Olmesartan medoxomil loaded niosomal gel for buccal delivery: Formulation, optimization, and ex vivo studies

Short Title: Olmesartan niosomal gel for buccal delivery

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ABSTRACT
Objective: Olmesartan medoxomil (OLM) is a low bioavailability antihypertensive drug. This study was aimed to prepare and optimize an OLM niosomal gel and investigate the drug permeation via a chicken buccal pouch.

Methods: OLM loaded niosomes were prepared using a film hydration technique. The vesicle size, zeta potential, entrapment efficiency, and percentage cumulative drug release of niosomes were evaluated. The niosomes were incorporated into a Carbopol 974P (1.5 % w/v) gel and drug permeability of niosomal gel was evaluated. The formulations of the niosomal gel were optimized using the Box-Behnken design. The optimized formulation was further characterized using transmission electron microscopy (TEM) and FTIR analysis.

Results: The particle size and zeta potentials of optimized niosomal formulations were found to be 296.4 nm and -38.4 mV, respectively. Based on TEM analysis, the niosomes were found spherical in shape. The permeability, flux, and permeability coefficient of optimized niosomal gel was found to be 0.507 mg/cm $^2$, 0.083 mg/cm $^2$ x h, 041 cm/h, respectively. The histopathology evaluation revealed that the niosomal gel had better permeability compared to OLM gel.

Conclusion: Based on the results of the OLM niosomal gel, it can be concluded that the formulation can be beneficial in increasing the bioavailability, resulting in better therapeutic efficacy.

Key words: Box-Behnken design, Buccal delivery, Histopathology, Niosomal gel, Olmesartan medoxomil, Permeability

INTRODUCTION
The buccal route of drug administration is an alternative to the oral route, particularly for gastro irritants and drugs with low bioavailability. The high vascularisation of the buccal mucosa allows for direct blood flow to the systemic circulation via the jugular vein, avoiding drug metabolism through the gastrointestinal and liver routes. Niosomes are novel drug delivery systems in which the drug is encapsulated in a bilayer of non-ionic surface active agents consisting of a vesicle. They can accommodate both hydrophobic and hydrophilic drugs and act as reservoirs for sustained release of drugs. Niosomes also help increase targeted drug delivery and oral bioavailability of poorly bioavailable drugs, therapeutic efficacy, and minimize drug toxicity. Niosomes can resolve some drawbacks associated with liposomes i.e., leakage, aggregation, stability, even though they are structurally similar. The mucoadhesive films containing niosomes can improve drug permeation, reduce skin irritation, and prevent the first-pass effect. Profound penetration of nanovesicles into the buccal mucosa can be achieved due to their small size of the particles and surface properties. Due to the small size of niosomes and lipid nature, the drug permeation in buccal mucosa can be improved while comparing with plain drug. Buccal delivery of niosomal formulations has been reported by various researchers to improve the bioavailability as well as local action of drug like metoprolol, benzocaine, and lornoxicam. Various formulations such as self-micro emulsifying drug delivery system (SMEDDS), nano capsules, nanostructured lipid carriers, nanosuspension, nanocrystals, and liquisolid Compacts of Olmesartan medoxomil (OLM) improved oral bioavailability. OLM belongs to the class of drugs known as angiotensin II receptor antagonists. It inhibits the action of certain natural substances that stiffen blood vessels, enabling better blood flow and heart pumping. The OLM is a poorly bioavailable drug (28%) through the oral route. Because of its low aqueous solubility (8 µg/ml) and high lipophilicity (log P 4.31), OLM is classified as a BCS Class II drug. The absorption potential of the buccal mucosa is influenced by the lipid solubility and molecular weight of the drug. The molecular weight of OLM is 558.5 g/mol and proper elimination half life ($t_{1/2}$ = 13 h) make it a suitable candidate for administration by buccal route. During
gastrointestinal absorption, OLM convert to Olmesartan by ester hydrolysis. Thus, the buccal mucosa has been explored as a potential location for the delivery of drugs because of its excellent accessibility, low enzymatic activity and avoids first-pass hepatic metabolism. Although, works have been done for improving the bioavailability of OLM by administering oral route but OLM loaded niosomal buccal gel has not done so far. So an attempt was made to develop OLM loaded niosomal gel as a carrier for buccal delivery, which can improve drug permeation and reduce the pre systemic metabolism of the drug. The aim of this research work was to prepare and optimize niosomal gel for buccal delivery of OLM and evaluate drug permeation through chicken mucosa.

**MATERIALS AND METHODS**

**Materials**

OLM was obtained gift sample from Glenmark pharmaceuticals, Mumbai. Sorbitan monostearate (span 60), dialysis bag was purchased from Himedia, Mumbai. Aloevera oil (AO), Carbopol 974P were procured from Yarrow chem, India. Cholesterol was purchased from SD fine chem, Mumbai. The chemicals used in the study were all of an analytical grade.

**Preparation of the OLM calibration curve**

Stock solution of olmesartan for UV determination was prepared at concentration of 50 μg/ml in 10 % (v/v) methanol in phosphate buffer (pH 6.8). The working standard solutions were prepared by diluting the stock solution in the concentration range from 2.5 to 25 μg/ml. The solutions were scanned in a UV-Visible spectrophotometer (Shimadzu UV-1800). The samples were analyzed for their respective absorbance at a λmax of 257 nm. The experiment was performed three times for each sample. The limit of detection (LOD) and limit of quantification (LOQ) for OLM by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y intercept of regression equation.

**Preparation and optimization of OLM loaded niosomes**

Niosomes were prepared using the lipid film hydration method with slight modification. Span60, cholesterol, and AO were dissolved in 10 ml of chloroform and methanol (2:1 V/V ratio) in a round bottom flask (Table 1 and Table 2). To the above mixture 40 mg of OLM was added and mixed properly. The solvent was evaporated from the round bottom flask using a rotary flash evaporator (R-3 Rotavapour, Buchi) under a vacuum of 10 mbar at a temperature of 50 °C until a smooth, dry lipid film was obtained. Then, the dried film was hydrated with 10 ml of 7.4 phosphate buffer saline and sonicated for 1 min at 50 % and 40 pulse using ultra sonicator (Model 300 V/T ultrasonic homogenizer, Biologics) to obtain niosomal dispersion. The niosomal dispersion was kept at 2-8 °C overnight. The niosomal formulations were optimized using a Box–Behnken design (Design-Expert version10; Stat-Ease Inc.). The independent variables such as span 60, cholesterol, and AO were used at low, medium, and high levels for preparing 17 formulations and depicted in Tables 1 and 2. Vesicle size (Y1), cumulative drug release (Y2), and permeability (Y3) were chosen as dependent variables. Additionally, response surface 3D graphs were plotted to show the effects of the predetermined variables on the measured responses.

**Characterization of niosomes**

**Particle size and zeta potential**

The mean vesicle size and zeta potential were measured using dynamic light scattering techniques (Horiba SZ 100, Japan). The measurement was done at an angle of 90° in 10 mm diameter cells at temperature of 25 °C. The measurements of vesicle size and zeta potential were carried out three times.

**Entrapment efficiency**

The ultracentrifugation method was used to assess the entrapment efficiency of niosomal formulations. Niosomal suspension (10 ml) were poured into a centrifuge tube and centrifuged at a speed of 25000 relative centrifugal force (RCF) by a cooling centrifuge for 90 min at 4 °C and then filtered to obtain clear fraction by using Whatman filter paper. The free drug was analysed using a UV-Visible spectrophotometer (Shimadzu UV-1800) at 257 nm on the clear fraction, and the entrapment efficiency was estimated using the formula.

\[ \text{Entrapment efficiency} \left( \% \right) = \frac{W_t - W_f}{W_t} \times 100 \]

Where, \( W_t \) = total amount of drug, \( W_f \) = amount of free drug

**Cumulative drug release studies**

The dialysis bag was washed with distilled water. The niosomal dispersion (5 ml) was transferred into a dialysis bag and both ends were sealed and the dialysis bag was put in a beaker containing 100 ml phosphate buffer (pH 6.8). The beaker was then positioned above the magnetic stirrer. 3 ml samples were taken out and substituted with fresh medium at different time intervals up to 24 h. Samples were diluted properly and quantified the drug using UV-visible spectrophotometer (Shimadzu UV-1800) at 257 nm. The % cumulative drug release (CDR) from different formulation was calculated. The % CDR of each formulation was performed three times.

**Formulation of niosomal gel**

The known volume of niosomal formulation was centrifuged for 90 min at 4°C and 25000 RCF in a cooling centrifuge. The highly viscous portion of niosomes was collected by decanting the supernatant and added to the 1.5 % Carbopol 974P gel base. The gel containing OLM niosomes was mixed properly using a mortar and pestle. Afterward, glycerine (1 %
W/W) and sucrose (q.s.) were added to the gel, while it was continuously triturated. Triethanolamine was used to adjust the pH to buccal pH.

**Evaluation of niosomal gel**
The calibration of pH meter was performed before measuring the pH of the gel and measurements were obtained by immersing the glass electrode in the gel formulations. The spreadability of gel formulations was performed by taking 1 g of gel on the lower slide and positioned the upper slide on the top of the gel. The weight (500 g) was placed on the upper slide and the diameter of the spread gel was measured in cm. The content uniformity of gel was performed by taking the gel from three parts of beaker. The gel (1 g) was added to methanol and sonicated for 15 min. The filtrate was collected after filtration of the mixture using Whatman filter paper and OLM concentration was analysed using UV–visible spectrophotometer at 257 nm after proper dilution with methanol.

**Ex vivo permeation study of niosomal gel**
Permeation studies on chicken buccal mucosa were performed employing a Franz diffusion cell with an effective diffusion area of 3.14 cm² and a receiver compartment capacity of 60 ml. The mucosa was tied on donor compartment and placed the phosphate buffer (pH 6.8) in the receiver compartment. The Franz diffusion cell was positioned on a magnetic stirrer that rotated at 50 rpm while maintaining a temperature of 37 ± 0.5 °C. The niosomal gel (1 g) was transferred to donor compartment and covered with aluminium foil. Two ml of samples were taken out at specific intervals up to 6h and quantified the drug content by UV–visible spectrophotometer. The drug permeability (mg/cm²) versus time graph was plotted and compared with the OLM gel. The flux (J), Permeability coefficient (P), at 6 h and the enhancement ratio (ER) were estimated using the following equations:

\[
\text{Flux (J)} = \frac{\text{Amount of drug permeated}}{\text{Surface area of buccal mucosa} \times \text{Time}}
\]

\[
\text{Permeability coefficient (P)} = \frac{\text{Concentration}}{\text{Flux}}
\]

\[
\text{Enhancement ratio (ER)} = \frac{\text{Flux of niosomal gel formulation}}{\text{Flux of gel formulation}}
\]

**FT IR study**
FT-IR (Bruker, Alpha-E, Germany) was utilized to analyse OLM, span 60, cholesterol and niosomes ranging from 4000 to 600 cm⁻¹ at room temperature.

**Transmission electron microscopy (TEM)**
Niosomal formulation was stained with a 1% phosphotungstic acid and monitored the shape of niosomes under transmission electron microscope (JEM 2100, Jeol, Japan).

**Histopathology**
After the application of gel, the cross sectioned chicken mucosa was stained with Haematoxylin and eosin for observing the histological alterations. The results were compared with control chicken mucosa.[7]

**Statistical Analysis**
The results were expressed as mean ± SD (n=3). The group means were compared using a student's t-test. A value of p < 0.05 was used to denote statistical significance.

**RESULTS AND DISCUSSION**

**Calibration curve of OLM**
The calibration curve of OLM was plotted using drug concentration on X-axis and absorbance on Y-axis. The calibration curve of OLM is depicted in Figure 1. The calibration plot of OLM showed good linear relationship with standard regression equation, \( y = 0.037x -0.003 \) in 10 % (v/v) methanol in phosphate buffer (pH 6.8) medium over the concentration range studied. The correlation coefficient (R² = 0.9997) was indicative of high significance.. The linear regression data for the calibration plot is indicative of a good linear. The LOD for OLM was found to be 0.46 μg/ml while the value of LOQ was found to be 1.39 μg/ml. The LOD and LOQ were found to be in the microgram level indicating the sensitivity of the method.

**Characterization of niosomes**
The size of niosome vesicles was found to range from 265.3 ± 5.3 nm to 344.6 ± 4.2 nm. The Polydispersity index (PDI) of niosomes was found ranged from 0.21 ± 0.06 to 0.33 ± 0.09. PDI show the homogeneity of vesicle size. The lower value of PDI shows formulation is more homogeneous in nature. The zeta potential was found in the range between -32.6 ± 1.8 and -38.4 ± 2.3 mV. Stable formulations have a zeta potential between +30 mV and -30 mV. The entrapment efficiency was found between 69.34 ± 1.9 % and 86.23 ± 2.7 %. Higher concentrations of span 60 may increase the possibility of vesicle aggregation, which frequently lowers the possibility of forming a stable film surface. As a result, there is drug leaking, which lowers the drug entrapment efficacy. It was found that raising the cholesterol level improved the effectiveness of drug entrapment.

**Analysis of design**
Three levels i.e. high, middle, and low were used for investigating each independent factor in Box-Behnken design. In this study, the manufacturing process of niosomal formulations was optimized by considering three independent variables at three levels (Table 2), as well as their binary interactions and polynomial outcomes. The three independent variables were optimized in 17 formulations, with 5 replicates of the center point. Based on the above evaluation studies, the vesicle size, % CDR, and permeability were chosen as responses for optimizing niosomal formulation.

**Vesicle size**
The effect of A, B, and C represent the average result of changing one variable at a time from its low level to its high level. From the above formula, it is stated that the high concentration of Span 60 (A; p < 0.6789 and F-value 1.865) showed a prominent effect on vesicle size than the cholesterol concentration (B; p < 0.0021 and F-value 22.66) and AO (C; P < 0.0275 and F value 7.70). The p-value (< 0.0001) indicated that cholesterol (B), AO (C) and their combination respectively, have a synergistic and antagonistic effect on vesicle size as a response variable. The combination of AB (span60 and cholesterol), AC (span60 and AO) had a greater negative effect on vesicle size, where BC (cholesterol and AO) witnessed positive effect. It is asserted by respective p-value and coded equation. In addition to that the coded factor claims that a synergistic effect was observed in binate amount of constrained independent variable such as A and C. The ANOVA table of vesicle size, % CDR, and permeability of niosomal formulations is depicted in Table 3. The interaction between span 60 and AO had significantly shown negative effect on vesicle size. The interaction between span 60 and cholesterol had significantly shown negative effect on vesicle size. The vesicle size was found the range from 265.3 nm to 344.6 nm. Based on the results, cholesterol played a significant role in particle size. It was predicted that at low cholesterol levels, nonionic surfactant and cholesterol would pack tightly together, increasing curvature and shrinking in size.\(^9\) Graph claims that term BC (cholesterol and AO) is associated with positive effects on vesicle size under constrained conditions of increasing cholesterol from low concentration (0.125 mmol) to high concentration (0.25 mmol) and AO at moderate level (0.375 ml) with constant level of span 60 (0.25 mmol) shown antagonistic effect of response factor. It is possible that at higher levels of cholesterol, it can directly cause vesicle fusion and reduce the vesicle size from 301 nm to 259 nm, as shown in graph. On the other hand, in case of high AO concentration on constrained conditions, the cholesterol concentration increased and vesicle size increased. As AO concentration increases, high surfactant charge increases vesicle aggregation and also increases cohesive force, by reducing interfacial tension between phases.\(^20\) This mechanism influences of increasing of vesicle size. Based on the analysis of the three (AB, AC, BC) second order of interactions BC shown greater influence on response factor (vesicle size). When the concentration of cholesterol decreases, the hydrophobicity of the bilayer membrane increases resulting increment of the vesicle size to achieve a more thermodynamically stable shape.\(^21\) At low levels of AO and increasing the cholesterol concentration, it was found that the vesicle size was decreased but at a high level of AO and increasing the cholesterol concentration, it was found that the vesicle size was increased (Figure 2). It may be due to AO occupied in the space of surfactant molecule influencing to increase the vesicle size. It was determined that vesicle size increased as Span 60 concentration increased, possibly because stronger contraction caused vesicle aggregation. The response surface plot revealed that the independent variables Span 60, cholesterol, and AO showed significant influence on vesicle size (Figure 3). The quadratic equation of vesicle size was generated as follows:

\[
\text{Vesicle size} = + 272.56 - 0.51A - 5.51B + 3.21C - 12.50AB - 5.35AC + 13.22BC + 32.51A^2 - 22.23B^2 - 27.68C^2
\]

% CDR
It was stated that, AO (C; p < 0.0001 and F value 545.18) showed a prominent effect on cumulative drug release compared to the non-ionic surfactant (A; p < 0.003 and F value 19.67) and cholesterol (B; p < 0.019 and F value 9.0). AB, AC, and BC terms were tested for their effects on % CDR using the factor tool. The results of the study claim that AC does not obey additive fashion. Instead, AC (span 60 and AO) showed negative effect on decreasing % CDR with constrained conditions AO at low level to high level (0.25 ml to 0.5 ml). The % cumulative drug release was found between 65.13 % and 96.22 %. On further increment of span 60 (A) the graph witnessed decline of % CDR as 77.34 %. By increasing the level of AO (C) term and low level of span 60 (A) term shown % CDR as 13.12B \(^2\) + 27.68C\( ^2\). The quadratic equation of % CDR was generated as follows:

\[
\% \text{ CDR} = + 72.96 - 2.10 A - 2.39B + 8.29C - 2.53AB - 6.21AC + 3.80BC + 9.58B^2 + 5.89C^2
\]

Permeability
It was stated that, AO (C; p < 0.0001, F-value 698.44) had an eminent effect while compared with surfactant concentration (A; p < 0.0003, F-value 44.94) and cholesterol concentration (B; p < 0.0001, F-value 9.00). The cholesterol and AO concentration (C; p < 0.0001, F-value 698.44) showed a positive effect on permeabilities as a result the permeation increases from 0.421 mg/cm\(^2\) to 0.507 mg/cm\(^2\) at constrained condition as AO (C) at high level (0.5 ml) and temperature at room condition. Cholesterol influences the fluidity of the membrane; at low temperatures it increases fluidity. In addition to that AO at high level enhance the permeability by facilitated permeation i.e. transient reduction in barrier resistance of SC (stratum corneum). Composition of chicken buccal mucosa and AO also alters lipid bilayer fluidity. The above observations show that there is a possibility of high drug permeation by AO, as evidenced by the dekeratinization of coenocytes in the Chicken buccal mucosa (SC).\(^26\) Nonionic surfactant concentration showed a promising effect on permeability. The addition of surfactant, which helps to solubilize lipid in the mucosa and allows for...
high vesicle penetration, may have higher drug permeability. High permeability of the drug obtained with high concentrations of cholesterol and AO. High concentrations of nonionic surfactant had a negative effect on permeability. The interaction between nonionic surfactant and AO showed a negative effect on permeability. The drug permeation was found to range from 0.321 mg/cm² to 0.507 mg/cm². The permeability study of formulations is depicted in Table 4 and Figure 5. The presence of AO, increased drug permeation could be attributed to the disruption of the structural arrangement of the lipid sequences in the mucosa, which promotes lipid fluidity. At a high level of AO concentration, the permeability of the drug was increased by increasing the concentration of cholesterol. The quadratic equation of permeability was generated as follows

\[
\text{Permeability} = +0.3796 -0.0085A +0.0058B +0.0448C -0.0318AC +0.0278BC -0.0112A^2 +0.0263B^2 +0.0198C^2
\]

The effect of any two variables on the response parameter was used to generate the response surface plot of Vesicle size, % CDR, and Permeability and is depicted in Figure 3.

**Formulation optimization**

In all responses, the predicted R² values were found to be in good agreement with the adjusted R². It was preferable to have a signal to noise ratio greater than 4 (Table 4). The trial runs were fitted in design of experiment software and analyzed by ANOVA. The niosomal gel formulation was optimized based on the particle size, CDR and permeability studies. Based on the desirability value obtained by the software and closest to 1 was chosen as the optimized formulation. Based on the analysis, NF8 where, span 60 (0.375 mmol), cholesterol (0.25 mmol), and AO (0.5 ml) (desirability = 0.89) was considered the optimized formulation.

**TEM**

It was revealed that the niosomes were spherical shape and uniform size which was confirmed by TEM study (Figure 5). The vesicle size of niosomes was found in the TEM study showed good agreement with dynamic light scattering method.

**FTIR**

The FTIR spectra of OLM, Span60, cholesterol, AO, and niosomal formulations are depicted in Figure 6. The FT-IR spectra of OLM were found peaks at 2995.37 cm⁻¹, 2923.16 cm⁻¹ due to C-H stretching, 1708.12 cm⁻¹, 1831.99 cm⁻¹ due to C-O stretching and 3299.12 cm⁻¹ due to N-H stretching. The same peaks were found in niosomal formulation and there were not significant changes in the wave number in the formulations. Thus, it can be confirmed that drug was entrapped in the formulations.

**Evaluation of niosomal gel**

The drug content of OLM niosomal gel and OLM gel was found to be 97.9 ± 3.5 % and 98.60 ± 3.2 % respectively. The pH of the OLM niosomal gel and OLM gel was found to be 6.5 and 6.7 respectively, which could be within tolerable limits. Spreadability is responsible for supplying the right dose to the intended place and adding it to the substrate rapidly. The results of spread ability studies, OLM niosomal gel found to be 5.6 ± 0.3 cm and revealed significantly higher (P < 0.05) than the OLM gel (4.3 ± 0.5 cm).

**Ex vivo permeation studies**

Chicken pouch mucosa is believed a suitable model for ex vivo permeation studies because of it is widely available and offers an alternative to the keratinized mucosa of rats and partially keratinized rabbits mucosa. Ex vivo investigations could alter it to mimic human mucosa.

The ex vivo buccal permeation of OLM niosomal gel and OLM gel is shown in Table 5 and Figure 7. After 6 hours, the results showed that ex vivo mucosal permeation was greater in the case of OLM niosomal gel compared to OLM gel loaded with an equivalent amount of drug. It was probably presence of the surfactant and AO in niosomal gel. The smaller size range of niosomes also accounted for this penetration enhancement. The results showed that the drug permeation characteristics of the optimized niosomal gel (0.507 mg/cm²) were significantly (P < 0.05) better than the plain gel (0.261 mg/cm²). The lower permeation of OLM from the suspension than niosomal formulation may be due to higher log p value of OLM. Based on ER results, it was found that OLM from niosomal buccal gel permeates significantly (P < 0.05) faster (approximately more than 2 times) compared to OLM suspension (Table 6). Niosomes can alter the drug transport through the mucosa because of adsorption on the surface of mucosa, which results in a high thermodynamic activity gradient of the drug at the interface helps act as a driving force for permeating the lipophilic drug. The ability of vesicles to enhance penetration is related to decrease the barrier properties of mucosa by niosomes. It may be explained by the superiority of niosomal carriers, which have high permeation in mucosal layers due to carrier portion integration with mucosa lipids. Surfactants in vesicular form reduce the crystallinity of the skin's intracellular lipid bilayer, improving drug permeation.

**Histopathology**

Chicken buccal mucosa without gel, OLM gel and niosomal gel application is shown in Figure 8. Histological observation revealed that control the buccal mucosa lined by stratified squamous epithelium with ducts in submucosa. A layer of smooth muscles was noticed below the submucosa. OLM gel treated buccal showed no severe damage to the buccal mucosa integrity compared to untreated control. A slight thinning of epithelium, less number of ducts in smooth mucosa and thin layer of smooth muscle was noticed in case of niosomal gel treated with OLM gel. Reduced layers of epithelium, no ducts in sub mucosa, thin layer of the submucosa and smooth muscles were noticed in case of buccal mucosa treated with OLM niosomal gel which could be an additional evidence of the enhanced permeability of drug.

**CONCLUSION**

The Box-Behnken design was used to optimise the OLM niosomal gel formulations. Noisome formulation (NF8) was chosen as an optimized formulation of the niosomal gel, for its small vesicle size (296.4 ± 3.9 nm, PDI = 0.21 ± 0.06)
and high % CDR (96.22 ± 2.9 %). The permeability study of niosomal gel formulations were performed using chicken buccal pouch. The optimized niosomal formulations showed higher permeation rates (0.507±0.017 mg/cm²) than plain OLM gel (0.261±0.013), which may be useful in increasing the systemic presence of OLM in the body. The niosomal gel exhibited significant permeation with almost 2.05 fold increased flux compared to OLM gel. Thus, the buccal administration of niosomal gel could help for improving the OLM bioavailability.

CONFLICT OF INTEREST: Authors declare no conflict of interest


REFERENCES


Figure 1: Calibration curve of OLM in 10 % (v/v) methanol in phosphate buffer (pH 6.8).
**Figure 2:** Interaction of (A) Vesicle Size (nm), (B) CDR (%), and (C) Permeability (mg/cm²).

**Figure 3:** Response surface plot of (A) Vesicle Size (nm), (B) CDR (%), and (C) Permeability (mg/cm²).
**Figure 4:** Cumulative drug release (%) of optimized niosomal formulation (NF8). Data presented as mean ± SD (n=3)

**Figure 5:** TEM study of the optimized niosomal formulation (NF8)
Figure 6: FT-IR study of (A) OLM, (B) Span60, (C) Cholesterol, (D) AO, and (E) Niosomes (NF8)

Figure 7: Ex vivo permeation studies of (A) NF1-NF6, (B) NF7-NF12, (C) NF13-NF17, and (D) OLM gel. Data presented as mean ± SD (n=3)
Figure 8: Histopathology of chicken buccal mucosa after 6 h of permeation study (A) Control, (B) OLM gel, and (C) OLM niosomal gel

Table 1: Process variables in Box-Behnken design for niosomal formulations

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Table 2: Formulation and Characterizations of niosomal formulations. Data presented as mean ± SD (n=3)

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<th>Response Niosomal gel</th>
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<td></td>
<td>A:Span 60 mmol</td>
<td>B:Cholesterol mmol</td>
<td>C:Aloevera oil mL</td>
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<tr>
<td>NF15</td>
<td>0.375</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>NF16</td>
<td>0.25</td>
<td>0.1875</td>
<td>0.5</td>
</tr>
<tr>
<td>NF17</td>
<td>0.375</td>
<td>0.1875</td>
<td>0.375</td>
</tr>
</tbody>
</table>

Table 3: ANOVA table of Vesicle size, CDR, and Permeability

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F-value</th>
<th>p-value</th>
<th>significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model</td>
<td>10268.63</td>
<td>9</td>
<td>1140.96</td>
<td>106.37</td>
<td>&lt; 0.0001</td>
<td>significant</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Vesicle size (nm)</th>
<th>CDR (%)</th>
<th>Permeability (mg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.9927</td>
<td>0.9959</td>
<td>0.9930</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td>0.9834</td>
<td>0.9907</td>
<td>0.9840</td>
</tr>
</tbody>
</table>

**Table 4:** Data generated from Box-behnken design analysis of niosomal formulations and predicted and observed values of the optimized formulation (NF8)
Predicted R² | 0.9331 | 0.9905 | 0.9516
---|---|---|---
Adeq Precision | 30.5098 | 45.6792 | 44.1961
Predicted value of optimized formulation (NF8) | 291.56 | 97.11 | 0.498
Observed value of optimized formulation (NF8) | 296.4 | 96.22 | 0.507

Table 5: *Ex vivo* permeation studies of niosomal gel formulations (Data presented as mean ± SD (n=3))

<table>
<thead>
<tr>
<th>FC</th>
<th>Permeability (mg/cm²)</th>
<th>Flux (J) (mg/cm²h)</th>
<th>Permeability coefficient (P) (cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>0.385±0.015</td>
<td>0.057±0.003</td>
<td>0.028±0.002</td>
</tr>
<tr>
<td>NF2</td>
<td>0.372±0.023</td>
<td>0.051±0.006</td>
<td>0.025±0.003</td>
</tr>
<tr>
<td>NF3</td>
<td>0.411±0.013</td>
<td>0.063±0.004</td>
<td>0.032±0.002</td>
</tr>
<tr>
<td>NF4</td>
<td>0.366±0.019</td>
<td>0.057±0.005</td>
<td>0.028±0.002</td>
</tr>
<tr>
<td>NF5</td>
<td>0.362±0.026</td>
<td>0.054±0.007</td>
<td>0.027±0.004</td>
</tr>
<tr>
<td>NF6</td>
<td>0.321±0.025</td>
<td>0.048±0.006</td>
<td>0.024±0.003</td>
</tr>
<tr>
<td>NF7</td>
<td>0.392±0.023</td>
<td>0.060±0.006</td>
<td>0.030±0.003</td>
</tr>
<tr>
<td>NF8</td>
<td>0.507±0.017</td>
<td>0.083±0.005</td>
<td>0.041±0.003</td>
</tr>
<tr>
<td>NF9</td>
<td>0.38±0.021</td>
<td>0.057±0.006</td>
<td>0.028±0.003</td>
</tr>
<tr>
<td>NF10</td>
<td>0.434±0.019</td>
<td>0.067±0.005</td>
<td>0.033±0.002</td>
</tr>
<tr>
<td>NF11</td>
<td>0.394±0.026</td>
<td>0.062±0.007</td>
<td>0.031±0.004</td>
</tr>
<tr>
<td>NF12</td>
<td>0.385±0.022</td>
<td>0.058±0.005</td>
<td>0.029±0.002</td>
</tr>
<tr>
<td>NF13</td>
<td>0.39±0.018</td>
<td>0.062±0.004</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td>NF14</td>
<td>0.384±0.026</td>
<td>0.062±0.006</td>
<td>0.031±0.003</td>
</tr>
<tr>
<td>NF15</td>
<td>0.4±0.021</td>
<td>0.055±0.005</td>
<td>0.027±0.002</td>
</tr>
<tr>
<td>NF16</td>
<td>0.474±0.018</td>
<td>0.076±0.004</td>
<td>0.038±0.002</td>
</tr>
<tr>
<td>NF17</td>
<td>0.376±0.025</td>
<td>0.057±0.007</td>
<td>0.028±0.004</td>
</tr>
</tbody>
</table>

Table 6: *Ex Vivo* permeation study of OLM gel and OLM niosomal gel (NF8). Data presented as mean ± SD (n=3)

<table>
<thead>
<tr>
<th>FC</th>
<th>Permeability (mg/cm²)</th>
<th>Flux (J) (mg/cm²h)</th>
<th>Permeability coefficient (J/C) (cm/h)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLM gel</td>
<td>0.261±0.013</td>
<td>0.040±0.002</td>
<td>0.020±0.001</td>
<td>-</td>
</tr>
<tr>
<td>OLM niosomal gel (NF8)</td>
<td>0.507±0.017</td>
<td>0.083±0.005</td>
<td>0.041±0.003</td>
<td>2.05±0.18</td>
</tr>
</tbody>
</table>