Introduction
Nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLN) are used to deliver the drug via different routes like parenteral, topical, and oral routes by controlling drug release of administered drug. But there are few drawbacks of SLNs, like limited drug loading due to min solubility of drug, leakage, and concentration in the aqueous dispersion were eliminated by formulating a nanoparticle with a controlled nanostructure are nanostructured lipid carriers.

Nitrofurantoin is effective against many urinary tract pathogens. It acts as bacteriostatic and/or bactericidal by inhibiting DNA-RNA protein and cell wall synthesis. Nanostructured lipid carriers (NLCs) of nitrofurantoin (NFT) were prepared by hot homogenization process. Glycerol monostearate and miglyol 812 were heated at 80°C temperature on hot plate. In the melted lipid, drug was added with continuous stirring. Formulation NLC12B has % entrapment efficiency 89.1 ± 0.5, polydispersity index (PDI) 0.11 ± 0.01, and mean particle size 237 ± 7 nm represents narrow particle size distribution. Spherical feature of NLCs with better uniformity without aggregation of nitrofurantoin loaded NLC was confirmed by transmission electron microscopy (TEM). Moreover, efficient miscibility of drug in lipids was confirmed by the absence of intense and characteristic peak of NFT in X-ray powder diffraction (XRPD). After 6 month storage at 2 to 8°C, there were no significant changes in the PDI, as well as, mean particle size.

Abstract
Nitrofurantoin is effective against many urinary tract pathogens. It acts as bacteriostatic and/or bactericidal by inhibiting DNA-RNA protein and cell wall synthesis. Nanostructured lipid carriers (NLCs) of nitrofurantoin (NFT) were prepared by hot homogenization process. Glycerol monostearate and miglyol 812 were heated at 80°C temperature on hot plate. In the melted lipid, drug was added with continuous stirring at high-speed homogenization. Formulation NLC12B has % entrapment efficiency 89.1 ± 0.5, polydispersity index (PDI) 0.11 ± 0.01, and mean particle size 237 ± 7 nm represents narrow particle size distribution. Spherical feature of NLCs with better uniformity without aggregation of nitrofurantoin loaded NLC was confirmed by transmission electron microscopy (TEM). Moreover, efficient miscibility of drug in lipids was confirmed by the absence of intense and characteristic peak of NFT in X-ray powder diffraction (XRPD). After 6 month storage at 2 to 8°C, there were no significant changes in the PDI, as well as, mean particle size.
infrared-spectroscopy (FTIR) analysis was performed to investigate the compatibility of excipients with drug. The physicochemical properties of NFT loaded NLCs, such as, mean particle size, zeta potential, drug entrapment efficiency, stability, and in vitro drug release profile were investigated in detail. Characterization of optimized formulation was confirmed by TEM and XRPD, stability study.

**Materials and Methods**

**Materials**

Nitrofurantoin was received as a gift sample from Hetero Labs Limited, poloxamer 188, poloxamer 407, glyceryl behenate, glyceryl monostearate, miglyol 840, miglyol 812, HPMC E3, mannitol, and tween 80 received from Sun Pharma, Vadodara. Millipore’s purified water, dimethylformamide, dichloromethane, sodium hydroxide, acetone, phosphate buffer, and remaining all ingredients were of analytical grade.

**Methods**

*Development of Analytical Method by High-Pressure Liquid Chromatography with UV Detector (HPLC-UV)*

System: LC-10AD/20AD with 254 nm UV detector.

Mobile phase: 6.8 grams of monobasic potassium phosphate was dissolved in 500 mL water. Add about 30 mL 1 N NaOH sufficiently to adjusting pH 7.2 and dilute with water to 1,000 mL.

Injection volume: 10 μL.

Stock solution: NFT in dimethylformamide + 50 mL internal standard solution.

Internal standard: 1 mg/mL acetanilide in water mobile phase and sonicated. 100 ppm solution was prepared from stock solution.

*Physical Compatibility Study*

Compatibility of drug with excipients was done by infrared spectroscopy. It was done out by mixing 1:1 part of excipients with the NFT and packed in glass vials, closed with rubber stopper, and kept for 1-month at 40°C/75% RH; 25°C/60% RH. During the study, physical observations were done at regular intervals. KBr pellet of the drug sample was prepared by mixing 2 mg of drug sample with IR-grade potassium bromide (KBr). Potassium bromide pellet was used as a blank for background, then the sample was scanned. The spectra were collected in the 400 cm⁻¹ to 4,000 cm⁻¹ region with 8 cm⁻¹ resolution. Average of 10 scans were taken to evaluate the molecular states of the pure drug and mixture of drug with excipients to check any changes in the chemical structure of the drug.

*Preparation of NFT-loaded NLCs*

Hot homogenization process was used to formulate NFT loaded nanostructured lipid carriers. Lipid layer was prepared by melting glyceryl monostearate and miglyol 812 on hot plate at 80°C temperature. In melted lipid, add nitrofurantoin with continuous stirring with high-speed homogenization at 10,000 rpm for 5 minutes. Aqueous medium was prepared by dissolving poloxamer 188 in double-distilled water and heated to same temperature of oil phase at 80°C. Hot aqueous surfactant solution was added to hot lipid phase using a high-speed homogenizer at 10,000 rpm for 10 minutes. During homogenization, temperature was maintained above/at 80°C. The resulted dispersion was cooled to room temperature and centrifugation performed at 10,000 rpm for 10 minutes, which were diluted with purified water as per requirement. NFT loaded SLNs were stored at 2 to 8°C to precede ahead.

*Optimization of the Composition and Process Parameters*

**Preliminary Development Batches for Process Feasibility**

In this study, preliminary development batches were manufactured to check the process feasibility and analytical method feasibility. Composition and process parameters are mentioned as per Table 1.

| Batch No. | Ingredients                  | NLC1 | NLC2 |
|-----------|------------------------------|------|------|
| 1         | Nitrofurantoin               | 0    | 1.2  |
| 2         | Glyceryl monostearate        | 10   | 8.8  |
| 3         | Miglyol 812                  | 50   | 50   |
| 4         | Poloxamer 188                | 40   | 40   |
| 5         | Purified water*              | qs   | qs   |
| Total     |                              | 100% | 100% |

| S. No. | Parameter                                                                 | NLC1 | NLC2 |
|--------|---------------------------------------------------------------------------|------|------|
| 1      | High-speed homogenization of lipid phase (rpm for 5 minutes)              | 10,000| 10,000|
| 2      | High-speed homogenization during mixing of aqueous and lipid phase (rpm for 10 minutes) | 10,000| 10,000|
| 3      | Centrifugation (rpm for 10 minutes)                                       | 10,000| 10,000|

*Quantity sufficient to prepare 5% w/w solution of poloxamer 188 throughout the process
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Formulation Variables
In present study, formulations were optimized for surfactant and lipid concentration. Surfactant (poloxamer 188) was increased from 30 to 70% and gradually decreases in lipid (miglyol) from 60 to 20%. Process parameters of high-speed homogenization were kept constant at 10,000 rpm for 10 minutes. The effects of surfactant and lipid concentration on the NFT loaded NLCs were evaluated. Composition and process parameters are given in the following Table 2.

Process Variables (High-Speed Homogenization)
In present study, high-speed homogenization process was optimized by considering the process variables to achieve the defined quality target product. During the mixing of lipid and aqueous phase time and speed of high-speed homogenization was increased from 2,500 rpm, 2 minutes to 10,000 rpm for 10 minutes. Composition and process parameters were given in Table 3.

Freeze Drying Process
Diluted NLCs dispersion of batch no. NLC12 was divided into batch no. NLC12A, NLC12B, and NLC12C equally shown in Table 4. The lyophilization cycle was carried out for 24 hours. The condenser temperature was allowed to get reduced to -70°C. The vials were kept on the surface of condenser. The vials were equilibrated at -70°C, then immediately transferred to the shelf with the slotted rubber stopper in position, and vacuum was then allowed to reach at 100 mTorr. After completion of cycle vials were stoppered and sealed.

In vitro Drug Release Profile to Optimize Formulation
All batches NLC2, NLC3, NLC4, NLC5, NLC6, NLC7, NLC10, NLC11, NLC12, NLC12B, and NLC12C were packed in capsule. In vitro drug release study was performed (Fig. 1) in USP-II apparatus with sinker using 900 mL of phosphate buffer (pH 7.2) at 50 rpm, 37 ± 0.5°C for 12 hours. First for 2 hours dissolution was performed in 0.1 N HCl followed by phosphate buffer pH 7.2 for 12 hours. Samples were withdrawn at predefined time interval and were analyzed.

Response Surface Methodology
The objective was to apply response surface methodology optimization model to optimize NFT loaded NLCs. Two formulation factors, i.e., concentration of lipid

Table 2: Composition and process parameter for formulation optimization batches

| S. No. | Ingredients       | NLC3 | NLC4 | NLC5 | NLC6 | NLC7 |
|-------|------------------|------|------|------|------|------|
| 1     | Nitrofurantoin   | 1.2  | 1.2  | 1.2  | 1.2  | 1.2  |
| 2     | Glycerol monostearate | 8.8  | 8.8  | 8.8  | 8.8  | 8.8  |
| 3     | Miglyol 812      | 60   | 50   | 40   | 30   | 20   |
| 4     | Poloxamer 188    | 30   | 40   | 50   | 60   | 70   |
| 5     | Purified water   | qs   | qs   | qs   | qs   | qs   |
| Total (%) |                  | 100  | 100  | 100  | 100  | 100  |

Table 3: Composition and process parameter for process optimization batches

| S. No. | Parameter                       | NLC8 | NLC9 | NLC10 | NLC11 | NLC12 |
|-------|--------------------------------|------|------|-------|-------|-------|
| 1     | High-speed homogenization of lipid phase (rpm for 5 minutes) | 10,000 | 10,000 | 10,000 | 10,000 | 10,000 |
| 2     | High-speed homogenization during mixing of aqueous and lipid phase (for 10 minutes) | 10,000 | 10,000 | 10,000 | 10,000 | 10,000 |
| 3     | Centrifugation (for 10 minutes) | 10,000 | 10,000 | 10,000 | 10,000 | 10,000 |
(miglyol 812) and surfactant (poloxamer 188), were selected as independent variables, and mean particle size and % entrapment efficiency (EE%) as responses. Design expert software was used for the DOE study. Statistical validity of the polynomials was established on the basis of ANOVA. Level of significance was considered at p < 0.05. Statistical parameters including the coefficient of variation (CV), the multiple correlation coefficient (R²), adjusted multiple correlation coefficient (adjusted R²) were used to identify best fitting mathematical model. Method of manufacturing and process parameter kept similar to formulation NLC12B. Experimental trial was performed using all possible combinations as per design layout shown in below Table 5.

For mean particle size; The predicted R² of 0.1868 is not as close to the adjusted R² of 0.8205 shown in Table 6, the difference is more than 0.2. Signal to noise ratio more than 4 is required. Here, it was of 8.584 indicates an adequate signal. This model can be used to navigate the design space; For % EE: The predicted R² of 0.7928 is in reasonable agreement with the adjusted R² of 0.9436; here, single to noise ratio is 17.237 indicates a proper signal. This model can be used to navigate the design space.

A statistical model describes interactive and polynomial terms are used to measure the response variables using Design Expert 12 version shown in Table 7. For factor: here, lipid and surfactant were taken as factor A and B; the coded value corresponding with the low actual setting used by the math engine to perform the analysis was 20 and high coded was 60; similarly, for surfactant it was 30 and 70; the mean value for miglyol is 40, and for poloxamer were 50

Values of “Prob > F” less than 0.05 indicate model terms are significant; values greater than 0.1000 indicate the model terms are not significant; model F value 11.97 reveals that the model is significant shown in Table 8. p values less than 0.0500 indicate model terms are significant; in this case A, A² are significant model terms; values greater than 0.1 shows the model terms are not significant; lack of fit F value of 4.29 implies there is a 9.65% chance that a lack of fit F value this large could occur due to noise.

### Table 4: Optimization of freeze-drying process

| S. No. | Freeze drying parameter | NLC12A | NLC12B | NLC12C |
|--------|-------------------------|--------|--------|--------|
| 1      | Temperature (condenser) | -70°C  | -70°C  | -70°C  |
| 2      | Vacuum (mTorr)          | 100    | 100    | 100    |
| 3      | Cycle time (hr)         | 12     | 18     | 24     |

### Table 5: Optimization by response surface methodology

| Standard | Run | Factor1 | Factor2 | Response1 | Response2 |
|----------|-----|---------|---------|-----------|-----------|
| 11       | 1   | 40      | 50      | 191       | 91.5      |
| 13       | 2   | 40      | 50      | 203       | 87.8      |
| 6        | 3   | 45      | 50      | 217       | 84.7      |
| 8        | 4   | 40      | 55      | 197       | 88.1      |
| 10       | 5   | 40      | 50      | 195       | 88.8      |
| 3        | 6   | 45      | 45      | 201       | 81.3      |
| 2        | 7   | 40      | 45      | 217       | 73.1      |
| 12       | 8   | 40      | 50      | 205       | 92.7      |
| 5        | 9   | 40      | 50      | 200       | 87.9      |
| 1        | 10  | 35      | 45      | 249       | 49.7      |
| 7        | 11  | 35      | 55      | 253       | 57.3      |
| 4        | 12  | 35      | 50      | 243       | 55.6      |
| 9        | 13  | 45      | 55      | 213       | 91.1      |

### Table 6: Fit statistics of mean particle size and % entrapment efficiency

| Mean particle size | % entrapment efficiency |
|--------------------|-------------------------|
| Std. Dev.          | 8.95                    | 3.61                  |
| R²                 | 0.8953                  | 0.8205                |
| Mean               | 214.15                  | 79.2                  |
| Adjusted R²        | 0.8205                  | 0.9436                |
| CV%                | 4.18                    | 4.56                  |
| Predicted R²       | 0.1868                  | 0.7928                |
| Adeq precision     | 8.584                   | 17.2372               |
Values of “p > F” less than 0.05 indicate model terms are significant; values more than 0.1 shows the model terms are not significant shown in Table 9; model F value is 41.13 reveals that the model is significant; p values less than 0.05 shows model terms are significant; in this case, A, B, A² are significant model terms; values more than 0.1000 shows the models are not significant; lack of fit F value of 4.79 implies there is an 8.22% chance that a lack of fit F value this large could occur due to noise.

In Fig. 2(A) blue region has mean particle size around 191 nm, which is increased from blue to red region. Red region has mean particle size around 253 nm, whereas in Fig. 2(B) red region have % entrapment efficiency around 92.7%, which is decreased from red to blue region. Blue region have % entrapment efficiency around 49.7%.

**EVALUATION AND CHARACTERIZATION OF NLCs**

**Evaluation of Nanostructured Lipid Carriers (NLCs)**

**Measurement of Particle Size and Polydispersity Index**
Zeta potential was carried out at room temperature using photon correlation spectroscopy by Zetasizer Nano ZS (Malvern, UK).[16] The cell of the nano track was cleaned and background was taken with double distilled water. For clear visibility sample was diluted with doubled distilled (DD) water before particle size analysis.[20,21]

**Entrapment Efficiency (%)**
Concentration of unentrapped free drug was used to measure entrapment efficiency (EE%), Drug-loaded sample was taken in a microcentrifuge tube and centrifuged at 50,000 rpm at 25°C for 2 hours, using Sorval mX 150 microcentrifuge (Thermo scientific, USA) and analyzed by HPLC. It was measured by calculating the amounts of non encapsulated NFT in aqueous surfactant solution, against the total amount of drug added to the formulation using following formula[20]:

\[
\text{% entrapment efficiency} = \left(\frac{\text{Amount of NFT in NLCs}}{\text{Total wt of NFT}}\right) \times 100
\]

**Assay**
Assay was calculated by measuring concentration of NFT in 100 mg freeze-dried NLCs against the lipid content of SLN by following formula. 10 ppm solution were prepared by diluting freeze-dried NLCs in dimethylformamide.

\[
\text{Assay} = \frac{\text{NFT in supernatant after centrifugation}}{\text{Total weight of NFT}} \times 100
\]

**Zeta Potential Determination**
Zeta potential of suitably diluted NLCs was measured using Zetasizer Nano ZS (Malvern Instruments, UK). Charge on NLCs and their mean zeta potential value with standard deviation of three measurements were obtained.

**Characterization of NLCs**

**TEM and XRPD Study of Optimized Batch**
The TEM study of NFT loaded NLCs of optimized batch were done by transmission electron micrograph (Hitachi, Japan, H 7500 ID) at high-resolution. X-ray diffractometry of lyophilized sample and NFT was done by using Xpert MPD–XRD Philips, Holland. By on keeping the graticule in vertical position sample was spread on a graticule and exposed for radiation.[17, 22]

**Stability Study of Optimized Batch**
In order to determine the change in physicochemical parameters and *in vitro* release profile on storage, a stability study was carried out.[23] Stability study for the optimized formulation of NFT loaded NLCs was carried out. NLCs were packed and sealed in class-I glass vials.

![Fig. 2: Counter plot for mean particle size](image)

(A) Mean particle size  (B) Entrapment efficiency (%)

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**Table 7: Design summary**

| File version | 12.0.8.0 | Design type | 3-level factorial |
|--------------|----------|-------------|-------------------|
| Study type   | Response surface | Design model | Quadratic |
| Factor   | Name | Units | Type | Min | Max | Coded low | Coded high | Mean | Std. Dev. |
| A    | Miglyol 812 | % w/w | Numeric | 35 | 45 | -1 = 20 | +1 = 60 | 40 | 3.535534 |
| B    | Poloxamer 188 | % w/w | Numeric | 45 | 55 | -1 = 30 | +1 = 70 | 50 | 3.535534 |
| Response | Name | Units | Observations | Analysis | Min | Max | Mean | Std. Dev. | Ratio | Model |
| R1    | Mean particle size | nm | 13 | Polynomial | 191 | 253 | 214.15 | 21.12 | 1.324607 | Quadratic |
| R2    | Entrapment efficiency | % | 13 | Polynomial | 49.7 | 92.7 | 79.2 | 15.21 | 1.865191 | Quadratic |
These freshly prepared freeze-dried NLCs were kept at 2 to 8°C and 25°C/60% RH for 6 months in stability chamber. The samples were analyzed for mean particle size, zeta potential, PDI, % entrapment efficiency, and % assay.

**In vitro Drug Release Study of Stability Batch**

Formulation of stability batch was performed for *in vitro* drug release profile. Freeze-dried formulation containing 10 mg NFT in USP-II apparatus using 900 mL of phosphate buffer (pH 7.2) at 50 rpm, 37 ± 0.5°C for 12 hours. First for 2 hours dissolution was performed in 0.1 N HCl followed by phosphate buffer pH 7.2 for 12 hours. Samples were withdrawn at predefined time interval and were analyzed by HPLC.[24]

### RESULTS AND DISCUSSION

#### Preformulation Study

**Result of Analytical Method**

The standard curve of concentration from 0 to 100 μg/mL was generated shown in Fig. 3. The solubility of nitrofurantoin in various solvent was checked in water and various aqueous buffer solutions. It is very slightly soluble in alcohol and in water. It is soluble in dimethylformamide (DMF). The results are shown in Fig. 1.

**Discussion for Drug-Excipients Compatibility Studies**

Compatibility study of drug with excipients was done at initial, after 2 weeks, and 1-month at 25°C/60% RH. Changes in appearance and molecular structure were observed by infra-red (IR). Observations of appearance at regular intervals are illustrated in Table 10.

#### Table 8: ANOVA response for mean particle size

| Source                  | Sum of squares | df | Mean square | F value | p value | -         |
|-------------------------|----------------|----|-------------|---------|---------|-----------|
| Model (quadratic) (R² = 0.8953) | 4,791.19      | 5  | 958.24      | 11.97   | 0.0025  | significant |
| A-miglyol 812           | 2,166         | 1  | 2,166       | 27.05   | 0.0013  | -         |
| B-poloxamer 188         | 2.67          | 1  | 2.67        | 0.03    | 0.8604  | -         |
| AB                      | 16            | 1  | 16          | 0.2     | 0.6684  | -         |
| A²                      | 1,977.59      | 1  | 1,977.59    | 24.7    | 0.0016  | -         |
| B²                      | 39.02         | 1  | 39.02       | 0.49    | 0.5077  | -         |
| Residual                | 560.51        | 7  | 80.07       | -       | -       | -         |
| Lack of fit             | 427.71        | 3  | 142.57      | 4.29    | 0.0965  | not significant |
| Pure error              | 132.8         | 4  | 33.2        | -       | -       | -         |
| Cor total               | 5,351.69      | 12 | -           | -       | -       | -         |

#### Table 9: ANOVA response for % entrapment efficiency

| Source                  | Sum of squares | df | Mean square | F value | p value | -         |
|-------------------------|----------------|----|-------------|---------|---------|-----------|
| Model: quadratic R² = 0.9671 | 2,683.52       | 5  | 536.7       | 41.13   | < 0.0001 | significant |
| A-miglyol 812           | 1,488.38       | 1  | 1,488.38    | 114.07  | < 0.0001 | -         |
| B-poloxamer 188         | 174.96         | 1  | 174.96      | 13.41   | 0.0081  | -         |
| AB                      | 1.21           | 1  | 1.21        | 0.09    | 0.7696  | -         |
| A²                      | 648.43         | 1  | 648.43      | 49.7    | 0.0002  | -         |
| B²                      | 65.57          | 1  | 65.57       | 5.03    | 0.0599  | -         |
| Residual                | 91.34          | 7  | 13.05       | -       | -       | -         |
| Lack of fit             | 71.44          | 3  | 23.81       | 4.79    | 0.0822  | not significant |
| Pure error              | 19.89          | 4  | 4.97        | -       | -       | -         |
| Cor total               | 2,774.86       | 12 | -           | -       | -       | -         |
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Table 10: Observation of drug with excipients compatibility study

| Run no. | Sample name          | Initial                  | 2 weeks 25°C/60% RH | 1-month 25°C/60% RH |
|---------|----------------------|--------------------------|---------------------|---------------------|
| 1       | Nitrofurantoin (NFT) | Yellow-colored powder    | Yellow-colored powder | Yellow-colored powder |
| 2       | NFT + poloxamer 188  | Yellow-colored powder    | Yellow-colored powder | Yellow-colored powder |
| 3       | NFT + glyceryl monostearate | Yellow-colored powder | Yellow-colored powder | Yellow-colored powder |
| 4       | NFT + miglyol 812    | Yellow-colored powder    | Yellow-colored powder | Yellow-colored powder |

Table 11: Results of the preliminary development batches

| S. No. | Evaluation                        | NLC1         | NLC2         |
|--------|-----------------------------------|--------------|--------------|
| 1      | Mean particle size (nm)           | 302 ± 16     | 229 ± 11     |
| 2      | Polydispersity index              | 0.23 ± 0.03  | 0.16 ± 0.01  |
| 3      | Zeta potential (-mV)              | -31.1 ± 3.8  | -35.4 ± 2.5  |
| 4      | % entrapment efficiency           | -            | 83.3 ± 1.6   |
| 5      | Assay                             | -            | 89.8 ± 1.2   |

Table 12: Effect of formulation variables on evaluation parameters

| S. No. | Evaluation                        | NLC3         | NLC4         | NLC5         | NLC6         | NLC7         |
|--------|-----------------------------------|--------------|--------------|--------------|--------------|--------------|
| 1      | Mean particle size (nm)           | 335 ± 19     | 218 ± 13     | 193 ± 12     | 215 ± 25     | 222 ± 29     |
| 2      | PDI (polydispersity index)        | 0.12 ± 0.01  | 0.11 ± 0.01  | 0.14 ± 0.02  | 0.72 ± 0.07  | 0.95 ± 0.09  |
| 3      | Zeta potential (-mV)              | -27.3 ± 2.3  | -34.1 ± 1.8  | -29.4 ± 1.3  | -15.3 ± 4.2  | -17.7 ± 3.3  |
| 4      | % entrapment efficiency           | 88.1 ± 0.9   | 87.4 ± 1.1   | 89.2 ± 1.1   | 59.8 ± 5.6   | 41.2 ± 5.1   |
| 5      | Assay                             | 89.4 ± 1.3   | 94.1 ± 1.6   | 92.1 ± 1.2   | 84.6 ± 1.5   | 93.1 ± 0.8   |

Fig. 4a: IR of drug (nitrofurantoin)

Fig. 4b: IR of drug with excipients (overlay)

After 1-month's observation, it was found that there was no change in the physical characters in the mixture of the drug with excipients.

Fig. 4a is the IR of nitrofurantoin plain drug. It shows principal peak at 1,729, 1,519, 1,427, 1,337, 1,109, and 1016 cm\(^{-1}\). Fig. 4b is the IR spectrum of mixture of drug with glyceryl monostearate, poloxamer 188, and miglyol 812. It was found that there was no alteration in the identical peaks of figure-print region 1729, 1519, 1427, 1337, 1109, and 1016. After 1-month of accelerated study, it was found that there was no significant change in the appearance of the binary mixture and its molecular structure. Hence, it reveals that selected excipients are compatible with drugs.

Selection of Preliminary Development Batches for Process Parameter and Compositions

Preliminary Development Batches for Process Feasibility

Preliminary batches were manufactured as per the composition and process parameter mentioned in Table 1. Results are mentioned in Table 11.

Batch no. NLC1 is placebo batch, which was manufactured to check the process feasibility and measurement of zeta potential and mean particle size. Even though mean particle size is around 300 nm, the process seems to be satisfactory to move ahead with the batch containing nitrofurantoin.

Batch no. NLC2 is active batch (batch containing API), which was manufactured to check the process feasibility, determination of mean particle size, zeta potential, and analytical method feasibility. % EE and assay of the formulation seems to be acceptable. Therefore, further batches were planned to optimize the formulation variable.

Evaluation of Formulation Variables

Ideal NLC should have narrow particle size distribution with small particle size. Higher EE and PDI < 1% depends on the process parameters and formulation composition. In this study concentration of lipid (miglyol 812) was decreased from 60 to 20%, and surfactant (poloxamer188) was increased from 30 to 70%. Composition of formulation and process parameter are as mentioned in Table 2.
Influences of the concentration of lipid on NLCs were evaluated.

The effects of the concentration of the lipid on the particle size and % entrapment efficiency are illustrated in Fig. 5.

The % lipid content affects viscosity of system and size distribution of the NLC. Wide difference among particle size of NLC leads to issue of content uniformity; also, it is responsible for the release of drug from the formulation. Increasing % lipid content resulted in more uniform distribution of particle size (decrease in the PDI). % EE was increased from 41.2 ± 5.1% to 88.1 ± 0.9% by increasing lipid concentration from 20 to 60% and decrease in surfactant. Formulation NLC3, NLC4, and NLC5 has % EE around 85 to 90, and for formulation NLC6 and NLC7 were 59.8 ± 5.6 and 41.2 ± 5.1%, respectively, as shown in Table 12. PDI of formulation NLC3, NLC4, and NLC5 is around 0.11 to 0.14, which indicates very narrow particle size distribution as shown in Fig. 6. Formulation NLC3 has the particle size of 335 ± 19 nm, and NLC4 has 218 ± 13 nm.

**Evaluation of Process Variables**

Composition of batch no. NLC5 and process parameters are as mentioned in Table 3. Outcome of the optimized batches are mentioned in Table 13.

Improper mixing of the aqueous and lipid phase separation was observed in formulation NLC8 and NLC9 due to low rpm and time of the high-speed homogenization. NLCs of less than 200 nm particle size with PDI < 1 were achieved by optimizing the process parameter as shown in Fig. 7, which indicates narrow particle size distribution. By keeping fix time and increasing in speed formulation no. NLC10 gave particle size of 173 ± 19 nm. Increase in speed of homogenization cycle resulted in decrease in particle size due to increase of applied shear forces. Time was increase by keeping rpm 10,000. Formulation NLC11 and NLC12 show NLC12 having particle size of 192 ± 7 nm with a PDI of 0.09 ± 0.01 as shown in Fig. 8, and zeta potential was about −37.3 ± 0.14 mV. So, batch no. NLC12 was taken for further process.

**Evaluation of Freeze Drying**

To enhance the stability of the formulation, freeze-drying is an essential process. Appearance and the water content of the formulated cake were the key evaluation parameter. The final diluted NLC dispersion of batch no. NLC12 was divided into batch no. NLC12A, NLC12B, and NLC12C, equally. These were kept for lyophilization process results are mentioned in Table 14.

Batch no. NLC12A shows the higher water content due to insufficient cycle time of 12 hours. Batch no. NLC12B and
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Table 14: Results of the freeze-drying process

| S. No. | Evaluation                  | NLC12A | NLC12B | NLC12C |
|--------|-----------------------------|--------|--------|--------|
| 1      | Mean particle size (nm)     | -      | 237 ± 7| 225 ± 9|
| 2      | PDI (polydispersity index)  | -      | 0.11 ± 0.01| 0.09 ± 0.01|
| 3      | Zeta potential (-mV)        | -      | -35.4 ± 0.14| -29.7 ± 1.8|
| 4      | % entrapment efficiency     | -      | 87.5 ± 1.1| 87.3 ± 1.5|
| 5      | Assay                       | -      | 89.1 ± 0.5| 90.2 ± 0.5|
| 6      | Water content               | 11.2 ± 0.3| 2.7 ± 0.3| 2.6 ± 0.2|

Table 15: Stability data of the batch no. NLC12B [capsules containing NLCs in high-density polyethylene (HDPE) bottle]

| S. No. | Evaluation parameter         | Target specification | Initial | 1M at 25°C/60% RH | 2M at 2–8°C | 3M at 2–8°C | 6M at 2–8°C |
|--------|------------------------------|----------------------|---------|-------------------|-------------|-------------|-------------|
| 1      | Description                  | Capsule containing yellow color powder | Complies | Sticky powder | Complies | Complies | Complies |
| 2      | Mean particle size (nm)      | NMT 350.0 nm         | 237 ± 7 | -     | 229 ± 5 | 241 ± 8 | 235 ± 10 | 243 ± 5 |
| 3      | Polydispersity index         | NMT 1.0              | 0.11 ± 0.01 | -  | 0.12 ± 0.02 | 0.11 ± 0.01 | 0.13 ± 0.03 | 0.14 ± 0.05 |
| 4      | Zeta potential (-mV)         | NMT -20.0            | -35.4 ± 0.14 | -     | -35.3 ± 0.08 | -38.2 ± 0.11 | -34.9 ± 0.12 | -36.1 ± 0.13 |
| 5      | % entrapment efficiency      | NLT 80.0             | 87.5 ± 1.1 | -     | 91.7 ± 0.7 | 90.8 ± 1.2 | 88.2 ± 1.5 | 90.0 ± 1.3 |
| 6      | % assay                      | NLT 85.0             | 89.1 ± 0.5 | -     | 90.3 ± 0.5 | 91.7 ± 0.8 | 89.2 ± 0.7 | 91.1 ± 1.1 |

NLC12C are acceptable. There is no significance difference between batch no. NLC12B and NLC12C. Moreover, in vitro drug release study to optimize formulation (Fig. 1) shows that batch no. NLC12B gave desirable drug release for 12 hours. Hence, the formulation of batch no. NLC12B was characterized by TEM, XRPD study, stability study, and its drug release profile.

Characterization of NLCs

From the above evaluation parameters formulation no. NLC12B was optimized and characterized by TEM, XRPD, stability study, and in vitro drug release for stability.

TEM and XRPD Study

The TEM image (Fig. 9) of the NFT loaded NLCs indicates that NLCs are not aggregated, having spherical feature with better uniformity and there is no remarkable change in the sphericity. It also shows that there is no change in surface morphology and size during stability study.

The XRPD (Fig. 10) shows intense peaks of nitrofurantoin, which is crystalline in nature. Efficient miscibility of NFT in lipids and amorphous form are characterized by the absence of intense and characteristic peaks of NFT in NLCs. This further shows molecular level dispersion of drug in lipid matrix after NLCs formation, which turn in the crystalline reduction of drug and lipids. Also, there is no significant change upon the stability.

Stability Study of Optimized Batch (Batch No. NLC12B)

From above evaluation parameters formulation no. NLC12B was selected for further characterization by stability study. In order to determine the change in physicochemical parameters and in vitro release on storage, a stability study was carried out. Capsule
containing freeze-dried nanostructured lipid carriers of optimized batch no. NLC12B were filled in capsule and packed in sealed bottles with proper labeling and evaluated for stability study for 6 months at 2 to 8°C and 25°C/60% RH.

It was found that NLCs kept at 25°C/60% RH were sticky so not taken for further study. Stability study of NFT loaded NLCs at 2 to 8°C was continued and tested for mean particle size (nm), polydispersity index, zeta potential, % EE, and % assay was carried out during initial, 1, 2, 3, and 6 months. Stability data are mentioned in Table 15. From this study, it was found that NLC12B showed no significant change in mean particle size, as well as, drug release profile after 6 months. Hence, it reveals that the formulation was stable under the required storage conditions of 2 to 8°C for 6 months.

**In vitro Drug Release Characterization of Stability Batch No. NLC12B**

Capsule containing NLC were evaluated for drug release profile. Characterization of stability batch no. NLC12B was done by in vitro drug release in phosphate buffer (pH 7.2) at 50 rpm for 12 hours. Drug release in 0.1 N HCl after 2 hours was not more than (NMT) 5% and around 90% at the end in phosphate buffer as mentioned in Fig. 11.

From in vitro drug release at defined time interval, it was found that homogenous dispersion of lipid matrix of NFT loaded NLCs gave slow drug release. There was no significant change in drug release profile after 6 months of stability as mentioned in Fig. 11. This indicates that freeze-dried nanostructured lipid carriers remained stable up to 6 months at 2 to 8°C.

**Conclusion**

In the current study, NLCs of nitrofurantoin were successfully formulated by high shear homogenization process. NLCs were optimized for different composition and process parameters and were successfully evaluated. The desired quality target product was achieved by optimized formulation. The good entrapment efficiency was observed for drug due to complete miscibility of NFT with surfactant and lipids. Increase in GI uptake of NFT-NLC reveals improvement in bioavailability. After 6 months stability data of 2 to 8°C conditions shows that NLCs of nitrofurantoin reveals that there is no remarkable change in the optimized formulation. These storage conditions of NLCs were found appropriate for drug delivery. This research opens the door for cost-effective NLCs of nitrofurantoin at the commercial level.

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