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Original Article

Preparation and Evaluation of Clotrimazole Matrix Type Patch: Effect of Olive Oil on Drug Penetration Across Rabbit Skin

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Abstract: The present study was conducted to formulate and evaluate transdermal patch of Clotrimazole for local as well as systemic therapy of severe to moderate fungal infections. The penetration effect of naturally occurring oil [Olive oil] was investigated on the *in vitro* penetration enhancement of Clotrimazole across synthetic membrane as well as albino rabbit skin. Clotrimazole was evaluated for its particle size and particle size distribution analysis by using Particle size analyzer [Horiba LA 300]. Clotrimazole patches were formulated by solvent evaporation method for *in vitro* drug penetration studies. Olive oil was added to some selected formulations at different concentrations i.e. 0.5, 1, 1.5, 2, 2.5 and 3% to see their effect on drug release patterns.The formulated patches were evaluated for various physico-chemical parameters to check their suitability for topical application. Franz diffusion cell Apparatus [Perm Gear, USA] was used for *in vitro* drug release studies having phosphate buffer pH 7.4 as receptor solvent.

Keywords: Clotrimazole; transdermal patch; Franz diffusion cell; olive oil.

NTRODUCTION

The transdermal drug delivery system is oftenly comprised of low bioavailability of the drug because of the barrier functions of the skin [1], but the drug release from this route is constant because of the avoidance of first-pass metabolism by the GIT. The amount of drug required for therapeutic effect in transdermal drug delivery systems as compared to oral routes is less bioavailable but the amount available is sufficient for therapeutic uses [2]. Transdermal drug delivery systems are used to treat several diseases but with limitations due to poor penetration through the skin. Topical formulations like creams, ointments,gels and patches can improve the delivery of certain drugs; this fact could be of great importance in succeeding the transdermal therapeutic approaches. The goal of improving the penetration of certain drugs via transdermal route can be achieved through penetration enhancers [3]. The poor absorption of the drug could be presumed by its physico-chemical properties but the drug's penetration can be enhanced by the modification of medicinal chemists and nature of physiological barriers. To overcome the physiological barriers great investigative studies have been carried out by scientists so far and came to know that penetrative properties of drugs via transdermal route could be enhanced by using certain natural and synthetic compounds [2]. The use of certain systems that has greater chemical potential then that of corresponding saturated solutions is an alternative method and also that can promote the penetration of the drug from vehicle to the skin. Transdermal patches proved the efficacy of such studies. Drug loaded monolayer transdermal patch can be easily prepared by fast solvent evaporation [1].

MATERIAL AND METHODS

Clotrimazole (Leads Pharma Islamabad, Pakistan), Carboxy polymethylene (Sigma Chemicals, USA), Triethanolamine, PVP K30 (Merk, Germany), Ethyl cellulose, Polyethylene glycol 400, Potassium dihydrogen phosphate, Sodium hydroxide (Merk, Germany), Ethanol, Olive oil [Sigma Aldrich, Germany], Magnetic stirrer, pH meter, Weighing balance, UV-Visible

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Spectrophotometer (Shimadzu, Japan), Particle size analyzer (Horiba LA 300), Franz diffusion cell Apparatus (Perm Gear, USA).

Preformulation Studies

In preformulation studies of Clotrimazole, the particle size and particle size distribution was studied by a particle size analyzer (Horiba LA300). Freshly prepared distilled water was used as circulating solvent for Clotrimazole particle and particle size distribution analysis and was set at an ultrasonic circulation for 5 min. For this purpose the refractive index used was 1.438-1.441.The data obtained was plotted using MS Excel Office.

Standard Calibration Curve of Clotrimazole

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 artificial/rabbit skin. For this purpose a stock solution was prepared by dissolving 20 mg of Clotrimazole in phosphate buffer pH 7.4. The prepared solution was kept in ultra sonifier until the complete dissolution of the drug. From this stock solution suitable dilutions were prepared at the rate 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.0062 mg/mL. all the dilutions were analyzed by UV-Visible Spectrophotometer (UVIDEC-1601 Shimadzu, Japan) at 262 nm. The values of absorbance were recorded and a standard curve was constructed by plotting absorbance against concentration. From this curve a regression line equation was obtained in MS Excel format.

Table 1. Concentration versus absorbance of clotrimazole in pH 7.4 phosphate buffer

S.No	Concentration	Absorbance
1	0.1 mg/mL	1.231
2	0.05 mg/mL	0.596
3	0.025 mg/mL	0.286
4	0.0125 mg/mL	0.164
5	0.0062 mg/mL	0.086

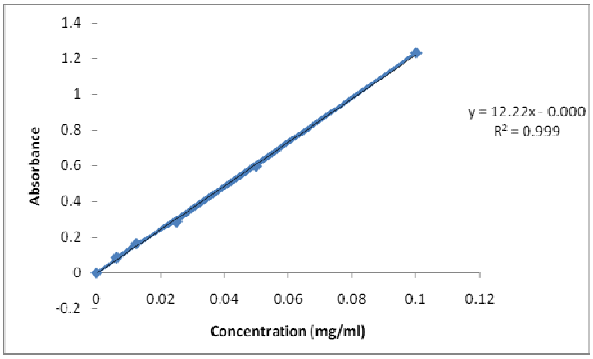

Fig. 1. Standard calibration curve of Clotrimazole.

Development of Clotrimazole Patch

Clotrimazole patch was prepared in order to study the penetration of drug across synthetic/rabbit membrane and to see the effect of different concentrations of olive oil as penetration enhancer on the *in vitro* drug penetration into the systemic circulation. First step in the development of Clotrimazole patch was the development of a backing membrane or supporting membrane which act as a supporting layer for the drug matrix. For backing membrane 4 gm PVA was dispensed in 100 mL of distilled water and was heated to a temperature of 80°C so as to evaporate 25% of the solution and the remaining 75% solution was stirred to dissolve any lump or particulate present in the solution. From this resultant solution, 15 mL was taken and poured into a petri dish with an area of 23 cm^2. The petri dish was covered with an inverted funnel and was dried in open air for 24 hrs and then was stored for further use. A drug matrix solution was prepared by taking a weighed amount of drug, polymers [Ethocel and Eudragit in 1:1 ratio], propylene glycol and different concentrations of enhancer and was dispensed in 10 mL solution of ethanol: acetone [1:1 ratio] in a conical flask. The solution was shaken well until a homogeneous mixture was obtained. This mixture was poured into petri dish containing backing membrane and the solvent from the petri dish was evaporated in open air. Transdermal patch was collected from the petri dish after drying and was cut down into small pieces each having a diameter of 1.5 cm^2. These developed patches were evaluated for physico-chemical parameters.

Table 3. Formulation of Clotrimazole transdermal Patch.

S. No.	Clotrimazole	Eudragit	Ethocel	PG (30% of polymer)	Olive oil (Enhancer)	Ethanol + Acetone (1:1 ratio)
1	20 mg	100 mg	100 mg	60 mg	-	QS to 10 mL
2	20 mg	100 mg	100 mg	60 mg	[0.5 %] 1 mL	QS to 10 mL
3	20 mg	100 mg	100 mg	60 mg	[I %] 2 mL	QS to 10 mL
4	20 mg	100 mg	100 mg	60 mg	[1.5%] 3 mL	QS to 10 mL
5	20 mg	100 mg	100 mg	60 mg	[2%] 4 mL	QS to 10 mL
6	20 mg	100 mg	100 mg	60 mg	[2.5%] 5 mL	QS to 10 mL
7	20 mg	100 mg	100 mg	60 mg	[3%] 6 mL	QS to 10 mL

Physico-chemical Evaluation of Transdermal Patches

Patch thickness

Clotrimazole transdermal patches were evaluated for their thickness tests by using vernier caliper. The thickness was measured at three different places of the same patch [4].

Weight variation tests

The synthesized patches were subjected to weight variation tests by individually weighing each patch. Weight variation tests were performed for each formulation [5].

Flexibility or Tensile strength determination

The flexibility or tensile strength of the patches was determined by means of a pulley system. The patch was pulled in opposite direction with the help of two small catchers by gradually increasing the force until the patch was broken. The tensile strength was noted from the scale of pulley in kg/cm^2 [5].

Folding endurance tests

This test was performed for randomLy selected patches. The selected patch was repeatedly folded at the same place until it broke. The number of time taken by each patch until its breakage was noted as folding endurance [6].

Moisture loss tests

The moisture loss test was performed for three patches. The patches were weighed accurately and were placed in desiccator along with calcium chloride for 24 hrs at constant temperature of 37^0C. After 24 hrs the patches were again weighed and the percent moisture loss was determined by subtracting the final weight from initial weight [5].

Drug content determination

For drug content determination a piece of 1 cm^2 was cut from the patch and added to a beaker containing 100 mL phosphate buffer pH 7.4. It was then stirred for 4-5 hrs with the help of a magnetic stirrer. The solution was filtered through a membrane filter of pore size 0.45 µm and was analyzed for drug content at 262 nm detection wavelength [5].

Preparation of Rabbit Skin for*ex-vivo* Studies

Rabbit skin was used for *ex-vivo* studies of Clotrimazole patch. A healthy albino rabbit with no known skin diseases was selected. Anesthesia was given to rabbit and hairs from the dorsal region were shaved carefully. Rabbit was scarified and the shaved skin was excised by using special surgical blades. The subcutaneous fats from the epidermis was removed by scalpel and then the epidermis itself was removed by dipping in hot water of 60°C for a while and teasing the epidermis carefully from the dermis. The excised skin was washed with distilled water and was covered with aluminum foil to prevent it from any harm [7].

In vitro drug penetration studies protocolfor patch

To study the *in vitro* drug release of Clotrimazole from transdermal patch, a modified Franz Cell apparatus (Perm Gear, USA) was fabricated [8]. In first step the artificial membrane/albino rabbit skin was fixed in between the two donor compartment and receptor compartment of Franz Cell apparatus. As a receptor solvent, phosphate buffer pH 7.4 was used. Each receptor compartment was filled with 5 mL of the receptor solvent. The skin was cut into such piece that the diffusion area was 1.5cm^2.The transdermal patch was fixed on the

rabbit skin in such a way that the drug layer of the patch was facing to the epidermis of the rabbit skin and was then fixed in between the donor and receptor compartments of the Franz Cell apparatus. The temperature of receptor solvent was maintained at 37°C ± 1 °C with constant circulation of hot water. Samples of each 2 mL were withdrawn from the receptor compartments at specific time interval and were immediately replaced with fresh receptor solvent already maintained at the same temperature. The samples were filtered through a membrane filter [0.45µm]and were analyzed for the drug concentrations by using UV-Visible spectrophotometer at a detection wave length of 262 nm [2].

In vitro drug release kinetics

Kinetic model was applied to the *in vitro* release profiles of Curcumin patches in order to investigate the way of drug release mechanism. The *in vitro* drug release mechanism of Curcumin was determined by putting the *in vitro* releasevalues in Korsmeyer Pappas kinetic model.

$$M_t / M\infty = K_5 t^n$$

Where $M_t / M\infty$ is fractional drug release from formulation into the receptor solvent, K is drug delivery constant and [n] is diffusion coefficient. The value of (n) in equation indicates the release mechanism of particular drug in solvent. The value of [n] if equal to 0.5 indicates quasi-Fickian diffusion mechanism, if (n>0.5) then anomalous or non-Fickian diffusion mechanism exists and if its value is (=1) then Zero order release mechanism exists.

RESULTS AND DISCUSSION

Prior to Clotrimazole patch preparation, the particle size and particle size determination of Clotrimazole was performed using Particle size analyzer (Horiba LA300). The particle size and particle size distribution is shown in the figure 2 below. It could be seen from the figure that the maximum percentage i.e. 60 % was of the particles having a diameter of 21.305 µm and the minimum concentration was of the particles having a diameter of 0.256 µm. The median of the Clotrimazole particles was 18.15 µm. It could be observed that the particle size of Clotrimazole was too small and was a good candidate for transdermal drug delivery systems.

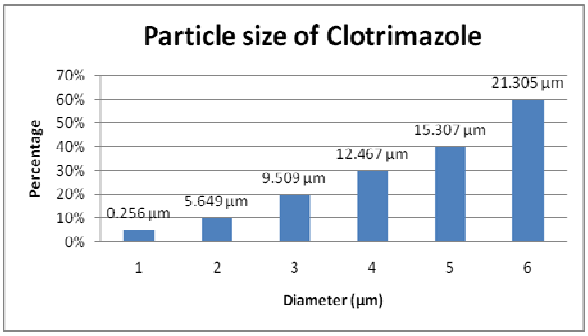

Fig. 2. Particle size and particle size distribution of Clotrimazole.

Clotrimazole Patch evaluation

Clotrimazole transdermal matrix patches were prepared by solvent evaporation technique. Different formulations of Clotrimazole patch were prepared with/without penetration enhancer at different concentrations in order to optimize the drug release patters. A combination of two polymers (Ethocel and Eudragit) was used in the patch formulations to extend the release patterns of Clotrimazole across synthetic membrane as well as rabbit skin. The synthesized transdermal patches of Clotrimazole were transparent, smooth, uniform and flexible. The values of different parameters applied to transdermal patches could be seen in the table 4 below. All the values of different parameters suggest that the patches were suitable for topical application.

Table 4. Results of physico-chemical parameters applied to Clotrimazole transdermal patch.

S. No.	Parameters	Clotrimazole patch (Blank)	Clotrimazole Patch with olive oil at different concentrations					
			0.5%	1%	1.5%	2%	2.5%	3%
1	Thickness (mm)	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
2	Weight ariation (mg)	0.361 ± 0.03	0.370 ± 0.07	0.363 ± 0.04	0.367 ± 0.05	0.369 ± 0.06	0.365 ± 0.05	0.366 ± 0.05
3	Tensile strength	12.98 ± 0.02	13.10 ± 0.02	13.07 ± 0.02	13.77 ± 0.01	13.54 ± 0.02	12.41 ± 0.01	13.98 ± 0.02
4	Folding endurance	>140	>178	>148	>144	>150	>151	>167
5	% Moisture loss	3.423 ± 0.033	3.591± 0.021	3.837 ± 0.052	3.697 ± 0.043	3.611 ± 0.049	3.430 ± 0.021	3.587 ± 0.037
6	% Drug content	99.1	98.5	98.8	99.2	98.7	98.2	98.4

In vitro drug release studies from patch

To all Clotrimazole patch formulations, Korsmeyer Pappas equation was employed to find out the release mechanism of Clotrimazole from transdermal gels and patches into systemic circulation across synthetic membrane and albino rabbit skin. In Korsmeyer equation as mentioned above, the value of (n) represents the mechanism of drug release, therefore by fitting the *in vitro* penetration data in Korsmeyer equation the values of [n] were ranging between 0.592 to 1.274 which indicates both non-Fickian (anomalous) and Zero order release mechanism (0.5<n>1).

In vitro drug release studies were performed for different formulations of Clotrimazole patch by using Franz Cell diffusion apparatus. *In vitro* penetration of drug was studied for 24 hrs across synthetic membrane and rabbit skin. It could be seen from the Fig. 3 and 4 given below that the percent penetration value of Clotrimazole without penetration enhancer was much less as compared to the formulations having different ratios of penetration enhancer and as the ratio of enhancer was increased the drug release/ penetration profiles were enhanced significantly, so by increasing the concentration of olive oil as penetration enhancer the drug penetration was enhanced which could be clearly in the figure. It could also be observed that the percent cumulative penetration was more across rabbit skin than that across synthetic membrane i.e. 92% drug was penetrated in 24 hrs across synthetic membrane while 97% dug was penetrated across rabbit skinin 24 hrs. It could be due to the reason that the rabbit skin is thinner than synthetic membrane which could effect the penetration of drug. It could also be due to the reason that rabbit skin has a large number of hair follicles which acts as a bearer to drug to penetrate into the systemic circulation [9].

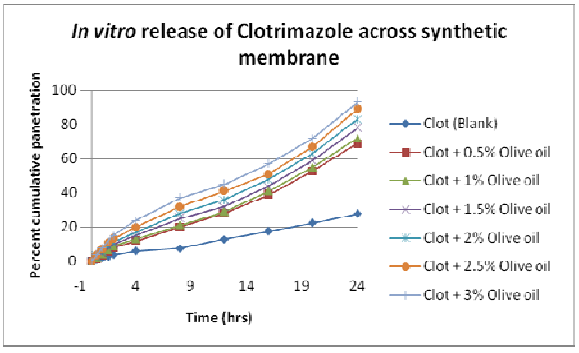

Fig. 3. Release profiles of Clotrimazole from topical patch having different concentrations of enhancer across synthetic membrane.

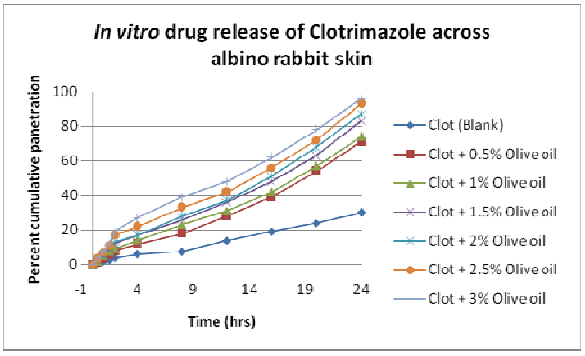

Fig. 4. Release profiles of Clotrimazole from topical patch having different concentrations of enhancer across albino rabbit membrane.

The best formulation among all was Clotrimazole patch with 3% olive oil which gave the greatest penetration enhancement.

CONCLUSION

From the present study, it is concluded that Clotrimazole can be successfully formulated for topical delivery to treat topical as well as systemic fungal infections. The topical patch of Clotrimazole was soft and non-irritant to skin which suggests its suitability for safe topical treatment of fungal infections. Further more, olive oil was found to be an effective enhancer in transdermal drug delivery systems, its concentration up to 3% of the formulation was found to be the best one.

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