Effect of Single and Combined Permeation Enhancers on the Skin Permeation of Ketoprofen Transdermal Drug Delivery Systems

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ABSTRACT

There are two main approaches to improve the efficacy of transdermal drug delivery systems (TDDSs): chemical and physical enhancements. Chemical substances, known as permeation enhancers, can be incorporated into the formulation and promote the skin permeation of several drugs. In this study, ketoprofen (KP) TDDS was prepared as the monolithic drug-in-adhesive. Eudragit®NE30D and Eudragit®E100 were used as acrylate pressure sensitive adhesives. The effects of several permeation enhancers on the skin permeation of KP across excised abdominal rat skin were investigated. Modified Franz®diffusion cells were used. KP transdermal patch was placed on the epidermal side of the rat skin and mounted between donor and receptor compartments of the diffusion cell. Isotonic phosphate buffer, pH 7.4, was added into the receptor compartment and stirred constantly at 32±1°C. Sample solution was withdrawn at specific time interval up to 30 hr. Permeated KP was analyzed by high pressure liquid chromatographic method. Single and combined permeation enhancers, selected from fatty acids and/or pyrrolidone derivatives, were incorporated into the formulation. It was shown that among four types of fatty acids; lauric acid, capric acid, caprylic acid and oleic acid, oleic acid was the most effective enhancer. In the meantime, among four types of pyrrolidone derivatives; N-methyl-2-pyrrolidone, 2-pyrrolidone, 1-(2-hydroxyethyl)-2- pyrrolidone and 1-ethyl-2-pyrrolidone, N-methyl-2-pyrrolidone and 2-pyrrolidone had a trend to act as more powerful enhancers than the others. Furthermore, oleic acid combined with 2-pyrrolidone was the excellent paired-enhancer when compared with the other combinations. In conclusion, monolithic drug-in-adhesive TDDS of KP containing oleic acid and 2-pyrrolidone as the combined permeation enhancer was the most effective formulation.

Key words: Ketoprofen, Transdermal drug delivery system, Skin permeation, Permeation enhancers

INTRODUCTION

Ketoprofen is a potent non-steroidal anti-inflammatory agent, widely used for the symptomatic treatment of inflammatory syndromes such as rheumatoid arthritis, osteoarthritis and acute gouty arthritis. Because of its gastric irritation after oral administration, many topical formulations of ketoprofen such as cream and gel were developed (Porzio et al., 1998; Kalia and Guy, 2001; Sweetman, 2002). Transdermal drug delivery system (TDDS) is one of the novel drug delivery systems. It has many advantages over the other dosage forms such as providing extended therapy with a single application, thus leading to good patient compliance (Allen et al., 2005). Generally, there are four types of TDDS: monolithic drug-in-adhesive, multilaminate drug-in-adhesive, liquid reservoir and polymer matrix. The simplest TDDS is the monolithic drug-in-adhesive. This system composes of three layers: the backing membrane, the drug with pressure sensitive adhesive (PSA) layer and the release liner. Among three different PSAs commonly used in the TDDS: acrylates, silicones and polyisobutylenes, acrylates are the most popular PSA. They offer advantages of good compatibility with a wide range of drugs and excipients, ease of processing and flexibility in tailoring the polymer properties (Tan and Pfister, 1999; Cantor and Wirtanen, 2002). In this study, KP-TDDSs were prepared as the monolithic drug-in-adhesive by using Eudragit®E100 and Eudragit®NE30D as acrylate presssure sensitive adhesives.

The main barrier of the drug permeation through the skin is stratum corneum which is located at the outermost layer of the epidermis (Chien, 1992). Various strategies have been employed in attempts to improve the efficacy of topical delivery of drugs, including iontophoresis, electroporation, occlusion and ultrasound. An alternative approach is to use chemicals, known as permeation enhancers (PEs), which are materials that can partition into, and interact with, skin constituents to induce a temporary and reversible decrease in the skin barrier properties (Roberts and Walters, 1998). Based on the chemical structure, PEs can be categorized into several groups such as fatty acids, fatty alcohols, fatty acid esters, terpenes and pyrrolidone derivatives (Osborne and Henke, 1997). Saturated and unsaturated fatty acids have been widely used in the enhancement of transdermal permeation of various drugs (Aungst et al. 1986, 1990; Barry, 1987; Ogiso and Shintani, 1990; Lee et al., 1993; Morimoto et al., 1996; Bhatia et al., 1997; Tanojo et al., 1997; Bhatia and Singh, 1998; Kandimalla et al., 1999). The addition of fatty acids, especially 5% lauric acid, in KP solution promoted the penetration of KP through rat skin (Kim et al., 1993). 2-Pyrrolidone and N-methyl-pyrrolidone are the most widely studied derivatives of the naturally-occurring pyrrolidone carboxylic acid. They are effective in enhancing the permeation of several drugs such as hydrocortisone, mannitol and indomethacin (Walters and Hadgrafts, 1993). In this study, two groups of PEs: fatty acids and pyrrolidone derivatives were focussed on because they are common constituents of topical formulations. Morever, because of their different theoretical enhancing mechanism (Barry, 1988), they were estimated to have the synergistic enhancing effect when added in the same formulation.

The aim of the present study was to develop ketoprofen transdermal drug delivery system, using various skin permeation enhancers. Both single and combined PEs were used in order to obtain the high potential delivery of KP through the abdominal rat skin.

MATERIALS AND METHODS

Materials

Ketoprofen was provided by BioLab Co.Ltd. (Thailand) as a generous gift sample. Eudragit® NE30D and Eudragit®E100 were purchased from Röhm GmbH & Co.KG (Germany), Triethyl citrate from Merck (Germany), Lauric acid and Capric acid from Sigma (USA), Caprylic acid, Oleic acid, N-Methyl-2-pyrrolidone and 2-Pyrrolidone from Fluka Chemie AG (Switzerland), 1-Ethyl-2-pyrrolidone and 1-(2-Hydroxyethyl)-2-pyrrolidone from Aldrich (USA). All other chemicals and solvents were of analytical grade and were obtained commercially. The backing membrane and the release liner were kindly gifted by the Neoplast Co.Ltd. (Thailand).

Fabrication of Ketoprofen Transdermal Drug Delivery System

Table 1. Composition of KP-TDDS formulations containing single permeation enhancer.

Ingrdients (%w/w)	Formulation No.								
	A	B	C	D	E	F	G	H	I
Ketoprofen	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Eudragit® NE30D	27.4	27.4	27.4	27.4	27.4	27.4	27.4	27.4	27.4
Eudragit®E100 solution	27.4	27.4	27.4	27.4	27.4	27.4	27.4	27.4	27.4
Triethyl citrate	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
Propylene glycol	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Lauric acid*	-	√	-	-	-	-	-	-	-
Capric acid*	-	-	√	-	-	-	-	-	-
Caprylic acid*	-	-	-	√	-	-	-	-	-
Oleic acid*	-	-	-	-	√	-	-	-	-
N-methyl-2-pyrrolidone#	-	-	-	-	-	√	-	-	-
2-Pyrrolidone#	-	-	-	-	-	-	√	-	-
Hydroxyethyl Pyrrolidone#	-	-	-	-	-	-	-	√	-
2 Ethyl Pyrrolidone#	-	-	-	-	-	-	-	-	√
Isopropanol:Acetone (3:2 w/w) q.s.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

N.B. * = 5%w/w of total solid polymer was added in the formulation
= 10%w/w of total solid polymer was added in the formulation

Table 2. Composition of KP-TDDS formulations containing combined permeation enhancers.

Ingrdients (%w/w)	Formulation No.			
	J	K	L	M
Ketoprofen	2.5	2.5	2.5	2.5
Eudragit® NE30D	27.4	27.4	27.4	27.4
Eudragit®E100 solution	27.4	27.4	27.4	27.4
Triethyl citrate	8.1	8.1	8.1	8.1
Propylene glycol	0.7	0.7	0.7	0.7
Oleic acid*	√	√	√	√
Lauric acid*	√	-	-	-
Caprylic acid*	-	√	-	-
N-methyl-2-pyrrolidone#	-	-	√	-
2-Pyrrolidone#	-	-	-	√
Isopropanol:Acetone (3:2 w/w) q.s	100.0	100.0	100.0	100.0

N.B. * = 5%w/w of total solid polymer was added in the formulation
= 10%w/w of total solid polymer was added in the formulation

Firstly, Eudragit®E100 solution (19%w/w in the mixture of isopropanol and acetone, 3:2 w/w) was mixed with Eudragit®NE30D, using a magnetic stirrer. Secondly, propylene glycol, triethyl citrate and permeation enhancer(s) was/were added in the Eudragit® mixture. Finally, ketoprofen was added into the mixture and well stirred until the homogeneous mixture was obtained. The mixture was poured directly and spread uniformly on the backing membrane with the TLC spreader. The release liner was placed on the surface of the drug-adhesive layer after drying at 64°C for 15 min. Thirteen KP-TDDS formulations (formulation A-M) were prepared with different compositions as shown in Tables 1 and 2.

In vitro Permeation Studies of Ketoprofen Transdermal Drug Delivery System

Abdominal Rat Skin Preparation

Male Wistar rats (National Laboratory Animal Center, Nakornpathom, Thailand) weighing 180-220g, were used in this study. On the day before removing the skin from the rat, the hair of the abdominal area was removed with an electric razor. On the next day, the rat was sacrificed by cervical dislocation. The abdominal skin was excised and the adherent fat and subcutaneous tissue were gently removed. The excised skin was rinsed with the normal saline solution and wiped carefully with tissue. The skin was then kept at -40°C until usage. Skin Permeation Studies

The rat skin was thawed at room temperature and cut into small pieces. The release liner was removed from the patch and KP-TDDS was fixed on the stratum corneum surface. The KP-TDDS with the skin was mounted between donor and receptor compartments and both compartments were then clamped together. The receptor compartment volume was 12.0

ml. and the effective surface area available for permeation was 1.7679 cm^2. All studies were performed at 32±1°C and stirred at 600 rpm. Isotonic phosphate buffer, pH 7.4, was used as the receptor solution. The samples were withdrawn, 500 μl, at fixed time intervals and the same volume of fresh receptor medium was replaced periodically up to 30 hr. The KP concentrations in the samples were determined by high pressure liquid chromatography. The cumulative amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. Each formulation was carried out in triplicate.

Analysis of Ketoprofen

Analysis of KP in KP-TDDS

Initial amount of KP in KP-TDDS was analysed by using isopropanol as a solvent. The mixture of KP-TDDS was shaken at room temperature for 3 hr. and filtered through the membrane filter 0.45 μm. The absorbance of the appropiate diluted filtrate was measured with UV-visible spectrophotometer (Spectronic 1001, Milton Roy), at 254 nm. Linearity was demonstrated from 2.0 to 16.0 μg/ml ($r^2 > 0.9900$).

Analysis of KP in Isotonic Phosphate Buffer

The amount of KP permeated through the abdominal rat skin was filtered through 0.45 μm membrane filter and determined, using HPLC (HP 1100, Hewlett Packard). The chromatographic analysis was carried out with a reverse phase Nucleosil 100–5 C_{18}column (5 μm, 250x4.6mm.i.d.) at 40°C and χ_{max} 254 nm. The mobile phase was an acetonitrile/pH 3.5 phosphate buffer mixture (55:45v/v) with a flow rate of 1.0 ml/min. The retention time of KP was 2.73 min. Linearity was demonstrated from 0.6 to 13.0 μg/ml ($r^2 > 0.9900$).

Data Analysis

Firstly, the permeation profiles of KP permeated through rat skin over the permeation period (up to 30 hr.) between different formulations were statistically analyzed by repeated measurement of general linear model. Secondly, the cumulative amounts of KP permeated were plotted as a function of time. The flux of KP was obtained from the slope of the linear portion of the plot. All flux data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-test to test the statistical significance of differences among formulations. Data were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Fabrication of Ketoprofen Transdermal Drug Delivery System

In general, soft or flexible film can be obtained when Eudragit®NE30D and Eudragit®E100 were used without any plasticizers in the formulation. However, in this study, optimal adhesive property of KP-TDDS was required to ensure that the patch remaining on the skin all the time during usage. Without plasticizers, no adhesive property of TDDS was obtained. For this reason, different concentrations of a plasticizer, triethyl citrate (TEC), was added in the formulation. Their adhesive properties were determined by the thumb tack test (Banakar and Osborne, 1995) (data not shown). It was indicated that the concentration of TEC which gave the appropriate adhesiveness of the transdermal patch was 8.1 %w/w as shown in Tables 1 and 2.

***In vitro* Skin Permeation Studies of Ketoprofen Transdermal Drug Delivery System Effect of Single Permeation Enhancer**

Figure 1 shows the permeation profiles of KP-TDDS containing various types of fatty acid. From the repeated measurement of statistical analysis, there were no significant differences between the permeation profiles of KP permeated from these formulations. Fluxes were calculated from the slopes of the straight lines (coefficient of correlation, r^2 = 0.9220-0.9875) and shown in Table 3. From the ANOVA statistical analysis; 1) there were no significant differences between the fluxes of KP permeated from the formulations with various types of fatty acid (formulation B-E) and the flux without fatty acid (formulation A), 2) there were no significant

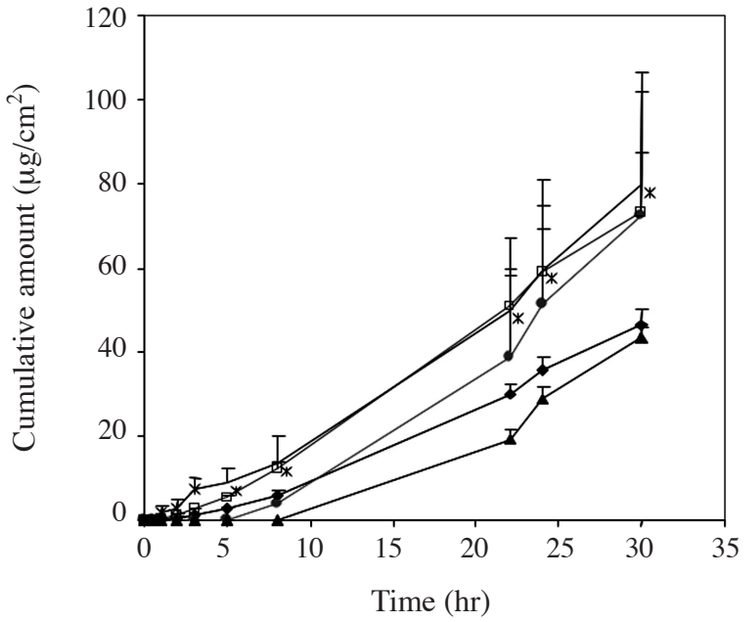

Figure 1. Effect of fatty acids on the permeation of KP from formulation A (◆), formulation B (●), formulation C (▲), formulation D (□) and formulation E (*). Data are reported as mean±S.D.(three replications) of the cumulative amount of KP in the receptor compartment (μg/cm^2) at the sampling time (0, 0.5, 1, 2, 3, 5, 8, 22, 24, 30 hr.).

Table 3. Flux and lag time of KP-TDDS containing single permeation enhancer across the abdominal rat skin.

Formulation No.	Type of Permeation Enhancer	Flux (ug/cm^2-hr.)	Lag time (min.)
A	none	1.57±0.23	106.44±16.55
B	LA	2.35±2.04	147.65±15.14
C	CPA	1.30±0.16	162.70±5.15
D	CPRY	2.53±0.94	87.93±18.13
E	OA	2.58±1.73	53.66±41.50
F	NMP	2.11±0.44	122.96±20.09
G	PYR	2.01±1.11	126.15±26.21
H	HEPYR	1.73±0.16	117.92±13.75
I	EPYR	1.46±0.03	120.68±21.12

Each value represents the mean±S.D. of three experiments. LA, lauric acid; CPA, capric acid; CPRY, caprylic acid; OA, oleic acid; NMP, N-methyl-2-pyrrolidone; PYR, 2-pyrrolidone; HEPYR, 1-(2-hydroxyethyl)-2-pyrrolidone; EPYR, 1-ethyl-2-pyrrolidone.

differences among the fluxes of KP permeated from the formulations with different fatty acids (formulation B-E). However, there was a trend which indicated that the formulation containing OA or CPRY (formulation E or D) showed the higher flux than the other formulations. In addition, when compared among the lag times, calculated from the x-intercept of the linear fit of the plot and established in Table 3, the formulation containing OA (formulation E) showed the shortest lag time than the other formulations. Thus, in this study, OA is the most powerful permeation enhancer. It contained suitable alkyl chain length and a double bond in its chemical structure (Barry, 1987). More specifically, when the stratum corneum was treated with oleic acid and examined by differential scanning colorimetry and infrared spectroscopy, OA decreased the phase transition temperatures of the lipid domain of the stratum corneum and increased motional freedom or fluidity of the lipid (Golden et al., 1987).

Figure 2 shows the permeation profiles of formulations containing different types of pyrrolidone derivatives. From the repeated measurement of statistical analysis, there were no significant differences between the permeation profiles of KP permeated from these formulations. As previously mentioned, fluxes of KP were calculated from the slopes of the straight lines (coefficients of correlation, $r^2 = 0.9447$-0.9835) and shown in Table 3. From the ANOVA statistical analysis, there were no significant differences among the fluxes of KP from these formulations. Moreover, the lag times of all formulations, as shown in Table 3, were not different. Nevertheless, there was a trend which suggested that the formulation containing NMP or PYR showed the higher flux than the other formulations. Thus, the functional group at the 1-position of their chemical structures may play an important role in

the enhancing effect of KP permeation through rat skin. Shorter functional group at the 1-position, hydrogen of PYR and methyl of NMP, had a trend to produce better enhancement than longer functional group, ethyl of EPYR and hydroxyethyl of HEPYR. These results were not in agreement with the previous studies by several researchers (Sasaki et al., 1988, 1990; Aoyagi et al., 1991; Godwin et al., 1997; Yoneto et al., 1998). They found that there was an increase in penetration enhancement with increasing alkyl chain length at the 1-position of alkyl pyrrolidone derivatives. All these findings have been investigated by using wide range of alkyl chain length at the 1-position , up to butyl, octyl or dodecyl functional group. For this reason, the results of the present study do not agree with other studies.

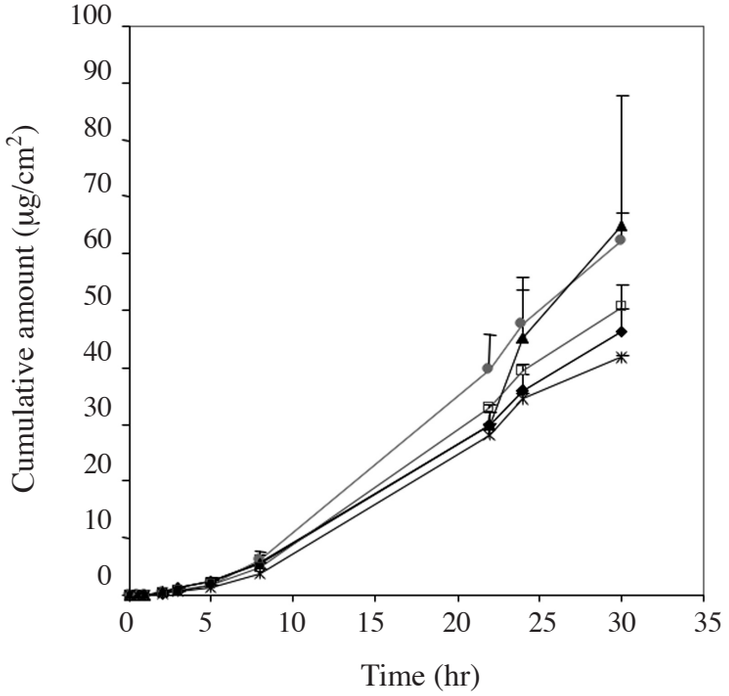

Figure 2. Effect of pyrrolidone derivatives on the permeation of KP from formulation A (◆), formulation F (●), formulation G (▲), formulation H (□) and formulation I (*). Data are reported as mean±S.D. (three replications) of the cumulative amount of KP in the receptor compartment (μg/cm^2) at the sampling time (0, 0.5, 1, 2, 3,5, 8, 22, 24, 30 hr.).

Effect of Combined Permeation Enhancer

According to the previous results of this study and also the effort to improve the flux of KP across the skin, combined permeation enhancers were also investigated. The effect of four different combined permeation enhancers on the permeation of KP is presented in Figure 3.

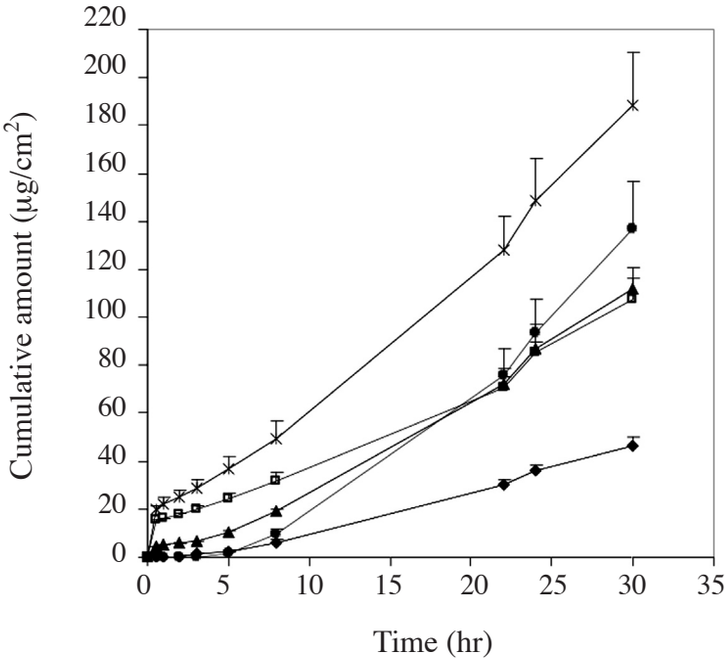

Figure 3. Effect of combined permeation enhancers on the permeation of KP from formulation A (◆), formulation J (●), formulation K (▲), formulation L (□)and formulation M (*). Data are reported as mean±S.D. (three replications) of the cumulative amount of KP in the receptor compartment (μg/cm^2) at the sampling time (0, 0.5, 1, 2, 3, 5, 8, 22, 24, 30 hr.).

From the repeated measurement of statistical analysis, there were significant differences between the permeation profiles of KP permeated from these formulations. Fluxes of KP were calculated from the slopes of the straight line (coefficients of correlation, $r^2 = 0.9609–0.9889$). From the ANOVA statistical analysis followed by Tukey test, there was significant difference when comparing among the flux of KP permeated from formulation without permeation enhancer (formulation A) with the flux of KP permeated from the formulation containing either OA and PYR (formulation M) or OA and LA (formulation J). In contrast, there was no significant difference among the fluxes of drug permeated from all formulations containing different combined permeation enhancers.

The enhancement ratios (ERs), calculated from the flux of each formulation containing single or combined permeation enhancers divided by the flux of the formulation without permeation enhancer, are shown in Table 4. The combination of OA and LA (ER = 2.80) was more effective than the combination of OA and CPRY (ER = 2.32). This may be due to the longer alkyl chain of LA (C_{12}, saturated alkyl chain) than CPRY (C_8, saturated alkyl chain). Generally, saturated fatty acid containing C_{10}- C_{12} alkyl chain lengths yields a potent enhancer (Williams and Barry, 2004). In comparison between the combination of OA and PYR with the combination of OA and NMP, OA combined with PYR (ER = 3.65) had much higher enhancing activity than OA combined with NMP (ER = 1.99). This may be due to the hydrogen group of PYR at the 1-position which had greater solubilizing and skin permeation effect on KP than the methyl group of NMP. Additionally, the combination of OA and PYR showed higher enhancing effect than the combination of OA and LA. This may be due to

their different mechanism of action on the skin permeation. OA interacts with and modifies the lipid domains of the stratum corneum (Willliams and Barry, 2004) while PYR increases the diffusivity of the drug (Godwin et al.,1997). This finding is very useful as an alternative approach to promote the skin permeation of KP from KP-TDDS formulation.

Table 4. The enhancement ratios of KP-TDDS containing single and combined permeation enhancers

Formulation No.	Type of Permeation Enhancer	Enhancement Ratio
A	none	1.00
B	LA	1.49
D	CPRY	1.61
E	OA	1.64
F	NMP	1.34
G	PYR	1.28
J	OA and LA	2.80
K	OA and CPRY	2.32
L	OA and NMP	1.99
M	OA and PYR	3.65

Each value calculated from the mean of three experiments. LA, lauric acid; CPRY, caprylic acid; OA, oleic acid; NMP, N-methyl-2-pyrrolidone; PYR, 2-pyrrolidone.

A comparison of the formulations containing single fatty acid with combined OA and LA or CPRY and also a comparison of the formulations containing single pyrrolidone derivative with combined oleic acid and NMP or PYR, in terms of the enhancement ratio, are also presented in Table 4. It was indicated that in both comparisons, the combined permeation enhancer exhibited higher enhancing activity than the single permeation enhancer.

CONCLUSION

In our study, the combined permeation enhancers, especially oleic acid and 2- pyrrolidone, acted as an effective permeation enhancer in promoting the skin permeation of KP from KP-TDDS through the abdominal rat skin.

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