doi.org/10.36721/PJPS.2021.34.1.SUP.245-255.1

Formulation and optimization of dimenhydrinate emulgels for topical delivery using response surface methodology

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Abstract: Development of dimenhydrinate (DMN) emulgel formulation has been described in this work with enhanced permeation for transdermal delivery of DMN for effective management of motion sickness. Various DMN emulgel formulations were prepared using central composite design in response surface methodology. Propylene glycol and olive oil were used in varying ratios as permeation enhancers along-with carbopol-934 as gelling agent. Prepared formulations were evaluated by physico-chemical properties, stability and Fourier transform infrared spectroscopy (FTIR) studies. *In-vitro* drug release was studied using cellophane membrane. Formulation F2 showed maximum drug permeation following diffusion-based release mechanism and was used in further studies. Rat skin was used in Franz cell for *ex-vivo* studies to determine various permeation kinetic parameters. FTIR studies provided no evidence of chemical interaction between DMN and polymers used, whereas molecular docking revealed formation of a stable complex in the presence of aqueous environment with stable intermolecular binding and the complex was well hydrated. No evidence of skin irritation was observed in human volunteers following application of the optimized formulation. Histopathology data of the rat skin showed a decreased proliferation of the lymphocytes whereas monocytes were induced. In conclusion, combination of propylene glycol and olive oil was successfully employed for delivery of DMN through transdermal route with good permeability and prolonged release time that can be highly beneficial in treating motion sickness in unusual circumstances.

Keywords: Dimenhydrinate, emulgel, motion sickness, molecular docking, response surface methodology, permeation.

INTRODUCTION

Motion sickness is an uncomfortable condition that occurs when individuals are imperiled to motion or the perception of motion, especially during travelling or riding. Common symptoms include malaise, nausea and wooziness (Frett *et al*., 2020). Motion sickness is caused by the mixed signals sent to the brain by the eyes and the inner ear. It cannot be cured by medication but can be prevented. Females have increased risk of motion sickness compared to males (Koslucher *et al*., 2016). The children under 12 years of age are also at high risk of motion sickness (Huppert *et al*., 2019). Medications used to relieve symptoms include promethazine, scopolamine, cyclizine, meclizine and dimenhydrinate (Golding, 2006, Golding and Gresty, 2015). Except scopolamine transdermal patches, limited research has been reported on other drugs for the development of some suitable topical dosage forms offering ease in self-medication and better patient compliance.

Topical and transdermal drug delivery can be defined as the application of a drug formulation to the skin or the mucosa to treat cutaneous disorders or to get systemic effects (Shahzad *et al*., 2013c). Topical drug delivery benefits include the capability to deliver drug substance to a specific site, avoiding first-pass effect, enhanced patient compliance, low chances of drug interaction, minimal side effects, immediate withdrawal, minimized frequency of dosing, prolonged duration of action and improved self-medication (Shahzad *et al*., 2013c). An ideal drug for topical formulation should have a molecular weight less than 500 Dalton and possess both hydrophobic as well as hydrophilic features in order to traverse the stratum corneum and to penetrate the aqueous epidermis respectively (Stanos, 2007, Shahzad *et al*., 2013b). The vehicle in which the active ingredient is delivered plays a significant role as it affects the depth of skin penetration and rate of absorption into the epidermis (Burger *et al*., 2017). Amongst these, the emulgels may be a suitable candidate for the incorporation of drugs for the prevention and treatment of different ailments.

Emulgels are topical formulations comprising of emulsion and gel (Alexander *et al*., 2013, Kashif and Akhtar, 2019). The oil phase, gelling agent, aqueous phase and emulsifying agent constitute the major components of an

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emulgel system and significantly affect the rate and extent of drug release from the formulation (Noronha *et al*., 2020, Sohail *et al*., 2018). Several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water soluble, more stable, bio-friendly, transparent and pleasant appearance are the excellent attributes of emulgels. The advantages include easy incorporation of hydrophobic drugs using oil-in-water emulsion, increased stability, better loading capacity and controlled drug release (Shahin *et al*., 2011, Stanos, 2007). During recent years, emulgels have been used for the preparation of both oil as well as water soluble drugs of varying pharmacological effects such as anti-emetic, non-steroidal anti-inflammatory drugs, anti-fungal agents, anti-viral, antibacterial and local anesthetics (Khullar *et al*., 2011, Ashara *et al*., 2017).

Dimenhydrinate (DMN) is a combination of diphenhydramine and 8-chloro-theophylline (Leichner *et al*., 2019). Its solubility in water is 3mg/mL and its molecular weight is 469 Dalton (Windholz *et al*., 1976). It directly inhibits the stimulation of certain nerves in the brain and inner ear to suppress nausea, vomiting, dizziness, and vertigo in motion sickness when taken 30 minutes before starting journey or ride (Sanger and Andrews, 2018). Despite its efficacy in motion sickness, no prior research has been conducted to formulate topical DMN emulgel. Thus, the present study was aimed to develop emulgel formulations for topical delivery of DMN utilizing statistical optimization using response surface methodology (Shahzad *et al*., 2013a). The formulated emulgels were extensively characterized using a variety of analytical tools and the optimized emulgel was subjected to permeation studies using a synthetic skin mimic and excised rat skin. Furthermore, molecular dynamics of the drug within the formulation was studied using an *in-silico* software package to gain further insights into drug and polymer interactions.

MATERIALS AND METHODS

Materials

DMN was generously donated by Star Laboratories (Pvt.) Ltd Lahore, Pakistan. Propylene glycol (PG), carbopol-934, olive oil, Tween 20, Span 20, liquid paraffin, methanol and propyl parabens were obtained from Merck (Pvt.) Ltd, Germany. Double distilled water prepared at in-house facility was used in all experiments. All the reagents were of analytical grade and used without further purification.

Pre-formulation solubility studies

The excess amount of DMN was mixed with 5mL of phosphate buffer at pH 5.8 and 7.4 in two separate beakers and stirred using a thermo-regulator stirrer at 37± 1°C for about 96 h. Later on, both the aforesaid mixtures were centrifuged at 13000 rpm for 10 min. Suitable dilutions were prepared from the filtered supernatant. Absorbance was measured by UV-VIS Spectrophotometer (Perkin Elmer, Lambda 25) at 278 nm to calculate the quantity of drug in each solvent system using calibration curve that was constructed through a series of dilutions in the linear range of 50-500µg/mL with correlation coefficient (R^2) value of 0.999.

Central composite design

A computer optimization technique in Design-Expert software (Version 7.0.0) based on the principle of polynomial equation and response surface methodology (RSM) was used to optimize the emulgel formulation. A central composite design (CCD) was employed as per the standard protocol in the said methodology (Pinto *et al*., 2019). The amounts of PG and olive oil at three levels (-1, 0, +1) each were selected as the independent variables A and B, respectively. The central point (0, 0) was studied in quintuplicate. All other formulation and process variables were kept invariant throughout the study. The polynomial equation together with quadratic terms was generated for response by using multiple linear regression analysis (MLRA) approach. Contour and 3D surface plots were also employed to select the formulation variables required to generate appropriate values (Shahzad *et al*., 2013a).

Dimenhydrinate loaded emulgel preparation

Emulgel formulations of 1% DMN were prepared using varying ratios of variables A (PG) and B (olive oil) as given in table 1. Composition of 100 g of each DMN emulgel formulation is given in table 2. Briefly, oil phase was prepared by weighing and mixing desired quantity of olive oil, span 20 and liquid paraffin. Mixture was heated at 60-70°C for 10-15 min. In second step, aqueous phase preparation was carried out by mixing propylene glycol, tween 20 and propyl paraben. A separately prepared hydro-alcoholic solution of DMN in methanol and double distilled water (70:30 v/v) was incorporated into this aqueous phase. This mixture was heated at 60-70°C for 10-15 min. Uniform emulsion was prepared by slowly adding the prepared oil phase into the aqueous phase along with continuous mixing at 25±0.5°C (Phase I). Simple gel was prepared by carefully weighing carbopol-934 and soaking in distilled water for 2 h followed by continuous stirring using homogenizer at 1000 rpm for 10 min (Phase II).

The DMN emulgel formulations were obtained by slowly adding the prepared Phase I into the phase II with continuous stirring followed by homogenization at 2000 rpm for 20 min. pH was adjusted in the range of 5-6 by adding 2 % triethanolamine aqueous solution dropwise.

Physico-chemical characterization of DMN emulgel formulations

The physical examination of the prepared DMN emulgel formulations was carried out visually to examine the color, texture and homogeneity. The results were recorded accordingly.

pH of all thirteen DMN emulgel formulations was recorded using pre-calibrated digital pH meter (Milwaukee MI 151). Briefly, 1 g of the formulation was dissolved in 25 ml of double distilled water and the probe of the pH meter was dipped in to it to record the reading. The experiment was conducted in triplicate.

Table 1: Coded levels of variables A (PG) and B (olive oil) to prepare thirteen (13) different formulations of DMN emulgel as obtained from design expert software.

Formulation Code	A	B
F1	0	0
F2	-1	1
F3	0	1
F4	1	0
F5	1	1
F6	-1	-1
F7	1	-1
F8	0	-1
F9	-1	0
F10	0	0
F11	0	0
F12	0	0
F13	0	0

CCD Variable Level	-1	0	+1
A (PG) g	25	27.5	30
B (olive oil) g	3	3.5	4

Table 2: Composition of DMN emulgel formulation (100 g)

Ingredients	Amount
Dimenhydrinate	1 %
Carbapol-934	2 %
Olive Oil	3, 3.5, 4 %
Propylene Glycol	25, 27.5, 30 %
Liquid paraffin	7.5 %
Span 20	0.92-0.98 %
Tween 20	0.4-0.8 %
Propyl paraben	0.01%
Double distilled water	qs to prepare 100 g emulgel

The viscosity of DMN emulgel preparations was determined using Brookfield digital viscometer (Ametek Brookfield). The formulation was placed in a small beaker and the S-06 spindle was allowed to rotate freely in the emulgel at a rotation rate of 2.5 rpm and the temperature was maintained at 25±1°C (Khullar *et al*., 2012). The experiment was conducted in triplicate.

Determination of spreadability

Spreadability of the formulations was measured in terms of change in diameter of the emulgel under the influence of the applied weight (Shahzad *et al*., 2013b). In this method, 1g of the formulation was placed in the middle of a glass slide within the marked circle of 1cm diameter; afterwards, another glass slide was placed over it and weight of 100g was applied. After 5 min, when no further spreading was observed, change in diameter of formed circle was measured. Spreadability ($g.cm.min^{-1}$) was calculated by using following equation (Shahzad *et al*., 2013a);

$$S = ml/t \quad (1)$$

Where 'S' is spreadability, m is weight (g) of the upper plate plus weight (g) applied on the plate, '*l*' is the difference in diameter (cm) of the spreading emulgel after application of weight and 't' is the time (min).

Drug content estimation

Drug content for all the prepared DMN emulgel formulations was calculated by dissolving an accurate 100 mg quantity of the formulation in 100mL of double distilled water. The solution was filtered and the absorbance was recorded at 278 nm by UV-VIS spectroscopy after suitable dilutions and quantified through already constructed calibration curve.

In vitro drug permeation through cellophane membrane

Permeation of DMN from the formulated emulgels through cellophane membrane was performed by using phosphate buffer at pH 7.4 as a release medium at 37±0.5 °C in USP Type-II dissolution apparatus (ERWEKA DT 820). Each formulated DMN emulgel (1g) was taken in cellophane membrane with cut off size of 14KD and tied with the help of thread to the paddle of dissolution apparatus having 250 mL of release medium. The paddle rotation was fixed at 100 rpm. 5 mL aliquot was collected at predetermined time intervals, filtered and the drug content was calculated by measuring absorbance at 278nm using UV-VIS spectrophotometer. After taking each sample, dissolution media was replenished with similar amount of freshly prepared phosphate buffer to maintain sink conditions. The concentration of DMN released from all the formulations was calculated by using calibration curve of DMN in phosphate buffer. The results are reported as mean ± standard deviation of minimum three experiments.

The drug release data was fitted to various kinetic models including zero order, first order, Higuchi, and Korsemyer-Peppas to elucidate the release kinetics of DMN from emulgels. Following equations (Eq 2 to 5) were applied for the aforementioned models respectively;

(Zero-order) $Q_t = K_o t$ (2)

(First-order) $\log Q_t = \log Q_o - k_1 t$ (3)

(Higuchi) $Q_t = k_H t^{1/2}$ (4)

(Korsmeyer-Peppas) $Q_t / Q_\infty = K.t^n$ (5)

Where Q_t indicates the quantity of drug released at time 't' and 'K_o' is the zero-order release rate constant in equation 2. 'K_1' is the first order release rate constant and Q_t is the amount of drug remained in the dosage form in equation 3. 'k_H' represents Higuchi dissolution rate constant in

equation 4. 'Q_∞' is the amount of drug released at ∞ and 'k' is the Korsmeyer-Peppas rate constant whereas 'n' is diffusional coefficient showing release mechanism for the drug in equation 5.

Ex-vivo permeation across rat skin

Permeation of the DMN across rat skin was determined using euthanized Sprague-Dawley rat (male weighing 250 – 280 g) skin following ethical guidelines approved by ethical committee of faculty of Pharmacy Bahauddin Zakariya University Multan, Pakistan vide reference NO. 01/PEC/2017 dated 05/06/2017. The rats were obtained from animal house of the Faculty of Pharmacy, Bahauddin Zakariya University and the animals were kept in 12 h light/dark cycles with ad libitum supply of food and water prior to euthanizing. Animals were euthanized using pentobarbitone at dose of 100-150 mg/Kg through intra-peritoneal route. Hair removed abdominal skin tissue was excised and mounted firmly between the two compartments of Franz diffusion cell to perform the ex-vivo permeation study (Ahmad *et al*., 2020). The donor compartment was loaded with 1 g of the optimized DMN emulgel formulation F2 while the receiver compartment was filled with phosphate buffer at pH 5.8. Continuous stirring was achieved with a magnetic stirring bar and the temperature was maintained at 32±0.5°C. Permeation of DMN from the donor compartment to phosphate buffer in the receiver compartment was measured by taking 1 mL sample from the receiver compartment at pre-decided time intervals followed by UV-VIS Spectrophotometry at 278 nm. The sink conditions were maintained by replenishing the same amount of the freshly prepared buffer at the time of each sampling. Quantification of the DMN was carried out with the help of already constructed standard curve.

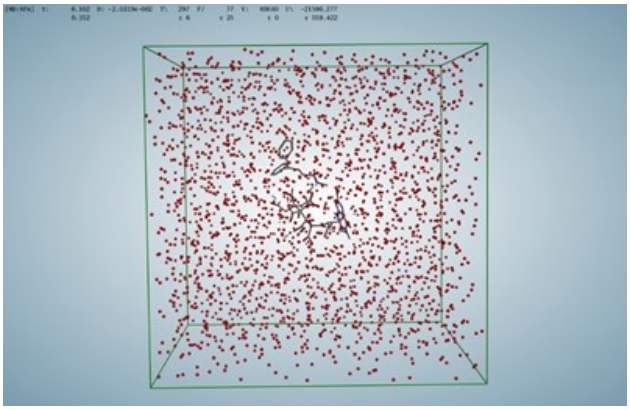

Fig. 1: The MD simulation assembly showing the periodic boundary conditions.

Histopathology of rat skin

After the completion of permeation study, the skin tissue was washed in ice-cold saline followed by blotting with paper towel to remove any excess fluid. 10% buffered formalin was used to fix the skin tissue. Afterwards, skin tissues were processed in gradually increasing concentration of ethanol up till 100% using tissue processor for 24 h to remove all water. The processed tissue was embedded in paraplast block using tissue embedder. Cross-sections of 5 µm thickness were cut from processed tissue using microtome. Tissue slides were stained using Hematoxylin and Eosin (H & E) followed by examination using a compound microscope (Usman *et al*., 2020).

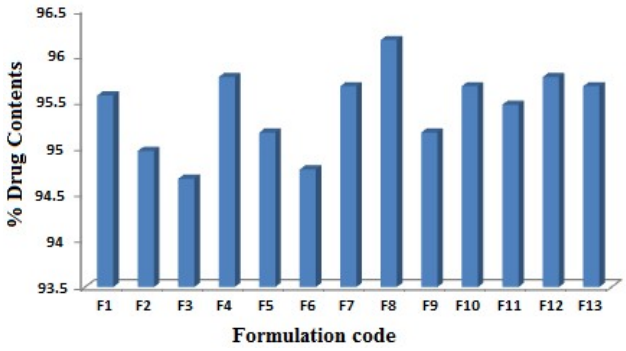

Fig. 2: % Drug content of the DMN formulations (F1 – F13)

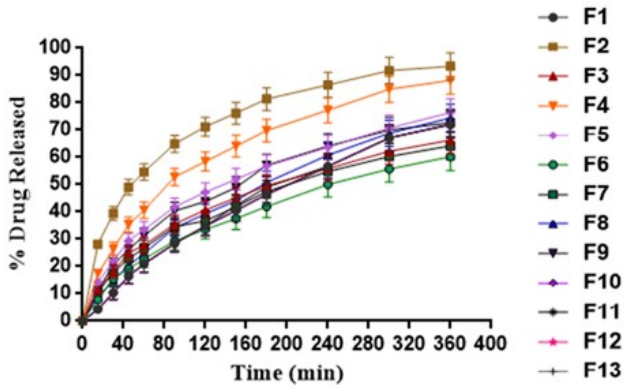

Fig. 3: DMN release from emulgel formulations

Skin irritation studies

Skin irritation studies were carried out on Human Volunteers following ethical guidelines approved by ethical committee of faculty of Pharmacy Bahauddin Zakariya University Multan, Pakistan vide reference NO. 05/PEC/2017 dated 05/07/2017. The forearm skin of the volunteers was shaved and the area was divided into four portions of 1 square inch each. First portion A and second portion B were used as positive and negative controls, respectively. Whereas 1g each of blank emulgel and 1% DMN loaded emulgel were applied to the third (C) and fourth (D) portions, respectively for 4 h. The skin was observed for any signs of allergic reaction, redness or rashes (Javed *et al*., 2018).

Accelerated stability studies

All DMN emulgel formulations were subjected to accelerated conditions of temperature and relative humidity i.e., 40±2°C and 75±5%, respectively in a

stability chamber for six months according to ICH guidelines (Q1C 1996). Various parameters including physical appearance, homogeneity, pH and drug content of the formulations were checked and recorded at specified intervals.

ATR-FTIR spectroscopy

ATR-FTIR spectroscopy was performed for carbopol-934, DMN and the optimized DMN loaded emulgel to determine the possible linkage of the components in the emulgel formulation as well as the possibility of drug and polymer interactions. All the FTIR studies were carried out in the range of 400-4000 cm^{-1} at a resolution of 2 cm^{-1} using Bruker Platinum ATR-FTIR (China) spectrophotometer (Mehmood *et al*., 2019).

Molecular docking (MD) studies

Low mode MD simulation was carried out to obtain the plausible chemical interactions driving the emulgel formulation. The chemical structures were built using the builder module in MOE v. 2018.0101 followed by charge application using MM94x force field for small molecules and energy minimization using Powell gradient method (gradient = 0.1 RMS/kcal/mol/$Å^2$). The compounds were then subjected to Low Mode MD (500ps) simulation to comprehend the process of complexation. The light bonds were constrained with periodic boundary conditions as depicted in fig. 1 (Gaikwad and Jadhav, 2019).

STATISTICAL ANALYSIS

All the data was analyzed and presented as mean ± standard deviation (S.D.) taking at least three observations per sample using Microsoft Excel, version 2016. Statistical differences between 13 different formulations were determined using the multiple linear regression analysis (MLRA) and one-way analysis of variance (One-way ANOVA) with $p<0.05$ as a minimal level of significance using SPSS (v16).

RESULTS

Dimenhydrinate loaded emulgel preparation using experimental design

The CCD resulted in 13 formulation combinations with varying contents of the PG and olive oil as independent variables as shown in table 1. The emulgel formulations were then prepared according to the ratios given by the response surface experimental design. It is noteworthy that all formulation combinations resulted in DMN loaded emulgels (F1-F13), which were stored at room temperature and used for further studies.

Physico-chemical characterization of DMN emulgel formulations

The physical properties including homogeneity, color and texture of formulated DMN emulgels were recorded. The results showed that all formulated emulgels had milky white appearance with smooth creamy texture. The formulations were homogenous and had no evidence of any phase separation, as mentioned in table 3.

Solubility of DMN was studied as mentioned above and was calculated by using UV-VIS Spectrophotometry. The solubility of DMN was found to be 7.26 ± 0.2 mg/mL and 10.01 ± 0.4 mg/mL in phosphate buffer at pH 7.4 and 5.8, respectively.

The pH values of all formulated emulgels were recorded as shown in table 3. It was found that all the formulations had values in the range 5.5-6.0 that lie in the normal pH range of skin. The viscosity of all the prepared DMN emulgel formulations (F1-F13) was recorded as described in the method and the readings were noted (table 3). The values were in the range of 181700 to 224000 cP. Spreadability of all formulations were in the range of 403.9 to 557.5g.cm.min^{-1}, which is a suitable range for topical application (table 3).

Determination of drug content

Percent drug content for all the prepared DMN emulgel formulations was calculated using UV-VIS Spectroscopy. The results were in the range of 94.6 to 96.2% depicting the homogeneity of the drug in all formulations, as shown in fig. 2.

In vitro drug release through cellophane membrane

Drug release study for the formulated DMN emulgel formulations (F1-F13) across cellophane membrane was carried out for 6 h in phosphate buffer at pH 7.4 and the results are shown in fig. 3. In general, the emulgel formulations described a higher flux through the synthetic membrane over 90 minutes which was followed by relatively slower drug release during the subsequent period of study. Maximum drug was released from formulation F2 (93.3%) over a period of 6 hrs. The release was more than 65% for all formulations except F6 and F7 where it was 60.2 and 64.2%, respectively. The increased drug release by formulation F2 may be a response attributed to high concentration of olive oil (Rastogi *et al*., 2015). The variability in the drug release behavior was studied by applying different kinetic models to develop an understanding about the structure of emulgel matrix. The results of data fitting to kinetic models are described in table 4. The results of goodness of fit revealed acceptable values of correlation coefficient R^2 (value close to 1) and mean square error (lowest amongst the fit models) for Korsmeyers-Peppas model. The value of release exponent (n) was less than 0.45 for F2, suggesting a diffusion-based drug release from the formulation. For other formulations the value was greater than 0.45, suggesting a non Fickian drug release mechanism. Furthermore, the results indicated a predictable drug release from F2 which was considered as optimized formulation and was used in further studies.

Table 3: Viscosity, pH, spreadability, color, texture and phase separation of the prepared DMN formulations (n =3; Mean ± S.D).

Formulation	Viscosity (Cp)	pH	Spreadability (g.cm.min^{-1})	Color	Texture	Phase separation
F1	210400±1.53	5.6±0.10	457.5±1.81	White	Smooth	No
F2	204400±1.53	5.8±0.15	490.2±1.60	White	Smooth	No
F3	224000±1.71	5.5±0.25	403.9±1.94	White	Smooth	No
F4	184000±2.31	5.7±0.20	506.5±2.37	White	Smooth	No
F5	213600±0.58	5.9±0.21	490.2±4.09	White	Smooth	No
F6	182800±1.15	5.4±0.25	541.2±1.64	White	Smooth	No
F7	196000±0.58	5.8±0.15	473.9±2.21	White	Smooth	No
F8	181700±1.15	6.0±0.25	557.5±1.25	White	Smooth	No
F9	200400±0.58	5.7±0.15	473.9±3.25	White	Smooth	No
F10	213900±1.15	5.6±0.15	424.8±2.86	White	Smooth	No
F11	211300±0.58	5.5±0.31	457.5±1.18	White	Smooth	No
F12	212200±1.73	5.7±0.21	441.2±2.70	White	Smooth	No
F13	209700±0.58	5.6±0.19	448.6±4.43	White	Smooth	No

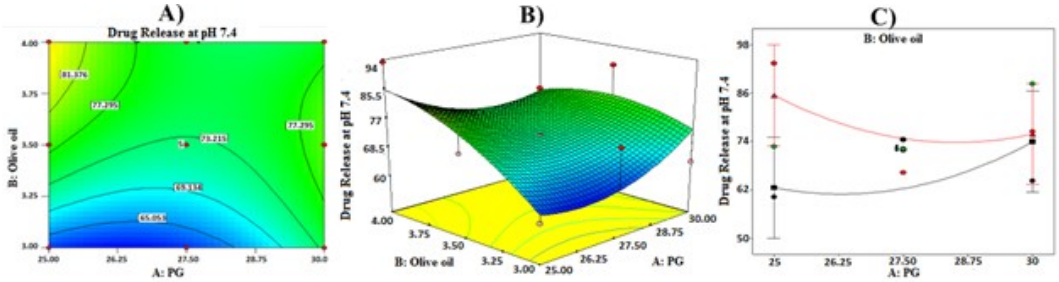

Fig. 4: Plots obtained using design expert software for the variables of the DMN emulgel formulations; A) Contour B) 3D Surface C) Interactive

RSM optimization

RSM optimization was carried out using Design Expert software (version 7). The polynomial equation was employed in terms of coded factors as given below:

$$Y_1 = b_o + b_1 A + b_2 B + b_{12} AB + b_1^2 A^{2+} b_2^2 B^2 \quad (6)$$

Drug release through cellophane membrane was determined using the aforesaid polynomial equation as;

$$\text{Drug release } (Y_1) = 72.74 + 0.43\ A + 6.20\ B - 5.24\ AB + 5.92\ A^2 - 4.21\ B^2 \quad (7)$$

The polynomial equation showed that the factors A (PG) and B (olive oil) have positive (synergistic) impact on the drug permeation in phosphate buffer pH 7.4 when used alone, whilst they have negative (antagonistic) impact when used in combination, as depicted in fig. 4 (A). Furthermore, it is evident from the equation that the quadratic value of PG (A^2) had a positive impact on drug release while the quadratic value of olive oil (B^2) negatively impacted the drug release. 3D surface plot also showed the similar pattern of impact on the response as shown in fig. 4 (B). Interactive plot as obtained from the aforesaid software is given in fig. 4 (C). Multiple linear regression analysis for DMN at pH 7.4 is given in table 5.

Accelerated stability studies

All the DMN emulgel formulations were subjected to stability studies under stress conditions. The formulations were examined for various physico-chemical parameters like pH, color, texture, any evidence of phase separation as well as contents of the DMN at the specified time intervals according to ICH guidelines. There was no significant change in any of the studied parameter during the storage period. The drug contents after six months were in the range of 80.5 to 91.8%, thus showed a minimal degradation. No change in physical appearance was observed, thus endorsing the stability of emulgels.

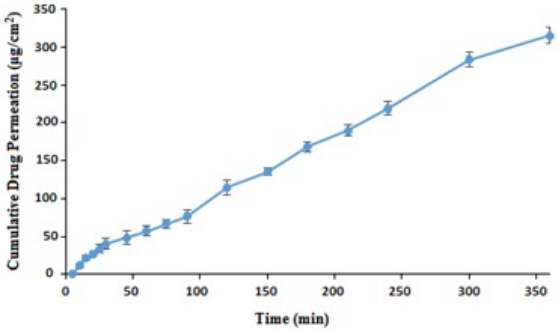

Fig. 5: Cumulative permeation of DMN from optimized emulgel (F2) through rat skin.

Ex-vivo permeation across rat skin

The cumulative amount of drug permeation across rat skin was calculated using Franz- diffusion cell for the

Table 4: Mathematical models for drug release kinetic parameters at pH 7.4

Kinetic Parameters		DMN Emulgel Formulations												
		F1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12	F 13
Zero Order	K_0	0.23	0.36	0.23	0.32	0.27	0.20	0.22	0.25	0.26	0.23	0.23	0.23	0.23
	R^2	0.92	0.62	0.54	0.36	0.47	0.72	0.59	0.83	0.57	0.92	0.91	0.92	0.92
First Order	K_1	0.004	0.012	0.004	0.007	0.005	0.003	0.004	0.004	0.005	0.004	0.004	0.004	0.004
	R^2	1.00	0.89	0.87	0.95	0.89	0.92	0.88	0.99	0.92	1.00	1.00	1.00	1.00
Higuchi	K_H	3.47	5.79	3.60	5.01	4.15	3.13	3.45	3.75	4.02	3.48	3.51	3.47	3.48
	R^2	0.93	0.82	0.99	0.98	0.99	0.99	0.99	0.97	0.99	0.93	0.93	0.93	0.93
Korsmeyer-peppas	K_{KP}	1.10	13.53	3.69	6.37	4.73	2.29	3.26	1.98	3.93	1.09	1.16	1.10	1.09
	R^2	0.99	0.98	0.99	0.99	1.00	1.00	0.99	1.00	0.99	0.99	0.99	0.99	0.99
	n	0.72	0.34	0.50	0.45	0.48	0.56	0.51	0.62	0.50	0.72	0.71	0.72	0.72
Hixon Crowell	K_{HC}	0.001	0.003	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	R^2	0.99	0.77	0.80	0.88	0.81	0.87	0.81	0.96	0.85	0.99	0.99	0.99	0.99

optimized formulation F2 in phosphate buffer at pH 5.8. The permeation kinetic parameters were calculated and the results revealed a consistent drug permeation at a flux rate 1.851±0.05 μg/cm^2/min after a time lag of 25minutes (fig. 5). Cumulative drug permeation for 6h was noted to be 336.81μg/cm^2. While the permeability coefficient was found to be $19\times10^{-4} \pm 01\times10^{-4}$ (cm/min).

Table 5: Multiple linear regression analysis of DMN emulgel formulations

Regression Coefficient	Response Y_1 (at pH 7.4)
Model	Quadratic
Intercept	72.74
A	0.43
B	6.20
AB	-5.24
A^2	5.92
B^2	-4.21
Model(P-Value)	0.4992
Co-efficient of variation (%)	11.46
R^2	0.4758
Adj R^2	0.1013
Pre R^2	-4.2681
PRESS	4990.66
F-value	0.77
SD	8.42
Mean	73.53
Adeq. precision	4.041

Skin irritation studies

Optimized DMN emulgel formulation (F2) was used for the skin irritation studies. The results showed no evidence of irritation, redness or any allergenic symptoms following the application as compared with blank emulgel and control. Pictorial evidence of the skin before and after the study is presented in fig. 6.

Histopathology of rat skin

No evidence of inflammatory response was observed in control group of the skin under investigation (fig. 7A). Histopathological examination of the excised skin samples after 6 h of the permeation study using drug loaded optimized DMN emulgel formulation (F2) showed that the monocytes count was induced whereas lymphocytes proliferation was decreased. Decrease in connective tissue was also observed (fig. 7B). Following 24 h of application, fibroblasts were observed in abundance for helping the initiation of regeneration of the rat skin (fig. 7C & D).

ATR-FTIR spectroscopy

FTIR spectra of DMN, carbapol-934 and formulated optimized DMN emulgel formulation are shown in fig. 8. No evidence of chemical interaction was observed in FTIR. All bands observed in DMN were also visible in the FTIR spectra of DMN emulgel formulation indicating

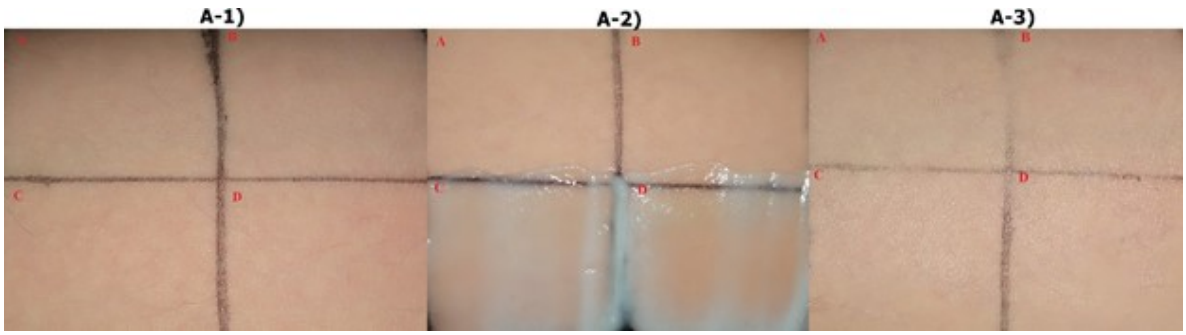

Fig. 6: Skin irritation test results; A-1) Before application of DMN emulgel formulation; A-2) Application of DMN emulgel formulation for 4 h; A-3) After removal of applied DMN formulation.

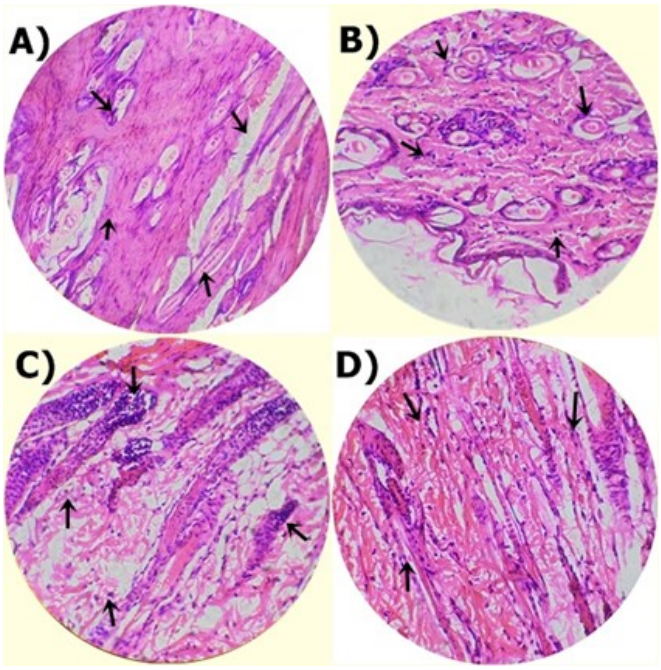

Fig. 7: Histopathology results; A) Normal rat skin tissue; B) 6 h after application of DMN emulgel formulation; C & D) 24 h after application of DMN emulgel formulation

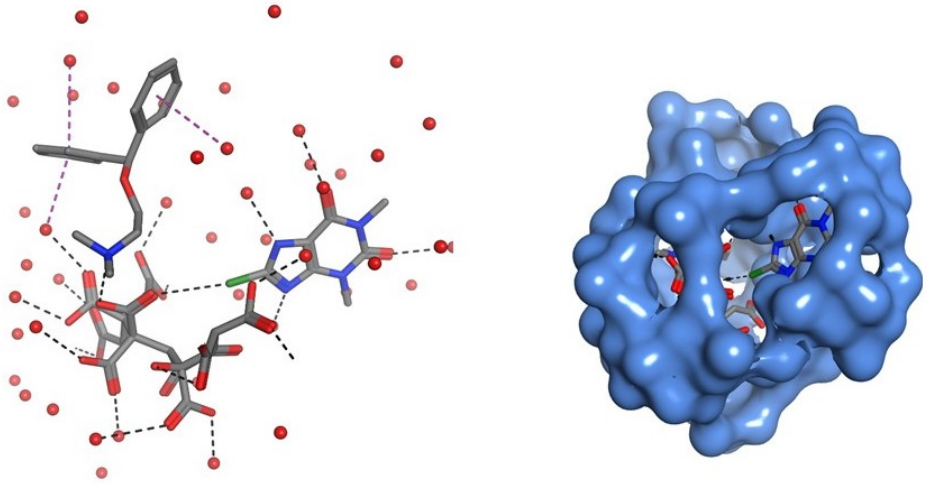

Fig. 8: MD simulation (A) van der Waals interactions between drug and polymer (B) position of drug within polymer cavity with electroscopic surface map.

chemical stability of the DMN after being formulated as emulgel. FTIR spectra of Carbapol-934 showed a prominent peak between 1750-1700 cm^{-1} due to carbonyl (C=O) stretching vibration, whereas peak appearing at 1450-1400 cm^{-1} was attributed to O-H. Bands at 1250-1200 suggest C-O-C of acrylates. Peaks in the range of 3500-3400 cm^{-1} represent OH-stretching vibrations (intra-molecular hydrogen bonding). In case of DMN, characteristics peaks at 1100 cm^{-1} belong to C-Cl stretch. Peaks around 1650 cm^{-1} may be due to C=O and C=C bond. In case of DMN emulgel formulation, all the major peaks of drug and polymer were retained. FTIR spectra showed a peak at 3000-2950 cm^{-1} range representing OH stretching vibration and intra-molecular hydrogen bonding (Nejati *et al*., 2018). Similarly, prominent band between 3500-3400 cm^{-1} assigned to hydrogen bonding by single bridge whereas bands from 1650-1600 cm^{-1} show carbonyl stretching vibration. FTIR data suggested DMN stability in the emulgel formulation and possible linkage between drug and polymer in the DMN emulgel (Barreiro-Iglesias *et al*., 2002).

Molecular docking (MD) studies

To comprehend the molecular forces governing complexation, low mode MD simulation was carried out using MOE software. Molecular docking studies suggested formation of stable complex in the presence of aqueous environment linked by weak van der Waals interaction fig. 8A &B.

DISCUSSION

This study presents formulation and characterization of DMN emulgel using RSM. Carbopol-934 was used to prepare water soluble gel base while different levels of input variables, namely PG and olive oil were used to obtain the desired DMN emulgel formulations. The resultant formulations were pleasant looking, smooth and stable. pH of all the formulations was in the range of 5.4 to 6 indicating suitability for application through topical route. Spreadability values indicated that the developed DMN emulgel formulations were easily spreadable by application of minimum shear force and thus the emulgels are suitable for topical applications.

In-vitro RSM optimization data revealed a linear relationship for both PG and olive oil with significant synergistic impact ($p<0.05$) on the drug release. Conversely, a non-synergistic impact on drug release was imposed in case of AB and B^2. This shows a remarkable behavior of both PG and olive oil in enhancing the drug release from the emulgel. The *in-vitro* membrane release profiles were modeled with quadratic equation and an optimal emulgel formulation (F2) was identified. The optimized formulation F2 had maximum concentration of olive oil and minimum concentration of for PG.

The *ex-vivo* permeation data revealed a satisfactory performance of the optimized formulation as it provides a lag time (t_{lag}) of 24.846±4.19 min, which is well below the desired limit of 30 minutes for an acceptable response. Furthermore, a constant gradient of the skin permeation warrants a persistent effect of the formulation following transdermal application. Moreover, the use of olive oil and PG combination facilitated the drug permeation across the rat skin. Similar results have been reported in previous research where olive oil and PG individually enhanced the drug permeation across the skin (Hashmat *et al*., 2020, Ghiasi *et al*., 2019).

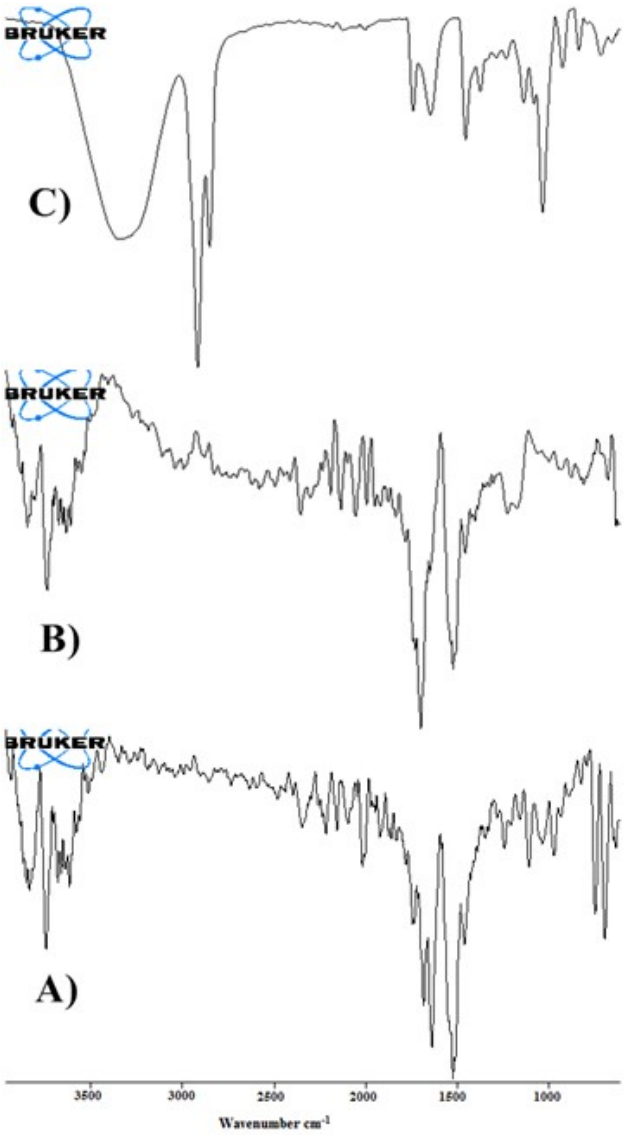

Fig. 9: ATR-FTIR spectra of A) carbapol-934, B) DMN and C) optimized DMN emulgel formulation

In molecular docking studies, DMN emulgel complex demonstrated stable intramolecular bonding, which was consistent with our FTIR results, thus suggesting possible OH stretching thereby resulting in stable hydrogen bonding. The benzene ring in the drug was found to mediate Hp-π interactions with the water molecules which serve as a bridge between the DMN and carbopol-934.

Apart from these interactions the polymer also exhibited hydrogen bonding with the drug molecules; however, the chemical characteristics of both the molecules remained consistent throughout the simulation. In this study a halogen bond between the chloride moiety of the drug and carbopol-934 was also observed (fig. 8). Taken together, these results suggested that DMN and carbopol-934 is mostly driven by polar interactions within the aqueous environment, which are in line with the observed FTIR results (fig. 9).

ATR-FTIR studies gave no evidence of chemical interaction between DMN and the polymers used. Had the drug and polymer interacted, the functional groups in FTIR spectra would have exhibited band shifts and broadening compared to spectra of pure drug and the polymers. FTIR spectra of DMN emulgel exhibited peaks which were on summation of characteristic peaks obtained from pure drug and polymers used, which showed no evidence of chemical interaction of drug even in the emulgel formulation during the method of preparation. The increase in the concentration of PG and olive oil did not initiate any drug polymer interaction indicating stability of DMN emulgel formulations that was also evident in stability data. In case of DMN emulgel formulation, all the major peaks of drug and polymer were retained. FTIR spectra showed a peak at 3000-2950 cm^{-1} range representing OH stretching vibration and intra-molecular hydrogen bonding. Similarly, prominent band between 3500-3400cm^{-1} assigned to hydrogen bonding by single bridge whereas bands from 1650-1600cm^{-1} showed carbonyl stretching vibration. FTIR data suggested DMN stability with similar findings from the molecular docking, as mentioned earlier (Belgamwar *et al*., 2009, Jadhav *et al*., 2013).

In histopathology data from animal studies, major response was observed from inflammasomes (inflammatory monocytes) with huge quantity of fibroblast cells showing no evidence of toxicity for fibroblast cells, thus making it suitable for topical application. DMN formulations were also safe on application to human skin following 4 h of study with no visible inflammatory response in the human volunteers under study.

CONCLUSION

DMN emulgel formulations were successfully prepared using varying concentrations of propylene glycol and olive oil along-with carbapol-934 using response surface methodology. Prepared formulations were stable following 6 months long accelerated stability studies. Optimized formulation (F2) depicted diffusion-based release mechanism with good permeation through rat skin. The optimized formulation showed persistent skin permeation with a flux rate of 1.851±0.05 µg/cm^2/min after a lag time of 25 minutes. Molecular docking studies confirmed the formation of stable complex in the presence of aqueous environment, which was well hydrated and depicted intermolecular hydrogen bonding with the same being observed in FTIR studies. No evidence of toxicity was observed in histopathology studies and DMN emulgel formulation was also found safe following application to human volunteers.

ACKNOWLEDGMENT

The authors would like to thank Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan for providing facilities to conduct this research.

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