the inner most cores of the solid lipids would take time and thus the release of the drug was slowed down significantly. Zhuang *et al.* [27] had reported similar kind of results for SLNs and NLCs formulation of vinpocetine. The comparative study of SIM suspension showed that almost 40 % drug was released in first 1 h and at the end of 8 h, 92%

drug was released. Drug release profile was found to follow first order drug release kinetics (r^2 = 0.927) [28].

XRD study: In XRD study, the graph for SIM shows sharp peaks at a diffraction angle (2θ) of 10.93°, 15.60°, 16.98°, 17.21°, 17.67°, 18.79°, 19.36°, 22.52°, 25.83° and 28.33° are present, and it reveals that drug is present in crystalline form. The absence of characteristic peaks of SIM in the NLCs graph suggests incorporation of drug into the matrix of NLCs which also indicates the amorphization of drug. Results obtained for XRD as shown in Fig. 4.

DSC study: The DSC thermograms of SIM, Compritol, SIM-Comp physical mixture and SIM NLCs formulation are as shown in fig. 5. SIM shows a sharp melting endotherm at 140 ºC which corresponds to its melting point. This melting endotherm was absent in DSC of physical mixture and NLCs formulation. Complete solubilisation of SIM in the lipid matrix could be a reason for the absence of SIM from the DSC of physical mixture and NLCs formulation.

Pharmacokinetic study: Bioavailability study involved measurement of drug concentration in plasma by developed bioanalytical method. Fig. 6 represents graph of plasma drug concentration at different time intervals for SIM after oral administration of SIM NLCs (Batch B5) and plain SIM. The plasma concentration of SIM increased and attained maximum after which the drug concentration declined. The pharmacokinetic parameters such as C_{max}, T_{max}, and AUC are given in Table 3. The difference of bioavailability between NLCs and plain drug was very significant. Mean plasma concentration profile of SIM NLCs indicated prolonged release and its subsequent *in vivo* absorption. In case of plain SIM, there was a quick absorption and a sharp elimination phase T_{max} was reached within 1.20 h of administration. Whereas after the release of drug from NLCs, the absorption phase was slow and prolonged. The post absorption phase after C_{max} for NLCs could be attributed to a combination of elimination of already absorbed drug and continued absorption from the slow release of SIM from NLCs *in vivo*. The mean T_{max} value for NLCs was found to be 4.52 h. This showed that NLCs were effective in delaying the peak plasma concentration, thus indicating prolonged plasma concentration of SIM *in vivo*. The mean peak plasma concentration (C_{max}) of NLCs was found to be 2.83 times more as compared to the plain drug. This showed that the lipid nanoparticulate formulation effectively reduced the amount of drug released and consequently absorbed *in vivo* in the initial phase. *In vivo* intestinal absorption of lipid nanoparticles has been reported by Zhang *et al.* [29] for SIM SLNs. They have studied absorbtion of SLNs into the enterocytes of intestinal lumen by endocytosis with the help of markers. It was hypothesised that rapid absorption of SLNs into the intestine could be useful for enhancing SIM bioavailability after oral administration before it gets degraded into the intestinal lumen.

The mean biological half-life ($t_{1/2}$) of SIM from NLCs and plain SIM was found to be 9.4 h and 1 h respectively. This indicates that the declining phase of the plasma concentration–time curve involves an input function in addition to the elimination function, i.e., it is not a true elimination phase. The plasma half-life values are the same for the same drug substance, regardless of the dosage form. The difference observed here is due to prolonged absorption of the NLCs; there is prolonged continuous introduction of SIM into the blood stream. Therefore, lipid nanoparticulate formulation was found to have a longer plasma half-life, i.e., the drug stays in the plasma for a longer time than the plain SIM. Also it has been reported that lipids enhance the lymph formation and fasten the lymph flow rate. They are used as carrier to decrease the hepatic first pass metabolism by way of intestinal lymphatic transport of the drug via the thoracic lymph duct to the systemic circulation at the junction of the jugular and left subclavian vein, causing enhancement in bioavailability. SLNs formulation of lovastatin has shown to increase in bioavailability of lovastatin through the same mechanism of lymph transport [30, 31].

The rate of elimination (K_e) for SIM NLCs was found to be (0.073 h^{-1}) less than that of plain SIM (0.693 h^{-1}), which implies that the drug was slowly released and absorbed from the NLCs. Results of the *in vitro* drug release study also confirm that SIM was released in a sustained manner from the lipid carrier system. It has been reported earlier that incorporation of SIM into lipidic carrier system increases its solubility approximately to 2 mg/ml from 15 ng/ml and this helps in the improvement of bioavailability of SIM [29, 32]. The mean area under plasma time curve AUC_{0-t} of NLCs was 1285.08 ng h/ml, while the mean AUC_{0-t} of plain SIM was 142.87 ng h/ml. Thus, the overall absorption of SIM from NLCs was 8.99 times higher than plain SIM which shows enhancement of bioavailability at the same dose. Based on these results, it can be concluded that the greater bioavailability obtained from SIM NLCs is due to its sustained release nature imparted by the solid lipid nanoparticulate structure and the slower release from the matrix of the lipid nanoparticles.

CONCLUSION

Lipid nanoparticles have been used as one of the systems of choice for increasing the oral bioavailability of poorly water soluble drugs having high first pass metabolism. In the present study SIM NLCs were successfully developed by HSH coupled US method which is the simplest method of preparation of SLNs. Formulation developed using Compritol 888ATO, Oleic acid and Tween 80 gave high EE, satisfactory particle size distribution. DSC and XRD studies proved the favorable crystalline behavior of NLCs for protection and entrapment of SIM. Pharmacokinetic studies also suggested the increased bioavailability and absorption of drug in a sustained manner in accordance with the results of *in vitro* drug release study. These results suggested use of SIM NLCs as a novel approach for treatment of hyperlipidemia and also a promising drug delivery system to enhance the oral bioavailability of poorly water soluble drugs.

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Conflict of Interest: There is not any conflict of interest in this study.

Table 1: Composition of SIM NLCs

Batch No	**Compritol 888 ATO (mg)**	**Oleic acid (mg)**	**Tween 80 (mg)**	**Water (ml)**
B1	110	-	275	20
B2	110	30	275	20
B3	110	50	275	20
B4	110	70	275	20
B5	160	30	300	20

Table 2: Results for particle size, EE And polydispersity index for batch B1-B5

Sr. No.	**Particle Size (nm)**	**EE (%)**	**Polydispersity index**
B1	153.53 ± 64.50	79.20 ± 2.00	0.230±0.0919
B2	165.52 ± 27.66	83.42 ± 1.66	0.104±0.0121
B3	188.60 ± 26.79	75.55 ± 1.95	0.432±0.0712
B4	175.14 ± 24.15	70.03 ± 2.03	0.557±0.0257
B5	200.19 ± 15.83	89.79 ± 2.25	0.314±0.0063

Table 3: Pharmacokinetic parameters for comparison of plain SIM and SIM NLCS

Parameter	**Plain SIM**	**SIM NLCs**
T_{max} (h)	1.20	4.53
C_{max} (ng/ml)	12.23	34.69
Absorption half life (h)	0.7	1.4
Absorption rate constant (K_a) (h^{-1})	0.99	0.495
Elimination half life (h)	1	9.4
Elimination rate constant (K_e) (h^{-1})	0.693	0.073
AUC_{0-t} (ng h/ml)	142.87	1285.08

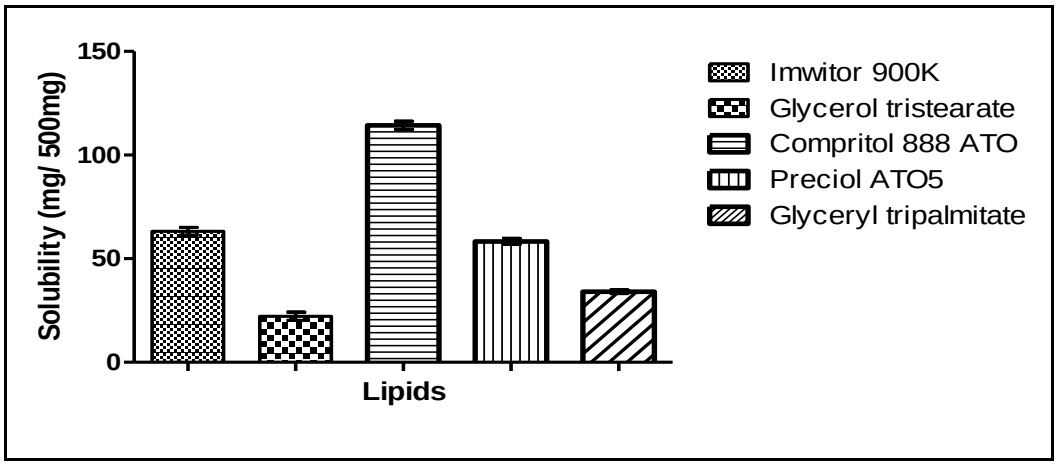

Fig 1: Comparative drug solubility in different lipids

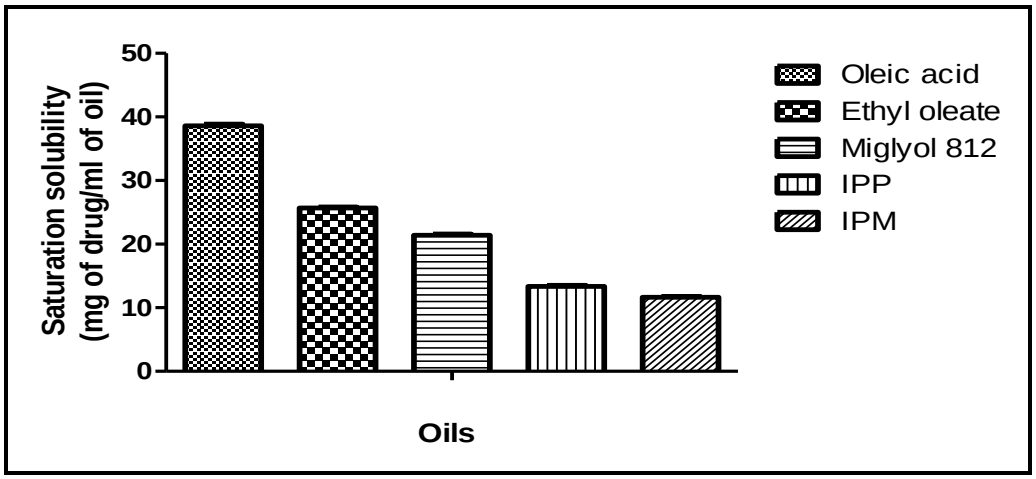

Fig 2: Saturation solubility of SIM in different oils

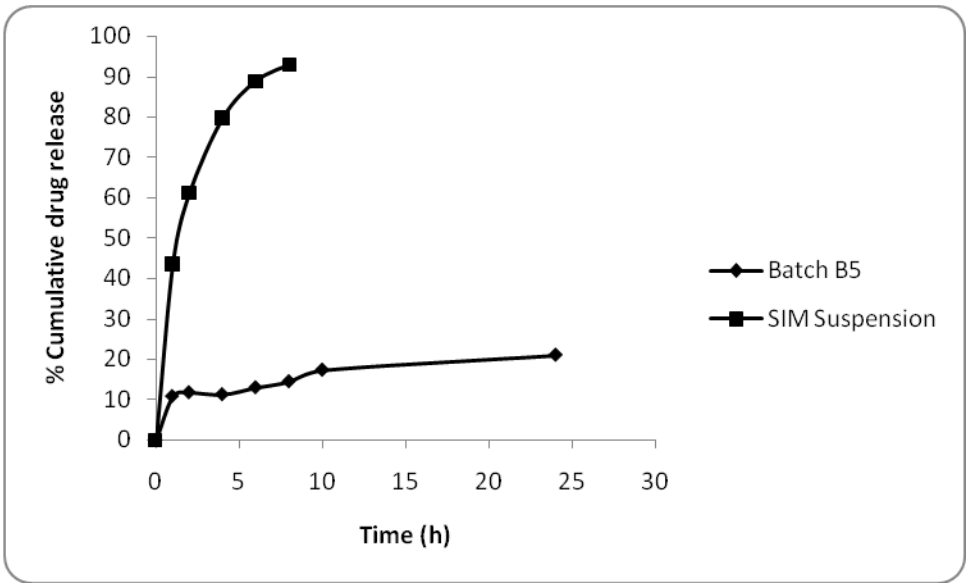

Fig 3: *In vitro* drug release of optimized NLCs formulation (batch b5) and SIM suspension

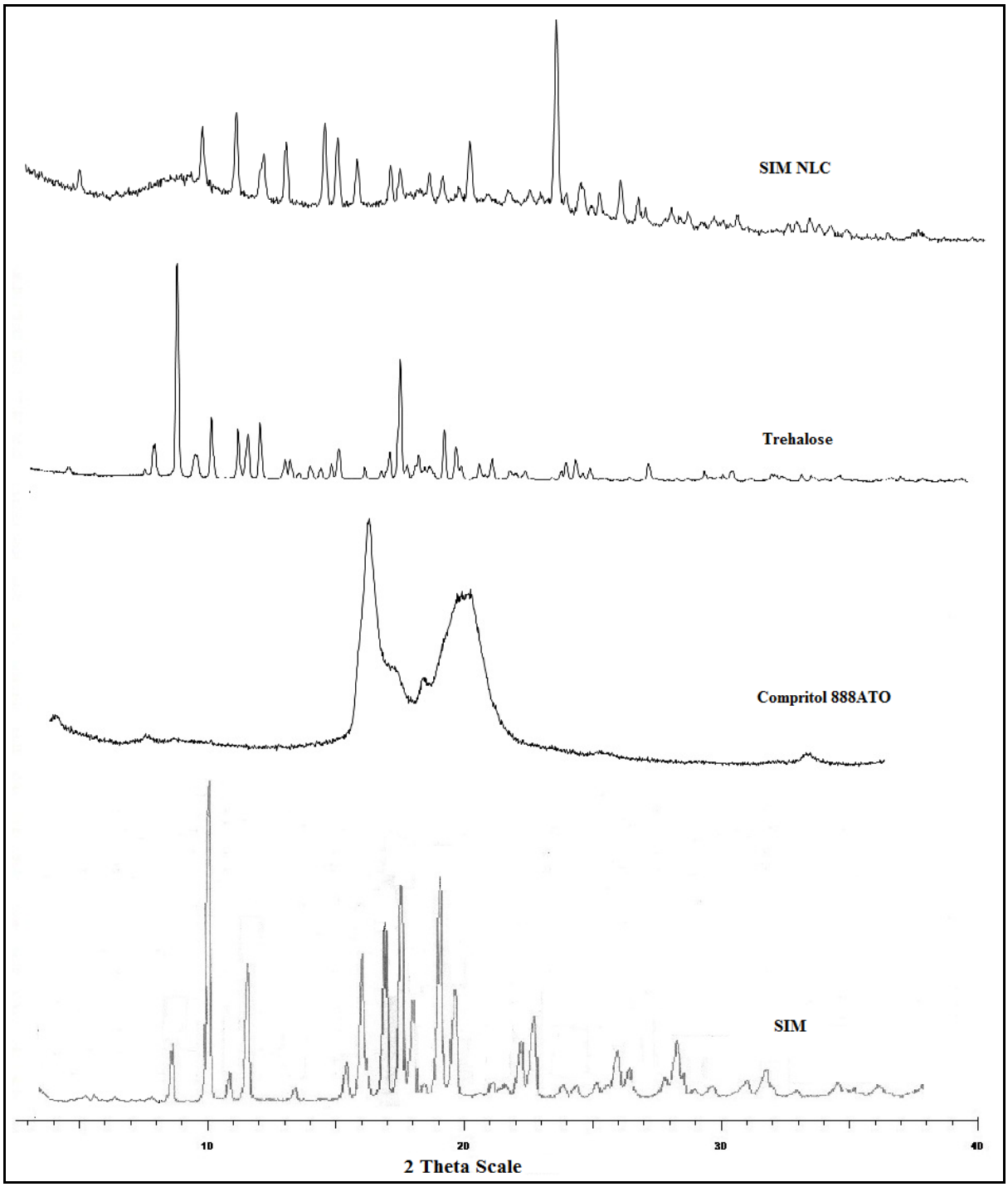

Fig 4: XRD patterns for SIM, Compritol 888ATO, trehalose and SIM NLCs

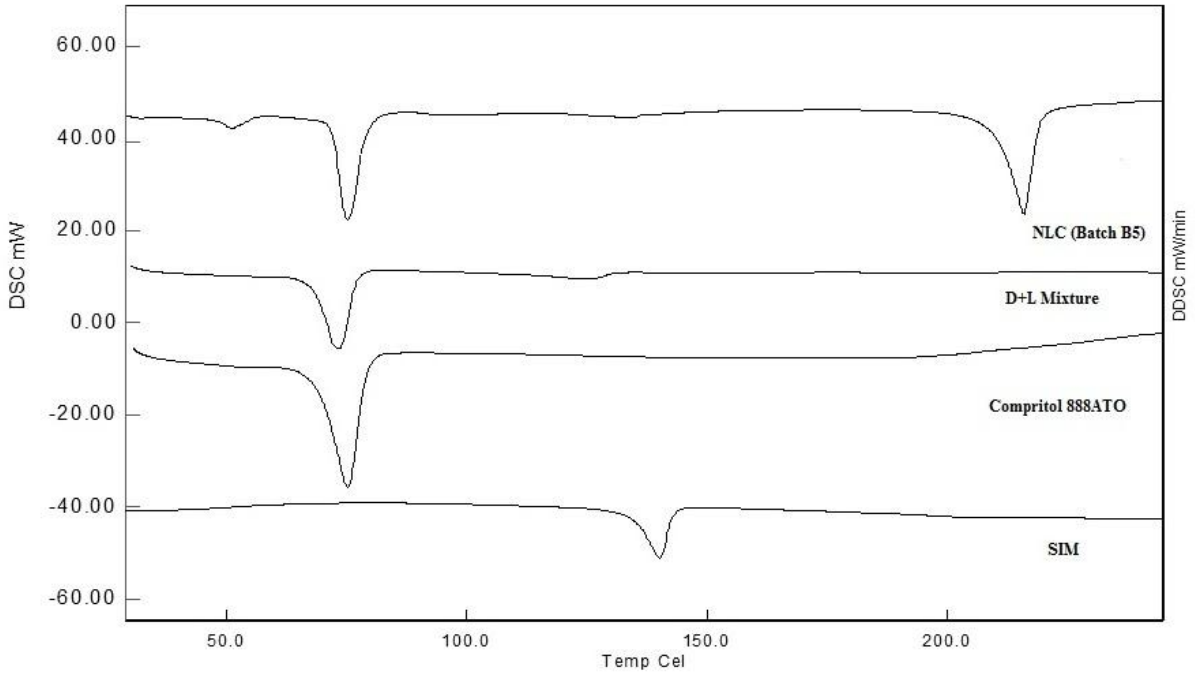

Fig 5: DSC scans for SIM, Compritol 888ATO, D+L physical mix and NLCs (batch B5)

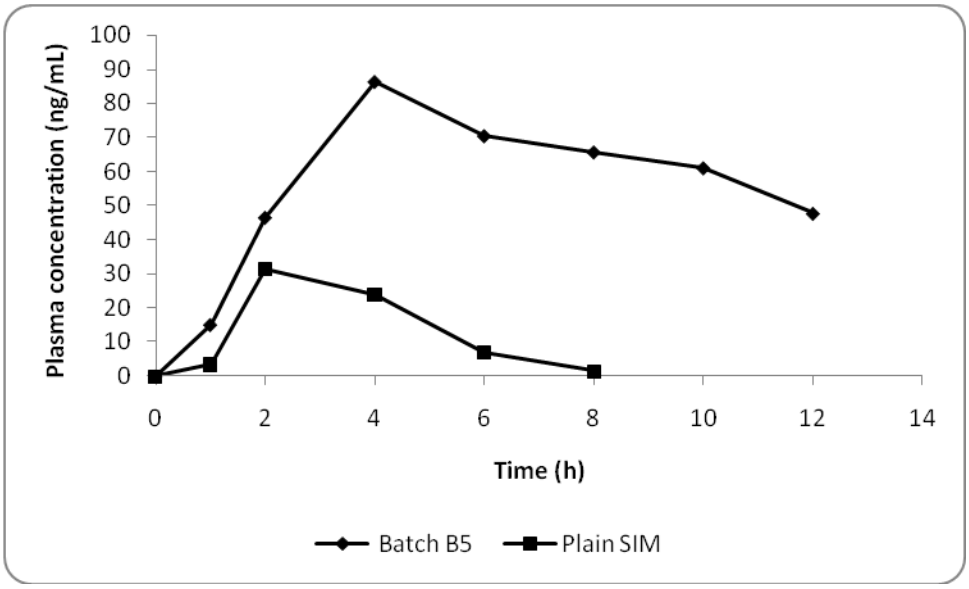

Fig 6: Mean plasma concentration of SIM after oral administration of plain SIM and SIM NLCs

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