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Preparation and characterization of nitrendipine solid lipid nanoparticles

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Nitrendipine, a dihydropyridine calcium channel blocker, has very poor oral bioavailability (10–20%) due to first pass effect. Solid lipid nanoparticle (SLN) delivery systems of nitrendipine have been developed using various triglycerides (trimyristin, tripalmitin and tristearin), soy phosphatidylcholine 95%, poloxamer 188 and charge modifiers stearylamine and dicetyl phosphate. SLNs were prepared by hot homogenization of melted lipids and aqueous phase followed by ultrasonication at temperatures above the melting point of lipids. Optimization studies of process and formulation variables were carried out. Particle size and zeta potential were measured by photon correlation spectroscopy (PCS) using Malvern zetasizer. Differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) studies were performed to characterize state of drug and lipid modification. *In vitro* release studies were performed in phosphate buffer pH 6.8 using modified Franz diffusion cell. Stable nitrendipine SLNs of mean size range 79 to 213 nm and zeta potential −38.2 to +34.6 mV were developed. About 99% nitrendipine was entrapped in SLNs and were stable on storage at 4 and 25 °C. DSC and PXRD analyses revealed that nitrendipine is dispersed in SLNs in an amorphous state. The release pattern of drug is analyzed and found to follow Weibull distribution rather than first order and Higuchi equation.

1. Introduction

Drug therapeutic index could be maximized while incidence of adverse reactions or side effects could be minimized by formulating the drug into nanotechnology based novel drug delivery systems. Since the beginning of nineties, attention from various research groups has focused on an alternative to polymeric nanoparticles, the solid lipid nanoparticles (SLNs). SLNs combine the advantages of different colloidal carriers, for instance these are physiologically acceptable like emulsions and liposomes, like colloidal drug delivery systems, SLNs are suitable for peroral administration for controlled and targeted delivery (Műller et al. 2000; Mehnert and Mäder 2001). In the literature, promising sustained release and targeting of camptothecin to brain was shown after oral administration of camptothecin loaded SLNs coated with poloxamer 188 (Yang et al. 1999). In recent years, much work has been focused in the development of SLNs as delivery systems for anticancer drugs, peptides, genetic material, cosmetics, etc (Olbrich et al. 2001; Wissing and Műller 2002; Zara et al. 2002a; Hu et al. 2004a). SLNs offer a new approach to improve the oral bioavailability and to minimize variations in bioavailabilities of poorly soluble drugs (Hu et al. 2004b; Luo et al. 2006; Műller et al. 2006). Intraduodenal administration of idarubicin SLNs and nitrendipine SLNs to rats showed improved bioavailability compared to corresponding solution/suspension (Zara et al. 2002b; Venishetty et al. 2007). Our earlier reports showed that clozapine and nitrendipine SLNs of different triglycerides after their duodenal administration to rats improved the bioavailability of corresponding drugs (Manjunath and Venkateswarlu 2005, 2006).

Present investigations are aimed at developing solid lipid nanoparticles (negatively and positively charged) of nitrendipine, and characterization of crystallinity of drug and lipids. Triglycerides used in the SLNs preparation may exhibit polymorphism in nature. Degree of crystallinity of the nanoparticles is a function of the nature of the lipid, surfactants and stabilizers used in the preparation and also of preparation technique. DSC quantifies the enthalpic changes during endothermic or exothermic effects. DSC utilizes the fact that different lipid modifications possess different melting points and melting enthalpies. DSC and PXRD analyses are used to investigate the status of the lipid and drug. Characterization of the degree of lipid crystallization and lipid modification are helpful in understanding the drug incorporation and release pattern. *In vitro* release studies are performed in evaluation of sustained and prolonged release potentials of dispersion systems. Charge modifiers such as stearylamine and dicetylphosphate are used with the intention that presence of either positive or negative charge on nanoparticles could be able to positively alter the *in vitro* stability factors. Effect of charge modifiers, stearylamine and dicetyl phosphate, on drug release are studied. Much attention is given to investigate release kinetics of nitrendipine from SLNs.

2. Investigations, results and discussion

2.1. Preparation of SLNs

Various researchers employed different methods (high-pressure homogenization, microemulsion, solvent emulsification, solvent diffusion techniques and solvent injection method) to prepare solid lipid nanoparticles (Gasco 1993; Műller et al. 1995; Dingler and Gohla 2002; Hu et al. 2002). In our previous study, we had developed an economical and simple method for the preparation of clozapine SLNs i.e. hot homogenization followed by ultrasonication (Venkateswarlu and Manjunath 2004). The same method is used in the present study after optimizing formulation and process variables. In order to optimize the lipid to drug ratio, different amounts (2, 3, 4 and 5% w/v) of lipids were selected and formulated with constant amount of drug (0.1% w/v). At lower lipid concentration (2 to 4%), drug deposition at the bottom was observed indicating insufficient encapsulation efficiency. However, at the lipid 5% w/v concentration, there was no such deposition in all three lipid based formulations. Based on these results lipid concentration 5% w/v was optimized for further studies. The solvent system chloroform:methanol (1:1) was efficient to disperse the nitrendipine homogeneously in the lipid phase. Rotoevaporation at 400 m bar, 60 °C followed by nitrogen purging ensures the complete removal of the traces of organic solvent. Homogenization of melted lipid phase with hot aqueous poloxamer solution for 3 min was sufficient to produce a coarse emulsion with particles of 3.15 to 3.19 μm. Further increase in homogenization time did not cause any significant decrease in particle size (3.08 to 3.15 μm). Thus a homogenization time of 3 min was optimized for all formulations. To further reduce the particle size below 1 micron, a probe ultrasonicator was used. Sonicating the coarse emulsion for 20 min produced particles between 79 to 213 nm with narrow size distribution (polydispersity index 0.259 to 0.265). Further increase in sonication time did not lead to a significant decrease in particle size.

2.2. *Measurement of size*

Sizes of nitrendipine loaded SLNs of different triglycerides with different percentages of poloxamer 188 (at 1.0% w/v of phosphatidylcholine 95%) are shown in Fig. 1. In three lipid based nitrendipine SLNs, optimum size (173.6 to 192.2 nm) was obtained at 1.5% w/v poloxamer 188 concentration. In case of blank SLNs optimum size obtained was 115.1 to 138.6 nm. Similarly, sizes of nitrendipine loaded SLNs of different triglycerides with different percentages of phosphatidylcholine (at 1.5% w/v of poloxamer 188) are shown in Fig. 2. As the concentration of phosphatidylcholine increases the size of the nitrendipine SLNs were decreased. Least particle size 113.9 to 127.6 nm of nitrendipine SLNs were obtained at 2.0% w/v phosphatidylcholine concentration. Least particle size range obtained in blank SLNs was between 85.4 and 102.5 nm.

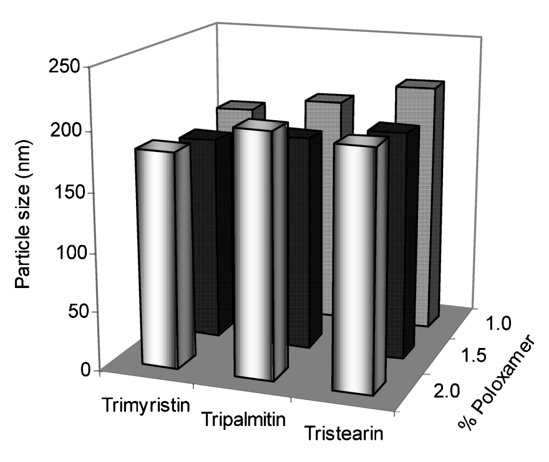

Fig. 1: Effect of poloxamer 188 concentration (1.0, 1.5 and 2.0% w/v) on particle size of nitrendipine SLNs of different triglycerides (trimyristin, tripalmitin and tristearin) (n = 3)

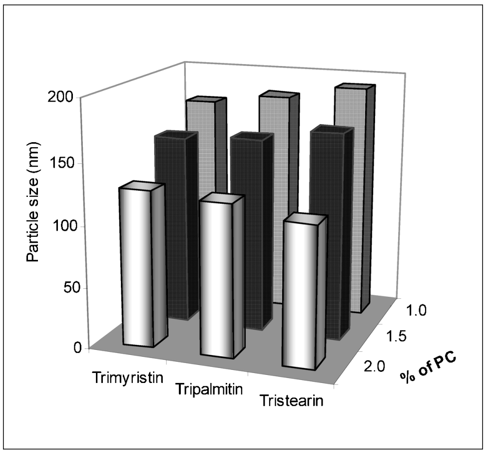

Fig. 2: Effect of phosphatidylcholine concentration (1.0, 1.5 and 2.0% w/v) on particle size of nitrendipine SLNs of different triglycerides (trimyristin, tripalmitin and tristearin) at constant 1.5% w/v of poloxamer 188 (n = 3)

Blank and nitrendipine SLNs of trimyristin (prepared with poloxamer 1.5% w/v and phosphatidycholine 2.0% w/v) showed particle sizes of 105.4 ± 0.9 and 127.6 ± 0.8 nm, respectively. In presence of dicetyl phosphate (0.05% w/v), particle sizes were reduced to 83.5 ± 1.5 ($p < 0.001$) and 109.1 ± 1.4 nm ($p < 0.01$), respectively. Similarly, in presence of stearylamine (0.05% w/v) the particle size reduced to 85.1 ± 1.5 ($p < 0.001$) and 110.0 ± 2.0 nm ($p < 0.01$) in case of blank and nitrendipine SLNs, respectively. A similar trend was observed in case of negatively and positively charged blank and nitrendipine SLNs of tripalmitin and tristearin (Table 1). Dicetyl phosphate and stearylamine induced negative and positive charges to the blank and nitrendipine solid lipid nanoparticles, respectively, by covering their surfaces effectively. Thus, similarly charged nanoparticles repulse with adjacent nanoparticles, thereby coalescence is minimized and the particles obtained were smaller than those of blank and nitrendipine SLNs without charge modifiers.

The present investigation was aimed to develop suitable (smaller sized) nitrendipine SLNs intended for improvement of oral bioavailability of the drug. On duodenal administration of drug loaded SLNs, nanoparticles are taken up by the lymphatic system (Bargoni et al. 1998; Cavalli et al. 2003). Optimum size for lymphatic uptake is between 10 and 100 nm (Swartz 2001). However, uptake becomes more selective and slower as the particle size increases. Therefore, in this study, formulations containing 1.5% w/v of poloxamer 188 and 2.0% w/v of phosphatidylcholine are optimized which produced smaller sized particles and these are used in further studies.

2.3. *Measurement of Zeta potential*

Zeta potential of blank and drug loaded SLNs of different triglycerides (TM, TP and TS) ranged from −16.8 ± 0.4 to −19.8 ± 0.1 mV (Table 1). In order to alter the zeta potential of blank and nitrendipine SLNs charge modifiers, stearylamine and dicetyl phosphate were included in the formulations individually. Addition of stearylamine (0.05% w/v) increased the zeta potential in all three lipid based SLNs (+32.8 ± 0.3 and +34.6 ± 2.3 mV for blank and nitrendipine SLNs, respectively). Addition of dicetyl phosphate (0.05% w/v) decreased the zeta potential in all three lipid based SLNs (−38.2 ± 0.4 and −35.1 ± 0.5 mV for blank and nitrendipine SLNs, respectively)

Table 1: Average sizes and zeta potentials of SLNs of different triglycerides (TM, TP and TS) with and without charge modifiers (1.5% poloxamer 188 and 2.0% phosphatidylcholine) (mean ± S.D.; n = 3)

SLN	Size (nm)					
	Without charge modifier		With dicetylphosphate (0.05% w/v)		With stearylamine (0.05% w/v)	
	BL	NDP	BL	NDP	BL	NDP
Trimyristin	105.4 ± 0.9	127.6 ± 0.8	83.5 ± 1.5***	109.1 ± 1.4***	85.1 ± 1.5***	110.0 ± 2.0***
Tripalmitin	107.0 ± 1.3	123.5 ± 1.4	79.3 ± 3.3***	107.2 ± 1.4**	84.4 ± 2.1***	108.6 ± 1.9**
Tristearin	102.5 ± 2.4	113.9 ± 1.8	81.8 ± 2.1***	104.9 ± 1.6*	89.4 ± 2.7**	101.9 ± 2.1**
	Zeta potential (mV)					
Trimyristin	−18.9 ± 0.6	−16.8 ± 0.4	−36.9 ± 0.4	−34.3 ± 1.2	+32.8 ± 0.3	+34.6 ± 2.3
Tripalmitin	−19.8 ± 0.1	−18.2 ± 0.3	−37.1 ± 0.3	−35.1 ± 0.5	+31.6 ± 0.8	+33.1 ± 0.2
Tristearin	−19.5 ± 0.2	−18.3 ± 0.2	−38.2 ± 0.4	−34.9 ± 0.8	+32.4 ± 0.5	+34.5 ± 0.5

Statistical significances with charge modifiers versus without charge modifier are
BL = Blank solid lipid nanoparticles
NDP = Nitrendipine solid lipid nanoparticles
***P < 0.001
**p < 0.01
*p < 0.05

(Table 1). This clearly indicates orientation of stearylamine and dicetyl phosphate in the monolayer along with phosphatidylcholine and poloxamer 188.

2.4. *Assay and entrapment efficiency*

The retention time of drug was 9.85 min. The average calibration curve ($y = 351849x - 16078$) was linear ($R^2 = 0.9992$) in the concentration range of 0.25–10 µg/ml in phosphate buffer pH 6.8. The inter- and intra-day accuracy and precision were within an R.S.D. ≤ 5.0%. Assay results showed that concentration of nitrendipine in the total system ranged from 0.97 to 1.01 mg/ml for different formulations. As the percentage of poloxamer 188 increased (1.0 to 2.0% w/v), entrapment efficiency was decreased very slightly (99.9 to 99.5%). This may be due to a slight increase in solubility of nitrendipine in the aqueous phase as the percentage of poloxamer 188 increased. However, there were no changes in entrapment efficiency of nitrendipine SLNs at different percentages of phosphatidylcholine. Even after the addition of charge modifiers, there were no changes of entrapment efficiency of nitrendipine SLNs (Table 2).

2.5. *Stability data*

After six months storage at 4 °C, the particle size of nitrendipine SLNs of different triglycerides (TM, TP and TS) without charge modifiers increased by maximum 48.1 nm, whereas sizes of nitrendipine SLNs with dicetyl phosphate and stearylamine increased by 41.3 nm and 92.6 nm, respectively. Storage at 25 °C, the particle size of nitrendipine SLNs of different triglycerides (TM, TP and TS) without charge modifiers increased by maximum 131.3 nm. Whereas size of nitrendipine SLNs with dicetyl phosphate and stearylamine increased by 89.6 nm and 172.6 nm, respectively. The increased size at 4 and 25 °C was in the following order NDP-SA > NDP (without charge modifiers) > NDP-DCP (Table 3). Increase in size is observed prominently in case of positively charged SLNs containing stearylamine when compared to SLNs negatively charged and without charge modifiers. This greater increase in size of positively charged SLNs might be caused by changes in shape of the particles due to interaction of stearylamine with the phospholipid. It has been previously shown that the phosphatidylcholine polar head groups strongly interact with the positively charged stearylamine head groups in the electrical double bilayer (Korner et al. 1994). Helder et al. (2000) identified that triglyceride based emulsions are able to form unexpected biocompartmental structures with different morphology when stearylamine is included in the formulation. Increase in size of blank SLNs of different triglycerides, with and without charge modifiers was also observed at 4 and 25 °C after six months storage. The increase in size of blank SLNs was in accordance with increase in size observed in case of drug loaded SLNs. Therefore, the increase in size of nitrendipine SLNs was due to inherent nanoparticle formulation rather than drug incorporation.

Entrapment efficiencies of SLNs of different triglycerides (TM, TP and TS) without charge modifiers and with dicetyl phosphate were lowered by 1.6% after six months of storage at 4 °C (Table 3), whereas it was lowered by 2.5% in case of SLNs with stearylamine. However, at 25 °C, entrapment efficiencies of SLNs of different triglycerides (TM, TP and TS) without charge modifiers and with dicetyl phosphate were lowered by 3.3%, compared to 3.7% in case of SLNs with stearylamine. Though increase in particle size and decrease in entrapment efficiency were observed, the values were still adequate for the stability of SLNs. In the preparation of SLNs, lipid components were being melted and subsequently cooled, hence, lipid components can solidify in several modifications of the crystal lattice. After

Table 2: Entrapment efficiency and content of nitrendipine SLNs of different triglycerides (TM, TP and TS) with and without charge modifiers (at 1.5% poloxamer 188 and 2.0% phosphatidylcholine) (mean ± S.D.; n = 3)

SLN	Without charge modifier		With dicetylphosphate (0.05% w/v)		With stearylamine (0.05% w/v)	
	Entrapment efficiency (%)	Assay (mg/ml)	Entrapment efficiency (%)	Assay (mg/ml)	Entrapment efficiency (%)	Assay (mg/ml)
Trimyristin	99.8 ± 0.12	0.97 ± 0.02	99.8 ± 0.15	0.99 ± 0.03	99.9 ± 0.06	1.01 ± 0.03
Tripalmitin	99.8 ± 0.06	0.99 ± 0.02	99.7 ± 0.12	0.97 ± 0.04	99.8 ± 0.06	0.98 ± 0.02
Tristearin	99.8 ± 0.15	0.99 ± 0.04	99.7 ± 0.18	0.98 ± 0.02	99.8 ± 0.23	0.97 ± 0.03

Table 3: Effect of storage temperature (4 and 25 °C) on particle size and entrapment efficiency of nitrendipine SLNs of different triglycerides (TM, TP and TS) with and without charge modifiers (mean ± S.D.; n = 3)

SLN	Size (nm)				Entrapment efficiency (%)	
	0 day	1 month	2 months	6 months	0 day	6 months
at 4 °C						
Without charge modifier						
NDP-TM	127.6 ± 0.8	135.1 ± 2.5	145.2 ± 1.5	162.0 ± 2.6	99.8 ± 0.1	98.4 ± 0.06
NDP-TP	123.5 ± 1.4	136.2 ± 4.2	141.4 ± 2.3	166.6 ± 1.9	99.8 ± 0.06	98.7 ± 0.8
NDP-TS	113.9 ± 1.8	129.5 ± 1.5	139.5 ± 2.5	162.0 ± 2.3	99.8 ± 0.06	98.2 ± 0.8
With dicetylphosphate (0.05%)						
NDP-TM-DCP	109.1 ± 1.4	118.8 ± 2.4	127.4 ± 1.4	138.5 ± 3.7	99.8 ± 0.06	98.8 ± 0.5
NDP-TP-DCP	107.2 ± 1.4	119.9 ± 1.3	132.5 ± 2.3	145.5 ± 4.1	99.7 ± 0.15	99.1 ± 0.5
NDP-TS-DCP	104.9 ± 1.6	121.5 ± 2.4	139.0 ± 4.1	146.2 ± 1.9	99.7 ± 0.1	98.7 ± 0.6
With stearylamine (0.05%)						
NDP-TM-SA	110.0 ± 2.0	129.8 ± 1.3	156.5 ± 1.7	175.3 ± 3.5	99.9 ± 0.06	97.5 ± 0.9
NDP-TP-SA	108.6 ± 1.9	138.2 ± 2.2	158.2 ± 2.6	182.6 ± 4.7	99.8 ± 0.15	97.6 ± 1.5
NDP-TS-SA	101.9 ± 2.1	136.6 ± 1.4	167.2 ± 1.4	194.5 ± 3.3	99.8 ± 0.06	97.3 ± 0.6
at 25 °C						
Without charge modifier						
NDP-TM	127.6 ± 0.8	151.5 ± 1.3	157.5 ± 3.6	222.4 ± 3.2	99.8 ± 0.1	96.6 ± 1.0
NDP-TP	123.5 ± 1.4	149.5 ± 3.5	161.5 ± 3.9	234.1 ± 2.2	99.8 ± 0.06	96.7 ± 0.4
NDP-TS	113.9 ± 1.8	162.3 ± 2 5	172.5 ± 4.5	245.2 ± 3.8	99.8 ± 0.06	97.2 ± 0.9
With dicetylphosphate (0.05%)						
NDP-TM-DCP	109.1 ± 1.4	123.3 ± 1.4	146.1 ± 1.7	179.1 ± 4.7	99.8 ± 0.06	97.1 ± 1.6
NDP-TP-DCP	107.2 ± 1.4	135.8 ± 4.2	153.5 ± 4.2	185.0 ± 3.9	99.7 ± 0.15	96.8 ± 0.6
NDP-TS-DCP	104.9 ± 1.6	137.6 ± 3.2	165.2 ± 3.1	194.5 ± 4.2	99.7 ± 0.1	96.4 ± 0.8
With stearylamine (0.05%)						
NDP-TM-SA	110.0 ± 2.0	162.0 ± 2.5	195.8 ± 2.4	245.0 ± 1.9	99.9 ± 0.06	96.5 ± 0.8
NDP-TP-SA	108.6 ± 1.9	175.7 ± 3.1	207.0 ± 3.4	256.8 ± 2.6	99.8 ± 0.15	96.4 ± 0.9
NDP-TS-SA	101.9 ± 2.1	182.0 ± 5.5	215.9 ± 4.9	274.5 ± 4.4	99.8 ± 0.06	96.1 ± 0.9

completion of preparation, particles crystallize at least partially in higher energy modifications α and β'. During storage, these modifications can transform to the lower energy, more ordered β modification. Due to its high degree of order, the number of imperfections in the crystal lattice is reduced thus leading to drug expulsion. Triglycerides tend to form a metastable α-form upon rapid cooling from the melt, which transforms to more stable and crystalline β-form as a function of time (Siekmann and Westesen 1994). Transitions of dispersed lipid from metastable forms to a stable form might occur slowly on storage due to small particle size and presence of emulsifier that may lead to drug expulsion from solid lipid nanoparticles (Mehnert and Mäder 2001; Westesen et al. 1993; Westesen and Bunjes 1995). Therefore, lower entrapment efficiency observed on storage may be due to drug expulsion during lipid modification.

2.6. *Differential Scanning Calorimetry (DSC)*

Fig. 3 shows DSC curves of nitrendipine, triglycerides, $PM_{1:50}$, $PM_{1:1}$, $SM_{1:50}$ of nitrendipine with triglycerides and lyophilized nitrendipine SLNs. A sharp melting peak of nitrendipine at 158.4 °C indicates its crystalline nature. The thermograms of the lyophilized NDP-TM-SA, $SM_{1:50}$ and $PM_{1:50}$ did not show the melting peak for the nitrendipine. Nitrendipine to lipid ratio in the SLNs was 1:50. Absence of melting point of nitrendipine in thermograms of lyophilized NDP-TM-SA, $SM_{1:50}$ and $PM_{1:50}$ might be due to low amounts of drug and solubility of nitrendipine in molten lipid during heating up the sample. Further, the presence of melting peak of nitrendipine in $PM_{1:1}$ of nitrendipine with trimyristin indicates that the very low amount of drug made it difficult to record the melting peak. An endothermic peak of trehalose trihydrate used as cryoprotectant was observed at 100.7 °C in case of lyophilized nitrendipine SLNs thermograms. A similar trend was observed in case of nitrendipine mixtures with tripalmitin and tristearin. There was no melting peak for nitrendipine in lyophilized NDP-TP-SA and NDP-TS-SA (Fig. 3). However, to investigate the state of drug in lyophilized SLNs, PXRD studies were carried out further which showed the amorphous state of the drug.

Polymorphic transitions after crystallization of triglyceride nanoparticles are slower for longer chain triglycerides than for shorter chain triglycerides (Bunjes et al. 1996) whereas these transitions are faster for small size of crystallites (Westesen et al. 1997). The degree of crystallinity of lyophilized SLNs was calculated by comparing enthalpy of SLNs with enthalpy of bulk lipid (zurMűhlen et al. 1998; Freitas and Műller 1999). Crystallinity of SLNs were in the following order NDP-TS-SA (89.5%) > NDP-TP-SA (82.4%) > NDP-TM-SA (74.1%). Degree of crystallinity of trimyristin in the mixtures are in the following order: $PM_{1:50}$ (90.4%) > $SM_{1:50}$ (87.6%) > $PM_{1:1}$ (86.1%) > lyophilized NDP-TM-SA (74.1%). A similar order was observed with tripalmitin and tristearin mixtures for their crystallinities (Table 4).

Melting points of TM, TP and TS in case of corresponding lyophilized SLNs were 56.2, 58.4 and 65.8 °C, respectively. These melting points were depressed when compared to the corresponding bulk triglycerides (Table 4). This suggests that triglycerides in SLNs might be in the β' form. However, there was no depression of melting point in corresponding PMs and SMs. PXRD results showed that peak intensities for nitrendipine in SM were reduced, this indicates fewer chances for drug to crystallize separately from the lipid. In spite of this there was no depression of melting point in SMs, therefore we expect melting point depression is due to the small particle size (nanometer

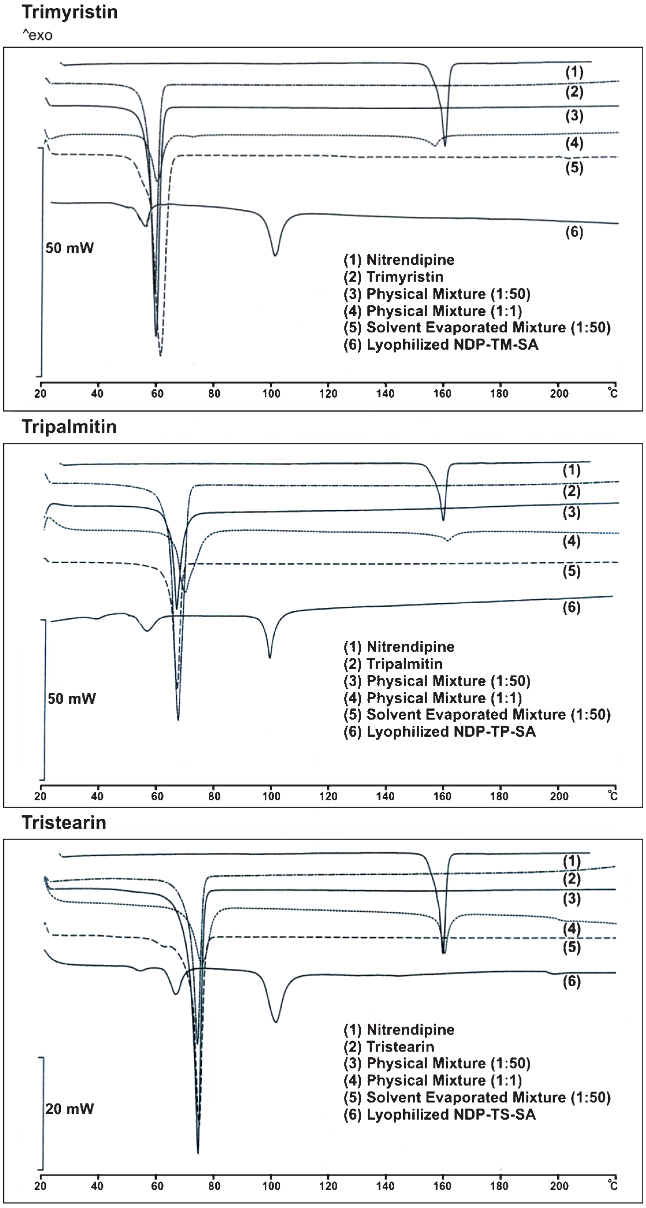

Fig. 3: Overlaid DSC thermograms of nitrendipine, triglycerides, physical & solvent evaporated mixtures of triglyceride and drug and lyophilized nitrendipine SLNs

range), their high specific surface area and presence of surfactant. Therefore, this melting point depression can be attributed to the Kelvin effect (Jenning et al. 2000). As stated by Kelvin, small isolated particles would melt at a reduced temperature relative to melting temperature of bulk materials. Depression of melting point is in proportion to the curvature 1/r of a spherical nanoparticle according to Gibbs-Thomson equation (Jesser et al. 2004).

2.7. Powder X-ray Diffractometry (PXRD)

Overlaid PXRD patterns of nitrendipine, triglycerides, $PM_{1:50}$, $SM_{1:50}$ and $PM_{1:1}$ of drug and triglycerides and lyophilized nitrendipine SLNs are shown in Fig. 4. PXRD pattern of nitrendipine exhibits sharp peaks at 2θ scattered angles 9.8, 11.4, 13.1, 24.2 and 27.5 indicates a crystalline nature of nitrendipine. The peak intensities for nitrendipine in $PM_{1:1}$, $PM_{1:50}$ and $SM_{1:50}$ were reduced at 2θ scattered angles 9.8 and 11.4, respectively. This showed that the degree of crystallinity of nitrendipine was reduced in physical and solvent evaporated mixtures. Degrees of crystallinity were compared on the basis of peak intensities. However, there were no characteristic peaks for nitrendipine in lyophilized NDP-TM-SA at corresponding 2θ scattered angles. This suggests that nitrendipine was not in a crystalline state in lyophilized NDP-TM-SA. Similarly, the degree of crystallinity of nitrendipine was reduced in case of PMs and SMs of TP and TS. Absence of characteristic peaks for nitrendipine indicated that nitrendipine was in amorphous form in lyophilized NDP-TP-SA and NDP-TS-SA. In our previous studies it was shown that clozapine in SLN formulation was in an amorphous state (Venkateswarlu and Manjunath 2004). In the preparation of solid lipid nanoparticles, lipids and nitrendipine were dissolved in a mixture of solvents and subsequently solvents were evaporated. This allowed a homogeneous dispersion of drug in the lipid. This confirms that our method of preparation (homogenization followed by ultrasonication) and the presence of surfactants could not allow the drug to crystallize. Similar results were reported by Cavalli et al. (1995, 1997) stating that rapid quenching of the microemulsion does not allow the drug to crystallize. DSC analysis of camptothecin SLNs prepared by high pressure homogenization showed that camptothecin was in an amorphous state (Yang and Zhu 2002).

PXRD pattern of TM shows sharp peaks at 2θ scattered angles 2.5, 19.5, 23.2 and 24.0 (Fig. 4) indicating a crystalline state of trimyristin. These characteristic peaks were reduced in PMs and SM. In case of lyophilized NDP-TM-SA the peaks were further reduced. Degrees of crystallinity of TM in mixtures were in the following order $PM_{1:50} > SM_{1:50} > PM_{1:1} >$ lyophilized NDP-TM-SA. This indicates that trimyristin was in a crystalline state in physical and solvent evaporated mixtures and in lyophilized NDP-TM-SA. Similarly, TP and TS were in crystalline form in PMs, SM and lyophilized SLNs. This clearly indicates that degrees of crystallinity of triglycerides in mixtures were in the following order: $PM_{1:50} > SM_{1:50} > PM_{1:1} >$ lyophilized SLNs. A similar trend was observed in DSC studies (Table 4).

Table 4: Melting peaks, enthalpies and crystallinity of bulk triglycerides, PMs (1:50 and 1:1), SMs and lyophilized SLN

	Parameter	Bulk	$PM_{1:50}$	$PM_{1:1}$	$SM_{1:50}$	Lyophilized SLN
Trimyristin	Melting peak (°C)	57.5	57.2	58.8	60.9	56.2
	Enthalpy (j/g)	174.8	154.9	75.2	150.2	16.7
	Crystallinity (%)	100.0	90.4	86.1	87.6	74.1
Tripalmitin	Melting peak (°C)	65.1	66.2	66.9	65.6	58.4
	Enthalpy (j/g)	209.2	203.2	99.4	198.2	22.3
	Crystallinity (%)	100.0	99.1	95.1	96.6	82.4
Tristearin	Melting peak (°C)	72.9	72.6	74.7	73.12	65.8
	Enthalpy (j/g)	192.2	187.5	93.1	185.4	22.2
	Crystallinity (%)	100.0	99.4	96.8	98.4	89.5

Physical mixture ($PM_{1:50}$) and solvent evaporated mixture ($SM_{1:50}$) contain 98% triglyceride; Physical mixture ($PM_{1:1}$) contains 50% triglyceride; Lyophilized SLN contains 12.94% triglyceride. Degree of crystallinity of PMs, SM and Lyophilized SLN were calculated by comparing their enthalpy with enthalpy of bulk triglycerides

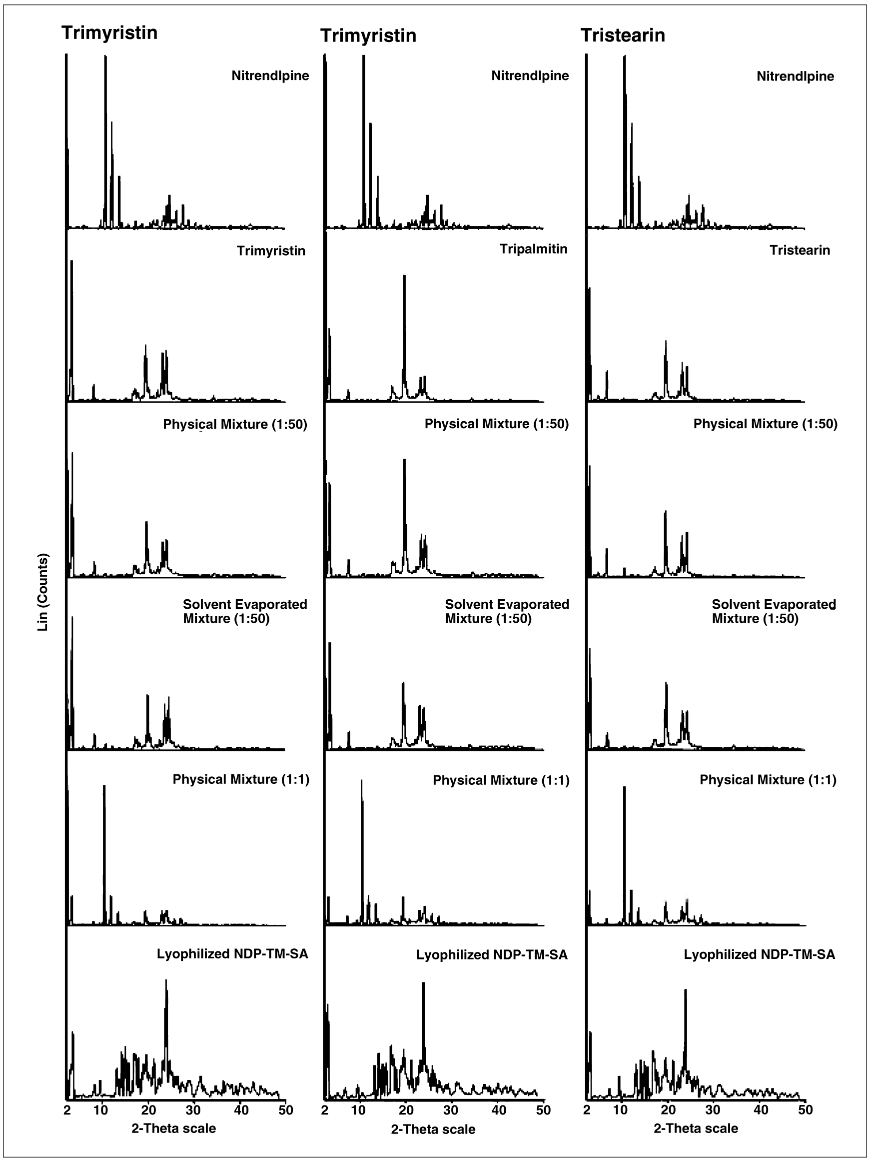

Fig. 4: Overlaid PXRD patterns of nitrendipine, triglycerides, physical & solvent evaporated mixtures of triglyceride and drug and lyophilized nitrendipine SLNs

2.8. *In vitro* release kinetics of nitrendipine SLNs

In vitro release studies for nitrendipine SLNs of different triglycerides were carried out using modified Franz diffusion cells with a dialysis membrane (pore size 2.4 nm). Dialysis membranes retained nanoparticles and allowed the transfer of the drug into the receiver compartment. *In vitro* release studies were carried out using phosphate buffer pH 6.8 as dialysis medium. Average percentages of drug released in phosphate buffer pH 6.8, from SLNs of different triglycerides with and without charge modifiers are shown in Fig. 5. All the SLNs exhibited a sustained release with less than 10% drug released up to 24 h. *In vitro* release of the drug from the SLN is affected by nature of lipid matrix, surfactant type and concentration, and

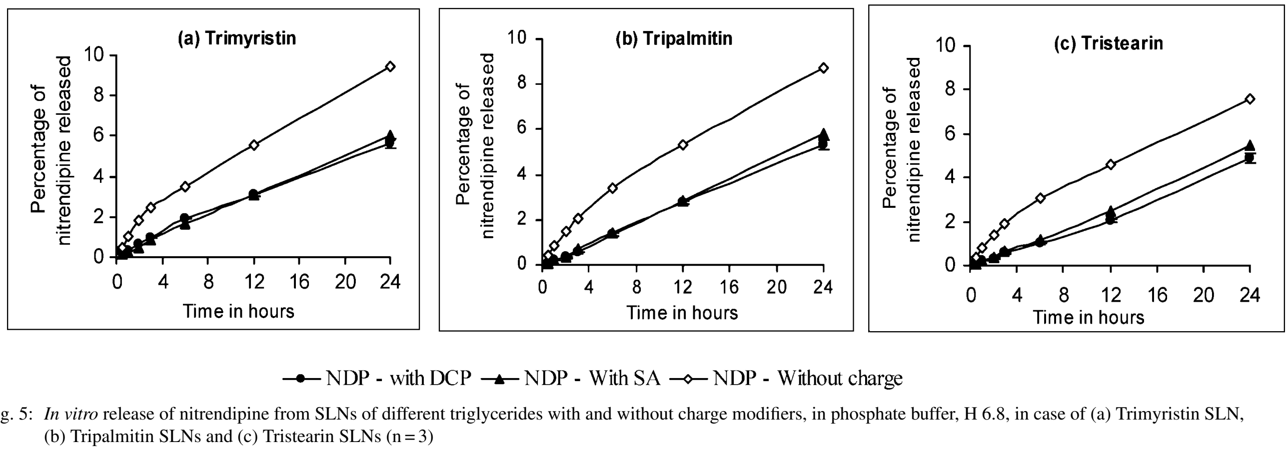

Fig. 5: *In vitro* release of nitrendipine from SLNs of different triglycerides with and without charge modifiers, in phosphate buffer, H 6.8, in case of (a) Trimyristin SLN, (b) Tripalmitin SLNs and (c) Tristearin SLNs (n = 3)

Table 5: Non-linear fits of nitrendipine released from SLNs of different triglycerides (TM, TP and TS) with and without charge modifiers in phosphate buffer pH 6.8

SLN	Equations			R^2		
	First order $\ln(Q_0\text{-}Q)$ Vs t	Higuchi Q Vs $\sqrt{t}$	Weibull ln ln{1/(1-Q)} Vs ln t	First	Higuchi	Weibull
NDP-TM	$y = -0.0037x - 0.0089$	$y = 0.0201x - 0.0113$	$y = 0.7310x - 4.6455$	0.9875	0.9887	0.9910
NDP-TM-DCP	$y = -0.0024x - 0.0021$	$y = 0.0129x - 0.0109$	$y = 0.8902x - 5.6535$	0.9940	0.9797	0.9976
NDP-TM-SA	$y = -0.0025x - 0.0003$	$y = 0.0138x - 0.0132$	$y = 0.9731x - 5.9001$	0.9966	0.9624	0.9996
NDP-TP	$y = -0.0036x - 0.0077$	$y = 0.0195x - 0.0121$	$y = 0.7664x - 4.7924$	0.9842	0.9941	0.9975
NDP-TP-DCP	$y = -.0023x + 0.0006$	$y = 0.0123x - 0.0127$	$y = 1.1026x\text{-}6.3779$	0.9973	0.9594	0.9993
NDP-TP-SA	$y = -0.0024x + 0.0008$	$y = 0.0132x - 0.0137$	$y = 1.1073x - 6.3335$	0.9965	0.9545	0.9996
NDP-TS	$y = -0.0031x - 0.0074$	$y = 0.0171x - 0.0101$	$y = 0.7650x - 4.8918$	0.9808	0.9927	0.9955
NDP-TS-DCP	$y = -.0020x + 0.0009$	$y = 0.0109x - 0.0114$	$y = 0.9820x - 6.2690$	0.9921	0.9218	0.9932
NDP-TS-SA	$y = -0.0023x + 0.0011$	$y = 0.0123x - 0.0129$	$y = 1.0334x - 6.2596$	0.9961	0.9333	0.9968

Q_0 = Drug to be released at zero time (mg)
Q = Amount of drug released at time t (mg)
t = Time in hours

production parameters (Műller et al. 2000; Wissing et al. 2004). Based on literature data three drug incorporation models (solid solution, drug-enriched shell and drug-enriched core) were proposed for SLN (Műller et al. 2000). Melting point of nitrendipine (158.4 °C) is higher than the melting point of triglycerides (57.5 to 72.9 °C) used in the preparation. Cooling of hot nanoemulsion during the preparation leads to supersaturation of the drug in the liquid lipid and subsequent drug precipitation prior to lipid crystallization. Therefore, a drug enriched-core model is proposed for nitrendipine SLNs. The drug enriched-core is surrounded by a practically drug free lipid shell. Thus, slow release (less than 10%) from nitrendipine SLNs after 24 h (Fig. 5) is due to the increased diffusional distance and hindering effects by the surrounding solid lipid shell.

Percentages of nitrendipine released from SLNs up to 24 h were in the following order: NDP-TM (9.4%) > NDP-TM-SA (6.1%) > NDP-TM-DCP (5.7%). SLNs with charge modifiers (NDP-TM-SA and NDP-TM-DCP) released a lower percentage of drug than NDP-TM (Fig. 5a). Dicetyl phosphate and stearylamine contain lipophilic hydrocarbon chain (16 and 18 carbons, respectively), which are accommodated in the lipid core and makes the interphase more lipophilic and hence slow release of the drug. A similar type of slow release of nitrendipine from SLNs of TP and TS with charge modifiers was observed (Fig. 5b and c). Thus the obtained release data was put into first order, Higuchi and Weibull equations. Release of nitrendipine from SLNs followed Weibull distribution rather than first order and Higuchi equations (Table 5). Nitrendipine, which is in amorphous form (confirmed by DSC and PXRD studies) dissolves in lipid, diffuses to the surface and undergoes partitioning between lipid and aqueous interphase. Soluble nitrendipine is partitioned into aqueous phase from which it is dialyzed into the dialysis medium.

2.9. *Conclusions*

It could be confirmed that homogenization followed by ultrasonication method is suitable to produce solid lipid nanoparticles of 79 to 213 nm size ranges. Lipophilic drugs like nitrendipine can successfully be loaded in the triglycerides (TM, TP and TS). Stability studies revealed that there were no severe changes on size and entrapment efficiency of nitrendipine SLNs. Stearylamine and dicetyl phosphate induced optimum zeta potential to nitrendipine SLNs. DSC and PXRD analyses revealed the amorphous state of nitrendipine and possible β' form of triglycerides in SLNs. Positively and negatively charged SLNs showed slower release of nitrendipine and release followed Weibull distribution rather than first order and Higuchi equation. Thus nitrendipine solid lipid nanoparticles can be suitably employed for modified release of drug. The developed solid lipid nanoparticles could be useful to overcome bioavailability problems of nitrendipine (Manjunath and Venkateswarlu, 2005), which has low bioavailability (10–20%) due to first pass metabolism. Solid lipid nanoparticles after their oral administration could be favourably taken up by payer's patches that leads to enhance their lymphatic transport, this might avoids first pass metabolism and thereby improves bioavailability.

3. Experimental

3.1. *Materials*

Nitrendipine was a kind gift from U.S. Vitamins, Mumbai, India. Trimyristin (TM) (Dynasan 114), tripalmitin (TP) (Dynasan 116) and tristearin (TS) (Dynasan 118) were generously supplied by Sasol, Witten, Germany. Soy phosphatidylcholine 95% (Epikuron 200) was donated by Degussa Texturant Systems, Hamburg, Germany. Poloxamer 188 (Pluronic F 68) and dialysis membrane−70 (molecular weight cut-off between 12000 – 14000) were purchased from HiMedia, Mumbai, India. Stearylamine and dicetyl phosphate were obtained from Sigma, St. Louis, MO, USA. Centrisart filters (molecular weight cut-off 20000) were purchased from Sartorius, Goettingen, Germany. The other chemicals were of analytical reagent grade.

3.2. *Preparation of SLNs*

SLNs were prepared by hot homogenization followed by ultrasonication method as described previously (Venkateswarlu and Manjunath 2004). Nitrendipine (0.1% w/v), triglycerides (2–5% w/v) and phosphatidylcholine 95% (1–2% w/v) were dissolved in 10 ml of mixture of chloroform and methanol (1:1). Organic solvents were completely removed using a Bűchi rotoevaporator (400 m bar, 60 °C). Nitrogen was blown onto the lipid layer for 30 min to remove traces of vapours of organic solvent, if any. Drug embedded lipid layer was melted by heating at 5 °C above the melting point of the lipid. An aqueous phase was prepared by dissolving poloxamer 188 (1–2% w/v) in double distilled water (sufficient to produce 10 ml of preparation) and heated to same temperature of oil phase. Hot aqueous phase was added to oil phase and homogenization was carried out (at 6,000 rpm and a temperature of 70 °C) using a Diax 900 homogenizer (Heidolph, Germany) for 3 min. Coarse hot oil in water emulsion so obtained was ultrasonicated (12T-probe) using Sonopuls (Bandelin, Germany) for 20 min. Nitrendipine solid lipid nanoparticles (NDP) were obtained by allowing hot nanoemulsion to cool to room temperature. Further, NDP were prepared using different amounts of emulsifiers (i.e., 1.0, 1.5 and 2.0% w/v of phosphatidylcholine 95% and poloxamer 188). Blank solid lipid nanoparticles (BL) were prepared in a similar way without drug. Blank and nitrendipine solid lipid nanoparticles prepared with trimyristin, tripalmitin and tristearin are abbreviated as BL-TM, BL-TP, BL-TS and NDP-TM, NDP-TP, NDP-TS, respectively. Stearylamine or dicetyl phosphate (0.05% w/v) were used as surface charge modifiers and dissolved in organic solvents along with lipids and drug. Nitrendipine solid lipid nanoparticles prepared with trimyristin using stearylamine and dicetylphosphate are abbreviated as NDP-TM-SA and NDP-TM-DCP, respectively. In a similar way nitrendipine SLNs prepared with tripalmitin and tristearin are abbreviated as NDP-TP-

SA, NDP-TP-DCP, NDP-TS-SA and NDP-TS-DCP. Prepared solid lipid nanoparticles were stored at 4 °C.

3.3. Measurement of size and Zeta potential of SLNs

Size (z-average) and zeta potential of SLNs were measured by Photon Correlation Spectroscopy (PCS) using zetasizer 3000 *HSA* (Malvern, U.K.). Samples were diluted appropriately to get 150 kcps with the aqueous phase of the formulation for measurements. The pH of diluted samples ranged from 6.8 to 7.0. Zeta potential measurements were done at 25 °C and the electric field strength was around 23.2 V/cm. The zetasizer measures the zeta potential based on the Smoluchowski equation as follows,

$$\zeta = U_E \eta / \varepsilon \qquad (1)$$

Where 'ζ' is zeta potential, 'U_E' is electrophoretic mobility, 'η' is viscosity of the medium and 'ε' is dielectric constant.

3.4. Statistical analysis

Size of SLNs with and without charge modifiers were compared using Student's t-test. Statistical analyses were performed using statistical package for social sciences (SPSS version 11.0).

3.5. Assay and entrapment efficiency

The prepared nitrendipine SLNs (0.2 ml) were diluted to 10 ml with chloroform: methanol (1:1). final dilution was made with mobile phase and nitrendipine content was determined by HPLC.

The entrapment efficiency of the drug was determined by measuring the concentration of free drug in the dispersion medium. Ultrafiltration was carried out using Centrisart which contains a filter membrane (Molecular weight cut-off 20,000 Da) at the base of the sample recovery chamber. About 1 ml of undiluted sample of SLNs dispersion was placed in the outer chamber and the sample recovery chamber was placed on top of the sample. The unit was centrifuged at 8,000 × g for 30 min. The solid lipid nanoparticles along with encapsulated drug remained in the outer chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of the nitrendipine in the aqueous phase was estimated by HPLC and the entrapment efficiency was calculated by the following equation.

$$\mathit{Entrapment\ efficiency}\,(\%) = \frac{\begin{array}{c}\mathit{Wt.\ of\ the\ drug\ in\ the\ formulation}\\ -\ \mathit{Wt.\ of\ the\ drug\ in\ the\ aqueous\ phase}\end{array}}{\mathit{Wt.\ of\ the\ drug\ in\ the\ formulation}} \times 100 \qquad (2)$$

3.6. Nitrendipine estimation by HPLC

Estimations of nitrendipine in aqueous phase of the system and in phosphate buffer of pH 6.8 were done by reversed-phase HPLC. The chromatographic system consisted of a Shimadzu LC–10AT solvent delivery pump equipped with a 20 µl loop and rheodyne sample injector. A Wakosil II 5C18RS (SGE) (25 cm × 4.6 mm ID) analytical column was used. The detector used was SPD-10A VP dual wavelength UV-Visible detector (Shimadzu) operated at 235 nm. Mobile phase was; acetonitrile:double distilled water:glacial acetic acid 60:40:0.1; flow rate was kept at 1 ml/min. The data was recorded using Winchrome software.

3.7. Stability studies

Blank and nitrendipine SLNs of different triglycerides (TM, TP and TS) with and without charge modifiers were stored in amber colour glass vials at 4 and 25 °C for six months. Average size and entrapment efficiency were determined. Number of samples estimated in each case were three.

3.8. Characterization by Differential Scanning Calorimetry (DSC)

DSC analysis was performed using Mettler DSC 822e/200 (Mettler Toledo). The instrument was calibrated with indium (calibration standard, purity > 99.999%) for melting point and heat of fusion. A heating rate of 10 °C/min was employed in the range of 20–220 °C. Analysis was performed under a nitrogen purge (50 ml/min). Standard aluminum sample pans (40 µl) were used. About 10 mg sample was taken for analysis. An empty pan was used as a reference.

Preparation of mixtures of SLN components for thermal analysis:

- Physical mixtures: $PM_{1:50}$ (drug: triglycerides; 1:50 w/w), $PM_{1:1}$ (drug: triglycerides; 1:1 w/w).
- Mixtures obtained by solvent evaporation ($SM_{1:50}$): Nitrendipine and triglycerides were dissolved in a mixture of chloroform and methanol (1:1) and the solvents were removed using rota evaporator.
- Lyophilized SLNs: 5 ml of SLNs dispersion was taken in a round bottom flask, 30% trehalose solution (5 ml) was added and cooled in ice-salt mixture and freeze dried at –45 °C with the help of lyophilizer (Virtis company Inc).

PMs, SMs, lyophilized nitrendipine SLNs, nitrendipine and triglycerides were subjected to same thermal cycles.

3.9. Powder X-ray Diffractometry (PXRD)

Powder X-ray diffractometer, Siemen's D-5000, was used for diffraction studies. PXRD studies were performed on the samples by exposing them to Cuk_α radiation (40 kV, 30 mA) and scanned from 2 to 50°, 2θ at a step size of 0.045° and step time of 0.5 s. Samples used for PXRD analysis were the same as those used for DSC analysis. The amount of pure drug taken for PXRD analysis was equivalent to that present in $PM_{1:50}$ and $SM_{1:50}$.

3.10. In vitro release kinetics of nitrendipine SLNs

In vitro release studies were performed using a modified Franz diffusion cell at 37 °C. Dialysis membrane having a pore size of 2.4 nm was soaked in double distilled water for 12 h before mounting in a Franz diffusion cell. Nitrendipine SLNs dispersion (1 ml) was placed in the donor compartment and the receptor compartment was filled with dialysis medium (12 ml of phosphate buffer pH 6.8). At fixed time intervals, 100 µl of the sample were withdrawn from the receiver compartment through a side tube. Fresh medium was replaced to maintain a constant volume. Samples were analyzed by HPLC as described above. Three experiments were carried out for each preparation.

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