Research Article

Nano Biomed Eng

Integrating the Quality-by-Design (QbD) Approach in the Development of Febuxostat-loaded Proniosomal Gel for Topical Delivery

Rajeshwar V. Kshirsagar[1], Sujata R. Rajewar[1], Shreya S. Kokil[1], Matte Kasi Viswanadh[2], Ramling G. Patrakar[3], Datta Maroti Pawde[4]

[1] School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India
[2] KL College of Pharmacy, Koneru Lakshmaiah Education Foundation Deemed to be University (KLEF), Green Fields, Vaddeswaram, Guntur, India
[3] Shree Santkrupa College of Pharmacy, Ghogaon, Tal. Karad, Satara, Maharashtra, India
[4] Department of Pharmaceutical Sciences, Dr. Vishwanath Karad MIT World Peace University, Pune

Corresponding author. E-mail: dattapawde4@gmail.com

Received: May 8, 2024; **Revised:** Jul. 16, 2024; **Accepted:** Aug. 8, 2024

Citation: R.V. Kshirsagar, S.R. Rajewar, S.S. Kokil, et al. Integrating the Quality-by-Design (QbD) approach in the development of febuxostat-loaded proniosomal gel for topical delivery. *Nano Biomedicine and Engineering*, 2024, 16(4): 625−637.

http://doi.org/10.26599/NBE.2024.9290101

Abstract

Febuxostat (FXT) is useful in treating hyperuricemia and chronic gout. However, it has limited bioavailability orally due to its low solubility and short half-life. This results in the need for more frequent and larger doses, increasing the likelihood of adverse effects. Against this background, the current study was established to prepare and optimize an FXT-loaded proniosomal gel using the coacervation-phase separation method. A Quality-by-Design (QbD) methodology was used to ensure the quality of the finished product by assessing how critical process parameters and critical material attributes (CMAs) affected the proniosomal gel's critical quality attributes. Box–Behnken design was employed to explore the effect of CMAs such as the amount of cholesterol, Span 40, and Span 60 on particle size and entrapment efficiency. The optimized proniosomes had a particle size and percentage entrapment efficiency of 193.7 ± 2.26 nm and 85.3% ± 0.89%, respectively. Scanning electron microscopy was used to examine the surface morphology of the optimized formulation. The developed formulation was also subjected to *in vitro* drug release and *ex vivo* permeation studies, which established the efficiency of the prepared proniosomal gel. Stability studies also established the fact that the prepared formulation is stable to variations in temperature for up to 6 months.

Keywords: febuxostat; proniosomal gel; Quality-by-Design (QbD); drug delivery

Introduction

Febuxostat (FXT) is a potent selective xanthine oxidase inhibitor. The oxidization of purines (hypoxanthine and xanthine) to uric acid is carried out with the aid of the xanthine oxidase enzyme [1]. It is useful in treating hyperuricemia and chronic gout, but has limited bioavailability orally due to its low solubility and short half-life. This results in the need for more frequent and larger doses, increasing the likelihood of adverse effects [2]. Several studies have reported that proniosomal gels may increase the drug's solubility and bioavailability [3–5]. Indeed, many formulations have been developed to improve these

variables. For instance, Rangaraj et al. formulated self-nanoemulsifying delivery systems using Capmul MCM C8 NF, labrasol, and transcutol HP as an oil phase, surfactant, and co-surfactant, respectively [6]. Similarly, Londhe et al. developed a liquid self-micro-emulsifying drug delivery system (SMEDDS) of FXT in different capsule shells using Capryol 90, labrasol, and PEG 400 in a liquid SMEDDS formulation based on the phase diagram and drug loading [7]. Meanwhile, Sohn et al. formulated an FXT-loaded solid dispersion by screening different solubilizers, pH agents, and carriers, using a solvent evaporation method [8]. However, they developed these formulations for the oral delivery of FXT, which, although improving solubility, did not significantly reduce the dose. In addition, the development of liposomes is not viable owing to phospholipids' poorer stability. The drug-loaded proniosomal gel; however, does not have this limitation as it requires nonionic surfactants and not phospholipids in its preparation [9]. Therefore, proniosomal gel was formulated in the current study with the aim of enhancing the solubility and bioavailability of FXT, thereby reducing the required dose and dosage regimen. By optimizing the process parameters, these proniosomes would mitigate the adverse effects associated with the conventional high oral dosage and would improve the therapeutic outcomes.

To attain the intended product profile, it is necessary to conduct numerous trials to optimize the formulation parameters for the desired properties [10]. The QbD method has received significant attention in the optimization of formulations [11]. In contrast to the conventional "One factor at a time" method, the systemic orientation provides formulation optimization in a manner that is affordable and effective [12]. To ascertain the potential association between various parameters, the QbD method requires only a limited number of experiments [13]. This offers a depth of process expertise, producing the desired product profile with minimal investment of money, time, and labor [14]. QbD-based pharmaceutical product development is a systematic process that necessitates multivariate analysis utilizing process analytical technology and other tests to select the critical quality attributes (CQAs) based on risk assessments. The QbD begins with predefined objectives and requires an understanding of how product quality is influenced by formulation and process variables. Here, the interaction/relationship among formulation and process factors was investigated using the QbD methodology (Fig. 1), in accordance with the International Conference on Harmonization (ICH) guidelines and Design Expert software [14, 15]. The formulation optimization was accomplished using

Fig. 1 Different steps comprising the QbD approach

design space after evaluation of the critical quality attributes (CQAs). Furthermore, the FXT-loaded proniosomes and formulated gel were characterized.

Material and Methods

Material

A sample of FXT was donated by Precise Biopharma Pvt. Ltd. (Navi Mumbai, India). Soya lecithin (26195), cholesterol (13640), Span 40 (18987) and 60 (18988), and triethanolamine (26763) were purchased from Molychem Pvt. Ltd. (Navi Mumbai, India). Carbopol 934 (56092) was purchased from SD Fine Chemicals (Mumbai, India).

Methods

Components of QbD for formulation development

Outlining the quality target product profile (QTPP): The first and most important phase in developing a product/formulation based on QbD is classification of the QTPP. The QTPP involves quality criteria that ideally lead to the end product's efficacy and safety. It is a predefined potential overview of the qualities of a formulation that are necessary to ensure efficacy with the intended level of safety. It also aids in identifying the product's CQAs [16, 17]. The QTPP of proniosomal gel containing FXT is described in Table 1.

Defining critical quality attributes (CQAs): The second step in the QbD concept is the identification and selection of CQAs. CQAs are the most important product quality characteristics, chosen from the QTPP of the proniosomal gel, which most strongly influence the dosage form performance. They are used directly to develop new products and procedures. Additionally, CQAs determine an extent or threshold for approval of the quality formulation to guarantee the required level of formulation attributes [16]. It is thus necessary to investigate and track the impact of CQAs [17]. The CQAs of the FXT-loaded proniosomal gel are listed in Table 1.

Identification of the critical material attributes (CMAs) and critical process parameters (CPPs): The first category of variables that can affect CQA variability is known as CMAs, which are linked to the formulations' composition. CPPs are another class of potential factors that are related to the formulation-preparation process and could impact CQA variability [17].

Risk analysis and screening out factors: Initial risk assessment studies were conducted to recognize probable components that might have a greater influence than others on the formulation's quality [18]. As a result, various material attributes (MAs) and process parameters (PPs) were selected. The cause-and-effect links were established using the Ishikawa fish-bone diagram (Fig. 2), which was created to identify multiple variables that can lead to formulation/product failure [19].

Table 1 QTPP and CQAs of the FXT-loaded proniosomal gel.

QTPP Elements		Target	Justification
Dosage form		Proniosomal gel	Elevates permeation into the skin, avoids first-pass metabolism, and also improves bioavailability
Route of administration		Topical	Better patient compliance and reduced systemic side effects
Stability		24 months	The product should have sufficient shelf life
Drug product quality attributes	Particle size	<400 nm	A smaller particle size enhances permeation into the stratum corneum
	PDI	Minimize	Monodisperse particle size distribution
	Zeta potential	>± 30	Decide the stability of the formulation and prevent agglomeration
	Drug content	Maximum (85%–95%)	Impacts safety and efficacy, to fulfill therapeutic requirements
	Entrapment efficiency	Maximum without affecting the required size range	Maximum entrapment of drug supports in enhanced permeation
Critical Quality Attributes			**Justification**
Particle size			A smaller particle size increases drug penetration into the skin
Particle size distribution			For uniform drug release and distribution of the drug
Entrapment efficiency			Entrapment efficiency affects drug loading; the capture efficiency should be maximal to increase drug loading
Stability			The shelf life of the product depends on its stability; an increase in the stability of the formulation/product increases the product's shelf life

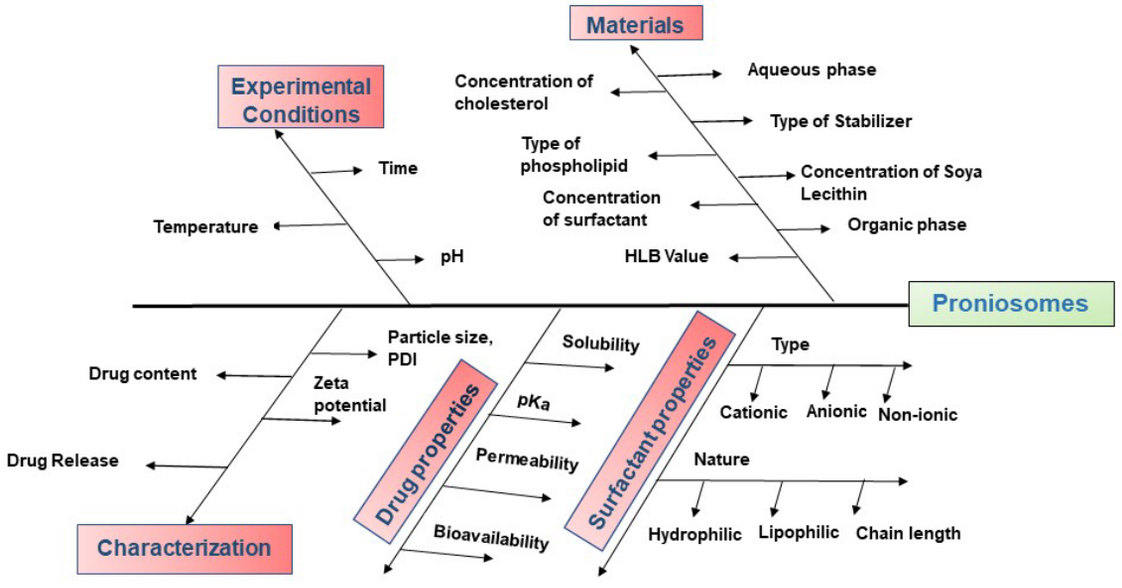

Fig. 2 Ishikawa fish-bone diagram for determining various material attributes and formulation parameters that impact product quality.

Employing the risk estimation matrix (REM), the components/factors were prioritized in accordance with the amount of risk these factors pose during the formulation phase. REM was used to choose CMAs and CPPs depending on the potential for low, medium, and high risk of each of the components [20]. This was pursued by screening variables to be considered when undertaking optimization studies (Table 2). Accordingly, a failure mode effect and analysis (FMEA) was conducted, in which each of the REM-assessed factors was quantitatively tested in terms of "occurrence," "severity," and "detectability," with a ranking score from 1.0 to 10.0, and the overall risk priority number (RPN) was computed (Table 3) in accordance with Eq. (1) [20].

$$\text{RPN} = \text{severity} \times \text{detectability} \times \text{occurrence} \quad (1)$$

Formulation development and optimization

Proniosomes were formulated using a slightly modified version of the coacervation-phase separation approach reported by Perrett et al. and Vora et al. In brief, in a clean glass vial, surfactants (Span 40 and 60), soya lecithin, cholesterol, and drug were combined [21, 22]. To dissolve all of the components, 1 mL of ethanol was used in a glass vial. To avoid solvent loss, the glass vial was sealed. The vials were then placed in a water bath and heated for approximately 5 min at 60ºC–70ºC, until the surfactants were completely dissolved. This was then heated in a water bath with the addition of 1 mL of phosphate buffer, pH 7.4, until a clear solution was formed. For the dispersion to transform into proniosomal gel, the glass vials were left to cool at room temperature. Then, 1% Carbopol-934 gel and proniosomal gel were mixed at a 1:1 ratio [23, 24].

Design of experiment for the optimization of formulations

To optimize the selected factor as per the Box–Behnken design of the FXT-loaded proniosomes, Design-Expert software 13.0 was used

Table 2 Risk estimation matrix (REM) by qualitative analysis for the initial risk assessment of various material attributes and process parameters.

Critical quality attributes(CQAs)	Material attributes and process parameters					
	Conc. of cholesterol	Conc. of soya lecithin	Conc. of SPAN 40	Conc. of SPAN 60	Temperature	Organic solvents for dissolution of lipophilic excipients
Particle size	High	Medium	High	High	Medium	Low
PDI	High	Low	High	High	Low	Low
Entrapment efficiency	High	Medium	High	High	Medium	High
Drug release	High	Low	High	High	Low	High

Table 3 Failure mode evaluation and analysis (FMEA) rank order score observed regarding the impact of MAs and PPs on mean particle size and % drug entrapment efficiency of prepared blank proniosomes assessed as per two diverse levels of individual factors [17].

MAs and PPs	Severity (S)	Detectability (D)	Occurrence (O)	RPN score
Concentration of the cholesterol	6	5	6	180
Concentration of soya lecithin	7	5	7	245
Concentration of Span 40	8	7	6	336
Concentration of Span 60	8	7	6	336
Temperature	2	1	2	4
Organic solvents for the dissolution of lipophilic excipients	2	1	1	2

[25]. The effect of these factors on the response variables, such as mean particle size (Y_1) and percentage drug entrapment efficiency (Y_2), was explored. Table 4 describes the experimental design together with the factors and responses, while Table 5 shows the actual values of 15 experimental runs for the coded factors.

Characterization of the FXT-loaded proniosomal gel

Determination of the particle size and polydispersity index (PDI)

A fixed volume (100 µL) of FXT-loaded proniosomes was diluted with Milli-Q water (900 µL) and sonicated before analysis to ensure that the proniosomes were properly dispersed. The zeta potential of the proniosomes under an applied electric field was measured using electrophoretic light scattering. Measurement was performed at 25 °C using a Malvern Zeta Sizer (ZS-2000 series; Malvern Instruments, UK). The mean ± standard deviation was calculated from three separate measurements [25, 26].

Determination of entrapment efficiency (%) and content uniformity

FXT-loaded proniosomes (0.2 g) and formulated gel (0.25 g) were dispersed in 10.0 mL of phosphate buffer (pH 7.4). The aqueous solution was then subjected to a 10-min sonication process. The unentrapped drug was separated from drug-loaded proniosomes by centrifugation at 25 000 r/min for 35 min at 20 ºC. The supernatant of the FXT-loaded proteosomes was diluted with methanol. The samples were analyzed in triplicate using a double-beam ultraviolet (UV) spectrophotometer (Shimadzu Co., Kyoto, Japan) at 315 nm. The entrapment efficiency was calculated using Eq. (2) [27]:

$$\text{EE\%} = ((\text{Total drug} - \text{Free drug})/\text{Total drug}) \times 100 \quad (2)$$

Morphology of the FXT-loaded proniosomal gel

Scanning electron microscopy (SEM, Supra 40; Zeiss, Germany) under normal atmospheric conditions and a voltage of 20 kV was employed to image the surface morphology of the FXT-loaded proniosomal gel [28]. Samples were placed on glass slides after diluting five times with distilled water and spread evenly, followed by vacuum drying. The slides were carbon-coated and the images were obtained at 40 000× magnification.

In vitro drug release study

A dialysis membrane with a molecular weight cut-off of 12–14 kDa (HiMedia, India) was used to conduct *in vitro* release tests on FTX-loaded proniosomes.

Table 4 Experimental design: independent variables (factors) and dependent variables (responses).

Independent variables (factors)	Levels		
	+1.0	0.0	−1.0
X_1: Amount of cholesterol (mg)	150	175	200
X_2: Amount of Span 40 (mg)	300	350	400
X_3: Amount of Span 60 (mg)	300	350	400
Dependent variables (responses)		**Target**	
Y_1: Mean particle size (nm)		Minimize	
Y_2: Drug entrapment efficiency (%)		Maximize	

Table 5 Design matrix: coded and real values for the Box–Behnken design.

Run	Coded values			Real values (mg)		
	X_1	X_2	X_3	Amount of cholesterol	Amount of Span 40	Amount of Span 60
1	−1.0	−1.0	0.0	150	300	350
2	1.0	1.0	0.0	200	400	350
3	0.0	0.0	0.0	175	350	350
4	0.0	0.0	0.0	175	350	350
5	0.0	−1.0	−1.0	175	300	300
6	−1.0	0.0	1.0	150	350	400
7	0.0	0.0	0.0	175	350	350
8	1.0	−1.0	0.0	200	300	350
9	−1.0	0.0	−1.0	150	350	300
10	−1.0	1.0	0.0	150	400	350
11	0.0	1.0	−1.0	175	400	300
12	0.0	1.0	1.0	175	400	400
13	1.0	0.0	1.0	200	350	400
14	0.0	−1.0	1.0	175	300	400
15	1.0	0.0	−1.0	200	350	300

The sample (2 mL) was added in a dialysis bag and properly sealed on both sides. Furthermore, the dialysis bag was immersed in 20 mL of pH 7.4 phosphate-buffered saline. It was stirred at 400 r/min and 37ºC ± 0.5ºC. Two milliliter aliquots were taken out at fixed intervals and an equal volume of fresh medium was added to retain the sink conditions [29]. The withdrawn samples were analyzed using a double-beam UV spectrophotometer (Shimadzu Co.) at 315 nm.

Determination of viscosity

The viscosity of the FXT-loaded proniosomal gel was estimated using a Brookfield viscometer (Spindle No. 21, Model DV-II). Briefly, the gel was placed in an appropriate beaker and positioned below the spindle. The r/min was set, the motor of the viscometer was switched on, and the readings were recorded. The procedure was repeated three times.

Spreadability study

The optimal spreadability is important to ensure that the proniosomal gel can spread on the applied area of the skin with minimal effort. To measure the spreadability, a measured sample (50 mg) was covered on top of a slide. Then, a weight of 50 g was placed on top of it for 2 min. Subsequently, the weight was removed and the diameter of the spread gel was measured. The spreadability factor was calculated using the following equation.

$$S_f = A/W \tag{3}$$

where S_f is the spreadability factor, A is the area of gel spread, and W is the slide weight.

Ex vivo permeation study

Using fresh goat ear skin, a comparative *ex vivo* permeation study of the proniosomal gel containing FXT and the FXT solution in 0.2% Tween80 was conducted on a Franz diffusion cell apparatus. Goat ear skin was selected because drugs permeate through it slowly compared with through rat/mouse skin [30]. The animal skin was stripped of its muscle layer and subcutaneous fat before being rinsed with phosphate-buffered saline (pH 7.4). The receptor chamber of the Franz diffusion cell was filled with phosphate-buffered saline (pH 7.4). Furthermore, the cleaned skin was mounted on the cell. Through the end of the donor chamber, the drug-loaded gel sample and FXT solution were applied separately to the skin previously equilibrated with solvent. The aliquots were withdrawn at scheduled time intervals and the same volume of fresh medium was added to the receptor chamber to retain the sink condition. The collected samples were evaluated for FXT content by UV–Vis spectrophotometry (Shimadzu Co.) at

315 nm [27].

Stability studies

The prepared proniosomal gel was subjected to stability studies under different temperature conditions as per ICH guidelines for 6 months. During these studies, samples were withdrawn at predefined time intervals and evaluated for particle size and zeta potential.

Results and Discussion

Risk identification and risk assessment screening

Identification of the critical material attributes, critical process parameters, and critical quality attributes

The QTPP and the CQAs of proniosomal gel containing FXT are described in Table 1. The CPPs and CMAs influencing the product/formulation performance and quality were assessed and are presented in Table 3. It was found that the material attributes (MAs) influencing the particle size and size distribution were the quantities of soya lecithin, cholesterol, and surfactant. The drug release and entrapment efficiency rates were impacted by the particle size and size distribution. Meanwhile, the proniosome size and size distribution were impacted by the temperature used during proniosome preparation. The various material attributes and formulation parameters that were determined to impact product quality are presented in Fig. 2 as an Ishikawa fish-bone diagram.

Risk assessment examination and screening of parameters

From the primary risk assessment studies, various parameters such as the concentrations of cholesterol, soya lecithin, and Span 40 and 60, temperature, as well as organic solvents for dissolving lipophilic excipients were found to be the MAs impacting drug entrapment efficiency, *in vitro* release, particle size, and size distribution [31, 32]. As indicated in Table 3, these were qualitatively assessed and rated as per their potential for risk using REM exploration.

Lather et al. showed the influence of soya lecithin and cholesterol on the entrapment efficiency and particle size [27]. In another study, Sambhakar et al. demonstrated the impact of the concentrations of surfactant, cholesterol, and lecithin on entrapment efficiency [33]. Elsewhere, Mokale et al. depicted the influence of surfactant and cholesterol on entrapment efficiency and drug release [34]. A few trials were carried out prior to the selection of critical material properties.

The published articles and obtained outcomes revealed that the concentrations/amounts of cholesterol and Span 40 & 60 could be the CMAs that are particularly important for the proniosome formulation. The goal of this investigation was to formulate proniosomes with a lower amount of surfactant and to produce the finished formulation with the required characteristics. In accordance with the FMEA guidelines, an additional risk assessment was conducted, as displayed in Table 4. RPN scores of 100.0 and above were regarded as reflecting CMAs/CPPs. Separating the possible risk of the MAs/PPs and then sorting out/screening for variables was made possible by the rank-order scoring of various attributes.

Optimization and statistical analysis of experimental results using software developed by design experts

The Box–Behnken design was executed for 15 runs. The achieved responses for each run are listed in Table 6. The mean particle size for all runs was found to be in the range of 176–421.2 nm and the drug entrapment was found to be in the range of 73%–84%. As the concentration of cholesterol increased, the particle size also increased, but there was a nonsignificant decrease in % entrapment (Table 6), which may have been attributable to the increased robustness facilitated by cholesterol. Similarly, as the quantities of surfactants were increased, an increase in particle size and decrease in drug entrapment were noted because a higher concentration of surfactants made the proniosomal wall harder [30].

The selected responses were analyzed using a reduced linear model on the basis of R^2, predicted R^2, and adjusted R^2 values (Table 6). The selected factors include concentration/amount of cholesterol and Span 40 and 60. The ANOVA outcomes for various factors on mean particle size and DEE (%) are presented in Table 7 and were assessed on the basis of significance

Table 6 Box–Behnken design matrix with obtained results for the responses and results of different models for regression analysis of dependent variables (responses)

Run	Coded values			Response values	
	X_1	X_2	X_3	Mean particle size (nm)	Drug entrapment efficiency
1	−1.0	−1.0	0.0	176	83%
2	1.0	1.0	0.0	397.8	75%
3	0.0	0.0	0.0	275.4	79%
4	0.0	0.0	0.0	221	77%
5	0.0	−1.0	−1.0	235.8	84%
6	−1.0	0.0	1.0	339.8	79%
7	0.0	0.0	0.0	246.9	80%
8	1.0	−1.0	0.0	292.5	82%
9	−1.0	0.0	−1.0	326	81%
10	−1.0	1.0	0.0	421.2	75%
11	0.0	1.0	−1.0	398.2	81%
12	0.0	1.0	1.0	348.1	78%
13	1.0	0.0	1.0	419.9	73%
14	0.0	−1.0	1.0	293.9	77%
15	1.0	0.0	−1.0	314.8	80%
Responses	R^2	**Adjusted R^2**	**Predicted R^2**	**Standard deviation**	**Coefficient of variation (%)**
Mean particle size	0.505 9	0.467 9	0.372 1	54.95	17.51
DEE	0.593 4	0.525 6	0.305 7	2.15	2.73

Table 7 ANOVA table for the mean particle size and drug entrapment efficiency (DEE%)

Mean particle size						
Source	**Sum of squares**	**df**	**Mean square**	***F*-value**	***p*-value**	**Significance**
Model	40 200.30	1	40 200.30	13.31	0.002 9	Significant
B-Span 40	40 200.30	1	40 200.30	13.31	0.002 9	Significant
Residual	39 257.90	13	3 019.84			
Lack of Fit	37 777.10	11	3 434.28	4.64	0.1906	Not significant
Pure Error	1 480.81	2	740.40			
Cor Total	79 458.20	14				
Drug entrapment efficiency						
Source	**Sum of squares**	**df**	**Mean square**	***F*-value**	***p*-value**	**Significance**
Model	81.25	2	40.62	8.75	0.004 5	Significant
B-Span 40	36.13	1	36.13	7.79	0.016 3	Significant
C-Span 60	45.12	1	45.12	9.72	0.008 9	Significant
Residual	55.68	12	4.64			
Lack of Fit	51.02	10	5.10	2.19	0.354 4	Not significant
Pure Error	4.67	2	2.33			
Cor Total	136.93	14				

being defined by a p-value of < 0.05. B-Span 40 was found to have a significant effect on mean particle size. Span 40 (B) and 60 (C) were found to have significant effects on the DEE (%).

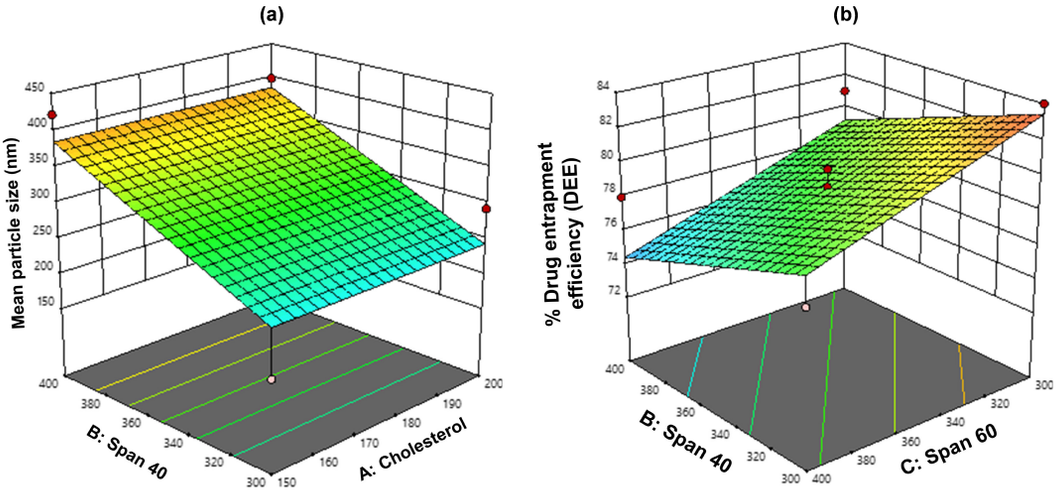

Fig. 3 3D response surface plots for the impact of different significant factors on the responses related to the mean particle size and DEE%.

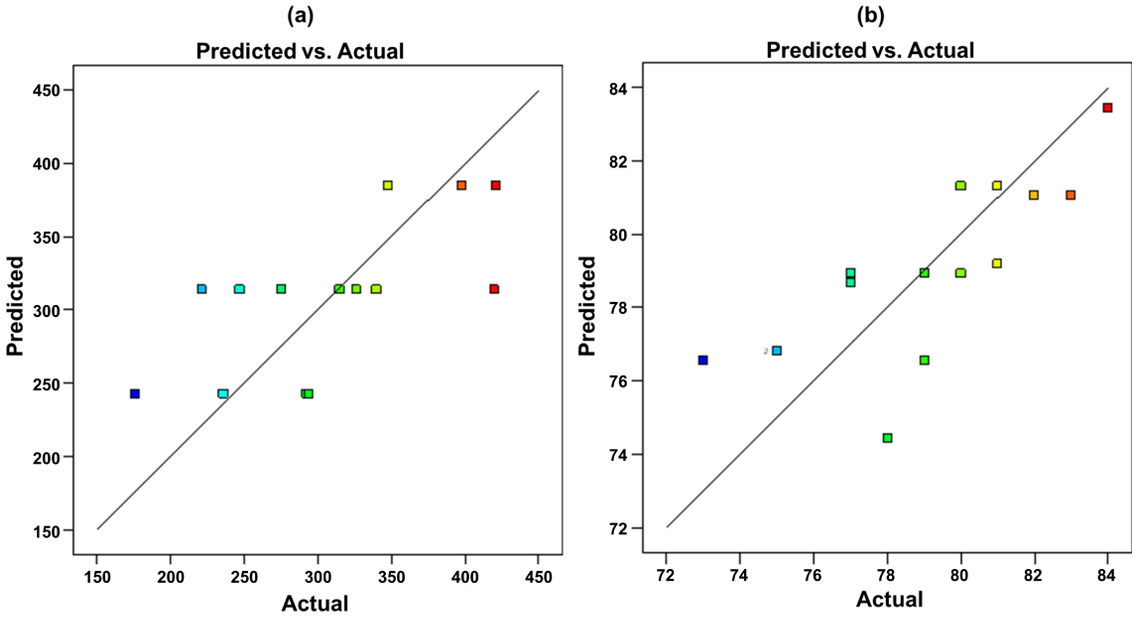

Fig. 4 Actual vs. predicted plots for responses related to mean particle size and DEE%.

The 3D response surface plots for the impact of different significant factors on the responses related to the mean particle size and DEE% are shown in Fig. 3. In Fig. 4, the actual vs. predicted plot is presented for each response parameter. This provides insights about the even distribution of data points near the straight line, demonstrates the models' predictive ability, and contrasts the measured responses for all proniosome formulation runs in these investigations with the average responses anticipated by the model.

Optimization of the model

To create a product with predetermined responses for the desired CQAs, optimization was performed. The solution for the current study was developed with the goal of producing a particle size of 200 nm or less and maximal drug entrapment efficiency. The design space is indicated in yellow in Fig. 5. The predicted values for the optimized formulation and the obtained values of the confirmation runs are shown in Table 8 [35].

Characterization of the FXT-loaded proniosomal gel

Determination of the particle size and polydispersity index

Particle size is critical in the improved pharmacokinetics of proniosomes. The mean particle

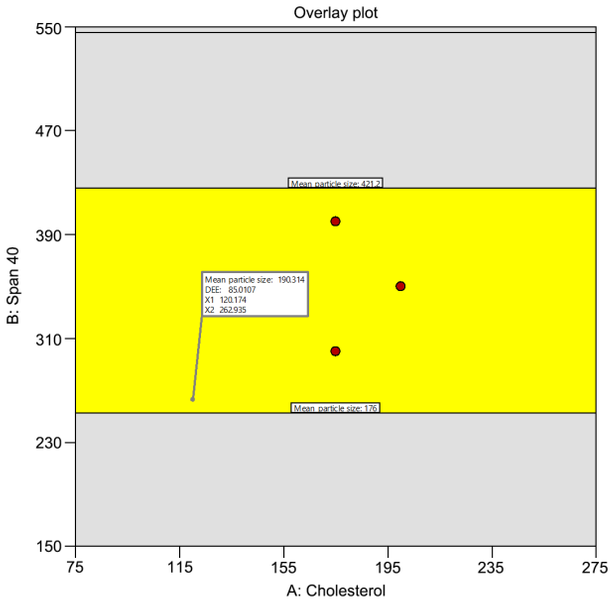

Fig. 5 The design space for the optimized condition of the FXT-loaded proniosomal gel is displayed in yellow.

size of vesicular systems should be in the range of 100–150 nm to achieve improved bioavailability. Using the principle of dynamic light scattering (DLS) on the Malvern zeta sizer, the particle size and zeta potential were measured for the prepared proniosomes. The range of particle size of the FXT-loaded proniosomes was found to be 176–421 nm and the polydispersity index was approximately 0.25. The particle size was in line with the cholesterol content used in the formulation. The proniosomal particles in this nano-size range provide a huge surface area of contact with the skin, for enhanced drug permeation. In association with surfactants and lipids, these nanosized vesicles have been reported to help improve the solubility of poorly soluble FXT [36, 37].

Determination of entrapment efficiency and content uniformity

The entrapment efficiency of FXT-loaded proniosomes was found to be in the range of 73%–85%. The content uniformity of the FXT-loaded proniosomal gel was 98.32% ± 0.83%. The observed drug content was within the limit, so the dose should be delivered accurately. The high content of Span 60 indeed improved the entrapment efficiency because surfactants with a long-chain hydrocarbon structure contribute to higher hydrophobicity, which would increase the EE% of poorly water-soluble drugs. At the same time, the cholesterol contributes to the stability of the proniosomal structure, which helps the nonionic surfactant to improve the overall entrapment efficiency [38].

Morphology of the FXT-loaded proniosomal gel

Morphological evaluation of the prepared proniosomes was carried out using SEM, with the corresponding micrograph shown in Fig. 6. The image of the FXT-loaded proniosomal gel revealed that the particles were spherical in shape with a uniform size and even surface. Moreover, the particle size data obtained from DLS were in fair agreement with the electron microscopy findings.

In vitro drug release study

The *in vitro* drug release from the prepared formulations was carried out using the dialysis bag diffusion method. From the results, a sustained-release pattern from the FXT-loaded proniosomes was observed. The formulation showed 85.26% cumulative release over a 24-h period (Fig. 7). The higher surfactant content, in addition to improving the solubility, also made the bilayer more rigid and maintained the drug release for up to 24 h.

Determination of the viscosity and spreadability

The viscosity of the prepared FXT-loaded proniosomal gel was measured using a Brookfield viscometer, which was found to be 33 094± 664.91 poise, while the gel's spreadability was 12.23 ± 0.34.

Ex vivo permeation study

Using a Franz diffusion cell with goat ear skin as a membrane barrier for permeation, *ex vivo* FXT permeation studies from the prepared proniosomal gel were conducted. In comparison to the aqueous solution of FXT (0.2% w/v solution of Tween80), the FXT-loaded proniosomal gel achieved higher drug

Table 8 The predicted values for the optimized formulation and the obtained values of the confirmation runs.

Independent variables (factors)	**Dependent variables (responses)**			
X_1: Amount of cholesterol = 120 mg	Mean particle size (nm)		Drug entrapment efficiency (%)	
X_2: Amount of Span 40 = 262 mg	Predicted	Observed	Predicted	Observed
X_3: Amount of Span 60 = 300 mg	190	193.7 ± 2.26	85%	85.3% ± 0.89%

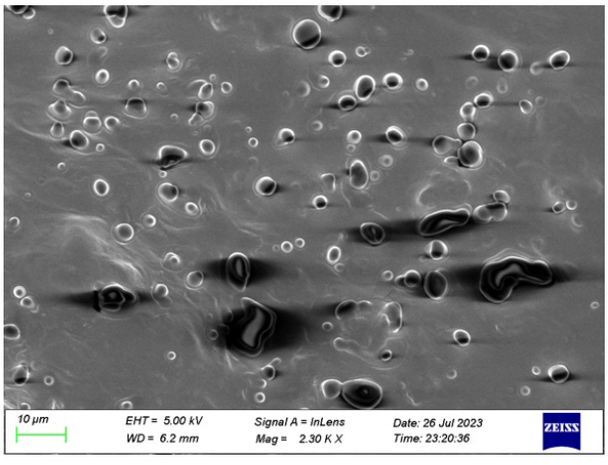

Fig. 6 SEM image of the FXT-loaded proniosomal gel.

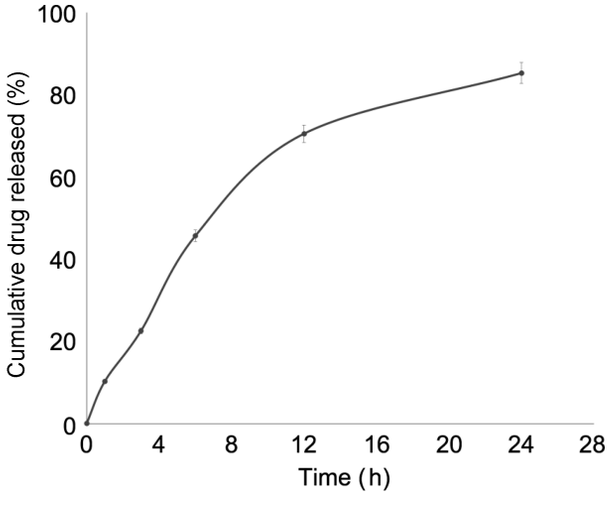

Fig. 7 *In vitro* drug release study of FXT-loaded proniosomes.

permeation through the skin. The aqueous solution of FXT and the FXT-loaded proniosomal gel showed drug permeation rates of 17.27% ± 1.12% and 85.45% ± 6.78%, respectively, within 24 h. Many mechanisms have been proposed to explain the improved permeability of drugs from proniosomes. The improved solubility of drugs in lipid vesicles is one mechanism. In addition, the presence of nonionic surfactants and lipids might have improved the permeability by temporarily altering the skin's barrier properties. Another mechanism that might have contributed to the improved permeability was the fusion of vesicles with the skin, thus bringing the solubilized drug in direct contact with the skin [34, 39].

Stability studies

Stability studies to assess the strength of the proniosomes to withstand the changes in temperature, humidity, and other variables were conducted in an environmental stability chamber and the samples collected at various time intervals were evaluated for particle size and zeta potential. The obtained values are shown in Table 9. The values clearly show that the formulation remains stable for 6 months with negligible variations in particle size and zeta potential, even after exposure to different temperature conditions [39, 40].

Conclusions

An FXT-loaded proniosomal gel with minimal particle size and maximal drug entrapment efficiency was successfully prepared by employing the coacervation-phase separation method and optimized by QbD methodology. Following the identification of the QTPP and CQAs, CMAs and CPPs were screened for further formulation optimization on the basis of preliminary investigations and risk analyses. To ensure the quality of the FXT-loaded proniosomal gel, we first established QTPP and selected the particle size and drug entrapment efficiency as CQAs. Using the Box–Behnken design, the formulation was optimized. The formulation comprising 120 mg of cholesterol, 262 mg of Span 40, and 300 mg of Span 60 was the optimized one. The optimized nanoparticles were shown to have particle size and entrapment efficiency of 193.7 ± 2.26 nm and 85.3% ± 0.89%, respectively. The model was validated by the agreement between the actual and predicted data. The formulation showed 85.26% cumulative release over a 24-h period. In comparison to an aqueous solution of FXT, the FXT-loaded proniosomal gel

Table 9 Stability studies

Formulation	Storage condition (°C)	Particle size (nm)			Zeta potential (mV)			Physical stability at the end
		0 days	3 months	6 months	0 days	3 months	6 months	
Febuxostat-loaded proniosomal gel	25 ± 2	164 ± 29.3	170 ± 6.2	188 ± 20.5	−16±1.21	−13.24 ± 3.13	−11.45 ± 1.13	The formulation remains stable
	40 ± 2	165±18.7	177.31 ± 9.41	193.45 ± 29.2	−15±3.28	−10.77 ± 2.15	−7.06 ± 2.14	The formulation remains stable
	2 –8	164±21.4	173.32 ± 12.19	178.67 ± 8.22	−16± 2.74	−11.68 ± 1.47	−9.70 ± 3.17	The formulation remains stable

showed fivefold permeation. The results of stability studies have established that the prepared proniosomal gel was stable for up to 6 months when exposed to different temperatures. The outcome of this study established that the FXT-loaded proniosomal gel could be developed as a promising transdermal drug delivery system after proper evaluation of its *in vivo* pharmacokinetics and efficacy in animals, along with further clinical evaluation.

CRediT Author Statement

Rajeshwar Vishwanathrao Kshirsagar- Experimental work, conceptualization, supervision, **Sujata Rameshwar Rajewar-** Experimental work, review and editing, **Shreya Sharad Kokil-** Experimental work, review and editing, **Matte Kasi Viswanadh-** formal analysis, review and editing, **Ramling G. Patrakar-**formal analysis, review and editing, **Datta Maroti Pawde-** conceptualization, supervision, review and editing.

Conflict of Interests

The authors declare that with regard to the reported data in the manuscript, the authors have no conflict of interest with respect to any entity or individual.

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