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Development of Clotrimazole Gels for Enhanced Transdermal Delivery

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ABSTRACT – To develop a topical bioadhesive formulation of clotrimazole for enhanced transdermal delivery, hydroxypropyl methylcellulose gel containing permeation enhancer was formulated and permeation studies were carried out. The release characteristics of the drug from the gel formulation were examined according to the receptor medium, drug concentration, and temperature. The rate of drug release from the gel increased with increasing drug concentration and temperature. The activation energy (Ea) of drug permeation, which was calculated from the slope of log P versus 1/T plots, was 14.41kcal/mol for a 1%(w/w) loading dose. The enhancer, such as saturated, unsaturated fatty acids, pyrrolidones, propylene glycol derivatives, glycerides, and non-ionic surfactants, were incorporated onto the gels to increase the amount of drug permeation into the skin. Among the enhancers used, polyoxyethylene 2-oleyl ether showed the highest level of enhancement. These results show that clotrimazole gels containing polyoxyethylene 2-oleyl ether could be used for the enhanced transdermal delivery of clotrimazole.

Key words – Clotrimazole, Gels, Penetration enhancer, Permeation, Transdermal delivery

Fungal infections in humans range from superficial and common, such as dermatophytoses and onychomycoses, to deeply invasive and disseminated, such as candidasis and aspergillosis. Clotrimazole is a relatively non-toxic synthetic imidazole derivative with a broad-spectrum of antimicrobial activity. Topical application of clotrimazole preparations into the skin, mouth or vagina are appropriate but can washed off rapidly. Therefore, an increase in solubility and the rapid release of clotrimazole is essential for such preparations.[1)]

Transdermal drug delivery is a convenient method of drug administration enabling physicians to provide controlled drug delivery to patients with minimum discomfort. Compared to oral and parenteral routes, the transdermal route of drug administration has the advantages of virtually no gastrointestinal side effects or drug degradation.[2)] Initially the drug must be delivered from a suitable vehicle from which the substance can partition into the skin. The drug must then diffuse through the hydrophilic and/or lipophilic environment of the stratum corneum to the deeper epidermal layers and to the dermis.[3)] However, the stratum corneum acts as a barrier to chemicals entering the human body. The greatest obstacle is the stratum corneum, which is the uppermost layer of the skin that provides the rate-limiting step for drug transport.

The use of a penetration enhancer as a chemical additive with a drug is an effective and simple method for reducing the barrier function of the skin. Therefore, many compounds have been investigated as transdermal penetration enhancers. It has been reported that dermal penetration can be improved using compounds that have been proven to be effective enhancers on the skin. Chemical methods involve the incorporation of specific chemicals in topical drug formulations to increase the level of the drug penetration. Penetration enhancers facilitate the absorption of a penetrant through the skin by temporarily increasing the permeability of the skin.[4)] The most widely implemented technique is the use of chemical enhancers. These agents allow drug permeation through the skin at the appropriate rate and for a sustained period of time.[5)]

Controlled drug release from hydrogels has been studied extensively over the past three decades. Hydrogels are insoluble, crosslinked polymer network structures consisting of hydrophilic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water and retain their shape without dissolving. Bioadhesive gels can be applied to the skin, and be removed easily. In recent years, the use of hydrophilic polymers, particularly cellulose derivatives, has attracted considerable attention for the development of controlled release technology in formulations of pharmaceutical products owing to their ability to form gels in aqueous media. In this study, hydroxypropyl methyl cellulose (HPMC) was used in gel formulations. HPMC belongs to a family of inert

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hydrophobic non-ionic polymers that are used widely in oral and topical pharmaceutical formulations, and is available in several grades, which vary in viscosity, molecular weight, and the extent of substitution.

The aim of this study was to formulate HPMC gels containing clotrimazole for enhanced transdermal delivery by examining its in vitro release characteristics. The feasibility of using HPMC as a gelling agent in the development of a clotrimazole gel for enhanced transdermal delivery was evaluated. The effects of temperature and drug concentration on the rate of drug release as well as the effect of penetration enhancers on the drug permeability from the HPMC gels across excised rat skin were assessed.

Materials and Methods

Materials

Clotrimazole was supplied by Kyungnam Pharm Ltd. (Seoul, Korea). Hydroxypropyl methylcellulose (K100M, MW: 100,000) was obtained from DOW chemical Co., Ltd. (Midland, MI, USA). Lauric acid, oleic acid, and caprylic acid were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). 2-pyrrolidone was purchased from Acros organics (USA). Myristic acid, linoleic acid, 1-methyl-2-pyrrolidone, polyoxyethylene-2-stearyl ether (Brij 72), polyoxyethylene-23-lauryl ether (Brij 35) and polyoxyethylene-2-oleyl ether (Brij 92) were purchased from Sigma-Aldrich Co., (St. Louis, MO, USA). Oleyl macrogol-6 glyceride, caprylocaproyl macrogol-8 glyceride, propylene glycol laurate, and propylene glycol monolaurate were kind gift from Gattefose (St. Priest, France). Stearic acid was purchased from Hayashi Pure Chemical Industries Ltd. (Japan) and palmitic acid was obtained from Kanto Chemical Co., Inc. (Japan). Methanol was of HPLC grade and supplied by Merck Co. (Darmstadt, Germany). All reagents of analytical grade were used without further purification.

Determination of Drug Solubility in Various Concentrations of PEG 400

Excess amounts of clotrimazole were added to various percentages of a PEG 400 solution and each solution was shaken at 37°C for 8 hr in a shaking incubator. The solutions were filtered through a 0.45 μm filter membrane. The concentration of clotrimazole was assayed using a UV/Vis spectrophotometer at 225 nm.

Preparation of HPMC Gels Containing Clotrimazole

Two grams of hydroxypropyl methylcellulose (HPMC) were dissolved in hot water to make 35 g. Approximately 1 g of clotrimazole was dissolved in 20 mL of PEG 400 in a glass beaker and heated with constant stirring. The clotrimazole solution was poured onto a polymer solution and water was added to the above mixed solutions with vigorous stirring to make a total mass of 100 g.

Permeation of Clotrimazole from the HPMC Gels

The in vitro release of clotrimazole through the cellulose membrane was examined using the Keshary-Chien cells. The diameter of the cell was 2 cm, providing an effective constant area of 3.14 cm^2. The flux of clotrimazole from the HPMC gels was determined using 40% PEG as a receptor. The synthetic cellulose membrane was mounted on the receptor compartment of the diffusion cell. Five grams of the prepared HPMC gels containing clotrimazole were placed in intimate contact with the cellulose membrane and the donor cap was covered with parafilm and clamped. The sampling port was sealed with parafilm to prevent evaporation of the receptor medium. The receptor solution was maintained at 37°C with a circulating water jacket and stirred at 150 rpm with a magnetic stirring bar. Before the experiment, the system was tested to remove any air bubbles remaining in the receptor site. The donor compartment was maintained at 25±1°C. At predetermined time, the entire solution was removed from the receptor cell and replaced with a fresh solution. The cumulative amount of clotrimazole released from the HPMC gels was determined at 225 nm using a UV spectrophotometer. The effect of the drug concentration on its release from the gels was examined at drug concentrations of 0.5, 1, 1.5 and 2% (w/w), and the effects of temperature on drug release was performed at 27, 32, 37 and 42°C in the thermostated water bath. The total samples (20 mL) from the receptor compartment were withdrawn at predetermined intervals to maintain a sink condition, and replaced immediately with the same amount of fresh PEG 40%.

HPLC Determination of Clotrimazole

Clotrimazole was assayed by HPLC. The HPLC system consisted of a pump (Knauer, DE/K-120, USA.), ultraviolet detector (Waters 484, USA), RESTEK C_{18} column (250×4.6 mm, 5 μm), degaser, and an integrator (D520A, Youngin scientific Co. Ltd. Korea). The mobile phase consisted of a mixture (75:25, v/v) of methanol and water. A flow rate of 1.0 mL/min yielded an operation pressure of ~ 1000 psi. The UV detector was operated at 225 nm. Under these conditions, the clotrimazole peak appeared at a retention time of 5.3 min.

Data Treatment for Drug Release Studies

Two mathematical equations were proposed by Higuchi to

describe the kinetics of drug release based on the state of the drug in the vehicle: release from solutions and release from suspensions. In the present study, the drug release rates were evaluated using the simplified Higuchi diffusion equation (1), depicting the release of a drug from one side of a semisolid layer in which the drug is dissolved.

$$Q = 2\ C_0\ (D_t/\pi)^{1/2} \quad (1)$$

where Q is the amount of drug released onto the receptor medium per unit area of exposure, C_0 is the initial drug concentration in vehicle, D is the apparent diffusion coefficient of drug and t is the time elapsed since the start of drug release.

In the case of passive diffusion, the steady-state flux through the unit area of a membrane is given by Fick's law,

$$J = P\ (C_d - C_r) \quad (2)$$

where J is the flux per unit area, P represents the permeability coefficient, and C_d and C_r are the concentrations in the donor and receptor solutions, respectively. In the case that the sink conditions are maintained on the receptor side, $(C_d - C_r)$ can be replaced by C_d.

$$J = P\ C_d \quad (3)$$

The permeability coefficient (P) is constant for a given drug under the same experimental conditions, and there should be a linear relationship between the flux and donor concentration.

Skin Preparation

A male Sprague Dawley rat was sacrificed by snapping the spinal cord at the neck. The hair of the abdominal area was removed carefully with electric clippers. A square section of the abdominal skin was excised. After the excision, the adhering fat and other visceral debris in the skin were removed carefully from the undersurface with tweezers. The excised skin was used immediately.

The experiments were carried out in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The Animal Care Committee of Chonnam National University (Gwangju, Republic of Korea) approved the design (2007-14) and conduct of this study.

Effect of Enhancer on the Permeation of Clotrimazole from the HPMC Gels through Rat Skin

The freshly excised full-thickness skin sample was mounted on the receptor side of the diffusion cell with the stratum corneum side facing upwards onto the donor compartment and the dermal side facing downwards onto the receptor compartment. The 1% clotrimazole gels were mixed with 5% (w/v) enhancer. The control samples without the enhancers were prepared in a similar manner. An appropriate amount of the gels (2 g) was placed on the stratum corneum side and covered with a round glass plate and clamped. The receptor medium was 40% PEG 400 to achieve the sink condition and was maintained 37°C using a circulating water bath. The total samples were withdrawn at predetermined times and replaced immediately with an equal volume of fresh medium. The enhancers used were saturated fatty acids, unsaturated fatty acids, pyrrolidones, propylene glycol derivatives, glycerides, and non-ionic surfactants. The enhancer might affect the fluidity of the stratum corneum structure allowing the drug to permeate better though the rat skin. The permeation quantities of clotrimazole were analyzed by HPLC at 225 nm. Each datum represents the average of three determinations.

Calculations

The cumulative amount of the drug permeated through the rat skin was plotted as a function of time (min). A linear profile was observed over a 16 hr period and the slope of the linear portion of the curve was determined by linear regression. The effectiveness of the penetration enhancer was determined by comparing the flux of clotrimazole in the presence or absence of the enhancer, and was defined as the enhancement factor (EF). The EF was calculated using the following equation:

EF = (flux of HPMC gels containing enhancers) / (flux of the control)

Results and Discussion

Solubility of Clotrimazole

The aqueous solubility of clotrimazole is extremely low and can be improved by adding a water-miscible hydrophilic polymer, such as PEG 400 into the aqueous solution as a solubilizer for clotrimazole. PEG 400 was reported to be an excellent solubilizer for many antifungal agents. The aqueous solubility of clotrimazole increased greatly with increasing volume fraction of PEG 400 with the highest solubility being observed at 50% PEG 400 (Fig. 1).

Effect of PEG 400 on the Release of Clotrimazole through Cellulose Membrane

The effect of PEG 400 on the diffusion of clotrimazole was examined. In order to obtain a sink condition, it is desirable for the receptor medium to dissolve the drug. The effect of the receptor medium on drug release across a synthetic cellulose membrane was examined using the prepared 1% clotrimazole

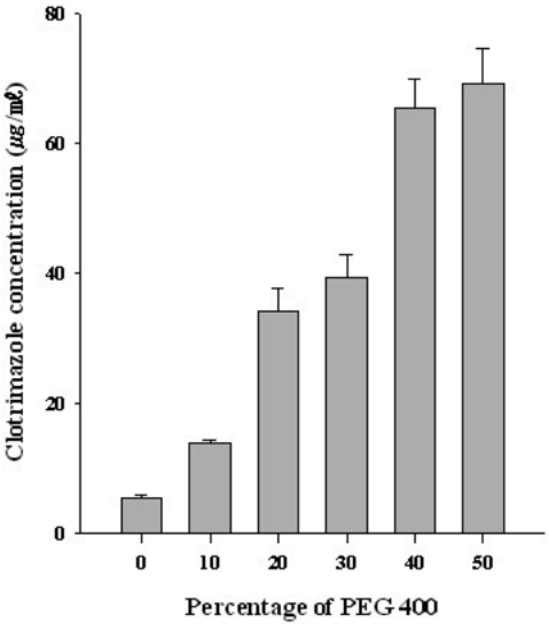

Figure 1—Solubility of clotrimazole in PEG 400 solutions at various concentrations.

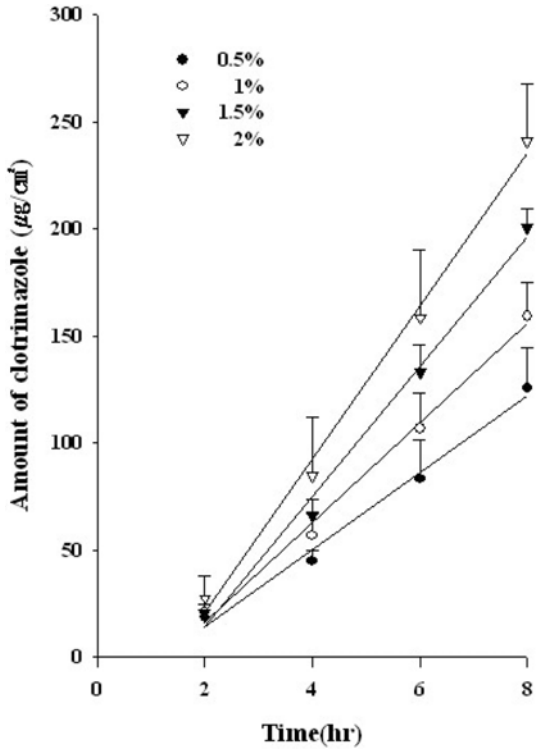

Figure 2—Effect of clotrimazole concentration on drug release through a cellulose membrane from HPMC gels.

gels at 37±0.5°C. The cumulative amount of clotrimazole released as a function of time showed good linearity (Figure, not shown). There was a rapid increase in the rate of drug release as the PEG 400 solution in the receptor medium was increased to approximately 40%, and only a slight increase with further increases in concentration. Therefore, the release of clotrimazole was examined using the 40% PEG solution.

Effect of Clotrimazole Concentration on the Drug Release

The effect of the clotrimazole concentration on rate of drug release across a synthetic cellulose membrane was examined using a 40% PEG solution from the prepared HPMC gels at 37±0.5°C. The concentrations tested were 0.5, 1, 1.5 and 2%, respectively. Fig. 2 shows the flux of clotrimazole from the gel formulation through the synthetic cellulose membrane (SPECTRA/POR, MW 12-14,000) for 8 hr. The release of clotrimazole from the gels increased with increasing drug concentration. The concentration of clotrimazole in many products on the markets, such as creams and lotions, is usually 1%. Therefore, formulation of 1% drug gel was used.

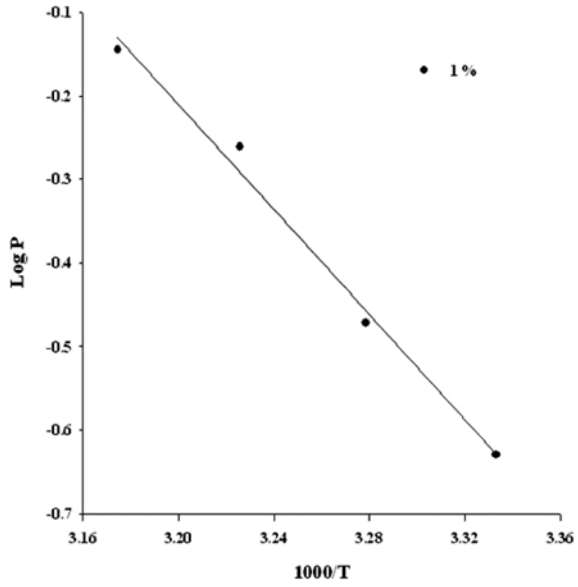

Figure 3—Effect of temperature on drug release from HPMC gels containing a 1% loading dose.

Effect of Temperature on Drug Release

The effect of temperature on the release of clotrimazole from a 1% drug gel was evaluated at 27, 32, 37 and 42°C. All experiments were carried out at least in triplicate. Fig. 3 shows the temperature dependence of the release of clotrimazole to the receptor as a function of time. The slope and intercept were used, respectively, to calculate the activation energy (E_a) for drug diffusion and the pre-exponential factor. The relationship between the permeation coefficient and temperature was

examined. The permeability coefficient can be defined as follows:

$$P = \frac{Flux}{Solubility} \quad (3)$$

$$P = P_0 \cdot e^{-\frac{Ea}{RT}} \quad (4)$$

$$LogP = LogP_0 - \frac{Ea}{R \cdot 2.303 \cdot 1000} \frac{1000}{T} \quad (5)$$

A linear relationship was observed between the logarithm of the permeability coefficient (P) and the reciprocal temperature. The apparent diffusion coefficient of clotrimazole increased with increasing temperature.

As expected from Equation 5, a plot of log P versus 1000/T yielded a straight line (Fig. 3), the slope of which was used to estimate the E_a (Equation 7).

$$Slope = -\frac{E_a}{R \cdot 2.303} \cdot \frac{1}{1000} \quad (6)$$

$$Ea = -Slope \times R \times 2.303 \times 1000 \text{ cal} = -Slope \times 1.987 \times 2.303 \text{ kcal} \quad (7)$$

The Ea of drug permeation was 14.41 kcal/mol for a 1% loading. This suggests that drug release from the gels is an energy-linked process.[6] However, 37°C was chosen for the permeation experiments to reflect the human temperature.[7]

Effect of Enhancers on the Permeation of Clotrimazole Across the Rat Skin

The effect of various enhancers on the permeation of clotrimazole across rat skin was examined. The permeation of clotrimazole from the HPMC gels containing an enhancer showed better permeation than without an enhancer (Table I). The mechanism of the penetration enhancers can be explained as an interfacial saturation phenomenon. For the stratum corneum lipids to be dissolved, it is essential that enhancers, such as the surfactant, accumulate at the lipid/liquid interface after penetrating the tissue. An effective penetration enhancer may increase the diffusion coefficient of the drug in the stratum corneum (i.e. disrupt the barrier nature of the stratum corneum). The enhancer might also affect the fluidity of the stratum corneum structure, which can improve the permeation of the drugs through the rat skin.

Selective perturbation of the intercellular lipid bilayers in the stratum corneum appears to be the major mode of enhancing the activity of fatty acids.[8] Percutaneous drug absorption has been increased by a wide variety of long chain fatty acids.[9] Unsaturated fatty acids have been used as potent enhancers for many drugs.[10] Oleic acid, once incorporated into the skin lipid, disrupts the molecular packaging and alters the level of hydration, thereby allowing drug penetration.[11] Oleic acid was found to increase the epidermal permeability through a mechanism involving the stratum corneum lipid membranes. Saturated fatty acids have been used successfully as penetration enhancers for many drugs.[12] It appears that saturated alkyl chain lengths of approximately C_{10}-C_{12} attached to a polar head group are potent enhancers.[9] Lauric acid has a significant effect on the skin permeation of several compounds,[12-14] possibly due to an imbalance of the partition coefficient and the affinity of lauric acid to the hydrophobic groups of the skin. Among the saturated fatty acid groups, lauric acid had the greatest enhancing effects. Among the unsaturated fatty acid groups, oleic acid caused a significant increase in the rate of clotrimazole permeation through the skin.

Surfactants have been reported to enhance the permeability of drugs,[12-18] and have been added to formulations to dissolve the lipophilic active ingredients. Therefore, they have the potential to dissolve lipids within the stratum corneum.[9] In recent years, they have been used to enhance the permeation rates of several drugs.[13] Skin pre-treated with a non-ionic surfactant showed that the stratum corneum is loosely layered

Table I-*Enhancement factor according to various enhancers (Mean ± S.D., n = 3)*

Enhancer	Flux (μg/cm²/hr)	EF
Control	0.23±0.14	1.00
Myristic acid	0.26±0.08	1.13
Stearic acid	0.26±0.16	1.13
Palmitic acid	0.27±0.18	1.18
Caprylic acid	0.32±0.21	1.37
Lauric acid	0.33±0.15	1.44
Linoleic acid	0.22±0.19	1.07
Oleic acid	0.31±0.12	1.32
Polyoxyethylene 2-stearyl ether	0.32±0.13	1.35
Polyoxyethylene 23-lauryl ether	0.34±0.14	1.46
Polyoxyethylene 2-oleyl ether	0.41±0.15	1.75
Caprylocaproyl macrogol-8 glycerides	0.27±0.19	1.13
Oleyl macrogol-6 glycerides	0.37±0.25	1.54
Propylene glycol laurate	0.25±0.09	1.14
Propylene glycol mono laurate	0.30±0.17	1.34
Polyvinyl-pyrrolidone	0.25±0.20	1.03
N-methyl-2-pyrrolidone	0.30±0.20	1.26
2-pyrrolidone	0.38±0.19	1.59

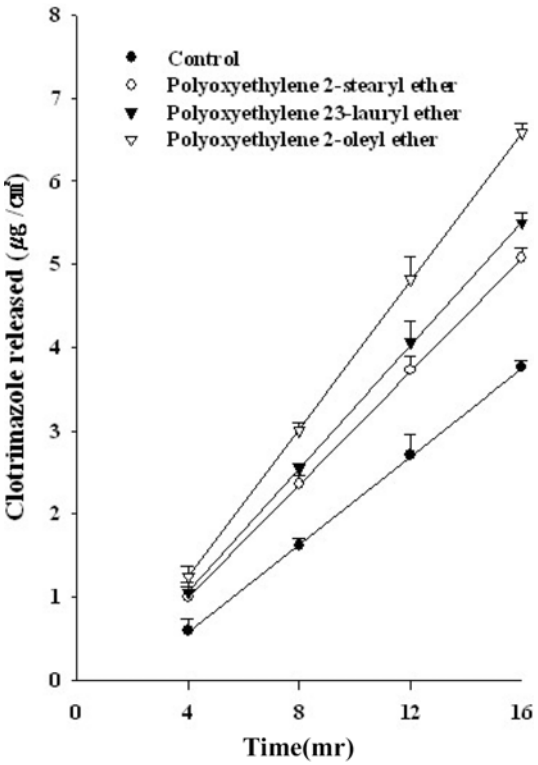

Figure 4–Effect of the non ionic surfactants on the permeation of clotrimazole from the HPMC gels through the excised rat skin.

with wide intercellular spaces.[13] Non-ionic surfactants are widely regarded as being safe. Surfactants generally have low chronic toxicity, and most have been shown to enhance the flux of materials permeating through biological membranes.[8,9] Among the non-ionic surfactants groups, polyoxyethylene 2-oleyl ether showed the highest permeation rate.

Caprylocaproyl macrogol-glyceride (Labrasol) increased the passive transport of drug molecules, and showed. high tolerance and low toxicity. It was included as a pharmaceutical excipient in European Pharmacopoeia in 1998. Oleyl macrogol-6 glyceride (Labrafil) is a PEG derivative, and is used as a co-surfactant in pharmaceutical systems, such as microemulsions. This substance is biocompatible and biodegradable.[14] Among the glycerides, oleyl macrogol-6 glyceride showed significant permeation of clotrimazole.

Propylene glycol (PG) is widely used as a vehicle for penetration enhancers. PG permeates well through the human stratum corneum. Permeation of the solvent through the tissue can alter the thermodynamic activity of a drug in the vehicle, which can in turn modify the driving force for diffusion. The solvent may partition into the tissue facilitating the uptake of the drug into skin but there may be some minor disturbances to the intercellular lipid packing within the stratum corneum bilayers.[9]

Pyrrolidones have been used as penetration enhancers in human skin for hydrophilic (e.g. mannitol, 5-fluorouracil and sulfaguanidine) and lipophilic (betamethasone-17-benzoate, hydrocortisone and progesterone) permeants.[9] Pyrrolidone and its derivatives have been reported to interact with both keratin and lipids in the skin.[8,9] A range of pyrrolidones and structurally-related compounds have been investigated as potential penetration enhancers in human skin. 2-pyrrolidone (2P) and N-methyl-2-pyrrolidone (NMP) are the most widely studied enhancers in this group. It is a clear liquid at room temperature and miscible with most common solvents, including water and alcohols.[9] NMP has been reported to increase the solubility of poorly soluble drugs and improve their skin penetration.[19,20] Among the pyrrolidone groups, 2-pyrrolidone improved significantly the permeation rate of clotrimazole from the HPMC gel.

The permeation of clotrimazole from the gels containing an enhancer showed a better enhancing effect than that without an enhancer (Table I). Among the enhancers used, polyoxyethylene 2-oleyl ether showed the highest level of enhancement considering the enhancement factors.

Conclusion

An increase in drug concentration and temperature increased the rate of drug release. The activation energy of release was estimated to be 14.41 kcal/mol at a 1% concentration. Among the enhancers used, polyoxyethylene 2-oleyl ether showed the greatest enhancing effects on drug permeation through excised rat skin. Overall, 1% clotrimazole gels containing an enhancer can be developed for enhanced transdermal drug delivery.

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