International Journal of Pharmaceutics 385 (2010) 12–19

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A novel transdermal patch incorporating meloxicam: *In vitro* and *in vivo* characterization

Young-Chang Ah [1], Jin-Kyu Choi [1], Yang-Kyu Choi, Han-Moi Ki, Joon-Ho Bae *

Amorepacific Co., R&D Center, 314-1, Bora-dong, Giheung-gu, Yongin-si, Gyeonggi-do 449-729, Republic of Korea

ARTICLE INFO

Article history:
Received 22 June 2009
Received in revised form
11 September 2009
Accepted 5 October 2009
Available online 13 October 2009

Keywords:
Meloxicam
Sodium methoxide
Transdermal delivery
Solubility
Patch

ABSTRACT

A monolithic drug-in-adhesive (MDIA) type patch containing meloxicam (MX) was designed with an acrylic adhesive, a solubility modulator increasing MX solubility, and enhancers. MDIA patches having one adhesive layer between the backing and the release liner give high productivity and improve patient compliance. The biggest problem to prepare MDIA patch including MX was poor solubility of MX. In this research, solubility modulators to increase solubility of MX and acrylic adhesives and skin permeation enhancers were investigated through solubility tests, *in vitro* skin permeation tests, and stability tests. Consequently, the composition of sodium methoxide (SM), an acrylic adhesive containing poly(vinyl pyrrolidone) blocks (MAS683), polyoxyethylene cetylether (BC-2), and diisopropanolamine (DIPA) made it possible for MX to be contained in an adhesive layer at a concentration of as much as 15 wt% without MX crystal and with high skin permeation over 400 μG/cm^2. Finally, the patch formulation containing MX (MX-patch) selected through our *in vitro* study was characterized by *in vivo* using an animal study to acquire pharmacokinetic (PK) parameters and to confirm the anti-inflammatory efficacy of MX-patch. In the animal study, MX-patch was compared with a commercially available piroxicam patch (PX-patch). The amount of MX delivered from MX-patch to the skin surface was believed to be higher than the amount of MX diffused from the skin tissue to circulatory system because the plasma concentration of MX continuously increased up to 32 h, the end time of PK study, although the patch samples were detached at 24 h. PX-patch produced a C_{max} at 8 h. MX-patch showed better significant efficacy than PX-patch in adjuvant arthritis model.

1. Introduction

Meloxicam {4-hydroxy-2-methyl-N-(5-methyl-2-thiazoyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, MX}, is a non-steroidal anti-inflammatory drug (NSAID) of the enolic acid class, and has two pKa values (pKa_1 = 1.09, pKa_2 = 4.18) (O'Neil et al., 2006). MX is an efficient drug for the treatment of joint diseases such as rheumatoid arthritis and osteoarthritis. MX inhibits the cyclo-oxygenase-2 (COX-2) isozyme more potently than the COX-1 isozyme (Pairet et al., 1998). It has been reported that MX shows similar efficacy for reducing pain and inflammatory symptoms but lower toxicity than the other NSAIDs (Engelhardt et al., 1995; Lipscomb et al., 1998; Degner et al., 2000). Additionally, MX is a drug having a low probability of inducing allergic reactions relating to NSAID intolerance (Bavbek et al., 2003; Senna et al., 2003; Prieto et al., 2007). Thus, MX is a good alternative drug for patients who are intolerant to other NSAID drugs. Although MX is relatively safer than other NSAIDs, adverse effects relating to the gastro-intestinal (GI) tract are still a weak point of MX (Gambero et al., 2005). To suppress these adverse effects in the GI tract while sustaining the therapeutic efficacy of MX, an alternative drug delivery method might be useful.

We selected transdermal drug delivery (TDD) as a method for reducing the adverse effects of MX that derive from oral administration. Delivering NSAIDs through skin is an effective strategy for evading NSAIDs' adverse effects in the GI tract and for increasing patient compliance (Grahame, 1995; Martens, 1997; Galer et al., 2000; Heyneman et al., 2000). Of course, TDD is not always a good choice. TDD can cause skin irritation due to direct contact between a drug and the skin. MX was reported as a drug that can be applied to the skin and mucosa because MX has lower tissue toxicity than piroxicam, ketoprofen, indomethacin, dichlofenac, and ibuprofen (Seti et al., 1996).

However, MX has big weak point as a drug candidate for TDD. MX is a zwitterionic drug showing a high melting temperature, a low solubility regardless of solvent polarity, and lipophilicity because of a large intramolecular multipole moment. Such characteristics of a zwitterionic drug make it unsuitable for transdermal delivery (Mazzenga and Berner, 1991). Many investigators

* Corresponding author. Tel.: +82 31 280 5940; fax: +82 31 284 3995.
E-mail address: rachisis@amorepacific.com (J.-H. Bae).
[1] These authors equally contributed to this work.

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.10.013

Fig. 1. Schematic diagram of monolithic drug-in-adhesive type patch.

have tried to overcome the weak points of MX as a zwitterionic drug for TDD. Introducing promoters (enhancers) in formulations (Jantharaprapap and Stagni, 2007; Yuan et al., 2006), complexation with cyclodextrine (Naidu et al., 2004), and forming MX salts with alkaline materials (Chang et al., 2007; Ki and Choi, 2007; Wang et al., 2008) have been used to increase the solubility or skin permeation of MX. In case of the salt formation, alkylammoniums, alkylamines, and, alkanolamines are well known counter parts to make an ion pair of zwitterionic materials (Hatanaka et al., 2000; Megwa et al., 2000; Cheong and Choi, 2002; Kamal et al., 2007).

MX has been studied as a drug candidate in TDD formulations such as gels (Gupta et al., 2002; Jantharaprapap and Stagni, 2007; Yuan et al., 2007), and microemulsions (Yuan et al., 2006) for TDD. The formulations in previous studies have weaknesses in controlling the delivery amount and delivery time regulation. Additionally, the formulations in previous studies can create unexpectedly a problem revealed from ultra violet (UV) exposure of the applied site. In the case of NSAIDs, photosensitization can be caused by UV exposure of skin TDD formulation applied on (Bastien et al., 1997). In contrast, patch system can give patients a well controlled dosage and better compliance than formulations in previous studies. UV exposure can be avoided by the backing in a patch.

In the present study, we tried to make a patch including MX that showed efficacy in reducing pain and inflammation caused by osteoarthritis (OA) or rheumatoid arthritis (AA), and to design a patch having simple structure that can be easily applied in an actual manufacturing process. Thus, a monolithic drug-in-adhesive matrix (MDIA) type patch (Fig. 1) was selected as the target since the structure of an MDIA patch is very simple and easy to be applied in manufacturing. Solvent casting was selected as the processing method to prepare an MDIA patch in this study. The main steps of preparing an MDIA patch using solvent casting are solvent mixing, coating, drying and backing lamination. The solubility of MX was a big obstacle to the preparation of an adhesive layer containing a high amount of MX without crystallization. Thus, increasing the solubility of MX in the solvent mixing step and in the adhesive layer after the drying step was the most important topic in this research.

2. Materials and methods

2.1. Chemicals

MX was purchased from Alembic Ltd. (India). A cross-linking type acrylic adhesive having functional groups such as carboxylic acid and hydroxy moieties (trade name: 87-2074) and a non-functional acrylic adhesive (trade name: 87-900A) were obtained from National Starch Co. (USA). Poly(2-ethylhexylacrylate-co-vinyl pyrrolidone) (MAS683) was purchased from Cosmed Co. (Japan). Three different kinds of polyoxyethylene cetyl ether (trade name: BC-2, BC-7, BC-40) were acquired from Nikkol Co. (Japan). Piroxicam (PX), sodium methoxide (SM), diethanolamine (DEA), diisopropanolamine (DIPA), 2-hydroxyprophyl-β-cyclodextrine (β-CD), carrageenan, monosodium-iodoacetate (MIA), and incomplete Freund's adjuvant (ICFA) were obtained from Sigma–Aldrich Co. (USA). *Mycobacterium butyricum* was bought from Difco Laboratories (USA). Propylene glycol monolaurate (PGML), isopropyl myristate (IMP), diethylene glycol monoethyl ether (Transcutol P) was purchased from Gattefosse Co. (France). Glycerol monolaurate (GML) was bought from Kanto Chemical Co. (Japan). A patch containing piroxicam (PX-patch, 48 mg/20 cm^2 as Trast®) was manufactured by SK Chemical Co. (South Korea). Polyethylene film (Cotran™ 9720) was supplied by 3 M Co (USA). HPLC grade acetonitrile (ACN), methanol (MeOH), ethylacetate (EA), tetrahydrofuran (THF) and n-hexane were bought from J.T. Baker (USA). N-methyl pyrrolidone (NMP) was purchased from Junsei Co. (Japan). All reagents were used without additional purification.

2.2. Animals

Hairless mice (age: 6–7 weeks) were used for the *in vitro* skin permeation test and Lewis rats (age: 5–6 weeks) were used for the adjuvant arthritis test induced by CFA. Sprague–Dawley (SD) rats (rat, age: 6–7 weeks) were used for the pharmacokinetic test, the carrageenan induced edema test, and the MIA induced osteoarthritis test. New Zealand white rabbits (male, weight: 2.0–2.5 kg) were used for primary skin irritation test. All animals were housed in a temperature- (23 ± 2 °C) and relative humidity-controlled ($50 \pm 10\%$) room. Lighting was adjusted automatically to give a cycle of 12 h light and 12 h dark. Throughout the study, the animals had free-access to the laboratory diet (Purina Co., Korea) and tap water. A 1-week acclimatization period was allowed before the test commenced. Both routine animal maintenance procedures and the protocols used in this study were in accordance with Institutional Animal Care and Use Committee (IACUC) of our center.

2.3. Solubility test

MX and an equimolar solubility modulator to MX were introduced into MeOH since the solubility modulators such as β-CD, DEA or SM selected in this study were not compatible with EA and n-hexane. The MeOH solution was stirred by magnetic stirrer for 2 days. When sedimentation was observed in MeOH solution, additional MeOH was added to dissolve the sediment completely. The MeOH solution was dried using a rotary evaporator (Eyela, N-1000, Tokyo Rikakikai Co., Japan) and additionally dried under vacuum to get dried mixture of MX and a solubility modulator (MX 2). In the case of water and MeOH, excess MX and an equimolar solubility modulator to MX were directly dissolved, but the other solvents were mixed with MX 2. The solvent mixtures having MX were stored in vials sealed with a screw cap on a water bath at 25 °C for 2 days. After 2 days, the solvent mixtures in the vials were filtered through polyvinyldifluoride (PVDF) membranes (pore size: 0.45 μm), and analyzed with high performance liquid chromatography (HPLC).

2.4. Patch formulation

MX or MX and solubility modulators was completely dissolved in methanol (Mix 1). Adhesive and enhancers were added to Mix 1 and stirred until the mixture was converted to a clear solution (Mix 2). When Mix 1 or Mix 2 did not make a clear solution, additional MeOH or THF were mixed to Mix1 or Mix 2. Air bubbles in Mix 2 were removed at room temperature under atmospheric pressure. After bubbles in Mix 2 were completely removed, Mix 2 was coated on release liner, poly(ethylene terephthalate) (PET) film using a knife coater (LC-100, Cheminstrument, USA). The coating thickness was determined on the basis of dried thickness. The thickness of dried Mix 2 was from 10 to 60 μm. The Mix 2 coated on the release liner was dried in a convection oven at 80 °C for 10 min, the thickness of the dried adhesive film was measured using a thickness gauge (ID-C112, Mitutoyo Co., Japan). The backing film, Cotran™ 9720 was laminated on the dried Mix 2 coated on the release liner film.

2.5. Evaluation of drug content and crystallization

Each patch was stored in a chamber (J-100S, Jisico Co., Korea) whose temperature was maintained at 60 °C for 1 month to observe crystallization of MX. MX crystallization in the adhesive layer was visually observed weekly during the month. MX in the patches was extracted to MeOH to evaluate the amount of MX in the patch. Patch samples were immersed in 100 ml of MeOH and stirred for 2 h. MeOH solution was stored in a refrigerator until analysis. The size of a patch sample was 3 cm × 3 cm (9 cm^2). The release liner was separated from the adhesive layer before extraction.

2.6. In vitro skin permeation test

In vitro skin permeation tests of samples were performed using a vertical Franz diffusion cell whose diffusion area was 0.785 cm^2, and hairless mouse skin. The skin was excised and the subcutaneous fat and other extraneous tissues were trimmed. The skin was mounted on the Franz diffusion cells with the stratum corneum (SC) facing the donor compartment. The receptor compartment was 5 ml in volume, and filled with pH 7.4 phosphate buffer solution (PBS) whose temperature was maintained as 32 °C. The receptor solution of a Franz diffusion cell was fully replaced with fresh PBS at 8 and 24 h, while stirring at 600 rpm. The collected PBS was subjected to high performance liquid chromatography (HPLC) to determine the content of the MX in PBS. The size of each patch sample for skin permeation was 1.5 cm × 1.5 cm.

2.7. Analytical methods

2.7.1. HPLC condition to analyze drug amount

Analysis for drug amount was carried out using an HPLC system. The HPLC system was Agilent 1100 (Hewlett-Packard, USA) consisted with a pump (Isopump, G1310A, Hewlett-Packard), autosampler (ALS, G1313A, Hewlett-Packard), and UV–visible detector (VWD, G1314A, Hewlett-Packard). The HPLC system was controlled by a computer program, Chemstation (Hewlett-Packard). The column for this analysis was Luna C18(2) (5 μm, 150 mm × 4.60 mm, Phenomenex). The eluant was analyzed at 355 nm. The mobile phase was a 1:1 mixture of 50 mmol sodium acetate solution and acetonitrile. The pH of the sodium acetate solution was adjusted to 3.3 with glacial acetic acid.

2.7.2. Sample preparation of HPLC analysis

The solution collected from the receptor of a Franz diffusion cell and MeOH solution obtained from the drug evaluation test were filtered through a PVDF membrane (pore size: 0.45 μm) and injected as much as 20 μl. The plasma samples obtained from the pharmacokinetic study were treated and analyzed according to the published method to measure MX content in plasma (Dasandi et al., 2002). 200 μl of plasma samples were mixed with 50 μl of internal standard solution, and vortexted for 10 s. Additionally, 50 μl of protein precipitating solution, a mixture of acetonitrile and perchloric acid (70%) in a 1:1 (v/v) ratio, was added to the plasma sample mixed with internal standard solution. After addition of protein precipitation solution, the mixtures were vortexed for 1 min, and centrifuged at 5000 rpm for 10 min. After centrifugation, 100 μl of supernatant was injected to HPLC system. MX and PX were used as internal standards in the pharmacokinetic study. PX solution (2 μg/ml) was introduced into a plasma sample gathered from an animal medicated with MX, and MX solution (2 μ/ml) was introduced into a plasma sample gathered from an animal medicated with PX.

2.7.3. Validation of HPLC analysis

Analyzing MX and PX in plasma by HPLC resulted in good separation with no interfering peaks. The linear range was 0.05–10 μm/ml (R^2 = 0.9999 for MX and R^2 = 0.9996 for PX), with the limit of quantitation (LOQ) of HPLC analysis in this study was 0.05 μm/ml. The precision and accuracy of MX and PX were determined at plasma concentrations of 0.05, 0.5, 1, 5, and 10 μm/ml. Intraday precision and accuracy were determined by analyzing three standard sample sets per an each drug on the same day. Interday validation was not done for 3 days. The results of precision and accuracy of HPLC are presented in Table 1.

2.7.4. Measuring adhesion properties

To determine the adhesion properties of MX-patch prepared in this research, peel adhesion force, tackiness, and shear strength were measured. The adhesion properties of MX-patch were compared with PX-patch. Texture analyzer (TAXT2i, Stable Micro System, UK) was used for the determination of peel adhesion force and tackiness. In the case of peel adhesion force, 180° peel adhesion test method was applied to the measurement. Patches were cut into strips 2.5 cm wide, and applied to an adherent plate made of stainless steel, and smoothened with 2 kg roller 3 times, and pulled from the substrate at 180° angle at a rate of 5 mm/s. Probe tack test method was used to measure the tackiness of patches. Patches were attached to a stainless steel plate with the backing facing the plate, and the release liner on adhesive layer was delaminated just before probe tack test. The stainless steel plate was firmly fixed with vice. The probe was ball shape, and its diameter was 1 in. The contact force was 4.5 N, and the contact time was 0.1 s. After contact the probe and the adhesive layer, probe move back at a rate of 0.1 mm/s, and the maximum strength was recorded. 8 bank oven shear tester (HT-8, ChemInstruments, USA) was used to measure shear strength of patches. Patches were cut into 1.25 cm × 5 cm, and the one side of a patch sample 1.25 cm × 1.25 cm was attached on the plate, and the other side was hung on a weight of 500 g. All test to measure the adhesion properties of patches were carried out at 24 ± 2 °C, and all samples and plates were stored at 24 ± 2 °C for 24 h before measurement of adhesion properties.

2.8. Animal tests

2.8.1. Pharmacokinetic test

SD rats were divided into three groups. One group was prepared for oral administration of MX (MX oral group), and the other two groups were for transdermal administration of MX and PX (MX-patch group and PX-patch group). MX was dissolved in pH 7.4 PBS for oral administration. For transdermal medication, the hairs on the back of SD rat were carefully shaven using a hair clipper and an electric razor. The dose of MX for oral medication was 1 mg/kg and the amounts of MX and PX in patch samples were 2.4 and 9.6 mg. The area each patch sample was 2 cm × 2 cm. Patch samples were applied on the shaved site of the back. Patch attachment was sustained for 24 h and patch samples were detached after 24 h. Blood samples were withdrawn at 0, 0.5, 1, 2, 4, 8, 24, 28, and 32 h post-dose. Blood samples were centrifuged to yield plasma and plasma samples were stored at −70 °C until analysis. The patch samples detached after 24 h were immersed into 100 °C of methanol and stirred for 2 h to extract the residual MX and PX in the samples.

2.8.2. Carrageenan induced edema model

The effect of MX-patch on acute edema was determined according to the methods of Swingle et al. (1969) with slight modifications. One day before the experiment, the left hind thigh of each animal was shaved without damaging the skin. The patch samples were

Table 1
Intraday and interday validation of HPLC analysis of MX and PX in rat plasma.

Concentration (ng/ml)	Intraday				Interday			
	MX ($n=3$)		PX ($n=3$)		MX ($n=3$)		PX ($n=3$)	
	Precision (%CV)	Accuracy (%)	Precision (%CV)	Accuracy (%)	Precision (%CV)	Accuracy (%)	Precision (%CV)	Accuracy (%)
50 (LOQ)	9.09	98.63	9.99	100.67	9.76	102.39	2.92	92.34
500	2.93	100.19	5.71	97.77	5.70	104.88	3.86	95.50
1,000	2.22	102.27	5.13	96.58	1.82	99.91	5.12	95.82
5,000	2.87	100.02	2.83	97.59	3.82	96.26	4.47	99.10
10,000	1.84	101.23	1.64	99.73	2.53	100.10	2.74	100.48

applied to the shaved area in the left hind thigh, and were covered with polyurethane tape. For the control group, only urethane tape without the patch was applied. 4 h after patch application, 0.1 ml of 1% carrageenan was injected into the left hind paw. The volume of the left hind paw was measured using a displacement plethysmometer (Ugo Basile 7240, Comerio, Italy). The size of each patch sample was 1 cm × 2 cm.

2.8.3. Adjuvant-induced arthritis model

This test was conducted according to the modified method of Matsuura et al. (2000). Arthritis was induced by injection of 0.1 ml of a 1% suspension of heat-killed *M. butyricum* mixed in the incomplete Freund's adjuvant into the base of tail. The patches were applied to the site for 6 h a day during 6 days. Patch application was started from 14 days after the injection of the adjuvant. Paw volumes were measured using a displacement plethysmometer. The size of each patch sample for this test was 1 cm × 2 cm.

2.8.4. Osteoarthritis pain model

This test was conducted according to the methods of Bove et al. (2003). For induction of MIA-induced arthritis, rats were given a single intra-articular injection of 1 mg MIA through the intrapatellar ligament of the right knee. MIA was dissolved in physiologic saline and administered in a volume of 50 μl. 14 days post-MIA injection, the hind limb withdrawal threshold to noxious mechanical stimulation was determined using a modified Randall Selitto test. Using an Ugo Basile analgesymeter (Type 7200, Varese, Italy), a mechanical force was applied to the left hind paw. During each measurement the mechanical stimulus force increased at a constant rate and the force in grams producing a withdrawal response was determined. The cut-off was 250 g. The measures were done at 0, 4 and 6 h after patch application to the hind paw. The size of each patch sample was 1 cm × 2 cm.

2.8.5. Primary skin irritation test

The primary irritation test was conducted according to the methods of Draize et al. (1944) with slight modifications. Six male New Zealand white rabbits (weighing 2.0–2.5 kg) were clipped free from hair with electric clippers and shaved. Prior to the application of the patches, the left side of the rabbit was stripped using adhesive tape (3M transpore®) to enhance the absorption of test material. The patches were applied in the area of 2 cm × 2 cm. The application site was covered and wrapped with elastic adhesive bandage (3M transpore®). Approximately 24, 48 and 72 h after application, animals were examined for signs of irritation. The skin reactions were evaluated in accordance with the following Draize method (1944): (1) erythema and eschar formation: Score 0, no erythema; Score 1, very slight erythema; Score 2, well-defined erythema; Score 3, moderate to severe erythema; Score 4, severe erythema and slight eschar formation and (2) edema formation: Score 0, no edema; Score 1, very slight edema; Score 2, slight edema; Score 3, moderate edema; Score 4, severe edema.

3. Results

It was difficult to prepare a homogenous adhesive solution having high MX content because MX has low solubility in solvents regardless of their polarity. Especially, MX was not completely soluble in n-hexane which is a remarkable solvent of rubbers, and the saturation concentration of MX in EA which is a main solvent for acrylic adhesives was just 1.28 mg/ml. 3 kinds of materials, DEA, β-CD, and SM were evaluated as solubility modulators increasing MX solubility in solvents dissolving adhesive polymers. The effect of a solubility modulator in EA and n-hexane was measured with MX 2 because these solubility modulators were not dissolved in EA and n-hexane. Among these three materials, SM was most effective at increasing the solubility of MX in MeOH and organic solvents. Rubber was not used for MDIA patch formulation due to poor solubility of MX and no effect of solubility modulators in n-hexane (Table 2). Even if MX was converted to MX 2s, the MX solubility was not enlarged in n-hexane.

MX or the mixture of MX and a solubility modulator in MeOH was introduced into an acrylic adhesive solution to evaluate the effect of an adhesive and a solubility modulator on skin permeation and stability of an adhesive layer. The concentrations of MX used were 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 wt% on the basis of the dried mass of MX and adhesive. Patch samples made with MX, MAS683, and SM had superior stability to the other patch samples including NSC 87-2074 or NSC 87-900A. By using MAS683 and SM, MX could be incorporated up to 15 wt% in an adhesive layer without crystallization. Patch samples were tested for skin permeation of MX. All these patch samples for skin permeation had adhesive layers containing 15 wt% MX and 60 μm thick. As the MX content, 15 wt% was selected to give same driving force depending on the concentration gap to saturation concentration. MAS683 showed the best results in the skin permeation test and had the greatest compatibility with MX (Table 3).

The effect of skin permeation enhancers (Table 4) was evaluated as patch formulation including MAS683, enhancers, and SM. A skin permeation enhancer was mixed into a patch formulation up to 10 wt% on the basis of the total dried mass of MAS683, MX, and an enhancer. In the case of the patch sample containing BC-2 and DIPA, the amounts of BC-2 and DIPA were maintained at 5 wt%

Table 2
The solubility of MX obtained from the test to evaluate the effect of solubility modulators. The amount of a solubility modulator was fixed as 1:1 molar ratio to MX. "IS" means "insoluble" and "X" means that test was not done.

Solvents	Solubility of meloxicam (mg/ml)			
	MX (a)	MX + DEA	MX + β-CD	MX + SM
DIW	0.06	3.2	0.29	4.2
EA	1.28	2.9	1.8	4.95
n-Hexane	IS	IS	IS	IS
MeOH	0.43	38	14.8	306
NMP	92.7	X	X	490.3
THF	21.91	X	X	67.9

Table 3
The concentration limit of meloxicam in an adhesive layer and the cumulative amount of MX permeated for 24 h under *in vitro* skin permeation test ($n = 5$).

	Adhesives								
	87-2074			900A			MAS683		
	MX	MX/DEA	MX/SM	MX	MX/DEA	MX/SM	MX	MX/DEA	MX/SM
Concentration limit (wt%)	< 1	<3	<5	< 1	<3	<3	< 3	<9	<17
Cumulative amount ($\mu g/cm^2$)	5.5[a] (2.0)[b]	9.2 (1.3)	18.5 (4.8)	4.3 (1.3)	11.5 (2.8)	16.9 (3.8)	22.2 (2.9)	74.4 (18.9)	55.9 (5.6)

[a] Mean.
[b] Standard deviation.

Table 4
Information of enhancers; these information was obtained from enhancer suppliers.

Enhancers	Symbols	HLB values	EO chain lengths (numbers of EO unit)	Hydro carbon numbers
Transcutol P	T	4.2	2	2
PGML	P	4.5	0	11
GML	G	5.2	0	11
BC-2	B2	8	2	15
IPM	I	11.5	0	13
BC-7	B7	11.5	7	15
BC-40	B40	20	40	15
DIPA	D	-	0	1

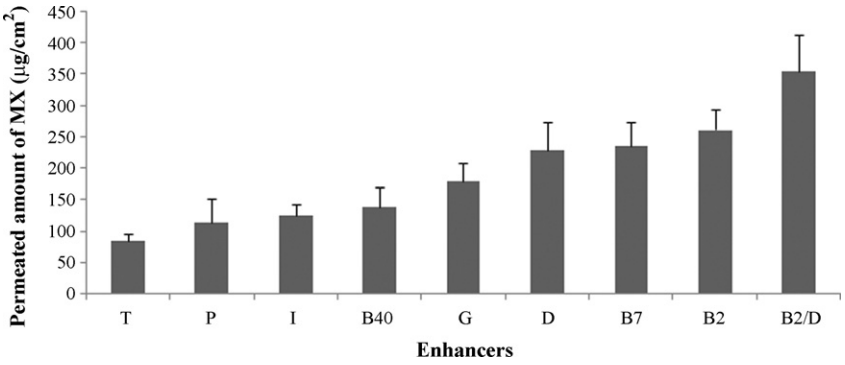

Fig. 2. The cumulative amount of MX permeated through skin for 24 h ($n = 5$). The thicknesses of the adhesive layers in all samples were 60 μm. Data are presented as means ± standard deviation.

each. The amounts of MAS683 and MX were fixed at 75 and 15 wt%, respectively, in all samples. SM was introduced into a sample concentration as high as a 1:1 mole ratio to MX. BC-2 and BC-7 were equally better than other enhancers, and when DIPA was mixed with BC-2, skin permeation of MX was the highest (Fig. 2).

The thickness of the adhesive layer is one of the important variables in the preparation an MDIA patch. When the concentration of drug and the other additives is same, a thicker adhesive matrix, which serves as a drug reservoir, is able to deliver higher amount of drug to skin over relatively longer application time because the amount of drug in an MDIA patch is controlled by the thickness of the adhesive layer. By increasing the thickness up to 30 μm, the cumulative amount of MX that permeated through skin for 24 h was increased. However, adhesive layers thicker than 30 μm did not cause additional increase in the cumulative amount of MX that permeated (Fig. 3A). The adhesive layer composition of patches in the thickness effect test was the same as in the sample denoted as B2/D in Fig. 2. Additionally, the amount of SM having the biggest effect on the solubility of MX was determined in order to maximize skin permeation. When SM was introduced as much as 0.5:1 molar ratio to MX, the skin permeation of MX was the highest (Fig. 3B). MX-patch for animal tests was prepared on the basis of these *in vitro* test results. The patch formulation containing MX for animal test (MX-patch) consisted of an adhesive layer (dried thickness: 40 μm) including MX at 15 wt%, DIPA at 5 wt%, BC-2 at 5 wt%, MAS683 at 75 wt%, and SM which was maintained at 0.5:1 molar ratio to MX. The amount of MX in the patch was 0.6 mg/cm^2.

The adhesion properties, peel adhesion, tack and shear strength are the important properties of patches for the application like wearing and removing. These three kinds of adhesion properties were measured to confirm the adhesion performance of MX-patch and compared with PX-patch. MX-patch for animal test showed similar peel strength and tackiness to PX-patch, but shear holding time for the MX-patch was shorter than PX-patch (Table 5).

Pharmacokinetic (PK) parameters were measured using SD rats administrated orally and transdermally. Plasma concentrations of MX in the MX oral group showed C_{max} (4.2 μg/ml) at 8 h after administration, and MX concentration decreased to 2 μg/ml at 32 h. The plasma concentration of MX of MX-patch group continuously increased to the end of the experiment (C_{32}: 3 μg/ml). C_{max} (1.4 μg/ml) of PX-patch group appeared at 8 h, and plasma concentrations of PX gradually decreased since then. The AUC_{0-32} for MX oral group, MX-patch group and PX-patch group were 101.0, 85.1, and 36.1 μg/ml/h, respectively (Fig. 4). The patch samples detached

Table 5
Adhesion properties of MX-patch tested in animal study and PX-patch ($n = 5$).

Patches	Peel adhesion (g/2.5 cm)	Tack (g)	Shear strength (min)
MX-patch	710 ± 82[a]	260 ± 51[a]	10.5 ± 3.4[a]
PX-patch	840 ± 65	240 ± 84	18.9 ± 6.1

[a] Mean ± standard deviation.

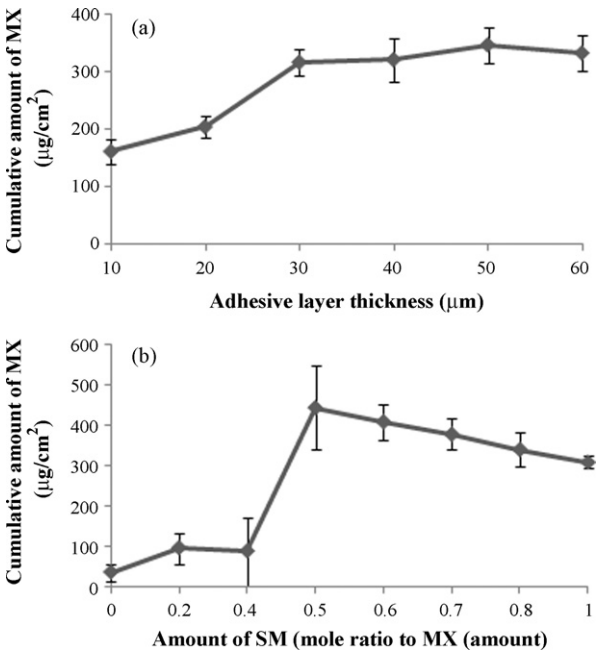

Fig. 3. The results of *in vitro* skin permeation test. (a) Permeated amount of MX according to the adhesive layer thickness ($n = 5$). The content of SM in these samples was fixed at 1:1 molar ratio to MX amount in adhesive layer. (b) Permeated amount of MX depending on SM amount ($n = 5$). The thicknesses of samples were evenly set at 40 μm. Data are presented as means ± standard deviation.

after PK study were immersed into methanol, and the residual drug amount of the patch samples were evaluated. The residual MX content was 2.13 ± 0.11 mg/4 cm^2, and residual PX in PX-patch was 9.45 ± 0.11 mg/4 cm^2.

In animal tests to confirm the efficacy of patches, all patch samples were attached to the shaved area of the hind thigh (Fig. 5). Foot edema induced by carrageenan was effectively suppressed by MX-patch (Fig. 6A). The MX-patch suppressed pain above 65% in the osteoarthritis pain model, but was not significantly different from PX-patch (Fig. 6B). In the adjuvant-induced arthritis (AA) model, only MX-patch significantly suppressed the increase in foot volume (Fig. 6C). Results of efficacy tests were statistically compared with *T*-test ($p = 0.05$).

Since patch is applied on skin, the local safety, skin irritation should be confirmed. The safety of MX-patch was evaluated with primary skin irritation study. The primary irritation index (PII) of MX-patch was 0.3 and PII of PX-patch was 0.25.

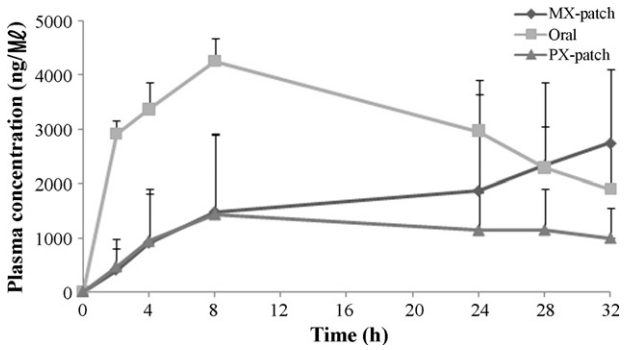

Fig. 4. Plasma concentration–time profile after oral administration of MX (1 mg/kg), and transdermal delivery of MX (MX-patch, 2.4 mg/4 cm^2) and PX (PX-patch, 9.6 mg/4 cm^2) ($n = 5$).

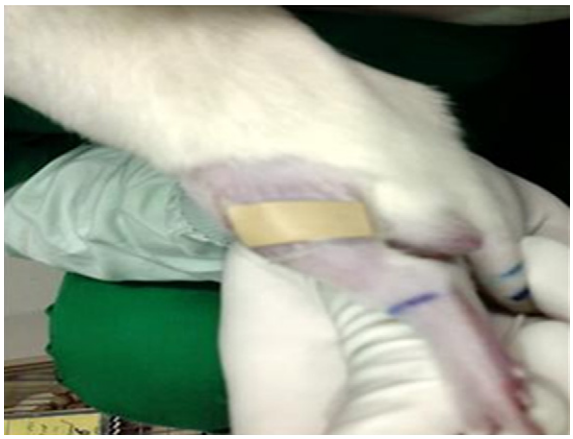

Fig. 5. The patch adhesion site in efficacy tests.

4. Discussion

MX solubility was increased by solubility modulators like β-CD, DEA, and SM, but β-CD was inferior to DEA and SM (Table 2). β-CD did not improve the MX solubility in EA. The effect of β-CD in EA can be explained with the MX solubility of MX/β-CD complex completely depended on the solubility of β-CD. β-CD was not miscible with EA. The similar effect of DEA and SM on MX solubility in water would be attributable to a conformational change of MX since DEA and SM can easily create alkaline conditions in water. MX is easily converted to the anionic form in alkaline conditions, and the solubility in water can be largely increased (Tsai et al., 1993; Luger et al., 1996). DEA was not effective as SM in MeOH and EA although DEA is an alkanolamine that is a well known counter-part easily forming salts with oxicams. The salts consisted of oxicams and alkanolamine showed progressive solubility in various non-aqueous materials (Cheong and Choi, 2002, 2003; Ki and Choi, 2007). It was not clearly examined why DEA had weaker effect to increase MX solubility in EA and MeOH than SM, but an interference can be deduced from the results in this study and an article reporting that ethanolamine was more effective than DEA and triethanolamine to MX solubility improvement (Ki and Choi, 2007). The reason of difference between DEA and SM in EA and MeOH might be alkaline strength of DEA and SM. Actually, the pH of water solution containing SM, ethanolamine, DEA, and triethanolamine was 13, 12.05, 11, and 10.5 at 0.1N in DIW, respectively (Merck Index, 2006). SM also increased MX solubility in polar aprotic solvents like THF and NMP, but SM made higher rate of MX solubility increase in polar protic solvents like DIW and MeOH. n-Hexane, a non-polar aprotic solvent could not solubilize MX even if SM was mixed with MX. Thus it is possible to make one conclusion that MX solubility in polar media can be increased with an alkaline material and the rate of solubility increase depends on the alkali strength.

SM made it possible to use high MX saturation concentrations over 15 wt% in an acrylic adhesive, and also improved MX permeation through skin (Table 3). Such solubility change can be explained by hydrophilicity of adhesives and conformational change of MX to the anionic form in non-aqueous phase under alkaline condition (Tsai et al., 1993). 87-900A was a non-functional acrylic adhesive, 87-2074 had carboxylic acid and hydroxy moiety, and MAS683 was a typical acrylic polymer containing a hydrophilic monomer, vinyl pyrrolidone in the main chain. Vinyl pyrrolidone is introduced to increase hydrophilicity of an acrylic polymer (Satas, 1989a,b). The order of hydrophilicity of these acrylic adhesives could be MAS683 > 87-2074 > 87-900A. Thus, greatest improvement in the solubility of MX, which was found for MAS683, is believed to be caused by a synergistic effect between the

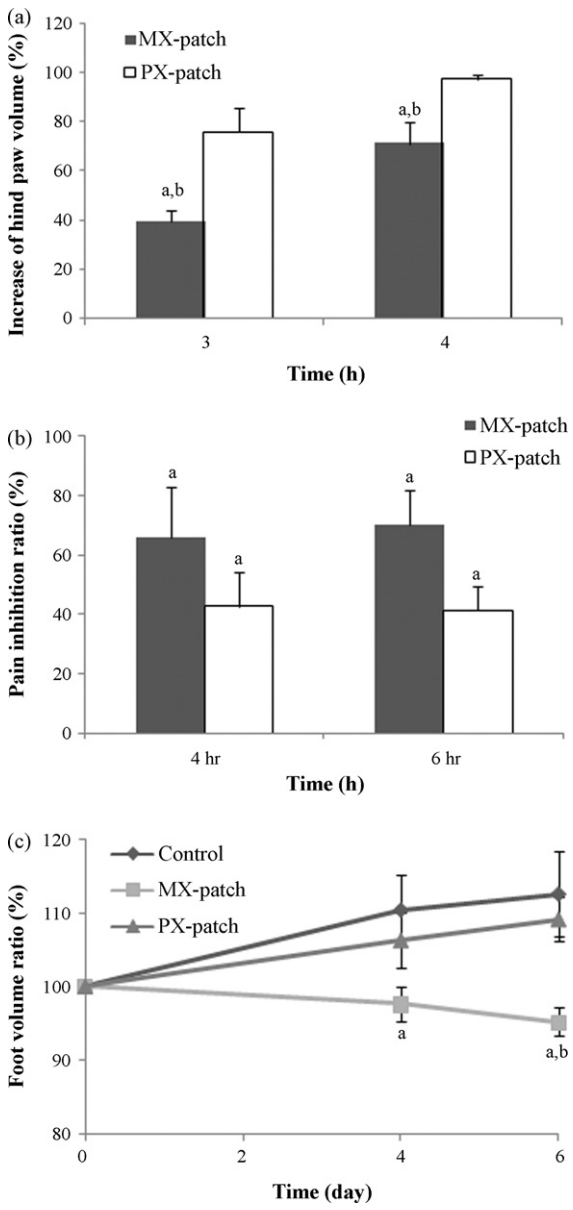

Fig. 6. Animal test results: (a) efficacy of patches suppressing hind paw edema induced in SD rat by carrageenan injection ($n = 5$); (b) efficacy of patches to reduce pain relating to osteoarthritis induced in SD rat by MIA injection ($n = 5$); (c) foot volumes of AA using Lewis rats ($n = 8$); the MX-patch (1.2 mg/2 cm^2) or the PX-patch (4.8 mg/2 cm^2) was applied on the shaved area of hind thigh. Data are presented as means ± standard error. The results of efficacy tests were examined statistically with *T*-test. a: $p < 0.05$ compared to control group; b: $p < 0.05$ compared to the PX-patch group.

hydrophilicity of MAS683 and the ability of SM leading anionic conformation of MX. The improved skin permeation results could be explained by the improvement in solubility of MX in skin. A study of salts of zwitterionic drugs showed that the salts were more permeable across epidermis because of the increased solubility in skin (Mazzenga et al., 1992).

In skin permeation test to select enhancers, BC-2, BC-7 and DIPA made very progressive results (Fig. 2). BC-2 and BC-7 have an ethylene oxide chain and are ranked intermediate hydrophilic lipophilic balance (HLB) values, which are 8 and 11.5. On the other hand, BC-40, Transcutol P, and IPM were inferior to BC-2 and BC-7. Such difference between good enhancers and poor enhancers could be explained by the existence of ethylene oxide chain in an enhancer and HLB. BC-40 has a long ethylene oxide chain, and Transcutol P has the same number of ethylene oxide chain as BC-2, and IPM is ranked in medium HLB (11.5), but the HLB values of BC-40 and Transcutol P were 20 and 4.2, and IPM does not have an ethylene oxide chain (Table 4). Thus, an enhancer having an ethylene oxide chain and ranked in medium HLB around 10 could be expected to enlarge the skin permeation of MX. This result is consisted with the study reporting enhancer effects to skin permeation of MX (Ki and Choi, 2007). In the case of DIPA, the enhancing mechanism might be increasing MX solubility in epidermis because DIPA can induce a high pH in water present in epidermis. The work relating to optimization of thickness of the adhesive layer and SM amount made the patch applicable over 24 h without drug depletion and maximized skin permeation of MX (Fig. 3A and B). The cause of the increase in skin permeation of MX derived by reduction in the amount of SM may be the concentration gap to saturation concentration of MX in the adhesive layer. MX concentrations in the test to screen enhancers and the test to evaluate the effect of adhesive layer thickness were fixed as much as 15 wt%, but the saturation concentration of MX in the adhesive layer having MAS683, MX, and SM was located between 15 and 17 wt% (Table 3). Introducing DIPA in the adhesive layer would contribute enlarging saturation concentration of MX since DIPA could form an ion pair with MX and increase MX solubility. In such state, reducing the SM amount should decrease MX solubility and increase chemical potential which is the driving force related to concentration gap to the saturation concentration of MX.

MX-patch showed similar peel strength and tackiness to PX-patch which is one commercial NSAID patch, but lower shear holding time than PX-patch. Such peel strength and tackiness mean that MX-patch has sufficient adhesion ability on skin. Shorter shear holding time of MX-patch indicated the possibility that MX-patch could be detached with residual adhesive on skin or applied shorter duration than PX-patch. This lower shear strength could be derived from the nature of MAS683 which is a non-crosslinked polymer. Non-crosslinked adhesive like MAS683 is easy to make low shear strength (Satas, 1989a,b).

Although patch samples were removed from SD rats at 24 h, MX concentrations in plasma continued to increase to the end of blood gathering (Fig. 4). Such result was also obtained in the *in vivo* skin permeation test of the MX sodium gel (Chang et al., 2007). The MX solubility in epidermis, especially SC might be increased by SM forming an MX sodium salt and DIPA increasing local pH in the epidermis. The prolonged elevation of plasma concentration of MX after patch removal at 24 h confirms that MX solubility in the epidermis was highly increased with SM and DEA. Thus, it is possible to explain the skin permeation behavior of MX-patch as increasing accumulation of a drug in epidermis. Accumulation of a drug in epidermis is controlled by partition into epidermis and diffusion to dermis and blood vessel. In the case of MX-patch tested in this PK study, MX was well partitioned into the epidermis of SD rat because of the increased MX solubility in water and lipid, and the diffusion speed to dermis should be slower than partition speed. The AUC of MX-patch group was 2.35 times higher than PX-patch group, although the drug amount in MX-patch (2.4 mg/4 cm^2) was less than PX-patch (9.6 mg/4 cm^2). Thus, it is clear that the bioavailability (BA) of MX-patch was better than PX-patch. Actually, the BA of MX-patch was 11.25% and PX-patch was just 1.56%. It was reported that the half life of MX in male rats orally medicated was 49.9 h (Busch et al., 1998). For PX in male rats orally medicated, the half life was 10.7 h (Kimura et al., 1997). Regarding the half life of drugs in the previous articles, and PK profiles in this research, the amount of PX delivered from PX-patch obviously decreased after 8 h, but MX-patch could continuously deliver MX up to 24 h. The efficacy of MX-patch in reducing inflammation was superior to PX-patch even if the plasma concentrations of MX and PX from the two patches were the same until for 8 h and patches were applied for 6 h

in efficacy tests. MX-patch had superior efficacy in the carrageenan induced edema model (Fig. 5A), and this result is consistent with the previous report comparing MX and PX in rat (Engelhardt et al., 1995; Gupta et al., 2002). In osteoarthritis model using MIA, the MX-patch showed higher efficacy than PX-patch, but MX-patch did not make significant difference from PX-patch (Fig. 5B). In the case of AA model to confirm anti-rheumatoid arthritis efficacy, the paw edema in MX-patch group was well suppressed and the result showed statistically significant difference to the control group and PX-patch group (Fig. 5C). Similar result was presented in an article comparing anti-inflammatory efficacy of gels containing MX, PX, and diclofenac (Gupta et al., 2002). The enlarging efficacy of MX-patch according to the number of patch application times in AA model could be also explained by the half life of MX and PX in male rats. MX concentration in plasma should be increased according to repetitive application of MX-patch. Below PII 0.5 is non-irritating level, thus, MX-patch is expected to be applied on skin without skin irritation or with minimal irritation.

Acknowledgement

This research was done with the assistance of the Preclinical Research Center in the Amorepacific R&D Center.

References

Bastien, M., Milpied-Homsi, B., Baudot, S., Dutartre, H., Litoux, P., 1997. Ketopofen-induced contact photosensitivity disorders; 5 cases. Ann. Dermatol. Venereol. 124, 523–526.

Bavbek, S., Dursun, A.B., Dursun, E., Eryilmaz, A., Misirligil, Z., 2003. Safety of meloxicam in aspirin-hypersensitive patients with asthma and/or nasal polyps. A challenge-proven study. Allerg. Immunol. (Paris), 393–396.

Bove, S.E., Calcaterra, S.L., Brooker, R.M., Huber, C.H., Guzman, R.E., Juneau, P.L., Schrier, D.J., Kilgore, K.S., 2003. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage 11, 821–830.

Busch, U., Schmid, J., Heizel, G., Schmaus, H., Baierl, J., Huber, C., Roth, W., 1998. Pharmacokinetics of meloxicam in animals and the relevance to humans. Drug Metab. Dispos., 576–584.

Chang, J.S., Huang, Y.B., Hou, S.S., Wang, R.J., Wu, C.W., Tsai, Y.H., 2007. Formulation optimization of meloxicam sodium gel using response surface methodology. Int. J. Pharm. 338, 48–54.

Cheong, H.A., Choi, H.K., 2002. Enhanced percutaneous absorption of piroxicam via salt formation with ethanolamines. Pharm. Res. 19, 1375–1380.

Cheong, H.A., Choi, H.K., 2003. Effect of ethanolamine salts and enhancers on the percutaneous absorption of piroxicam from a pressure sensitive adhesive matrix. Eur. J. Pharm. Sci. 18, 149–153.

Dasandi, B., Shivaprakash, Saroj, H., Bhat, K.M., 2002. LC determination and pharmacokinetics of meloxicam. J. Pharm. Biomed. Anal. 28, 999–1004.

Degner, F., Sigmund, R., Zeidler, H., 2000. Efficacy and tolerability of meloxicam in an observational, controlled cohort study in patients with rheumatic disease. Clin. Ther. 22, 400–410.

Draize, J.H., Woodard, G., Calvey, H.O., 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82, 377–390.

Engelhardt, G., Homma, D., Schlegel, K., Utzmann, R., Schnitzer, C., 1995. Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. Inflamm. Res. 44, 423–433.

Galer, B.S., Rowbotham, M., Perander, J., Devers, A., Friedman, E., 2000. Topical dichlofenac patch relieves minor sports injury pain: results of multicenter controlled clinical trial. J. Pain. Symptom Manag. 19, 287–294.

Gambero, A., Becker, T.L., Zago, A.S., de Oliveira, A.F., Pedrazzoli Jr., J., 2005. Comparative study of anti-inflammatory and ulcerogenic activities of different cyclo-oxygenase inhibitors. Inflammopharmacology 13, 441–454.

Grahame, R., 1995. Transdermal non-steroidal anti-inflammatory agents. Br. J. Clin. Pract. 49, 33–35.

Gupta, S.K., Bansal, P., Bhardwaj, R.K., Jaiswai, J., Velpandian, T., 2002. Comparison of analgesic and anti-inflammatory activity of meloxicam gel with dichlofenac and piroxicam gel in animal models: pharmacokinetic parameters after topical application. Skin Pharmcol. Appl. Skin Physiol. 15, 105–111.

Hatanaka, T., Kamon, T., Morigaki, S., Katayama, K., Koizumi, T., 2000. Ion pair skin transport of a zwitterionic drug, cephalexin. J. Control. Release 66, 63–71.

Heyneman, C.A., Lawless-liday, C., Wall, G.C., 2000. Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs 60, 555–574.

Jantharaprapap, R., Stagni, G., 2007. Effects of penetration enhancers on *in-vitro* permeability of meloxicam gels. Int. J. Pharm. 343, 26–33.

Kamal, A.A.H.M., Iimura, N., Nabekura, T., Kitagawa, S., 2007. Enhanced skin permeation dichlofenac by ion-pair formation and further enhancement by microemulsion. Chem. Pharm. Bull. 55, 368–371.

Ki, H.M., Choi, H.K., 2007. The effect of meloxicam/ethanolamine salt formation on percutaneous absorption of meloxicam. Arch. Pharm. Res. 30, 215–221.

Kimura, E., Bersani-Amado, C.A., Sudo, L.S., Santos, R.J., Oga, S., 1997. Pharmacokinetic profile of piroxicam β-cyclodextrin in rat plasma and lymph. Gen. Pharmacol. 28, 695–698.

Lipscomb, G.R., Wallis, N., Armstrong, G., Rees, W.D.W., 1998. Gastrointestinal tolerability of meloxicam and piroxicam: a double-blind placebo-controlled study. Br. J. Clin. Pharmacol. 46, 133–137.

Luger, P., Daneck, K., Engel, W., Trummlits, G., Wagner, K., 1996. Structure and physicochemical properties of meloxicam, a new NSIAD. Eur. J. Pharm. Sci. 4, 175–187.

Martens, M., 1997. Efficacy and tolerability of a topical NSAID patch (local action transcutaneous flurbiprofen) and oral dichlofenac in the treatment of soft-tissue rheumatism. Clin. Rheumtol. 16, 25–31.

Matsuura, M., Imayoshi, T., Okumoto, T., 2000. Effect of FTY720, a novel immunosuppressant, on adjuvant- and collagen-induced arthritis in rats. Int. J. Immunopharmacol. 22, 323–331.

Mazzenga, G.C., Berner, B., 1991. The transdermal delivery of zwitterionic drugs I: the solubility of zwitterion salts. J. Control. Release 16, 77–88.

Mazzenga, G.C., Berner, B., Jordan, F., 1992. The transdermal delivery of zwitterionic drugs I: the flux of zwitterion salts. J. Control. Release 20, 163–170.

Megwa, S.A., Cross, S.E., Whitehouse, M.W., Benson, H.A., Roberts, M.S., 2000. Effect of ion pairing with alkylamines on the *in-vitro* dermal penetration and local tissue disposition of salicylates. J. Pharm. Pharmacol. 52, 929–940.

Naidu, N.B., Chowdary, K.P.R., Murthy, K.V.R., Satyanarayana, V., Hayman, A.R., Becket, G., 2004. Physicochemical characterization and dissolution properties of meloxicam-cyclodextrin binary systems. J. Pharm. Biomed. 35, 75–86.

O'Neil, M.J., Heckelman, P.E., Koch, C.B., Roman, K.J., Kenny, C.M., D'Arecca, M.R., 2006. The Merck Index, 14th ed. Merck & Co., INC, NJ, USA.

Pairet, M., van Ryn, J., Schierok, H., Mauz, A., Trummlitz, G., Engelhardt, G., 1998. Differential inhibition of cyclooxygenasse-1 and -2 by meloxicam and its 4′-isomer. Inflamm. Res. 47, 270–276.

Prieto, A., De Barrio, M., Martin, E., Fernandez-Bohorqez, M., De Castro, F.J., Ruiz, F.J., Herrero, T., Tornero, P., Rubio, M., 2007. Tolerability to nabumetone and meloxicam in patients with nonsteroidal anti-inflammatory drug intolerance. J. Allergy Clin. Immunol. 119, 960–964.

Satas, D., 1989a. Handbook of Pressure Sensitive Adhesive Technology, 2nd ed. Van Nostrand Reinhold, New York, p. 109.

Satas, D., 1989b. Handbook of Pressure Sensitive Adhesive Technology, 2nd ed. Van Nostrand Reinhold, New York, p. 403.

Senna, G.E., Passalacqua, G., Dama, A., Crivellaro, M., Schiappoli, M., Bonadonna, P., Canonica, G.W., 2003. Nimesulide and meloxicam are a safe alternative drugs for patients intolerant to nonsteroidal anti-inflammatory drugs. Eur. Ann. Allergy Clin. Immunol. 35, 393–396.

Seti, P., Kruss, B., Wiegleb, J., Trach, V., 1996. Local tissue tolerability of meloxicam, a new NSAID: indications for parental, dermal and mucosal administration. Br. J. Rheumatol. 35, 44–50.

Swingle, K.F., Grant, T.J., Jaques, L.W., Kvam, D.C., 1969. Interactions of anti-inflammatory drugs in carrageenan-induced foot edema of the rat. J. Pharmcol. Exp. Ther. 172, 423–425.

Tsai, R.S., Carrupt, P.A., Tayar, N.E., Giroud, Y., Andrade, P., Testa, B., 1993. Physicochemical and structural properties of non-steroidal anti-inflammatory oxicams. Helv. Chim. Acta. 76, 842–854.

Wang, R.J., Wu, P.C., Huang, Y.B., Tsai, Y.H., 2008. The effects of iontophoresis and electrophoration on transdermal delivery of meloxicam salts evaluated *in-vitro* and *in-vivo*. J. Food Drug Anal. 16, 41–48.

Yuan, Y., Li, S.M., Mo, F.K., Zhong, D.F., 2006. Investigation of microemulsion system for transdermal delivery of meloxicam. Int. J. Pharm. 321, 117–123.

Yuan, Y., Chen, X., Zhong, D., 2007. Determination of meloxicam in human plasma by liquid chromatography–tandem mass spectrometry following transdermal administration. J. Chromatogr. B 852, 650–654.