RESEARCH ARTICLE

Diagnostic Analysis and Graphical Optimization of Fenoprofen Calcium-loaded Nanostructured Lipid Carriers using Design of Experiments

Ishrat Zahoor[1], Neelam Sharma[2], Tapan Behl[3], Sukhbir Singh[2*]

[1]*Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab, India*
[2]*Department of Pharmaceutics, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India,*
[3]*School of Health Sciences and Technology, University of Petroleum and Energy Studies, Dehradun, Uttarakhand, India*

Received: 30th June, 2022; Revised: 02nd August, 2022; Accepted: 29th August, 2022; Available Online: 25th September, 2022

ABSTRACT

The present research aims to investigate optimized parameters for the formulation of Fenoprofen calcium-loaded nanostructured lipid carriers (FEN-NLC) by emulsion evaporation-solidification at low temperature using Compritol 888 as solid lipid and oleic acid as liquid lipid. Box-Behnken design was utilized for optimization of formulation and process variables like solid lipid: liquid lipid (X1), stirring speed (rpm) (X2), and emulsifier concentration (% v/v) (X3) using Design-Expert software. The Pareto chart demonstrated that X1 and X2 produced significant synergistic effects. At the same time, X3 had a significant antagonistic effect on percentage entrapment efficiency (Y1), while X1, X2, and X3 exerted significant synergistic influence on flux (µg/cm^2/hr) (Y2) and percentage yield (Y3) (*$p < 0.05$). The model fit summary statistical analysis revealed that the quadratic model was the best model for Y1-Y3 as this model showed significant sequential p-value (*$p < 0.05$), insignificant lack of fit p-value ($p > 0.05$), least standard deviation and maximum R^2 value for Y1-Y3. Diagnostic analysis of response parameter values authenticated the competence and reliability of their actual and predicted values as the residual values were exceptionally fitted within the limit of ± 4. Conclusively, Design-Expert software generated an optimization ramp report to explore optimized FEN-NLC with a desirability function of 0.874. The optimization ramps revealed that the coded values of optimized FEN-NLC were 0.561, 0.999, and 0.999 for X1, X2, and X3, respectively, with predicted values of 72.98%, 136.19 µg/cm^2/hr and 92.889% for Y1, Y2 and Y3, respectively.

Keywords: Desirability function, Fenoprofen calcium, Nanostructured lipid carriers, Optimization ramp, Pareto chart.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.3.03

How to cite this article: Zahoor I, Sharma N, Behl T, Singh S. Diagnostic Analysis and Graphical Optimization of Fenoprofen Calcium-loaded Nanostructured Lipid Carriers using Design of Experiments. International Journal of Pharmaceutical Quality Assurance. 2022;13(3):240-246.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Fenoprofen calcium (FEN) is a non-steroidal, anti-inflammatory, analgesic, and antipyretic agent having IUPAC name calcium 2-(3-phenoxy phenyl) propanoate dihydrate. FEN is generally prescribed in osteoarthritis and rheumatoid arthritis-related pain, inflammation, and stiffness.[1-3] The recommended adult dosage of FEN is 200 mg every 4–6 hours. FEN is generally well-tolerated but can cause a few side effects like headache, gastrointestinal upset, nausea, dizziness, diarrhea, abdominal discomfort, and hypersensitivity reactions. Fenoprofen is extensively metabolized by the liver.[4,5] Nanostructured lipid carriers (NLCs) are a new generation of lipid nano-sized systems with considerable potential as drug delivery systems for providing drug protection, dose reduction, increased bioavailability and prolonged drug circulation. In recent years, lipid-based drug delivery systems have emerged as up-and-coming nanotechnology, which is primarily attributable to specific characteristics of lipid materials like biocompatibility with the biological surface, lipophilicity, and enormous potential to penetrate through physiological barriers.[6,7] NLCs are the second generation of lipid nanoparticles and can facilitate the incorporation of liquid lipids into solid lipid matrices. This creates a void inside their crystal lattice; consequently, the drug molecule accommodates better inside the NLCs. In addition, this strategy tends to improve the efficiency of drug encapsulation, provide higher drug loading capacity, reduce the drug lost during the storage period and mitigate the disadvantage of drug expulsion from the lipid phase.[8-10] These nanocarriers can be used to deliver drugs that are both lipophilic and hydrophilic. NLCs have become considered as a viable carrier system for the delivery of drugs by oral, ocular, topical, pulmonary, parenteral, and transdermal

*Author for Correspondence: singh.sukhbir12@gmail.com

routes. NLCs have nanometer-sized particles that enable them to touch intimately with the skin tissues and increase drug penetration through the skin.[11-13] Most lipids used as excipients in the manufacturing of NLCs have been considered biocompatible to the skin. Solid lipids are responsible for sustained drug release from NLCs, and additionally, lipids generate a film with occlusive properties that help keep moisture in the skin for longer on topical application.[14,15]

This research synthesized Fenoprofen calcium-loaded nanostructured lipid carriers (FEN-NLCs) through emulsion evaporation-solidification at low temperatures.[16] The compritol 888 was used as solid lipid, while oleic acid was used as liquid lipid during NLCs manufacturing. Box-Behnken design was applied as design of an experimentation tool for formulation optimization. The present research's objective was to investigate the effect of formulation and process parameters on percentage entrapment efficiency, flux ($\mu g/cm^2/hr$), and percentage yield of FEN-NLC. The statistical analysis of response parameters was executed to investigate the best fit model. The diagnostic analysis was executed in order to verify the chosen model's reliability and finally optimization formulation of FEN-NLC was explored by Design-Expert software.

MATERIALS AND METHODS

Materials

Fenoprofen Calcium was purchased from Suven Pharmaceuticals Limited, Hyderabad, Telangana. Sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), tween 80, oleic acid, Brij 30, and ethanol were purchased from Loba Chemicals Private Limited, Mumbai, Compritol 888 was purchased from Gattefosse SAS, France. All the chemicals utilized were of analytical grade.

Production of Fenoprofen Calcium-loaded Nanostructured Lipid Carriers

A total of seventeen formulations of FEN-NLC were manufactured as per the 3-factor 3-level Box-Behnken design layout as generated by Design-Expert software (Stat-Ease Inc., MN).17-19 The formulation and process variables (X1, X2, and X3) investigated during nanostructured lipid carriers manufacturing are mentioned in Table 1. The FEN-NLCs were developed by emulsion evaporation-solidification at low-temperature technique.[16,20-22]

Evaluation of Fenoprofen Calcium-loaded nanostructured lipid carriers

Percentage entrapment efficiency (Y1)

Accurately weighed amount of for FEN-NLCs was dissolved in phosphate buffer (pH, 6.8) for 24 hours and centrifuged at 3500 rpm for ten minutes. The supernatant was collected and estimated for drug concentration using UV spectrophotometer at 270 nm. Each measurement was performed in triplicate.[23-25] The percentage entrapment efficiency of FEN-NLC was determined using the following equation.

$$\%\ \text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Free drug in supernatant}}{\text{Total amount of drug}} \times 100$$

Table 1: The levels of formulation and process variables used during the manufacturing of Fenoprofen calcium-loaded nanostructured lipid carriers

Factors	Low (-1)	Medium (0)	High (+1)
Solid lipid: Liquid lipid (X1)	75:25	80:20	85:15
Stirring speed (rpm) (X2)	1500	2000	2500
Emulsifier Concentration (% v/v) (X3)	1.5	2	2.5

Flux (Y2)

A dialysis membrane with 12000-14000 Dalton molecular weight and surface area of 2.54 cm^2 was placed between acceptor and donor compartments of Franz diffusion cell kept at constant at temperature of 37 ± 0.5°C. The acceptor compartment was filled with 20 mL phosphate buffer (pH, 6.8) and continuously stirred at 200 rpm speed using a magnetic bar to decrease stagnant surfaces. The dispersion of FEN-NLCs was placed in the donor compartment. About 0.5 mL of samples were collected at intervals of 0.5, 1, 2, 4, 6, 8, 12, and 24 hours from the acceptor compartment, replenished with an equal volume of buffer, and subsequently, samples were analyzed using a UV spectrophotometer at 270 nm. The total quantity (µg) of the permeated drug was calculated by multiplying the drug concentration (µg/mL) in the buffer by the volume (mL) of the buffer in the receptor chamber. The slope of the linear part of the curve between the quantities of drug permeated per unit area ($\mu g/cm^2$) versus time (hour) represented flux value ($\mu g/cm^2/h$).[26,27]

Percentage yield (Y3)

The quantity of for FEN-NLCs obtained for formulation batches were precisely weighed individually. The % yield (w/w) of NLCs was determined by the following equation.[28,29]

$$\%\text{Yield} = \frac{\text{Actual weight of recovered nanostructured lipid carrier}}{\text{Weight of drug} + \text{weight of solid lipid} + \text{weight of liquid lipid}} \times 100$$

Pareto Chart Analysis of Response Variables (Y1-Y3) for FEN-NLCs

The effect of solid lipid: liquid lipid (X1), stirring speed (rpm) (X2), and emulsifier concentration (% v/v) (X3) on percentage entrapment efficiency (Y1), flux (Y2) and percentage yield (Y3) of for FEN-NLCs was portrayed in the form of Pareto chart. The bar length in the plot represents the value of the regression coefficient for X1, X2, and X3, depicted as b1, b2, and b3, respectively. The factor which significantly affected the response parameters has been marked with an asterisk ($*p < 0.05$).[30]

Fit Summary and Model Summary Statistical Analysis

Fit summary statistical analysis was applied to the values of response parameters (Y1-Y3) for FEN-NLCs to reveal the values of sequential p-value, lack of fit p-value, adjusted R^2 and predicted R^2 for linear, two-factor interaction (2-FI) and quadratic models. The model summary statistical study was also executed for percentage entrapment efficiency (Y1), flux

Table 2: Fit summary and model summary statistics data for response parameters of FEN-NLC

Source	Sequential *p*-value	Lack of Fit *p*-value	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS
Percentage entrapment efficiency (Y1)							
Linear	< 0.0001	0.0138	1.36	0.9758	0.9702	0.9538	45.74
2FI	0.7066	0.0090	1.45	0.9788	0.9661	0.9097	89.39
Quadratic	0.0048	0.0890	0.7239	0.9963	0.9915	0.9528	46.66
Flux (Y2)							
Linear	< 0.0001	0.0042	3.89	0.9415	0.9280	0.8788	408.49
2FI	0.0280	0.0114	2.87	0.9755	0.9608	0.8793	406.55
Quadratic	0.0143	0.0609	1.68	0.9963	0.9865	0.9216	264.29
Percentage yield (Y3)							
Linear	< 0.0001	0.0035	3.68	0.9270	0.9101	0.8548	350.01
2FI	0.9615	0.0018	4.14	0.9290	0.8864	0.6670	802.67
Quadratic	< 0.0001	0.6928	0.7725	0.9983	0.9960	0.9903	23.37

(Y2), and percentage yield (Y3) of FEN-NLCs to disclose standard deviation, R^2-value and predicted residual error sum of squares (PRESS) value.[30-32]

Diagnostic Analysis of Response Variables for FEN-NLCs

The diagnostic plots were generated by Design-Expert software for the values of percentage entrapment efficiency (Y1), flux (Y2), and percentage yield (Y3). Plots were acquired in various combinations of run number, actual value, predicted value, residuals, and externally studentized residuals to examine the value of Y1-Y3 were lying within the specifications in proximity to normal or zero-axis.[30,33,34]

Exploring Optimization Ramps and Graphical Overlay for an Optimized Batch of FEN-NLC

Design-Expert software constructed an optimization ramp report and graphical overlay for investigating an optimized batch of fenoprofen calcium-loaded nanostructured lipid carriers. The optimized composition variables, *i.e.*, solid lipid: liquid lipid (X1) and emulsifier concentration (% v/v) (X3) and process variables, *i.e.*, stirring speed (rpm) (X2) for FEN-NLC manufacturing explored on the set criterion of maximizing percentage entrapment efficiency (Y1), flux (Y2) and percentage yield (Y3).[35,36]

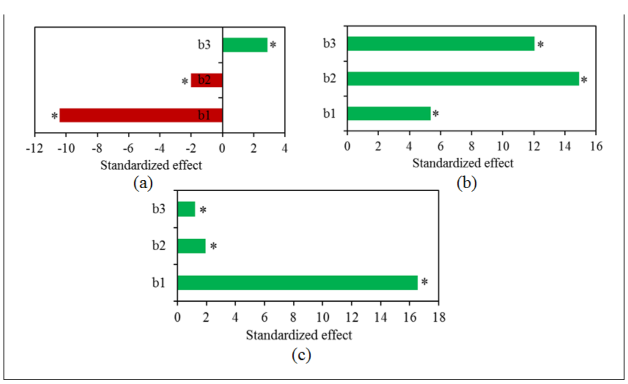

Figure 1: Pareto chart for response parameters (a) % entrapment efficiency, (b) flux and (c) % yield (*$p < 0.05$)

RESULTS AND DISCUSSION

Pareto Chart

The Pareto chart, as shown in Figure 1a, demonstrated that solid lipid: liquid lipid (X1) and stirring speed (rpm) (X2) generated a significant (*$p < 0.05$) synergistic effect (green color bars). In comparison, emulsifier concentration (% v/v) (X3) produced significant (*$p < 0.05$) antagonistic effect (red color bars) on percentage entrapment efficiency (Y1). Figure 1b and Figure 1c revealed that solid lipid: liquid lipid (X1), stirring speed (rpm) (X2) and emulsifier concentration (% v/v) (X3) produced significant synergistic influence (*$p < 0.05$) on flux (Y2) and percentage yield (Y3).[26,30,37,38]

Fit Summary and Model Summary Statistical Analysis

The model satisfying the criterion of the significant sequential *p*-value (*$p < 0.05$) and insignificant lack of fit *p*-value ($p > 0.05$) was best fitted in the design model. Therefore, based on values as depicted in Table 2, the quadratic model was chosen as the bets fit model for response parameters, *i.e.*, %entrapment efficiency (Y1), flux (Y2), and percentage yield (Y3). Furthermore, the selected quadratic model should have a minimum standard deviation, the highest R^2, the difference between adjusted R^2, predicted R^2 should be less than 0.2, and a minimum PRESS value estimated by model summary statistics Design-Expert software. The standard deviation values for linear, two-factor interaction (2-FI) and quadratic models for Y1 were 1.36, 1.45 and 0.7239; for Y2 were 3.89, 2.87 and 1.68 and for Y3 were 3.68, 4.14 and 0.7725, respectively. Therefore, the quadratic model was a superlative model for Y1-Y3.30,39-41 Furthermore, the quadratic model had the highest R^2 value among other models (Table 2).

Diagnostic Analysis

The actual value predicted value, residual, and externally studentized residuals for run number 1-17 (FEN-NLC-1 to FEN-NLC-17) for estimated response parameters *i.e.*, % entrapment efficiency (Y1), flux (Y2), and percentage yield (Y3) are depicted in Tables 3-5, respectively. The actual value of Y1, Y2, and Y3 for FEN-NLC, as depicted in Tables 3 to 5, ranged from 62.10–89.97%, 77.28–134.23 µg/cm^2/hr, and 55.67–92.89%, respectively. In normal probability plots, the values of Y1-Y3 were positioned over or in close proximity to the normality line, as indicated by the red line in Figures 2 to 4. The values of residuals and externally studentized residuals for Y1, Y2, and Y3 for FEN-NLC lies within the limit of ±4 as depicted graphically in Figures 2 to 4. The slightest difference between actual and predicted values of Y1-Y3 was observed, as shown in Figures 2 to 4.[30,42]

Exploring Optimization Ramps and Graphical Overlay for an Optimized Batch of FEN-NLC

The desirability function of optimized FEN-NLC was found 0.874. The explored optimized batch of FEN-NLC was comprised of solid lipid: liquid lipid (X1), stirring speed (rpm)

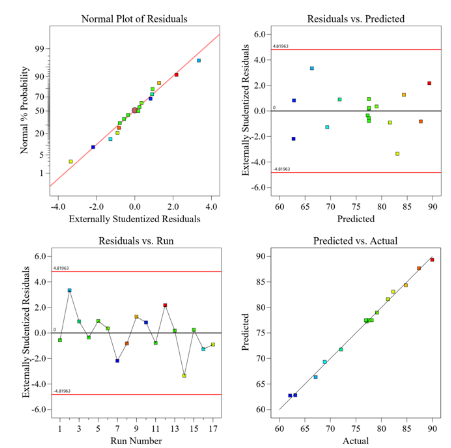

Figure 2: Diagnostic plot for % entrapment efficiency of FEN-NLC

Table 3: Actual value, predicted value, and residuals for percentage entrapment efficiency of FEN-NLC

Run	Actual value	Predicted value	Residual	Externally studentized residuals
	87.36	87.66	-0.3050	-0.823
	67.11	66.34	0.7725	3.344
	82.32	83.09	-0.7725	-3.344
	63.12	62.82	0.3050	0.823
	84.78	84.34	0.4412	1.272
	62.10	62.74	-0.6363	-2.178
	89.97	89.33	0.6363	2.178
	68.89	69.33	-0.4413	-1.272
	77.12	77.26	-0.1363	-0.352
	72.10	71.77	0.3312	0.903
	81.28	81.61	-0.3312	-0.903
	79.14	79.00	0.1362	0.352
	77.63	77.50	0.1280	0.184
	77.12	77.50	-0.3820	-0.560
	76.98	77.50	-0.5220	-0.784
	78.11	77.50	0.6080	0.930
	77.67	77.50	0.1680	0.241

Table 4: Actual value, predicted value, and residuals for flux values for FEN-NLCs

Run	Actual value	Predicted value	Residual	Externally studentized residuals
	86.27	84.40	1.87	3.756
	98.12	98.01	0.1137	0.125
	116.98	117.09	-0.1138	-0.125
	123.12	124.99	-1.87	-3.756
	87.10	87.46	-0.3550	-0.395
	98.21	96.81	1.40	1.973
	108.76	110.16	-1.40	-1.973
	122.65	122.30	0.3550	0.395
	77.28	78.79	-1.51	-2.261
	98.87	98.40	0.4687	0.527
	92.19	92.66	-0.4688	-0.527
	134.23	132.72	1.51	2.261
	103.12	104.15	-1.03	-0.657
	104.24	104.15	0.0880	0.054
	105.27	104.15	1.12	0.716
	103.24	104.15	-0.9120	-0.576
	104.89	104.15	0.7380	0.462

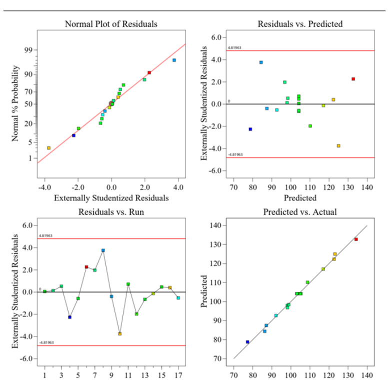

Figure 3: Diagnostic plot for flux values for FEN-NLCs

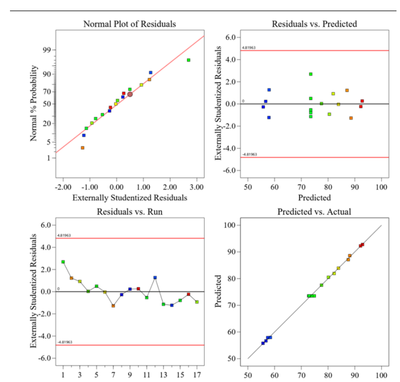

Figure 4: Diagnostic plot for % yield for FEN-NLC

Table 5: Actual value, predicted value, and residuals for % yield for FEN-NLC

Run	Actual value	Predicted value	Residual	Externally studentized residuals
	55.67	55.78	-0.1100	-0.265
	87.56	87.10	0.4575	1.226
	57.43	57.89	-0.4575	-1.226
	92.89	92.78	0.1100	0.265
	56.78	56.68	0.0963	0.232
	88.13	88.60	-0.4712	-1.273
	58.43	57.96	0.4713	1.273
	92.16	92.26	-0.0962	-0.232
	77.56	77.55	0.0138	0.033
	82.29	81.93	0.3613	0.926
	80.14	80.50	-0.3612	-0.926
	83.89	83.90	-0.0137	-0.033
	72.76	73.53	-0.7680	-1.134
	74.88	73.53	1.35	2.691
	73.14	73.53	-0.3880	-0.532
	73.89	73.53	0.3620	0.495
	72.97	73.53	-0.5580	-0.785

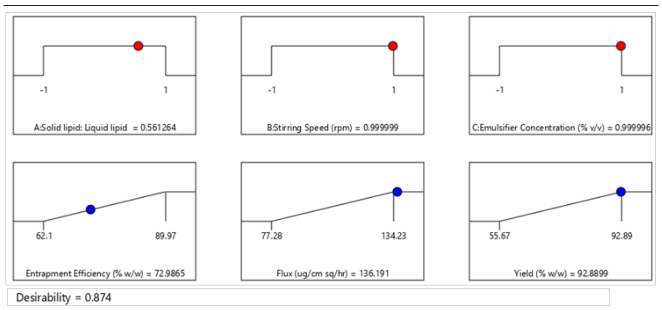

Figure 5: Optimization ramps showing optimum values of formulation and process variables with the value of desirability function predicted by Design-Expert software

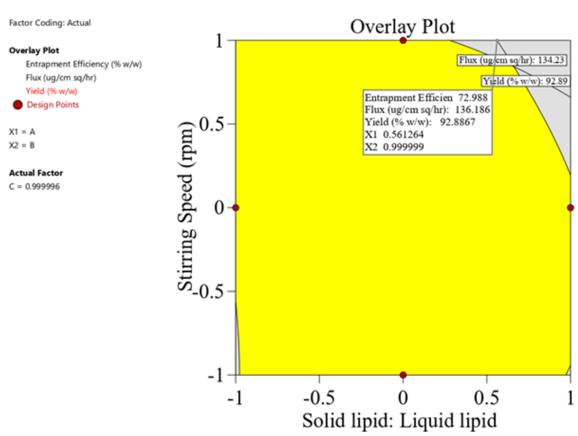

Figure 6: Graphical overlay showing optimum values of independent and response variables predicted by Design-Expert software

(X2), and emulsifier concentration (% v/v) (X3) at coded values of 0.561, 0.999 (~1) and 0.999 (~1), respectively, which indicated that medium level of X1 while a high level of X2 and X3 were required for the formulation of an optimized batch of FEN-NLC. The predicted values of Y1-Y3 for an optimized batch of FEN-NLC with specified values of X1-X3 were 72.98% of entrapment efficiency (Y1), 136.19 μg/cm^2/hr of flux (Y2) and 92.889 % of yield (Y3) (Figures 5 and 6).[35,43,44]

CONCLUSIONS

Fenoprofen calcium-loaded nanostructured lipid carriers (FEN-NLC) were successfully synthesized by emulsion evaporation-solidification at low temperature using Compritol 888 and oleic acid and optimized by Box-Behnken design. Solid lipid: liquid lipid (X1), stirring speed (rpm) (X2), and emulsifier concentration (% v/v) (X3) were studied as an independent variable, and percentage entrapment efficiency (Y1), flux (μg/cm^2/hr) (Y2) and percentage yield (Y3) were response parameters. The quadratic model was found to be the best model for Y1-Y3 with a significant sequential *p*-value ($*p < 0.05$) and insignificant lack of fit *p*-value ($p > 0.05$). Diagnostic analysis of Y1-Y3 authenticated the reliability of actual and predicted values since the values of residuals were less than ±4. In conclusion, the optimized FEN-NLC with a desirability function of 0.874 was explored by Design-Expert software with coded values of 0.561, 0.999, and 0.999 for X1, X2, and X3, respectively, and predicted values of 72.98%, 136.19 μg/cm^2/hr and 92.889% for Y1, Y2 and Y3, respectively.

ACKNOWLEDGEMENTS

The authors wish to thank Chitkara College of Pharmacy, Chitkara University, Punjab, India, for the infrastructural support of this research study. The authors would also like to thank the Department of Pharmaceutics, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India 133207 for providing facilities for the compilation of this research article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Farghaly DA, Aboelwafa AA, Hamza MY, Mohamed MI. Topical delivery of fenoprofen calcium via elastic nano-vesicular spanlastics: Optimization using experimental design and in vivo evaluation. AAPS PharmSciTech 2017; 18:2898-2909.
2. Mohamed ME, Frag EY, Hathoot AA, Shalaby EA. Spectrophotometric determination of fenoprofen calcium drug in pure and pharmaceutical preparations. Spectroscopic characterization of the charge transfer solid complexes. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2018; 189:357-365.
3. Zhu H, Xu J, Varlashkin P, Long S, Kidd C. Dehydration, hydration behavior, and structural analysis of fenoprofen calcium. Journal of Pharmaceutical sciences 2001; 90:845-859.
4. Ammar HO, Makram TS, Mosallam S. Effect of Polymers on the Physicochemical Properties and Biological Performance

of Fenoprofen Calcium Dihydrate-Triacetyl-β-Cyclodextrin Complex. Pharmaceutics 2017; 9:23.
5. Farghaly DA, Aboelwafa AA, Hamza MY, Mohamed MI. Microemulsion for topical delivery of fenoprofen calcium: in vitro and in vivo evaluation. Journal of Liposome Research 2018; 28:126-136.
6. Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). European Journal of Pharmaceutics and Biopharmaceutics 2008; 70:633-640.
7. Qumber M, Alruwaili NK, Bukhari SN, Alharbi KS, Imam SS, Afzal M, Alsuwayt B, Mujtaba A, Ali A. BBD-based development of itraconazole loaded nanostructured lipid carrier for topical delivery: in vitro evaluation and antimicrobial assessment. Journal of Pharmaceutical Innovation 2021; 16:85-98.
8. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Advanced Drug Delivery Reviews 2007; 59:522-530.
9. Salvi VR, Pawar P. Nanostructured lipid carriers (NLC) system: A novel drug targeting carrier. Journal of Drug Delivery Science and Technology 2019; 51:255-2367.
10. Salunkhe SS, Bhatia NM, Pokharkar VB, Thorat JD, Bhatia MS. Topical delivery of Idebenone using nanostructured lipid carriers: evaluations of sun-protection and anti-oxidant effects. Journal of Pharmaceutical Investigation 2013; 43:287-303.
11. Patel D, Dasgupta S, Dey S, Roja Ramani Y, Ray S, Mazumder B. Nanostructured lipid carriers (NLC)-based gel for the topical delivery of aceclofenac: preparation, characterization, and in vivo evaluation. Scientia Pharmaceutica 2012; 80:749-764
12. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. International Journal of Pharmaceutics 2008; 346:124-132
13. Ghate VM, Lewis SA, Prabhu P, Dubey A, Patel N. Nanostructured lipid carriers for the topical delivery of tretinoin. European Journal of Pharmaceutics and Biopharmaceutics 2016; 108:253-261.
14. Agrawal Y, Petkar KC, Sawant KK. Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. International Journal of Pharmaceutics 2010; 401:93-102.
15. Iqbal MA, Md S, Sahni JK, Baboota S, Dang S, Ali J. Nanostructured lipid carriers system: recent advances in drug delivery. Journal of drug targeting 2012; 20:813-830.
16. Chen-yu G, Chun-fen Y, Qi-lu L, Qi T, Yan-wei X, Wei-na L, Guang-Xi Z. Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. International Journal of Pharmaceutics 2012; 430:292-298.
17. Moghddam SM, Ahad A, Aqil M, Imam SS, Sultana Y. Optimization of nanostructured lipid carriers for topical delivery of nimesulide using Box–Behnken design approach. Artificial Cells, Nanomedicine and Biotechnology 2017; 45:617-624.
18. Thapa C, Ahad A, Aqil M, Imam SS, Sultana Y. Formulation and optimization of nanostructured lipid carriers to enhance oral bioavailability of telmisartan using Box-Behnken design. Journal of Drug Delivery Science and Technology 2018; 44:431-439.
19. Jazuli I, Nabi B, Alam T, Baboota S, Ali J. Optimization of nanostructured lipid carriers of lurasidone hydrochloride using Box-Behnken design for brain targeting: in vitro and in vivo studies. Journal of Pharmaceutical Sciences 2019; 108:3082-3090.
20. Chen H, Wang Y, Zhai Y, Zhai G, Wang Z, Liu J. Development of a ropivacaine-loaded nanostructured lipid carrier formulation for transdermal delivery. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2015; 465:130-136.
21. Chen P, Zhang H, Cheng S, Zhai G, Shen C. Development of curcumin loaded nanostructured lipid carrier based thermosensitive in situ gel for dermal delivery. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2016; 506:356-362.
22. Fan X, Chen J, Shen Q. Docetaxel–nicotinamide complex-loaded nanostructured lipid carriers for transdermal delivery. International Journal of Pharmaceutics 2013; 458:296-304.
23. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, Pan YS, Tang X. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. International Journal of Pharmaceutics 2010; 394:179-185.
24. Pezeshki A, Ghanbarzadeh B, Mohammadi M, Fathollahi I, Hamishehkar H. Encapsulation of vitamin A palmitate in nanostructured lipid carrier (NLC)-effect of surfactant concentration on the formulation properties. Advanced Pharmaceutical Bulletin 2014; 4:563.
25. Sharma N, Singh S, Laller N, Arora S. Application of Central Composite Design for Statistical Optimization of Trigonella foenum-graecum Phytosome-Based Cream. Evaluation 2020; 71:63-32.
26. Aslam M, Aqil M, Ahad A, Najmi AK, Sultana Y, Ali A. Application of Box–Behnken design for preparation of glibenclamide loaded lipid based nanoparticles: Optimization, in vitro skin permeation, drug release and in vivo pharmacokinetic study. Journal of Molecular Liquids 2016; 219:897-908.
27. Ricci M, Puglia C, Bonina F, Di Giovanni C, Giovagnoli S, Rossi C. Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. Journal of Pharmaceutical Sciences. 2005; 94(5):1149-1159.
28. Awadeen RH, Boughdady MF, Meshali MM. Quality by Design Approach for Preparation of Zolmitriptan/Chitosan Nanostructured Lipid Carrier Particles-Formulation and Pharmacodynamic Assessment. International Journal of Nanomedicine 2020; 15:8553.
29. Shah NV, Seth AK, Balaraman R, Aundhia CJ, Maheshwari RA, Parmar GR. Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: Design and in vivo study. Journal of Advanced Research 2016; 7:423-434.
30. Singh S, Singla YP, Arora S. Statistical, diagnostic and response surface analysis of nefopam hydrochloride nanospheres using 35 box-behnken design. International Journal of Pharmaceutical Sciences and Research 2015; 7:89-101.
31. Pinto F, de Barros DP, Reis C, Fonseca LP. Optimization of nanostructured lipid carriers loaded with retinoids by central composite design. Journal of Molecular Liquids 2019; 293:111468.
32. Kovács A, Berkó S, Csányi E, Csóka I. Development of nanostructured lipid carriers containing salicyclic acid for dermal use based on the Quality by Design method. European Journal of Pharmaceutical Sciences 2017; 99:246-257.
33. Myers WR. Response surface methodology. In: Chow, S.C. (Eds.), Encyclopedia of biopharmaceutical statistics. New York: Marcel Dekker; 2003. p. 858-69.
34. Gade S, Patel KK, Gupta C, Anjum MM, Deepika D, Agrawal AK, Singh S. An ex vivo evaluation of moxifloxacin nanostructured lipid carrier enriched in situ gel for transcorneal permeation on goat cornea. Journal of Pharmaceutical Sciences

2019; 108:2905-2916.

35. Attia M, Enan ET, Hashish AA, MH El-kannishy S, Gardouh AR, K. Tawfik M, Faisal S, El-Mistekawy A, Salama A, Alomar SY, H. Eltrawy A. Chemopreventive Effect of 5-Flurouracil Polymeric Hybrid PLGA-Lecithin Nanoparticles against Colon Dysplasia Model in Mice and Impact on p53 Apoptosis. Biomolecules 2021; 11:109.
36. Ghanem HA, Nasr AM, Hassan TH, Elkhoudary MM, Alshaman R, Alattar A, Gad S. Comprehensive Study of Atorvastatin Nanostructured Lipid Carriers through Multivariate Conceptualization and Optimization. Pharmaceutics 2021; 13:178.
37. Patil TS, Deshpande AS. Design, development, and characterisation of clofazimine-loaded mannosylated nanostructured lipid carriers: 33-Box-Behnken design approach. Materials Technology 2021; 36:460-475.
38. Fahmy UA, Ahmed OA, Badr-Eldin SM, Aldawsari HM, Okbazghi SZ, Awan ZA, Bakhrebah MA, Alomary MN, Abdulaal WH, Medina C, Alhakamy NA. Optimized nanostructured lipid carriers integrated into in situ nasal gel for enhancing brain delivery of flibanserin. International Journal of Nanomedicine 2020; 15:5253.
39. Sharma N, Singh S. Central composite designed ezetimibe solid dispersion for dissolution enhancement: synthesis and in vitro evaluation. Therapeutic Delivery 2019; 10:643-658.
40. Sharma N, Singh S, Arora S, Madan J. Quality-by-design approach for development and optimization of nefopam hydrochloride loaded poly-(epsilon-caprolactone) and poly-3-hydroxybutyrate microspheres. International Journal of Pharmaceutical Sciences and Research 2017; 8:5111-5121.
41. Sukhbir S, Yashpal S, Sandeep A. Development and statistical optimization of nefopam hydrochloride loaded nanospheres for neuropathic pain using Box–Behnken design. Saudi Pharmaceutical Journal 2016; 24:588-599.
42. Beg S, Saini S, Bandopadhyay S, Katare OP, Singh B. QbD-driven development and evaluation of nanostructured lipid carriers (NLCs) of Olmesartan medoxomil employing multivariate statistical techniques. Drug Development and Industrial Pharmacy 2018; 44:407-420.
43. Montgomery DC. Design and analysis of experiments. 7th ed. New York: John Wiley & Sons; 2008.
44. Sreeharsha N, Naveen NR, Anitha P, Goudanavar PS, Ramkanth S, Fattepur S, Telsang M, Habeebuddin M, Anwer MK. Development of Nanocrystal Compressed Minitablets for Chronotherapeutic Drug Delivery. Pharmaceuticals 2022; 15:311.