Electrically-Assisted Skin Permeation of Two Synthetic Capsaicin Derivatives, Sodium Nonivamide Acetate and Sodium Nonivamide Propionate, *via* Rate-Controlling Polyethylene Membranes

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The objective of this study was to examine the transdermal delivery of sodium nonivamide acetate (SNA) using iontophoresis and electroporation with ultra high molecular weight polyethylene membranes (Solupor®) to achieve controlled transdermal drug delivery. A derivative of SNA, sodium nonivamide propionate (SNP), was also used as a model drug in this investigation. Iontophoresis increased the transdermal permeation of SNA as compared to passive diffusion. Most Solupor membranes were rate-limiting for the iontophoretic permeation of SNA except for Solupor 8P07, which showed negligible resistance to SNA delivery. The tortuosity (Gurley number), pore size, and the current density-induced attachments on the surface of the Solupor membranes may have been important for their rate-controlling effect. The trends for inhibiting or controlling SNA permeation were similar for both iontophoretic and electroporation applications. The higher molecular size and lower hydrophilicity of SNP compared to SNA resulted in lower permeation of SNP using electrically-assisted methods. Moreover, the various types of Solupor membranes showed similar trends for both SNA and SNP. This present study indicates that Solupor membranes act as rate-limiting membranes for controlling the release and skin permeation of both SNA and SNP by electrically-assisted methods.

Key words sodium nonivamide acetate; sodium nonivamide propionate; transdermal delivery; iontophoresis; electroporation; Solupor®

Sodium nonivamide acetate (SNA; $C_{19}H_{28}NO_5Na$) is a non-pungent derivative of capsaicin which is synthesized by alkylation of the phenolic hydroxyl group of nonivamide with bromoacetic acid.[1)] The antinociceptive potency of SNA is 1.75 and 27.5 times than that of capsaicin and indomethacin, respectively.[2)] Transdermal drug delivery is preferable for SNA to avoid the short half-life (16.80 min) with intravenous administration.[3)] Methods for transdermal enhancement by iontophoresis (electric current density application) have been utilized to overcome the poor permeability of SNA.[4—6)]

A common aim of the development of transdermal devices is the controlled delivery of drugs, so that the rate of drug input into the bloodstream is predictable and reproducible. When drugs applied to skin *in vivo*, the semisolid forms or the solution forms incorporated with a membrane (patches) were practically preferable to restrict the applied site in a certain area. Transdermal therapeutic systems, which utilize rate-controlling membranes, provide more-reproducible delivery compared to the skin alone, and produce smaller inter- and intra-subject variations.[7)] Solupor® is a microporous polymeric membrane composed of a unique combination of randomly oriented thick and thin fibrils of ultra high molecular weight (MW) polyethylene of 5—7$\times 10^6$ Da. Solupor can be used as a substrate through which a liquid or gas can be released in a controlled manner. It has good chemical resistance, high tortuosity, and favorable biocompatibility.[8)] Hence Solupor may be suitable as a membrane for transdermal drug delivery systems.

The aim of this study was to investigate the influence of Solupor membranes on the transdermal iontophoretic delivery of SNA. SNA permeation across various types of skin or artificial membranes was elucidated to explain the possible mechanisms. Electroporation is also an electrically-assisted technique which involves the application of short, high-voltage electric field pulses to create transient elevation of the permeation of lipid bilayer membranes. SNA permeation by electroporation application was performed to compare with data from iontophoresis. Sodium nonivamide propionate (SNP; $C_{20}H_{30}NO_5Na$) is another derivative of capsaicin. The difference in the structures between SNA and SNP is that the ether-linked group of the phenol is acetate and propionate, respectively. Another purpose of this present study was to investigate the influence of different molecular sizes on the iontophoretic permeation of drugs.

MATERIALS AND METHODS

Materials The synthesis procedures for SNA and SNP were performed in our laboratory and were reported earlier.[1)] A series of Solupor® membranes was kindly provided by Teijin Solfill Ltd. (Tokyo, Japan), which is authorized by DSM N.V. (Heerlen, the Netherlands). The CelluSep® cellulose membrane (with a MW cutoff of 6000—8000) was obtained from Membrane Filtration Products (Seguin, TX, U.S.A.). All other chemicals and solvents were of analytical grade.

***In Vitro* Permeation Procedures** The *in vitro* permeation rates of SNA and SNP were determined using horizontal glass diffusion cells. The dorsal skin of excised female nude mice (7—8 weeks old) was used as the model membrane. To obtain stratum corneum (SC)-stripped skin, adhesive tape was applied to the intact skin with uniform pressure and then removed. This procedure was repeated 20 times. The Solupor membrane was placed on the SC side of the skin if necessary. The receptor phase contained 8 ml of pH 7.4 (0.06 M) citrate-phosphate buffer. The donor compartment was filled with 8 ml of pH 4.2 (0.06 M) buffer containing 0.02% (w/v) SNA or SNP. The available diffusion surface

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area was 0.785 cm^2. Cells were agitated by magnetic stirrers at 600 rpm. The 300-μl samples were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh receptor solution. Samples were assayed by HPLC.[1)]

Iontophoresis Assembly A pair of Ag/AgCl wires, having an effective length of 15 mm, was used as the electrodes by immersing them in the diffusion cells, with the cathode in the donor and the anode in the receptor cell. The electrodes were connected to a current power supply (Model 7651, Yokogawa, Japan). A current density of 0.5 mA/cm^2 was applied for 3 h.

Electroporation Assembly Electroporation was performed using an exponential decay pulse generator (Electro Cell Manipulator 630, Genetronics, U.S.A.). Platinum electrodes (0.5×1.5 cm^2) were used, each located 3 cm from the skin in the diffusion cell. The cathode was positioned in the donor cell, while the anode was in the receptor cell. The electroporation protocol consisted of one pulse per 30 s, applied for 10 min. The pulse voltage was 300 V, and pulse length was 200 ms. Voltages were expressed as applied values, not as transdermal values.

Scanning Electron Microscopic (SEM) Examination The Solupor membranes before and after the experiments were affixed with gold-palladium in an ion coater and examined by SEM (Hitachi S-2400, Japan). All analyses were performed in a blinded fashion.

Statistical Analysis The statistical analysis of differences between various treatments was carried out using unpaired Student's *t*-test. The 0.05 level of probability was taken as the level of significance. The ANOVA test was also utilized in this study.

RESULTS AND DISCUSSION

Transdermal Delivery of SNA across Various Skin Types Figure 1 shows the cumulative amount-time profiles of the passive and iontophoretic permeation of SNA across intact skin, SC-stripped skin, and the artificial cellulose membranes. Slopes of the resulting linear plots from 0 to 6 h were calculated, and fluxes (μg/cm^2/h) were determined from the slopes. The values of flux are shown in the legend of Fig. 1. The concentration of SNA in the receptor cell during the 6 h of passive diffusion was found to be undetectable using HPLC. This result indicates that the passive diffusion of SNA across a lipophilic biological membrane is limited due to its ionic nature. Figure 1 shows that iontophoresis for 3 h greatly enhanced the permeation of SNA. After cessation of the applied current, the permeated amount of SNA still continued to rise as shown in Fig. 1. When the electric field was discontinued, the skin became depolarized, but the resistance of the skin did not immediately recover. Another reason for this observation may have been the existence of a drug reservoir inside the skin since the permeant desorbs from the skin until the drug reservoir is empty.[4)]

The passive permeation of SNA across SC-stripped skin was much higher than the value across intact skin, suggesting that the SC layer is the rate-limiting structure for the passive diffusion of SNA. For the permeation of SNA across SC-stripped skin, the iontophoretic flux was 5.13-fold higher than the passive flux (*t*-test, $p<0.05$). Moreover, the iontophoretic permeation of SNA across SC-stripped skin was higher (*t*-test, $p<0.05$) than that across intact skin. The data indicate that the rate-limiting characteristics of the SC on the passive diffusion of SNA could be partially, but not completely, overcome by application of iontophoresis.

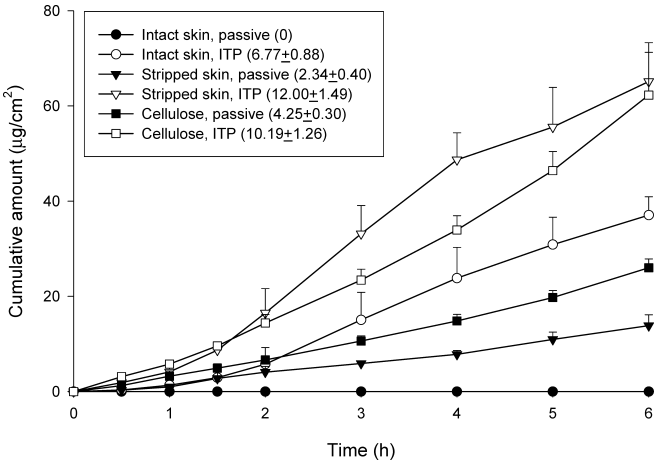

Fig. 1. Cumulative Amount–Time Profiles of Transdermal SNA Delivery across Various Types of Skin and Membranes with or without Iontophoretic Application

Each value represents the mean±S.D. ($n=4$).

Diffusion of SNA across the cellulose membrane was also carried out as a control to validate the results from the excised skin membranes. The MW cutoff value for the cellulose membrane is 6000—8000, and thus drug molecules can freely diffuse across it. As shown in Fig. 1, the higher flux for SNA through the cellulose membrane (*t*-test, $p<0.05$) demonstrates that the skin indeed exhibited barrier properties in these permeation studies. The passive diffusion of SNA across the cellulose membrane was significantly higher (*t*-test, $p<0.05$) than that across SC-stripped skin, indicating that not only the SC layer but also viable epidermis/dermis contributed to the barrier functions against SNA delivery. Although SNA possesses negative charges as ionic molecules, the long alkyl chain in the structure may cause SNA to possess certain hydrophilic characteristics. This may have resulted in hindrance to permeation of SNA across the hydrophilic epidermis/dermis.

The diffusion of SNA across the cellulose membrane was significantly enhanced (*t*-test, $p<0.05$) by iontophoresis as shown in Fig. 1. This indicates that the electrically repulsive force produced by the electric field is a predominant mechanism for enhancing ionic SNA permeation during iontophoresis. The direction of the iontophoresis-induced electro-osmotic flow of water is toward the cathode, which may reduce the enhancing effect of iontophoresis on anionic molecules. The contribution of electro-osmosis appears to have been negligible with the artificial membranes used since the cellulose membrane does not have an electric charge.[9)] Hence the enhancement ratio of 2.40 after iontophoretic application to the cellulose membrane may simply indicate the contribution of the electrically repulsive force.

Influence of Solupor Membranes on Transdermal Iontophoresis of SNA A series of Solupor membranes was incorporated on the SC side of the skin to examine the effect of these membranes on the topical delivery of SNA *via* iontophoresis. Solupor membranes are composed of very high

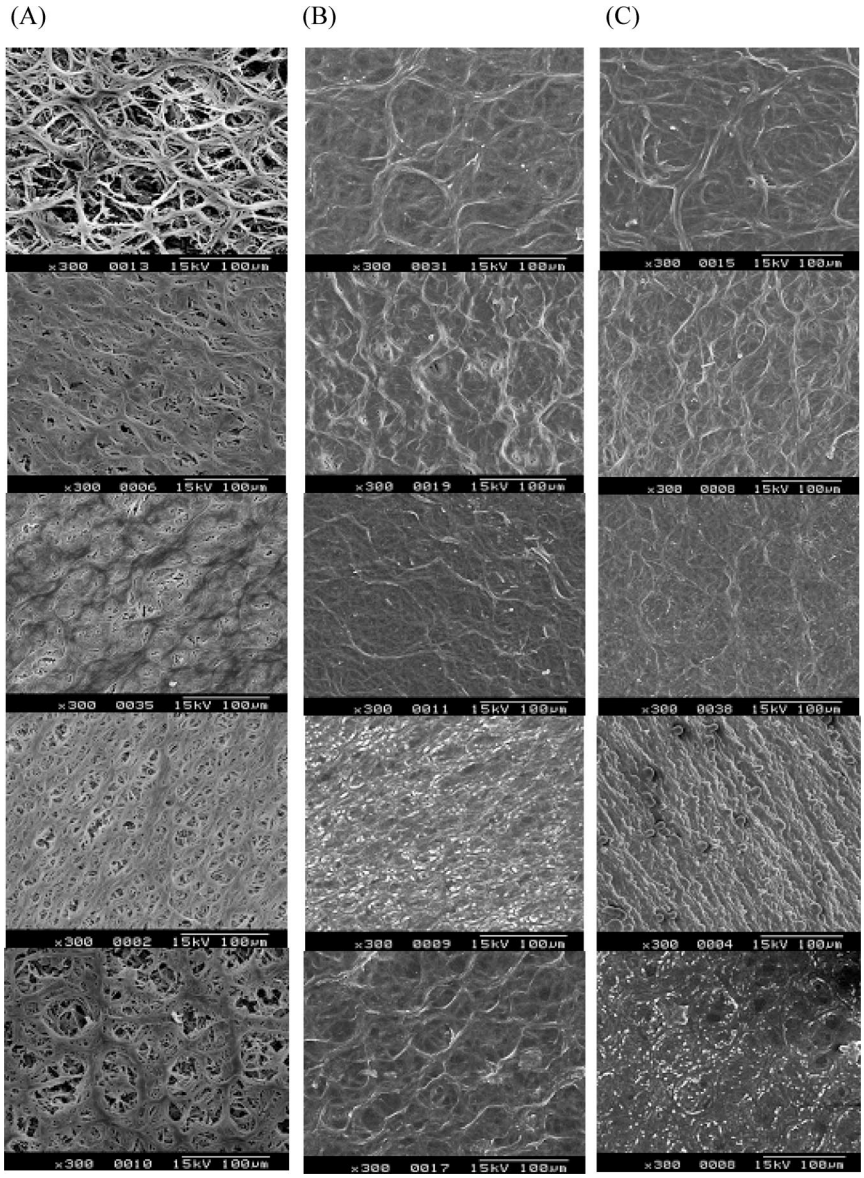

Fig. 2. SEM Images (Magnification ×300) of Solupor® Membranes before (A) and after Treatment with Passive Diffusion (B) and Iontophoresis (C) in the *in Vitro* Permeation Experiments

up to bottom: Solupor 7P03, 8P07, 10P01, 10P05, and 16P05 membranes.

MW polyethylene. The polyethylene film has microporous characteristics as shown in Fig. 2A. Transport of a drug through this kind of membrane occurs by diffusion through liquid-filled pore media.[7] Profiles of the iontophoretic flux of SNA, which had permeated across nude mouse skin with the Solupor membrane, are shown in Table 2. The cumulative amount–time profiles are also depicted in Fig. 3. The passive flux of SNA across the combination of a Solupor membrane and skin was negligible for all five of these membranes tested. The iontophoretic flux of SNA was reduced after the Solupor membrane was added onto skin except for Solupor 8P07. The Solupor 8P07 membrane showed lower resistance to the iontophoretic permeation of SNA across the skin (*t*-test, $p<0.05$). As shown in Table 1, it seems that there is no single physicochemical characteristic of Solupor which predominated the trends of SNA permeation *via* iontophoresis. Hence, the complicated mechanisms which influence the transport of SNA across the Solupor membranes should be

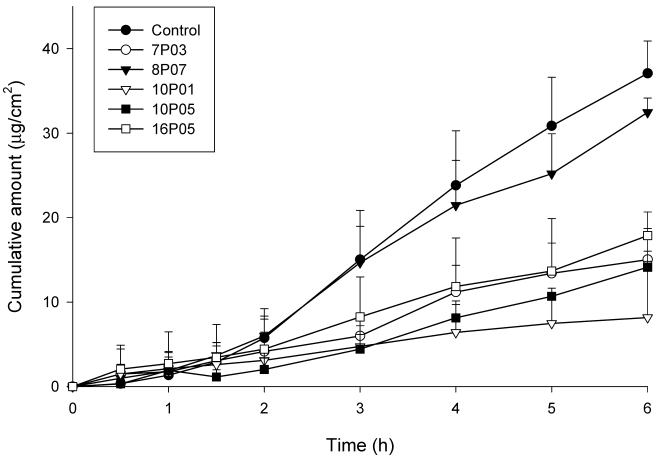

Fig. 3. Cumulative Amount–Time Profiles of Transdermal SNA Delivery across Solupor® Incorporated with Skin by Iontophoresis

Each value represents the mean±S.D. ($n=4$).

Table 1. The Physicochemical Properties of Solupor

Property	7P03	8P07	10P01	10P05	16P05
Total weight/surface (g/m^2)	7	8	11	10	16
Thickness (μm)	50	50	21	60	120
Porosity (%)	85	85	47	83	87
Gurley number (s/50 ml)	15	2	100	4	3
Coulter pore size (μm)	0.3	0.7	0.08	0.5	0.5

Table 2. Effect of Various Solupor Membranes on the Flux of SNA across Nude Mouse Skin

Membrane	Iontophoretic flux (μg/cm^2/h)	Electroporation flux (μg/cm^2/h)
None	6.77±0.88	0.66±0.18
7P03	2.65±0.49	0.09±0.03
8P07	5.72±0.67	0.88±0.06
10P01	3.33±1.30	0
10P05	2.38±0.17	0
16P05	2.91±0.92	0

Each value represents the mean±S.D. (n=4).

discussed.

As shown in Table 2, SNA permeation across skin with the Solupor 8P07 membrane was comparable to that across skin alone. This may have been due to the reduced thickness of this membrane, with a shorter path length across which SNA had to diffuse (Table 1). However, this phenomenon was not observed for Solupor 7P03, which is the same thickness as Solupor 8P07. Hence other mechanisms may also contribute to the low resistance of Solupor 8P07. The larger pore size of Solupor 8P07 than the other membranes may be another reason. The Gurley number (s/50 ml) is proportional to τ^2, where τ is the tortuosity. Tortuosity indicates the effective path length through the pores inside the membrane, while the Gurley number is an indicator of the permeability of a membrane.[8] Table 1 shows that Solupor 8P07 has a much lower Gurley number, and therefore a more-open microstructure than the other membranes.

A clear reduction in the SNA iontophoretic flux with Solupor 7P03 was detected compared to the respective flux across skin alone (Table 2). A possible reason was that Solupor 7P03 has a high Gurley number of 15 s/50 ml, resulting in longer paths for the permeation of SNA. A much-smaller pore size may also have contributed to the barrier properties of this membrane. The same phenomenon was demonstrated for Solupor 10P01, which has the smallest pore size of 0.1 μm (Table 1). The lowest porosity and highest Gurley number of Solupor 10P01 among all membranes tested may also contribute to this barrier property although it showed the thinnest thickness. There was no significant difference (ANOVA test, $p>0.05$) among the SNA fluxes across Solupor 7P03, 10P01, 10P05, and 16P05 membranes (Table 2). The barrier property of Solupor 16P05 may result from its great thickness. Porosity (%) is an indicator of the void volume of a membrane. Although a highest porosity was possessed by Solupor 16P05, it did not produce negligible resistance to SNA permeation. Moreover, the porosity of these five membranes did not correlate well with SNA permeation, indicating that the porosity was not important in controlling the drug permeation across Solupor membranes.

As depicted in Table 1, the tortuosity values of Solupor 10P05 and 16P05 are not large. The physicochemical profiles also indicated a relatively larger pore size for Solupor 10P05 and 16P05 than for 7P03 and 10P01. Nevertheless, the reduction in SNA flux was comparable for these membrane types. Solupor membranes were examined by SEM to determine the appearance after passive diffusion and iontophoretic stimulation. As shown in Fig. 2B, some particles had adhered to the surface of the Solupor 10P05 membrane after immersion in pH 4.2 buffer during passive diffusion. This phenomenon was more noticeable after iontophoresis had been applied for 3 h (Fig. 2C). A similar result was determined for Solupor 16P05, but not for the other membranes. These attached particles may have originated from the donor solution such as buffer species or SNA molecules. However, we cannot explain why these attachments were only found on Solupor 10P05 and 16P05 membranes under the current conditions. Further studies should be performed to verify this effect. These attached particles may form an additional barrier to drug permeation, resulting in the reduction of SNA flux after iontophoretic application. When the original magnification of SEM was amplified to 2000× as shown in Figs. 4B and C, the attached particles can again be seen on Solupor 10P05 and 16P05 membranes. Another observation was that the margins of the orifices of Solupor membranes presented a fibril-like morphology before treatment (Fig. 4A). After treatment with passive diffusion or iontophoresis, the margins of the orifices had become smoother, and the fibril-like materials had vanished.

Effect of Electroporation on the Transdermal Delivery of SNA The transdermal delivery of SNA across Solupor membranes and skin was also investigated by applying electroporation. Figure 4 shows the cumulative amount–time profiles of SNA permeation across various skin types with electroporation. The application of 300-V pulses (200 ms) for 10 min increased the SNA flux from 0 to 0.66 μg/cm^2/h. The mechanism of transport differs between iontophoresis and electroporation.[10] Iontophoresis is known to act primarily on drug molecules through iontophoretic drift. The pathways for iontophoresis of SNA appeared mainly to be follicles and other shunts.[4] Electroporation involves the application of high-voltage pulses which create transient aqueous pathways in lipid bilayers which permit transport of drug molecules across these pathway.[11] According to a previous study,[6] however, the contribution of electroporated lipid bilayers was negligible for SNA transport across the skin, while electrophoretic movement during 10 min of electroporation was important for SNA. Since the direct electrophoretic force by pulsing was the main mechanism acting on SNA's permeation, this may suggest that the enhancement due to transdermal iontophoresis was great, and the enhancement due to 10 min of electroporation was low in comparison (Figs. 1, 5).

As shown in Fig. 5, the permeated amount of SNA remained elevated after 10 min of electroporation. This elevated post-pulse permeation may come from an SNA reservoir created within the skin during pulsing. Due to the long alkyl chain of SNA's structure, a high affinity for the lipophilic environment of the skin may be responsible for the drug reservoir formation. Electroporation using SC-stripped skin enhanced the SNA flux by 2-fold (Fig. 5). The constant increase in SNA permeation after electroporation was also

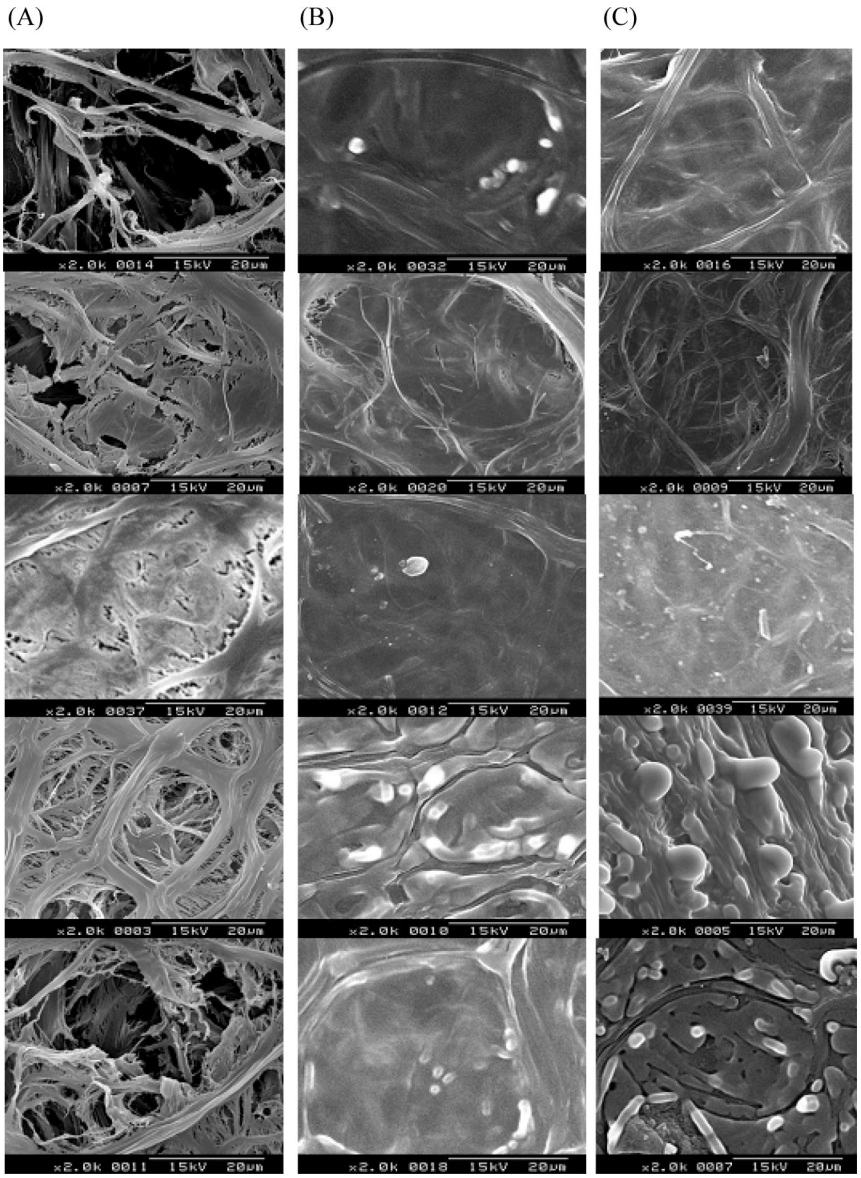

Fig. 4. SEM Images (Magnification ×2000) of Solupor® Membranes before (A) and after Treatment with Passive Diffusion (B) and Iontophoresis (C) in the *in Vitro* Permeation Experiments

up to bottom: Solupor 7P03, 8P07, 10P01, 10P05, and 16P05 membranes.

observed for SC-stripped skin. This result confirms that micropores formed within the lipid bilayers of the SC are not important for SNA delivery. The formation of a large drug reservoir may be the predominant mechanism for this enhancement. Figure 5 shows that the application of electroporation through the cellulose membrane did not produce increased SNA transport relative to that by passive diffusion. This indicates that electrophoretic drift induced by 10 min of electroporation was not sufficient to increase SNA permeation. Moreover, this phenomenon again indicates the importance of the skin reservoir with electroporation.

Table 2 demonstrates the influence of Solupor membranes on the transdermal electroporation of SNA. The results of electroporation were similar to those of iontophoresis. There were no significant differences (*t*-test, $p>0.05$) between SNA fluxes across skin with and without the Solupor 8P07 membrane. The other membranes including Solupor 7P03, 10P01, 10P05, and 16P05 showed lower SNA permeation values as compared to the control. This may suggest that electrophoresis, the common mechanism for both iontophoresis and electroporation, predominates SNA permeation across Solupor membranes. The Solupor 10P01, 10P05, and 16P05 membranes showed significant barrier properties, producing a negligible SNA permeated amount within 6 h. The permeation of SNA across skin by electroporation was slightly retained after the addition of a Solupor 7P03 membrane (Table 2). This suggests that the high tortuosity of the membrane did not overly hinder SNA's delivery *via* electroporation.

Comparison between the Transdermal Deliveries of SNP and SNA When transdermal iontophoresis is administered, the molecular size can influence the effect of current density on drug permeation. The effect of the iontophoresis of SNP, a derivative of SNA, was selected for examination in this study. As shown in Table 3, iontophoresis increased SNP permeation across intact skin from 0 to 0.81 μg/cm^2/h. However, this enhancement was less than that for SNA. The same

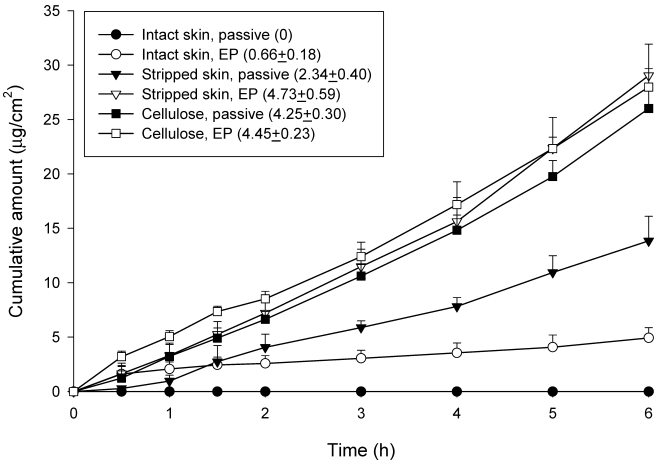

Fig. 5. Cumulative Amount–Time Profiles of Transdermal SNA Delivery across Various Types of Skin and Membranes with or without Electroporation Application

Each value represents the mean±S.D. (n=4).

Table 3. Effect of Various Solupor Membranes on the Flux of SNP across Various Skin Types

Membrane type	Application mode	Flux (μg/cm^2/h)
Intact skin	Passive	0
Intact skin	Iontophoresis	0.81±0.29
Stripped skin	Passive	0.80±0.06
Stripped skin	Iontophoresis	1.52±0.19
Cellulose	Passive	1.20±0.08
Cellulose	Iontophoresis	1.33±0.15
Skin+Solupor 7P03	Iontophoresis	0.44±0.07
Skin+Solupor 8P07	Iontophoresis	0.84±0.15
Skin+Solupor 10P01	Iontophoresis	0.34±0.01

Each value represents the mean±S.D. (n=4).

result was detected when using SC-stripped skin. These results indicate that the application of iontophoresis has a more-pronounced enhancement effect on the permeation of SNA than of SNP. This result was in accordance with our previous study,[12] showing iontophoresis had a limited effect on SNP permeation. Electroporation was also carried out to increase the permeation of SNP. Nevertheless, application of high-voltage pulsing did not change the negligible permeation of SNP across intact skin. In contrast to SNA, iontophoresis did not increase the diffusion of SNP across the cellulose membrane. This may suggest lower iontophoretic mobility of SNP during iontophoresis.

According to the permeation data of various skin types, the enhancement by iontophoresis (and electroporation) was more significant for SNA. This was possibly due to the effect of the molecular size and hydrophilicity of the permeants. A compound's MW, calculated molar volume, and solute radius have been shown to be inversely related to its iontophoretic mobility.[13] The iontophoretic mobility of a permeant is assumed to be proportional to the fractional volume of the space that is accessible to the ion sphere.[14] So the iontophoretic permeation of an ion solute has been shown to be directly related to the molar volume of the solute. The molar volume of an ion is related to its MW. Yoshida and Roberts reported that the logarithm of iontophoretic permeation declines linearly with increasing molecular volume.[15] The relationship for negatively charged solutes appears to be steeper than that for positively charged solutes. Although SNP has a higher molecular volume than SNA by just a single $-CH_2$ group, the anionic inherence of both molecules may result in the decisive difference observed between the iontophoretic permeation rates of SNA and SNP.

The application of iontophoresis has a more-pronounced enhancement effect on the permeation of more-hydrophilic molecules. This observation is consistent with the mechanistic model developed by Kontturi *et al*. to describe the transdermal transport of drugs under iontophoresis.[16] In their model, flux is divided into the contributions through the lipid matrix and through aqueous pores; iontophoresis enhances only the aqueous pathway:

$$J_{if}=J_w \cdot E+J_0;$$

where E is the enhancement factor, and J_{if}, J_w, and J_0 are the fluxes under iontophoresis, through the aqueous pathways, and through the lipid matrix, respectively.

Solupor 7P03, 8P07, and 10P01 membranes were selected as rate-controlling membranes to examine SNP permeation. As shown in Table 3, the trends of SNP permeation across these three membranes *via* iontophoresis were consistent with those for SNA. Solupor 7P03 and 10P01 inhibited one-half the value of the iontophoretic flux of SNP across intact skin. This ratio was in accordance with the results for SNA. This suggests that the difference in molecular size did not affect the rate-limiting level of Solupor. That is, it is possible to predict the permeation rate of various permeants after incorporation of this rate-controlling membrane.

CONCLUSIONS

This study illustrates the influence of Solupor membranes on the transdermal iontophoresis and electroporation of SNA. Most of the Solupor membranes examined in this study reduced and controlled the iontophoretic permeation of SNA across nude mouse skin, except Solupor 8P07, which presented only negligible resistance to the diffusion of SNA. Some attached particles on the surface of Solupor 10P05 and 16P05 membranes after iontophoretic application may also have contributed to the resistance to SNA permeation. The enhancement by electroporation of SNA delivery was weaker than that by iontophoresis. The rate-limiting property of Solupor membranes with electroporation showed a similar trend as compared to the results with iontophoresis. The permeation of SNP was significantly lower than that of SNA after application of either iontophoresis or electroporation. The molecular size and hydrophilicity may be the determinants of this difference. The same trend was observed for the rate-controlling properties of Solupor membranes for both SNP and SNA.

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