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Transdermal delivery of capsaicin derivative-sodium nonivamide acetate using microemulsions as vehicles

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Abstract

The objective of this study was to prepare sodium nonivamide acetate (SNA) microemulsion for topical administration. Microemulsions consisted of a mixed surfactant of Tween 80 and Span 20 as surfactant, ethanol as cosurfactant, isopropyl myristate (IPM) as an oil phase and water as an external phase. The effect of composition of microemulsion including the ratio of oil phase/surfactant/aqueous phase, various cosurfactant and polymer on the character and permeability of microemulsion were evaluated. The mean droplet size of SNA microemulsions ranged from 64 to 208 nm. Microemulsions showed potent enhancement effect for SNA transdermal delivery by a 3.7–7.1-fold increase when compared with the control group. Microemulsion containing ethanol as cosurfactant had the highest enhancement effect. With incorporated polymer, the viscosity of microemulsions increased resulting in the decrease in penetration rate of SNA. However, the permeability of SNA delivered from microemulsion was higher than SNA from volatile vehicles (pH 4.2 buffer containing 25% ethanol) reported in an earlier study, therefore microemulsions could be an effective vehicle for topical delivery of SNA.

Keywords: Sodium nonivamide acetate; Microemulsion; Transdermal delivery; Isopropyl myristate; Viscosity

1. Introduction

Capsaicin can stimulate the release of vasoactive neuropeptides such as substance P and calcitonin gene-related peptide from C-fiber nerve terminals and has a variety of pharmacological activities on cardiovascular, respiratory and nervous systems (Monsereenusorn et al., 1982; Manzini et al., 1989). Moreover, capsaicin is used in topical therapy for a variety of disorders such as rheumatism, lumbago and sciatica. However, capsaicin has strong pungency leading to limitation in clinical use. In addition, it has very poor aqueous solubility resulting in difficulties in the design of pharmaceutical formulations.

A newly designed derivative of capsaicin is sodium nonivamide acetate (sodium *N*-nonanoyl vanillyamide-4′-*O*-acetate; SNA) (Fig. 1) which was synthesized by alkylation of the phenolic hydroxyl group of nonivamide (*N*-nonanoyl vanillyamide) with bromoacetic acid (Fang et al., 1995). SNA demonstrates marked antinociceptive activity (1.75 and 27.50 times that of capsaicin and indomethacin) without producing an overt pungent sensation and irritation characteristic which has been found in capsaicin (Chen et al., 1992; Fang et al., 1996). Therefore, it is suggested that SNA be extensively used in clinical therapy (Yang et al., 1992; Fang et al., 1999). In this study, the transdermal delivery system of SNA was prepared and evaluated.

The most difficult aspect of a transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. The methods for improvement of drug permeation through the skin are to use penetration enhancers, iontophoresis and ultrasound. Iontophoresis and ultrasound are not frequently used due to the requirement of qualified staff for their application. Microemulsion systems have received increasing attention during the past years, because of having several advantages such as ease of manufacturing, thermodynamic stability, enhanced drug solubilization and increased drug

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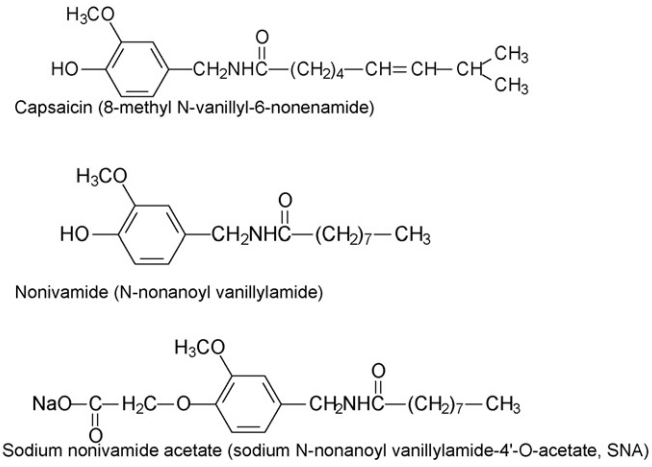

Fig. 1. The structures of capsaicin (8-methyl *N*-vanillyl-6-nonenamide), nonivamide (*N*-nonanoyl vanillylamide) and sodium nonivamide acetate (sodium *N*-nonanoyl vanillylamide-4′-*O*-acetate, SNA).

permeation rate (Laurence and Rees, 2000; Paolino et al., 2002; Peltola et al., 2003), therefore, they are useful as vehicles for topical delivery of drugs such as triptolide, apomorphine, estradiol, indomethacin and 8-methoxsalen (Baroli et al., 2000; Ho et al., 1996; Peira et al., 2001; Paolino et al., 2002; Peltola et al., 2003; Chen et al., 2004). In this study, the SNA microemulsions were prepared and evaluated for their enhancement effect of drug percutaneous absorption through rat skin.

2. Materials and methods

2.1. Materials

The following reagents were used: sorbitan monolaurate (Span 20), nonivamide (NVA) (Tokyo Chemical Industry, Japan), polyoxyethylene sorbitan monooleate (Tween 80) (Showa, Japan), isopropyl myristate (IPM), 1,3-propandiol, isopropanol and PEG 400 (Merck Chemicals, USA). Sodium nonivamide acetate (SNA) was synthesized by our laboratory and reported earlier (Fang et al., 1995). All other chemicals and solvents were of analytical reagent grade.

2.2. Construction of phase diagrams and SNA microemulsion preparation

Pseudo-ternary phase diagrams were constructed using the H_2O titration method at ambient temperature to ascertain the concentration range of components for the existing range of microemulsions. Combinations of Tween 80 and Span 20 at ratios of 1/4, 1/1 and 3/2 weight ratios were prepared and used as mixed surfactant. Ethanol at a level of 40% aqueous phase was used as cosurfactant. The mixtures of oil (IPM) and mixed surfactant at certain weight ratios (1/9, 2/8, 4/6, 6/4 and 8/2) of 1 g were diluted with aqueous phase dropwise, under moderate agitation for 1 min. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions or crude emulsions.

After the microemulsion regions in the phase diagrams were identified, the drug-loaded microemulsion formulations were selected from Tween 80 and Span 20 at the ratio of 3/2. The compositions of SNA microemulsion are listed in Table 1.

2.3. Microemulsion characterization

The average particle sizes of SNA microemulsions were determined by photo correlation spectroscopy by laser light scattering (Zetasizer 3000HSA, Malvern, UK) using a helium–neon laser with a λ of 633 nm. Samples were loaded into 1 cm^2 cylindrical cuvettes and placed in a thermostated scattering chamber. Light scattering was monitored at a fixed angle of 90° and fixed temperature of 25 °C.

The electrical conductivity of the microemulsions was measured by a handheld conductivity meter (WTW Cond 315i, SUNTEX) at 25 ± 2 °C.

For viscosity measurement, the microemulsion was placed in a cone-and-plate viscometer (Brookfield, Model DV-2, USA) and maintained at 37 °C. Reading was carried out 30 s after measurement was made, when the level had stabilized.

2.4. In vitro skin permeation experiments

The permeability of SNA from microemulsions was determined using a modified Franz glass diffusion cell fitted with

Table 1
Composition, particle size (PS), polydispersity index (PI), electrical conductivity (EC) and permeation parameters of SNA microemulsions

	Surfactant	Oil phase	Water phase	Particle size (nm)	PI	EC (μs/cm)	Viscosity (mPa s)	Cumulative amount (μg/cm^2)	Flux (μg/cm^2 h)	Lag time
Control			100			1.7		9.1 ± 3.8	0.41 ± 0.17	4.0 ± 2.0
F01	35	52	13	71.1 ± 0.2	0.25 ± 0.00	2.4	61.6	68.6 ± 8.8	2.74 ± 0.30	0.5 ± 0.0
F02	35	13	52	130.0 ± 1.2	0.64 ± 0.01	82.6	41.0	73.0 ± 7.4	2.91 ± 0.09	0.7 ± 0.3
F03	40	50	10	101.1 ± 0.9	0.46 ± 0.01	0.9	61.6	66.6 ± 4.9	2.80 ± 0.13	0.5 ± 0.0
F04	40	40	20	64.0 ± 0.4	0.49 ± 0.00	10.7	61.6	57.7 ± 20.9	2.36 ± 0.76	1.0 ± 0.0
F05	50	40	10	143.6 ± 1.4	0.49 ± 0.01	1.2	82.0	55.5 ± 6.1	2.43 ± 0.33	2.3 ± 0.6
F06	50	25	25	208.3 ± 4.5	0.44 ± 0.04	14.2	82.0	54.1 ± 25.2	2.40 ± 1.27	2.0 ± 0.0
F07	50	20	30	177.5 ± 2.0	0.41 ± 0.04	26.6	82.0	58.1 ± 3.5	2.84 ± 0.11	3.0 ± 0.0
F08	60	30	10	193.7 ± 1.8	0.53 ± 0.01	2.6	102.0	42.4 ± 15.3	2.30 ± 0.65	0.5 ± 0.0
F09	60	20	20	192.6 ± 2.7	0.42 ± 0.02	8.8	102.0	57.6 ± 12.3	2.54 ± 0.63	2.0 ± 0.0
F10	70	20	10	190.7 ± 0.9	0.46 ± 0.02	2.8	143.0	27.0 ± 6.9	1.51 ± 0.24	6.0 ± 2.0

Surfactant, oil phase and water phase were Tween 80/Span 20 = 3/2, IPM and 40% ethanol, respectively. Ethanol as 40% of aqueous solution was used as cosurfactant.

abdominal skin of excised Wistar rat. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 mL of SNA microemulsions and occluded by paraffin. The receptor compartment was filled with 20 mL of alcoholic phosphate buffer (pH 7.4) and its temperature was maintained at $37 \pm 0.5\,^{\circ}C$ by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Approximately 0.5 mL of the receptor medium was withdrawn at predetermined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. The sample withdrawn from the receptor compartment was then analyzed by HPLC as described previously (Tsai et al., 1994). Each data point represented the average of three determinations.

2.5. Data analysis

From in vitro study, the cumulative amount of the drug permeation through rat skin was plotted as a function of time and a linear regression analysis was used to determine the flux of the drug. Lag time was defined as the first time of detected drug. To discuss the effect of microemulsions as carrier on transdermal administrations, enhancement ratio (ER) is determined by the following equation:

$$\mathrm{ER} = \frac{\text{flux from microemulsion formulation}}{\text{flux from control vehicle}}.$$

3. Results and discussion

3.1. Phase studies

According to previous studies (Trotta et al., 1999; Wu et al., 2001), it is reported that short chain alcohol could decrease the hydrophilicity of the polar solvent and is capable of solubilizing high water content and then promoting the microemulsion form, therefore, ethanol was incorporated into the aqueous phase as a cosurfactant for phase diagrams in this study.

Microemulsions were made up of IPM, a mixed surfactant of Tween 80 and Span 20 and an aqueous phase in the presence cosurfactant (ethanol). Fig. 2 presents the pseudo-ternary phase diagrams with various weight ratios of Tween 80/Span 20. The translucent microemulsion region is presented in phase diagrams. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. From Fig. 2, it was found that the area of microemulsion isotropic region slightly increased in size with the increasing ratio of Tween 80/Span 20. Wu et al. (2001) reported a similar result by which the isotropic regions tended to narrow down with increasing Span 80/Tween 80 ratios in the oil-poor part of the pseudo-ternary diagram.

3.2. Characteristics of microemulsion

The drug-loaded microemulsions were selected from the phase diagram at Tween 80/Span 20 at a ratio of 3/2. The physico-chemical parameters of microemulsions containing SNA of 1 mM were measured and are listed in Table 1. The droplet size of microemulsion vesicles was small with all the formulations having a mean vesicle size between 64 and 208 nm. Furthermore, the particle size had no significant change after 1 month of storage at room temperature (data not shown).

The electrical conductivity of IPM (oil phase), doubled water, aqueous containing cosurfactant and drug free microemulsion (F02) were 0, 0.6, 1.7 and 114.2 μs/cm, respectively, showing that microemulsion could increase electrical conductivity (Bumajdad and Eastoe, 2004). As shown in Table 1, the electrical conductivity of all drug-loading microemulsions ranged from

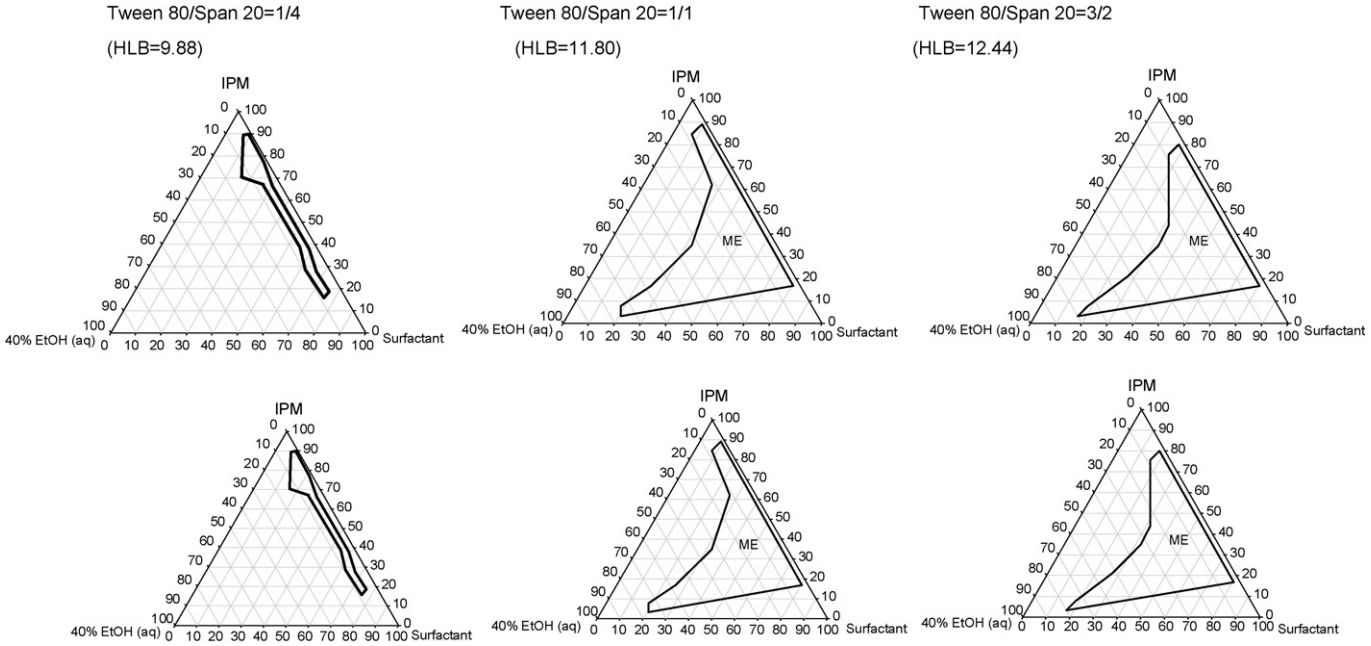

Fig. 2. Pseudo-ternary phase diagrams of microemulsion (ME) composed of IPM, mixed surfactant (Tween 80/Span 20), cosurfactant (ethanol) and aqueous solution.

0.9 to 82.6 μs/cm. The electrical conductivity of microemulsions was significantly higher than oil phase. Moreover, electrical conductivity of microemulsions increased by the increase in the level of aqueous phase. The increase in electrical conductivity might be due to the increase in dissociation of surfactant as a function of water content (Baker et al., 1984). These results were in accordance with a previous study (Sintov and Shapiro, 2004) and indicated that the microemulsions were oil-in-water type. The solubility of SNA in IPM and aqueous phase containing cosurfactant were 0.5 and 88.58 μg/mL, showed that most parts of SNA dissolved in the continuous phase.

The viscosity of microemulsions at 37 °C ranged from 41 to 143 mPa s. It was found that the viscosity increased with an increasing amount of surfactant.

3.3. In vitro skin penetration experiments

In the present study, microemulsions consisted of IPM, mixed surfactant of Tween 80 and Span 20, cosurfactant (ethanol) and aqueous phase. IPM is an effective penetration enhancer (Goldberg-Cettina et al., 1995). Moreover, the surfactant and cosurfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancers (Peltola et al., 2003). Therefore, the effect of composition of microemulsion on SNA permeation through skin was evaluated. The excised Wistar rat skin was used as the model membrane since the permeability of SNA through rat skin was more similar to that through human skin than other skin types (Fang et al., 1995). The permeation profiles of SNA microemulsions through rat skin are shown in Fig. 3. The permeation parameters including the cumulative amount at 24 h, flux and lag time were calculated followed zero-order model ($R^2 > 0.9920$) and are listed in Table 1. The cumulative amount ranged from 27.0 ± 6.9 to 73.0 ± 7.4 μg/cm^2, flux ranged from 1.51 ± 0.24 to 2.91 ± 0.09 μg/cm^2 h and lag time ranged from 0.5 to 6 h indicating that the permeation parameters of SNA from microemulsions were markedly influenced by the composition microemulsions. The enhancement ratios (ER) of microemulsions were 3.7–7.1-fold higher than the control group (SNA dissolved in 40% ethanol aqueous solution). Among these microemulsions, all except F10 could shorten the lag time. These results demonstrated that microemulsion had a potent enhancement effect for transdermal delivery (Wu et al., 2001; Peltola et al., 2003).

In additional, a trend was observed for decreasing flux and prolonging lag time with increasing the amount of surfactant. The result might be due to a decreased thermodynamic activity of the drug in microemulsion at higher concentrations of surfactant (Rhee et al., 2001). Another possibility was that drug diffusion through the double layer microemulsion might be a rate-determining step, as the viscosity plays an important role in controlling the release of the drug into the receptor (Ho et al., 1996). However, the formulation F02 showed the highest penetration rate (2.91 ± 0.09 μg/cm^2 h) and lower lag time (0.7 h). The permeability of SNA delivered from microemulsion was higher than SNA from volatile vehicles (pH 4.2 buffer containing 25% ethanol) (Fang et al., 1999).

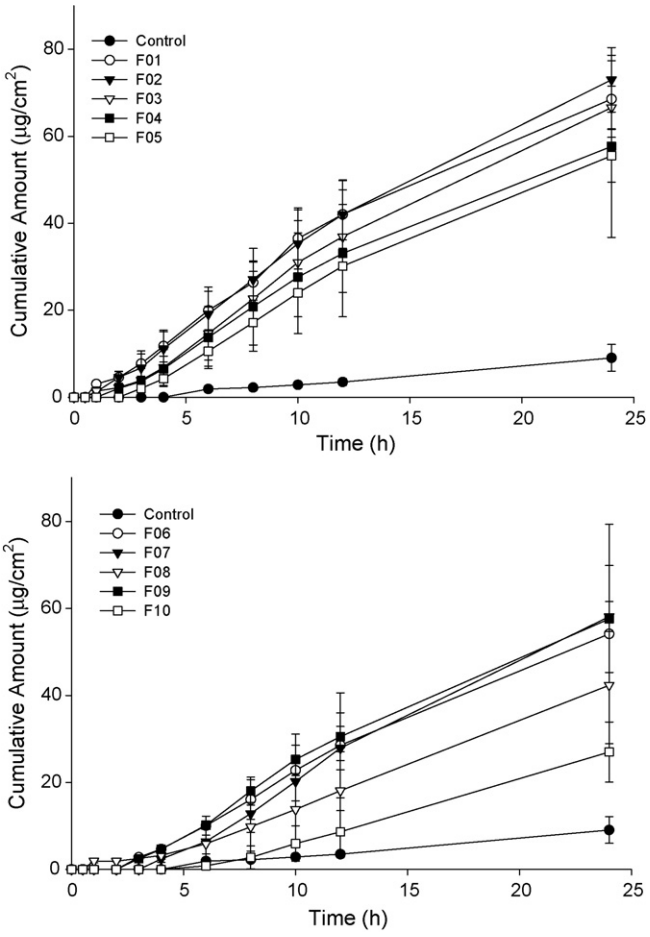

Fig. 3. In vitro penetration–time profile of SNA microemulsions through rat skin ($n = 3$).

In comparison to the effect of the ratio of Tween 80/Span 20 on the permeation of SNA, it was found that the microemulsions could not form while the ratio was below 1/4 (HLB = 9.9) (Table 2). The particle size of microemulsion containing Tween 80/Span 20 at the ratio of 2/3 (HLB = 11.2) was 330.6 nm and significantly higher than other microemulsions. The viscosity increased with increase of the HLB value of surfactant. The microemulsion containing surfactant at ratio of 3/2 (HLB = 12.4) had the highest penetration effect and shortest lag time. These results demonstrated that an appropriate ratio of Tween 80/Sapn 20 was necessary for SNA transdermal delivery.

According to earlier reports (Trotta et al., 1999; Wu et al., 2001), the cosurfactant lowers the interfacial tension of the surfactant file in microemulsions, resulting in a more flexible and dynamic layer. The drug in this energy-rich system can diffuse across the flexible interfacial surfactant film between the phases; a thermodynamic process that increases partitioning and diffusion into the stratum corneum. Therefore, the influence of various cosurfactants on microemulsion formation and penetration absorption of SNA was also evaluated in this study. Various short chain alcohols instead of ethanol were used to prepare the microemulsion (F02) with high water content. The microemulsion could not be formed by using glycerin, propylene glycol and 1,3-propandiol as the cosurfactant. The cumulative

Table 2
Characteristics and permeation parameters of SNA microemulsion containing various ratio of Tween80/Span80

Surfactant	Particle size (nm)	Viscosity ($\times 10^3$ cps)	Flux ($\mu g/cm^2$ h)	Lag time (h)
Tween80/Span20 = 5/0 (HLB = 15.0)	23.3 ± 3.5	205.0	0.81 ± 0.05	3.67 ± 0.58
Tween80/Span20 = 4/1 (HLB = 13.7)	16.5 ± 0.3	82.0	1.33 ± 0.10	1.33 ± 0.58
Tween80/Span20 = 3/2 (HLB = 12.4)	130.0 ± 1.2	41.0	2.91 ± 0.09	0.67 ± 0.29
Tween80/Span20 = 2/3 (HLB = 11.2)	330.6 ± 4.1	31.0	1.98 ± 0.10	1.00 ± 0.00
Tween80/Span20 = 1/4 (HLB = 9.9)	–	–	–	–
Tween80/Span20 = 0/5 (HLB = 8.6)	–	–	–	–

(–): the microemulsion cannot form.

Table 3
Characterizes and permeation parameters of SNA microemulsion without and with different type of polymer at 1%

Control	Particle size (nm)	Viscosity ($\times 10^3$ cps)	Flux ($\mu g/cm^2$ h)	Lag time (h)
F02	130.0 ± 1.2	41.0	2.91 ± 0.09	0.67 ± 0.29
F02 + CMC	147.0 ± 0.2	82.0	1.23 ± 0.23	0.67 ± 0.29
F02 + HPC	142.8 ± 0.6	82.0	1.29 ± 0.29	1.67 ± 0.58
F02 + PF127	138.8 ± 0.9	61.6	1.56 ± 0.17	1.00 ± 0.00
F02 + PF68	141.6 ± 0.8	61.6	1.41 ± 0.16	2.00 ± 1.00

amount of SNA at 24 h from microemulsions with different cosurfactants were 73.0 ± 7.4, 32.9 ± 6.4 and 29.2 ± 9.0, 34.4 ± 7.6 and 13.33 ± 2.88 $\mu g/cm^2$ for ethanol, 1-propanol, 1,2-propandiol, isopropanol and PEG 400, respectively, indicating that the penetration capacity of SNA was influenced by various cosurfactants. The cumulative amount and flux of SNA microemulsions increased with the increase in the polarity of solvent. However, the microemulsion containing ethanol had highest permeability (higher flux and shorter lag time). In additional, the microemulsion (F02) containing 52% aqueous phase could not be formed at lower levels of ethanol from 0 to 30%. The result was in accordance with a previous study which pointed out that it is not possible to obtain high water incorporation in the absence of cosurfactants (Ho et al., 1996; Trotta et al., 1999).

In additional, previous studies (Gallarate et al., 1990;Gasco et al., 1991) reported that the release of azelaic acid from the viscosized microemulsion was higher than that from a fluid microemulsion, most likely as a consequence of the different permeability of the two barriers. Hence, the different type of 1% polymers including CMC, HPC, Pluronic F127 and Pluronic acid F68 were incorporated into microemulsions. As shown in Table 3, viscosity increased, then flux decreased and lag time was prolonged when 1% polymers were incorporated. The result is not in accordance with earlier studies (Gallarate et al., 1990; Gasco et al., 1991) and might be attributed to the gel formation in the microemulsion that will increase its viscosity and further decrease the permeation in the skin.

4. Conclusion

A microemulsion consisting of IPM as oil phase, a mixture of Tween 80 and Span 20 as surfactant, ethanol as cosurfactant and an aqueous solution could be an effective vehicle for topical delivery of SNA.

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References

Baker, R.C., Florence, A.T., Ottewill, R.H., Tadros, T.H.F., 1984. Investigation into the formation and characterization of microemulsions II. Light scatting conductivity and viscosity studies of microemulsion. J. Colloid Interf. Sci. 100, 332–349.

Baroli, B., Lopez-Quintela, M.A., Delgado-Charro, M.B., Fadda, A., Blanco-Mendez, J., 2000. Microemulsions for topical delivery of 8-methoxsalen. J. Control. Rel. 69, 209–218.

Bumajdad, A., Eastoe, J., 2004. Conductivity of water-in-oil microemulsions stabilized by mixed surfactants. J. Colloid Interf. Sci. 274, 268–276.

Chen, I.J., Yang, J.M., Yeh, J.L., Wu, B.N., Lo, Y.C., Chen, S.J., 1992. Hypotensive and antinociceptive effects of ether-linked and relatively non-pungent analogues of *N*-nonanoyl vanillylamide. Eur. J. Med. Chem. 27, 187–192.

Chen, H., Chang, X., Wang, T., Zhao, X., Gao, Z., Yang, Y., Xu, H., Yang, X., 2004. A study of microemulsion systems for transdermal delivery of triptolide. J. Control. Rel. 98, 427–436.

Fang, J.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H., 1995. In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. Int. J. Pharm. 126, 119–128.

Fang, J.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H., 1996. In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases: pharmacokinetic analysis in rabbits. Int. J. Pharm. 128, 169–177.

Fang, J.Y., Kuo, C.T., Huang, Y.B., Wu, P.C., Tsai, Y.H., 1999. Transdermal delivery of sodium nonivamide acetate from volatile vehicles: effects of polymers. Int. J. Pharm. 176, 157–167.

Gallarate, M., Gasco, M.R., Rua, G., 1990. In vitro release of azelaic acid from oil-in-water microemulsions. Acta Pharm. Yugosl. 40, 541–545.

Gasco, M.R., Gallarate, M., Pattarino, F., 1991. In vitro permeation of azelaic acid from viscosized microemulsions. Int. J. Pharm. 69, 193–196.

Goldberg-Cettina, M., Liu, P., Nightingale, L., Kurihara-Bergstrom, T., 1995. Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int. J. Pharm. 114, 237–245.

Ho, H.O., Hsiao, C.C., Sheu, M.T., 1996. Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs. J. Pharm. Sci. 85, 138–143.

Laurence, M.Y., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 45, 89–121.

Manzini, S., Perretti, F., De Benedetti, L., Pradelles, P., Maggi, C.A., Geppetti, P., 1989. A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of the guinea pig: involvement of substance P and calcitonin gene-related peptide release. Br. J. Pharmacol. 97, 303–312.

Monsereenusorn, Y., Kongsamut, S., Pezalla, P.D., 1982. Capsaicin—a literature survey. Crit. Rev. Toxicol. 10, 321–339.

Paolino, D., Ventura, C.A., Nistico, S., Puglisi, G., Fresta, M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous absorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21–31.

Peira, E., Scolari, P., Gasco, M.R., 2001. Transdermal permeation of apomorphine through hairless mouse skin from microemulsions. Int. J. Pharm. 226, 47–51.

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T.M., Urtti, A., 2003. Microemulsions for topical delivery of estradiol. Int. J. Pharm. 254, 99–107.

Rhee, Y.S., Choi, J.G., Park, E.S., Chi, S.C., 2001. Transdermal delivery of ketoprofen using microemulsions. Int. J. Pharm. 228, 161–170.

Sintov, A.C., Shapiro, L., 2004. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability on vivo. J. Control. Rel. 95, 173–183.

Trotta, M., Gallarate, M., Pattarino, F., Carlotti, M.E., 1999. Investigation of the phase behaviour of systems containing lecithin and 2-acyl lysolecithin derivatives. Int. J. Pharm. 190, 83–89.

Tsai, Y.H., Huang, Y.H., Fang, J.Y., Wu, P.C., 1994. Percutaneous absorption of capsaicin and its derivatives. Drug Dev. Ind. Pharm. 20, 719–730.

Wu, H., Ramachandran, C., Weiner, N.D., Roessler, B.J., 2001. Topical transport of hydrophilic compounds using water-in-oil nano emulsions. Int. J. Pharm. 220, 63–75.

Yang, J.M., Wu, B.N., Chen, I.J., 1992. Depressor response of sodium nonivamide acetate: a newly synthesized non-pungent analogue of capsaicin. Asia Pac. J. Pharmacol. 7, 95–102.