Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 Issue 2, 2014

Research Article

FORMULATION AND *IN VITRO* STUDY OF KETOPROFEN PSEUDOLATEX GEL FOR TRANSDERMAL DRUG DELIVERY SYSTEMS

JIRAPORNCHAI SUKSAEREE*, CHAOWALIT MONTON, APIRAK SAKUNPAK, AND TOSSATON CHAROONRATANA

Faculty of Pharmacy and Sino-Thai Traditional Medicine Research Center (Cooperation between Rangsit University, Harbin Institute of Technology, and Heilongjiang University of Chinese Medicine), Rangsit University, Pathum Thani 12000, Thailand.
Email: jirapornchai.s@rsu.ac.th (J. Suksaeree)

Received: 11 Jan 2014, Revised and Accepted: 26 Feb 2014

ABSTRACT

Objective: This study was to prepare ketoprofen pseudolatex gel for transdermal delivery systems which could enhance solubility and permeability of ketoprofen by using different enhancers: isopropyl palmitate, tween 80, and oleic acid, with or without ethanol.

Methods: The ketoprofen pseudolatex gel was made from ethyl cellulose and deproteinized natural rubber latex in a ratio of 1:1. Polyvinyl pyrrolidone, glycerine, and dibutyl phthalate were used as a channeling agent, skin humectant, and plasticizer, respectively. Polyvinyl alcohol was used as a polymeric surfactant. The ketoprofen pseudolatex gel was prepared using a homogenization and solvent removal method.

Results: The ketoprofen pseudolatex gel formulations were homogeneous and smooth in texture, and elegant in appearance. The pH of ketoprofen pseudolatex gel formulations was 6.08 – 7.02, and the particle size ranged between 685.39 and 703.29 nm. The viscosity and spreadability showed the good characteristics. Stable colloidal dispersion was formed with a zeta potential of -40.32 to -43.29 mV. Zeta potential studies confirmed the stability of the ketoprofen pseudolatex gel preparations, as indicated by the negative charges obtained for all the preparations. The *in vitro* studies of ketoprofen were also evaluated. The enhancer could increase the release and permeation of ketoprofen from the pseudolatex gel. The formula containing tween 80 with ethanol showed the best permeation through pig skin.

Conclusion: This was the most suitable ketoprofen pseudolatex gel as it provided a controlled release and suitable permeation patterns of the ketoprofen. Thus, these tween 80 systems can be used and prepared for transdermal drug delivery systems.

Keyword: Pseudolatex gel, Ketoprofen, Transdermal drug delivery systems, Enhancer.

INTRODUCTION

Ketoprofen is a potent non-steroidal anti-inflammatory drug and is practically insoluble in water. It is commonly used for the treatment of musculoskeletal disorders such as osteoarthritis and rheumatoid arthritis, as well as for symptoms of trauma [1]. Administration via the dermal route can bypass these disadvantages, and may maintain relatively consistent plasma levels for long term therapy from a single dose. However, the barrier function of the skin limits its formulation as a transdermal dosage form and makes this challenging [2, 3]. Solid dispersion is an effective technique which can easily enhance the dissolution rate of drugs. Transdermal delivery of the ketoprofen drug, such as nano-emulsion, gel formulation, or patch system, has previously been used for topical treatments to minimize side effects associated with use of oral anti-inflammatories [2, 4-6]. However, the stratum corneum is a strong barrier to most exogenous substances, including drugs, due to its multilayered structure. One approach for drug delivery through skin is to reversibly reduce the barrier function of skin with penetration enhancers [3, 7]. Therefore, ketoprofen was selected as the transdermal formulation for a controlled-release dosage form.

Pseudolatex systems should possess suitable characteristics, such as colloidal aqueous dispersions that are water-based after solvent evaporation [8]. They must also be useful in mediating drug release [9-11]. Pseudolatex can be prepared by the emulsification – evaporation technique, by dissolving the polymer in a suitable solvent system, and introducing the organic phase into water in order to form an emulsion by employing surfactant as stabilizers. After homogenization, the solvent is removed by vacuum evaporation [9, 12].

The main objective of this work was to enhance the transdermal permeation of ketoprofen by using a different enhancer: isopropyl palmitate (IPP), tween 80 (TW 80), or oleic acid (OA), without or with ethanol. The ketoprofen pseudolatex gel was made from ethyl cellulose (EC) and deproteinized natural rubber latex (DNRL). Polyvinyl alcohol (PVA) was used as the surfactant and stabilizer of the pseudolatex systems. Glycerine was used as a skin humectant, dibutyl phthalate (DBP) as a plasticizer, and polyvinyl pyrrolidone (PVP) as the channeling agent. The ketoprofen pseudolatex gel formulations were homogenized to form the colloidal emulsion and vacuum evaporation was used to remove the organic solvent. The physical appearances, *in vitro* release, and skin permeation of ketoprofen were also studied.

MATERIALS AND METHODS

Materials

The DNRL was prepared in-house from fresh NRL collected from *Hevea brasiliensis* (RRIM 600 clone), and purified by enzyme deproteinization followed by centrifugation by W. Pichayakorn laboratory [13-15]. Ketoprofen (98 % purity, *Mw* = 254.28 g/mol), polyoxyethylene-20 oleyl ether, EC, PVP, glycerine, IPP, PVA (*Mw* = 31,000 g/mol), TW 80, OA, and DBP were obtained from Sigma-Aldrich (USA). The other chemicals were of analytical grade.

Preparation and characterization of pseudolatex systems

For the oil phase, 10% w/w EC and 10% w/w dry DNRL were dissolved in 500 ml of dichloromethane using a magnetic stirrer until it was a clear solution. After that, the 6% w/w DBP, and 2 g ketoprofen were mixed together into polymeric pseudolatex gel. The 5 % w/w penetration enhancer (IPP, TW 80, or OA) was mixed in this solution. For the water phase, 4% w/w PVA and 14% w/w PVP were dissolved in 100 mL of water and stirred until completely soluble.

Then, 6% w/w glycerine was mixed into this solution. The two phases were mixed together by pouring water phase into the oil phase, and then the homogenization method was used for 30 minutes until an emulsion-like system occurred. This was then poured into round bottom flask where the dichloromethane was removed by rotary evaporator with a controlling temperature of 40±2°C and a vacuum condition for 5 hours. Finally, these pseudolatex systems were adjusted to 100 mL with water. Thus, the ketoprofen pseudolatex gel had the ketoprofen content of approximately 20 mg/mL in each pseudolatex system. The pH of

the ketoprofen pseudolatex gel was measured by a S220 SevenCompact™ pH/Ion pH meter (Mettler Toledo, Switzerland) at room temperature. The pH meter was calibrated by using pH 4.0, 7.0, and 10.0 standard buffers.

The viscosity was measured by a Brook field viscometer (Brookfield engineering laboratories Inc, USA) at 25 ± 2 ºC.

The particle size, size distribution, and surface charge on the particles were measured by a ZetaPALS (Brookhaven, Germany) at 25 ± 2 ºC, and presented as the effective diameter, polydispersity index (PI), and zeta potential (ζ), respectively.

The spreadability values (g cm2/sec) were measured by pouring each of the formulations onto a glass plate. Then, each was pressed with another glass plate to expel air and form a complete film between the two plates. The film diameter during each interval was given as the area of complete film, which indicated the spreadability property. It was further calculated by the equation below:

$$S = \frac{W \times A}{T}$$

where S is the spreadability (g cm^2/sec), W is the weight of the sample (g), A is the area of the sample spread on the glass plate (cm^2), and T is the time taken to spread completely on the glass plate from each formula (sec) [16, 17].

Determination of ketoprofen content

The ketoprofen content in pseudolatex gel formulations were extracted with 0.5 w/v of polyoxyethylene-20 oleyl ether in isotonic phosphate buffer solution pH 7.4. They were immersed for 30 minutes and sonicated for 1 hour. They were filtered by using a 0.45 µm cellulose membrane. The ketoprofen content in each sample was determined by HPLC method.

***In vitro* studies**

The in vitro study of ketoprofen was performed using a modified Franz-type diffusion cell with a diffusion area of 1.77 cm2. The receptor compartment was filled with 12 mL of 0.5 w/v of polyoxyethylene-20 oleyl ether in isotonic phosphate buffer solution (pH 7.4), controlled with a water jacket at 37 ± 0.5ºC, and constantly stirred at 300 rpm with a magnetic stirrer. A 1 mL sample of isotonic phosphate buffer solution was withdrawn at 0.5, 1, 2, 4, 6, 8, and 12 hour time intervals, and an equal volume of fresh isotonic phosphate buffer solution was then added as a replacement [18]. For the in vitro release, the pure ketoprofen and ketoprofen pseudolatex gel formulations were applied to cellulose membrane (Mw cut-off 3,500 g/mol). The in vitro permeation of ketoprofen was determined using pig skin. The method for pig skin preparation has been described by Songkro et al [19]. The pig skin was placed on the top of the receptor compartment with the stratum corneum facing upwards to the donor compartment. The ketoprofen pseudolatex gel formulations were directly applied to the pig skin.

HPLC Condition

The samples collected from the *in vitro* study were analyzed by the HPLC system (Agilent 1260 series, USA) with an Agilent C18 analytical column 4.6 mm × 150 mm. The mobile phases used were 0.025% v/v trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B), and they were run at a gradient of 70:30 for 5 min, then 10:90 for 8 min followed by 0:100 (solvent A:B, respectively) with a flow rate of 1 mL/min. Ketoprofen content in the samples was determined at 255 nm.

Statistical analysis

The average value was calculated as a mean ± standard deviation value. All results were statistically analyzed by one-way analysis of variance followed by post hoc analysis. A *p*-value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Characteristics of pseudolatex gels

The latex is harvested using the tapping process from the Para rubber tree. It is very stretchy and flexible, and extremely waterproof [20, 21]. However, it is comprised of various types of proteins (Hev 1 – 14) which can cause allergic reactions to patients [22, 23]. Thus, the latex used in this study had the protein content reduced, as performed by Pichayakorn *et al* [14]. This low protein content latex is called DNRL. DNRL solution was safe enough to apply to the skin of New Zealand white rabbits, as shown by the Thailand Institute of Scientific and Technology Research [13, 14]. Therefore, the DNRL solution was deemed to be a suitably safe polymer to use in this work.

The polymeric pseudolatex dispersion as a new topical drug delivery system was prepared by using a solvent removal method first reported by Büyükyaylaci *et al* [24]. The preparation of each ketoprofen transdermal pseudolatex gel had three different enhancer types: IPP, TW 80, and OA. They could be easily prepared by homogenization. The ketoprofen solid forms could be completely dissolved in mixed solvents comprised of EC and DNRL polymers before adding any other ingredients, and all finished polymeric pseudolatexs exhibited equally sticky properties. They were all yellowish mixtures due to the color of ketoprofen, with good homogeneity and were smooth in texture (Fig. 1). The ketoprofen pseudolatex gels also showed good drying times for forming film (approximately 20 min). The different enhancer types were not affected significantly on drying times. When 20% of ethanol was added in ketoprofen pseudolatex gels, the formulations slightly reduced the drying time to 10-15 min for film formation. The ethanol is the greater easily volatility solvent compared to water. All of the pseudolatex gels produced complete films with no cracking and no flaking. They were suitable enough to be tested as transdermal drug delivery systems because skin movements had no effect on their integrity. Moreover, they might prevent abrasion on the film surface from environmental factors, for example by contact with cloth.

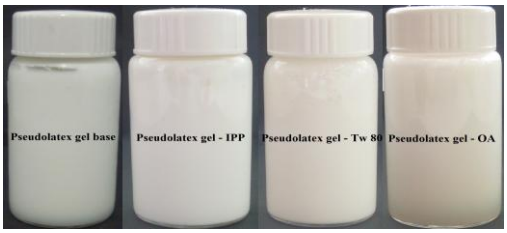

Fig. 1: Appearance of ketoprofen pseudolatex gel with different enhancers: IPP, TW 80, and OA

The pH, viscosity, effective diameter, ζ, and spreadability values of the pseudolatex systems are shown in Table 1-2 which were indicated their safety and ease to be applied directly on the skin. They had pH values ranging between 6.08 and 7.02, and they were safe for use on the skin with no irritation [25]. The viscosity, effective diameter, ζ, and spreadability values of ketoprofen pseudolatex gel base were 387.32 ± 29.21, 698.53 ± 37.29 (PI = 0.15), -40.32 ± 1.82, and 1.48 ± 0.26, respectively. When each enhancer (IPP, TW 80, or OA) was added to the ketoprofen pseudolatex gel base, their viscosity, effective diameter, ζ, and spreadability values were not affected significantly. The ζ values ranged between -60 and -30 mV. Moreover, the 20% ethanol mixed into the ketoprofen pseudolatex gel slightly affected their viscosity, effective diameter, ζ, and spreadability values (Table 1-2). This might be due to a slight agglomeration of the latex particle. This result was related to increasing viscosity and effective diameter values of the ketoprofen pseudolatex gel. Thus, the ketoprofen pseudolatex gel exhibited a very good physical stability during their shelf-life, as there were no significant changes to their physicochemical properties from the initial preparation (Table 1) to after storage for 3 months (Table 2).

Table 1: Physicochemical properties of the initial preparation of ketoprofen pseudo latex gel (mean ± SD, n=5)

Formulas	Initial preparation					
	pH	Viscosity (cps)	Effective diameter (nm)	PI	ζ (mV)	Spreadability values (g cm²/sec)
Ketoprofen pseudolatex gel base	6.08±0.18	387.32±21.78	698.53±37.29	0.15	-40.32±1.82	1.48±0.26
Ketoprofen pseudolatex gel base - IPP	6.13±0.14	365.37±1.44	685.39±41.56	0.12	-41.82±3.29	1.46±0.14
Ketoprofen pseudolatex gel base – TW 80	6.23±0.22	389.28±42.18	691.28±39.77	0.16	-43.28±4.58	1.45±0.24
Ketoprofen pseudolatex gel base - OA	6.49±0.18	375.28±38.92	687.39±32.92	0.14	-40.29±5.04	1.49±0.24
Ketoprofen pseudolatex gel base – IPP - ethanol	6.73±0.29	379.92±49.28	695.28±41.29	0.13	-43.29±3.29	1.54±0.21
Ketoprofen pseudolatex gel base – TW 80 - ethanol	6.72±0.18	403.82±35.22	703.29±39.28	0.19	-42.33±3.21	1.57±0.31
Ketoprofen pseudolatex gel base - OA - ethanol	6.89±0.25	402.12±41.43	692.43±50.27	0.18	-40.19±3.01	1.52±0.29

Table 2: Physicochemical properties of the ketoprofen pseudo latex gel after 3 months (mean ± SD, n=5)

Formulas	3 months					
	pH	Viscosity (cps)	Effective diameter (nm)	PI	ζ (mV)	Spreadability values (g cm²/sec)
Ketoprofen pseudolatex gel base	6.18±0.08	412.98±11.82	743.19±39.17	0.13	-39.79±3.29	1.38±0.17
Ketoprofen pseudolatex gel base - IPP	6.37±0.15	370.29±48.92	693.98±38.25	0.12	-42.19±2.98	1.40±0.13
Ketoprofen pseudolatex gel base – TW 80	6.59±0.24	392.78±38.29	702.11±49.28	0.14	-42.01±2.39	1.37±0.19
Ketoprofen pseudolatex gel base - OA	6.93±0.26	390.19±44.29	709.38±54.32	0.17	-43.293.29	1.42±0.22
Ketoprofen pseudolatex gel base – IPP - ethanol	7.02±0.21	400.39±50.29	719.44±49.28	0.17	-40.19±4.29	1.49±0.16
Ketoprofen pseudolatex gel base – TW 80 - ethanol	6.74±0.19	419.83±39.84	721.39±51.98	0.15	-42.91±3.29	1.51±0.22
Ketoprofen pseudolatex gel base - OA - ethanol	6.93±0.22	438.78±51.05	733.29±58.91	0.13	-41.92±4.59	1.47±0.19

Determination of ketoprofen content

All completed ketoprofen pseudolatex gel formulations were extracted with 0.5 w/v of polyoxyethylene-20 oleyl ether in pH 7.4 isotonic phosphate buffer solution to detect the ketoprofen in their formulation by HPLC method.

Table 3: Ketoprofen content of the pseudolatex system (mean ± SD, n=5)

Formulas	Initial preparation (mg/mL)	3 months (mg/mL)
Ketoprofen pseudolatex gel base	20.33 ± 2.31	20.38 ± 3.12
Ketoprofen pseudolatex gel base – IPP	19.39 ± 2.18	19.03 ± 2.45
Ketoprofen pseudolatex gel base – TW 80	21.82 ± 3.92	21.48 ± 2.39
Ketoprofen pseudolatex gel base – OA	20.81 ± 2.34	20.73 ± 2.23
Ketoprofen pseudolatex gel base – IPP – ethanol	20.64 ± 2.84	20.48 ± 2.58
Ketoprofen pseudolatex gel base – TW 80 – ethanol	19.94 ± 1.98	19.89 ± 2.98
Ketoprofen pseudolatex gel base - OA – ethanol	20.89 ± 2.56	20.57 ± 3.02

The percent content of the various ketoprofen pseudolatex gel formulations are shown in Table 3. The initial preparation of all formulations found the ketoprofen content in the range of 19.39 – 21.82 mg/mL. After all preparations were kept at 4°C for 3 months, the ketoprofen content was found to be in the range of 19.03 – 21.48 mg/mL.

The ketoprofen contents extracted from all formulations were not significantly different from initial preparation. From these results, it might be assumed that pseudolatex gel formulations dissolve and entrap the ketoprofen very well.

***In vitro* studies**

The ketoprofen pseudolatex gels were made from the mixture of drug dissolved into a pseudolatex base that comprised of a different type of enhancer: IPP, TW 80, or OA. Ketoprofen has low solubility in water and has dissolution problems [26]. It was found that the pseudolatex gel could enhance the aqueous solubility of this poor water soluble ketoprofen. When the pseudolatex gel systems were studied in the *in vitro* release of ketoprofen, they provided faster release of ketoprofen from pseudolatex gel, compared to the pure drug (Fig. 2).

The biphasic release of ketoprofen from the pseudolatex gel was found as the burst release in less than 1 hour, followed by a slow release upon formation of the film formation. However, the different type of enhancer: IPP, TW 80, or OA, increased the solubility of ketoprofen, which showed higher ketoprofen release compared with the ketoprofen pseudolatex gel base (Fig. 2).

The TW 80 increased both the solubility of ketoprofen and the release behavior, compared with IPP and OA.

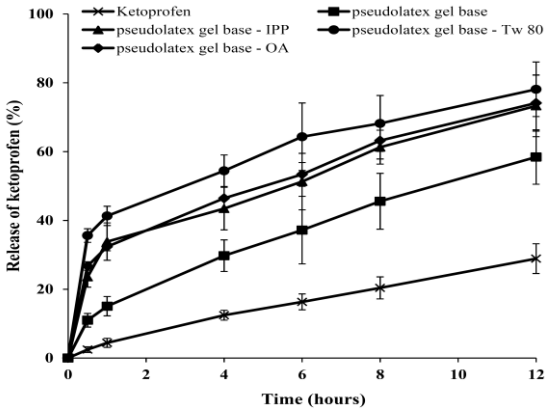

Fig. 2: The release of ketoprofen pseudolatex gel with different enhancers (mean ± SD, n=3)

In addition, the ethanol effects on the ketoprofen release patterns from the ketoprofen pseudolatex gel are presented in Fig. 3 – 5. The release behavior of ketoprofen from added ethanol preparations was higher than that without ethanol. This correlated with the increase of viscosity of these colloidal dispersions due to some agglomerations after ethanol dilution [27]. Ethanol has been the effective enhancer with many other transdermal formulations, and may be used as solvent to increase solubility of drug [28-30]. Thus, the ketoprofen also dissolved well in their pseudolatex gel formulation, as it is highly released from the formulation.

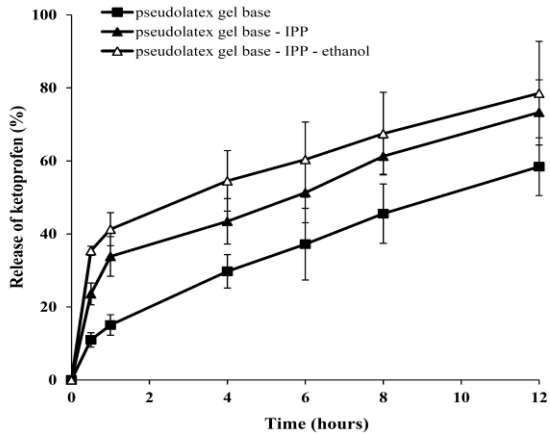

Fig. 3: The release of ketoprofen pseudolatex gel – IPP without/with ethanol (mean ± SD, n=3)

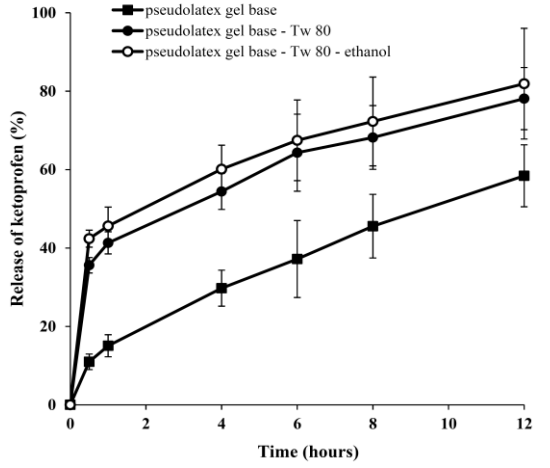

Fig. 4: The release of ketoprofen pseudolatex gel – TW 80 without/with ethanol (mean ± SD, n=3)

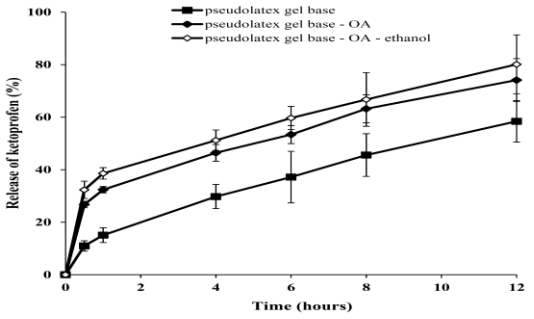

Fig. 5: The release of ketoprofen pseudolatex gel – OA without/with ethanol (mean ± SD, n=3)

The *in vitro* skin permeation using the pig skin as a barrier between formulation and the receptor medium was used due to the anatomical, physiological, and biochemical properties of the pig skin which resemble human skin [31, 32]. The ketoprofen pseudolatex gel significantly increased the *in vitro* skin permeation of ketoprofen (Fig. 6). The *in vitro* permeation patterns of ketoprofen were similar to *in vitro* release pattern, which depended on solubility of ketoprofen and enhancer: IPP, TW 80, or OA. This was because enhancer IPP, TW 80, or OA, reversibly decreased the barrier resistance of the stratum corneum, and allowed drugs to penetrate more readily to the viable tissues and the systemic circulation [33, 7]. The TW 80 produced a significantly higher *in vitro* skin permeation of ketoprofen, more so than IPP and OA (Fig. 6).

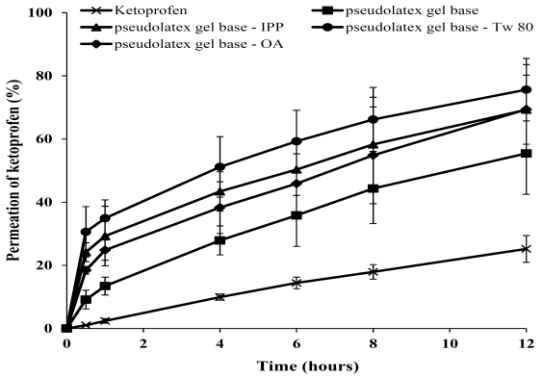

Fig. 6: The permeation of ketoprofen pseudolatex gel with different enhancers (mean ± SD, n=3)

Furthermore, the ethanol adding effect was also investigated on the *in vitro* skin permeation study, which provided the increasing ketoprofen permeation from their pseudolatex gel with different enhancers: IPP, TW 80, or OA (Fig. 7 – 9). Ethanol could act as a skin enhancer, as reported elsewhere [7]. It might extract and alter the solubility of the lipid fraction of the stratum corneum, and improve the flux of the drug molecules [28, 7].

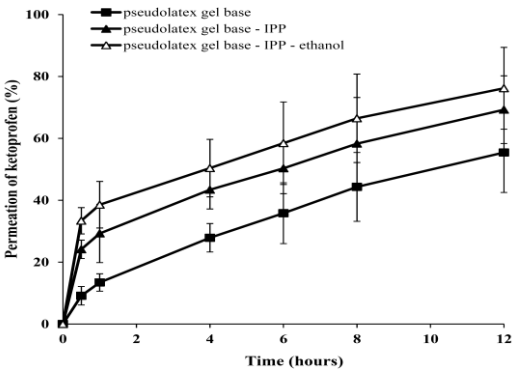

Fig. 7: The permeation of ketoprofen pseudolatex gel – IPP without/with ethanol (mean ± SD, n=3)

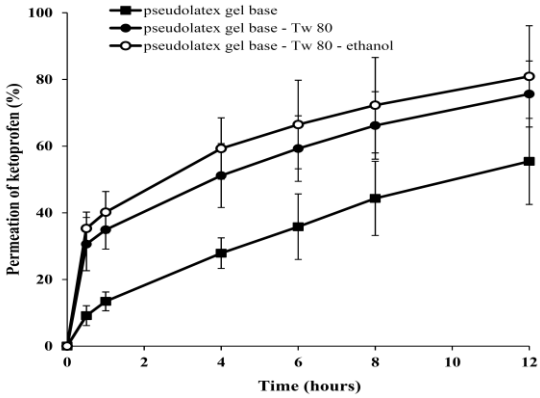

Fig. 8: The permeation of ketoprofen pseudolatex gel – TW 80 without/with ethanol (mean ± SD, n=3)

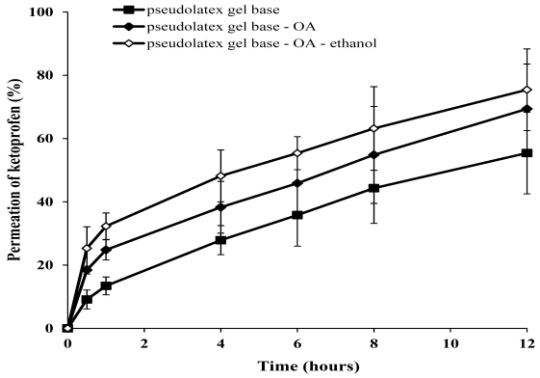

Fig. 9: The permeation of ketoprofen pseudolatex gel – OA without/with ethanol (mean ± SD, n=3)

CONCLUSIONS

In conclusion, the present study prepared the ketoprofen pseudolatex gel from EC and DNRL at ratio 1:1 by using different enhancers: IPP, TW 80, or OA. The pH, viscosity, effective diameter, ζ, and spreadability values of the pseudolatex gel systems indicated their safety and ease to be applied directly on the skin with no irritation. However, the IPP, TW 80, or OA were not significantly affected on these values of their pseudolatex gel formulation. But, the ethanol levels were was slightly affected on these values. This is due to the minor DNRL agglomeration in their formulations. Moreover, *in vitro* study results showed that different patterns were produced from enhancer effect and adding ethanol. The pseudolatex gel could improve the solubility of ketoprofen, the enhancer could be extracted and alter the solubility of the lipid fraction of the stratum corneum, and increase ketoprofen through the skin. The formula containing TW 80 with ethanol showed the best permeation through pig skin. This was the most suitable ketoprofen pseudolatex gel as it provided a controlled release and suitable permeation patterns of the ketoprofen. Thus, this work successfully used pseudolatex gel system to increase solubility, *in vitro* release, and *in vitro* skin permeation of ketoprofen, making it suitable for developing transdermal delivery systems.

ACKNOWLEDGEMENTS

The authors would like to acknowledge express their gratitude Associate Professor Dr. Prapaporn Boonme and Assistant Professor Dr. Wiwat Pichayakorn from Faculty of Pharmaceutical Sciences, Prince of Songkla University, Professor Dr. Krisana Kraisintu from Faculty of Oriental Medicine and Dr. Laksana Charoenchai from Faculty of Pharmacy, Rangsit University. The authors would like to express their gratitude to KI Tull from Rangsit University for assistance with the English in this paper. The authors would like to acknowledge the Faculty of Pharmacy, Rangsit University for financial supports.

REFERENCES

1. Sekiya I, Morito T, Hara K, Yamazaki J, Ju Y-J, Yagishita K, et al. Ketoprofen absorption by muscle and tendon after topical or oral administration in patients undergoing anterior cruciate ligament reconstruction. AAPS PharmSciTech 2010; 11(1): 154-158.
2. Mazières B. Topical ketoprofen patch. Drugs R D 2005; 6(6): 337-344.
3. Samy AM, Ghorab MM, Shadeed SG, Mazyed EA. Formulation and evaluation of different transdermal drug delivery systems of ketoprofen. Int J Pharm Pharm Sci 2013; 5(suppl. 2): 600-607.
4. Myung-Chul P, Hark K, Dae-Hwan P, Jae-Hun Y, Jin-Ho C. Ketoprofen-LDH nanohybrid for transdermal drug delivery system. Bull Korean Chem Soc 2012; 33(6): 1827-1828.
5. Sakeena MHF, Muthanna FA, Ghassan ZA, Kanakal MM, Elrashid SM, Munavvar AS, et al. Formulation and *in vitro* evaluation of ketoprofen in palm oil esters nanoemulsion for topical delivery. J Oleo Sci 2010; 59(4): 223-228.
6. Silion M, Hritcu D, Jaba I, Tamba B, Ionescu D, Mungiu O, et al. In vitro and in vivo behavior of ketoprofen intercalated into layered double hydroxides. J Mater Sci: Mater Med 2010; 21(11): 3009-3018.
7. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev 2004; 56(5): 603-618.
8. Sastry SV, Wilber W, Reddy IK, Khan MA. Aqueous-based polymeric dispersion: preparation and characterization of cellulose acetate pseudolatex. Int J Pharm 1998; 165(2): 175-189.
9. Chang R-K, Hsiao C. Eudragit RL and RS pseudolatices: Properties and performance in pharmaceutical coating as a controlled release membrane for theophylline pellets. Drug Dev Ind Pharm 1989; 15(2): 187-196.
10. Thioune O, Briançon S, Devissaguet JP, Fessi H. Development of a new ethylcellulose pseudolatex for coating. Drug Dev Res 2000; 50(2): 157-162.
11. Vyas SP, Gogoi PJ, Jain SK. Development and characterization of pseudolatex based transdermal drug delivery system of diclofenac. Drug Dev Ind Pharm 1991; 17(8): 1041-1058.
12. Suksaeree J, Charoenchai L, Monton C, Chusut T, Sakunpak A, Pichayakorn W, et al. Preparation of a pseudolatex-membrane for ketoprofen transdermal drug delivery systems. Ind Eng Chem Res 2013; 52(45): 15847-15854.
13. Pichayakorn W, Suksaeree J, Boonme P, Amnuaikit T, Taweepreda W, Ritthidej GC. Nicotine transdermal patches using polymeric natural rubber as the matrix controlling system: Effect of polymer and plasticizer blends. J Membr Sci 2012; 411-41281-90.
14. Pichayakorn W, Suksaeree J, Boonme P, Taweepreda W, Ritthidej GC. Preparation of deproteinized natural rubber latex and properties of films formed by itself and several adhesive polymer blends. Ind Eng Chem Res 2012; 51(41): 13393-13404.
15. Suksaeree J, Boonme P, Taweepreda W, Ritthidej GC, Pichayakorn W. Characterization, *in vitro* release and permeation studies of nicotine transdermal patches prepared from deproteinized natural rubber latex blends. Chem Eng Res Des 2012; 90(7): 906-914.
16. Barakat NS. Evaluation of glycofurol-based gel as a new vehicle for topical application of naproxen. AAPS PharmSciTech 2010; 11(3): 1138-1146.
17. Nagariya K, Jadon PS, Naruka PS, Chauhan CS. Formulation development and characterization of aceclofenac gel using poloxamer 407. J Chem Pharm Res 2010; 2(4): 357-363.
18. P. Shah P, R. Desai P, Channer D, Singh M. Enhanced skin permeation using polyarginine modified nanostructured lipid carriers. J Controlled Release 2012; 161(3): 735-745.
19. Songkro S, Purwo Y, Becket G, Rades T. Investigation of newborn pig skin as an *in vitro* animal model for transdermal drug delivery. STP Pharma Sci 2003; 13(2): 133-139.
20. Chen Y, Peng Z, Kong LX, Huang MF, Li PW. Natural rubber nanocomposite reinforced with nano silica. Polym Eng Sci 2008; 48(9): 1674-1677.

21. Rippel MM, Lee L-T, Leite CAP, Galembeck F. Skim and cream natural rubber particles: Colloidal properties, coalescence and film formation. J Colloid Interface Sci 2003; 268(2): 330-340.
22. Turjanmaa K, Alenius H, Mäkinen-Kiljunen S, Reunala T, Palosuo T. Natural rubber latex allergy. Allergy 1996; 51(9): 593-602.
23. Yeang HY, Arif SAM, Yusof F, Sunderasan E. Allergenic proteins of natural rubber latex. Methods 2002; 27(1): 32-45.
24. Büyükyaylaci S, Joshi Y, Peck G, Banker G, Polymeric pseudolatex dispersions as a new topical drug delivery system, in: Anderson J, Kim S (Eds.), Recent Advances in Drug Delivery Systems. Springer US, 1984, pp. 291-307.
25. Draize JH, Woodward G, Calvery OH. Method for the study of Irritation and toxicity of substance applied topically to the skin and mucous membrane. J Pharmacol Exp Ther 1944; 82377-390.
26. Gauri N, Aditi L, Shikhaa A, P. K. D. Solubility enhancement of a poorly aqueous soluble drug ketoprofen using solid dispersion technique. Der Pharmacia Sinica 2011; 2(4): 67-73.
27. Watcharakul N, Poompradub S, Prasassarakich P. *In situ* silica reinforcement of methyl methacrylate grafted natural rubber by sol–gel process. J Sol-Gel Sci Technol 2011; 58(2): 407-418.
28. Morimoto Y, Wada Y, Seki T, Sugibayashi K. *In vitro* skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. Biol Pharm Bull 2002; 25(1): 134-136.
29. Rhee Y-S, Huh J-Y, Park C-W, Nam T-Y, Yoon K-R, Chi S-C, et al. Effects of vehicles and enhancers on transdermal delivery of clebopride. Arch Pharm Res 2007; 30(9): 1155-1161.
30. Srisuk P, Thongnopnua P, Raktanonchai U, Kanokpanont S. Physico-chemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for *in vitro* transepidermal delivery targeting psoriasis treatment. Int J Pharm 2012; 427(2): 426-434.
31. Roberts MS, Walters KA, Human skin morphology and dermal absorption, in: Roberts MS, Walters KA (Eds.), Dermal Absorption and Toxicity Assessment, 2nd ed. Informa Healthcare, New York, 2007, pp. 1-87.
32. Simon GA, Maibach HI. The pig as an experimental animal model of percutaneous permeation inman: Qualitative and quantitative observations - An overview. Skin Pharmacol Appl Skin Physiol 2000; 13(5): 229-234.
33. Dhamecha DL, Rathi AA, Saifee M, Lahti SR, Dehghan MHG. Drug vehicle based approaches of penetration enhancement. Int J Pharm Pharm Sci 2009; 124-46.