Pakistan Journal of Pharmaceutical Research
Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan
ISSN: 2410-6275 Jan, 2018 DOI: 10.22200/pjpr.201811-14
Research Article

Design Expert based Optimization and Permeation Study of Novel Topically Applied Anti-acne gel

Syed Nisar Hussain Shah[1], Rabia Sumreen[2], Hina Javed*[2], Bushra Nasir[1], Rao Khurram Ayoub[2] and Atif Akber [3]

[1]Department of Pharmaceutics, Bahauddin Zakariya University, Multan Pakistan
[2]SNS Pharmaceutical Research Lab Multan, Pakistan
[3]Department of Statistics, Bahauddin Zakariya University, Multan-Pakistan

Received: May, 15, 2019
Revised: May, 16, 2019
Accepted: Jun, 29, 2019
Online:

Abstract

In present study, Propylene glycol (PG) and Polyethylene glycol (PEG) were used as permeation enhancers to increase percutaneous absorption and release of CCT gel through silicone membrane. Gel formulations containing Clarithromycin (Macrolide antibiotic) and Cetirizine HCl (a non-sedative, anti-histaminic H-1 receptor) were formulated and optimized by using Response Surface Methodology (RSM) containing different ratios of PG and PEG. PG(X_1) and PEG(X_2) were taken as two independent variables whereas cumulative amount of Clarithromycin was used to calculate dependent (response) variables (Y1…Y7). From all formulated gels, Clarithromycin was released and permeated through silicon membrane using Franz diffusion cell at 37±1℃. Fick's laws of diffusion were used to calculate permeation kinetic parameters like lag time (t_{lag}), diffusion co-efficient (D), K, permeation co-efficient (Kp), Flux (J), enhancement ratios (ER) and input rate (IR). The physical properties of all gel formulations like clarity, pH, viscosity, solubility, partition co-efficient (Ko/w), homogeneity, spreadability and Ex-vivo Panel test, Draize's irritation test were performed. Analysis of variance (ANOVA) was conducted to evaluate the results which showed that the independent variables have remarkable effects ($p < 0.05$) on dependent variables. Multiple linear regression analysis was used to compare the results among control and different formulations of CCT gels. Contour plots were also constructed to show the response between dependent and independent variables. Two gels G4 and G5 were optimized. In optimized gels maximum cumulative amounts permeated through silicon membrane were high as compared to Gc. It was found that permeation was increased with the increase in concentration of both permeation enhancers in combination i.e. PG and PEG. Thus it was concluded that PG and PEG can be successfully used in combination as permeation enhancers for transdermal delivery of CCT gel.

Keywords: Transdermal, RSM, Optimized, Permeation kinetic parameters, Silicon membrane.

Introduction

Percutaneous delivery is a term that can be defined as "The transport of drug into targeted tissues", with an attempt to avoid systemic adverse effects. The concept of percutaneous absorption of drug was first introduced. Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals (Ravi Kumar, 2008). Transdermal drug delivery (TDD) is the method in which a drug is applied to the skin in the form of a patch, cream, lotion or gels wherein the drug penetrates across the skin and reaches the bloodstream (Prausnitz et al., 2004). The percutaneous absorption of drug occurs via 2SNS Pharmaceutical Research Lab Multan, Pakistan. two consecutive processes: 1) the drug release from the topically applied formulation. 2) Absorption of the drug into the skin where it is applied (Zi et al., 2008). Therefore, the rate of percutaneous absorption increases as the rate of release of drug from the formulation increases. The rate of release of drug is also dependent on the physicochemical properties of the drug and the vehicle (Rasool et al., 2010). The permeation of drug depends on some factors i.e nature of the skin barrier, physicochemical properties of the membrane and the drug. Other Factors of significance are time of application of formulation, site of application, skin conditions, properties and types of drug formulations and finally the techniques used to aid the transdermal transport (Kandavilli et al., 2002). The extent of drug absorbed after transdermal

***Corresponding Author:** Hina Javed
Address: SNS Pharmaceutical Research Lab Multan, Pakistan.
Email address: j_hani2003@yahoo.com

drug delivery is determined by different means i.e. from blood urinary excereton Profile and from the clinical response of patients (Patel et al., 2002).

The term "Transdermal Drug Delivery System" (TDDS) includes all drug formulations that are administered topically and provide controlled drug delivery into systemic circulation. Topical drug administration is used mostly in topical infections of skin including acne, dermatophytosis, candidiasis, tineanigra and fungal keratitis. The drugs commonly used for treatment of acne are Benzoyl peroxide, Clindamycin, Tretinoin, Clarithromycin, Azithromycin, Erythromycin and Isotretinoin. The topically applied drug is absorbed in a sequential mode i.e. the drug releases from formulation, and is absorbed through stratum corneum. It passes through then epidermis and dermis and enters into blood circulation. For a best and successful transdermal formulation, it is necessary for the drug to penetrate through skin to underlying tissues and blood circulation without accumulation of drug in the skin layers. Looking at physicochemical, pharmacokinetic, and pharmacodynamic properties, Clarithromycin is a worthy candidate for topical drug delivery. It has a MW 747.96 Daltons and value of Log P (Octanol/Water) is 2.86±0.03. It is mostly metabolized by liver microsomal enzymes, which lowers its bioavailability. These properties favors its transdermal delivery approaches transdermally (Mohammadi et al., 2011). In the present study, Cetirizine used as second drug. Presently various research studies are being carried out for Cetirizine formulations, suggesting that a lot of work is to be done for making Cetirizine acceptable transdermally; a significant prospect for preferring the drug for this specific research (Waheed et al., 2014). Moreover, until now, no work has been done in the direction of skin permeation of Cetirizine gel alone or in combination which necessitate this formulation approach combination with antibiotics. Furthermore a hypothesis can be generated for this study plan; "PEG and PG may enhance the permeation rate through silicon membrane as well as solubility of Clarithromycin and Cetirizine HCl".

Experimental

Materials

Clarithromycin (Gift from Opal Laboratories, Karachi), Cetirizine (gift from Opal Laboratories, Karachi) , Isopropyl alcohol (IPA) (Merck, Germany) , Carbopol-940 (Merck, Germany), Ethanol (Merck, Germany) , Benzyl alcohol(Aldrich Chemical Co Ltd) , Ethylene glycol (Fluka, Germany), Triethanolamine (Merck, Germany), Menthol (Shama Laboratory chemical works Lahore), Propylene glycol (1,2-prpanol) (PG) (Merck, Germany), Polyethylene glycol (PEG-6000) (Fluka, Germany).

Preparation of Dilution solution

Dilution solution (D.S.) was prepared by mixing IPA and ethanol in ratio of 28.5:71.5.

Solubility Studies

Pure Clarithromycin in excess amount was added in separate Glass bottles containing 10 ml of three solvents each separately i.e. distilled water and ethanol. These mixtures were stirred in a thermostatically controlled stirrer keeping a constant temperature of 37oC ± 2 for 48 h. Then the mixtures were centrifuged at 13000 rpm for15 minutes and supernatant aliquot was taken out by a pipette and analyzed using UV-spectrophotometer at 265 nm to determine

the concentration in µg/ml (Waheed et al., 2014, Shah et al., 2012, Ayoub et al., 2015).

Partition Co-efficient Studies (Ko/w)

A small amount of Clarithromycin was dissolved in 10 ml of distilled water in separating funnel, shake it for 10 minutes. Then add 10 ml of Octanol in same separating funnel and shaked vigorously for 10 minutes. Allowed it to stand for 24 hours. Two layers were formed, the separated layers were separately collected in two different test tubes and analyzed by UV spectrophotometer at 265 nm and Octanol to water ratio were calculate. Each experiment was carried out in triplicate (n=3) (Shah et al., 2012).

Preparation of Hydro-alcoholic CCT gel

Hydro-alcoholic gels (20 gm each) of Clarithromycin and Cetirizine HCl having various concentrations of permeation enhancers i.e. PEG and PG were prepared after carefully weighing and measuring all of the ingredients, containing varying quantities as mentioned in Table 1.

Table 1: Formulation of gels (20 gm each)

Formulations (G)	Clarithromycin(g)	Cetirizine HCl (g)	X1=PG (ml)	X2=PEG (g)	Carbopol- 940 (g)	Isopropyl alcohol (ml)	Ethanol (ml)	Benzyl alcohol (ml)	Ethylene glycol (ml)	Triethanolamine (ml)	Water (ml)
G_1	0.2	0.2	6	1.0	0.5	3	5	3	0.1	0.125	2.5
G_2	0.2	0.2	6	1.0	0.5	2	5	3	0.1	0.125	2.5
G_3	0.2	0.2	4	2.0	0.5	2	5	3	0.1	0.125	3.87
G_4	0.2	0.2	6	2.0	0.5	2	5	3	0.1	0.125	3.0
G_5	0.2	0.2	3	1.5	0.5	3	5	3	0.1	0.125	4.7
G_6	0.2	0.2	7	1.5	0.5	2	5	3	0.1	0.125	1.5
G_7	0.2	0.2	5	2.5	0.5	2	5	3	0.1	0.125	4.5
G_8	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	1.5
G_9	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	2.7
G_{10}	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	2.7
G_{11}	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	2.7
G_{12}	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	2.7
G_{13}	0.2	0.2	5	1.5	0.5	3	5	3	0.1	0.125	2.7
G_C	0.2	0.2	0	0	0.7	4	6	3.5	1	0.125	4.5

In the preparation of hydro-alcoholic gels, first 500 mg Carbopol 940 was mixed in different amounts of PG and water. Approximately 2 ml of IPA and 5 ml of ethanol was taken in another beaker and 200mg Clarithromycin was dissolved in it then 200 mg Cetirizine was added in it and dissolved. The solution containing both drugs was added slowly in wetted Carbopol 940 and homogenized (Waheed et al., 2014, Rasool et al., 2010). In another beaker, PEG was dissolved in the contents containing Carbopol 940. Then added 0.1 ml of ethylene glycol and mixed. The pH of the gel was adjusted by the addition of 2-3 drops of triethanolamine. Finally, few drops of fragrance (menthol solution) were added to the gel and mixed. Then the gel was packaged in tubes and stored (Waheed et al., 2014, Ayoub et al., 2015).

Clarithromycin Assay (Calibration curve)

Stock solution was prepared by dissolving carefully weighed 100 mg of Clarithromycin in D.S in 100 ml volumetric flask, and final volume was made up to 100 ml. From this solution, dilutions of 50, 100,150, 200, 250,

300µg/ml were prepared. The resultant dilutions were then analyzed for UV absorbance and the maximum UV absorbance of Clarithromycin was found at 265 nm. The absorbance was measured for each sample at 265 nm with a UV spectrophotometer (Spectronic, Genesys 5). The Calibration curve for Clarithromycin is illustrated in the Figure 1.

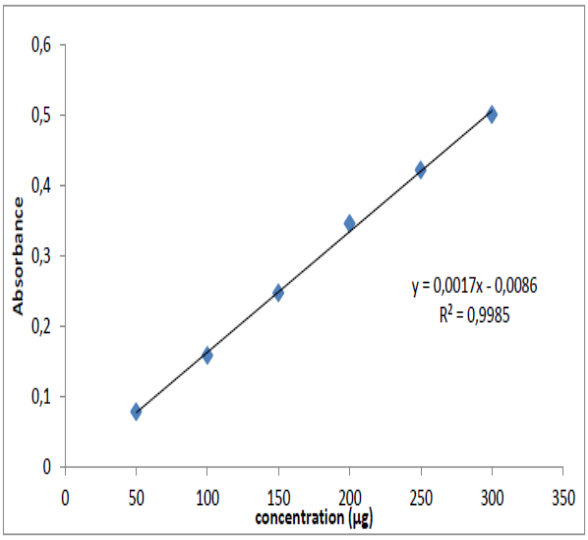


Figure 1: Calibration curve for Clarithromycin at 265 nm.

Y= 0.0017x - 0.0086 and R^2 = 0.9985; In which x was concentration (µg/ml) of Clarithromycin, y was absorbance at 265 λ max and R^2 correlation coefficient.

In vitro Diffusional Studies through Silicon Membrane

Franz-type diffusion cells were used for the diffusion studies across silicone membrane which has a diffusional area of ~0.788cm^2 and receptor phase volume of ~5 ml. Silicone membrane was cut to appropriate sizes in round-shape and soaked overnight in the receptor solution i.e. D.S. The membrane was then placed in between the two compartments, donor and receptor, of the diffusion cells. Before placing the membrane vacuum grease was applied on the inner surfaces (collar) of the two compartments to produce a leak-proof seal system. Furthermore the two compartments were clamped, after placing donor over receptor compartment. The receptor phase was filled in the receptor compartment through cell arm and was degassed in an ultrasonic bath to remove air bubbles and prevent the buildup of air pockets in the receptor phase. For uniform mixing of the receptor phase a magnetic stirrer that was placed in the receptor compartment .The opening of cell arm of receptor and circumference of the donor was covered with a parafilm, to prevent evaporation (El-Houssieny and Hamouda, 2010, Ayoub et al., 2015, Shah, 2012).

The diffusion cells were placed on a stirring bed immersed in a water bath at 37°C±2, to maintain a temperature of ~35°C at the membrane surface. After 1 hour the receptor phase was completely removed and refilled with pre-thermo stated receptor phase.

The donor compartment was charged with 1 ml of the saturated solution with excess solute present to maintain the saturation throughout the experiment. This ensures that the depletion of solute in the vehicle does not become the rate limiting step in diffusion. 0.2 ml of sample from receptor solution was drawn using micro pipette, at definite time intervals of 15, 30, 45, 60, 90, 120, 150 and 180 minutes. After each with drawl of sample, 0.2ml of pre-thermostated receptor phase was added to receptor to maintain the sink conditions. The samples were analyzed spectrophotometric at 265 nm wavelength to obtain the amount permeated through silicon membrane. Experiments were conducted in triplicate to obtain a statistically significant data (Waheed et al., 2014).

Experimental design

A computer optimization technique based on a response surface methodology (RSM) utilizing polynomial equation was used to search for the optimal Gel formulation and quantify the influences of formulation variables on the drug permeation. A central composite design (CCD) with α = 2 was employed as per the standard protocol. The amount of PG and PEG was selected as the factors, studied at five levels each. The central point (0, 0) was studied in quintuplicate as shown in Table 2. All other formulations and process variables were kept invariant throughout the study.

Table 2: Factor combinations as per chosen experimental design and translation of coded levels in actual units

	Coded Factor Levels	
Trial No.	**X_1**	**X_2**
I	–1	-1
II	1	–1
III	–1	1
IV	1	1
V	-2	0
VI	2	0
VII	0	–2
VIII	0	2
IX	0	0
X	0	0
XI	0	0
XII	0	0
XIII	0	0

Coded Level	**–2**	**–1**	**0**	**1**	**2**
X_1: PG (ml)	3	4	5	6	7
X_2: PEG (mg)	0.5	1	1.5	2	2.5

Physical Properties

The appearance and other physical properties, including clarity, homogeneity consistency and spreadability of the prepared Clarithromycin gel were inspected.

Homogeneity

Through visual appearance the homogeneity of prepared gels was checked after setting in the container. In order to check the presence of aggregates the prepared gels were carefully tested (Shah et al., 2012).

Spreadability test

In order to determine the spreadability of the prepared Clarithromycin gels, 0.1 g of the gel sample was put on the lower glass slide surface of the marked circle of two glass slides (5mm sides) and pressed between them by placing a weight of about 1 kg on the upper slide. Allow the apparatus to stand for about 5 minutes. The diameters of the spreaded gel around marked circle were measured in cm. The experiment was repeated in triplicate (Waheed et al., 2014, El-Houssieny and Hamouda, 2010).

Stability Studies

Stability studies were performed on the optimized gels i.e. G4 and G5.The formulations were packed in collapsible aluminum tubes (5g) and subjected to stability studies at 250C / 60 % RH and 400C / 65 % RH for a period of three months. Samples were withdrawn after specified time was evaluated for physical appearance, rheological properties and chemical assay. Both the gels were found stable under stated storage conditions (Ayoub et al., 2015).

Drug Content

The volumetric flask containing solution (10ml) was shaken well and filtered and estimated spectrophotometrically at 265nm using as blank.

Ex-Vivo Studies

Primary skin irritation test: (Draize's skin irritation test) In present study 11 human volunteers were selected to perform primary test for irritation for optimized gels and a small amount of optimized gel formulation was applied on an area of 2 cm^2 into the back of hand. The volunteers were then observed for lesions or irritation (Ayoub et al., 2015).

Panel Test

Questionnaire containing six questions was prepared and given to each volunteer (total of eleven volunteers of age 21±2 either sex) for sensory evaluation of the optimized gels and average points were calculated from the points assigned (Nine values from -4 to +4 indicating very bad to Excellent respectively) by each volunteer for each question (Ayoub et al., 2015).

Statistical Data Analysis

Microsoft Excel, version 2007 was utilized to perform Statistical Data Analysis. Statistically significant differences, between 13 different formulations, were verified using the regression analysis and analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance (Ayoub et al., 2015,).

Spreadability test

The diameters of the spreaded gel around the marked circle were measured in cm (Table 3) and three average readings were taken and calculated (El-Houssieny and Hamouda, 2010, Ayoub et al., 2015).

pH – value

The pH – value of the prepared gels were measured at 25 ± 1°C using Digital pH meter, WTW, pH 526 Germany (Table 3).

Viscosity

The viscosity of all prepared gels was determined and they were found within the limits (Table 3).

Homogeneity

All gels were tested for homogeneity by visual inspection after they have been set in the container. They were tested for their appearance and presence of any aggregates/precipitates.

Partition Co-efficient Studies

The value of partition coefficient (Ko/w) calculated is 2.86 ±0.03.

Results

Solubility Studies

The solubility of Clarithromycin in different solvents were determined i.e. 0.37 (µg/ml) ±0.01 in water, 14930 (µg/ml) ±0.011 in ethanol and 17690 (µg/ml) ±0.021 in IPA.

Physical properties

The appearance and other physical properties including clarity, homogeneity, pH, viscosity and spreadability of the prepared gels were given in the Table 3.

Table 3: Physical properties of CCT gels

Trails	Clarity	Homogeneity	Spreadability (cm)	pH	Viscosity Cps×10^3
G_1	Transparent	Good	2.2	4.95	130
G_2	Transparent	Good	3.1	5.90	132
G_3	Transparent	Good	3.3	5.05	130
G_4	Transparent	Good	3.5	5.30	138
G_5	Transparent	Good	3.4	5.01	135
G_6	Transparent	Good	3.1	5.25	136
G_7	Transparent	Good	3.8	5.02	135
G_8	Transparent	Good	4.4	5.25	138
G_9	Transparent	Good	4.1	5.05	135
G_{10}	Transparent	Fair	2.1	5.10	136

G_{11}	Less Transparent	Good	2.2	5.08	132
G_{12}	Less Transparent	Fair	2.0	5.08	132
G_{13}	Less Transparent	Good	2.1	5.03	133

Ex-Vivo Studies

Draize skin irritation test

Primary skin irritation test was performed for optimized gels i.e. G_4 and G_5 on 11 volunteers and they found no irritation or lesions as shown in Table 4.

Table 4: Skin irritation test for optimized CCT gels

Optimized Trails	1st day	7th day	14th day	30th day
	E/R/U	E/R/U	E/R/U	E/R/U
G_4	0	0	0	0
G_5	0	0	0	0

E= Erythema R= Redness U= Urticaria

Panel Test

Questionnaire containing six questions was prepared and given to each volunteer (total of eleven volunteers of age 21±2 either sex) for sensory evaluation of the optimized gels and average points were calculated from the points assigned (Nine values from -4 to +4 indicating very bad to Excellent respectively) by each volunteer for each question as shown in Table 5.

Table 5: Average values for panel test of the optimized gels at room temperature

Ease of application	Sense after application	Sense in long-term	Skin irritation	Shine on Skin	Sense of softness
3.65	3.21	3.76	00	1.33	3.78

Stability Studies

The optimized gels G_4 and G_5 were selected for stability testing for three months as per ICH guidelines at a temperature of 25±1°C. The optimized selected CCT gels were analyzed for the change in their appearance (C & T), pH and drug content determination as mentioned in Table 6.

Table 6: Stability study of the optimized gels at room temperature

Optimized Trails	Months	Appearance	pH	Drug content %
G_4	1	C & T	5.30	98.33
	2	C & T	5.30	98.24
	3	C & T	5.30	98.24
G_5	1	C & T	5.01	98.70
	2	C & T	5.01	98.70
	3	C & T	5.01	98.69

C=Clear T=Transparent

Diffusion Studies

The in-vitro permeation study of CCT gels in the donor compartment of the modified Franz diffusion cell was done (Waheed et al., 2014, Ayoub et al., 2015). The cumulative amount of Clarithromycin that has been passed through silicone membrane was estimated by UV spectrophotometer at 265 nm and analyzed statistically on MS Excel 2007 as represented in Table 7. All the permeation data obtained is presented here along with the corresponding graphical representations as shown in Table 8.

Data Analysis

Fick's second law of diffusion, the cumulative amount of drug (Q_t) appearing in the receptor solution in time t is expressed in Eq. 1:

$$Q_t = AKLC_0\left[\left(\frac{Dt}{L^2}\right)-\left(\frac{1}{6}\right)-\left(\frac{2}{\pi^2}\right)\Sigma\frac{(-1)^n}{n^2}\right]\times\exp\left(\frac{D^n 2\pi^2 t}{L^2}\right) \quad (1)$$

Table 7: Comparison of cumulative amounts of Clarithromycin (μg/cm^2) permeated through silicone membrane from various formulated gels (mean±SD, n=3)

Trial gels	Time (minutes)							
	15	30	45	60	90	120	150	180

G_1	1783.63 ±63.70	2164.24 ±57.65	2746.67 ±33.72	3458.93 ±44.38	4203.03 ±86.24	5015.43 ±74.39	5851.89 ±83.73	6736.56 ±87.35
G_2	1974.728 ±63.701	2152.002 ±62.93	2713.739 ±64.65	3415.997 ±74.96	4166.849± 57.87	4956.723 ±83.956	6234.626 ±95.76	7173.106 ±105.493
G_3	2356.933 ±63.701	2780.908 ±57.652	3499.523 ±43.202	4236.463 ±71.77	5015.845± 21.639	5821.807 ±100.082	6733.891 ±38.726	7702.873 ±68.284
G_4	2420.634 ±63.701	2743.322 ±45.273	3513.602 ±47.872	4324.454 ±38.451	5224.589± 57.523	6122.826 ±69.451	7084.25 ±60.216	8083.147 ±111.595
G_5	2314.466 ±36.778	2571.419 ±50.79	3257.873 ±63.198	4076.311 ±51.929	4949.119± 77.175	5934.63 ±13.841	6878.874 ±10.142	7866.879 ±12.481
G_6	2420.634 ±63.701	2659.556 ±40.493	3458.972 ±30.166	4349.598 ±21.478	5364.71 ±40.482	6398.815 ±53.248	7432.342 ±26.475	8686.503 ±37.446
G_7	2441.867 ±36.777	2697.287 ±50.789	3454.001 ±50.849	4422.387 ±56.464	5488.795± 51.755	6527.93 ±49.819	7761.519 ±61.735	8978.299 ±81.63
G_8	2356.933 ±63.701	2613.375 ±89.251	3373.509 ±47.345	4299.279 ±46.766	5313.732± 22.429	6378.01 ±6.8278	7466.913 ±64.408	8753.835 ±6.378
G_9	2484.335 ±63.701	2705.737 ±62.934	3427.161 ±36.826	4336.837± 175.174	5283.385± 100.207	6297.258 ±109.420	7414.638 ±150.154	8627.221 ±73.513
G_{10}	2335.699 ±36.778	2625.903 ±50.789	3424.66 ±53.427	4295.20 ±66.800	5286.000± 66.632	6325.968 ±105.026	7370.472 ±76.267	8488.165 ±71.759
G_{11}	2250.765 ±36.778	2592.251 ±33.285	3373.596 ±38.969	4236.577 ±30.166	5153.697± 49.015	6153.346 ±43.846	7223.886 ±61.694	8397.932 ±48.596
G_{12}	2293.232 ±63.701	2567.194 ±62.934	3355.059 ±64.651	4165.339 ±74.963	5183.725± 61.313	6166.107 ±64.908	7221.444 ±58.149	8444.859 ±11.706
G_{13}	2356.933 ±63.701	2613.375 ±40.493	3407.016 ±45.272	4290.975 ±71.896	5249.16 ±58.461	6263.925 ±16.709	7314.291 ±14.184	8476.468 ±26.847
Gc	1783.63 ±63.70	1963.2 ±62.93	2494.94 ±72.93	3083.43 ±87.86	3783.16 ±93.22	4509.61 ±103.1	5284.28 ±113.08	6108.95 ±123.07

Table 8: Permeation kinetic parameters of formulated gels with RSM factors and response variables (mean ±S.D; n=3)

Trial gels	**Coded levels**		**Actual Concentration**		**Response variables**		**Permeation parameters**	
	X_1	X_2	PG (ml)	PEG (g)	$[Y_1]$ t_{lag} (min)	$[_2Y]$ ER	Flux (μg/cm^2.min)	Diffusion coefficient (D_m) (10^{-4} · cm^2/min)
G_1	-1	-1	4.0	1.0	47.368±1.524	1.126	29.897±0.577	6.11±1.97E-05
G_2	1	-1	6.0	1.0	41.639±1.113	1.206	32.04±0.291	5.37±1.44E-05
G_3	-1	1	4.0	2.0	63.069±1.873	1.202	31.93±0.11	8.14±2.42E-05
G_4	1	1	6.0	2.0	56.871±1.972	1.299	34.513±0.713	7.34±2.54E-05
G_5	-2	0	3.0	1.5	51.877±2.177	1.289	34.25±0.332	6.7±2.81E-05
G_6	2	0	7.0	1.5	69.084±0.508	1.444	30.89±0.02	8.92±6.56E-06
G_7	0	-2	5.0	0.5	43.283±1.171	1.517	40.307±0.469	5.59±1.51E-05
G_8	0	2	5.0	2.5	43.187±1.819	1.474	39.163±0.293	5.57±2.35E-05
G_9	0	0	5.0	1.5	48.233±1.946	1.420	37.717±0.933	6.23±2.51E-05
G_{10}	0	0	5.0	1.5	46.588±0.299	1.422	37.777±0.359	6.01±3.87E-06
G_{11}	0	0	5.0	1.5	45.843±1.898	1.402	37.267±0.596	5.92±2.45E-05
G_{12}	0	0	5.0	1.5	45.056±2.279	1.411	37.503±0.304	5.82±2.94E-05
G_{13}	0	0	5.0	1.5	47.004±2.584	1.412	37.497±0.482	6.07±3.33E-05
Gc	0	0	5.0	-	50.219±1.453	-	26.563±0.375	6.48±1.88E-05

Where A, is the effective diffusion area, C_0, represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady state, it is expressed in Eq. 2:

$$\frac{Q_t}{A} = KLC_0\left[\left(\frac{Dt}{L^2}\right)-\left(\frac{1}{6}\right)\right] \quad (2)$$

The steady state flux (J) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area as a function of time, in the steady-state region where the drug would pass by constant rate. The lag time was determined from the x-intercept of the slope at the steady state. The flux is expressed in Eq. 3;

$$J = \frac{C_0 KD}{L} = C_0 K_P \quad (3)$$

From this relation the permeability coefficient was calculated using Eq. 4;

$$K_P = \frac{J}{C_0} \quad (4)$$

The effectiveness of penetration enhancers (enhancement ratio i.e. ER) was calculated from the ratio of (gels) flux in the presence and absence of enhancers.

The analysis of responses, namely lag time (t_{lag}) and enhancing ratio (ER) were performed using Minitab statistical software version 16. Linear, quadratic and cubic mathematical models were employed. The best fit model was selected based on the comparison of several parameters including the multiple correlation coefficients (R^2), adjusted multiple correlation coefficients (adjusted R^2), predicted residual sum of square (PRESS), and the lack of fit (p-value). Experimental design resulted in a quadratic polynomial equation which is expressed in Eq.5:

$$Y=\beta_0+ \beta_1X_1+ \beta_2X_2+ \beta_{12}X_1X_2 - \beta_1^2X_1^2- \beta_2^2X_2^2 \quad (5)$$

Where Y is the dependent variable (response), β_0 is a constant representing the mean of the dependent variable obtained in each experiment; X_1 and X_2 are the independent variables; X_1X_2 are the interaction terms; X_1^2 and X_2^2 are the quadratic term and β_1, β_2…are the coefficients. This expression gives an insight into the effect of the different independent variables. A positive sign of coefficient indicates a synergistic effect whereas a negative term indicates an antagonistic effect upon the response. Large coefficient means the causal factor has potent influence on the response. Afterwards contour and 3D-surface plots visualizing the simultaneous effect of the causal factors on the response were established (Shah et al., 2009).

The optimization and validation of experimental domain was performed by predicting optimum formulation using numerical optimizing provision of the Minitab software. The experimental response values and model predicted response values were compared and percentage predicted error was calculated. One-way ANOVA was applied to estimate the significance of the model ($p < 0.05$) as shown in table 9 & 10. All measured data are expressed as mean ± standard deviation (S.D.). Each measurement was executed in three replicates (n= 3).

RSM Optimization Results Mathematical modeling

Mathematical relationships in the form of polynomial equations are generated using MLRA for the studied response variables as expressed in Equation 6 & 7. The polynomial equations comprise the coefficients for intercept, first order main effects, interaction terms, and higher order effects. The sign and magnitude of the main effects show the relative impact of each factor on the response. Statistical validation of the polynomial equations generated by Design Expert and estimation of significance of the models was established on the basis of analysis of variance provision of the software as shown in Tables for Y_1 and Y_2 respectively. Using 5% significance level, a model is considered significant if the P-

value (significance probability value) is less than 0.05.

Effect of Enhancers on lag time values Y_1 (t_{lag})

Mathematical relationships generated using MLRA for the studied response tlag is expressed in equation 9 and analysis of variance is given in Table 11.

Analysis of variance (ANOVA) for Response t_{lag} (Y_1)

In Table 9, p-values for response Y_1 represent that the linear contributions (X_1) and (X_2) are non-significant. Also the cross product contribution (X_2X_2) is not significant; whereas lack of fit is significant.

Table 9: Analysis of variance (ANOVA) for Response Y_1 (t_{lag})

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Significance
Model	157.021	5	31.404	0.820	0.572	Non-Significant
X_1	42.312	1	42.312	1.105	0.328	NS
X_2	78.758	1	78.758	2.057	0.195	NS
X_1X_2	0.055	1	0.055	0.001	0.971	NS
X_1^2	13.618	1	13.618	0.356	0.570	NS
X_2^2	12.095	1	12.095	0.316	0.592	NS
Residual	267.971	7	38.282	—	—	—
Lack of Fit	262.200	3	87.400	60.580	0.0009	Significant
Pure Error	5.771	4	1.443	—	—	—

Polynomial equation

The equation below indicates that PG has a weaker but negative effect on lag time. But the PEG has a stronger and positive influence. The combined effects of both the factors have negative effect but the quadratic contribution of PEG has positive influence on t_{lag}. From the tlag equation 6, the two terms containing X_2 ($2.56X_2$-$0.73X_2^2$) showed that the t_{lag} decreased with increasing concentration of PEG.

$$t_{lag}=48.16-1.88X_1+2.56X_2-0.12X1X_2+0.77X_1^2-0.73X_2^2 \quad (6)$$

Contour plots for the response Y_1 (t_{lag})

Figures 2 A and B depict a nonlinear trend of tlag in an ascending pattern with an increase in the amount of each enhancer. However with PG and PEG the declining trend is observed at higher level, followed by invert plateau at lower levels. This may be explained on the basis of mathematical models generated for the response variable, t_{lag} (Eq. 6). It can be deduced from the model that at higher levels of PG and intermediate level of PEG the value of t_{lag} is increased. The higher-order terms e.g. (X_1^2 and X_2^2) tend to outweigh the linear contribution of the enhancer (X_2) alone.

Permeation enhancers and (B) Response surface plot showing the influence of PG and PEG on t_{lag}.

Effect of Enhancers on ER (Y_2)

Mathematical relationships generated using MLRA for the studied response ER is expressed in equation and analysis of variance is given in Table 11.

Analysis of variance (ANOVA) for Response ER (Y_2)

In Table 10, p-values for response Y_2 represent the quadratic contribution of PG has negative influence on ER. Also the cross product contribution (X_1X_2) is not significant; whereas lack of fit is significant.

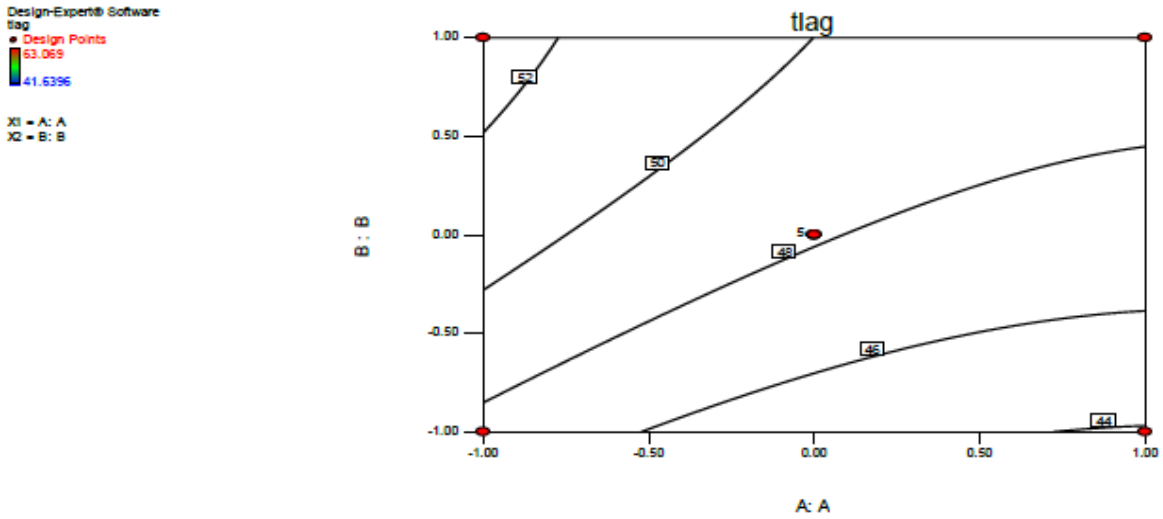
Polynomial equation

From the ER equation 7, the two terms containing X_1 ($0.041X_1$-$0.02X_1^2$) showed that the ER increased with increasing concentration of PG but decreased with further increasing concentration.

$$ER=1.35+0.041X_1+0.007X_2+0.004X_1X_2-$$

$0.02X_1^2+0.02X_2^2$ (7)

(A)



(B)

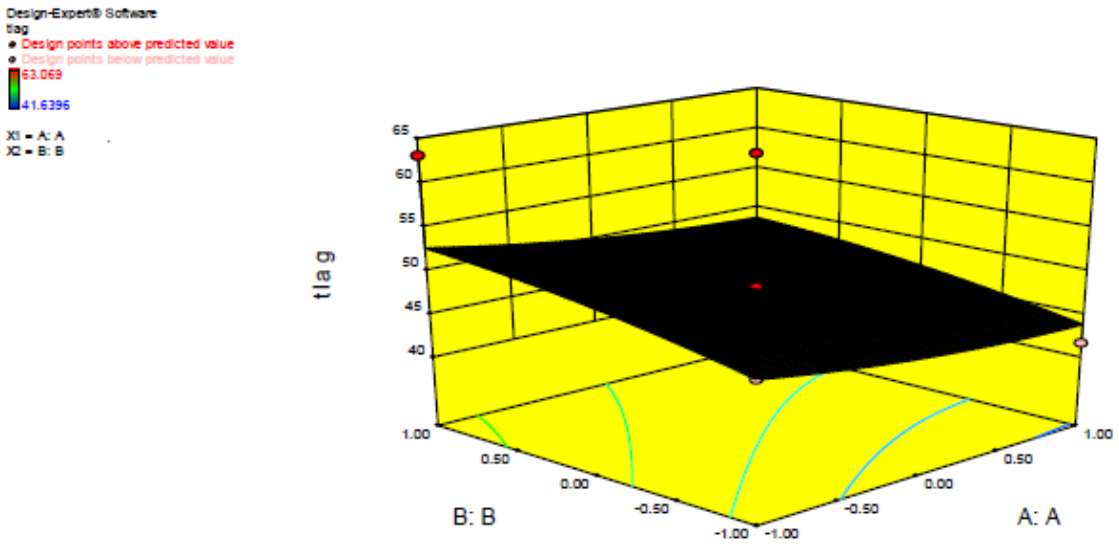


Figure 2: (A) Contour plot showing the relationship between various levels of 2.

Table 10: Analysis of variance (ANOVA) for Response Y_2 (ER)

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Significance
Model	0.0371	5	0.0074	0.387	0.844	Non-Significance
X_1	0.0198	1	0.0198	1.032	0.344	NS
X_2	0.0006	1	0.0006	0.030	0.867	NS
X_1X_2	6.86E-05	1	6.86E-05	0.004	0.954	NS
X_1^2	0.0054	1	0.0054	0.283	0.611	NS
X_2^2	0.0065	1	0.0065	0.339	0.579	NS
Residual	0.1345	7	0.0192	—	—	—
Lack of Fit	0.1343	3	0.0448	769.785	<0.0001	Significance
Pure Error	0.0002	4	5.82E-05	—	—	—

Contour plots for the responses Y_2 (ER)

Figures: 3 A and B depict a nonlinear trend of ER in a descending pattern with an increase in the amount of each enhancer. With PG and PEG the declining trend is observed at higher level as the concentration of each enhancer increases the value of ER decreases. By mathematical model generated for the response variable, ER (Eq. 7) shows that at higher levels of PG and intermediate level of PEG the value of ER increases. The higher-order terms $X_1(X_1^2$ and $X_2^2)$ tend to outweigh the linear contribution of the enhancer (X_1) alone. Plateaus were observed at higher levels of enhancers with increased ER.

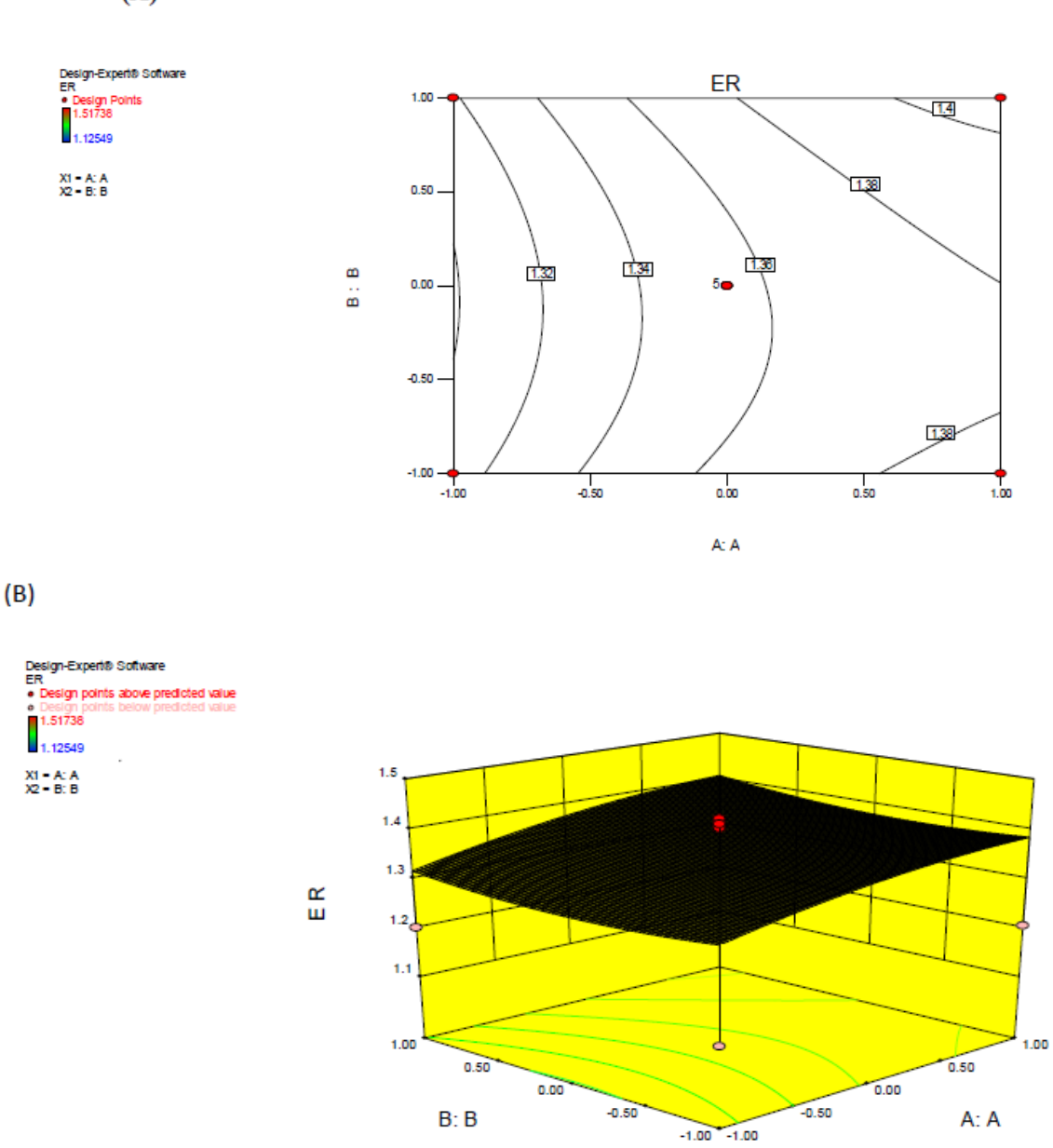


Figure 3: (A) Contour plot showing the relationship between various levels of 2 permeation. Enhancers and (B) Response surface plot showing the influence of PG and PEG on ER

The comparative values of R^2, adjusted R^2, PRESS, lack of fit (p-value) are summarized in Table 11.

Table 11: Summarized statistical parameters of each response variable determined by Multiple Linear Regression Analysis (MLRA)

Regression Coefficient	Coefficient Estimates	
	t_{lag}	*ER*
β°	48.16	1.35
β_1 (X_1) PG	-1.88	0.041
β_2 (X_2) PEG	2.56	0.007
β_{12} (X_1X_2)	-0.12	0.004
β_1^2 (X_1^2)	0.77	-0.02
β_2^2 (X_2^2)	-0.73	0.02
Model (p-value)	0.572	0.844
R^2	0.6248	0.5703
Adjusted R^2	0.3568	0.2634
PRESS	397.81	116.26
C.V %	26.24	25.61
Lack of Fit (p-value)	769.785	<0.0001

Discussion

The solubility of Clarithromycin in each solvent was compared to select the best solvent system for receptor phase for permeation diffusion study (Shah *et al.*, 2012). The Clarithromycin is not soluble in water whereas slightly soluble in ethanol and IPA as reported in this study. The significance of formulated gels variables on Clarithromycin permeation in the presence of enhancers PG and PEG was evaluated through multiple linear regression analysis (MLRA) using Design Expert version 16 and the comparative values of R^2, adjusted R^2, PRESS, lack of fit (p-value) are calculated. It seemed that experimental outcomes that enhance the effect of PG and PEG are not only due to the changing solubility of these formulations in the solvent system, but also due to the transport rate of the permeate (Clarithromycin) mostly by diffusion as concluded in earlier studies (Ayoub *et al.*, 2015, Kasting *et al.*, 1987, Shah, 2012). All physical evaluation has shown that clarithromycin gels possessed good homogeneity and stability for prolonged period of time. The gel formulations has shown no skin irritation so can be used safely on skin.

Conclusion

It can be concluded from this research study that PG and PEG are effective penetration enhancers and can be used in combination in the CCT gel formulations which may be effectively used because Clarithromycin is soluble in ethanol and IPA so can easily accumulate in SC of the skin/membrane. Cetirizine HCl is freely soluble in both methanol and water so due to combination of organic solvents both can show their synergistic effect in a significant pattern.

Acknowledgements

The authors acknowledge the support of Department of Pharmacy, Bahauddin Zakariya University, Multan for providing facilities to conduct this work.

Declaration of interest

The authors declare that there are no potential conflicts of interest associated with this study.

References

Ayoub, RK Murtaza, G Imran, M Khan, SA Mir, S Khan, AK Azhar, S Mehmood, Z Sajjad, A and Shah, SNH. (2015). Formulation and permeation kinetic studies of flurbiprofen gel. *Tropical Journal of Pharmaceutical Research,* **14**: 195-203.

El-Houssieny, BM and Hamouda, HM. (2010). Formulation and evaluation of clotrimazole from pluronic F 127 gels. *Drug discoveries & therapeutics,* **4**.

Kandavilli, S Nair, V and Panchagnula, R. (2002). Polymers in transdermal drug delivery systems. *Pharmaceutical technology,* **26**: 62-81.

Kasting, GB Smith, RL and Cooper, E. (1987). Effect of lipid solubility and molecular size on percutaneous absorption. *Pharmacol. Skin,* **1**: 138-153.

Mohammadi, G Nokhodchi, A Barzegar-Jalali, M Lotfipour, F Adibkia, K Ehyaei, N and Valizadeh, H. (2011). Physicochemical and anti-bacterial performance characterization of clarithromycin nanoparticles as colloidal drug delivery system. *Colloids and Surfaces B: Biointerfaces,* **88**: 39-44.

Patel, H Ten Berge, W and Cronin, MT. (2002). Quantitative structure–activity relationships (QSARs) for the prediction of skin permeation of exogenous chemicals. *Chemosphere,* **48**: 603-613.

Prausnitz, MR Mitragotri, S and Langer, R. (2004). Current status and future potential of transdermal drug delivery. *Nature reviews Drug discovery,* **3**: 115.

Rasool, BKA Abu-Gharbieh, E Fahmy, S Saad, H and Khan, S. (2010). Development and evaluation of ibuprofen transdermal gel formulations. *Tropical Journal of Pharmaceutical Research,* **9**.

Ravi Kumar, M 2008. Handbook of particulate drug delivery (2-Volume Set). American Scientific Publishers ISBN.

Shah, SNH. (2012). Developing an efficacious diclofenac diethylamine transdermal formulation. *Journal of Food and Drug Analysis,* **20**.

Shah, SNH Asghar, S Choudhry, MA Akash, MSH Rehman, NU and Baksh, S. (2009). Formulation and evaluation of natural gum-based sustained release matrix tablets of flurbiprofen using response surface methodology. *Drug development and industrial pharmacy,* **35**: 1470-1478.

Shah, SNH Salman, M Ahmad, M Rabbani, M and Badshah, A. (2012). Effect of oleic acid on the permeation kinetics of Diclofenac Diethylamine. *Journal of the Chemical Society of Pakistan,* **34**.

Waheed, S Murtaza, G Sharif, A Rao, KA and Shah, SNH. (2014). Comparative efficiency of propylene glycol and polyethylene glycol in enhancing percutaneous absorption and release of a drug through silicone membrane and rat skin. *Pakistan Journal of Zoology,* **46**.

Zi, P Yang, X Kuang, H Yang, Y and Yu, L. (2008). Effect of HPβCD on solubility and transdermal delivery of capsaicin through rat skin. *International journal of pharmaceutics,* **358**: 151-158.