

Formulation and *in vitro* evaluation of clotrimazole gel containing almond oil and Tween 80 as penetration enhancer for topical application

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Abstract: Achieving a desirable percutaneous absorption of drug molecule is a major concern in formulating dermal and transdermal products. The use of penetration enhancers could provide a successful mean for this purpose. The aim of this study was to develop Clotrimazole gel and to evaluate the effect of almond oil and tween 80 (in different concentrations), on the permeation of drug through rabbit skin *in vitro*. In order to investigate the effect of penetration enhancers used in this study on the permeation of Clotrimazole through sections of excised rabbit skin, Franz diffusion cell was employed. Sample solution was withdrawn at specific time interval up to 24 h. Significant difference in permeation among the eight formulations was seen in the study. The permeation profile of various formulations also showed that the added enhancers in individual batches affected the permeation of the drug. Drug permeation increased with increased concentration of Tween 80 and decreased concentration of almond oil. Furthermore, almond oil combined with tween 80 showed synergistic effect. The clotrimazole gels were successfully formulated and could be beneficial for topical use.

Keywords: Clotrimazole; almond oil; tween 80; penetration enhancer; transdermal drug delivery.

INTRODUCTION

Clotrimazole [bis-phenyl-2-(chloro-phenyl)-1-imidazole-5-ylmethane], a lipophilic, an imidazole derivative. Clotrimazole is a broad spectrum antimycotic agent (Pedersen *et al.*, 1998). It is also a promising agent for various diseases including sickle cell anemia and cancer, anti-inflammatory effect in patients with rheumatoid arthritis (Ning *et al.*, 2005) and neuroprotective effect (Wojtulewski *et al.*, 1980). Clotrimazole when administered orally exhibits poor bioavailability, due to low aqueous solubility and slow dissolution in water (Pedersen, 1993). Therefore to improve its bioavailability, an attempt was made to develop transdermal Clotrimazole gels.

Transdermal delivery system of drugs is a novel drug delivery system. This system breaks many barriers in drug therapy like need of assistance and uncomfortable administration. Transdermal delivery has many advantages over conventional modes of drug administration like it potentially decreases side effects, avoids hepatic first pass metabolism and improves patient compliance (Allen *et al.*, 2005 and Barry, 2002). Gels are semisolid systems in which a liquid phase is constrained within a three-dimensional polymeric matrix (consisting of natural or synthetic gums) in which a high degree of physical (or sometimes chemical) cross-linking has been introduced. The clarity range from clear to a whitish translucent. The polymers are used between 0.5-15 % and in most of the cases they are usually at the concentration

between 0.5-2%. Gels are usually clear transparent semisolid containing the solubilised active substances (Chandira *et al.*, 2010).

However, the skin, especially the stratum corneum provides resistance for drug absorption and it is the rate limiting step in percutaneous absorption. The permeation of drugs through the stratum corneum can be enhanced by physical methods and chemical modification or by the use of chemical penetration enhancers. Chemical penetration enhancers modify the barrier properties of the stratum corneum and hence increase the drug permeability across the skin. Chemical enhancer should be non-toxic, non-allergenic and also compatible with the drugs and excipients (Michniak *et al.*, 1993). Many chemicals have been investigated that act as penetration enhancers like azone, alcohols, pyrrolidones, sulfoxides. It was also observed that high concentration of olive oil was more effective by enhancing penetration of drug through stratum corneum (Hussain *et al.*, 2012). In the present study, attempts have been made to design, formulate and evaluate Clotrimazole gel and to explore the penetrating enhancing activity of almond oil and tween 80.

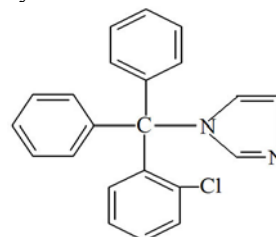


Fig. 1: Chemical structure of Clotrimazole

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MATERIALS AND METHODS

Materials

Clotrimazole (CLTZ) (Pearl pharma, Islamabad), Almond oil, Tween 80, Carboxy polymethylene (CPM) (Sigma Aldrich, Germany), Ethanol, Sodium Hydroxide, Potassium dihydrogenphosphate, Triethanolamine (TEA) (Merck, Germany), Hairless Rabbit skin and Distilled water. All the chemicals were used without further purification.

Preparation of Standard Curve

A standard calibration curve was constructed for Clotrimazole in order to obtain the linear equation which was further used to calculate the penetrated concentration of Clotrimazole across rabbit skin. For this purpose a stock solution was prepared by dissolving 20 mg of Clotrimazole in 100 ml of ethanolic phosphate buffer pH 7.4. The mixture was heated at 40°C in a water bath to facilitate solubilization using a sonicator. The mixture was then shaken for 48 hrs in an isothermal shaker and filtered through membrane filter (pore size 0.45 mm). The clear filtrate was diluted with ethanolic phosphate buffer pH 7.4 and was measured spectrophotometrically using a spectrophotometer

(UVIDEC-1601 Shimadzu, Japan) at 262 nm. The calibration curve was obtained by plotting absorbance of Clotrimazole (y-axis) against its concentration (x-axis) fig. 2. The return equation is: $Y = 12.22 X + 0.002$.

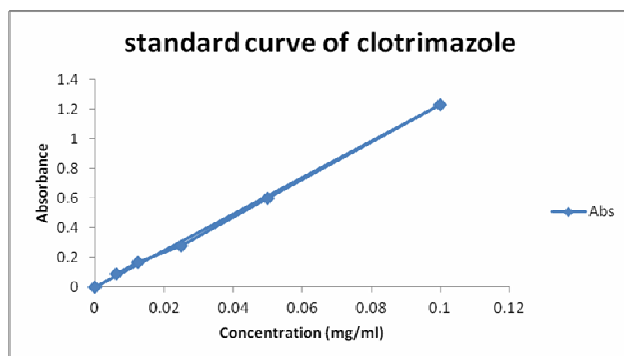


Fig. 2: Standard curve of Clotrimazole.

Table 1: Composition of different gel formulations

Formulation Code	Drug g	Polymer g	Ethanol ml	Tea g	Enhancers		Water q.s. to 100 ml
					Almond oil (g)	Tween80 (g)	
F 1	2	1	10	1	-	-	100
F 2	2	1	10	1	1	-	100
F 3	2	1	10	1	2	-	100
F 4	2	1	10	1	3	-	100
F 5	2	1	10	1	-	0.1	100
F 6	2	1	10	1	-	0.3	100
F 7	2	1	10	1	-	0.5	100
F 8	2	1	10	1	2	0.5	100

Preparation of clotrimazole gel

Gels were prepared by dispersing the polymer CPM (carboxy polymethylene) in water and stirred continuously at 300 rpm for 2 h. The Clotrimazole (2%) was dispersed in 10 ml of ethanol and then added to the carboxy polymethylene mixture. To this mixture, different concentrations of almond oil and tween 80 was added which behaves as penetration enhancers and mixed for 1 h. Finally the dispersion was neutralized and made viscous by the addition of triethanolamine. The gel was sonicated for 30 min on bath sonicator and kept overnight to remove air bubbles (Sastry *et al.*, 1995). The compositions of different gel table 1.

Evaluation of gels

The above formulated gels containing Clotrimazole were subjected to evaluation for the following parameters.

pH of gel

The pH of the various gel formulations was determined by using digital pH meter (Denver, USA) (table 2). Mean of two readings was recorded.

Viscosity

Viscosity of the different gel formulations was measured by Brookfield viscometer (model DV-1+, USA), using spindle No 04. Viscosity of gel was measured at different angular velocities at a temperature of 37°C. The spindle was rotated at 2.5, 5 and 10 rpm. The average of two readings was used to calculate the viscosity (Ikanth *et al.*, 2008).

Spreadability

Spreadability of gel was determined by wooden block and glass slide apparatus. The apparatus consisted of a wooden block with fixed glass slide and a pulley. About 20 g of gel was applied between the two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 min. Now, about 5 g of weight was added to the pan. The time required to separate the two slides i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (S) (Mitra *et al.*, 2006).

$S = M.L/T$ Where, S= spreadability in g.cm/s
M= weight tide to upper slid, L= length moved on the glass slide, T= time taken.

Extrudability

A collapsible tube was filled with sample gel and then pressed firmly at the crimped end. When the cap was removed, gel extruded until pressure dissipated, weight in grams required to extrude 0.5 cm ribbon of gel in 10 s was determined (Shinde *et al.*, 2005). Extrudability = Applied weight to extrude gel from tube (in gm)/Area (in cm^2)

Homogeneity

All the developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

Skin Irritation study

Three albino rabbits were selected for skin irritation study. The test sites were depilated on both sides of the spine and demarcated for the application of the formulation, 24 h prior to the test. The measured quantity of gel was applied to the test sites. The test sites were observed for the erythema and edema for 48 h after application.

Drug content

A specified quantity (100 mg) of the developed gel was taken and dissolved in 100 ml of ethanolic phosphate buffer pH 7.4. The volumetric flask containing gel solution was shaken for 2 h on mechanical shaker in order to get complete solubility of drug. The resultant mixture was filtered through membrane filter (pore size 0.45 μm). The absorbance of the sample was determined spectrophotometrically at 262 nm using ethanolic phosphate buffer pH 7.4 as a blank. The concentration of Clotrimazole was estimated from the regression equation of the calibration curve (Sera and Ramana., 2006).

Stability study

Stability study was carried out for selected gel formulations for three months at a temperature of 40°C/75 % RH. After three months formulations were evaluated for physicochemical properties and *in-vitro* permeation study (Harmonized Tripartite Guidelines, 2003).

Preparation of skin

Rabbit skin was used for the *in vitro* permeation study. The rabbit was anesthetized with chloroform and the abdomen region was carefully shaved with a razor after removal of hair by electric clipper. Full thickness skin (epidermis with *stratum corneum* and dermis) was excised from the shaved abdominal site. The dermal tissue and fat was surgically removed. The skin was washed immediately with phosphate buffered saline, wrapped in aluminum foil and stored at -20°C till further use. Used it within 1 week of preparation and before starting the

experiments, the skin was allowed to reach room temperature for 10 h (Narishetty and Panchagnula, 2004).

In vitro permeation study

The *in-vitro* drug permeation from gel formulations was studied across excised rabbit skin using Franz diffusion cell with effective diffusional surface area of 0.77 cm^2 and a receptor cell volume of 5 ml. The receptor compartment was filled with ethanolic phosphate buffer pH 7.4. The rabbit skin was fixed between the donor and receptor compartment of Franz cell in such a way that the epidermis is exposed to open air, while the inner surface faces the receptor compartment. The donor compartment was charged with 1 gm of sample gel and covered with a piece of aluminum foil to prevent drying out. The temperature of the cell was maintained at 37 °C by surrounding water in jacket and the medium was stirred by magnetic stirrer at 100 rpm. The samples were collected from the receptor compartment at predetermined intervals i.e. 0.5, 1, 1.5, 2, 4, 8, 12, 16, 20 and 24 h, and replaced with equal volume of fresh receptor solution to keep the volume constant. The amount of Clotrimazole in the samples was analyzed spectrophotometrically at 262 nm using ethanolic phosphate buffer pH 7.4 as a blank.

STATISTIC AL ANALYSIS

The results obtained from the permeation studies were analyzed statistically by one way ANOVA using SPSS software (version 17). The results were evaluated at probability level of 0.05.

RESULTS

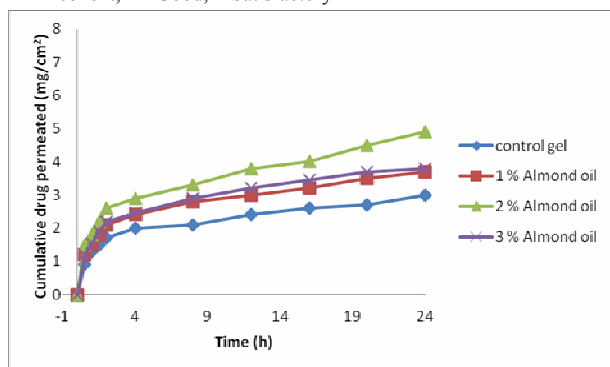
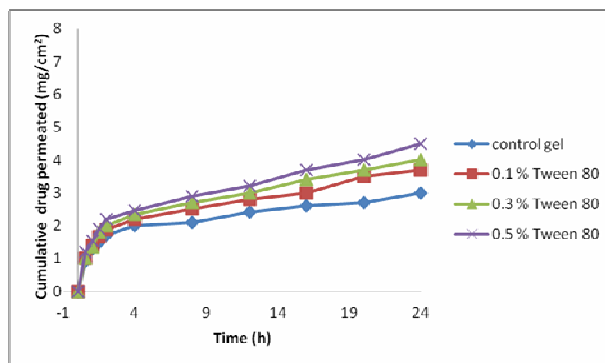
The physicochemical properties of the gel formulations are shown in table 2. The pH of the developed gel formulations was in the range of 6.7 to 7.2, which lies in the normal pH range of the skin and would not produce any skin irritation (Hussain *et al.*, 2012). The viscosity of gel formulation ranges from 16696 to 17489 cP showing its consistency (table 2). The consistency of different gel formulations can be ranked according to their viscosity values as follows: F 7 (viscosity 17489 cP) > F 4 (viscosity 17354 cP) > F 8 (viscosity 17279 cP) > F 5 (viscosity 17167 cP) > F2 (viscosity 17045 cP) > F 3 (viscosity 16987 cP) > F1 (viscosity 16870 cP) > F6 (viscosity 16696 cP). The results of spreadability varies from 5.43 to 7.30 g.cm/s (table 2), indicating that the gel is easily spreadable by small amount of shear. The extrudability of gel formulations from the collapsible tube varies from 181 to 195 g.

All the developed gel formulations showed good homogeneity with absence of lumps (table 2). The physical appearance of the gel formulations was white or opaque in nature. The drug content of the gel formulations

Table 2: Physicochemical evaluation of different gel formulations of Clotrimazole

Formulation Code	pH	% Drug content	Homogeneity	Viscosity (cP) at 10 rpm	Spreadability (g.cm/s)	Extrudability (g)
F 1	6.7	88.1%	+	16870	5.43	190
F 2	6.8	89.3%	+++	17045	5.91	184
F 3	6.9	90.4%	++	16987	6.13	179
F 4	6.7	90.7%	++	17354	5.90	181
F 5	7.1	92.9%	+++	17167	6.54	182
F 6	7.2	89.6%	++	16696	6.56	185
F 7	7.1	91.3%	++	17489	7.01	195
F 8	6.9	93.1%	+++	17279	7.30	189

+++ Excellent, ++ Good, + Satisfactory

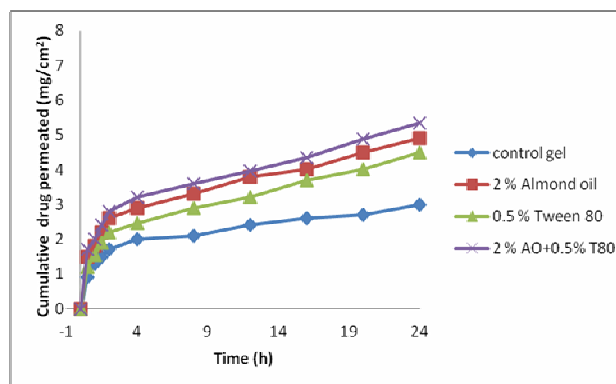
**Fig. 3:** Permeation profile of Clotrimazole 2% gel containing different concentrations of almond oil.**Fig. 4:** Permeation profile of Clotrimazole 2% gel containing different concentrations of tween 80.

was in the range of 88.1% to 93.1%, showing content uniformity. The skin irritation study of gel formulations was carried out on rabbit skin and no signs of redness or erythema observed for 48 h after application of the gel. During stability study, the gel was found to be stable at 40°C/75% RH with respect to their physical parameters and drug content.

Fig. 3 shows the permeation profile of Clotrimazole from gel containing 1, 2 and 3% almond oil. It can be seen from fig. 2 that at a 2% concentration, the cumulative permeation of Clotrimazole was the highest. The drug permeation increased significantly ($p < 0.05$) when the amount of almond oil in the gel formulation was increased from 1 to 2% w/w. However, at an almond oil concentration of 3% a significant reduction ($p < 0.05$) in permeation of Clotrimazole through rabbit skin, in comparison with the 2% almond oil concentration, was noted. This may be possibly due to complex formation between Clotrimazole and almond oil at high almond oil concentration, making the partitioning of Clotrimazole molecule out of gel base rather difficult, hence reducing the amount of drug molecules permeated through the skin.

Fig. 4 presents the penetration profile of Clotrimazole from gels containing 0.1, 0.3 and 0.5% tween 80. The drug permeation increased significantly ($p < 0.05$) when the amount of tween 80 in the gel formulation was increased from 0.1 to 0.5 %. This relationship between the

concentration of tween 80 and the drug permeation could be due to its hydrophilic nature and its concentration below critical micelle concentration (CMC), because concentration of surfactants above CMC could make micelles of the drug, which could be difficult to diffuse out from the gel base.

**Fig. 5:** Permeation profile of Clotrimazole 2% gel containing almond oil and tween 80.

A oil = Almond oil, T 80 = Tween 80

Fig. 5 illustrates the penetration profile of Clotrimazole from gels containing 2% almond oil and 0.5% tween 80. Although a moderate increase in permeation of the drug was achieved by incorporation almond oil and tween 80 alone in the gel, a definite increase in the permeation of the drug was exhibited following the co-application of

Table 3: Kinetic parameters of Clotrimazole permeation from different formulations

Formulation Code	Zero-order		First-order		Higuchi	
	k_0 (mg/h)	r^2	k_1	r^2	k_H	r^2
F 1	0.051	0.646	26.064	0.867	17.170	0.767
F 2	0.276	0.651	38.800	0.887	19.267	0.765
F 3	0.424	0.669	51.648	0.934	24.332	0.679
F 4	0.567	0.771	79.980	0.949	37.794	0.892
F 5	0.445	0.748	56.769	0.965	25.387	0.780
F 6	0.419	0.755	61.809	0.956	31.771	0.773
F 7	0.546	0.789	64.909	0.927	29.897	0.699
F 8	0.532	0.799	65.708	0.987	34.970	0.784

r^2 = correlation coefficient

almond oil and tween 80. One possible hypothesis to explain such an increase in cumulative amount is that almond oil could work synergistically with tween 80.

DISCUSSION

The use of penetration enhancers is among the various methods for improving the passage of drug molecules through the skin. The effects of the enhancers are specific and dependent on the drug, vehicle used, their concentration, and other factors. In this study the effect of almond oil and tween 80 was evaluated on the absorption of Clotrimazole through excised rabbit skin.

The examination of the correlation coefficient ' r^2 ' indicated that the drug release followed diffusion controlled mechanism from gels, as the values of ' r^2 ' for first order (ranged from 0.867 to 0.987) found to be higher in comparison to zero order (ranged from 0.646 to 0.799) and Higuchi's square root of time (ranged from 0.679 to 0.892) Table3. It was understood to be predominant first order release pattern.

CONCLUSION

A new Clotrimazole gel for topical application was developed with a high *in vitro* permeation rate and excellent properties. The formulation F 8 consisting of 2 % almond oil and 0.5% tween 80 was found to be better as compared to others based upon its physicochemical properties and *in vitro* permeation across rabbit skin. In conclusion, the present data confirm the feasibility of developing Clotrimazole transdermal gel.

Further studies, now in progress, will deal with the application of the presently reported findings to human skin permeation, involving *in vivo* testing.

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