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ORIGINAL ARTICLE

Olmesartan Medoxomil-Loaded Niosomal Gel for Buccal Delivery: Formulation, Optimization, and *Ex Vivo* Studies

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ABSTRACT

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

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

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

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

Keywords: 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 vascularization 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.[1] 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 drug release. Niosomes also help increase targeted drug delivery and oral bioavailability of poorly bioavailable drugs, therapeutic efficacy, and minimizing drug toxicity. Niosome can resolve some drawbacks associated with liposomes, *i.e.*, leakage, aggregation, and stability, even though they are structurally similar. Mucoadhesive films containing niosome can improve drug permeation, reduce skin irritation, and prevent the first-pass effect. Profound penetration of nanovesicles into the buccal mucosa can be achieved because of the small size of the particles and surface properties. Due to the small size of the noisome and its lipid nature, drug permeation in the buccal mucosa can be improved compared with that

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of the plain drug. Buccal delivery of niosomal formulations has been reported by various researchers to improve the bioavailability as well as local action of a drug like metoprolol, benzocaine, and lornoxicam.[2-4] Various formulations such as self-micro emulsifying drug delivery system, nanocapsules, nanostructured lipid carriers, nanosuspension, nanocrystals, and liquisolid compacts of olmesartan medoxomil (OLM) improved oral bioavailability.[5-10] 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. OLM is a poorly bioavailable drug (28%) through the oral route.[11] Owing to its low aqueous solubility (8 µg/mL) and high lipophilicity (log P 4.31), OLM is classified as a BCS class II drug.[12] 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 its proper elimination half-life ($t_{1/2}$ = 13 h) makes it a suitable candidate for administration by the buccal route. During gastrointestinal absorption, OLM is converted to olmesartan by ester hydrolysis.[13] 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 avoidance of first-pass hepatic metabolism.[14] Although works have been done to improve the bioavailability of OLM by administering oral route, OLM loaded niosomal buccal gel has not been performed so far. Therefore, an attempt was made to develop an OLM-loaded niosomal gel as a carrier for buccal delivery, which can improve drug permeation and reduce the pre-systemic metabolism of the drug. This study aimed 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 from a gift sample from Glenmark Pharmaceuticals, Mumbai. Sorbitan monostearate (Span 60), dialysis bag, were purchased from Hi Media, Mumbai. *Aloe vera* oil (AO) and Carbopol 974P were procured from Yarrow chem, India. Cholesterol was purchased from SD Fine Chemicals, Mumbai. The chemicals used in the study were all of analytical grade.

Preparation of the OLM calibration curve

Stock solution of olmesartan for ultraviolet (UV) determination was prepared at a 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 λ_{max} of 257 nm. The experiment was performed three times for each sample. The limit of detection (LOD) and limit of quantification (LOQ) for OLM using 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 the y-intercept of the regression equation.[15]

Preparation and optimization of OLM-loaded niosome

Niosome were prepared using the lipid film hydration method with slight modification.[16] Span 60, cholesterol, and AO were dissolved in 10 mL of chloroform and methanol (2:1 *v/v* ratio) in a round bottom flask (Tables 1, 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 bar at a temperature of 50 °C at 80 rpm until a smooth, dry lipid film was obtained. The dried film was then hydrated with 10 mL of 7.4 phosphate buffer saline and sonicated for 1 min at 50% and 40 pulses using an ultrasonicator (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 version 10; Stat-Ease Inc.). Independent variables such as Span 60, cholesterol, and AO were used at low, medium, and high levels for preparing 17 formulation sand are depicted in Tables 1 and 2. Vesicle size (Y1), cumulative drug release (CDR) (Y2), and permeability (Y3) were chosen as dependent variables. In addition, response surface 3D graphs were plotted to show the effects of the predetermined variables on the measured responses.

Characterization of the 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 performed at an angle of 90° in 10mm diameter cells at a temperature of 25 °C. The measurements of vesicle size and zeta potential were performed three times.

Entrapment efficiency

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

$$\text{Entrapment efficiency (\%)} = \frac{Wt\text{-}wf}{Wt\text{-}wf} \times 100$$

Where, Wt= total amount of drug and Wf= amount of free drug

CDR 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. The dialysis bag was placed in a beaker containing 100 mL phosphate buffer (pH 6.8). The beaker was then positioned above the magnetic stirrer. Three milliliter samples were taken out and substituted with fresh medium at different time intervals up to 24 h. Samples were diluted properly and the drug was quantified using a UV-

visible spectrophotometer (Shimadzu UV-1800) at 257 nm. The percentage CDR from different formulations was calculated. The CDR (%) of each formulation was calculated three times.

Formulation of the niosomal gel

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

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

Independent variable	Low (-1)	Medium (0)	High (+1)
Span 60 (mmol)	0.25	0.375	0.5
Cholesterol (mmol)	0.125	0.187	0.25
Aloe vera oil (mL)	0.25	0.375	0.5

Evaluation of the niosomal gel

The calibration of the 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 determined by placing 1 g of gel on the lower slide and positioning 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.[17] The content uniformity of the gel was determined by taking the gel from three parts of the 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 the OLM concentration was analyzed using a UV-visible spectrophotometer at 257 nm after proper dilution with methanol.

Ex vivo permeation study of the niosomal gel

Permeation studies on chicken buccal mucosa were conducted using a Franz diffusion cell with an effective diffusion area of 3.14 cm^2 and a receiver compartment capacity of 60 mL. The mucosa was tied to the donor compartment and phosphate buffer (pH 6.8) was placed in the receiver compartment. The Franz diffusion cell was positioned on a magnetic stirrer

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

FC	Independent variable			Gelling agent	Response niosomal suspension		Response niosomal gel
	Factor 1 (X1)	Factor 2 (X2)	Factor 3 (X3)		Response 1 (Y1)	Response 2 (Y2)	Response 3 (Y3)
	A: Span 60	B: Cholestrol	C: AO	Carbopol 974P	Vesicle size	CDR	Permeability
	mmol	mmol	mL	%	nm	%	mg/cm^2
NF1	0.375	0.1875	0.375	1.5	270.4 ± 3.2	73.54 ±2.6	0.385 ± 0.015
NF2	0.375	0.1875	0.375	1.5	274.5 ± 4.1	72.21 ±3.1	0.372 ± 0.023
NF3	0.25	0.25	0.375	1.5	298.3 ± 3.6	85.13 ±2.9	0.411 ± 0.013
NF4	0.5	0.1875	0.25	1.5	331.6 ± 2.9	73.1 ± 3.1	0.366 ± 0.019
NF5	0.375	0.25	0.25	1.5	265.3 ± 5.3	72.13 ± 2.6	0.362 ± 0.026
NF6	0.25	0.1875	0.25	1.5	325.7 ± 3.7	65.13 ± 3.2	0.321 ± 0.025
NF7	0.5	0.1875	0.5	1.5	329.1 ± 4.1	77.34 ± 3.1	0.392 ± 0.023
NF8	0.375	0.25	0.5	1.5	296.4 ± 3.9	96.22 ± 2.9	0.507 ± 0.017
NF9	0.375	0.1875	0.375	1.5	269.6 ± 3.1	74.14 ± 2.3	0.38 ± 0.021
NF10	0.375	0.125	0.5	1.5	282.5 ± 3.5	93.12 ± 2.3	0.434 ± 0.019
NF11	0.25	0.125	0.375	1.5	282.8 ± 4.3	85.11 ± 3.2	0.394 ± 0.026
NF12	0.375	0.1875	0.375	1.5	271.6 ± 3.9	73.54 ± 2.9	0.385 ± 0.022
NF13	0.5	0.125	0.375	1.5	310.6 ± 3.6	86.22 ± 3.2	0.39 ± 0.018
NF14	0.5	0.25	0.375	1.5	276.1 ± 4.1	76.11 ± 2.3	0.384 ± 0.026
NF15	0.375	0.125	0.25	1.5	304.3 ± 3.2	84.23 ± 3.1	0.4 ± 0.021
NF16	0.25	0.1875	0.5	1.5	344.6 ± 4.2	94.22 ± 1.9	0.474 ± 0.018
NF17	0.375	0.1875	0.375	1.5	276.7 ± 4.1	71.35 ± 2.9	0.376 ± 0.025

SD: Standard deviation, AO: Aloe vera oil, CDR: Cumulative drug release, FC: Formulation code

that rotated at 50 rpm while maintaining a temperature of 37 ± 0.5 °C. The niosomal gel (1 g) was transferred to the donor compartment and covered with aluminum foil. Two milliliters of samples were taken out at specific intervals up to 6 h and the drug content was quantified by UV-visible spectrophotometer. The drug permeability (mg/cm^2) versus time graph was plotted and compared with the OLM gel. The flux (J), permeability coefficient (P), at 6 h, and enhancement ratio (ER) were estimated using the following equations:

$$\text{Flux (J)} = \left(\frac{\text{Amount of drug permeated}}{\text{Surface area of buccal mucosa}}\right) \times \frac{1}{\text{Time}}$$

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

$$\text{ER} = \frac{\text{Flux of niosomal gel formulation}}{\text{Flux of gel formulation}}$$

Fourier transform infrared radiation (FTIR) study

FTIR (Bruker, Alpha-E, Germany) was used to analyze OLM, Span 60, cholesterol, and niosome ranging from 4000 to 600 cm^{-1} at room temperature.

Transmission electron microscopy (TEM)

Niosomal formulations were stained with 1% phosphotungstic acid and the shape of niosomes was monitored using a transmission electron microscope (JEM 2100, Jeol, Japan).

Histopathology

After the application of gel, the cross-sectioned chicken mucosa was stained with hematoxylin and eosin to observe the histological alterations. The results were compared with control chicken mucosa.[7]

Statistical analysis

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

RESULTS

Calibration curve of the OLM

The calibration curve of OLM was plotted using drug concentration on the X-axis and absorbance on the Y-axis. The calibration curve of the OLM is depicted in Figure 1. The calibration plot of OLM showed a good linear relationship with the 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 (R^2= 0.9997) indicated high significance. The linear regression data for the calibration plot are indicative of a good linear. The LOD for OLM was found to be 0.46 µg/mL, whereas the value of LOQ was found to be 1.39 µg/mL. The LOD and LOQ were found to be at the microgram level, indicating the sensitivity of the method.

Characterization of the niosomes

The size of the niosome vesicles ranged from 265.3 ± 5.3 nm to 344.6 ± 4.2 nm. The Polydispersity index (PDI) of niosome ranged from 0.21 ± 0.06 to 0.33 ± 0.09. The zeta potential was found in the range between -32.6 ± 1.8 and -38.4 ± 2.3 mV. The entrapment efficiency was found range between 69.34 ± 1.9% and 86.23 ± 2.7%.

Analysis of the design

Three levels, *i.e.* high, middle, and low, were used for investigating each independent factor in the 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 five replicates of the center point. Based on the above evaluation studies, vesicle size, CDR (%), and permeability were chosen as responses for optimizing niosomal formulation.

Vesicle size

The effects 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 0.1865) showed a more prominent effect on vesicle size than 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 synergistic and antagonistic effects on vesicle size as a response variable. The combination of AB (Span 60 and cholesterol) and AC (Span 60 and AO) had a greater negative effect on vesicle size, whereas BC (cholesterol and AO) had a positive effect. It is asserted by the respective p value and coded equation. In addition, the coded factor claims that a synergistic effect was observed in binate amounts of constrained independent variables such as A and C. The analysis of variance (ANOVA) table of vesicle size, CDR (%), and permeability of the niosomal formulations is depicted in Table 3. The interaction between Span 60 and AO had a significant negative effect on vesicle size. The interaction between Span 60 and cholesterol had a significant negative effect on vesicle size. The vesicle size ranged from 265.3 nm to 344.6 nm.

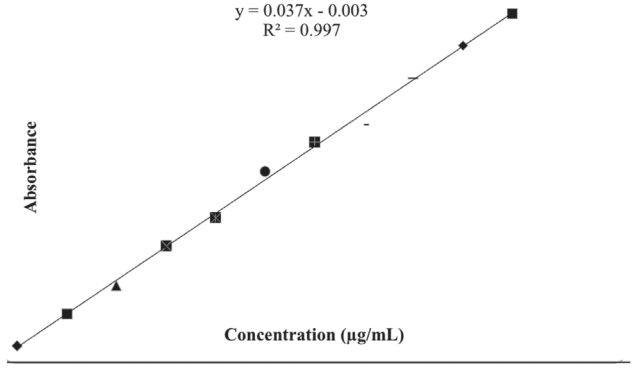

Figure 1. Calibration curve of OLM in 10% (v/v) methanol in phosphate buffer (pH 6.8)

OLM: Olmesartan medoxomil

Table 3. ANOVA table of vesicle size, CDR, and permeability

Parameters	Source	Sum of squares	DF	Mean square	*f* value	*p* value	
Vesicle size (nm)	Model	10268.63	9	1140.96	106.37	< 0.0001	Significant
	A-Span 60	2.00	1	2.00	0.1865	0.6789	
	B-cholestrol	243.10	1	243.10	22.66	0.0021	
	C-AO	82.56	1	82.56	7.70	0.0275	
	AB	625.00	1	625.00	58.27	0.0001	
	AC	114.49	1	114.49	10.67	0.0137	
	BC	699.60	1	699.60	65.22	< 0.0001	
	A^2	4449.42	1	4449.42	414.81	< 0.0001	
	B^2	724.50	1	724.50	67.54	< 0.0001	
	C^2	3226.61	1	3226.61	300.81	< 0.0001	
	Residual	75.08	7	10.73			
	Lack of fit	39.83	3	13.28	1.51	0.3415	Not significant
	Pure error	35.25	4	8.81			
	Cor total	10343.72	16				
CDR (%)	Model	0.0292	9	0.0032	110.56	< 0.0001	Significant
	A-Span 60	0.0006	1	0.0006	19.67	0.0030	
	B-cholestrol	0.0003	1	0.0003	9.00	0.0199	
	C-AO	0.0160	1	0.0160	545.18	< 0.0001	
	AB	0.0001	1	0.0001	4.50	0.0716	
	AC	0.0040	1	0.0040	137.22	< 0.0001	
	BC	0.0031	1	0.0031	104.82	0.0001	
	A^2	0.0005	1	0.0005	17.89	0.0039	
	B^2	0.0029	1	0.0029	99.30	< 0.0001	
	C^2	0.0017	1	0.0017	56.32	0.0001	
	Residual	0.0002	7	0.0000			
	Lack of fit	0.0001	3	0.0000	0.7895	0.5595	Not significant
	Pure error	0.0001	4	0.0000			
	Cor total	0.0294	16				
Permeability	Model	1344.10	9	149.34	189.78	< 0.0001	Significant
	A-Span 60	35.36	1	35.36	44.94	0.0003	
	B-cholestrol	45.55	1	45.55	57.89	0.0001	
	C-AO	549.63	1	549.63	698.44	< 0.0001	
	AB	25.65	1	25.65	32.60	0.0007	
	AC	154.38	1	154.38	196.18	< 0.0001	
	BC	57.76	1	57.76	73.40	< 0.0001	
	A^2	1.54	1	1.54	1.96	0.2047	
	B^2	386.59	1	386.59	491.26	< 0.0001	
	C^2	63.62	1	63.62	80.84	< 0.0001	
	Residual	5.51	7	0.7869			
	Lack of fit	0.2888	3	0.0963	0.0738	0.9709	Not significant
	Pure error	5.22	4	1.30			
	Cor total	1349.61	16				

ANOVA: Analysis of variance, CDR: Cumulative drug release, DF: Degrees of freedom, AO: Aloe vera oil

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 greater levels of cholesterol, it can directly cause vesicle fusion and reduce the vesicle size from 301 to 259 nm, as shown in the graph. On the other hand, in the case of high AO concentration under constrained conditions, the cholesterol concentration increased and vesicle size increased. Based on the analysis of the three (AB, AC, BC) second-order interactions, BC showed a greater influence on the response factor (vesicle size).

At low levels of AO and increasing cholesterol concentration, it was found that the vesicle size decreased, but at a high level of AO and increasing cholesterol concentration, it was found that the vesicle size increased (Figure 2). The response surface plot revealed that the independent variables Span 60, cholesterol, and AO significantly influenced vesicle size (Figure 3). The quadratic equation for vesicle size was generated as follows:

Vesicle size = + 272.56 - 0.51A - 5.51B + 3.21C - 12.50AB - 5.35AC + 13.22BC + 32.51A^2 -13.12B^2 + 27.68C^2

CDR (%)

It was stated that AO (C; $p < 0.0001$ and f value 545.18) showed a prominent effect on CDR 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 a negative effect on decreasing percentage CDR with constrained conditions AO at low level to high level (0.25 mL to 0.5 mL). The percentage CDR was found to be between 65.13% and 96.22%. At a higher level of AO (C) term and low level of Span 60 (A) term, showed CDR (%) as 94.22%. On further increment of Span 60 (A), the graph witnessed a decline of CDR (%) as 77.34%. A high concentration of cholesterol significantly influenced the CDR (%) and had a negative effect on CDR. The interaction between non-ionic surfactant and cholesterol had a negative effect on CDR (%). The interaction between non-ionic surfactant and AO had a negative effect on CDR (%). The CDR (%) of the optimized niosomal formulation is depicted in Figure 4. The quadratic equation of CDR (%) was generated as follows:

CDR (%)= + 72.96 - 2.10A - 2.39B + 8.29C - 2.53AB - 6.21AC + 3.80BC + 9.58B^2 + 3.89C^2

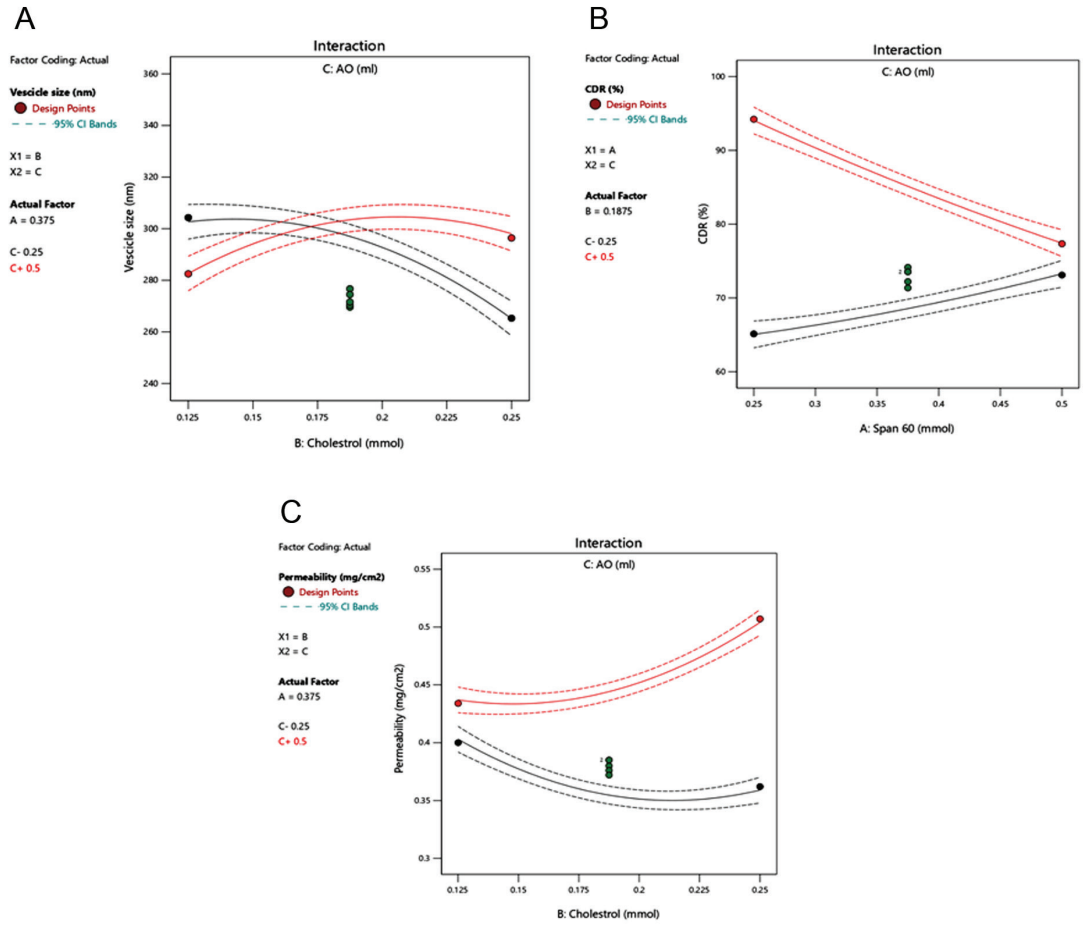

Figure 2. Interaction of (A) vesicle size (nm), (B) CDR (%), and (C) permeability (mg/cm^2)

CDR: Cumulative drug release

Permeability

It was stated that AO (C; $p < 0.0001$, f value 698.44) had an eminent effect 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 concentrations (C; $p < 0.0001$, f value 698.44) showed a positive effect on permeability as a result the permeation increases from 0.421 mg/cm^2 to 0.507 mg/cm^2 at the constrained condition as AO (C) at high level (0.5 mL) and temperature at room condition. High permeability of the drug obtained with high concentrations of cholesterol and AO. High concentrations of non-ionic surfactant had a negative effect on permeability. The interaction between the non-ionic surfactant and AO showed a negative effect on permeability. The drug permeation was found to range from 0.321 mg/cm^2 to 0.507 mg/cm^2. The permeability study of the formulations is depicted in Table 4 and Figure 5. The presence of AO and 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 AO concentration, the permeability of the drug was increased by increasing the cholesterol concentration. The quadratic equation of permeability was generated as follows:

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 which is depicted in Figure 3.

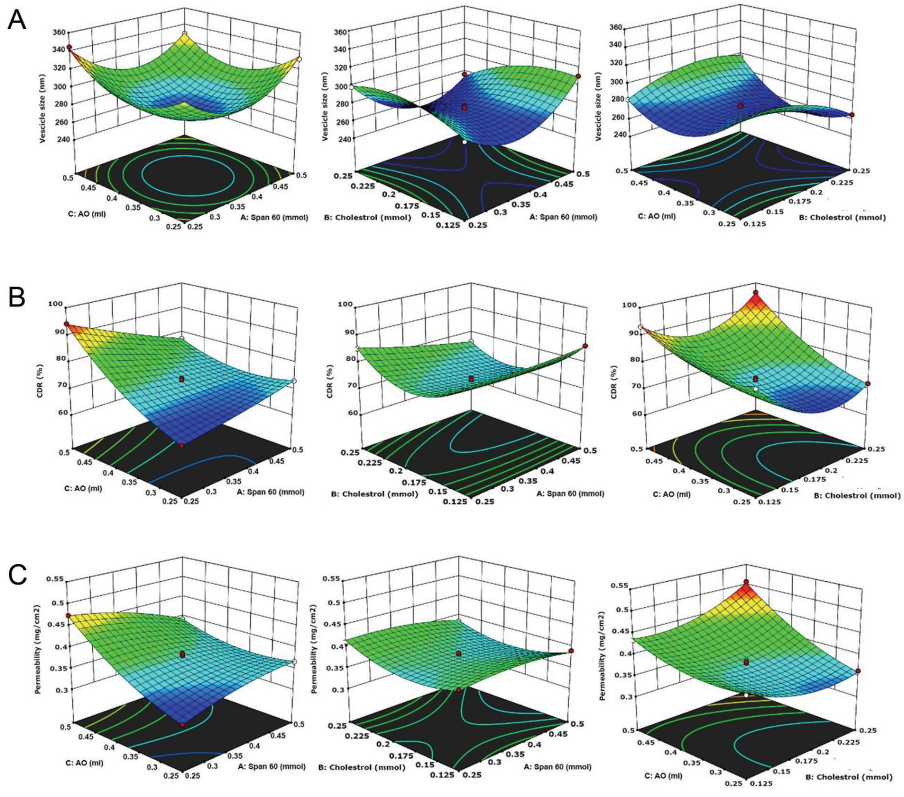

Figure 3. Response surface plot of (A) vesicle size (nm), (B) CDR (%), and (C) permeability (mg/cm^2)
CDR: Cumulative drug release

Table 4. Data generated from Box-Behnken design analysis of niosomal formulations and predicted and observed values of the optimized formulation (NF8)

Responses	Vesicle size (nm)	CDR (%)	Permeability (mg/cm^2)
R^2	0.9927	0.9959	0.9930
Adjusted R^2	0.9834	0.9907	0.9840
Predicted R^2	0.9331	0.9905	0.9516
Adeq precision	30.5098	45.6792	44.1961
Predicted value of the optimized formulation (NF8)	291.56	97.11	0.498
Observed value of the optimized formulation (NF8)	296.4	96.22	0.507

CDR: Cumulative drug release

Formulation optimization

In all responses, the predicted R^2 values were found to be in good agreement with the adjusted R^2. It was preferable to have a signal-to-noise ratio greater than 4 (Table 4). The trial runs were fitted in the design of the experiment software and analyzed by ANOVA. The niosomal gel formulation was optimized on the basis of particle size, CDR, and permeability studies. Based on the desirability value obtained by the software, the closest value 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) were considered the optimized formulation.

TEM

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

FT-IR

The FTIR spectra of the OLM, Span 60, cholesterol, AO, and niosomal formulations are depicted in Figure 6. The FTIR spectra of OLM showed 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 the niosomal formulation, and there were no significant changes in the wavenumber in the formulations. Thus, it can be confirmed that the 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. In the results of spreadability studies, OLM niosomal gel was 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 to be a suitable model for *ex vivo* permeation studies because it is widely available and offers an alternative to the keratinized mucosa of rats and partially keratinized rabbit mucosa.[27] Because of the non-keratinized nature of chicken mucosa, *ex vivo* investigations could alter it to mimic human mucosa.

The *ex vivo* buccal permeation of the OLM niosomal gel and OLM gel is shown in Table 5 and Figure 7. After 6 h, the results showed that *ex vivo* mucosal permeation was greater in the case of OLM niosomal gel than in the case of OLM gel loaded with an equivalent amount of drug. It was probably the presence of the surfactant and AO in the niosomal gel. The smaller size range of the niosomes also accounted for this penetration enhancement. The results showed that the drug permeation characteristics of the optimized niosomal gel (0.507 mg/cm^2)

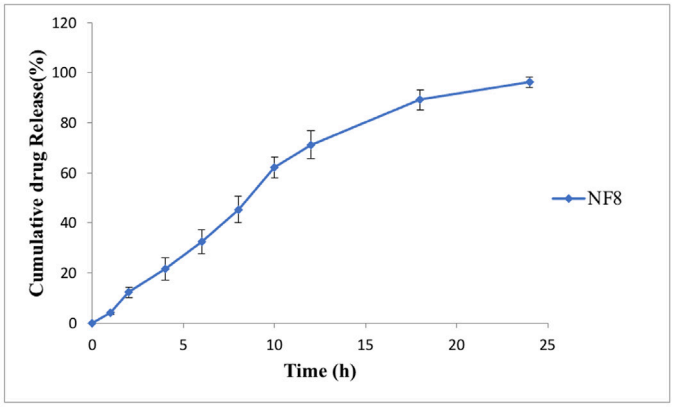

Figure 4. Cumulative drug release (%) of optimized niosomal formulation (NF8). Data are presented as mean ± SD (n= 3)

SD: Standard deviation

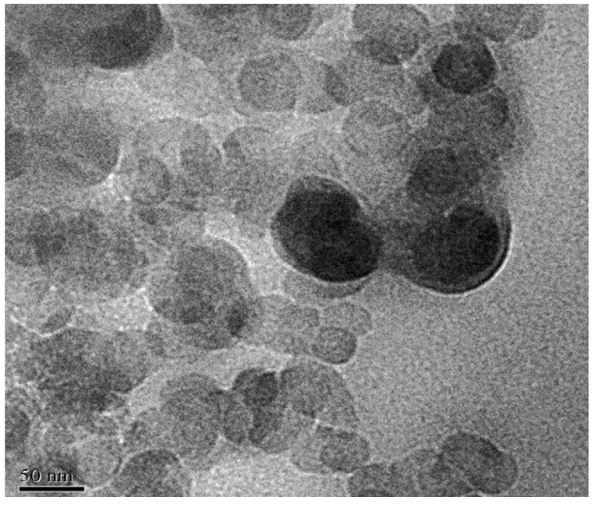

Figure 5. TEM study of optimized niosomal formulation (NF8)

TEM: Transmission electron microscopy

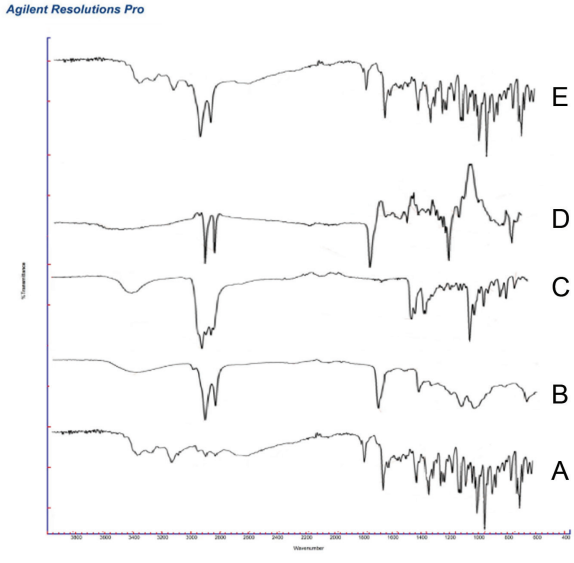

Figure 6. FT-IR analysis of (A) OLM, (B) Span 60, (C) cholesterol, (D) AO, and (E) niosomes (NF8)

FT-IR: Fourier transform infrared spectroscopy, OLM: Olmesartan medoxomil, AO: Aloe vera oil

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

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

SD: Standard deviation, FC: Formulation code

were significantly (p <0.05) better than those of the plain gel (0.261 mg/cm²). Based on the ER results, it was found that OLM from the niosomal buccal gel permeates significantly (p < 0.05) faster (approximately more than 2 times) compared to the OLM suspension (Table 6).

Histopathology

Chicken buccal mucosa without gel, OLM gel, and niosomal gel application are shown in Figure 8. Histological observation revealed that control of the buccal mucosa was lined by stratified squamous epithelium with ducts in the 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 with the untreated control. A slight thinning of the epithelium, fewer ducts in the smooth mucosa, and a thin layer of smooth muscle were noticed in the buccal mucosa treated with OLM gel.

DISCUSSION

PDI shows homogeneity of vesicle size. The lower value of PDI shows that formulation is more homogeneous in nature.[18] Based on the zeta potential results, the OLM niosomes showed sufficient dispersion stability. Stable formulations have a zeta potential between +30 mV and -30 mV.[19] 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 leakage, which lowers the drug entrapment efficacy. It was found that raising the cholesterol level improved the effectiveness of drug entrapment.[17] Based on these results, cholesterol played a

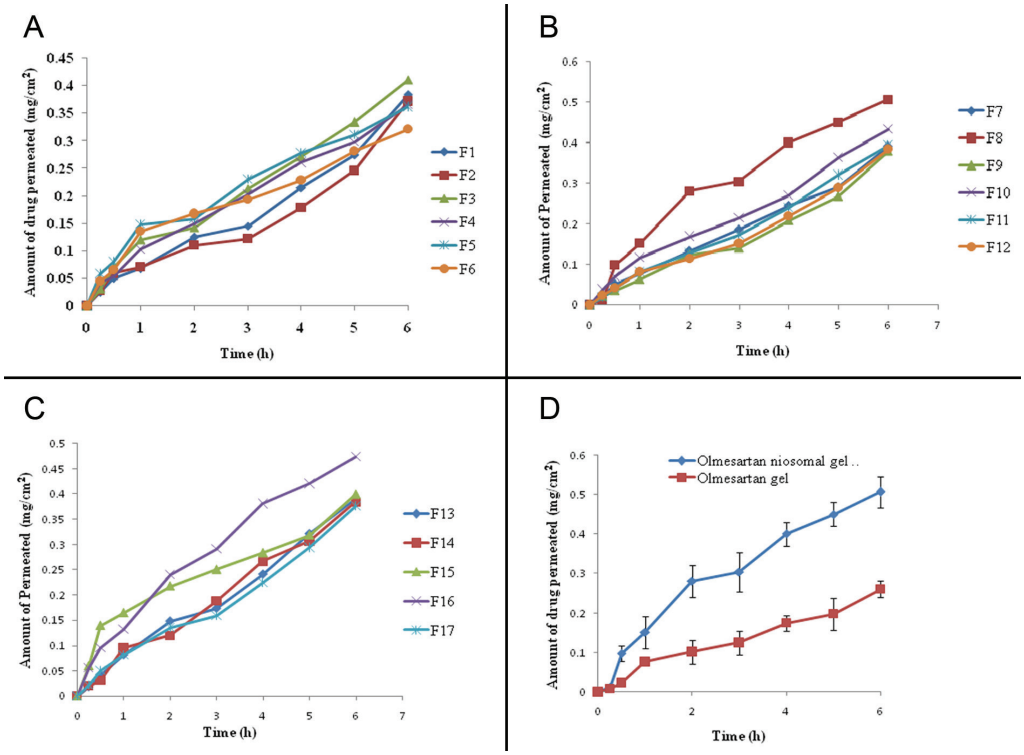

Figure 7. *Ex vivo* permeation studies of (A) NF1-NF6, (B) NF7-NF12, (C) NF13-NF17, and (D) OLM gels. Data are presented as mean ± SD (n= 3)
OLM: Olmesartan medoxomil, SD: Standard deviation

significant role in particle size. It was predicted that at low cholesterol levels, non-ionic surfactant and cholesterol would pack tightly together, increasing curvature and shrinking in size.[20] As the AO concentration increases, the high surfactant charge increases vesicle aggregation and cohesive force by reducing the interfacial tension between phases.[21] When the concentration of cholesterol increases, the hydrophobicity of the bilayer membrane increases, resulting in an increase in the vesicle size to achieve a more thermodynamically stable shape.[22,23] This mechanism influences the increase in vesicle size. This may be due to AO occupying the space of the surfactant molecule influencing the increase in vesicle size. It was determined that the vesicle size increased as the Span 60 concentration increased, possibly because stronger contraction caused vesicle aggregation. By increasing the level of surfactant, the formulation becomes more consistent and the diffusional path length of the vesicles increases.[24] In addition, the surfactant concentration acts as a depot, reducing drug leakage from niosomal to dissolution media. Drug release from niosomal vesicles was reduced as the concentration of Span 60 increased. This may be because an improvement in the surfactant concentration acts as a depot, reducing drug leakage from niosome to dissolution media.[25] Drug release was decreased by increasing the cholesterol concentration. This may be due to the rigidization of vesicles, resulting in the minimization of drug transport from the vesicles to the dissolution medium. Cholesterol influences the fluidity of the membrane; at low temperatures, it increases fluidity. In addition, AO at a high level enhances permeability by facilitated permeation, *i.e.* transient reduction in barrier resistance of stratum corneum (SC). The composition of chicken buccal mucosa and AO also alters lipid bilayer fluidity. The observations show that there is a possibility of high drug permeation by AO, as evidenced by the keratinization of coenocytes in the chicken buccal mucosa (SC).[26] Non-ionic surfactant concentration showed a promising effect on permeability.The addition of surfactant, which helps to solubilize lipids in the mucosa and allows for high vesicle penetration, may result in higher drug permeability.[17] The lower permeation of OLM from the suspension than from the niosomal formulation may be due to the higher log *p* value of OLM.[28] Niosome can alter drug transport through the mucosa because of adsorption on the surfaceof the mucosa, which results in a high thermodynamic activity gradient of the drug at the interface, which helps act as a driving force for permeating the lipophilic drug. The ability of vesicles to enhance penetration is related to a decrease in the barrier properties of the mucosa by niosome. This may be explained by the superiority of niosomal carriers, which have high permeation in mucosal layers due to carrier portion integration with mucosal lipids. Surfactants in vesicular form reduce the crystallinity of the skin's intracellular lipid bilayer, improving drug permeation.[29] Reduced layers of epithelium, no ducts in the submucosa, a thin layer of the submucosa, and smooth muscles were noticed in the buccal mucosa treated with OLM niosomal gel, which could be additional evidence of enhanced drug permeability.

CONCLUSION

The Box-Behnken design was used to optimize the OLM niosomal gel formulations. Noisome formulation (NF8) was chosen as an optimized formulation of the niosomal gel because of its small vesicle size (296.4 ± 3.9 nm, PDI= 0.21

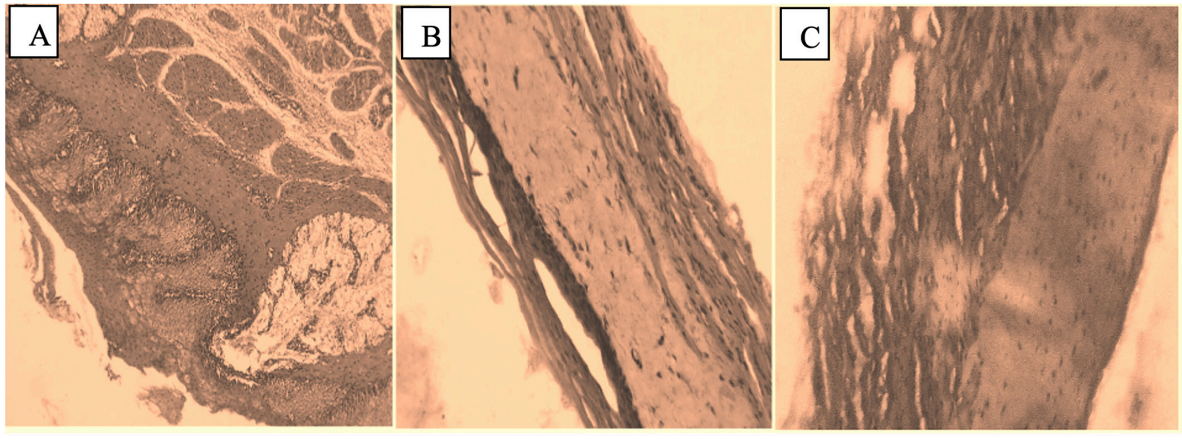

Figure 8. Histopathology of chicken buccal mucosa after 6 hours of permeation study (A) control, (B) OLM gel, and (C) OLM niosomal gel
OLM: Olmesartan medoxomil

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

FC	Permeability (mg/cm^2)	Flux (J) (mg/cm^2 h)	Permeability coefficient (J/C) (cm/h)	ER
OLM gel	0.261 ± 0.013	0.040 ± 0.002	0.020 ± 0.001	-
OLM niosomal gel (NF8)	0.507 ± 0.017	0.083 ± 0.005	0.041 ± 0.003	2.05 ± 0.18

OLM: Olmesartan medoxomil, ER: Enhancement ratio, FC: Formulation code

± 0.06) and high CDR (96.22 ± 2.9%). Permeability studies of niosomal gel formulations were performed using chicken buccal pouches. The optimized niosomal formulations showed higher permeation rates (0.507 ± 0.017 mg/cm^2) 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 an almost 2.05-fold increase in flux compared with the OLM gel. Thus, the buccal administration of niosomal gel could help improve OLM bioavailability.

Ethics

Ethics Committee Approval: The *ex vivo* permeation studies were conducted using chicken buccal mucosa. The buccal mucosa was procured from local slughter house. So this study does not require animal ethics approval.

Informed Consent: Not required.

Authorship Contributions

Concept: N.N.P, Design: N.N.P, B.C.M., Data Collection or Processing: N.N.P, J.R., Analysis or Interpretation: N.N.P, P.M.G., Literature Search: N.N.P, B.C.M., Writing: N.N.P, B.C.M.

Conflict of Interest: Authors declared no conflicts of interest.

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