ORIGINAL ARTICLE

Designing and Optimization of Lipid-Based Nanocarriers for Safer and Effective Oral Delivery of Tizanidine Hydrochloride

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Abstract

Introduction: The oral route is widely favored for drug delivery due to its simplicity. However, after oral administration, the effectiveness of tizanidine hydrochloride (TZN) is limited due to its low solubility and increased metabolism. The present study investigates the utility of nanostructured lipid carriers (NLCs) as an oral formulation for TZN to boost its therapeutic effectiveness. **Materials and Methods:** TZN-loaded NLCs were prepared by the solvent evaporation method using glyceryl monostearate and oleic acid as solid and liquid lipids, respectively, while the combination of tween 80 and poloxamer 188 was used as surfactants. The formulation optimization was carried out using a Box–Behnken design taking particle size and entrapment efficiency as response variables. The optimized formulation underwent evaluation for particle size, polydispersity index (PDI), ζ-potential, morphological studies, entrapment efficiency, permeability, and drug release characteristics. **Results:** The final optimized formulation exhibited all the necessary attributes, including a particle size of 320.6 ± 13.86 nm, PDI of 0.376 ± 0.031, a zeta potential of −25.8 mV, and entrapment efficiency of 78.33% ± 1.96%. *In vitro* release studies have also demonstrated substantial drug release profiles, highlighting the potential of NLCs for enhanced drug delivery with extended-release profiles. **Conclusion:** The above findings signify that the current optimization model can be used for the development of NLCs for safer and effective oral delivery of TZN which have all the necessary pharmaceutical attributes and may be a promising avenue for its safer and more effective delivery for such drugs.

Key words: Nanostructured lipid carriers, Tizanidine hydrochloride, Muscle relaxant, Design of experiment

INTRODUCTION

Recent estimates indicate that a significant proportion of new chemical entities entering drug development, ranging from 40% to as much as 70%, exhibit inadequate solubility in aqueous solutions. This limitation hinders their consistent absorption within the gastrointestinal tract at levels necessary for therapeutic effectiveness. About 40% of new drug candidates suffer from low water solubility, leading to common issues in oral drug delivery, including reduced bioavailability, substantial variability in absorption between and within individuals, and a lack of dose proportionality.[1]

The oral route of drug administration is one of the earliest and most popular methods for drug delivery in the body. Oral medication delivery is preferred over parenteral drug delivery due to its high patient compliance, non-invasiveness, low cost, and simplicity of administration. Moreover, oral administration allows for more precise dose frequency control and lowers the possibility of disease transmission.[2,3]

Tightened muscles, often due to brain or spinal cord damage, disrupt movement and speech and can cause discomfort or pain. This condition is called spasticity. Spasticity arises from impaired nerve communication between the brain or spinal cord and muscles, resulting in increased muscle tone that hinders movement, speech, and can generate discomfort.[4]

Tizanidine hydrochloride (TZN) is the hydrochloride salt of the drug tizanidine, a centrally acting adrenergic agonist,

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and imidazoline derivative similar to clonidine with muscle relaxant property is widely used as centrally-acting muscle relaxant.[5] It is used to treat spasticity caused by spinal cord injury, multiple sclerosis, or sickness and acts primarily on the spinal cord. TZN is categorized as a BCS class II drug, characterized by high permeability but low solubility. Due to the drug's substantial first-pass metabolic effects, it experiences limited oral bioavailability, with only around 21% of the drug entering the bloodstream. In addition, TZN has a relatively short average elimination half-life of approximately 3 h. As a result, it needs to be administered to patients regularly to maintain its therapeutic effects.[6]

NLCs are third-generation lipid-based nanoparticles that are developed based on solid lipid nanoparticles (SLNs). Nanostructured lipid carriers (NLCs) are colloidal drug delivery systems composed of physiological and biodegradable lipids (solid and liquid lipids).[7,8] To overcome the drawbacks of SLN incorporation of liquid lipid causes structural imperfection in crystalline arrangement giving the high loading capacity and averting drug leakage. In recent years, NLCs have emerged as the promising carrier system for delivery through different routes oral, parenteral, ocular, topical, and transdermal.[9] The present study aims to develop and optimize TZN-loaded NLCs to overcome the drawbacks associated with the conventional dosage form.

MATERIALS AND METHODS

Materials

TZN was received as a gift sample from Akums Laboratory. (India), glyceryl monostearate (GMS), oleic acid, tween 80, was provided by Shri G.S. Institute of Technology and Science, and poloxamer 188 gifted by BASF (India). Analytical-grade chemicals and solvents were utilized in the study.

Selection of lipids and surfactant

The selection of appropriate solid lipid used for the formulation of NLCs was selected based on the solubility of the drug. Various solid lipids were weighed at 500 mg, heated to 5°C above their respective melting points, and then the drug was gradually added [Table 1]. For the liquid lipid and surfactant [Tables 2 and 3], the drug was added to 1 mL of the corresponding liquid lipid and mixed using a vortex mixer.[10] This process continued until saturation was achieved, and then the mixture was left to stabilize for 12 h.[11] The goal is to ensure that no individual particles are visually observed.

Selection of solid-liquid ratio

To assess the miscibility between the solid and liquid lipids, various physical mixtures were prepared with increasing proportions, ranging from 60:40 to 90:10 of solid-to-liquid lipids.

Table 1: Selection of solid lipid

S. No.	Solid lipid	Solubility (Approximately)	Observation
1.	Stearic acid	2 mg	Turbid
2.	Compritol ATO 888	2 mg	Turbid
3.	Glyceryl monostearate	3 mg	Clear
4.	Tristearin	2 mg	Turbid

Table 2: Selection of liquid lipid

S. No.	Liquid lipid	Solubility (Approximately)	Observation
1.	Caprylic acid	2 mg	Slightly turbid
2.	Oleic acid	3 mg	Clear

Table 3: Selection of surfactant

S. No.	Surfactant	Solubility (Approximately)	Observation
1.	Tween-80	1 mg	Turbid
2.	Poloxamer 188	1 mg	Turbid

These mixtures were heated to 100°C ± 1°C for 1 h while being stirred at 200 rpm. Subsequently, the mixtures were allowed to cool to room temperature (20°C ± 1°C) for solidification. To determine the existence or absence of miscibility between the two lipids, a portion of each solidified mixture was placed on a filter paper.[12] Visual observation was then conducted to check for the presence of oil droplets, which would indicate a lack of miscibility between the lipids [Table 4].

Selection of surfactant ratio

The following procedure was used to select the optimum ratio for the surfactant to co-surfactant: 5 mL water and S_{mix} were taken in a 3% fixed ratio while changing the surfactant ratio in different proportions, namely 1:1, 1:2, 1:3, 2:1, and 3:1.[13] Results are mentioned in [Table 5]. The lipid accumulation study was conducted to determine the optimal ratio.

Preparation of TZN-loaded NLC

The tizanidine NLC was prepared using the solvent evaporation method; solid lipid (GMS) and liquid lipid (oleic acid), TZN, and soya phosphatidylcholine-90 (150 mg) dissolved in absolute ethanol (10 mL) as an organic phase. For the aqueous phase, tween 80 and poloxamer 188 (surfactant) in a ratio of 3:1 were dissolved in distilled water (10 mL), and both phases were kept in the water bath at 70 ± 5°C temperature till equilibrium. Then, the organic phase was filled in a glass syringe and added into the aqueous phase dropwise while continuously stirring at 1200 ± 25 rpm using a magnetic stirrer. The emulsion was

kept under stirring for 2 h and then transferred into 30 mL cold water (4°C) for 1 h under continuous stirring. After that, the NLCs were separated using lyophilization and stored in a closed container for further studies.[14,15]

Optimization of formulation using Box–Behnken design

The optimization of the NLC formulation was conducted using the "Box–Behnken design" with the assistance of "Design-Expert 12 software" from State-Ease Inc., Minneapolis, MN, USA. This design involved three independent factors, each with three levels. The selected independent variables were the concentration of the lipid (A) ranging from 200 mg to 400 mg, the amount of the drug (B) varying from 2 mg to 10 mg, and the concentration of the surfactant (C) ranging from 1.5% to 4.5%. The dependent variables considered in this study included the particle size (R1), the polydispersity index (PDI) (R2), and the entrapment efficiency (R3).

Optimization of process parameters

Several processing factors were optimized which influence the lipid film deposition methodology used to prepare NLCs.

The temperature of the water bath

At the time of manufacturing of microemulsion, the temperature should be kept at or above the lipid phase transition temperature, the temperature at which the organic solvent can evaporate is also considered. Batches of TZN-NLCs were prepared in a water bath using GMS and oleic acid at a ratio of 8:2 at different temperatures ranging from 50°C to 70°C [Table 6].

Stirring speed of the magnetic stirrer

The magnetic stirrer was used for the preparation of NLCs and its speed was also pre-optimized [Table 7].[16]

Particle size, PDI, and zeta-potential

The analysis of particle size, PDI, and zeta potential for the formulations were analyzed by the particle size analyzer (Horiba). To conduct the analysis, the NLC samples were appropriately diluted with Milli-Q water, and these measurements were performed in triplicate for accuracy.

Entrapment efficiency

The entrapment efficiency of TZN-loaded NLCs was determined by the column chromatography separation method. The formulation was poured in the Sephadex G-50 column for separating the entrapped and unentrapped drug followed by centrifugation of the column and lysis of the NLCs collected in the filtrate using triton X-100.[17-19] Subsequently, the supernatant samples were carefully pipetted, appropriately diluted, and then subjected to analysis using a "UV spectrophotometer" set at 320 nm.[20] The entrapment efficiency was determined using the following equation:

$$Entrapment\ efficiency = \frac{Amount\ of\ drug\ entrpped}{Total\ amount\ of\ drug\ taken} \times 100$$

Morphological characterization of NLC

The structural morphology of the optimized NLCs was observed by field emission scanning electron microscopy. A mail-out sample was placed on a stub and stuck with the help of carbon back tape and the optimized NLCs were coated with gold fumes. The sample was analyzed at 5 kV accelerated voltage and 50.7 KX magnification.[21]

Table 4: Selection of optimum ratio of solid and liquid lipid

S. No.	Lipid ratio (solid: liquid)	Lipid amount (mg)		Observation
		Solid lipid	Liquid lipid	
1.	60:40	60	40	Oil stain
2.	65:35	65	35	Oil stain
3.	70:30	70	30	No stain
4.	75:25	75	25	No stain
5.	80:20	80	20	No stain
6.	85:15	85	15	No stain
7.	90:10	90	10	No stain

Table 5: Selection of optimum ratio of surfactant and co-surfactant

S. No.	S_{mix} ratio	Surfactant weight (mg)		Lipid accumulated (μL)	Observation
		Poloxamer 188	Tween 80		
1.	1:1	75	75	6	Cloudy
2.	1:2	50	100	8	Cloudy
3.	1:3	37.5	112.5	10	Clear
4.	2:1	100	50	10	Turbid
5.	3:1	112.5	37.5	10	Turbid

Table 6: Effect of aqueous and organic phase temperature on NLC formation

S. No.	Temperature range (°C)	Observation
1.	50±5	The nanoparticles formed are irregular along with the presence of lipid crystals
2.	60±5	The nanoparticles formed are irregular along with the presence of lipid crystals
3.	70±5	The nanoparticles formed are regular and formed with no presence of any lipid crystals

NLC: Nanostructured lipid carriers

Table 7: Effect of RPM on NLCs

S. No.	RPM	Observation
1.	800±25	Large nanoparticles observed
2.	1000±25	Large nanoparticles observed
3.	1200±25	Comparable smaller nanoparticles observed

NLC: Nanostructured lipid carriers

Differential scanning calorimetry (DSC)

The results of DSC tests were obtained using DSC (Perkin Elmer DSC 6000) that was calibrated by Indium. The analysis of samples of pure drug, drug-loaded NLCs that have been optimized, and the sample taken 2.8 mg, was placed in a standard-grade aluminum pan and sealed. The analysis of the sample was carried out in the temperature range of 50–300°C at a 20°C/min scanning rate.[19]

In vitro drug release

The *in vitro* drug release of prepared pure drug solution and drug-loaded NLCs was carried out by dialysis method using a dialysis membrane using 12–14 kDa MWCO (Himedia®, West Chester, PA). 5 mL of the sample were filled in a dialysis bag while one end was closed and then both ends were closed with dialysis closure clips. Then dialysis bags were dipped in 50 mL phosphate buffer saline (pH 6.8) at 37°C ± 0.5°C and 300 RPM. 5 mL of sample were withdrawn and replaced with the equivalent amount of freshly prepared saline phosphate buffered buffer at every fixed interval. Then, the sample was analyzed using a UV-visible spectrophotometer (Shimadzu-1700®) at 320 nm.

RESULTS AND DISCUSSION

Screening of lipids and surfactant

The approximate solubility of TZN was determined to be 3 mg/mL in GMS, displaying clear solubility without any issues. However, when tested in other solid lipids such as stearic acid, compritol ATO 888, and tristearin, turbidity was observed at 2 mg/mL. In addition, TZN exhibited a solubility of 3 mg/mL in oleic acid and 2 mg/mL in caprylic acid. Based on these findings, it was evident that TZN had the highest solubility in GMS and oleic acid. Consequently, GMS was chosen as the solid lipid, and oleic acid was chosen as the liquid lipid for the NLCs formulation. For the selection of a non-ionic surfactant, factors such as low toxicity, high stability, compatibility, and pH-independence were taken into consideration. TZNs solubility was found to be the lowest in tween 80 (<1 mg) and in poloxamer 188 (<1 mg) compared to other non-ionic surfactants. Therefore, the combination of surfactants was selected for the NLCs formulation.

Selection of solid: liquid ratio

The combination of GMS and oleic acid exhibited excellent compatibility across various ratios. Furthermore, it is worth noting that the extent of drug molecule entrapment is influenced by the quantity of liquid lipid present. Consequently, the chosen lipid should be present in the maximum possible quantity. This is because a higher proportion of liquid lipid disrupts the well-organized crystalline structure of the carrier system, resulting in additional space for accommodating the drug. Therefore, a ratio of eight parts GMS to two parts oleic acid was employed.

Selection of surfactant ratio

The ratio of tween 80:poloxamer 188 at which it was clear and maximum lipid accumulated was selected for further studies which was 1:3. The optimum combination surfactant ratio is important to form a stable microemulsion.

Optimization of process parameters temperature and RPM

The effect of temperature and RPM on the size of NLC was studied which was observed using an optical microscope. The result showed that temperature range and RPM of 70 ± 5°C and 1200 ± 25, respectively, are the optimum temperature and RPM for the formulation of NLCs.

Optimization using Box–Behnken design

We employed a "Box–Behnken experimental design" with three independent variables, each at three different levels, to fine-tune the formulation. The chosen independent variables are the total lipid concentration (A), drug quantity (B), and surfactant concentration (C), and the dependent variables, including particle size (R1), PDI (R2), and entrapment efficiency (R3). To visualize the relationships, the 3D plots for all three responses, as illustrated in Figure 1.

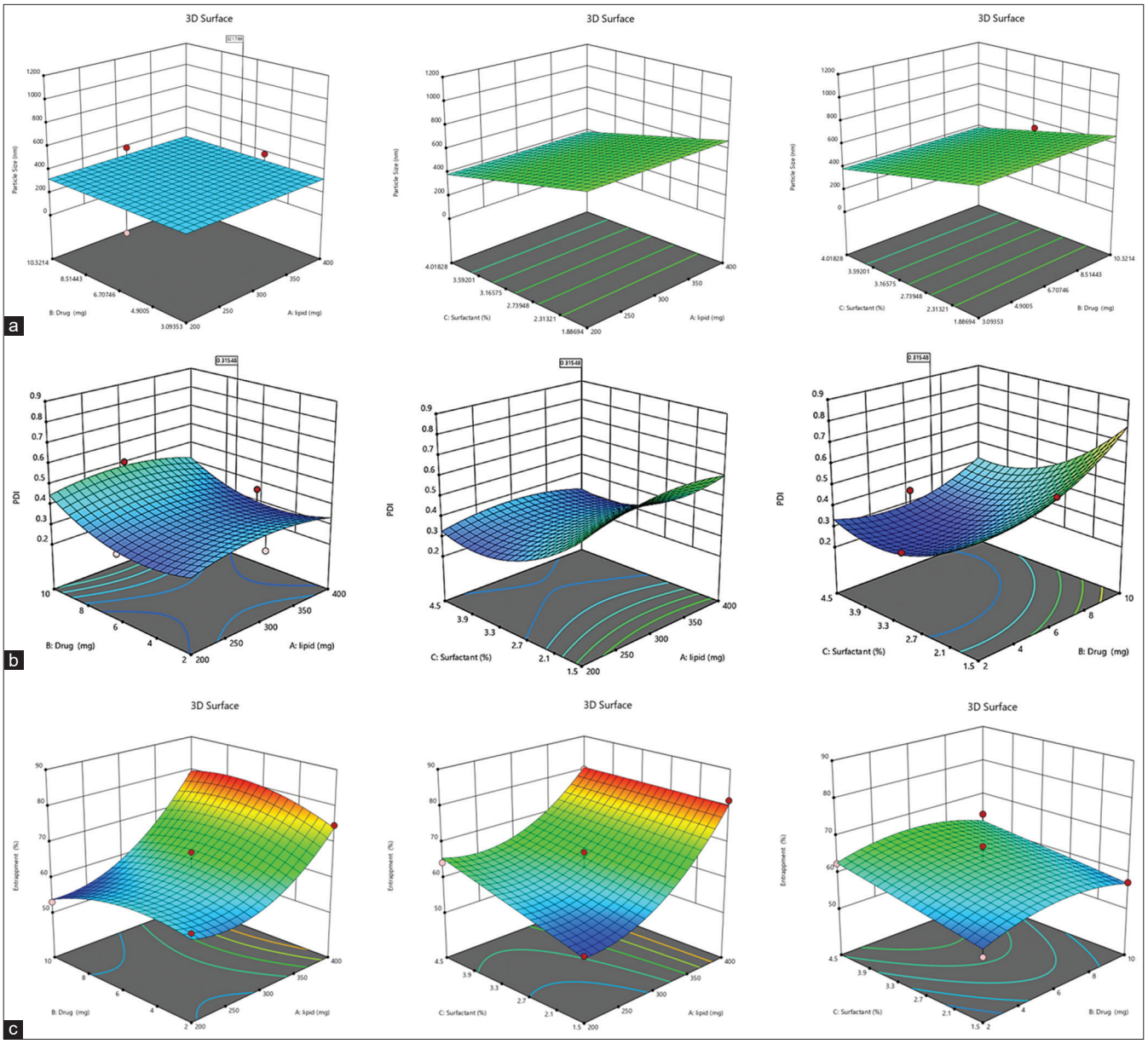

Figure 1: A 3D-response surface plot illustrates the impact of independent variables, including the total lipid concentration, drug amount, and surfactant concentration on (a) particle size, (b) polydispersity index, and (c) entrapment efficiency

The results obtained from the 15 experimental runs are presented in Table 8. The most suitable model for all three dependent variables, determined using non-linear software, was found to be the quadratic model. These models exhibited a high coefficient of correlation (R^2) close to 1. The polynomial equations of the dependent variable and the associated model-summary statistics can be found in Table 9.

Effect of independent variables on (R1)

The R^2 value and adjusted R^2 in fit statistics for particle size suggested by the model were found to be 0.5716 and 0.5387, respectively, which indicates the suitability of the model. Similarly, the predicted R^2 was 0.3851, which is in reasonable agreement with the adjusted R^2, i.e., the difference is <0.2. The model F-value of 17.35 signifies the significance of the model. When "Probability>F" values are <0.0500, it suggests that the model terms are indeed significant.[22]

The 3-D response surface plot illustrating the impact of the independent variables on particle size is displayed in Figure 1a. It was observed that an increase in the total lipid concentration (A) led to an increase in the particle size of TZN-loaded NLCs. In contrast, an increase in surfactant concentration (C) resulted in a reduction in NLC size. This phenomenon was explained by the presence of the surfactant, which created steric hindrance between the droplets of the microemulsion, this hindrance prevented smaller particles from coalescing into larger ones at the time of microemulsion formation.[23,24]

Table 8: Evaluation of optimized batches of tizanidine HCl-loaded NLCs

Batch code	Lipid concentration (mg)	Drug amount (mg)	Surfactant concentration (%)	Particle size	Polydispersity index	% Entrapment efficiency
N1	200	2	3	359.5	0.334	60.26
N2	400	2	3	496.2	0.310	74.81
N3	200	10	3	425.6	0.459	53.03
N4	400	10	3	571.4	0.396	78.15
N5	200	6	1.5	546.5	0.415	53.44
N6	400	6	1.5	607.5	0.564	81.56
N7	200	6	4.5	131	0.29	64.35
N8	400	6	4.5	382.5	0.36	80.12
N9	300	2	1.5	742.5	0.482	53.50
N10	300	10	1.5	1018.8	0.874	57.28
N11	300	2	4.5	365.4	0.305	62.52
N12	300	10	4.5	420.2	0.504	64.02
N13	300	6	3	541.2	0.265	59.70
N14	300	6	3	640.9	0.431	67.28
N15	300	6	3	607.7	0.324	62.23

NLC: Nanostructured lipid carriers, TZN: Tizanidine Hydrochloride

Table 9: Polynomial equation for each factor

Response variable	Polynomial equation
Particle size (Y1)	523.79-202.03*C
PDI (Y2)	0.3400+0.0165*A+0.1002*B-0.1095*C-0.0097*AB-0.0197*AC-0.0482*BC-0.0496*A+0.0844*B^2+0.1169*C^2
Entrapment efficiency (Y3)	63.08+10.32*A+0.1749*B+3.0*C+2.64*AB-2.83*AC-0.5710*BC+7.14*A^2-3.65*B^2-0.0947*C^2

PDI: Polydispersity index

Among the 15 formulations tested, formulation 7 exhibited the smallest particle size at 131.0 ± 14.5 nm, while formulation 10 had the largest particle size at 1018 ± 23.58 nm.

Effect of independent variables on (R2)

The model F-value of 3.08 indicates the significance of the model. When "Prob>F" values are <0.0500, it signifies that the model terms are indeed significant. The "lack of fit F-value" of 1.68 suggests that the lack of fit is not significant relative to the pure error, which is a positive outcome as it signifies a good model fit. The R^2 for the PDI was 0.8473, the adjusted R^2 was 0.5724, indicating an excellent fit, and the predicted R^2 was 0.8471, showing reasonable agreement with the adjusted R^2.

In the 3-D response plot depicting the impact of independent variables on the PDI [Figure 1], it was observed that an increase in the total lipid concentration led to an increase in the PDI of TZN-loaded NLCs.

Effect of independent variables on (R3)

The model F-value of 14.30 signifies the significance of the model. When the "Prob>F" values are <0.0500, it indicates that the model terms hold significance. In addition, the "Lack of Fit-value" of 0.41 suggests that the lack of fit is not significant, indicating a well-fitting model. The R^2 value of 0.9626 is in good agreement with the adjusted R^2 of 0.8953, confirming a strong model fit.

As depicted in Figure 1, an increase in the total lipid concentration led to an enhancement in the entrapment efficiency of the NLCs. This outcome can be attributed to the increased availability of lipids, allowing for the complete encapsulation of drug particles. In addition, the liquid lipid creates a less order arrangement which leads to higher entrapment efficiency.[25]

Particle size, PDI, zeta-potential, and entrapment efficiency of optimized formulation

The optimized formulation of TZN-loaded NLCs exhibited a particle size of 320.6 ± 13.86 nm and a PDI of 0.376 ± 0.031. The particle size and the size distribution curve of this optimized NLC formulation are illustrated in Figure 3. In addition, the zeta-potential curve of the

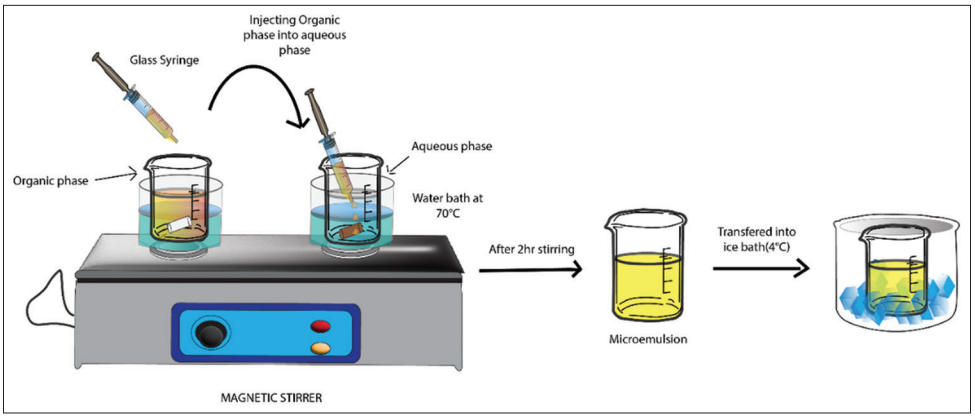

Figure 2: Schematic representation of method of preparation

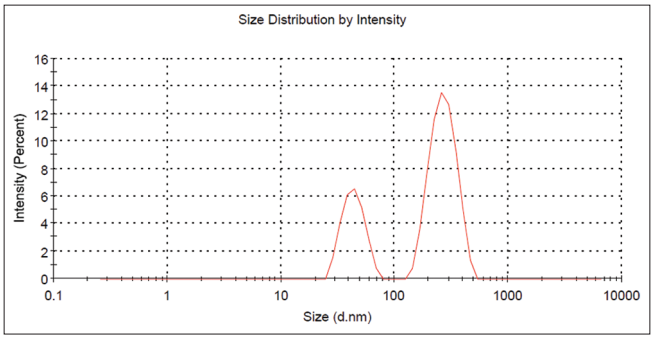

Figure 3: Particle size distribution of optimized tizanidine hydrochloride-loaded nanostructured lipid carriers

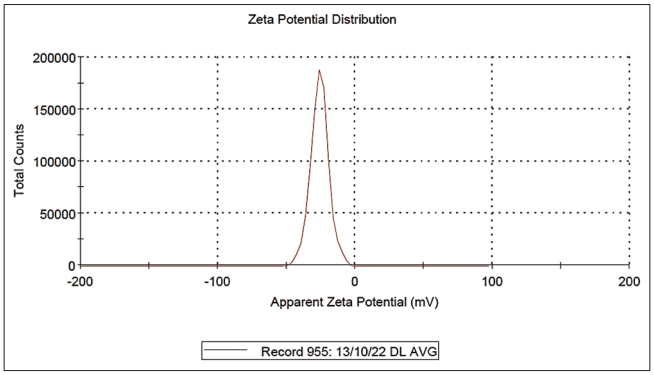

Figure 4: Zeta potential of nanostructured lipid carriers

optimized formulation can be seen in Figure 4, with the measured zeta potential of the TZN-loaded NLC being −25.8 mV. This negative zeta-potential value confirms the stability of the particles within the formulation. It is worth noting that zeta-potential values exceeding 30 mV or falling below −30 mV indicate complete electrostatic stabilization. Furthermore, the EE of the optimized TZN-loaded NLC formulation was determined to be 78.33 ± 1.96%.

Morphology of NLC

The optimized formulation of TZN-loaded NLCs was visualized using field emission scanning electron microscopy,

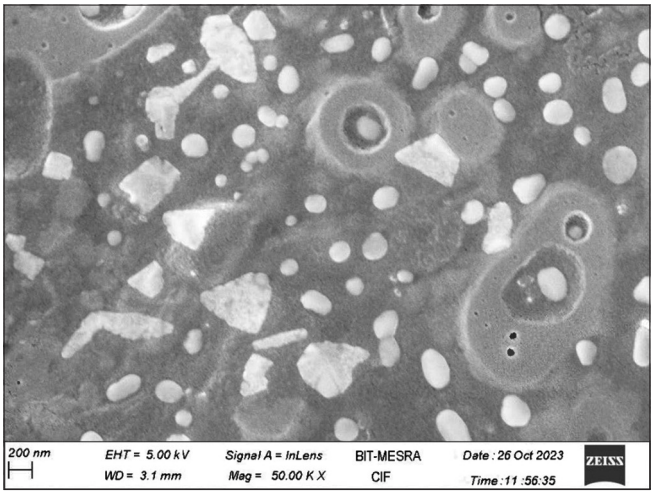

Figure 5: Field emission scanning electron microscopy image of optimized nanostructured lipid carriers

and the resulting image is presented in Figure 5. This image revealed the presence of circular structures and irregular structures.[26,27]

In vitro drug release

The comparative *in vitro* drug release study between pure drug and TZN-loaded NLC revealed that the initial release from pure drug solution was significantly higher than the cumulative amount of drug released from the NLC. This difference was particularly evident in simulated intestinal fluid (phosphate buffer, pH 6.8), as depicted in Figure 6. The initial percentage cumulative drug release from drug solution and NLCs was 33.3 ± 0.9% and 21.7 ± 1.1% at 30 min, and 96.4 ± 0.8% and 80.07 ± 1.6% at 480 min, respectively. Notably, the results indicated an initial burst release of the drug from the NLC, followed by sustained release after 120 min.

The release data for TZN from NLC were analyzed using various mathematical models, including the "zero-order," "first-order," "Higuchi," and "Korsmeyer–Peppas models."

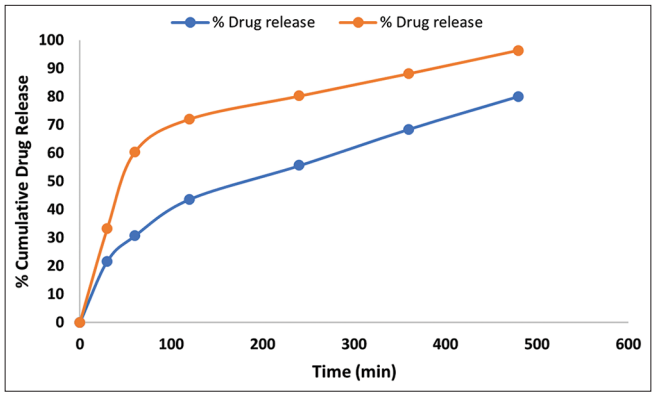

Figure 6: Cumulative % drug release of pure drug and tizanidine hydrochloride loaded- nanostructured lipid carriers

Among these, the "Korsmeyer–Peppas model" exhibited the highest R^2 values of 0.99 in the phosphate buffer at pH 6.8.

On analyzing the release data using the "Korsmeyer–Peppas equation," it was observed that the release exponent (n) had values of 0.4. This indicates that the mechanism of drug release from the NLC was in accordance with the "Fickian diffusion" mechanism.

CONCLUSION

TZN-loaded NLCs were successfully prepared using the solvent evaporation method, and the formulation was optimized through a Box–Behnken design. The size of the TZN-loaded NLCs was determined to be 320.6 ± 13.86 nm, with a PDI of 0.376 ± 0.031. The zeta potential for these NLCs was measured at −25.8 mV, and the entrapment efficiency was found to be 78.33% with a precision of ±1.96%. *In vitro* drug release studies were performed using the dialysis bag method, showing a cumulative release of 21.70 ± 1.1% within 30 min and 80.07 ± 1.6% within 480 min.

The encapsulation of tizanidine in NLCs presents a promising avenue for its safer and more effective delivery. By utilizing NLCs, the drug can potentially bypass gastric metabolism and exploit the lymphatic system for transportation. This strategy aims to enhance the overall effectiveness of tizanidine and improve therapeutic outcomes. NLCs offer a protective milieu for the drug, preventing early degradation in the stomach and facilitating its passage through the lymphatic system, thereby minimizing the risk of adverse effects and maximizing therapeutic benefits.

Collectively, these findings signify that the current optimization model can be used for the development of NLCs for safer and effective oral delivery of TZN which will have all the necessary pharmaceutical attributes.

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