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Research Article

Formulation, Optimization, and Characterization of Nanostructured Lipid Carrier of Nitrofurantoin

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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. Glyceryl 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.

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.[1,2] Nanostructured lipid carriers (NLCs) gaining attention in the recent trends of latest generation of lipid nanoparticles.[3,4]

Nitrofurantoin derived from furan by the addition of a hydantoin side chain and nitro group. It is a weak acid and pH will affect its solubility. It is mostly absorbed in the proximal small bowel, and it is well absorbed from the gastrointestinal tract.[5] Nitrofurantoin reduced to reactive intermediates by bacterial flavoproteins, which inactivate or alter bacterial ribosomal proteins and other macromolecules. This inhibits protein synthesis, DNA synthesis, RNA synthesis, and cell wall synthesis. Nitrofurantoin is the drug of choice for urinary tract infection (UTI), but it is having short biological half-life (0.3 to 1-hour) so it required multiple dosing in a day.[6] So, the development of novel formulation with decreasing in particle size, increases absorption of drug from gastrointestinal tract (GI)-tract and its bioavailability is important in the clinical use of the drug. Particle size also affects bioavailability. Both absorption and excretion of drug from urine is very slow for macrocrystalline form than the microcrystalline.[7]

The purpose of the present study was to find out the feasibility of preparing NLCs loading NFT by high shear hot homogenization technique.[8] The fourier transform

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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.[9]

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)[10]

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.[11,12] 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.[13,14]

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.

Table 1: Composition and process parameter for preliminary development batches

Batch No.		*NLC1*	*NLC2*
S. No.	*Ingredients*	*% w/w (solid content)*	
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

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)[14] concentration from 60 to 20%. Process parameters of high-speed homogenization were kept constant at 10,000 rpm for 10 minutes.[15] 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.[15,16] 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.[17]

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.[18] 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

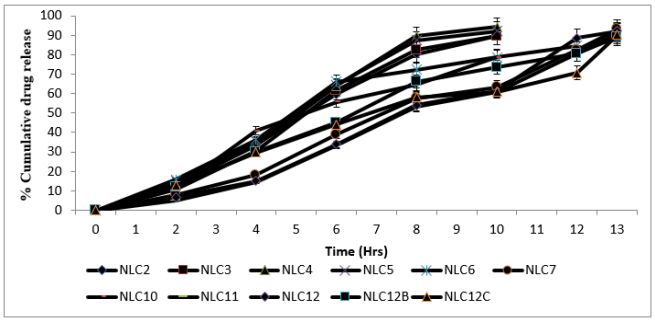


Fig. 1: *In vitro* drug release study to optimize formulation

Table 2: Composition and process parameter for formulation optimization batches

Batch No.		*NLC3*	*NLC4*	*NLC5*	*NLC6*	*NLC7*
S. No.	*Ingredients*	*% w/w (solid content)*				
1	Nitrofurantoin	1.2	1.2	1.2	1.2	1.2
2	Glyceryl 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
S. No.	*Parameter*	*NLC3*	*NLC4*	*NLC5*	*NLC6*	*NLC7*
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

Table 3: Composition and process parameter for process optimization batches

		Reference formula of batch no. NLC5				
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 aq. and lipid phase	2,500 rpm 2 min	5,000 rpm 2 min	10,000 rpm 2 min	10,000 rpm 5 min	10,000 rpm 10 min
3	Centrifugation (rpm 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.[19] Statistical validity of the polynomials was established on the basis of ANOVA. Level of significance was considered at $p < 0.05$.[19] 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 R^2 of 0.1868 is not as close to the adjusted R^2 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 R^2 of 0.7928 is in reasonable agreement with the adjusted R^2 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 is 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, A^2 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

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

Table 5: Optimization by response surface methodology

		Factor1	*Factor2*	*Response1*	*Response2*
Standard	*Run*	*Miglyol 812 (% w/w)*	*Poloxamer 188 (% w/w)*	*Mean particle size (nm)*	*% entrapment efficiency*
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	R^2	0.8953	Std. Dev.	3.61	R^2	0.9671
Mean	214.15	Adjusted R^2	0.8205	Mean	79.2	Adjusted R^2	0.9436
CV%	4.18	Predicted R^2	0.1868	CV%	4.56	Predicted R^2	0.7928
	Adeq precision		8.584		Adeq precision		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, A^2 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]:

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

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.

$$\text{Assay} = \frac{(\text{Amount of NFT in NLCs} + \text{Amount of NFT in supernatant after centrifugation})}{\text{Total weight of NFT}} \times 100$$

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.

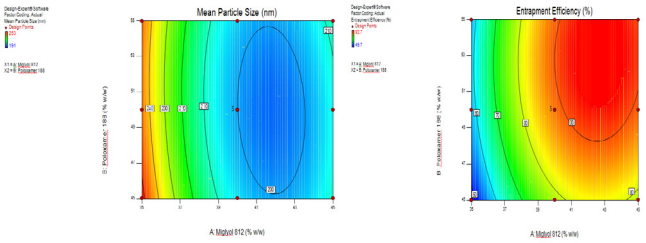
Characterization of NLCs

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.



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

Fig. 2: Counter plot for mean particle size

Table 7: Design summary

File version	12.0.8.0	*Design type*		3-level factorial					
Study type	Response surface	*Design model*		Quadratic		*Runs*	13	*Build time (ms)*	3.0
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]

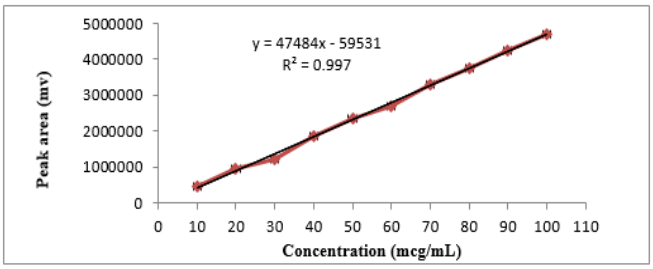


Fig. 3: Linearity curve of nitrofurantoin

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

Mean particle size						
ANOVA for response surface quadratic model						
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	-
A^2	1,977.59	1	1,977.59	24.7	0.0016	-
B^2	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

% entrapment efficiency						
ANOVA for response surface quadratic model						
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	-
A^2	648.43	1	648.43	49.7	0.0002	-
B^2	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	-	-	-	-

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

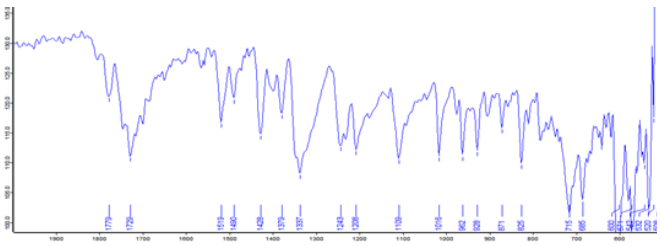


Fig. 4a: IR of drug (nitrofurantoin)

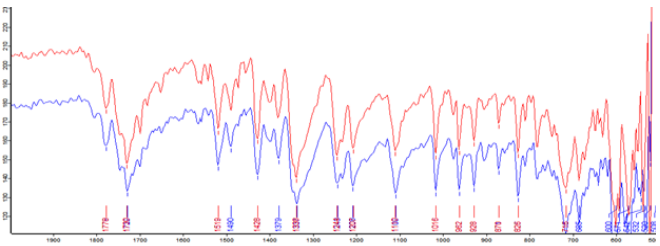


Fig. 4b: IR of drug with excipients (overlay)

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, 1337, 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.

Table 13: Results of the process variables on evaluation parameters

S. No.	*Evaluation*	*NLC8*	*NLC9*	*NLC10*	*NLC11*	*NLC12*
1	Mean particle size (nm)			173 ± 19	179 ± 12	192 ± 7
2	PDI (polydispersity index)			1.21 ± 0.08	0.49 ± 0.03	0.09 ± 0.01
3	Zeta potential (-mV)	Phase separation Observed		-29.2 ± 2.1	-26.7 ± 1.9	-37.3 ± 0.14
4	% EE (entrapment efficiency)			51.9 ± 3.1	71.3 ± 1.7	89.8 ± 0.6
5	Assay			90.8 ± 1.2	91.2 ± 0.5	92.0 ± 0.8

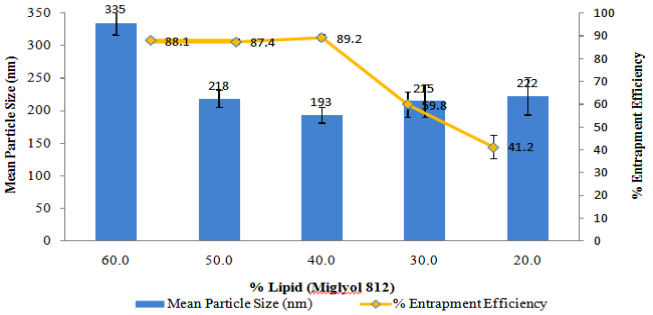


Fig. 5: Effect of the lipid concentration on particle size and % entrapment efficiency

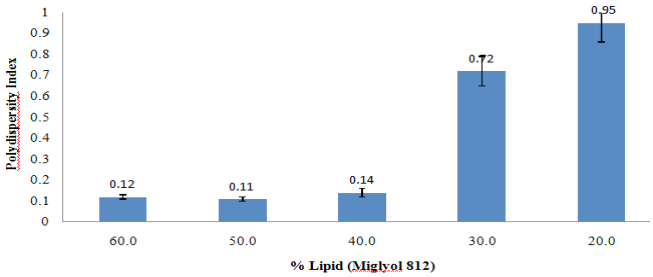


Fig. 6: Effect of lipid concentration on PDI

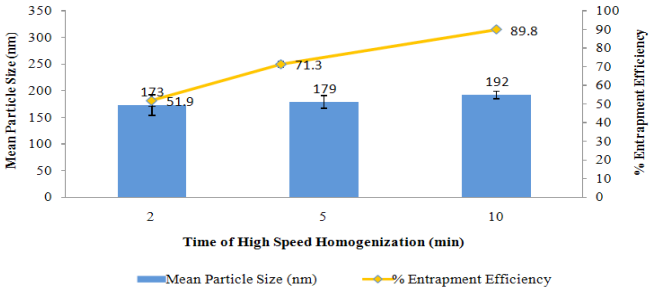


Fig. 7: Effect of high-speed homogenization time on entrapment efficiency and mean particle size

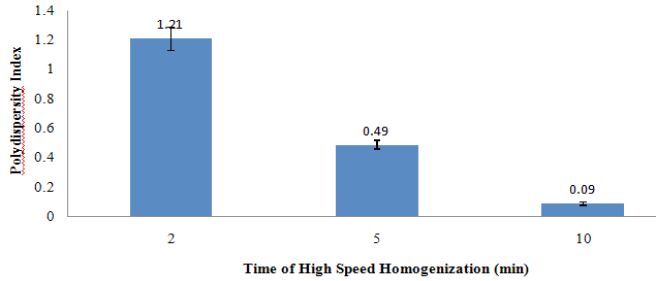


Fig. 8: Effect of high speed homogenization time on PDI

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

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]

			Condition					
S. No.	*Evaluation parameter*	*Target specification*	*Initial*	*1M at 25°C/ 60% RH*	*1M at 2–8°C*	*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	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

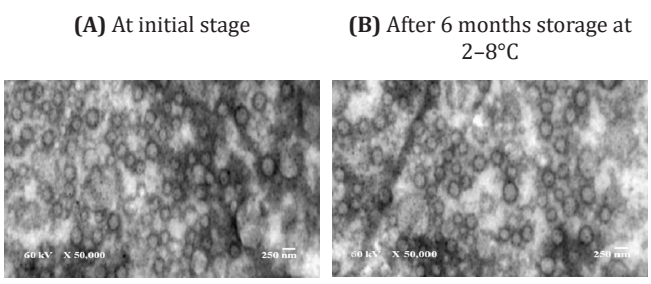


Fig. 9: TEM evaluation of the NLC

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.

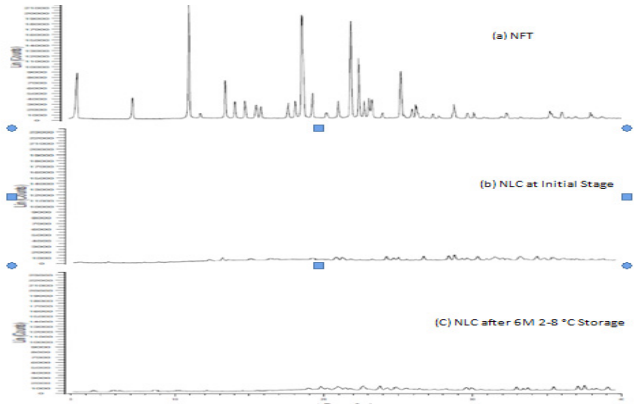


Fig. 10: XRPD spectra of (a) nitrofurantoin; (b) NLC at initial stage; (c) NLC

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

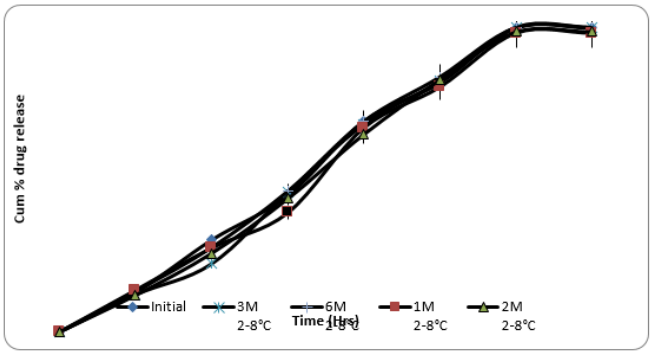


Fig. 11: *In vitro* characterization of stability batch no. NLC12B

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 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 NHCl 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 hot 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.

References

1. Praveen S, Gowda D, Srivastava A. Formulation and Evaluation of Nanostructured Lipid Carrier (NLC) For Glimepiride. Der Pharmacia lettre. 2016;8(7):251-256.
2. Fang Y-P, Lin Y-K, Su Y-H, Fang J-Y. Tryptanthrin-loaded nanoparticles for delivery into cultured human breast cancer cells, MCF7: The effects of solid lipid/liquid lipid ratios in the inner core. Chemical and Pharmaceutical Bulletin. 2011;59(2):266-271.
3. Sethuraman N, Shanmuganathan S, Anbarasan B. Design, development and characterization of nano structured lipid carrier for topical delivery of aceclofena. Indian Journal of Pharmaceutical Education and Research. 2018;52(4):581-586.
4. Souza G, Silva J, Martins L. Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. Eur. J Pharm Biopharm. 2011;79:189-196.
5. Gleckman R, Alvarez S. Drug therapy reviews nitrofurantoin. Am. J Hosp Pharm. 1979;36(3):342351.
6. Ayush G, Anshu S, Indrajeet S. Preparation and evaluation of sustained release film coated tablets of nitrofurantoin. Int. J. of Res. in Pharmacy and Pharmaceutical Sci. 2016;1(1):47-53.
7. Nerella A, Basava R, Aruna D. Formulation, Optimization and *in vitro* characterization of letrozole loaded solid lipid nanoparticles. International Journal of Pharmaceutical Sciences and Drug Research. 2014;6(3):183-188.
8. Naglakshmi S, Shanmuganathan S, Sandhya K, Anbarasan B. Design, development and characterization of nanostructured lipid carrier for of aceclofenac. Ind. J. of Pharmaceutical Edu. and Res. 2018;52(4):581-586.
9. Parveen S, Sahoo K. Polymeric nanoparticles for cancer therapy. J Drug Target. 2008;16:108-123.
10. Pawar RK, Lokhande R, Yadav R, Velankar S. A new HPLC assay method for determination and quantitation of nitrofurantoin. International Journal of Chemical and Pharmaceutical Analysis. 2016;4(1):1-7.
11. Gaur PK, Mishra S, Bajpai M, Mishra A. Enhanced oral bioavailability of efavirenz by solid lipid nanoparticles: *In vitro* drug release and pharmacokinetics studies. BioMed Research International Volume. 2014;ID 363404:9.
12. Poonia N, Kharb R, Lather V, Pandita D. Nanostructured lipid carriers: Versatile oral delivery vehicle. Future science OA. 2016;2(3):1-24.
13. Khosaa A, Reddia S, Saha R. Nanostructured lipid carriers for site-specific drug delivery. Biomedicine and Pharmacotherapy. 2018;108:598-613.
14. Patil D, Pattewar S, Sarvesh P, Gargi P, Sharma S. Nanostructured lipid carriers: A platform to lipophilic drug for oral bioavailability enhancement. Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):758-764.
15. Yiyin Chen, Xiaomin Yang, Lin Zhao, Vasil M, Aihua Zou. Preparation and characterization of a nanostructured lipid carrier fora poorly soluble drug. Colloids and Surfaces A: Physicochem. Eng. Aspects. 2014;455:36-43.
16. Dandagi PM, Gadad AP, Tigadi SG, Bolmal UB. Rosuvastatin loaded nanostructured lipid carrier: for enhancement of oral bioavailability. Indian Journal of Pharmaceutical Education and Research. 2016;50(4):605-611.