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Research article

Investigating the influence of lipids on nanostructured lipid carrier formulation

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ABSTRACT

The study aimed to evaluate the effect of different lipids on the properties of nanostructured lipid carrier (NLC) formulations. The particle size, zeta potential, polydispersity index, entrapment efficiency, and drug release at 24 hours were analyzed for formulations containing various lipid matrices. Among the formulations tested, N3 (Compritol 888 ATO and Softigen) exhibited the most favourable characteristics, including the smallest particle size, highest entrapment efficiency, sustained drug release, and good stability, as indicated by a high zeta potential. Other lipids, such as Witepsol H 32 and Beeswax, also showed desirable properties. The formulations containing Dynasan 114 and Acconon-C-44 EP/NF resulted in larger particle sizes, lower entrapment efficiencies, and slower drug release. Cholesterol exhibited distinct properties, with a lower zeta potential and moderate drug release. The findings highlight the importance of lipid selection in determining the performance and functionality of NLC formulations. Compritol 888 ATO and Softigen were identified as suitable lipids for further optimization of NLC formulations. These lipids contribute to the formation of stable and uniform NLC particles, which are desirable for efficient drug delivery systems. The study provides valuable insights for formulating NLCs with optimized characteristics, facilitating the development of effective drug delivery systems. Future research can focus on optimizing other factors to enhance the performance and therapeutic effectiveness of NLC formulations.

Keywords: Nanostructured Lipid Carrier, Surfactant, Hot homogenization technique, Particle size, Zeta potential.

INTRODUCTION

Lipids play a pivotal role in the formulation of Nanostructured Lipid Carriers (NLCs), serving as the primary constituent of the lipid matrix within NLCs. Their selection and combination significantly influence the properties and performance of NLC formulations, impacting aspects such as stability, drug loading capacity, and drug release characteristics. Here are several key roles that lipids play in NLC formulation:

Matrix Formation

Lipids act as the fundamental building blocks for NLCs, forming a solid or semi-solid framework to accommodate drugs and other active ingredients. The choice of lipids has a direct impact on the physical attributes of the lipid matrix, including properties like melting point, crystallinity, and viscosity, which, in turn, affect the stability and drug release behavior of NLCs.

Enhanced Drug Loadin

Lipids in NLCs enhance the capacity to load hydrophobic drugs. Certain lipids possess lipophilic properties that facilitate the solubilization and encapsulation of lipophilic drugs within the lipid

matrix. This leads to increased drug entrapment efficiency and higher drug payloads in NLC formulations

Improved Stability

Lipids contribute to the overall stability of NLCs by preventing drug leakage and particle aggregation. The solid lipid components play a crucial role in preserving the structural integrity of NLCs, preventing drug expulsion during storage or dilution, thereby enhancing the long-term stability of the formulation.

Controlled Drug Release

The choice of lipids can influence the release kinetics of drugs from NLCs. Lipids with varying physicochemical properties, such as melting point and crystallinity, can modulate the drug release profile. Solid lipids with higher melting points can provide sustained release, while lipid mixtures can create diffusion pathways and influence the rate of drug release.

Biocompatibility and Safety

Lipids commonly used in NLC formulations are known for their biocompatibility and safety in pharmaceutical applications. These lipids are typically derived from natural sources or have undergone extensive safety assessments, ensuring that NLC formulations based on them exhibit minimal toxicity and are suitable for in vivo applications.

The lipids play a critical role in NLC formulation by providing a stable lipid matrix, enhancing drug loading capacity, controlling drug release, and ensuring biocompatibility. The careful selection and optimization of suitable lipids are paramount for developing NLC formulations with desired properties and therapeutic efficacy. It's important to note that various methods and lipid combinations can be employed in NLC formulation, and the choice should consider the biological, chemical, and physical characteristics of the ingredients. The ultimate goal is to enhance bioavailability while ensuring consistent & reproducible processes and products [1-3].

NLCs are formulated by combining lipids (Solid and Liquid). The proportion of solid lipid to liquid lipid typically ranges from 70:30 to 99.9:0.1. The blending of these lipids results in a robust matrix at both body and room temperatures. The lipids used should be biocompatible, biodegradable, and physiological in nature, while the surfactant employed should have a size ranging from 10 to 500 nm [4-5]. Unlike pure solid lipids, oils have a lower melting point, allowing NLCs to contain up to 95% solid content. However, the development of SLNs is limited due to issues such as unpredictable gelation tendency, poor dispersion in aqueous medium (only 1-30% of particles), aggregation leading to lump formation, and low drug loading efficiency [6-8].

Carvedilol, a nonselective beta-adrenergic antagonist used to treat hypertension and angina pectoris, undergoes hepatic metabolism with limited bioavailability (25%). Carvedilol exhibits poor aqueous solubility, which is inversely proportional to pH [9-10].

The objective of the present investigation is to optimize the technique with lipid for formulating NLCs by carefully considering and optimizing various formulation techniques, while identifying and controlling the process and formulation variables involved. By doing so, the aim is to achieve an improved and efficient method for NLC formulation.

MATERIALS AND METHODS

The excipients utilized in the research of NLCs include Dynasan 114, Softigen, Stearic acid, Compritol 888 ATO, Witepsol H 32, Beeswax, Acconon-C-44 EP/NF, Cholesterol obtained from H-media laboratories in Chennai. Stearic acid is sourced from Spectrum Reagents & Chemicals in Cochin. Carvedilol, the active ingredient, is acquired from Saimira Pharma Pvt Ltd in Chennai. Various equipment utilized in this research include an Ultrasonicator from Q Sonica in Germany, a High-Speed Homogenizer from CAT in Germany, and a Zetasizer from Malvern.

Preparation of the NLC

The formulation of NLCs by homogenization involves the lipid phase, comprising Carvedilol (10 mg in each formulation) and a 1:1 proportion of liquid lipid (Softigen) and various solid lipid (Dynasan 114, Stearic acid, Compritol 888 ATO, Witepsol H 32, Beeswax, Acconon-C-44 EP/NF, Cholesterol). A temperature of 70°C is maintained throughout the process. To prepare the aqueous phase, Span 80 (1% w/v) as surfactants, along with Poloxamer 188 (0.5% w/v) as a cosurfactant, are dissolved in double-distilled water and heated to 70°C, similar to the oil phase. Table 1 provides a summary of the ingredients used in the formulation. The hot aqueous phase is then added to the prepared oil phase and transferred to a High-Speed Homogenizer (CAT, Germany). The mixture is homogenized at 15,000 RPM for 10 minutes at 70°C to obtain an oil-in-water (O/W) emulsion. Subsequently, the O/W emulsion is subjected to ultra-sonication using an Ultrasonicator (Q-sonica, Germany) for 5 minutes. The resulting nanoemulsion is then allowed to cool to room temperature. After Lyophilization process, the Carvedilol-loaded NLC is collected and stored for further studies [11,12].

Optimization of Formulation Variables

To prepare NLCs, various formulation variables need to be optimized, including the choice of technique, surfactant, cosurfactant, and lipids. The Table 1 shows the composition of the optimized variables. The impact of these variables on important parameters such as zeta potential (ZP), poly dispersity index (PI), and particle size (PS) was evaluated. The goal of optimizing formulation variables is to identify the best lipid that yields the desired characteristics of NLCs, such as appropriate surface charge, uniform size distribution of particle, and optimal stability. Through systematic evaluation. The

formulation variables that result in favourable ZP, PI, and PS values can be determined [13,14].

Table 1: Composition of ingredients in NLC formulation

Ingredients	F1	F2	F3	F4	F5	F6	F7
Carvedilol (mg)	10	10	10	10	10	10	10
Solid Lipid (mg)							
Dynasan 114 + Softigen	10						
Stearic acid + Softigen		10					
Compritol 888 ATO + Softigen			10				
Witepsol H 32 + Softigen				10			
Beeswax + Softigen					10		
Acconon-C-44 EP/NF + Softigen						10	
Cholesterol + Softigen							10
Liquid Lipid: Softigen (ml)	10	10	10	10	10	10	10
Surfactant: Span 80 (%)	1	1	1	1	1	1	1
Co-surfactant: Poloxamer 188 (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Homogenization	15,000 RPM for 10 minutes						
Ultrasonication	20 kHz for 5 min						

Evaluation of NLC Formulation

DLS - Dynamic Light Scattering Studies

The particle size distribution, average particle size (PS-Z average), Poly Dispersity Index (PI), and Zeta Potential (ZP) of the NLCs were optimized using the Malvern Zeta sizer. To ensure accurate measurements, the samples were appropriately diluted and filtered through a 0.45-micron membrane filter. The desired PS range for NLCs is between 10 and 1000 nm, with a Zeta Potential greater than 30 mV, and a low PI value (< 0.5) indicating a unimodal size distribution [18-22].

SEM studies

The surface morphology of the NLCs in the selected optimal formulations was examined using a Hitachi S-3000N SEM. Lyophilized NLC powder samples underwent a process where a thin layer of platinum was applied to them using a sputter coater. Subsequently, these samples were examined using a scanning electron microscope (SEM). During this analysis, the electron beam generated secondary electrons from the NLC particles, allowing for the observation of their surface morphology in the resulting images. This technique provides valuable insights into the structural characteristics of the NLCs [26-28].

Encapsulation efficiency studies

The EE of the NLCs was assessed using the centrifuge technique. Dialysis bags with a specific molecular weight cutoff and pore size were filled with NLC nanodispersion and sealed. These bags were then placed in centrifuge tubes containing pH 7.4 PO4 buffer and centrifuged at high speed to separate the free drug from the NLC carrier. After centrifugation, a sample of the buffer solution was collected, and the concentration of carvedilol was determined using a UV spectrophotometer at 241 nm. The percentage encapsulation efficiency was intended based on the total amount of drug and the amount of drug present in the collected sample [29-36].

***In-vitro* drug release studies**

NLC dispersion drug release was evaluated using the dialysis bag method. NLC dispersion was filled into dialysis membranes, tightly knotted, and immersed in a pH 7.4 PO4 buffer solution. The buffer solution was stirred at a specific speed, and samples were withdrawn at regular intervals. The withdrawn samples were replaced with afresh buffer solution to maintain a sink condition. The released carvedilol was quantified using a UV spectrophotometer at 241 nm. This study provides insights into the release kinetics and profile of carvedilol from the NLCs [37-41].

Stability Studies

Stability studies were conducted on the optimized Carvedilol NLC dispersion selected for this investigation. The formulation was divided into two batches, and each batch comprised three sets of samples designated as 3rd month, 6th month, and 12th month. These samples were individually placed in test tubes, tightly sealed, and covered with aluminum foil to safeguard against light-induced degradation. One batch was stored in a refrigerator at 2–6°C, while the other batch was stored at room temperature (25°C ± 2°C) with a relative humidity of 60%. Periodically, samples from both storage conditions were analyzed to assess particle size (nm), zeta potential, polydispersity index (PI), and entrapment efficiency (%). Rigorous measures were taken to ensure the reproducibility of results for each formulation [23,27,42].

RESULTS

Screening of Lipids

The screening of lipids was performed to determine the outcome of lipid compositions on the PS, ZP and PI of the NLC formulations. Formulations N1 to N7 were prepared using various lipid matrices, including both liquid and solid lipids. The particle size analyzer (Zetasizer Nano ZS) was used to measure the PS, ZP, and PI of the NLC formulations, and the results were analyzed. The outcome of lipids on the response parameters in the NLC formulations can be analyzed and discussed based on the provided data. The comparison and discussion in Table 2 and Figure 1-3:

Particle Size (PS)

N3 (Compritol 888 ATO) exhibited the smallest particle size (265.1 ± 9.2 nm) among all the formulations, indicating efficient lipid matrix formation and nanostructure formation. N4 (Witepsol H 32), N5 (Beeswax) also showed relatively smaller particle sizes (298.6 ± 12.6 nm and 275.2 ± 13.4 nm, respectively). N1 (Dynasan 114) and N6 (Acconon-C-44 EP/NF) had larger particle sizes (693.8 ± 14.4 nm and 733.2 ± 15.2 nm, respectively). The results are shown in table 2 and figure 1.

Zeta Potential (ZP)

Zeta potential values were negatively charged for all formulations, indicating good stability. N3 (Compritol 888 ATO) and N2 (Stearic acid) had the same zeta potential value (-34.7 ± 2.64 mV and -34.7 ± 3.86 mV, respectively), suggesting similar surface charge characteristics. N7 (Cholesterol) showed the least zeta potential value (-16.2 ± 2.96 mV), indicating a lower surface charge related to all other preparations. The results are shown in Table 2 and Figure 1.

Polydispersity Index (PI)

N3 (Compritol 888 ATO) exhibited the lowest polydispersity index value (0.401 ± 0.022), indicating a narrow PS distribution and high monodispersity. N4 (Witepsol H 32) and N5 (Beeswax) also showed relatively low polydispersity index values (0.369 ± 0.024 and 0.440 ± 0.022, respectively). N6 (Acconon-C-44 EP/NF) had the highest polydispersity index value (0.579 ± 0.022), indicating a broader particle size distribution and lower uniformity. The results are shown in Table 2 and Figure 1.

Entrapment Efficiency (EE %)

N3 (Compritol 888 ATO) exhibited the highest entrapment efficiency (93.54 ± 3.26%), indicating efficient encapsulation of the drug within the lipid matrix. N4 (Witepsol H 32), N5 (Beeswax), and N7 (Cholesterol) also showed relatively high entrapment efficiencies (82.68 ± 3.12%, 84.26 ± 2.88%, and 69.54 ± 5.34%, respectively). N6 (Acconon-C-44 EP/NF) had the lowest entrapment efficiency (53.54 ± 6.88%), suggesting lower drug encapsulation efficiency. The results are shown in Table 2 and Figure 1, 2.

Drug Release at 24 Hours (%ADR at 24 hours)

N3 (Compritol 888 ATO) exhibited the highest drug release at 24 hours (91.36 ± 3.14%), indicating sustained and prolonged release characteristics when compared to the marketed dosage form (Carvil®) and other formulations as shown in Table 2 and Figure 3. N4 (Witepsol H 32), N5 (Beeswax), and N7 (Cholesterol) also showed significant drug release at 24 hours (76.28 ± 3.12%, 78.76 ± 2.86%, and 63.62 ± 2.58%, respectively). N6 (Acconon-C-44 EP/NF) had the lowest drug release at 24 hours (49.68 ± 2.52%), suggesting slower release kinetics.

Stability Studies

The particle size remains within an acceptable range throughout the study at 4˚C. At 25˚C/60% RH, there is a slight increase in particle size over time, but it remains within the specified acceptance criteria (10-500 nm). Zeta potential values at both 4˚C and 25˚C/60% RH are within the acceptable range (±30 to 60 mV) throughout the study. PI values for both storage conditions are consistently below the specified acceptance criterion (<0.5), indicating a narrow size distribution. At 4˚C, entrapment efficiency remains above the acceptable threshold (> 85 %) throughout the study. At 25˚C/60% RH, there is a gradual decrease in entrapment efficiency, but it stays above the specified criterion.

DISCUSSION

The choice of different lipids in the NLC formulations influenced various response parameters. Lipids such as Compritol 888 ATO, Witepsol H 32, and Beeswax showed favorable features in terms of smaller particle size, higher entrapment efficiency, and significant drug release at 24 hours. Lipids like Dynasan 114 and Acconon-C-44 EP/NF resulted in larger particle sizes, lower entrapment efficiency, and slower drug release. Cholesterol exhibited distinct properties, with a lower negative zeta potential and moderate drug release. The selection of lipids is crucial in determining the overall performance and functionality of NLC formulations. The selection of perfect lipid was concluded based on the below specific results. The results, shown in Figure 1A-D and summarized in Table 2, revealed that the NLC formulations N1 to N7 exhibited varying PS, ZP, and PI values. The PI values ranged from 265.1 ± 9.2 to

733.2 ± 15.2 nm, the ZP values ranged from -16.2 ± 2.96 to -34.7 ± 2.64 mV, and the PI values ranged from 0.369 ± 0.024 to 0.579 ± 0.022. Based on the obtained results, formulation N3, which contained Compritol 888 ATO and Softigen as the lipid components, was chosen as the optimal formulation for NLC preparation. This decision was based on several factors: *High ZP value:* The high negative ZP value indicated that the NLC particles possessed a good surface charge potential, suggesting better stability.

Smallest particle size Formulation N3 exhibited the least PS (265.1 ± 9.2 nm), indicating effective particle size reduction and potential for enhanced drug delivery. *Optimal PI value:* The PI value of formulation N3 (0.401 ± 0.022) indicated a highly monodisperse particle size distribution, implying good uniformity in the NLC. The Carvedilol NLC N3 formulation demonstrated overall stability over the 12-month period as shown in Table 3.

The results suggest that storage at 4˚C is preferable for maintaining the desired characteristics of the formulation, as it showed minimal variations in particle size, zeta potential, polydispersity index, and entrapment efficiency. These findings are crucial for ensuring the pharmaceutical quality and efficacy of the Carvedilol NLC over an extended period, providing valuable insights for its potential use in therapeutic applications. Therefore, Compritol 888 ATO and Softigen were identified as suitable lipids for further optimization of other

independent factors in subsequent NLC formulations. It can be inferred that these lipids contribute to the establishment of stable and uniform NLC particles, which are desirable properties for efficient drug delivery systems. The results of this lipid screening study provide valuable insights for formulating NLCs with optimized characteristics, paving the way for the progress of active drug delivery systems.

Table 2: Estimation on outcome of Lipids on dependent variables in NLC.

Formulation	Solid lipid (mg/formulation)	PS(nm)	ZP (mV)	PI	EE%	%ADR at 24 hr
N1	Dynasan 114	693.8 ±14.4	-34.3 ± 4.64	0.498±0.024	68.24±3.12	66.28±2.54
N2	Stearic acid	466.2±22.2	-34.7 ± 3.86	0.453±0.020	70.86±2.80	68.78±2.88
N3	Compritol 888 ATO	265.1± 9.2	-34.7±2.64	0.401±0.022	93.54±3.26	91.36±3.14
N4	Witepsol H 32	298.6± 12.6	-28.1±2.99	0.369±0.024	82.68±3.12	76.28±3.12
N5	Beeswax	275.2 ± 13.4	-32.0±3.68	0.440±0.022	84.26±2.88	78.76±2.86
N6	Acconon-C-44 EP/NF	733.2 ±15.2	-30.3±4.50	0.579±0.022	53.54±6.88	49.68± 2.52
N7	Cholesterol	581.7±18.2	-16.2±2.96	0.532±0.020	69.54±5.34	63.62±2.58
Liquid Lipid - Softigen (10mg)						
Surfactant : Span 80 (1%) in all N1-N7 formulation						
Co-Surfactant : Poloxamer 188 (0.5%) in all N1-N7 formulation						

Figure 1: (A) Effects of various lipids on PS of NLC; (B) Effects of various lipids on PDI of NLC; (C) PS and PDI report of N3 formulation with optimized Compritol 188 ATO; (D) ZP report of N3 formulation with optimized Compritol 188 ATO

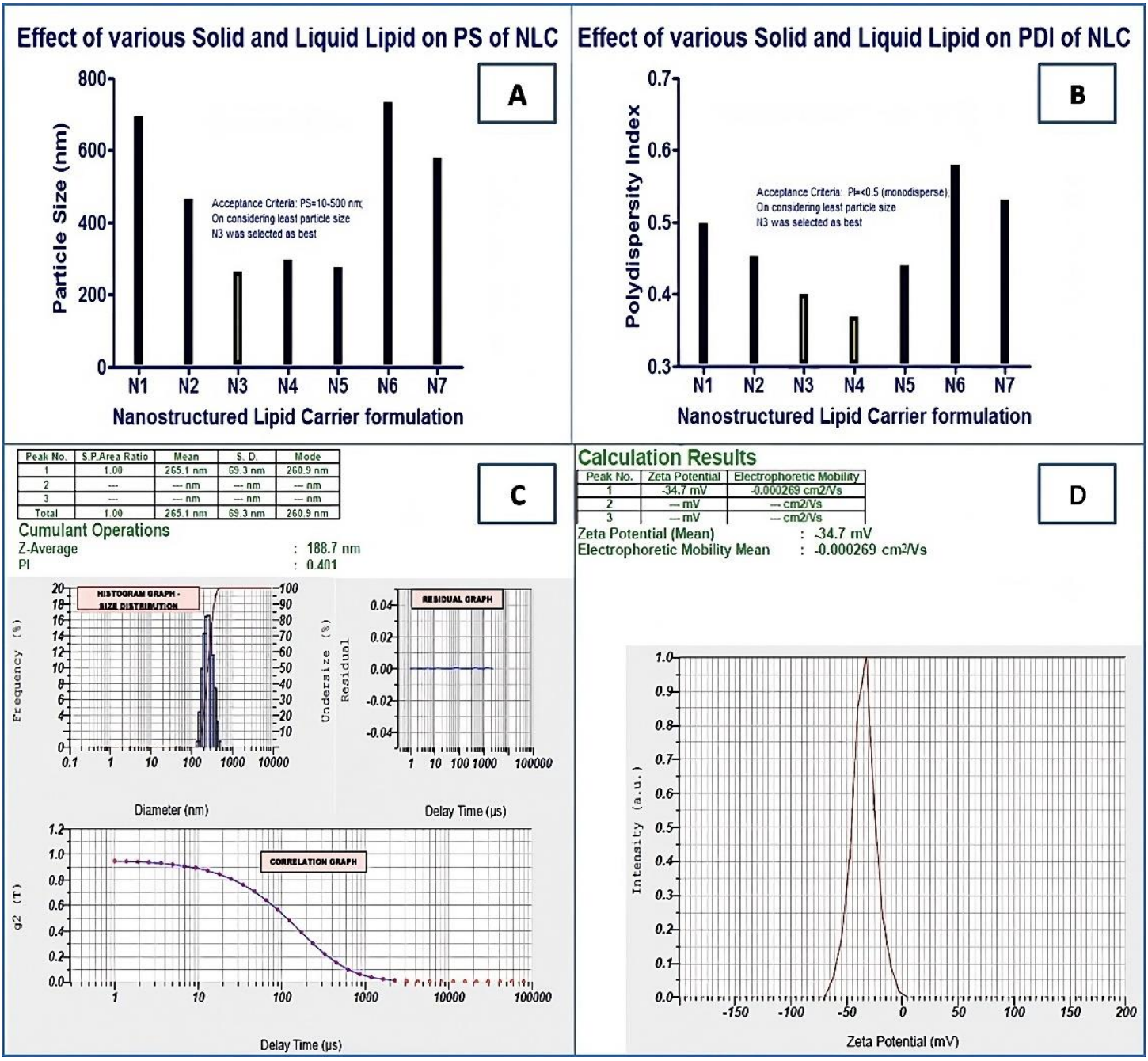

Figure 2: Optimized NLC SEM Report (A) NLCs SEM; (B) Group of NLC SEM

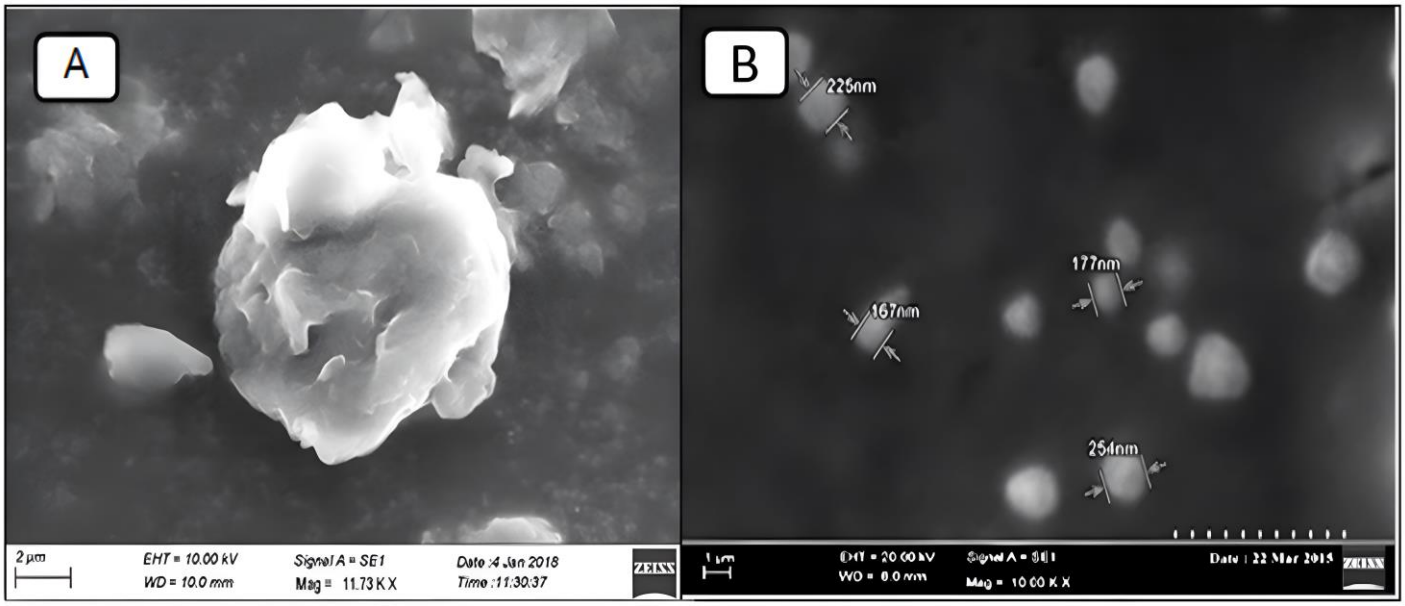

Figure 3: *In-vitro* drug release for marketed available dosage form vs. NLC (mean ± S.D., n=3)

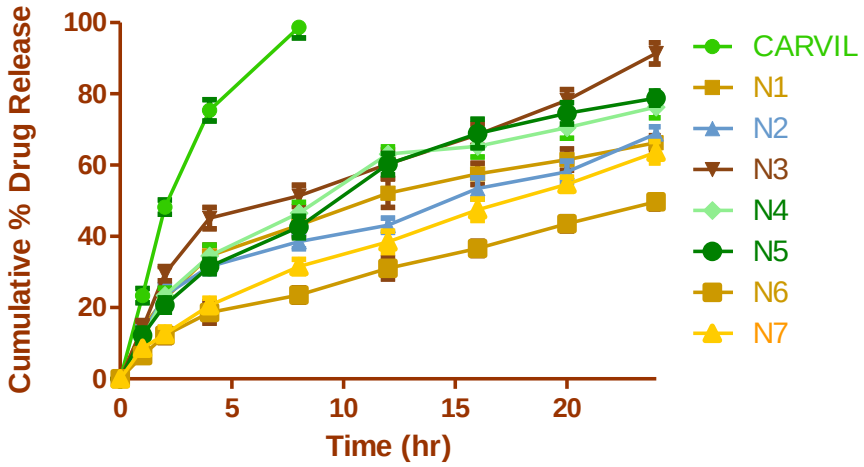

Table 3: Stability studies for optimized Carvedilol NLC by long term studies

Parameters	**Stability studies data's of Carvedilol NLC***				
N3 NLC	Temp	Initial	After 3 month	After 6 month	After 12 month
Particle Size (nm)	4˚c ± 2˚ c	265.1± 9.2	265.4±2.32	266.6±2.76	267.8±2.82
	25˚c ± 2˚c/ 60%RH	265.1± 9.2	265.8± 2.42	268.8±2.24	274.6±2.78
Zeta potential (mV)	4˚c ± 2˚c	-34.7±2.64	-34.7±2.84	-33.8±2.68	-32.6 ±2.24
	25˚c ± 2˚c/ 60% RH	-34.7±2.64	-33.6±2.22	-32.4±2.46	-30.8 ±2.86
PI	4˚c ± 2˚c	0.401±0.022	0.401±0.04	0.408±0.08	0.412±0.06
	25˚c ± 2˚c / 60%RH	0.401±0.022	0.412±0.06	0.422±0.06	0.424±0.08
EE %	4˚c ± 2˚c	93.54±3.26	93.94±2.44	92.80±3.62	91.68±3.88
	25˚c ± 2˚c/ 60%RH	93.54±3.26	91.98±2.46	90.98±3.42	88.46±3.28

CONCLUSION

Impact on The screening of lipids in NLC formulations showed that different lipids had a significant particle size, zeta potential, polydispersity index, entrapment efficiency and drug release at 24 hours. Among the formulations tested, N3 (Compritol 888 ATO) exhibited the most favourable characteristics, including the least PS, high entrapment efficiency, sustained drug release, and good stability, as indicated by a high negative zeta potential. Based on the overall performance and functionality of the NLC formulations, Compritol 888 ATO and Softigen were identified as suitable lipids for further optimization. These lipids contributed to the development of stable and uniform NLC particles, which have desirable properties for efficient drug delivery systems. This lipid screening study provides valuable insights for formulating NLCs with optimized characteristics, allowing for the progress of effective drug delivery systems. Further studies can focus on optimizing other independent factors in NLC formulations to enhance their performance and therapeutic efficacy.

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