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RESEARCH ARTICLE

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CONVERSION OF STAVUDINE LIPID NANOPARTICLES INTO DRY POWDER

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ABSTRACT

Freeze-drying has been considered as an advanced and reliable technique to improve the long-term stability of colloidal nanoparticles. Stavudine loaded solid lipid nanoparticles (SLN) suitable for parenteral administration were converted into dry product. The lipid matrix Trimyristin (2.0 w/w %) was stabilized using combination surfactant system. The protective effect of various types and concentrations of cryoprotectants (e.g. carbohydrates, polymers etc) was studied on stability of the particle size. The sugar trehalose proved to be most effective in preventing particle growth during freeze-drying process. Changes in particle size distribution during lyophilization could be minimized by optimizing the right concentration of cryoprotectants. Reconstituted lyophilized stavudine lipid nanoparticles were suitable for i.v. injection with regard to the size distribution. The mean particle size of freeze-dried lipid nanoparticles after reconstitution was significantly increased in comparison to that of the preparations before freeze-drying.

KEYWORDS

lipid nanocarriers, stability, lyophilization, particle size, sterilization

INTRODUCTION

Nanoparticulate drug delivery systems in general can be used to provide targeted (cellular/tissue) delivery of drugs, improve oral bioavailability, sustain drug effect in target tissue, solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation (1). As a particulate carrier system, solid lipid nanoparticles (SLN) are highly attractive with regard to toxicological considerations but generally face physical and chemical long-term stability problems. It has been shown that optimized systems are physically stable as an aqueous dispersion up to one year (2).

The major obstacle that limits the use of these nanoparticles is due to the physical instability (aggregation/particle fusion) and/or to the chemical instability, which are frequently noticed when these nanoparticle aqueous suspensions are stored for an extended periods (3). In order to improve the stability of these systems water has to be removed. The most commonly used process which allows to convert solutions or suspensions into solids of sufficient stability for distribution and storage in the pharmaceutical field is freeze-drying (lyophilization) (4). It is an industrial process which consists on removing water from a frozen sample by sublimation and desorption under vacuum. (5, 6)

Freezing is the first step of freeze-drying. During this step, the liquid suspension is cooled, and ice crystals of pure water forms. This highly concentrated and viscous liquid solidifies, yielding an amorphous, crystalline, or combined amorphous-crystalline phase (7). The small percentage of water that remains in the liquid state and does not freeze is called bound water. This high concentration of particulate system may induce aggregation and in some cases irreversible fusion of nanoparticles. Furthermore, the crystallization of ice may exercise a mechanical stress on nanoparticles leading to their destabilization. For these reasons, cryoprotectant (freezing stress) or lyoprotectant (drying stress) must be added to the suspension of nanoparticles before freezing to protect these fragile systems and enhance stability upon storage. The most popular cryoprotectants encountered in the literature for freeze-drying nanoparticles are sugars: trehalose, sucrose, glucose, mannitol and the concentration can be optimized by a trial and error without studying the scientific principles of this complex process. The freeze-drying of colloidal systems can be controlled in order to reach a shelf life of several years (8). The protective effect of cryoprotectants has been widely investigated (9). Generally, freezing must be carried out below Tg′ of a frozen amorphous sample or below Teu (eutectic crystallization temperature) which is the crystallization temperature of soluble component as a mixture with ice. If it is in a crystalline state in order to ensure the total solidification of the sample (8, 10, 11). The present study intended to convert bare and surface modified lipid nanoparticles in the presence of various cryoprotectants to ensure long time stability of the systems.

MATERIAL AND METHODS

Materials:

Polyvinyl pyrrolidone (PVP), molecular weight 40,000, Mannitol, Fructose, Lactose, Glucose, Sorbitol, Trehalose, Maltose and Sucrose were purchased by S.D. Fine Chemicals, India. Dynasan 114, poloxamer 188 and Solutol HS 15 (BASF, India) and Tween 80 from Uniquema, India. Stavudine was obtained as

generous gift samples from Alkem Laboratories, India.

Methods:

Solid lipid nanoparticles were produced by hot high-pressure homogenization (APV Homogenizer GmbH, Germany). The drug stavudine was dissolved in melted lipid and was poured into the hot surfactant solution (poloxamer 188) at 80°C and dispersed using a high-speed rotor-stator stirrer. The formed pre-emulsion was then homogenized at 200 bars for two cycles. Particle-size analysis was performed by using a photon correlation spectroscopy (PCS) (Beckmann Counter Coulter N5, UK). The surface of nanocarriers was modified using polyethylene glycol (PNP), polysaccharide (DNP) and albumin (BNP) using physical adsorption method.

All nanoparticles were lyophilized using a Supermodul 12K Freeze Dryer, England under high vacuum. The dispersions were diluted (1:1) with the cryoprotectant solutions before freezing, yielding a lipid content of 5% (w/w), of this diluted dispersion, 5 ml placed in 20 ml glass vials. Slow freezing was carried out on the shelves in the freeze-drier (shelf temperature - 40°C for 6 h). The samples were lyophilized for 24 h at a temperature of - 60°C for 48 h, followed by a secondary drying phase of 24 h at - 25°C and maximum vacuum. Reconstitution of the lyophilized products was performed by manual shaking with distilled water. Effect of lyophilization on drug loading was determined by using UV spectroscopy at 265 nm. The vials were closed under vacuum in the freeze-drier. In this study, total seven different carbohydrates and one polymer were tested in different concentrations.

Characterization of Nanoparticles:

A critical analysis of appearance of freeze-dried products of the cake was done visually. The desired characteristics of a freeze-dried pharmaceutical form include an intact cake occupying the same volume as the original frozen mass. An attentive examination of cake was carried out to detect any shrinkage or collapse of the formulation. pH of all the Stavudine nanoparticles was determined using calibrated pH meter. In general, freeze-dried product rehydrates immediately after the addition of water. The manual shaking was used to resuspend the freeze dried nanoparticles after the addition of water to ensure full re-suspension. The Syringability of the dispersions generally expressed as the time needed to completely fill a 10 ml syringe, equipped with a 40 mm needle with diameter of 21 G (0.8 mm). Formulations were also checked for clogging properties.

Particle size distribution

PCS is based on Dynamic Light Scattering technique suitable for application to particles ranging in size of 3 nm to 3 µm. Mean particle size and particle size distribution and polydispersity index (P.I) of developed lipid nanoparticles were determined using N5 Beckman Submicron Particle Size Analyzer at fixed angle of 90° at 20 degree temperature using double distilled water as dispersant.

Drug content:

The drug content in nanoparticles was determined by UV spectroscopy at 265 nm for stavudine lipid nanoparticles and compared to that before freeze-drying to detect any leakage of drug from nanoparticles during freeze-drying.

In-vitro drug release

In-vitro drug release was carried out to obtain quantitative and qualitative information on drug release and predict probable pattern of drug release *in-vivo*. Dissolution studies were carried out using modified USP dissolution apparatus. Phosphate buffer pH 7.2, (500 ml) maintained at 37 °C±2 °C at 50 rpm was used as dissolution medium. Dissolution studies were carried out in triplicate. Aliquots were

withdrawn at specified time intervals and replaced with respective dissolution medium and absorbance was measured by UV spectrophotometry at 265 nm. Total drug released (%) at respective time interval was calculated.

In-vitro plasma compatibility study

As a part of the study of toxicological effects, the studies of plasma compatibility are important and serve as regulatory requirement for all intravenously (IV) administered drug products even before Phase I clinical studies (12). This is due to the fact that there have been several problems caused by precipitation of drug molecules in blood vessels upon IV injection, especially water-insoluble drugs formulated with nonaqueous vehicles (13). The precipitated drug particles may lead to severe adverse events, such as phlebitis, thrombosis or even death (14). The *in-vitro* plasma compatibility of the formulation was evaluated under conditions that mimic intravenous injection. The lipid nanodispersions were diluted with 0.9 % w/v NaCl to make concentrations of 1.5 mg/ml which were further diluted with plasma in the ratio of 1:1 to simulate intravenous infusion rate and slide was observed under microscope for presence of any precipitation or aggregation (16X).

Radiation sterilization:

Selected batch lipid nanoparticles of stavudine was filled in sterilized clean glass vials and fitted with rubber closures and sealed with disinfected aluminum caps and subjected to gamma radiation chamber. Gamma sterilization was carried out at radiation dose of 5, 15 and 25 kGy respectively for unlyophilized and lyophilized product.

Stability Studies:

The stability study was carried out at refrigeration, 25°± 2°C and residual humidity of 60%, 30°C± 2°C and 65%RH and at photo stability conditions for six months. Every month, the size and drug loading etc. was evaluated to detect any instability in nanoparticles batches. An accelerated testing at 40 °C and residual humidity of 75% was also performed. (15).

RESULTS AND DISCUSSION

Preparation of lipid nanodispersions:

Trimyristin showed good solubility for stavudine which could be further enhanced by addition of surfactants. Combination of hydrophobic surfactants was found to stabilize the system more effectively than single surfactant system. The lowest mean particle size of 75 nm was obtained at 200 bar pressure and 2 cycles for stavudine loaded trimyristin lipid nanoparticles.

Lyophilization of lipid nanodispersions:

Developed formulations were converted into dry powder using lyophilization technique. The cryoprotectants were selected as per literature and their percentages were decided from previous experiments done. Both bare and surface modified nanoparticles were subjected to lyophilization with and without cryoprotectants. Different cryoprotectants were selected from class of carbohydrates and polymers. Table 1 lists the cryoprotectants and their respective concentrations used for converting nanoparticles into dry product. Each cryoprotectant was tried at three different levels (+1, 0, -1) and their effect on particle size was studied (Table 1). Most of cryoprotectants used showed formation of dry product at higher concentrations except few exceptions. The drug content and pH remained unchanged after lyophilization and was in range of 6.9 to 7.11 but drastic increase in particle size was observed as expected. Nanoparticles with and without cryoprotectants produced white color dry product. It was found that all of cryoprotectants used at lower concentration yielded sticky product. No any other sign of instability was observed..

Table 1
Cryoprotectants used for lyophilization of Nanoparticles

Cryo-protectants	Level (% w/w)	Batch	Final product	pH	Particle size (nm)	PDI	% Drug content
Dextrose	-1 (2%)	NPDX	Sticky Product	-	-	-	-
	0 (4%)	NPDX4	Slightly Sticky	7.11	535.4	0.433	94.65
	+1 (8%)	NPDX8	Dry Product	7.13	484.5	0.401	93.22
Lactose	-1 (2%)	NPLT2	Sticky Product	-	-	-	-
	0 (4%)	NPLT4	Slightly Sticky	7.11	529.2	0.408	95.03
	+1 (8%)	NPLT8	Dry Product	7.09	415.9	0.420	95.18
Trehalose	-1 (2%)	NPTL2	Slightly Sticky	-	-	-	-
	0 (4%)	NPTL4	Moist Product	7.13	388.4	0.363	94.11
	+1 (8%)	NPTL8	Dry Product	7.09	403.3	0.325	94.56
Mannitol(a)	-1 (2%)	NPMN 2	Sticky Product	-	-	-	-
	0 (4%)	NPMN 4	Slightly Sticky	7.03	501.3	0.355	93.08
	+1 (8%)	NPMN 8	Dry Product	7.01	450.6	0.367	94.15
Pearlitol (b)	-1 (2%)	NPPT2	Sticky Product	-	-	-	-
	0 (4%)	NPPT4	Slightly Sticky	7.04	638.9	0.430	91.22
	+1 (8%)	NPPT8	Dry Product	7.11	620.4	0.395	93.55
Sorbitol	-1 (2%)	NPSO2	Sticky Product	-	-	-	-
	0 (4%)	NPSO4	Sticky Product	-	-	-	-
	+1 (8%)	NPSO8	Slightly Sticky	-	-	-	-
Polyvinyl pyrollidone	-1 (2%)	NPPV2	Sticky Product	-	-	-	-
	0 (4%)	NPPV4	Sticky Product	7.01	728.2	0.439	94.75
	+1 (8%)	NPPV8	Dry Product	7.09	620.7	0.409	94.07
Fructose	-1 (2%)	NPFR2	Sticky Product	-	-	-	-
	0 (4%)	NPFR4	Slightly Sticky	7.12	532.8	0.423	93.62
	+1 (8%)	NPFR8	Dry Product	7.11	456.4	0.406	93.15
Sucrose	-1 (2%)	NPSU2	Sticky Product	-	-	-	-
	0 (4%)	NPSU4	Slightly Sticky	7.09	500.4	0.310	94.45
	+1 (8%)	NPSU8	Dry Product	7.11	482.7	0.323	93.13
Glucose	-1 (2%)	NPGL2	Sticky Product	-	-	-	-
	0 (4%)	NPGL4	Sticky Product	-	-	-	-
	+1 (8%)	NPGL8	Slightly Sticky	7.11	560.8	0.363	93.35

Sorbitol and glucose at all concentrations used failed to give dry product. At high sorbitol and glucose concentrations of 8% w/v concentrations it was sticky but could be easily redispersed in water for injection giving mean particle size of above 600 nm. Mannitol,

Pearlitol and fructose were able to produce dry product only at high concentration of 8% w/v. Both mannitol, i.e. (a) and (b) worked well at higher concentrations giving particle size of 450 and 620 nm, respectively. The Pearlitol 160, a coarse mannitol, gave larger particle size than that of mannitol of extra fine grade. This comparison suggests that nature and type of starting material (cryoprotectants in this case) could affect the final size of lyophilized particle after reconstitution. This may be due particle size differences in grades of mannitol supplied.

PVP resulted in formation of a dry product at 8% w/v concentration with mean particle size of 621 nm. Table sugar granulated to fine powder and fructose gave unexpected good results. The complete dried form was obtained at concentrations of 4 to 8% w/v. Concentrations in between 6 to 8% w/v would be sufficient for table sugar to act effectively as cryoprotective agent. Fructose behaved in similar way, giving lyophilized dry product with mean particle size of 457 nm at 8% w/v concentration.

Amongst all the cryoprotectants, trehalose gave best results having a mean particle size of 404 nm at 8% w/v concentration followed by lactose, mannitol, fructose, dextrose, PVP and sucrose. PVP, water soluble polymer used at 2 and 4% w/v concentration, gave sticky product but at higher concentration i.e. at 8% w/v produced dry product having a particle size range in between 50 to 2800 nm with a mean particle size of 631 nm. Trehalose was best as expected for converting lipid nanoparticles into dry form. As the particle size difference between trehalose and other carbohydrates was not so significant, they can be considered as effective cryoprotectants for lyophilization of stavudine nanoparticles in term of their cost effectiveness and easy availability.

Surface modified lipid nanocarriers were subjected to lyophilization using only trehalose 8% w/v as cryoprotectant. They were also freeze dried without any cryoprotectant for comparison. All surface modified nanoparticles with cryoprotectant yielded dried product. BNP (albumin coated lipid nanoparticles) lowest particle size of 429 nm. Lyophilized DNP (polysaccharide coated), PNP (PEG coated) with trehalose showed particle size of 515 and 528 nm, respectively after reconstitution. In absence of cryoprotectant batch NP, DNP and PNP DNP, PNP and NP resulted in sticky product but was easily dispersible in water for injection showing mean particle size of 361, 415, and 438 nm. Albumin produced extremely dry product without cryoprotectant with mean particle size of 363 nm which was comparatively less than that of lyophilized in presence of cryoprotectant trehalose. From experience it is suggested that 0.5% w/v of BSA would be sufficient for completely drying the lipid nanoparticles and with not much increase in particle size as compared to trehalose and other cryoprotectants. This may be due to additional layer of cryoprotectants formed around nanoparticles. In conclusion, albumin can be used as cryoprotectants at selected concentration. Mean particle sizes of various batches were shown in table 2 and size distribution of bare and surface modified nanoparticles were below 2500 nm as observed from PCS. Broader polydispersity index confirmed the formation of polydispersed phase. The particle size was significantly increased for both bare and surface modified lyophilized product. Thus the nature and amount of cryoprotectant added had significant effect on particle size. Thus cryoprotectants played important role in lyophilization of bare and surface modified stavudine lipid nanoparticles Drug content of reconstituted bare and coated nanodispersions stavudine were found to be in range of 95.84 ± 1.11%. Lyophilization did not showed any effect on drug content.

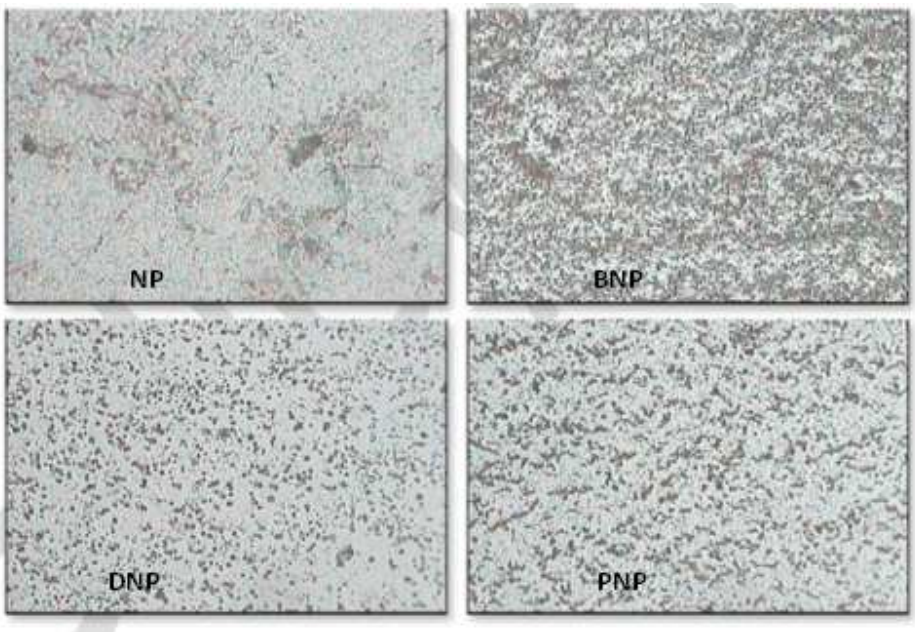
Table 2

Lyophilization of surface modified nanoparticles with and without cryoprotectants.

	Batch	Appearance	pH	Mean Particle size (nm)	PDI	% Assay
Trehalose 8%	BNPT	Dried product	6.9	428	0.312	94.32
	DNPT		6.8	515	0.425	95.63
	PNPT		7.1	528	0.468	94.15
No cryoprotectant	NP	Slightly Sticky	6.8	361	0.312	95.12
	BNP	Dried product	6.9	363	0.285	96.03
	DNP	Slightly Sticky	6.9	415	0.316	95.26
	PNP	Slightly sticky	6.99	438	0.328	94.34

Light microscopy:

Light microscopy picture were captured using Hund Microscope after freeze drying for bare and surface modified nanoparticles (Fig. 1). Batch DNP and BNP showed particles of similar characteristic in absence of cryoprotectants. Presence of aggregates was observed in absence of trehalose whereas in presence of cryoprotectant the particles were more segregated making them easily dispersible. Particle size was not determined as it was impossible to count the individual particle because of presence of clumps.



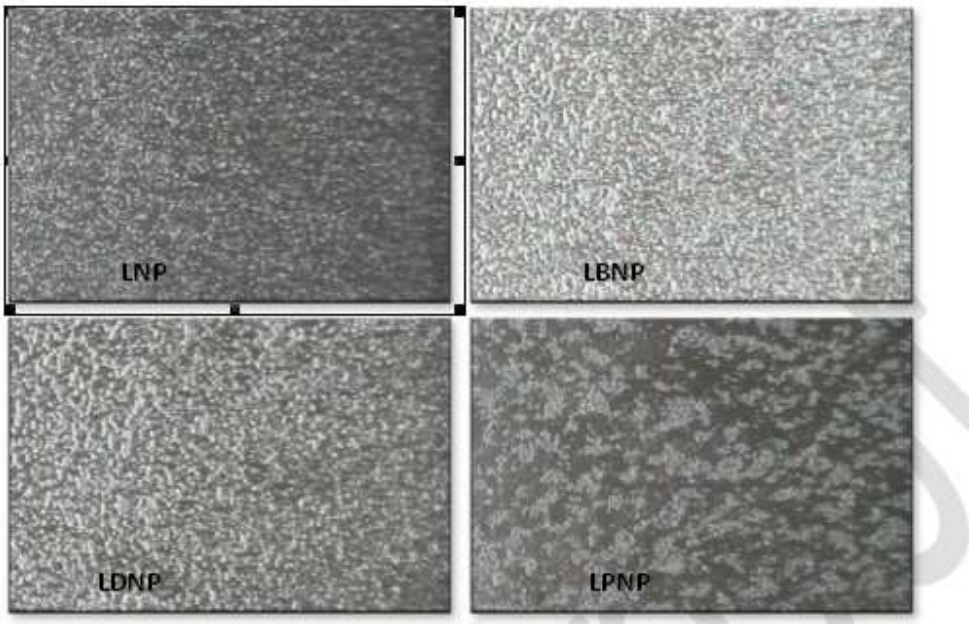


Fig 1
Light microscopy of bare and surface modified nanoparticles before and after lyophilization

In-vitro drug release:
In-vitro drug release profile for stavudine lipid nanocarriers with dialysis bag is shown in fig 2. Nanoparticles lyophilized using PVP, sucrose and trehalose showed around 79.01, 77.89 and 78.35% drug release at the end of 3 hours, respectively. Lactose lyophilized nanoparticles showed lowest drug release of 74.56% as compared with other batches at end of 3 h (Table 3). Batch NPPV8 showed longer $t_{50\%}$ of 0.983 h, followed by NPLT8 and NPSU8 which showed $t_{50\%}$ of 0.968 h and 0.933 h.

Table 3
Evaluation of selected cryoprotectants batches after lyophilization

Batch	Injectability	Syringability	*In-vitro* drug Release	
			$t_{50\%}$	$t_{75\%}$
NPDX8	Good	Good	0.929	3.623
NPLT8	Good	Good	0.968	3.718
NPTL8	Good	Good	0.829	3.523
NPMN8	Good	Good	0.912	3.611
NPPT8	Good	Good	0.895	3.423
NPPV8	Good	Good	0.983	3.983
NPFR8	Good	Good	0.920	3.333
NPSU8	Good	Good	0.933	3.523

Table 4

Evaluation of lyophilized surface modified nanoparticles with and without cryoprotectants.

	Batch	Injectability	Syringability	*% In-Vitro* Drug Release	
				$t_{50\%}$	$t_{75\%}$
Trehalose 8% w/w	BNP	Good	Good	0.245	2.033
	DNP	Good	Good	0.513	1.934
	PNP	Good	Good	0.958	3.963
No cryoprotectant	NP1	Good	Good	0.968	3.625
	BNP1	Good	Good	0.263	1.928
	DNP1	Good	Good	0.498	1.801
	PNP1	Good	Good	0.973	3.923

Surface modified nanoparticles dried in presence of trehalose showed faster drug release except PNPT (PEG coated nanoparticles in presence of trehalose) as compared with bare nanoparticles. BNPT and DNPT showed 82.39 and 86.98 % drug being release at the end of 3 h. PNPT showed similar pattern of drug release as that of bare lipid nanoparticles lyophilized using trehalose. Lyophilized batch PNPT showed prolonged $t_{50\%}$ of 0.958 whereas BNPT and DNPT showed short $t_{50\%}$ of 0.245 and 0.513 h as compared to $t_{50\%}$ of 0.829 h obtained from bare nanoparticles (Table 4). Batch BNP after lyophilization showed much faster drug release. This may be because of easy breakdown of trehalose coat making nanoparticle directly available to dissolution medium. For bare and surface modified lyophilized batches (in absence of cryoprotectants) showed comparable drug release to that of lipid nanodispersions with slight delay in attaining $t_{50\%}$ this may be due to the sticky nature of product. BNP lyophilized in presence of cryoprotectant showed faster drug release.

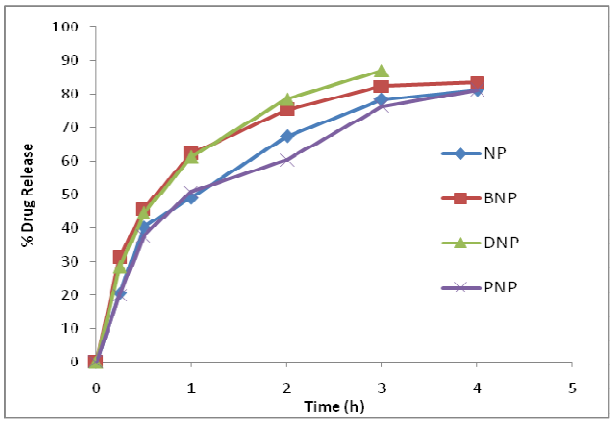


Fig 2

In-vitro drug release profile from lyophilized stavudine lipid nanoparticles.

In-vitro plasma compatibility:

The stavudine lipid nanoparticles would be administered intravenously. The dose of the drug is 30-40 mg, and the intended rate of injection is 0.4-0.5 ml/sec or more depending upon the viscosity. For 20 to 30 ml it would take around 30 min to 1 hour. Based on the calculated targeted dose and dosing time, the concentration of 1.5 mg/ml of dosing lipid nanoparticles was selected for evaluation. Nanoparticles were mixed with plasma at 1:1 ratio. Figure 3 shows the photomicrographs of bare lipid nanoparticles diluted with plasma and their interaction at different time intervals. At end of 5 min, formation of aggregates was seen while it was disappeared at end of 8 min and showed no aggregations at 10 min.

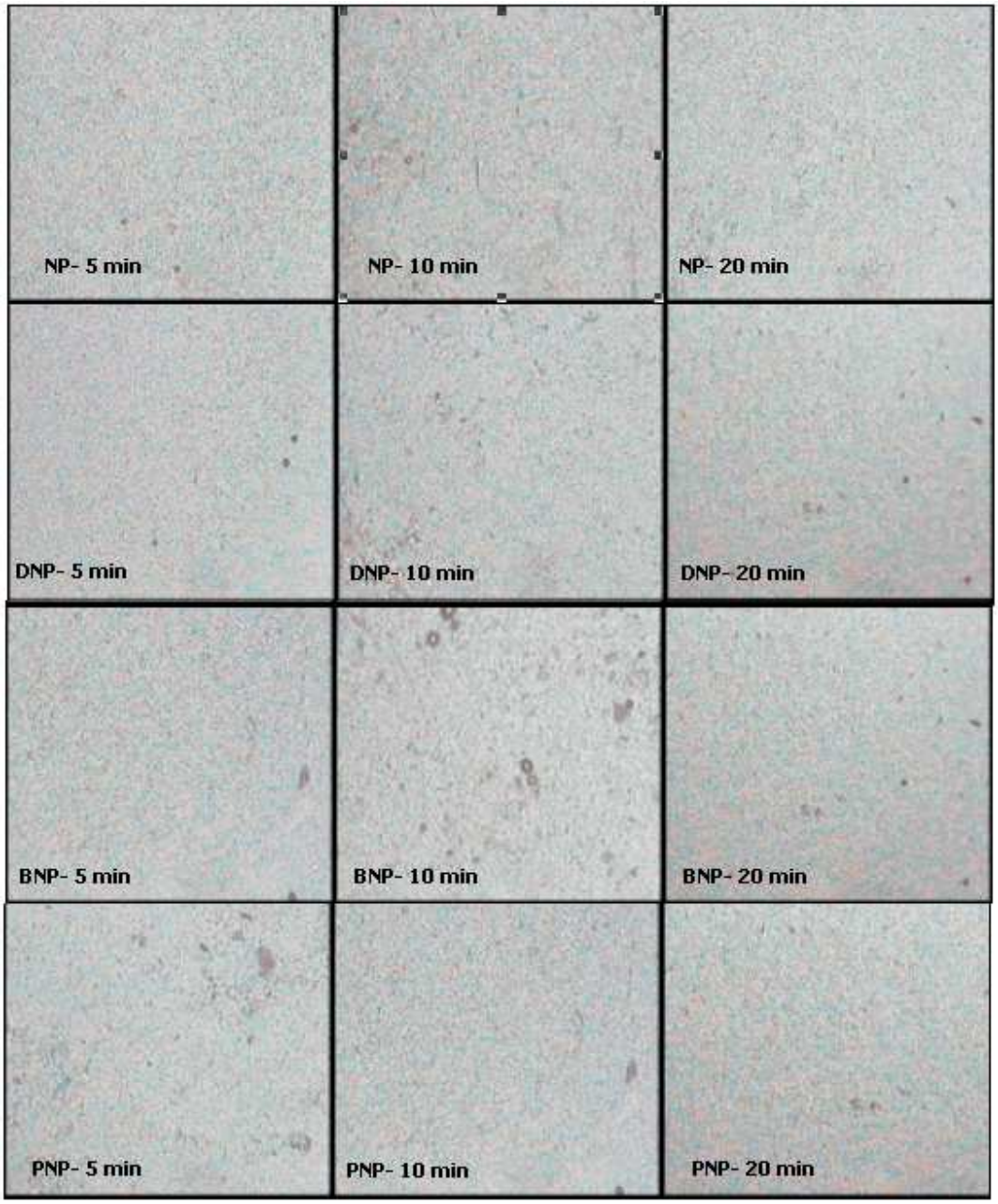


Figure 3
In-vitro plasma compatibility of lyophilized lipid nanoparticles.

In case of DNPT, the aggregation with plasma persistent till the end of 15 min but after that conditions get normalized with complete absence of aggregates. The aggregates were more prominent in case of BNP than that of NPT and DNPT. In case of PNPT, particle aggregation was observed in initial 5 min but they neutralized at the end of 10 min. But again after 15 min they showed some interactions with plasma leading to formation aggregates comparably larger than that of DNPT, BNPT and NPT but reduced at end of 20 min. Interaction get slower after reaching 20 min with particle aggregates of 1 micron.

However, the size of aggregated lipid nanoparticle with that of plasma was much smaller and were comparable to their initial size at end 20 minutes of incubation. There was no significant difference was observed in particle sizes. The results indicated that aggregation increased as a function of time especially in case of BNPT and PNPT, but reduced immediately after 10 minutes. This may be due to the interaction between adsorbed stabilizers from nanoparticle surface, leading to initial increase in aggregation, followed by adsorption of new stabilizers in the plasma. Furthermore, the aggregation pattern did not show significant difference between formulations with and without surface modification. This suggests that lyophilization did not caused any aggregation and can be used for IV injection as well. During the microscopic evaluation, it was observed that all the aggregates were lose aggregates, which can be easily broken down by mechanical force such as tapping on the slides. These aggregates should not have difficulty passing through blood vessel and become monomeric much easier than “solid” aggregates.

Terminal sterilization of lyophilized Nanocarriers:

Both bare and surface modified lyophilized nanoparticles were subjected for radiation sterilization at different radiation doses and their impact on various parameters of nanocarriers was studies and investigated. Various radiation doses employed for sterilization of lyophilized lipid nanodispersions of stavudine. Drug content of the nanodispersions remained unaffected after gamma radiation at all dose levels viz. 5, 15 and 25 kGy. However there was no significant change in particle size of lyophilized nanocarriers when subjected to increasing irradiation dose. After radiation sterilization at 5 kGy, bare lyophilized formulation showed particle size 413.3 nm which was further increased at 25 kGy up to 431 nm. No significant changes in appearance, color odour or particle size was observed for surface modified nanoparticles.

Lyophilized bare and surface modified formulations were subjected to plasma compatibility and found that bare and surface modified nanoparticles interacted with plasma by formation of loose clumps but immediately disappeared further showing good compatibility at the end of 20 min. Both lyophilized and unlyophilized bare nanoparticles showed excellent plasma compatibility.

This study revealed that there was no significant effect of radiation sterilization on plasma compatibility of developed formulation (fig. 4). Almost all lipid nanocarriers behaved in similar way as that of unsterilized nanoparticles. Hence the use of bare and surface modified nanoparticles in liquid or lyophilized form upon intravenous administration would not produce after injection effect and will easily be compatible with plasma. For lyophilized nanoparticles faster drug release after radiation at 25 kGy.

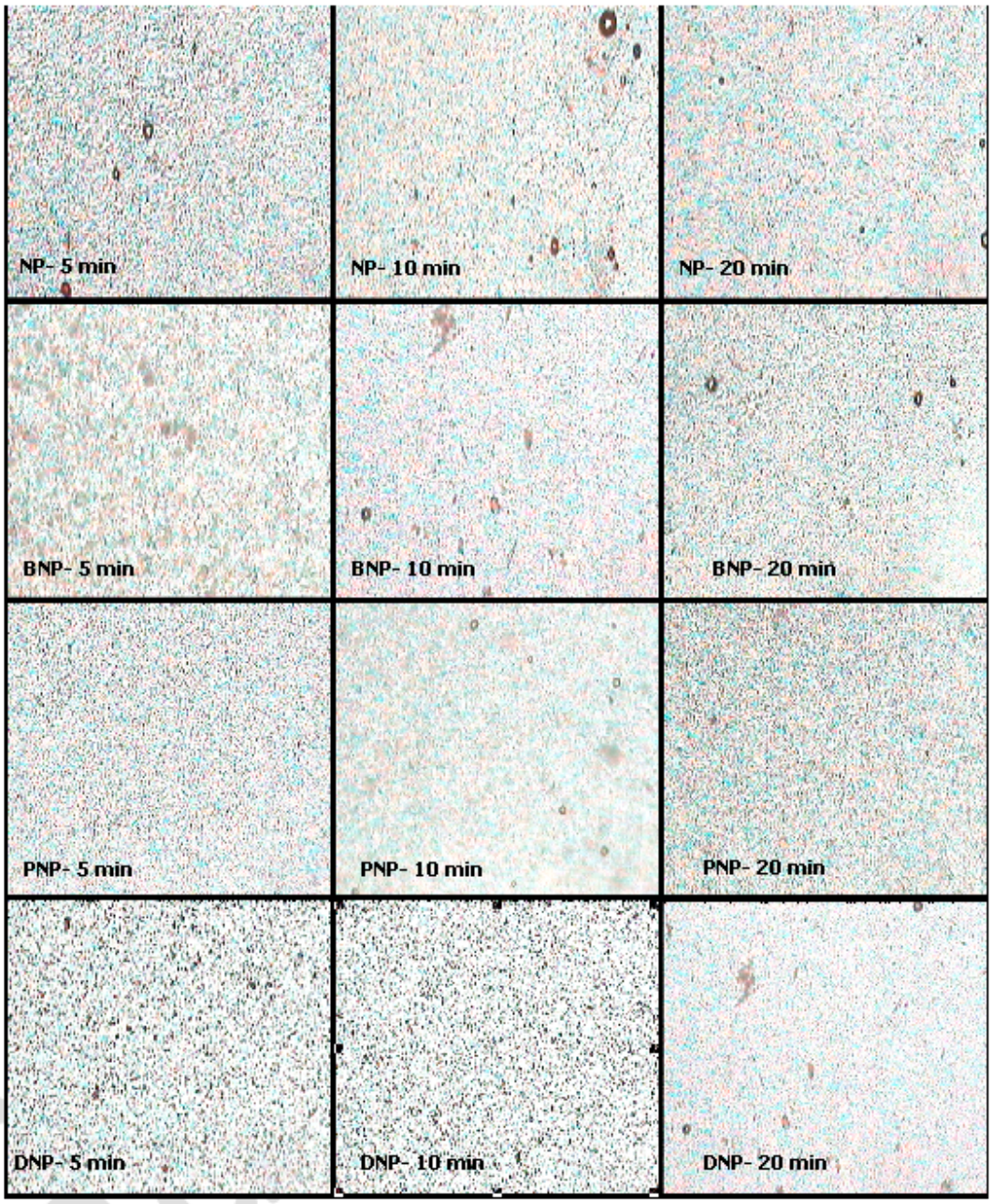


Figure 4
In-vitro plasma compatibility of lyophilized lipid nanoparticles after gamma sterilization (25 kGy).

Stability studies:
The nanoparticle samples stored at refrigeration were bluish transparent dispersions. The pH and drug content were remained almost constant. The syringability was good. Slight increase in particle size from 76 to 154 nm was observed

at end of 24 weeks. The polydispersity index was also increased slightly but still indicating narrow particle size distribution. Storage of lipid nanoparticle dispersion at refrigeration with stood the coalescence to the highest extent as obvious from the lowest growth rate of particle size. The lyophilized nanoparticle samples stored at refrigeration showed good redispersibility in water with a pH in range of 7.08 to 7.18 transparent by the end of 24th week. The nanoparticles were stable with very slight increase in mean particle size from 403 to 415 nm which is negligible. The particle size distribution was observed to be in the range of 10-2000 nm with polydispersity index (PI) of 0.32 to 0.45. The drug content was remained constant. All formulations showed good syringability till the end of 24 weeks.

At 25°C ± 2°C/60% ± 5% RH the lipid nanoparticles were transparent initially and gradually lost their appearance as time progresses. The mean particle size of the visibly stable nanoparticles increased from 76 to 174 nm. None of the particles exceeded 1000 nm after 20th week, the particle size distribution was however maintained at 10-700 nm. Slight increase in polydispersity index was noted. Whereas lyophilized nanoparticles resulted in translucent solution when dispersed in water for injection. Apparently stable increase in the particle size from mean particle size of 403 to 426 nm was noticed. The pH and drug content were stable 6.89 to 7.15 and 93.21 to 95.63%. All the formulations showed good syringability.

At 30°C ± 2°C/60% ± 5% RH storage condition, lipid nanoparticles were stable initially up to 4 week. After that, sudden marked increase in particle size was observed resulting in thick dispersions. Drug content was slightly low at end of the 4th week. In case of lyophilized nanoparticles, increase in mean particle size was observed to be from 403 to 571 nm and particle size distribution showed that none particles were above 1500 nm at end of 16 week at 30°C ± 2°C/60% ± 5% RH. The particle size distribution was observed to be in the range of 10-1500 nm with high PI indicting broad size distribution. The pH and drug content were remained almost constant. The syringability and viscosity remained unchanged. At 20th week the dry lyophilized product became sticky and it was not possible to redisperse properly in water.

The most deteriorating change was obtained at the extreme condition of 40°C ± 2°C/75% ± 5% RH with complete loss of initial appearance and with highest growth rate of particle size. The mean particle size increased within 1 week. Also drastic reduction in pH from 7.11 to 6.12 as well as in drug content from 94.45 to 80.08% was resulted at the end of first week. At the end of the 4th week complete solidification was observed. Lyophilized nanoparticles stored at 40°C ± 2°C/75% ± 5% RH remained dispersible in water till the end of 4th week after that they turned into sticky mass, it may be attributed to melting or softening of lipid. Increase in particle size was observed from 416 to 536 nm with 0.425 polydispersity index at the end of 4th week. No significant change drug content after reconstitution was observed.The nanoparticle samples stored under UV light showed that the particle dispersions were transparent by the end of 6 cycles. The nanoparticles were stable with very negligible increase in mean particle size from 76 to 94.3 nm. Particle size distribution showed that none particles were above 500 nm even after 6th cycle and at the end of 9 cycles, the particle size distribution was observed to be in the range of 10-500 nm with low PI indicting narrow size distribution. The pH and drug content were remained almost constant within 9 cycles respectively. The syringability and viscosity remained within range even after 9 cycles of 24 h. The lyophilized nanoparticle samples stored at photo stability condition showed good redispersibility in water with a pH in range of 7.08 to 7.11 and with a very slight increase in mean particle size from 403 to 421 nm which is negligible. Particle size distribution showed that none particles were above 1500

nm even after 6th cycle and at the end of 9 cycle. The particle size distribution was observed to be in the range of 10-1500 nm with PI of 0.32 to 0.36. The drug content were remained almost constant. The syringability and viscosity remained unchanged.

The limiting factor for i.v. administration is particle size, the particle size above 5 µm can potentially block capillaries. There are specifications in the pharmacopoeia regarding the limits of contamination by particulates from the packing material, e.g. in infusions. There are no definite limits specified for the number of microparticles in dispersions for i.v. administration, such as emulsions for parenteral nutrition. The present lyophilized nanoparticles maintained particle size much below as specified for i.v. administration. Now it is well studied that the various stages of lyophilization are based on very sound physical, chemical and engineering principles and can be controlled to the extent that the outcome of a given process performed on a given product can often be estimated to within fairly close tolerance, without the need for trial-and-error experimentation (4) Even more important, stable freeze-dried nanoparticles can be designed by matching an optimum nanoparticle formulation with its associated optimum drying process cycle.

CONCLUSIONS

Freeze-drying of stavudine SLN optimized conditions leads to a lyophilisate with excellent reconstitution properties. Optimized SLN formulation, after reconstitution was suitable for i.v. administration with regard to the size distribution. Further optimization of the lyophilization parameters to obtain an i.v. injectable product appears feasible. Freeze-drying of nanoparticles is a very complex process that requires a major investigation of the formulation and the process conditions. Many parameters of the formulation may decide the success of freeze-drying as the nanoparticles composition (type of polymer, type and concentration of surfactant, type and concentration of cryo and lyoprotectants, interaction between cryoprotectants and nanoparticles, surface modification of nanoparticles).

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