FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIERS BASED CAPSULE

Karan K. Lokhande[a], Pranali P. Malusare[b], Dhanashree P. Sanap[b]* and Kisan R. Jadhav[b]

(Received 23 January 2024) (Accepted 01 June 2024)

ABSTRACT

The objective of this investigation was to formulate an ideal nanostructured lipid carrier (NLC) for glibenclamide in oral capsules. Employing factorial design, the NLC formulation was achieved through high-pressure homogenization using Design-Expert® software, optimized using a 3^2-factor experimental design and response surface analysis. Comprehensive analyses were conducted to characterize NLCs and determine the optimal formulation. Rigorous evaluations, including drug content, *in vitro* diffusion and stability studies, were performed. Safety assessments, efficacy studies and optimization led to the identification of an optimal NLC formulation with specific lipid and surfactant percentages, resulting in a desirable particle size (nm) and a robust zeta potential indicative of strong physical stability.

Keywords: Nanostructured lipid carriers, glibenclamide, lipid nanoparticles, capsule, diabetes, factorial design

INTRODUCTION

Diabetes mellitus, a group of metabolic disorders is defined by deficiencies in insulin, leading to hyperglycemia. Globally, an estimated 382 million individuals, or 8.3 % of adults, are affected by diabetes. If these trends continue, one in ten adults, or approximately 592 million people, will have diabetes by 2035. There are presently thought to be 28 million women globally who have diabetes mellitus. Most of these ladies suffer from type II diabetes. There are many drawbacks to the current treatment for diabetes mellitus, including patient noncompliance and sporadic hypoglycemia. Additionally, oral administration has some major drawbacks, including first-pass metabolism, limited intestinal bioavailability, and the inability to manage drug distribution or adjust the pace of absorption. Researchers worldwide are interested in the transdermal technique because of its advantages over other routes. In general, efforts have been undertaken to create transdermal methods to get around the problems with traditional drug administration of antidiabetic medications. Nanostructured lipid carriers (NLCs), a recent advancement, have emerged as innovative lipid nanoparticles garnering substantial attention as "nanodrug delivery" for successful delivery of a drug. NLCs offer a multifaceted delivery approach, enabling the administration of medications through the transdermal route[1].

This becomes a crucial tool when it is required to provide the medication over an extended period, when systemic absorption must be decreased, and when the medication irritates at large doses. NLCs have been created to get around some of the limitations associated with solid lipid nanoparticles (SLNs), including constrained drug loading capacity and potential drug leakage during storage[2,3]. Adopting a straightforward strategy for the NLC was the goal of the current study. Variable lipid content formulations were investigated to enhance the formulation for maximum drug entrapment efficiency (EE). EE was not the only factor considered; particle size and drug release were as well. To identify the lipid type and size of the formulations, various characterization tests, such as zeta potential, FTIR and DSC were conducted[4].

The present investigation examined the viability of NLC, a new carrier system for glibenclamide administration with respect to the modification of glibenclamide utilizing both liquid and solid lipids. Formulations were developed and fine-tuned using a three-factor two-level approach. Subsequent assessments included particle entrapment

[a] Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, University of Mumbai, Navi Mumbai- 400 614, Maharashtra, India

[b] Department of Pharmaceutics, Bharati Vidyapeeth's College of Pharmacy, University of Mumbai, Navi Mumbai - 400 614, Maharashtra, India

*For Correspondence: E-mail: dhanashree.sanap@bvcop.in

https://doi.org/10.53879/id.61.07.14547

efficacy, size, shape *in vitro* skin penetration efficiency and *in vitro* studies[1].

MATERIALS AND METHODS

Materials

Glibenclamide was obtained from Flamingo Pharmaceuticals Ltd., India. Glyceryl monostearate, Tween™ 80, Tween™ 20 and triethanolamine were purchased from Loba Chemicals, India. Glyceryl palmitostearate/Precirol ATO™ 5 (Gattefosse, France), Capryol™ 90 (Gattefosse, France), Transcutol™ HP (Gattefosse, France), Trehalose (Sigma-Aldrich, India), Compritol 888™ ATO (Gattefosse, France), Dynasan™ 114 (CREMER OLEO GmbH & Co, Germany), Imwitor™ 491 (CREMER OLEO GmbH & Co, Germany), Imwitor™ 900 K (CREMER OLEO GmbH & Co, Germany) and isopropyl myristate (S.D. Fine Chemicals, Mumbai, India) were the other ingredients used.

Methods

Screening of solid lipids

To obtain high drug entrapment into the lipid matrix, the drug needs to be lipid soluble. As a result, glibenclamide's solubility in several lipids was assessed to identify the lipid with the greatest ability to solubilize the medication. 5 mg of glibenclamide was precisely weighed and introduced into a set of test tubes to investigate its solubility in solid lipids. Sequentially, increasing amounts of solid lipids were added, and the test tubes were subjected to controlled heating in a heating mantle set at 10 °C above the melting point of each lipid. Periodic vortexing using a cyclone mixer was performed, with careful inspection for any residual drug. Achieving complete solubilization of the medication required a specific quantity of lipid in its molten state[5].

Screening of liquid lipids

Based on the relative drug solubility, liquid lipid or oil was chosen for the manufacture of NLCs. The technique used to determine a drug's solubility in oil was comparable to that used to determine a substance's solubility in microemulsions or self-emulsifying systems[6]. Glass vials were used to take the set volume (2 mL) of each oil individually. Each vial received an extra dose of the drug that was added until the oil was saturated and then combined with a cyclone mixer. These vials were tightly sealed and placed on a mechanical shaker at room temperature, for 72 h. If complete dissolution of the drug in the designated oil occurred within this timeframe, an additional drug was added until 48 h. Following this, the mixture underwent centrifugation at 10,000 rpm for 30 min to remove any undissolved drug. The resultant supernatant was filtered through a 0.45µ membrane filter. The filtrate was suitably diluted with methanol, and absorbance was recorded at 230 nm. Solubility of glibenclamide (mg mL^{-1}) in oils was calculated using a back-calculation method.

Methods of preparation of NLCs dispersion[7,8,9]

To prepare the NLCs dispersion, high shear homogenization with ultrasonication was used. Preciprol ATO™ 5 and Gelucire® 50/13, along with oleic acid, were accurately weighed and combined with the drug and heated to 70–80 °C. Separately, Tween™ 20 was dissolved in double-distilled water and heated to approximately 70–80 °C. The heated aqueous surfactant solution, maintained to 70–80 °C, was added to melted lipid mixture containing drug (oil phase). This combined mixture was homogenized for 10 min at 6,000 rpm within a water bath at 70–80 °C. The resulting coarse (o/w) emulsion was further subjected to sonication using a Sonapros PR-250 M for 10 min at a power of 30 Watts. The resultant o/w nanoemulsions were kept in an ice bath at 2-4 °C to cool down along with being magnetically stirred for 10-15 min. At low temperatures, the lipid nanoparticles of melted lipids changed into solid nanoparticles, resulting in the creation of NLCs dispersion. Following cooling, the formulation was stored in a refrigerated environment to maintain a low temperature, since NLCs dispersion has a longer shelf life in a cool environment than at room temperature.

Design for optimizing formulation of NLCs dispersion

The optimization of NLCs was conducted using a 3^2-randomized complete factorial design. Initial experiments revealed that the pivotal factors influencing entrapment efficiency (% EE) and mean particle size (MPS) were surfactant's concentration [X2] and total quantity of lipid [X1]. To create an optimized formula for the highest % EE and minimal MPS using a 3^2-factorial design, these variables were chosen as independent variables. Thus, the impact of these independent factors on two dependent variables, i.e. Y1 and Y2, was examined. The programme Design-Expert® was used to perform the optimization. Considering the outcomes from the initial investigation, adjustments were made to the operational parameters, specifically the rpm and duration of the ultra-turrax, along with the time and power settings for ultrasonication during the preparation of NLCs dispersion. These parameters were subsequently kept consistent throughout the optimization batch runs. 10 mins of pre-emulsification using ULTRA TURRAX® at 9,000 rpm was

followed by 10 min of ultrasonication at 20 Watt. As stated in Table I, all 9 potential combinations were used in the experimental investigations. These nine formulations underwent evaluations for mean particle size and percent entrapment efficiency.

Table I: Formulation of NLCs dispersion using 3^2 factorial designs

Run	Coded level	Conc. of total lipid (mg) [X1]	Conc. of surfactant (mg) [X2]	DW qs to (mL)
1	-1, +1	350	150	10
2	0, +1	400	150	10
3	-1,0	350	100	10
4	-1, -1	350	50	10
5	+1, +1	450	150	10
6	0,0	400	100	10
7	0, -1	400	50	10
8	+1, -1	450	50	10
9	+1,0	450	100	10

Note: *Total lipid concentration (mg) (X1); low (-1) 350 mg; medium (0) 400 mg; high (+1) 450 mg concentration of surfactant (mg) (X2); low (-1) 50 mg; medium (0) 100 mg; high (+1) 150 mg*

The software analyzed suitable linear, quadratic and special cubic mathematical models within the mixture design. Statistical parameters were compared to evaluate the significance of the model. Higher adjusted R2 and forecast R2 values were used to determine which model was the best. No farther than 0.02 should separate the expected R2 from the amended R2. For the best model, PRESS value should be low. How well the model matches the data is shown by the PRESS value[10].

Formulation and evaluation of glibenclamide loaded NLCs based capsules

NLCs dispersion was freeze dried with the use of a suitable cryoprotectant[11,12] to turn the liquid nanoparticles into a dry powder that can be put into capsules to increase patient compliance. To enhance stability and prevent particle aggregation during storage, the lipid dispersion is transformed into a solid state through the process of freeze-drying, also known as lyophilization. The freeze-dried product of the dispersion was placed within a firm gelatin capsule of size 00. In capsules, there was enough freeze-dried powder to contain 5 mg of glibenclamide. Each dose's matching amount of powder was weighed individually, and the capsule was manually filled. Wearing hand gloves, these capsules were filled right away after freeze drying.

EVALUATION AND CHARACTERIZATION

Evaluation of nanostructured lipid carrier

% Entrapment efficiency (% EE)

The proportion of the medication that is both internal to and adsorbed onto the NPs is referred to as entrapment efficiency. Measurement of the amount of unentrapped medication in the lipidic dispersions was utilized to calculate the entrapment efficiency (%EE)[13,14]. Centrifugation was performed on a lipid dispersion that had been appropriately diluted for 30 minutes at 10,000 rpm. After the resulting dispersion was centrifuged (Remi Instruments, India), the quantity of unbound drug in the clear liquid was calculated by taking absorbance at 249 nm. The variance between the initial drug content and the unbound drug in the supernatant was used to calculate the amount of drug that was assimilated. The experiment was conducted three times or in triplicate.

Particle size analysis

Nanoparticle dispersion was adequately diluted with water (ultra pure) to provide a sufficient intensity using dynamic light scattering technique[15]. The cuvette containing the diluted NLCs dispersion was filled, set in the instrument's cuvette holder, and examined using Nano Prox software. Every measurement was made three times or in triplicate.

In vitro drug release study

The drug release from the optimized NLCs dispersion containing glibenclamide was investigated using a USP XXIII dissolution testing device type II, with a revolving paddle operating at 50 rpm, 37 ± 0.5 °C temp, and a dialysis membrane approach was employed in pH 7.4 phosphate buffer (900 mL)[16]. In the dissolution investigation, 10 mL of optimized NLC dispersions containing 5 mg of glibenclamide were utilized. The dialysis membrane, which had previously been soaked in double-distilled water for 24 h, was filled with the NLC dispersion. To uphold the sink condition, 5 mL sample was replaced with 5 mL of fresh buffer at designated intervals of 0.5, 1, 2, 4, 6, 8, 10 and 24 h and further filtered using syringe filters. A validated HPLC technique was used to assess the filtrate's glibenclamide content after injecting it onto the column. The percent release vs time graphs for optimized NLCs dispersion were generated by calculating the quantity of glibenclamide released at various time points.

Evaluation of NLC capsules[5]

Disintegration test

NLCs capsule disintegration tests were performed following IP protocol. Each tube should be filled with a disc and one capsule; subjected to 900 mL of distilled water (temp 37 ± 5 °C). If every capsule has broken down, the test is considered successful. Repeat the test on 12 more capsules if 1 or 2 do not dissolve. At least 16 of the total 18 capsules examined should be dissolved.

Assay

Six capsules content were combined. A 25 mL sample of mobile phase was used to extract a powder containing 5mg of glibenclamide in (Acetonitrile (ACN): Double distilled water (DDW) 90:10 V/V). Samples were diluted further and then filtered using a syringe filter. A validated HPLC technique was used to evaluate the filtrate for glibenclamide content at a maximum wavelength of 230 nm. Three sets of duplicates were run, and the average value and standard deviation were recorded.

Content uniformity

Content uniformity was performed for 30 capsules. If there are no capsule contents that exceed 85 to 115 % of the average content and no contents that exceed 75 to 125 % of the average content, it passes the test. If more than three individual contents deviate from the average content by less than 85 % or more than 115 %, or if one or more falls outside the range of 75 % to 125 % of the average content, the capsule is considered noncompliant with the test. Repeat the calculation using a different set of contents if two or three individual contents fall outside the range of 75 to 125 % but inside the range of 85 to 115 %. The preparation passes the test if no more than three of the total sample of 30 capsules have individual contents that falls outside the range of 85 % to 115 % of the average content, and if none exceed the range of 75 % to 125 %, the capsule is considered compliant with the test.

Weight variation

Weight variation of capsules was carried out as per IP. 20 capsules were taken individually. The intact capsule was initially weighed. Subsequently, without any loss of the shell's components, it was opened, and the contents were extracted as thoroughly as possible. The shell was then reweighed, and the difference in weight provided the measure of the contents. The procedure was repeated with the other 19 capsules. The determination of the average weight was conducted. As the average weight exceeds 300 mg, the test criteria are satisfied unless more than two individual weights deviate from the average by more than 7.5 %, with none exceeding a 15 % deviation[17,18].

In vitro drug release study

Glibenclamide release from a freeze-dried NLC capsule's optimized batch was performed in 900 mL of buffer 6.8, (USP XXIII) using a basket at 100 rpm and 37 ± 0.5 °C. The capsule was filled with freeze-dried NLC powder equivalent to 40 mg of glibenclamide. The *in vitro* study was conducted by retaining the capsules in the basket, preventing them from floating on the surface of the dissolution media. Aliquots of 5 mL were withdrawn from the dissolving medium at designated intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h and replaced with fresh media. Syringe filters were used to filter the withdrawn samples. A validated HPLC technique was used to analyze the filtrate for the presence of glibenclamide at the maximum wavelength of 249 nm. The quantity of glibenclamide released from the optimized NLC-filled capsules at various time intervals was graphed as a percent cumulative release vs time. With the use of the model-dependent (curve fitting) approach, the release data were assessed. Several kinetic models were employed to characterize the release kinetics and examine the *in vitro* release data.

Stability study testing

The optimized formulation batches underwent accelerated stability testing in accordance with ICH guidelines[19].

Protocol for stability testing

Time span: The capsules were packaged in aluminum foil with a PVC coating.

Sampling condition: According to ICH rules, the stability tests were carried out for a 1 month under the following storage circumstances. 30±2 °C / 65±5 % RH

Study: 1 month

Packaging: 40±2 °C / 75±5 % RH

Sampling points: Day 0 and day 30

Parameters evaluated: The stability batches were assessed for various parameters, including visual appearance, *in vitro* drug release, assay and particle size.

RESULTS AND DISCUSSION

Screening and selection of NLC components

Screening of solid lipids

Different lipids were used to test the lipid solubility. The smallest quantity of lipid needed to solubilize

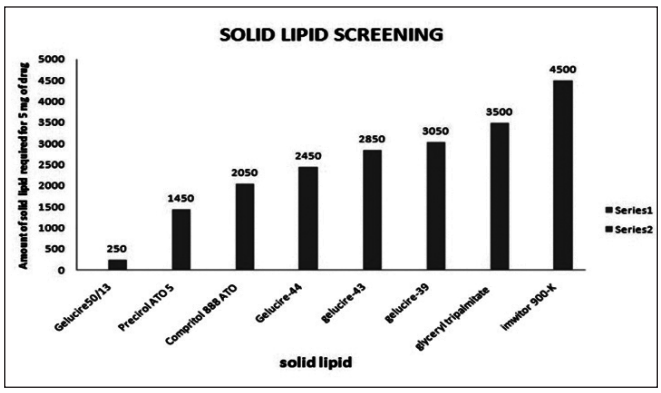

Fig. 1: Screening of solid lipids

glibenclamide was chosen Fig. 1 illustrates the quantity of different solid lipids needed to dissolve 5mg of glibenclamide. Gelucire® 50/13 demonstrated the maximum solubility of the different solid lipids tested. However, Gelucire® 50/13 by itself cannot be employed as a solid lipid due to its dispersible nature. A blend of Gelucire® 50/13 and Precirol® ATO™ 5 served as the solid lipid in formulation and development experiments, due to concerns that the dispersible property of gelucires could potentially impede the formation of a solid structure[20]. Based on the drug's solubility in lipids, Precirol ATO™ 5 and Gelucire® 50/13 were selected.

Screening of liquid lipid

Oleic acid demonstrated the maximum solubility for glibenclamide among the different liquid lipids evaluated, as illustrated in Fig. 2. As a result, oleic acid was thought to be the optimum option for making NLCs to optimize drug entrapment.

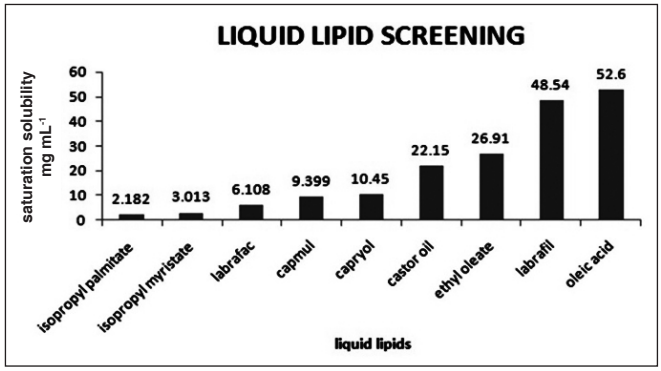

Fig. 2: Screening of liquid lipids

Screening of surfactants

To provide improved integration of glibenclamide in the lipid core, surfactants were chosen based on the parameters of lowest solubility. In contrast to other surfactants, glibenclamide had the lowest solubility in

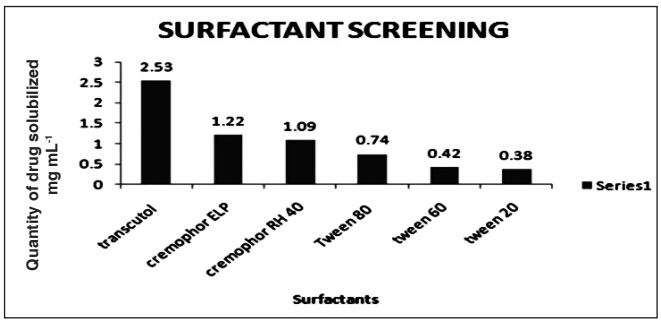

Fig. 3: Screening of surfactants

Tween™ 80, Tween™ 60 and Tween™ 20. Fig. 3 displays the amount of glibenclamide solubilized in different surfactants.

OPTIMIZATION AND CHARACTERIZATION OF NLC FORMULATIONS

Optimization of NLCs dispersion

The dispersion of NLCs was optimized using a 3^2 factorial design. For nine batches of dispersion, Stat-Ease Design-Expert® 9.0.4 statistical software, Response-1 (percent EE) and Response-2 (MPS), for entrapment efficiency (EE) and mean particle size (MPS), the target was established within the acceptable range using the levels chosen as given in Table II.

Table II: Formulation of NLCs dispersion by factorial design and their responses

Run	Coded level	Concentration of total lipid (mg)	Concen-tration of surfactant (mg)	% EE	MPS (nm)
1	-1, +1	350	150	92	132
2	0, +1	400	150	94	126
3	-1, 0	350	100	83	163
4	-1, -1	350	50	64	263
5	+1, +1	450	150	98	123
6	0, 0	400	100	87	156
7	0, -1	400	50	72	210
8	+1, -1	450	50	76	204
9	+1, 0	450	100	90	143

Contour plots and response surface plots proved valuable in examining the simultaneous interaction between two factors and a response in the study. The % EE rises when lipid and surfactant concentrations rise, as

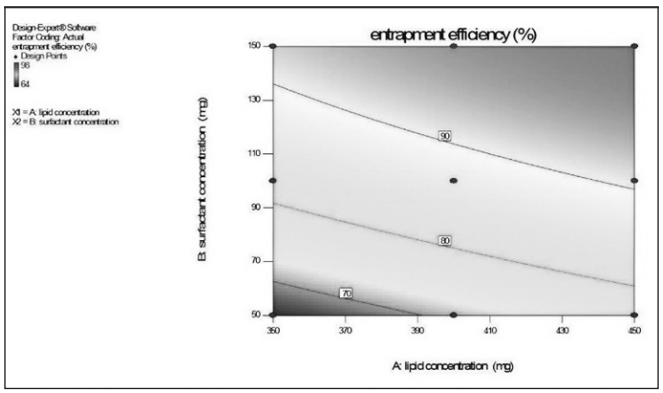

Fig. 4: Contour plots illustrating the impact of lipid and surfactant concentrations on the % EE

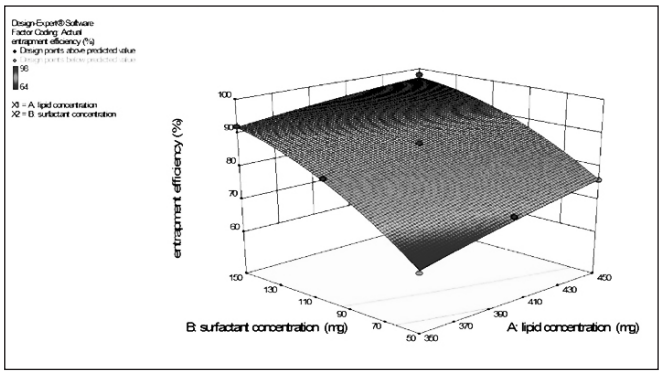

Fig. 5: Response surface plots demonstrating the influence of lipid and surfactant concentrations on the % EE

seen by the contour plot (Fig. 4) and response surface plot (Fig. 5). This may be because when lipid concentration rises, more drug is entrapped in the lipid, leaving less drug free for dispersion, which raises the percent EE. In contrast, as surfactant concentration rises, the NLC is stabilized, raising the percent EE. The best-fitting model recommended by the program was the linear model after

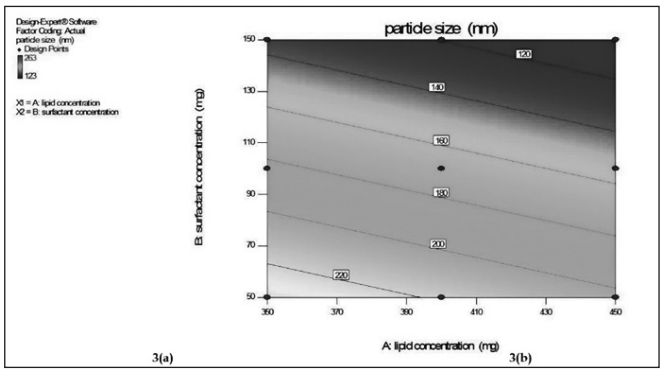

Fig. 6: Contour plots depicting the impact of lipid and surfactant concentrations on particle size

analyzing the various models for metrics like standard deviation (SD), adjusted R^2, projected R^2, etc.

The contour plot (Fig. 6) and response surface plot (Fig. 7) demonstrate that when lipid concentration rises, particle size increases because lipid tends to agglomerate at high concentrations. As the

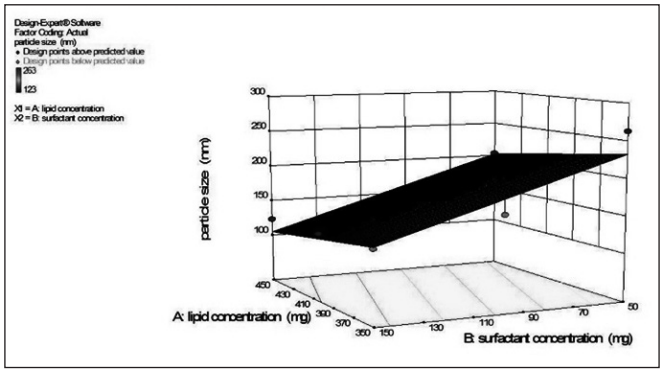

Fig. 7: Response surface plots illustrating the influence of lipid and surfactant concentrations on particle size

concentration of surfactant increases, the surface tension between the aqueous and lipid phases may decrease, leading to a reduction in particle size and the prevention of particle aggregation.

Evaluation and characterization of NLCs dispersion

Physical evaluation

There are two factors that affect the NLC dispersion's physical and chemical stability. Suspension stability, or the capacity of a suspension to maintain homogeneity, and lipid recrystallization prevention come first and second, respectively (carrier stability). Sedimentation, flocculation, creaming, or coalescence are the results of a suspension's failure to remain stable. Gelation results from the crystals cross-linking during recrystallization. So, for every such incident, the NLC dispersion was visibly observed. The mixture was kept in a refrigerator. It appeared milky white and lacked any signs of sedimentation, gelation or coalescence.

Evaluation by light microscopy

Light microscopy was used to continue monitoring the NLC dispersion. Under a microscope, diluted samples were examined, and microscopic images were recorded. To look for any sort of aggregation, drug crystals, lipid crystallisation etc., microscopy was used. A drop of diluted dispersion was placed on a glass slide with a coverslip and examined under 10X and 45X magnification. It

Table III: Selection of cryoprotectant for EPE loaded NLCs dispersion

Batch	Cryoprotectant	Concentration (%)	Appearance	% EE	Particle size (nm)	PDI
1	Trehalose	1	+	88%	198.87	0.433
2		1.5	++	89.54%	178.66	0.543
3		2	+++	90.76%	156.28	0.502
4	D-Mannitol	1	-	-	-	-
5		1.5	+	88.23%	400.34	0.498
6		2	++	87.91%	430.54	0.543
7	Sucrose	1	-	-	-	-
8		1.5	-	-	-	-
9		2	+	86.98%	580.98	0.449

displayed spherical uniform particle size and no signs of aggregation.

Drug entrapment efficiency

Efficiency in entrapment was done by indirect methods. All optimization batches' entrapment efficiency varied from 64 to 98 %. Entrapment efficiency was 91.83 % in the optimized batch (OB) with Lmix concentration of 350 mg and surfactant concentration of 150 mg.

In vitro drug release study

Fig. 8 illustrates the *in vitro* drug release pattern of an enhanced batch of EPE-loaded NLCs dispersion. For glibenclamide-loaded NLCs, an observed release pattern displayed biphasic characteristics, consisting of a burst release at the beginning and a continuous release at the end. While the sustained release profile indicated that glibenclamide was released from the lipid matrix's core into the release medium, the occurrence of burst release clearly shows that a specific amount of glibenclamide was located on the surface of NLCs. The percentage of medication released from the NLC formulation after 24 h was discovered to be 92 % in a controlled manner.

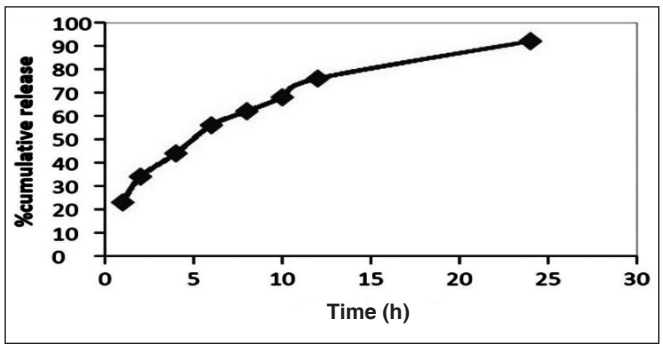

Fig. 8: The *in vitro* drug release profile of the optimized NLC dispersion of glibenclamide

Particle size

For all the optimization batches, average particle sizes fell between 116.56 nm and 134.22 nm. The optimum batch (OB) had a particle size of 134.22 nm and included 350 mg of Lmix and 150 mg of surfactant.

Formulation and evaluation of EPE loaded NLCs based capsules

Selection of cryoprotectant

The best results for glibenclamide loaded NLCs dispersion are obtained with 2 % trehalose. The appearance was cake-like and compact in character, and particle size decreased as the concentration of trehalose increased from 1 to 2 %, as indicated in Table III. In contrast, when D-mannitol and sucrose were used, the resultant dispersion did not adequately cake and shrank at reduced cryoprotectant concentrations. While the product was adequately caked at higher concentrations with just a small amount of shrinkage, the particle size achieved upon reconstituting the freeze-dried product was significantly larger than the dispersion of NLCs prior to freeze-drying. Hence, a concentration of 2% trehalose was determined to be the optimal choice for cryoprotectant during lyophilization. For freeze dried, EPE-loaded NLCs, the particle size and EE percentage were 156.28 nm and 90.76 % respectively. This shows that the particle size increased but the percent EE decreased slightly.

Disintegration test

Within 10 minutes, the entire batch of 6 glibenclamide-loaded NLC capsules disintegrates. Considering the IP method, it passes the disintegration test.

Assay of capsules

The optimized NLC capsule's assay result was 98.89 %.

Weight variation test

The observed average weight for all the 20 capsules was found to be more that 300 mg, thus it passes the weight variation test as per IP.

Flow properties

Table IV displays the NLC powder's physical characteristics. The NLC powder was found to have a percent compressibility and an angle of repose of 15.49 % and 21.9° respectively. These findings suggested that the NLC powder had good flow properties.

Table IV: Flow properties of NLC powder

Test	Results
Bulk density	0.68 g mL^{-1}
Tapped density	0.78 g mL^{-1}
% Compressibility (Carr's index)	15.49 %
Hausner ratio	1.1838
Angle of repose	21.9^0

X-ray diffraction study

The characteristic peaks observed for GBN (Fig. 9) in the XRD study and those for Lmix (a combination of Precirol ATO™ 5 and Capryol™ 90) in Fig. 10 were not discernible in the diffraction pattern of the GBN-loaded NLCs-based freeze-dried product, as depicted in Fig. 12. This suggests that the drug has been assimilated into the freeze-dried NLCs product, maintaining its amorphous state. X-ray diffraction pattern of blank freeze dried NLC is depicted in Fig. 11.

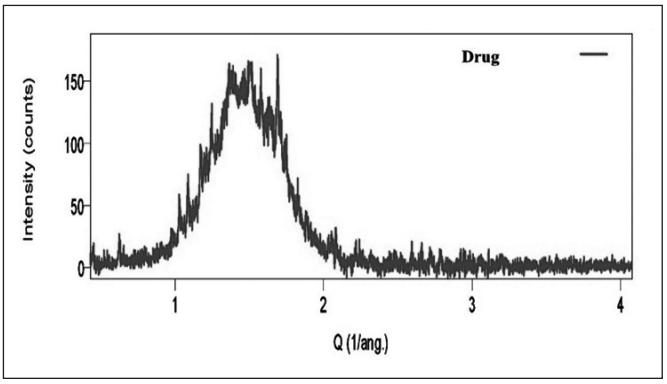

Fig. 9: X-ray diffraction pattern of pure glibenclamide (GBN)

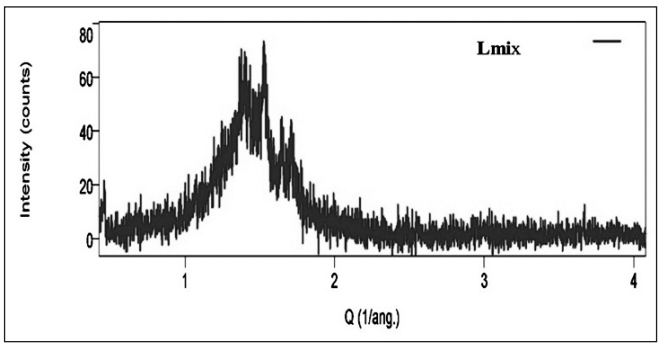

Fig. 10: X-ray diffraction pattern of Lmix

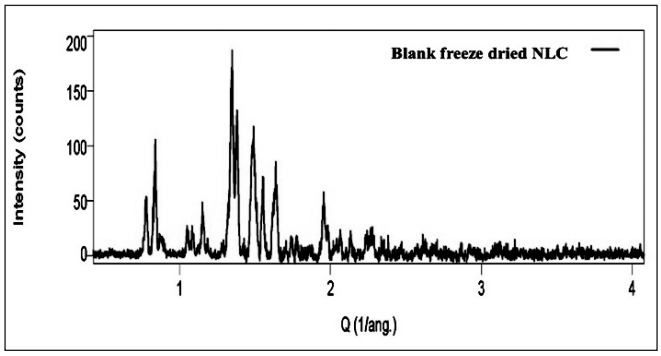

Fig. 11: X-ray diffraction pattern of blank freeze dried NLC

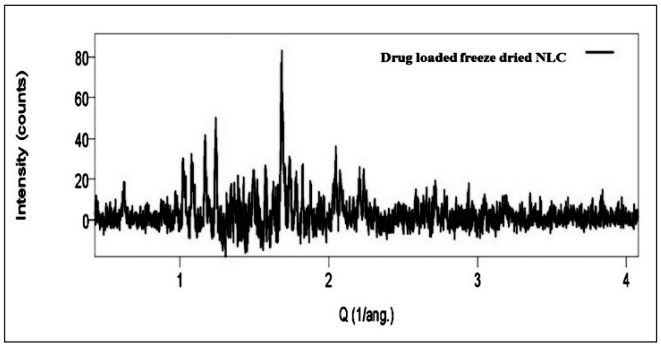

Fig. 12: X-ray diffraction pattern of drug loaded freeze dried NLC

DSC study

DSC tests were conducted to evaluate the drug's physical state within the NLC, crucial for both *in vitro* and *in vivo* drug release. Various drug/lipid combinations were examined, showing no trace of crystalline drug in the freeze-dried NLC, indicating either dissolved, amorphous or molecularly dispersed drug within the lipid matrix as shown in Fig. 13. Glibenclamide in the NLCs likely existed in an amorphous or molecularly dispersed state, evidenced by the absence of its endothermic peak. Peaks at 71.1°C and 213°C corresponded to the melting points of L_{mix} and the cryoprotectant in the NLC formulation, respectively.

Table V: Kinetics model fitting for NLC capsule

Formulation	Models (R^2 Value)				
	Zero order	First order	Higuchi	Hixson-Crowell	Korsemeyer Peppas
Glibenclamide loaded NLC based capsule	0.7688	0.9498	0.8493	0.8245	0.8907

Table VI: Optimized formulation of the stability study

Sr. No.	Parameter	Storage conditions			
		30 °C/ 65% RH		40 °C/ 75% RH	
		0 day	30 day	0 day	30 day
1	Visual characteristics	+++	+++	+++	+++
2	% Cumulative drug release at 24 h	88.10	87.32	91.23	88.75
3	Assay	98.23	98.34	98.43	98.10
4	Particle size	154.3	157.5	156.7	159.6

Note: Good (+++)

In vitro drug release study

After 24 h, it was observed that the drug release from NLC capsules reached 88%. It was also evident that a biphasic release pattern, consisting of a burst release at the beginning and a continuous release at the end, was seen in the case of the NLC capsule (Fig. 14), within the first 2 h, approximately 30 % of the loaded glibenclamide from the capsule was released. The burst release phenomenon distinctly illustrates the presence of a specific quantity of glibenclamide on the surface of NLCs, while the sustained release profile indicates the gradual release of glibenclamide from the core of the lipid matrix into the release medium.

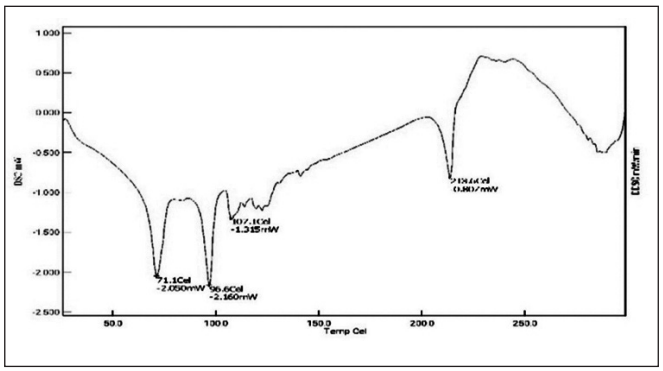

Fig. 13: DSC thermogram of drug loaded freeze dried NLC

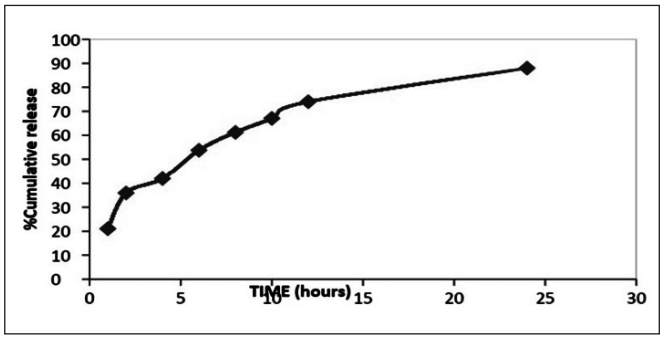

Fig. 14: Release of glibenclamide from NLC capsule

The regression coefficient was used to assess the model that best matches the release data (R2). R2 value used as a criterion for selecting the top model to explain drug release from formulations. The R2 value of the first order model was discovered to be the closest for the NLC capsule after comparing the R2 values of the various kinetic models. Thus, it can be said that the NLC capsule's drug release kinetics best match the first order model of drug release, as shown in Table V.

STABILITY STUDIES

Appearance

The freeze-dried NLC powder had excellent properties, as shown in Table VI, which suggested that the formulation was stable for a 30-day period.

In vitro drug release study

At first, it was discovered that 83.01 % of the medicine was released from the glibenclamide-loaded NLCs-based capsule after 24 h. At the conclusion of 30 days, the release profile for the capsule was identical for both

storage settings, as indicated in Table VI.

Assay

Initial analysis of the improved NLC capsule revealed a 98.89 % success rate. The results of the test after 30 days are listed in Table VI. NLC capsules' test was confirmed to be within acceptable limits.

Particle size

Initially, it was discovered that the optimal freeze-dried NLC's particle size was 270.02 nm. According to Table VI, the particle size rose somewhat after 30 days. However, it was determined to be within acceptable bounds.

CONCLUSION

Glibenclamide-loaded NLCs were created using high-shear homogenization and an ultrasonication approach. The formulation was optimized using design-specific software. Conclusively, the production of glibenclamide-loaded NLCs using high-shear homogenization and an ultrasonication process was effective in the current investigation, demonstrating that NLC capsules may be suggested as a sustained-release oral drug delivery system for the administration of glibenclamide. NLCs alter a drug's kinetics, release and distribution. These characteristics of NLCs might make them an innovative glibenclamide carrier. Thus, the goals outlined in this article were achieved.

REFERENCES

1. Aslam M., Aqil M., Ahad A., Najmi A.K., Sultana Y. and Ali A.: Application of Box–Behnken design for preparation of glibenclamide loaded lipid based nanoparticles: Optimization, *in vitro* skin permeation, drug release and *in vivo* pharmacokinetic study, **J. Mol. Liq**., 2016, 219, 897–908. https://doi.org/10.1016/j.molliq.2016.03.069.
2. Muller H.: Solid-liquid (semi-solid) lipid particles and method of producing highly concentrated lipid particle dispersions, **Eur. Pat. Appl.**, 2000, 45,199-203.
3. Muller R., Wissing A. and Kayser O.: Solid lipid nanoparticles for parenteral drug delivery, **Adv. Drug Deliv. Rev**., 2004, 56(9), 1257-1272. doi.10.1016/j.addr.2003.12.002
4. Dandagi P.M., Dessai G.A., Gadad A.P. and Desai V.B.: Formulation and evaluation of nanostructured lipid carrier (nlc) of lornoxıcam, **Int. J. Pharm. Pharm. Sci**., 2014, 6(2),73-77.
5. Patel K., Padhye S. and Nagarsenker M.: Duloxetine HCl lipid nanoparticles: preparation, characterization and dosage form design, **AAPS Pharm. SciTech**., 2012, 13(1), 125-133. doi:10.1208/s12249-011-9727-6
6. Wankhade V., Tapar K., Pande S. and Bobade N.: Design and evaluation of self nanoemulsifying drug delivery systems (SNEDDS) for gliclazide, **Der Pharmacia Lett.**, 2010, 2(4), 132-143.
7. Fang J.Y., Fang C.L., Liu C.H. and Su Y.H.: Lipid nanoparticles as vehicles for topical psoralen delivery: Solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC), **Eur. J. Pharm. Biopharm**., 2008, 70 (2), 633–640. doi: 10.1016/j.ejpb.2008.05.008
8. Sanna V., Caria G. and Mariani A.: Effect of lipid nanoparticles containing fatty alcohols having different chain length on the *ex vivo* skin permeability of econazole nitrate, **Powder Technol.**, 2010, 201(1), 32–36. doi:10.1016/j.powtec.2010.02.035
9. Chen C., Tsai T., Huang Z. and Fang J.: Effects of lipophilic emulsifiers on the oral administration of lovastatin from nanostructured lipid carriers: physicochemical characterization and pharmacokinetics, **Eur. J. Pharm. Biopharm**., 2010, 74, 474-482. https://doi.org/10.1016/j.ejpb.2009.12.008.
10. Huang Y.B., Tsai Y.H., Yang W.C., Chang J.S., Wu P.C. and Takayama K.: Once -daily propranolol extended-release tablet dosage form: formulation design and *in vitro*/*in vivo* investigation, **Eur. J. Pharm. Biopharm**., 2004, 58 (3), 607–614. https://doi.org/10.1016/j.ejpb.2004.03.037
11. Guan T., Miao Y., Xu L.,Yang S., Wang J., He H., Tang X., Cai C. and Xu H.: Injectable nimodipine-loaded nanoliposomes: preparation, lyophilization and characteristics, **Int. J. Pharm.**, 2011, 410 (1-2), 180–187. doi: 10.1016/j.ijpharm.2011.03.009.
12. Varshosaz J., Eskandari S. and Tabbakhian M.: Freeze-drying of nanostructure lipid carriers by different carbohydrate polymers used as cryoprotectants, **Carbohydr. Polym**., 2012, 88 (4), 1157–1163.doi:10.1016/j.carbpol.2012.01.051
13. Souto E.B., Wissing S.A., Barbosa C.M. and Müller R.H.: Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery**, Int. J. Pharm.,** 2004, 278 (1), 71-77. doi: 10.1016/j.ijpharm.2004.02.032.
14. Dixit R.P. and Nagersenker M.S.: Dry adsorbed emulsion of simvastatin: optimization and *in vivo* advantage, **Pharm. Dev. Technol.,**2007,12(5),495-504.Doi:10.1080/10837450701557246.
15. Bhaskar K., Anbu J., Ravichandiran V., Venkateswarlu V. and Rao Y. M.: Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, *in vitro*, *ex vivo* and *in vivo* studies**, Lipids Health Dis**., 200,8, 6-14. doi: 10.1186/1476-511X-8-6.
16. Kumar A.N., Varghese L., Ramesh S., Ansari L.T. and Kumar K.S.: Enhancement of dissolution rate of glibenclamide usıng liquisolid technique, **IJRPNS**, 2015, 4(4), 206 - 216.
17. Carstensen J.T.: Preformulation. In Modern Pharmaceutics; Banker, G.S., Rhodes, C.T., Eds.; Pub**- Marcel Dekker, New York.**, 2002, Vol. 40, pp 213.
18. Sinko P. J. and Martin A.N.: Micromeretics. In: Martin's physical pharmacy and pharmaceutical sciences. Baltimores, M.D., Ed; Lippincott Williams and Wilkins, 2001, pp 423-454. 109. ICH, Q1A, (R2) Stability testing guidelines: Stability of new drug substances and product: Methodology in processing of ICH Geneva, 2003.
19. ICH Q1A (R2): Stability testing guidelines: Stability of new drug substances and product: Methodology in processing of ICH Geneva, 2003.
20. Joshi M. and Patravale V.: Nanostructured lipid carrier (NLC) based gel of celecoxib. **Int. J. Pharm**., 2008, 346 ,124–132. doi: 10.1016/j.ijpharm.2007.05.060.