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Development and Optimization of Proniosomal Formulation of Irbesartan Using a Box−Behnken Design to Enhance Oral Bioavailability: Physicochemical Characterization and *In Vivo* Assessment

Md. Ali Mujtaba,[¶] Mohammed Kaleem,[¶] Ragini Chaware, Ashwini Ingole, Yahya I. Asiri, Mohd. Zaheen Hassan, Vidya Sabale,* Prafulla Sabale,* Md. Khalid Anwer, Danish Mahmood, and Mohammed F. Aldawsari

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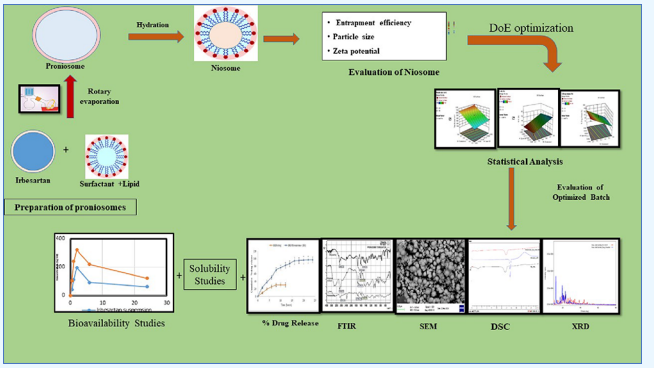
ABSTRACT: This research work aimed to develop and evaluate proniosomes for the oral delivery of the lipophilic drug Irbesartan (IRB) to improve its solubility and bioavailability. Proniosomes of Irbesartan were formulated using a lipid, surfactant, and carrier by a slurry method. Based on the prepared preliminary trial batches and their evaluation, the formulation was optimized by employing a Box−Behnken design (BBD) in which concentrations of span 60 (X_1), cholesterol (X_2), and mannitol (X_3) were used as three independent variables and the vesicular size (VS) (Y_1), % entrapment efficiency (% EE) (Y_2), and % cumulative drug release (% CDR) (Y_3) were used as dependent variables. The optimized batch B1 was obtained from the BBD experiment after validation of checkpoint analysis, and their characterization was done for VS, % EE, % CDR, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) analysis. The optimized batch showed a VS of 199 ± 5.4 nm, a % EE of 99.25 ± 2.24%, and a % CDR of 97.36 ± 1.13% at 24 h. Scanning electron microscopy (SEM) study showed a smooth surface of batch B1. DSC and XRD studies indicated the amorphous nature of the proniosomal formulation. The proniosomal formulation showed increased solubility (2.65 ± 0.2 mg/mL) in phosphate buffer, pH 6.8, as compared to water (0.059 ± 0.02 mg/mL). The pharmacokinetic study in rats confirmed the increased bioavailability of the drug in optimized proniosomal formulation compared with its pure drug suspension. C_{max}, T_{max}, and $AUC_{0\text{-}t}$ of the drug also increased by 2-fold compared to those of drug suspension. Thus, in conclusion, the proniosomal formulation proved to be an efficient carrier for improved oral delivery of Irbesartan by improving the solubility and bioavailability of the drug.



1. INTRODUCTION

Recently, new potent chemical entities having poor water solubility are increasing, but they possess many problems like slow drug release, poor membrane permeability, poor bioavailability, and thus less efficacy in patients.[1] Active pharmaceutical ingredients with poor water solubility require special attention for selecting the appropriate formulations for oral bioavailability enhancement. These formulation strategies include the use of cosolvents,[2] the formation of salts,[3] cyclodextrin complexation,[4] solid dispersions,[5] nanosized formulations like nanosuspension,[6] nanoparticles,[7] and nanocrystals,[8] and lipid-containing drug delivery systems like microemulsions,[9] self-emulsifying drug delivery system,[10] solid lipid nanoparticles (SLN),[11] nanostructured lipid carriers (NLC),[12] liposomes,[13] niosomes,[14] etc. Out of these formulation strategies, niosomes and liposomes are points of greater attention as alternative options for colloidal lipid carriers. However, there are several issues with liposome dispersions in various applications such as expensiveness, purity difference of phospholipids, and the need for a vacuum atmosphere during preparation.[15] Niosomes correspond to liposomes as they involve the use of non-ionic surfactants,

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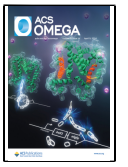

which are biodegradable and biocompatible. Niosomes are more advantageous in that they possess better chemical stability, are cheaper, and have an ease of selection of ingredients. However, they are physically unstable and can lead to aggregation. These problems can be solved by formulating proniosomes.[16] Proniosomes help to improve the bioavailability of lipophilic drugs, along with the improvement of the chemical and physical stability of the drug and formulations. The preparation of proniosomes involves coating of the drug with dry surfactants.[17] The proniosomal powder immediately becomes converted to niosomal dispersion when it comes in contact with hot water with stirring. The proniosomes also help to control the release rate of the drug and extend the circulation of the entrapped drug.[18]

About one-third of adults in the United States suffer from hypertension, a significant risk factor for heart disease.[19] India has reported similar circumstances.[20] Irbesartan (IRB) is an angiotensin-II receptor antagonist and is used to treat clinical conditions associated with hypertension. It exists in vascular smooth muscle and adrenal gland, results in smooth muscle relaxation, and prevents aldosterone secretion, resulting in reduced blood pressure. IRB is a Biopharmaceutic Classification System (BCS) class II drug (low solubility and high permeability), which causes major problems in the oral administration of the drug.[21]

The simplest and most convenient method of non-invasive delivery of drugs is oral delivery. Oral drug delivery, however, may not be effective for therapeutic compounds with low water solubility.

Nanotechnology has focused much of the interest on vesicular drug delivery systems. Proniosomes emerge as the most advantageous among them, in comparison to other vesicular carriers. Only a finite number of studies have been reported related to the formulation and evaluation of the proniosomal powder for oral administration. Most of the publications focused on transdermal delivery.[22] However, no relevant studies about Irbesartan proniosomal systems for oral administration have been reported yet. Therefore, the main objective of this research was to develop and optimize the proniosomal formulation of IRB using the Box−Behnken design. Independent variables were selected such as span 60 (X_1), cholesterol (X_2), and mannitol (X_3) to evaluate their combined and separate effect on vesicular size (VS) (Y_1), % entrapment efficiency (% EE) (Y_2), and % cumulative drug release (% CDR) (Y_3). Second, the optimized formulation was evaluated for various physicochemical characterizations, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM) analysis, and solubility study, and then evaluated the effect of proniosomal formulation on bioavailability in the animal model.

2. MATERIALS AND METHODS

Irbesartan was procured from Yucca Enterprises, Mumbai. Span 60, cholesterol, disodium hydrogen phosphate, potassium dihydrogen phosphate, ammonium acetate buffer, and mannitol were purchased from Loba Chemie, Mumbai. Chloroform and acetonitrile were procured from Merck Pvt., Ltd. All solvents used were of HPLC grade, and the ingredients and reagents used were of analytical grade. Distilled water was used for all of the experiments.

2.1. Preparation of Proniosomal Powder. In the present work, the slurry method was used for the formulation of IRB-loaded proniosomes for oral drug delivery as this method is simple and easy to scale up.[23,24] The amounts of each ingredient for preparing different batches of proniosomal formulations are mentioned in Table 2. The amount of IRB drug taken was 150 mg for each batch. The scheme of the method of preparation is as follows (Figure 1):

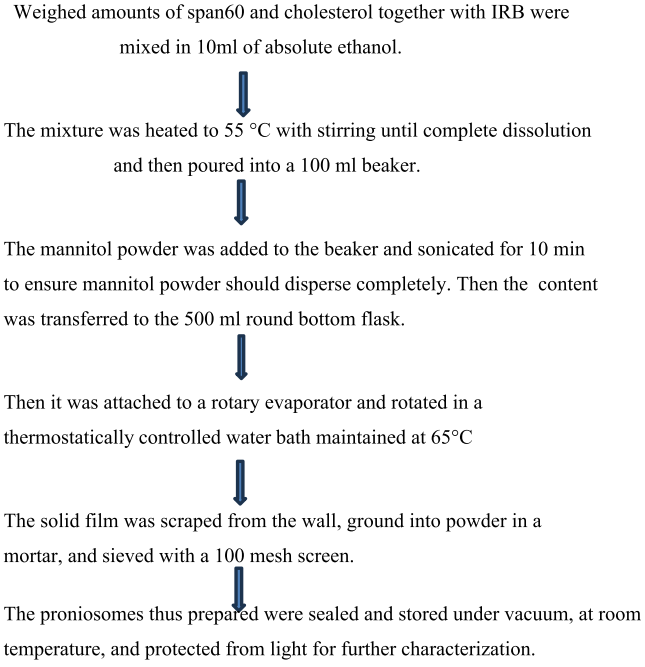

Figure 1. Scheme of the method of preparation of proniosomes.

2.2. Box−Behnken Experimental Design. When developing a complex formulation, traditional experiments need more effort, more material, and more time. A formulation that requires less testing and evaluates the relative importance of various factors can be developed with the use of a variety of experimental designs. A Box−Behnken design (BBD) is one of the experimental designs that can statistically optimize formulation parameters and also evaluate the various effects of independent variables like the main effect, quadratic effect, and interaction effects on the dependent variables of the formulation.[23] A BBD is a cost-effective technique for formulation optimization as it requires less number of experimental runs and less time for process optimization compared to D-optimal design, central composite design, and 3-level factorial design.[24,25]

A three-factor, three-level BBD was utilized to investigate the formulation variables that impact the study. Span 60 (X_1), cholesterol (X_2), and mannitol (X_3) were taken as three formulation variables at low (−1), intermediate (0), and high (+1) concentration levels (Table 1). To get a more consistent estimate of the prediction variance over the whole experimental design, this design required 15 runs with three replicated center points. The study considered three dependent variables: VS (Y_1), % EE (Y_2), and % CDR (Y_3). The BBD was built by Design-Expert software (version 13.0, Stat-Ease Inc., MN, USA), which produced and examined 15 trial runs.

2.3. Preparation of Niosomes from Proniosomes. Proniosomes were converted to niosomes by hydrating each batch with 25 mL of distilled water heated at 80 °C. To examine VS, PDI, zeta potential, and % EE, the dispersion was

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Table 1. Variables in BBD for Formulation Development

variable	level		
	low (−1)	medium (0)	high (+1)
independent variable			
X_1 = span 60 (g)	0.45	0.7225	0.995
X_2 = cholesterol (g)	0.1	0.35	0.6
X_3 = mannitol (g)	3.855	3.8775	3.9
dependent variable			
Y_1 = VS (nm)			
Y_2 = % EE			
Y_3 = % CDR			

first stirred for 2 min using a magnetic stirrer and then sonicated for 30 s using a sonicator (Bharat Biotech, India).

2.4. Characterization of Proniosomes. *2.4.1. Vesicular Size (VS), Polydispersity Index (PDI), and Zeta Potential (ZP) Measurement.* Using a particle size analyzer (Malvern Instruments, Malvern, UK), we determined the average VS, ZP, and PDI of niosomes. The hydration of proniosomal powder was done with phosphate buffer, pH 6.8, and the appropriately diluted sample was tested at 25 °C and a 90° detection angle. VS and PDI were measured as the mean ± SD. The polydispersity index (PDI) was determined as it represents the particle size distribution. The ZP of the sample of proniosome was measured using a zetasizer (Malvern Instruments, Malvern, UK) with the zeta meter framework.[26]

2.4.2. Entrapment Efficiency (% EE) Determination. For the determination of % EE, niosomal dispersion samples were centrifuged using a cooling centrifuge (Remi Elecktrotechnik Ltd., India) at 14,000 rpm for 40 min at 4 °C. The supernatants were collected and suitably diluted for UV assay of the free drug concentrations. Entrapment efficiency for IRB was determined using UV spectrophotometry at 227 nm.[27] The determination was done in triplicate, and % EE was determined using the following formula:

$$\%\ \mathrm{EE} = \frac{\text{total drug added} - \text{free drug}}{\text{total drug added}} \times 100$$

2.4.3. In Vitro Drug Release Study. The % cumulative drug release (% CDR) of reconstituted niosomal dispersion from all 15 batches was determined by the dialysis bag method under sink conditions. The dialysis bag was first immersed in the pH 6.8 phosphate buffer for 12 h before use. Ten milliliters of dispersion was added to the dialysis bag closed at both ends and attached to the shaft, immersed in a flask containing 900 mL of phosphate saline buffer, pH 6.8. The temperature was kept at 37 ± 0.5 °C, and the samples were withdrawn at predetermined intervals. Each sample was filtered using 0.45-μm cellulose nitrate filter and assayed using UV spectrophotometry. The cumulative % CDR was calculated and presented as the mean ± SD.[28]

2.5. Checkpoint Analysis. To validate the function of the contour plots and the resultant polynomial equation in response prediction, a checkpoint analysis was carried out. Based on the generated polynomial equations, the software optimization process predicts the values of the independent variables (X_1, X_2, and X_3) that reach optimal formulation with the desired responses. Each contour plot was used to obtain the values of the independent variables, which were then substituted into the polynomial equation to determine the theoretical values of the dependent factors. The recommended amount of each ingredient was used to generate the two optimal formulas, P1 and P2. To compare the measured values of VS, % EE, and % CDR with the predicted values derived from the equation, the two formulas were tested.

2.6. Optimization of Formulation and Characterization of the Optimized Proniosome. The purpose of using a BBD was to get a more thorough look at the selected parameters and their interaction effects. It consists of 15 experiments, in which three formulation parameters were varied. As per the design of experiments, the composition of proniosomes was optimized, and the final batch was prepared with the given concentration. Each response results in the development of contour plots, which split the plot surface into zones that are desirable and undesirable. Batch B1 was selected based on the design space and evaluated for various response variables. Batch B1 was also subjected to further characterization.

2.6.1. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. The study was conducted to check the compatibility of excipients like span 60, cholesterol, mannitol, and their physical mixture with Irbesartan, and also it helps to check the suitability of excipients. FTIR spectra were studied using a Shimadzu FTIR spectrometer (Shimadzu Corporation, Kyoto, Japan). The samples of Irbesartan, optimized batch, and excipients like span 60, cholesterol, mannitol, and physical mixtures were analyzed. The scanning range was kept from 4000 to 400 cm^{-1}.[29]

2.6.2. Differential Scanning Calorimetry (DSC) Analysis. Thermals analysis of the proniosomal formula was done using DSC (DSC, Germany, METTLER TOLEDO, DSC3), and thermograms were obtained by scanning the sample at 10 °C/min during a nitrogen purge, between 40 and 280 °C at the flow rate of 30 mL/min.[30]

2.6.3. Powder X-ray Diffraction (XRD) Analysis. XRD analysis of proniosomal powder and pure drug provides diffraction peak patterns. Analysis was conducted using an X-ray diffractometer (Bruker, USA) at 45 kV voltage and 40 mA current, an an X'celerator detector equipped with Cu Kα radiation, and a nickel-filtered graphite monochromator. The analysis was performed between 3° and 45° (2θ) at 1° (2θ) min^{-1}.[31]

2.6.4. Scanning Electron Microscopy (SEM) Analysis. SEM (S-4100, Hitachi, Japan) was used to characterize the surface morphology of optimized proniosome formulation. The analysis involved fixing the sample on a brass stub using double-sided adhesive tape and applying a film of gold to improve the conductivity of 15 keV voltage. Images were obtained to assess the proniosome particles' morphology during the studies, which were conducted at lower pressure (0.001 mmHg).[32]

2.7. Solubility Determination. The saturation solubility of IRB and optimized proniosome formula B1 was determined in various buffers and distilled water by shaking on an orbital shaker. Excess amounts of IRB and proniosomal powder were added into 8 mL of water and phosphate buffer, pH 6.6, 6.8, 7.2, and 7.4. The equilibration of the suspension was done for 72 h on the orbital shaker at 37 °C, and it was filtered through a membrane filter with a pore size of 0.2 μm and analyzed by UV spectrophotometry at 232 nm.[21]

2.8. Stability Studies. For 90 days, the proniosomal powder-optimized formula B1 was stored in a glass vial under two storage conditions, e.g., at room temperature and in a refrigerator (4 ± 2 °C). Samples were taken out at

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Table 2. Variables and Observed Responses in the BBD

formulation code	independent variable at different levels			dependent variable		
	X_1 = span 60 (g)	X_2 = cholesterol (g)	X_3 = mannitol (g)	Y_1 = VS (nm)	Y_2 = % EE	Y_3 = % CDR
B1	0.45	0.1	3.8775	199 ± 5.4	99.25 ± 2.24	97.36 ± 1.32
B2	0.995	0.1	3.8775	402 ± 3.6	98.12 ± 2.87	95.17 ± 2.63
B3	0.45	0.6	3.8775	645 ± 2.9	85.48 ± 2.36	75.87 ± 2.12
B4	0.995	0.6	3.8775	771 ± 3.8	86.89 ± 2.89	80.13 ± 3.24
B5	0.45	0.35	3.855	503 ± 5.2	92.32 ± 3.04	86.05 ± 1.42
B6	0.995	0.35	3.855	544 ± 2.9	95.66 ± 3.14	92.50 ± 2.51
B7	0.45	0.35	3.9	636 ± 3.6	90.88 ± 2.44	84.46 ± 1.38
B8	0.995	0.35	3.9	506 ± 3.1	97.66 ± 1.28	91.57 ± 3.63
B9	0.7225	0.1	3.855	316 ± 1.9	96.90 ± 1.54	94.50 ± 2.76
B10	0.7225	0.6	3.855	793 ± 1.1	89.22 ± 2.84	78.00 ± 2.40
B11	0.7225	0.1	3.9	468 ± 3.8	97.69 ± 1.09	90.18 ± 1.52
B12	0.7225	0.6	3.9	734 ± 2.2	87.43 ± 2.99	79.33 ± 3.82
B13	0.7225	0.35	3.8775	552 ± 4.7	95.53 ± 2.54	89.98 ± 2.32
B14	0.7225	0.35	3.8775	489 ± 2.8	95.25 ± 1.37	91.65 ± 1.93
B15	0.7225	0.35	3.8775	512 ± 1.8	96.80 ± 2.04	90.20 ± 2.47

Table 3. Statistical ANOVA Results for the Linear Model

response	*F*-value	*p*-value	R^2	adjusted R^2	predicted R^2	lack of fit	remark
VS	20.30	<0.0001	0.8470	0.8053	0.6811	6.00	significant
% EE	17.71	0.0002	0.8284	0.7817	0.6850	8.01	significant
% CDR	20.66	<0.0001	0.8492	0.8081	0.7165	12.82	significant

predetermined intervals, viz., 0, 30, 60, and 90 days, and hydrated with phosphate buffer, pH 6.8.[33] The samples were then assessed for % EE and % CDR.

2.9. *In Vivo* Pharmacokinetic Study. The bioavailability study was conducted in male Sprague−Dawley rats (n = 18). The institutional animal ethics committee (IAEC) gave their approval to the research protocol according to CPCSFA guidelines for bioavailability studies through project authorization numbers DBCOP/IAEC/1426/2022-23/P6. The animals were in the range of 200−250 g weight and were housed in a controlled condition at 22 ± 2 °C temperature and 50 ± 5% relative humidity. All rats were provided with adequate amounts of food and water. They were acclimatized for 7 days and were kept fasting overnight before the experiment. These rats were divided into three groups, each group containing six animals. Group 1 got drug suspension, while group 2 got proniosomal formulation at the dose per kg wt of body, and group 3 was kept as a blank group without treatment. All groups were kept separately in cages under laboratory conditions. The blood samples were withdrawn at different time intervals of 0 min, 30, min, 1 h, 3 h, 6 h, and 24 h and kept in heparinized microcentrifuge tubes. Centrifugation was used to separate the plasma sample, and the sample was stored at −20 °C and then analyzed using validated HPLC methods.[18,34] The sample must be at room temperature before bioanalysis, and the drug was separated by using the protein precipitation technique with acetonitrile. To precipitate the protein, 1 mL of acetonitrile (1 mL) was added to the plasma sample (1 mL). After 10 min of vortexing, the mixture was centrifuged for 10 min at 10,000 rpm. The HPLC method was utilized to determine the quantity of IRB present in every sample. To achieve a concentration range of 10 to 5000 ng/mL, different amounts of IRB were added to the blank plasma to establish the method's linearity. IRB was extracted and examined by using the previously described method. The linearity of the standard curve was measured at R^2 = 0.9991.

Model-independent analysis, also known as noncompartmental analysis, was used to calculate the pharmacokinetic parameters. For each subject in the group, the following pharmacokinetic parameters were determined individually: T_{max}, C_{max}, $T_{1/2}$, $AUC_{0\text{-}t}$, and MRT. The results are shown as the mean ± SD. Additionally, the pure drug suspension and improved proniosomal formulation B1 *in vivo* comparative bioavailability profiles were established. The pharmacokinetic data were statistically analyzed using GraphPad Prism (Instat 3.06, Software Inc., San Diego, CA, USA), and Student's t test was used to determine significant differences.

3. RESULTS AND DISCUSSION

3.1. Box−Behnken Design Experiments. A BBD was used to examine the effects of three independent factors: the amount of span 60 (X_1), cholesterol (X_2), and mannitol (X_3) at three levels of concentration on the dependent factors VS, % EE, and % CDR of the niosomal suspension prepared from proniosomes after hydration. The BBD produced a total of 15 formulation batches. Proniosome formulations of all batches (B1−B15) were made via the slurry method, also known as the thin-film method. Using this procedure, niosomal suspension was formed by hydrating the dry film with hot water at 80 ± 2 °C. This temperature was chosen for the hydration process as it is higher than the mean transition phase temperature of span 60 (50 °C). Mannitol is a safe, nontoxic material that dissolves well in water to facilitate hydration but is poorly soluble in the mixed solution. Consequently, it serves as a carrier to raise the hydration surface area and therefore increase the loading efficiency.[23] Cholesterol, a structural lipid, helps to improve the vesicles' entrapment efficiency and membrane stability. 2 shows a total of 15 formulated batches with their compositions and responses.

3.2. Evaluation of the Response Surface with the Polynomial Equation. Optimization was done based on the effect of independent variables, namely, span 60 concentration

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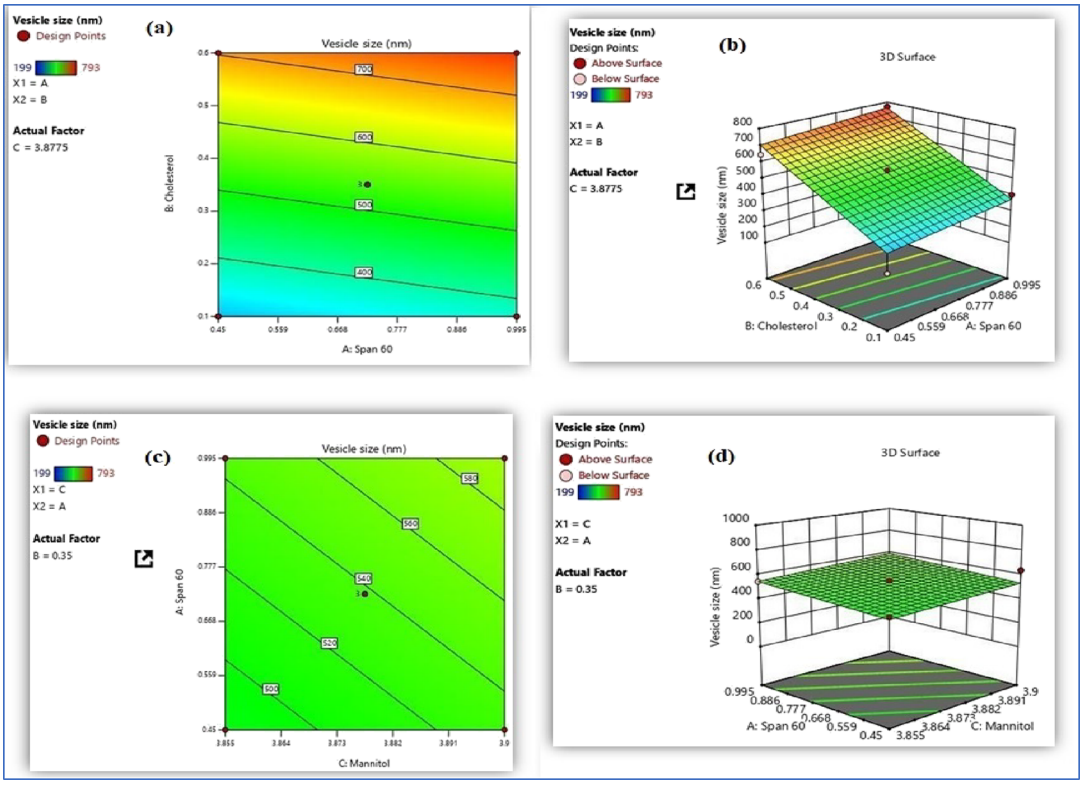

Figure 2. (a) Contour plot showing the effect of span 60 vs cholesterol on vesicle size, (b) 3D surface response plot showing the effect of span 60 vs cholesterol on vesicle size, (c) contour plot showing the effect of span 60 vs mannitol on vesicle size, and (d) 3D surface response plot showing the effect of span 60 vs mannitol on vesicle size.

(X_1), cholesterol concentration (X_2), and mannitol concentration (X_3) on dependent variables, i.e., VS, % EE, and % CDR.

3.2.1. Effect on VS. The average vesicle size (VS) of proniosome-derived niosomes ranged from 199 ± 5.4 to 793 ± 1.1 nm. The results of the VS analysis obtained from 15 batches are listed in Table 2. The average VS increases with increased HLB of the surfactant (span 60) as the free energy decreases with decreasing lipophilicity. Also, the cholesterol content results in an increase in VS. ANOVA was applied to determine the linear model on VS (Table 3). From the regression statistics, *p*-values were less than 0.0500, indicating that model terms are significant. The lack of fit was nonsignificant, which is desirable. The mathematical relationship between independent variables and VS for the proniosomal formulation is given by the following polynomial equation. The polynomial equation (eq 1) helps to determine the relationship between the variables and VS.

$$\mathrm{VS} = +\,538.00 + 30.00 \times A + 194.75 \times B + 23.50 \times C \quad (1)$$

From the above equation, it was observed that the coefficients of *A*, *B*, and *C* shown by the polynomial equation (eq 1) have a positive value that suggests a desired outcome. Additionally, it was found that only *B* had a substantial impact on VS ($p < 0.0001$). The effect of all independent variables is mentioned in Figure 2a−d with contour and 3D surface response plots.

3.2.2. Effect on % EE. The % EE of proniosome-derived niosomes was estimated by the UV-spectrophotometric method. % EE was found to be in the range of 85.48 ± 2.36 to 99.25 ± 2.24%. The maximum % EE achieved by batch B1 is 99.25 ± 2.24%. ANOVA was applied to determine the linear model on % EE as shown in Table 3. The model appears significant based on its *F*-value of 17.71. The lack of fit appears to be not significant, as indicated by the lack-of-fit *F*-value of 8.01. The mathematical relationship between independent variables and % EE for the proniosomal formulation is represented by the given polynomial equation. Finding the relationship between the components and % EE is made easier by the polynomial equation (eq 2).

$$\%\ \mathrm{EE} = +\,93.67 + 1.30 \times A - 5.37 \times B - 0.0550 \times C \quad (2)$$

where the independent variables are *A*, *B*, and *C*. From the above equation, it was observed that as the concentration of span 60 increases, % EE also increases. As the concentration of cholesterol increases, % EE decreases and there is not any major effect of the concentration of mannitol on % EE. There is less than 0.2 difference between the adjusted R^2 of 0.7817 and the predicted R^2 of 0.6857, indicating that there is acceptable agreement. The model helps the designer navigate the design area. The effect of all independent variables is mentioned in Figure 3a−d.

3.2.3. Effect on % CDR. % CDR was assessed to elucidate the release pattern of IRB from proniosome-derived niosomes. The % CDR at 24 h of all batches (B1−B15) ranged from 75.87 ± 2.12 to 97.36 ± 1.32 (Table 2). ANOVA was applied to determine the linear model on % CDR as shown in Table 3. The model *F*-value of 20.66 implies that the model is significant. Multiple regressions were used to determine the amount of drug released after 24 h at various values of the three components, yielding the following polynomial equation (eq 3).

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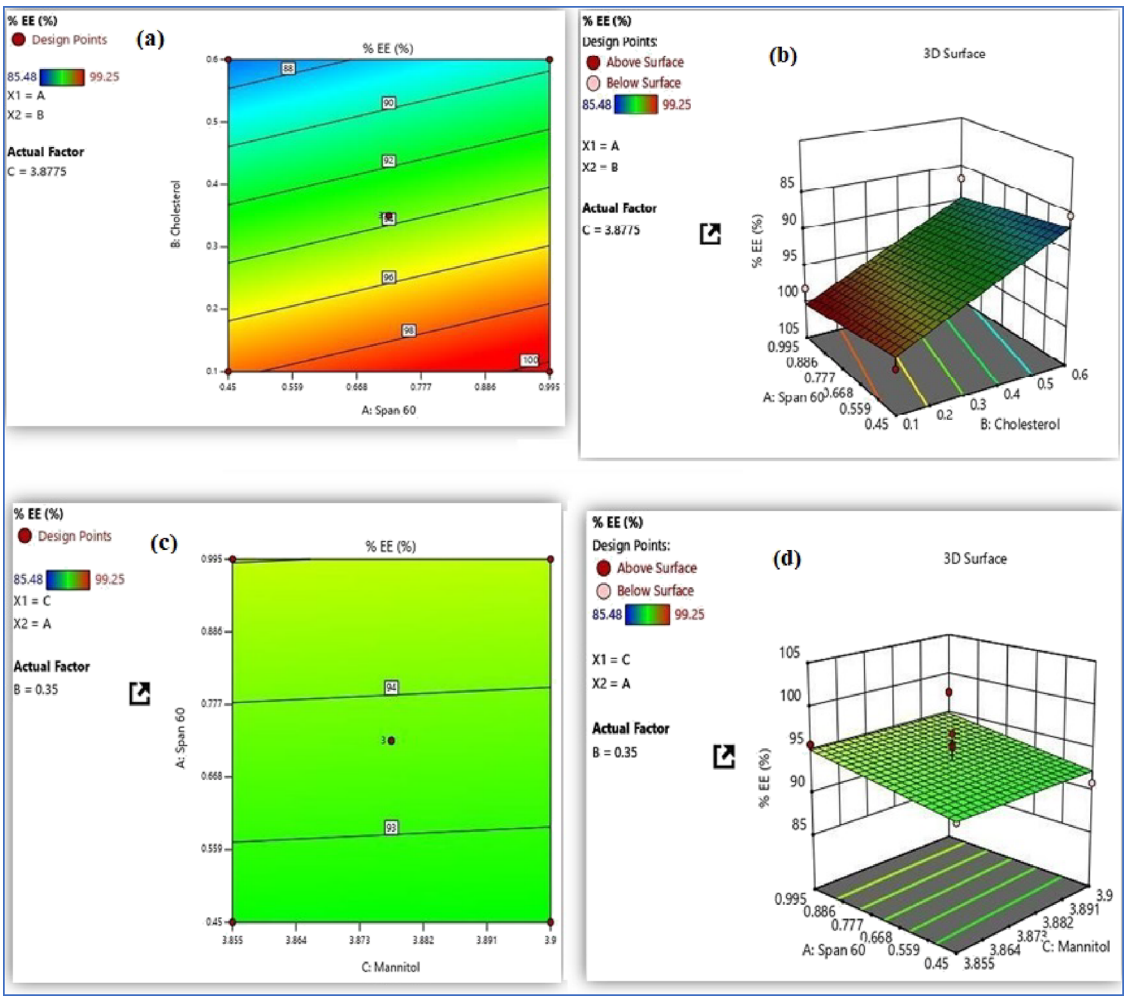

Figure 3. (a) Contour plot showing the effect of span 60 vs cholesterol on % EE, (b) 3D surface response plot showing the effect of span 60 vs cholesterol on % EE, (c) contour plot showing the effect of span 60 vs mannitol on % EE, and (d) 3D surface response plot showing the effect of span 60 vs mannitol on % EE.

$$\% \text{ CDR} = +87.80 + 1.95 \times A - 7.98 \times B - 0.6888 \times C \quad (3)$$

where the independent variables are A, B, and C. From the above equation, as A increases, % CDR increases. As B increases, % CDR decreases, and no major effect of C on % CDR was found. The adjusted R^2 of 0.8081 and the predicted R^2 of 0.7165 are reasonably in agreement; that is, the difference is less than 0.2. The signal-to-noise ratio is detected with adequate precision. The model helps to navigate the design area. The effect of all independent variables is mentioned in Figure 4a–d.

3.3. Check Point Analysis and Optimization of Formulation. Following an analysis of how the independent variables affected the responses, the values of these variables that produced the optimal responses were identified. The desirability of 0.918 had been obtained, which predicted a 91.80% chance of getting the desired response using optimized values of factors. Identification of the checkpoint batch from the overlay plot was done. Two checkpoint batches P1 and P2 were prepared and evaluated for vesicle size, % EE, and % cumulative drug release as shown in Table 4. When comparing these actual values with predicted values, the differences were found to be <5% of all the responses mentioned in Table 4.

The composition of proniosomes was optimized with design of experiments in the BBD using Design-Expert software, and the optimized formulation batch was prepared accordingly. To obtain a more comprehensive analysis of selected parameters and their interactions through the use of BBD, contour plots are developed for every response that divides the plot surface into desirable and nondesirable zones. The optimized batch B1 contained 0.45 g of span 60, 0.1 g of cholesterol, and 3.877 g of mannitol. Figure 5 shows the yellow area, which was the optimized area that provides a maximum design space for optimization, and the formulated batch B1 fell in the yellow region. Hence, batch B1 was selected as the optimized batch.

3.4. Characterization of Optimized Formulation. Niosomes were prepared from the proniosomes by hydration using phosphate buffer, pH 6.8, followed by sonication for 3 min. The resulting dispersion was evaluated for VS, PDI, ZP, % EE, and % CDR. The average VS of optimized proniosome-derived niosomes was 199.95 ± 5.4 nm, and the PDI was 0.212 ± 0.11 (Figure 6a). The PDI of the formulation determines the homogeneity of particles in the suspension. It represents the ability of particles to aggregate, and the value of PDI should be less than 0.300. The PDI values of the formulation are less than 0.300, which represents a homogeneous dispersion medium. The optimized batch B1 of proniosome-derived niosomes had

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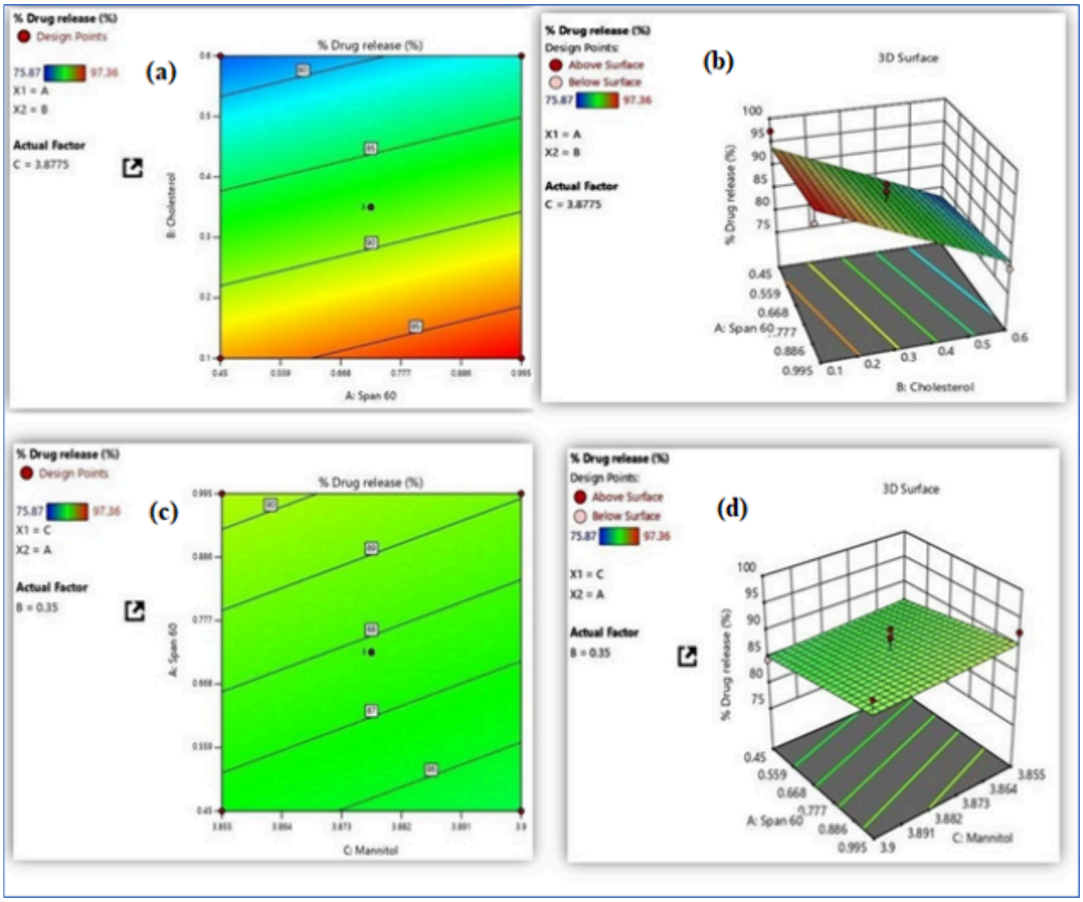

Figure 4. (a) Contour plot showing the effect of span 60 vs cholesterol on % cumulative drug release, (b) 3D surface response plot showing the effect of span 60 vs cholesterol on % CDR, (c) contour plot showing the effect of span 60 vs mannitol on % CDR, and (d) 3D surface response plot showing the effect of span 60 vs mannitol on % CDR.

Table 4. Predicted and Actual Responses of the Checkpoint Batch

	batch P1				batch P2			
evaluation parameter	predicted value	actual value	residual	% error	predicted value	actual value	residual	% error
Y_1: VS	348	357	−9	2.58	375	379	−4	1.06
Y_2: % EE	94.22	96.00	−1.78	1.88	96.95	97.32	−0.37	0.38
Y_3: % CDR	89.18	90.38	−1.2	1.13	93.27	97.12	−3.85	0.87

a zeta potential of −32.6 ± 0.40 mV, indicating the good stability of the prepared formulation, and they are less prone to flocculation (Figure 6b). The lower the PDI values (<0.4), the more homogeneous is the suspension.[30] The increased stability of niosomes is attributed to their highly positive or highly negative surface charge, which prevents colloidal particles in the niosomes from aggregating due to repulsion between similarly charged particles.[28] % EE achieved by batch B1 was 99.25 ± 2.24%. The drug release in the prepared formulation batches is shown in Figure 7. *In vitro* release study suggested that the prepared proniosomal formulation batch B1 showed maximum drug release compared to the drug and other batches. The % CDR of the optimized batch B1 was found to be 97.36% in 24 h.

3.4.1. FTIR Spectroscopy Analysis. FTIR spectra of IRB, span 60, cholesterol, mannitol, physical mixture, and proniosomal formulation batch B1 are illustrated in Figure 8. The characteristic absorption peaks of pure drug IRB were found at 1721.52 cm^{-1} (C═O stretch), 1618.28 cm^{-1} (N−H stretch), and 1623.28 cm^{-1} (C═C aromatic). The FTIR spectra of the physical mixture showed peaks at 1721.52 cm^{-1} (C═O stretch), 1613.48 cm^{-1} (N−H stretch), and 1613.48 cm^{-1} (C═C aromatic). The FTIR spectra of proniosome formulation also showed the characteristic peaks of 1613.48 cm^{-1} (C═O stretch), 1620.29 cm^{-1} (N−H stretch), and 1621.39 cm^{-1} (C═C aromatic). After the interpretation of the FTIR spectrum, it was observed that the peaks corresponding to the characteristic absorption bands and bonds of IRB were retained in the physical mixture and proniosome formulation. Thus, it can be concluded that the drug remained in the formulation and physical mixture and no signs of incompatibility were observed between the drug and excipients. All the peaks corresponding to the structure and functional groups of drugs and excipients like span 60, cholesterol, and mannitol were observed to be within the range.

3.4.2. Thermal Analysis by DSC. The pure drug and drug−excipient interactions were also evaluated by thermal analysis using DSC, and it was compared. The DSC thermogram of the drug IRB displayed a sharp endothermic peak near its melting point at 184 °C, indicating the crystalline nature of IRB. The

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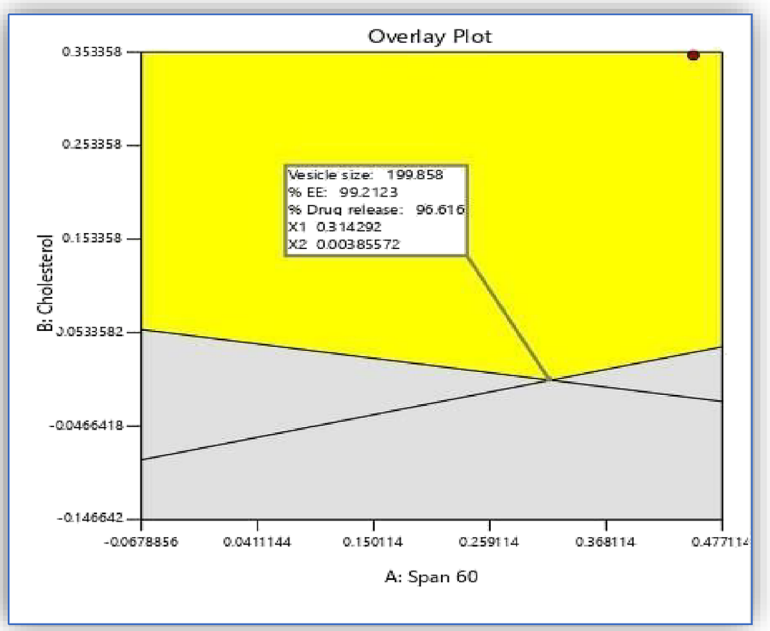

Figure 5. Overlay plot for the optimized batch B1.

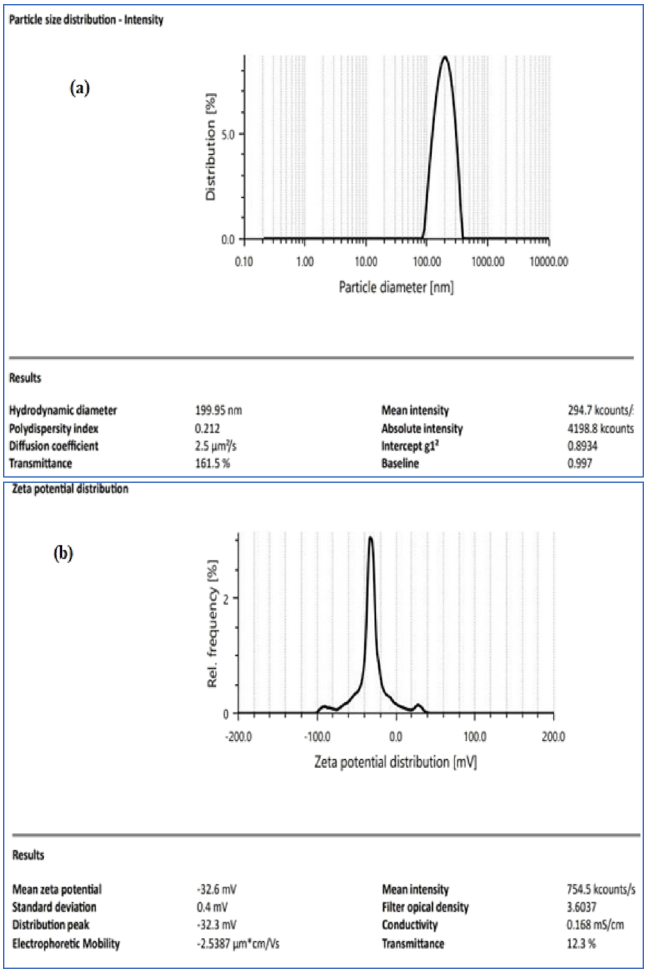

Figure 6. (a) Vesicular size and (b) zeta potential of the optimized batch B1.

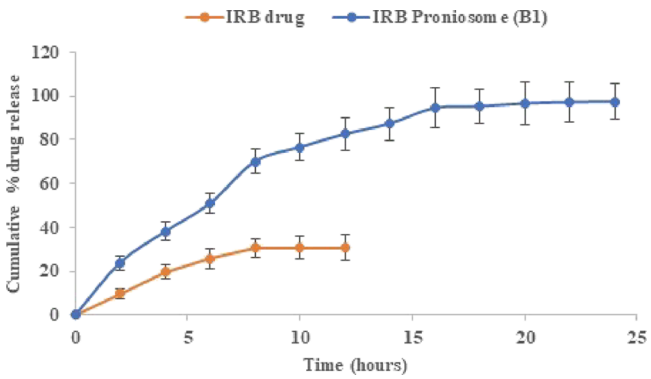

Figure 7. Drug release profile of the optimized batch B1.

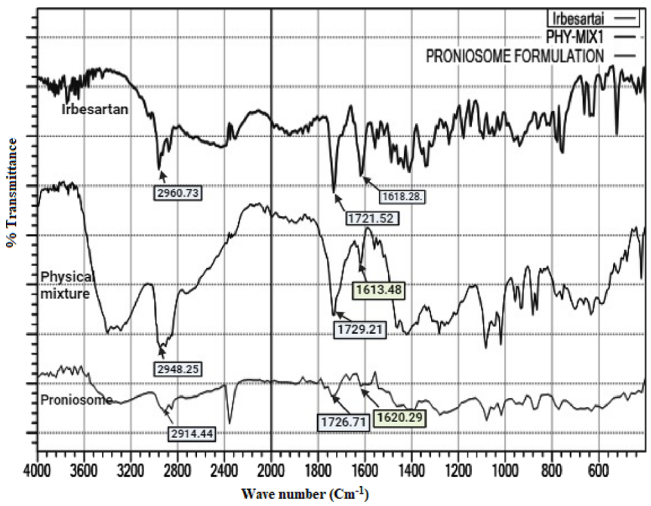

Figure 8. FTIR overlay spectra of Irbesartan, physical mixture, and proniosomal formulation batch B1.

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ACS Omega 2024, 9, 16346−16357

DSC thermogram of proniosomal batch B1 showed a less intense peak near 169 °C (Figure 9). When compared to the

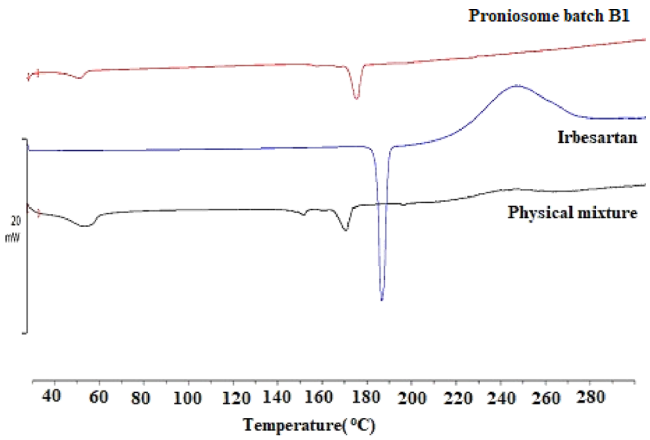

Figure 9. DSC thermogram of Irbesartan, physical mixture, and proniosome batch B1.

pure drug, the optimized formulation's melting point showed a slight shift in peak with reduced intensity, indicating that the drugs are converted into an amorphous form in the proniosomal formulation.[35]

3.4.3. XRD Analysis. XRD patterns of pure drug IRB and proniosomal powder were analyzed by an X-ray diffractometer. The sharp peak of the pure drug was observed at the diffraction angles, indicating the crystalline nature of the drug. The formulation showed a broad peak with less intensity, indicating the conversion into an amorphous state. The reduction in the intensity of crystalline peaks of IRB in the formulation is due to the molecular dispersion of the drug into excipients, and the conversion of the drug into the amorphous form takes place as shown in Figure 10.

3.4.4. SEM Analysis. The shape and surface morphology of proniosomal powder and proniosome-derived niosomes of batch B1 (i.e., an optimized batch) was analyzed by SEM. The photomicrograph indicated that the proniosomal surface was rough and crystalline due to the presence of mannitol as shown in Figure 11a. The photomicrograph of proniosome-derived

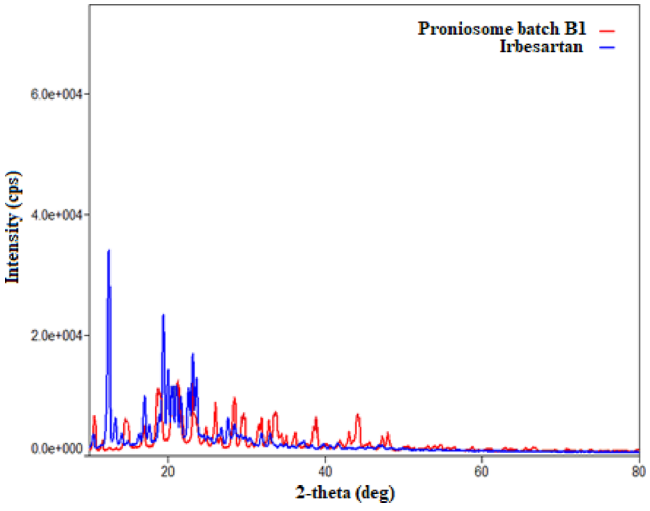

Figure 10. XRD overlay spectra of Irbesartan and optimized proniosomal formulation.

niosomes of the optimized batch B1 revealed that the niosomes are spherical as shown in Figure 11b.

3.5. Solubility Determination. The solubility of IRB and its proniosomal powder was determined in phosphate buffers of different pH values as shown in Table 5. The solubility of IRB was found to be 0.059 + 0.02 mg/mL, and that of its proniosomes was found to be 1.95 ± 0.3 mg/mL in water. The maximum solubility of IRB and proniosomes was found in phosphate buffer, pH 6.8, i.e., 0.912 ± 0.06 and 2.65 ± 0.2 mg/mL, respectively. Thus, it can be concluded that the proniosomes of Irbesartan converted the drug to an amorphous state at pH 6.8, and the solubility was observed to be increased considerably in the pH 6.8 buffer.

3.6. Stability Study. Stability testing is done on the prepared proniosomes to assess how environmental conditions, such as temperature, light, and humidity, will affect them. No major changes were observed in drug content, indicating the good stability of the optimized batch of proniosomal powder at 5 ± 2 and 25 ± 2 °C and 75 ± 5% relative humidity for 3 months. Thus, the results of the stability study showed that the proniosomal powder was found to be stable under the refrigerated condition as compared to room temperature and there were no major changes found in % EE and % CDR of the proniosomal powder for 90 days as shown in Table 6.

3.7. Pharmacokinetic Analysis. Pharmacokinetic study was conducted in rats to examine the *in vivo* bioavailability of the optimized IRB proniosome (batch B1) with respect to the pure drug suspension. Figure 12 depicts the mean plasma drug concentration versus the time curve following oral administration of proniosomal formulation and drug suspension, whereas Table 7 represents the calculated pharmacokinetic parameters. The peak plasma concentrations (C_{max}) of the IRB suspension and proniosomal formulation of batch B1 were found to be 196 ± 1.32 and 320 ± 1.74 ng/mL, respectively. The $AUC_{0\text{-}t}$ and half-life ($T_{1/2}$) values were 2133.75 ± 13.7 ng/mL h and 15.66 ± 1.7 h for IRB suspension and 4202.5 ± 17.92 ng/mL h and 17.88 ± 1.09 h for proniosome formulation, respectively. When compared to IRB suspension, IRB proniosome formulation demonstrated nearly 2-fold increases in C_{max} and $AUC_{0\text{-}t}$ ($p < 0.05$). Hence, the bioavailability of IRB was found to be increased by almost 2-fold. These findings showed that incorporating the IRB drug into proniosomes greatly increased its absorption, which in turn improved its bioavailability. The $T_{1/2}$ of the proniosome was found to be longer than that of the IRB suspension. Moreover, the proniosome formulation showed longer MRT than IRB suspension. The higher values of $T_{1/2}$ and MRT for proniosome formulation indicate the slow elimination rate of the drug, and this observation confirmed the sustained release of IRB from proniosome formulation compared with IRB suspension. The following factors may be responsible for the increased bioavailability of IRB-loaded niosomes. Similar to liposomes, niosomes first showed bioadhesion to the wall of the gastrointestinal tract before undergoing fusion or endocytosis. Furthermore, niosomes may promote lymphatic transport, hence reducing the first-pass effect and increasing bioavailability. The nanosized drug in the nanoproniosome may contribute to the increased bioavailability of the drug from the proniosome formulation. The surfactant (span 60) may be another factor contributing to the increased bioavailability. Surfactants have the potential to increase intestinal epithelial cell penetration efficiency by encouraging ultrastructural alterations and opening the tight junction.[23,36] The pharma-

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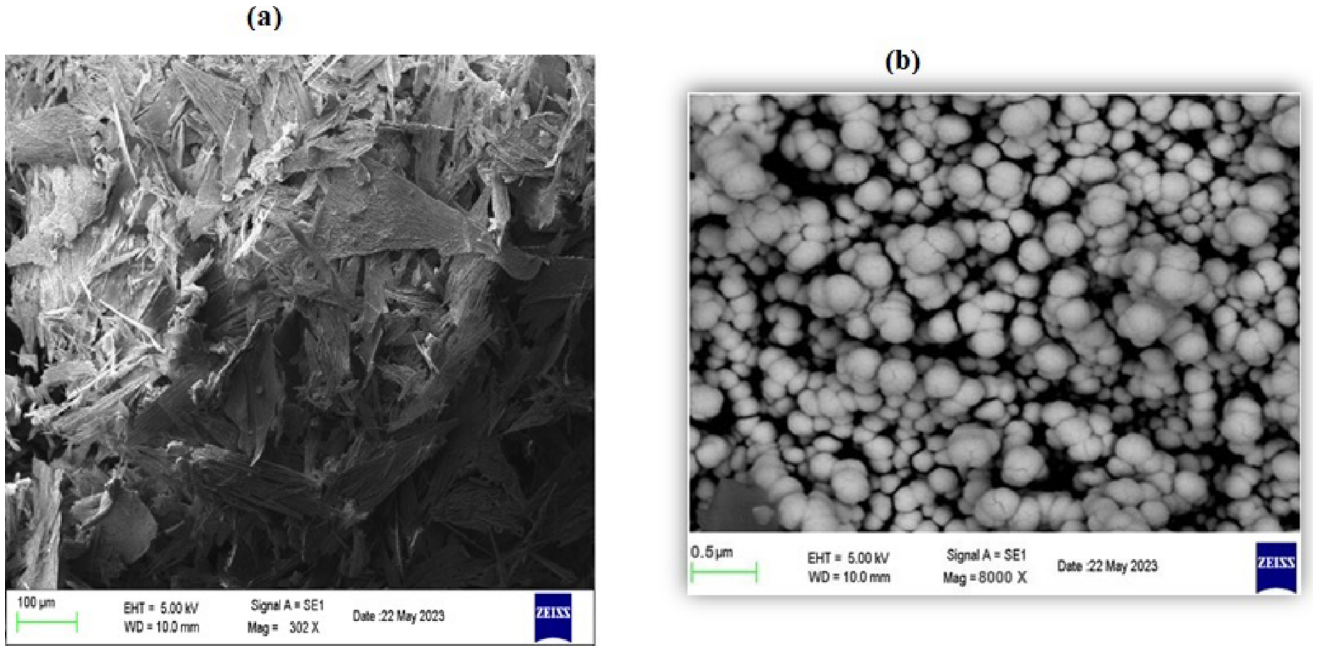

Figure 11. (a) SEM image of optimized proniosomal powder (batch B1) and (b) SEM image of optimized batch B1.

Table 5. Solubility of Irbesartan and Proniosomal Powder in Water and Different Phosphate Buffers

s. no.	solvent	solubility of Irbesartan (mg/mL)	solubility of proniosomal powder (mg/mL)
1.	water	0.059 ± 0.02	1.95 ± 0.3
2.	phosphate buffer, pH 7.2	0.387 ± 0.04	2.16 ± 0.1
3.	phosphate buffer, pH 7.4	0.632 ± 0.07	2.39 ± 0.5
4.	phosphate buffer, pH 6.6	0.848 ± 0.05	2.52 ± 0.3
5.	phosphate buffer, pH 6.8	0.912 ± 0.06	2.65 ± 0.2

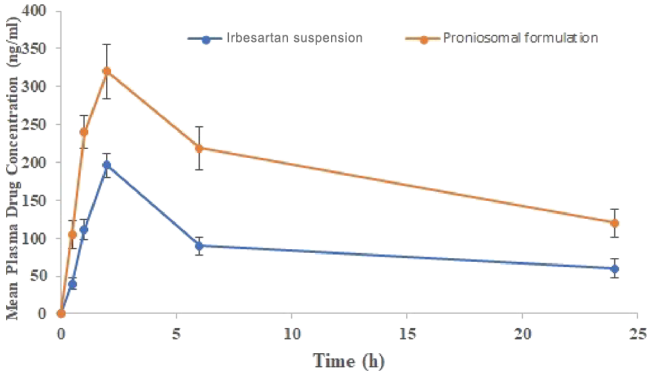

Figure 12. Plasma concentration−time curve of proniosomal formulation of Irbesartan and Irbesartan drug suspension.

cokinetic data indicate that the bioavailability of drugs with low solubility was enhanced by niosome suspension derived from proniosomes.

4. CONCLUSIONS

Irbesartan-loaded proniosome formulations were successfully prepared by the slurry method using span 60, cholesterol, and mannitol. The concentration of span 60 and cholesterol had a more significant effect on VS, % EE, and % CDR as compared with the concentration of mannitol. Solubility study showed that proniosomes improved the solubility of IRB in water and phosphate buffers. DSC and XRD analysis showed that the drug was in an amorphous form in formulation. SEM analysis of IRB-loaded proniosomes showed that vesicles are nearly uniform and spherical with smooth surfaces. *In vivo* bioavailability studies suggest that in comparison to the pure drug suspension, proniosomes enhanced the oral bioavailability of IRB by almost 2-fold. From the results, it can be concluded

Table 6. % EE and % CDR of Optimized Proniosomal Powder (B1) under Different Conditions[a]

		% entrapment efficiency		% cumulative drug release	
s. no.	days	refrigerator (5 °C)	room temperature (25 °C)	refrigerator (5 °C)	room temperature (25 °C)
1	0	99.25 ± 0.34	99.25 ± 0.34	99.36 ± 0.33	99.36 ± 0.33
2	15	99.25 ± 0.34	99.25 ± 0.37	99.36 ± 0.33	99.36 ± 0.31
3	30	99.25 ± 0.56	95.00 ± 1.39	99.15 ± 0.49	95.33 ± 1.56
4	45	99.00 ± 0.71	94.00 ± 2.78	99.03 ± 0.53	91.02 ± 3.23
5	60	98.90 ± 1.03	90.00 ± 2.23	98.70 ± 1.07	87.87 ± 2.98
6	75	98.50 ± 1.08	88.00 ± 3.01	98.54 ± 1.04	84.64 ± 1.90
7	90	98.50 ± 1.23	82.00 ± 1.89	98.13 ± 0.59	80.12 ± 2.59

[a]±SD; $n = 3$.

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ACS Omega 2024, 9, 16346−16357

Table 7. Pharmacokinetic Parameters after Oral Administration

s. no.	parameter	Irbesartan suspension	proniosomal formulation (B1)
1.	C_{max} (ng/mL)	196 ± 1.32	320 ± 1.74
2.	$T_{1/2}$ (h)	15.66 ± 1.7	17.88 ± 1.09
3.	AUC_{0-t} (ng/mL h)	2133.75 ± 13.7	4202.5 ± 17.92
4.	MRT (h)	23.83 ± 1.36	26.64 ± 1.16

that IRB proniosomes, a vesicular carrier system, are a helpful tool for increasing the oral bioavailability of lipophilic drugs.

AUTHOR INFORMATION

Corresponding Authors

Vidya Sabale − *Department of Pharmaceutics, Dadasaheb Balpande College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Nagpur 440034 Maharashtra, India;* orcid.org/0000-0001-9685-1341; Email: vidyasabale@gmail.com

Prafulla Sabale − *Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur 440033 Maharashtra, India*; Email: prafullasable@yahoo.com

Authors

Md. Ali Mujtaba − *Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University, Arar 91911, Saudi Arabia;* orcid.org/0000-0002-6372-890X

Mohammed Kaleem − *Department of Pharmacology, Dadasaheb Balpande College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Nagpur 440034 Maharashtra, India;* orcid.org/0000-0003-4681-2031

Ragini Chaware − *Department of Pharmaceutics, Dadasaheb Balpande College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Nagpur 440034 Maharashtra, India*

Ashwini Ingole − *Department of Pharmaceutics, Dadasaheb Balpande College of Pharmacy, Rashtrasant Tukadoji Maharaj Nagpur University Nagpur, Nagpur 440034 Maharashtra, India*

Yahya I. Asiri − *Department of Pharmacology, College of Pharmacy, King Khalid University, Asir 61421, Saudi Arabia*

Mohd. Zaheen Hassan − *Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Asir 61421, Saudi Arabia*

Md. Khalid Anwer − *Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*

Danish Mahmood − *Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah 52571, Saudi Arabia*

Mohammed F. Aldawsari − *Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c10506

Author Contributions

¶M.A.M. and M.K. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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