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Transdermal delivery of sodium nonivamide acetate from volatile vehicles: effects of polymers

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Abstract

The permeation of sodium nonivamide acetate (SNA), a newly designed analogue of capsaicin, from ethanol/pH 4.2 buffer solutions containing antinucleant polymers across rat skin, was investigated. The in vitro release of SNA was determined under an open condition at 25°C and 65% relative humidity. Therefore, the influence of the evaporation of vehicle components on the permeation of SNA was examined. Evaporation of the vehicle led to so drastic compositional changes that supersaturation is attained quickly. However, supersaturated solutions started to crystallize reducing the thermodynamic activity of SNA. Antinucleant polymers were used in the preparation of volatile vehicles in order to maintain the increased activity state of the drug. Methyl cellulose (MC) and hydroxypropyl cellulose (HPC) were both the efficient antinucleant polymers to increase the permeation of SNA. The permeation of SNA determined from volatile vehicles with 2% MC showed the result that the flux of SNA reached maximum at a certain ethanol proportion. A part of ethanol in the vehicle may penetrate into the skin causing the dehydration of stratum corneum and, therefore, the reduction of SNA permeation. The permeation of SNA was increased when ethanol in the volatile vehicle was replaced by *n*-propanol which could be due to the increased SNA solubility and reduction of diffusional barrier of stratum corneum in the presence of *n*-propanol.

Keywords: Sodium nonivamide acetate; Transdermal delivery; Volatile vehicle; Supersaturation; Antinucleant polymer

1. Introduction

Sodium nonivamide acetate (SNA; $C_{19}H_{28}NO_5Na$) is a newly designed derivative of capsaicin which was synthesized by alkylation of

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the phenolic hydroxyl group of nonivamide with bromoacetic acid (Fang et al., 1995). SNA reveals marked antinociceptive activity without producing overt pungent sensation and irritation that have been found in capsaicin (Chen et al., 1992; Fang et al., 1996c). Hence the improvement of patients' compliance can be completely achieved. Both penetration enhancers and iontophoresis have been used to enhance the permeation of SNA with varying success (Wu et al., 1995; Fang et al., 1996a,b, 1997). However, penetration enhancers alter the barrier properties of skin by exerting their effect on stratum corneum. Iontophoresis requires complex and expensive delivery devices. Penetration enhancement through the use of supersaturation provides an attractive alternative. Supersaturation is that state where drug concentration in a vehicle is greater than the saturated solubility. Therefore, supersaturated solutions increases the activity of a drug, so that it has a greater leaving tendency, producing an increased flux (Pellett et al., 1997). The main advantage of this method over other techniques is that it is inexpensive and does not greatly disturb the skin.

Supersaturated solutions are physically unstable by their very nature. The drug tends to crystallize upon preparation of the solution. The remarkable effects of antinucleant polymers in stabilizing supersaturated solutions have been utilized to demonstrate marked improvement in transdermal delivery from supersaturated systems (Kondo et al., 1987; Davis and Hadgraft, 1991). Many methods for preparing supersaturated solutions have been identified (Pellett et al., 1997). In this study, supersaturated solutions were prepared by the removal of volatile solvents (evaporation). Ethanol/pH 4.2 buffer co-solvent system as the volatile vehicle was mixed in different proportions in the presence of antinucleant polymers which stabilize the supersaturated solutions. The pH value of water phase was set at 4.2 because SNA showed higher permeation from pH 4.2 buffer than from the other buffers with different pH values (Tsai et al., 1994). The effects of supersaturation on the in vitro transdermal delivery of SNA across excised rat skin in an open condition are evaluated and reported here. 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 the other skin types (Fang et al., 1995).

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose (HPMC), 50 cps grade was obtained from Shin-Etsu Chemicals (Japan). Methyl cellulose (MC), 20–30 cps grade was purchased from Tokyo Kasei (Japan). Hydroxypropyl cellulose (HPC), 3–6 cps grade was supplied by Tokyo Kasei (Japan). Polyvinylpyrrolidone (PVP), MW 4000 was obtained from Tokyo Kasei (Japan). Eudragit was from Rohm Chemicals (Germany). The synthesis of SNA has been performed from our laboratory and reported earlier (Fang et al., 1995). All other chemicals and solvents were of analytical grade.

2.2. Solubility study

Excess SNA was added to a series of ethanol/pH 4.2 McIlvaine buffer co-solvent systems ranging from 0 to 40% (v/v). The suspensions were stirred using magnetic bars for 24 h in a water bath at 37 ± 1°C. After centrifugation for 10 min at 3000 rpm, the supernatant layer was directly injected into HPLC. The HPLC system for analyzing SNA was described previously (Tsai et al., 1994). Briefly, the mobile phase consisting of 60% pH 4 buffer and 40% acetonitrile was used at a flow rate of 1.0 ml/min. The C_{18} column effluent was passed through the fluorescence detector set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. The retention time of SNA and *p*-phenylphenol (internal standard) was found to be 2.9 and 7.6 min, respectively.

2.3. In vitro transdermal absorption study

The permeation of SNA was determined by

using Franz glass diffusion cell. The Wistar rat skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The release of SNA from volatile vehicles was carried out under the open condition at 25°C and 65% relative humidity of laboratory environment. The donor compartment was filled with 2 ml volatile vehicles with 0.02% SNA (w/v). Polymer with various types and concentrations was also incorporated into vehicles. A 20-ml aliquot of 1:1 (v/v) ethanol/pH 7.4 McIlvaine buffer was used as receptor medium to maintain a sink condition. The available diffusion area of cell was 2.54 cm^2. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 700 rpm. At appropriate intervals, 200-μl aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by HPLC.

2.4. Effect of ethanol on the permeation of SNA

The influence of ethanol proportions on SNA permeation through rat skin was assessed by the side-by-side diffusion cell under the close condition. The 15-ml ethanol/pH 4.2 buffer co-solvent systems ranging from 0 to 50% (v/v) with 0.02% SNA (w/v) were added to the donor phase. A 15-ml aliquot of 1:1 (v/v) ethanol/pH 7.4 McIlvaine buffer was used as receptor medium. The effective diffusion surface area was 2.00 cm^2. The other procedures were the same as those of the vertical diffusion cell experiment.

2.5. Evaporation of volatile vehicles

A tissue culture well plate with a flat bottom and a 2.54-cm^2 area was selected for the evaporation experiment. The plate is a non-absorbing material and thus all loss of the solvents is through evaporation. The plate was placed in an oven with a temperature of 25°C and 65% relative humidity. A 2-ml aliquot of vehicle was spread over the entire surface area of a plate and then weighed at determined intervals.

3. Results and discussion

3.1. Effect of antinucleant polymers on the permeation of SNA

The permeation of SNA from volatile vehicles (ethanol/pH 4.2 buffer; 25:75 v/v) was examined. As shown in Fig. 1, the volatile vehicle without polymers (control group) shows an initial increase in SNA permeation for 12 h follow-ed by an eventual decrease in the permeation. The initial increase of SNA permeation seen in this experiment is due to SNA partitioned from the volatile solution into the skin. As the solvent evaporated the thermodynamic activity of SNA increased and became greatest at permeation. However, the SNA permeation gradually leveled off, indicating that the initial high rate of permeation was not sustained. Presumably this was because of the further evaporation of volatile vehicle precipitated excess drug as a deposited film on the skin (Tsai et al., 1992). The precipitation limited the amount of SNA that could be absorbed and led to poor permeation of drug from the formulation because it could not effectively partition into the skin. The time dependencies of evaporation of volatile solution at 25°C are shown in Fig. 2. Ethanol evaporated rapidly causing a subsequent evaporation of water. In control group, $< 5\%$ of volatile vehicle remained after 4 h for all the volume applied. The permeated amount of SNA across skin obtained after 4 h may have been due to a depletion of the remaining drug deposited in the skin reservoir and a slight steady dissolution of the deposited drug film (Akhter and Barry, 1985).

It is assumed that membrane transport is directly proportional to the thermodynamic activity of a permeant in a vehicle. It has been reported that antinucleant polymers are excellent crystal growth retardants for some drugs and as such slow the transformation of the drug from its high energy state to the stable crystalline form (Kondo et al., 1987; Megrab et al., 1995a). Consequently the antinucleant polymers with a concentration of 2% (w/v) including MC, HPC, HPMC, PVP and Eudragit were used in the preparation of volatile vehicles in order to maintain the increased activity state of SNA. The permeation of SNA after the

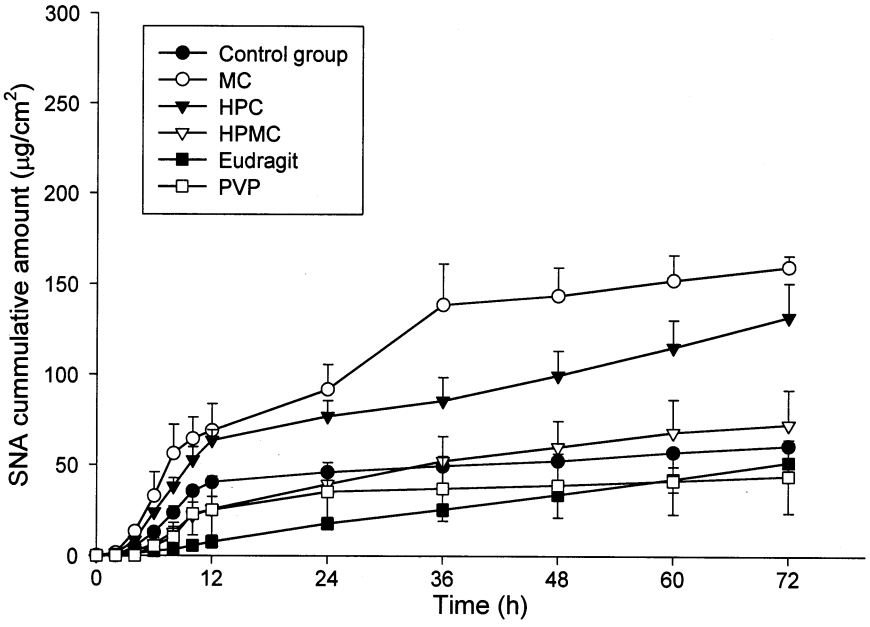

Fig. 1. In vitro permeation profiles of SNA from ethanol/pH 4.2 buffer volatile vehicle (25:75, v/v) in the presence of 2% polymers. All data represent the means of three experiments ± S.D.

addition of antinucleant polymers was examined as shown in Fig. 1. The permeation of SNA increased with the presence of cellulose polymers including MC, HPC and HPMC, with the first two showing a high degree of enhancement. Since SNA was administered onto the skin as a 2-ml dose of a 0.02% (w/v) concentration over an effective area of 2.54 cm^2. The administered dose of SNA may work out at 157 $\mu g/cm^2$. The curve of cumulative amount-time profile of SNA from MC-based formulation plateau at levels of about 160 $\mu g/cm^2$ after 36 h administration. This indicates that not only the precipitation limited the amount of SNA permeated through skin, but also the donor depletion contributed to this effect. One of the mechanism influencing the nucleation process is the adsorption of polymer on hydrophobic surface of crystals with regard to the stabilization of precipitates which result in the increase of thermodynamic activity of drug (Megrab et al., 1995a). MC shows the highest lipophilicity among three cellulose polymers (Narasimhan and Peppas, 1997). It would be expected that MC adsorbed to the hydrophobic surface of SNA more profoundly resulted in the higher thermodynamic activity of vehicle and permeation of SNA in the presence of MC.

In our in vitro permeation study design, the donor solution was not stirred and this raises the possibility that a polymer stagnant layer adjacent to the skin may contribute significantly to the overall diffusional resistance. The increase in the donor solution viscosity could have accentuated donor stationary layer effect (Megrab et al., 1995b). HPMC shows the most viscous characteristic among cellulose derivatives (Vazquez et al., 1992), which contributes to the lower SNA permeation as compared to MC and HPC. As shown in Fig. 1, there is no or negative effect of PVP and Eudragit on the permeation of SNA, indicating PVP and Eudragit may not to interact with SNA.

Fig. 2 shows that volatile solutions incorporated with polymers evaporate completely during 12 h application. Accordingly SNA released from dry film of polymer matrix after 12 h which, as can be seen in Fig. 1, contributes to a gradual increase in SNA cumulative amount after 12 h application. In comparison, there was no or negli-

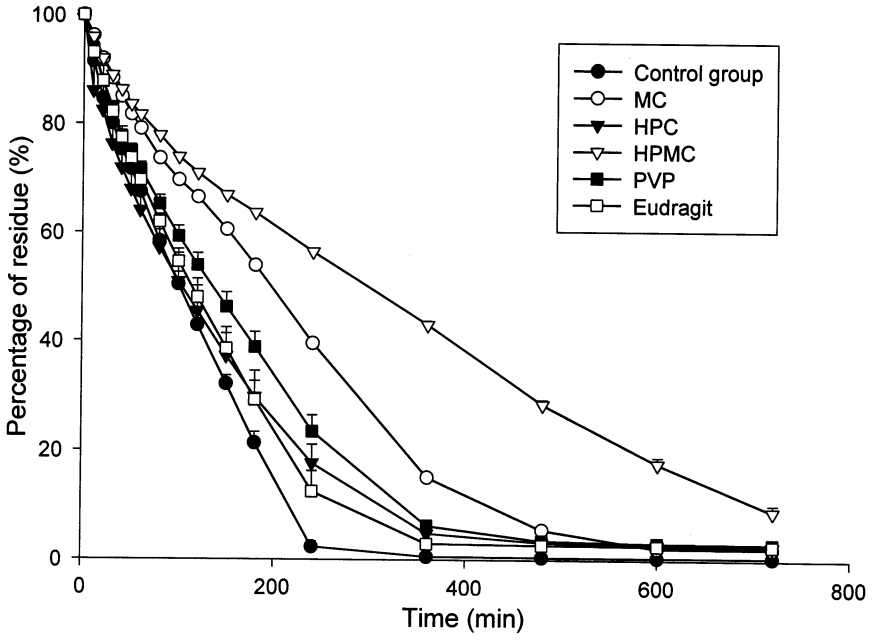

Fig. 2. Evaporation of ethanol/pH 4.2 buffer volatile vehicle (25:75, v/v) in the presence of 2% polymers at 25°C and 65% relative humidity as a function of time. All data represent the means of three experiments ± S.D.

gible increase in the cumulative amount of SNA for volatile solution without polymer (control group) after 12 h application because of the lack of vehicles. MC and HPC are the most efficient antinucleant polymer for SNA as determined in Fig. 1 and they are therefore selected for use of further studies.

3.2. Effect of MC and HPC concentrations on the permeation of SNA

The effect of MC and HPC concentrations on SNA permeation was investigated as shown in Fig. 3. SNA showed the lowest permeation in the presence of 4% MC during the first 12 h application, then the cumulative amount of SNA significantly increased and eventually surpassed the cumulative amount of SNA from 1 and 2% MC vehicles after 12 h (Fig. 3a). At the first 12 h, the viscosity of vehicles increased as the MC concentration rose which resulted in the increase of donor solution consistency and stationary layer effects (Megrab et al., 1995a). Accordingly the permeation of SNA reduced in the presence of 4% MC during the first 12-h application.

The slopes of SNA cumulative amount–time curves calculated from 12 to 72 h increased following the increase of MC concentration (1% MC, 1.27; 2% MC, 1.53; 4% MC, 2.64 $\mu g/cm^2/h$). Three parameters describe completely the adsorption of a polymer on drug: the amount of the adsorption per unit area of surface, the fraction of segments in contact with the surface and the distribution of segments in the vicinity of the surface (Hasegawa et al., 1988). Therefore the effect of adsorption became stronger with MC concentration, that is, higher concentration of MC may provide higher inhibition on the crystallization of SNA. This trend was consistent with those of the previous studies using sulfisoxasole and oestradiol as the model drugs with antinucleant polymers (Sekikawa et al., 1978; Megrab et al., 1995a).

The effect of HPC concentrations on the permeation of SNA were also studied (Fig. 3b). HPC showed the same effect on SNA permeation with MC did. However, there was a slighter change on SNA permeation when HPC concentration increased from 1 to 4% as compared to the result of MC.

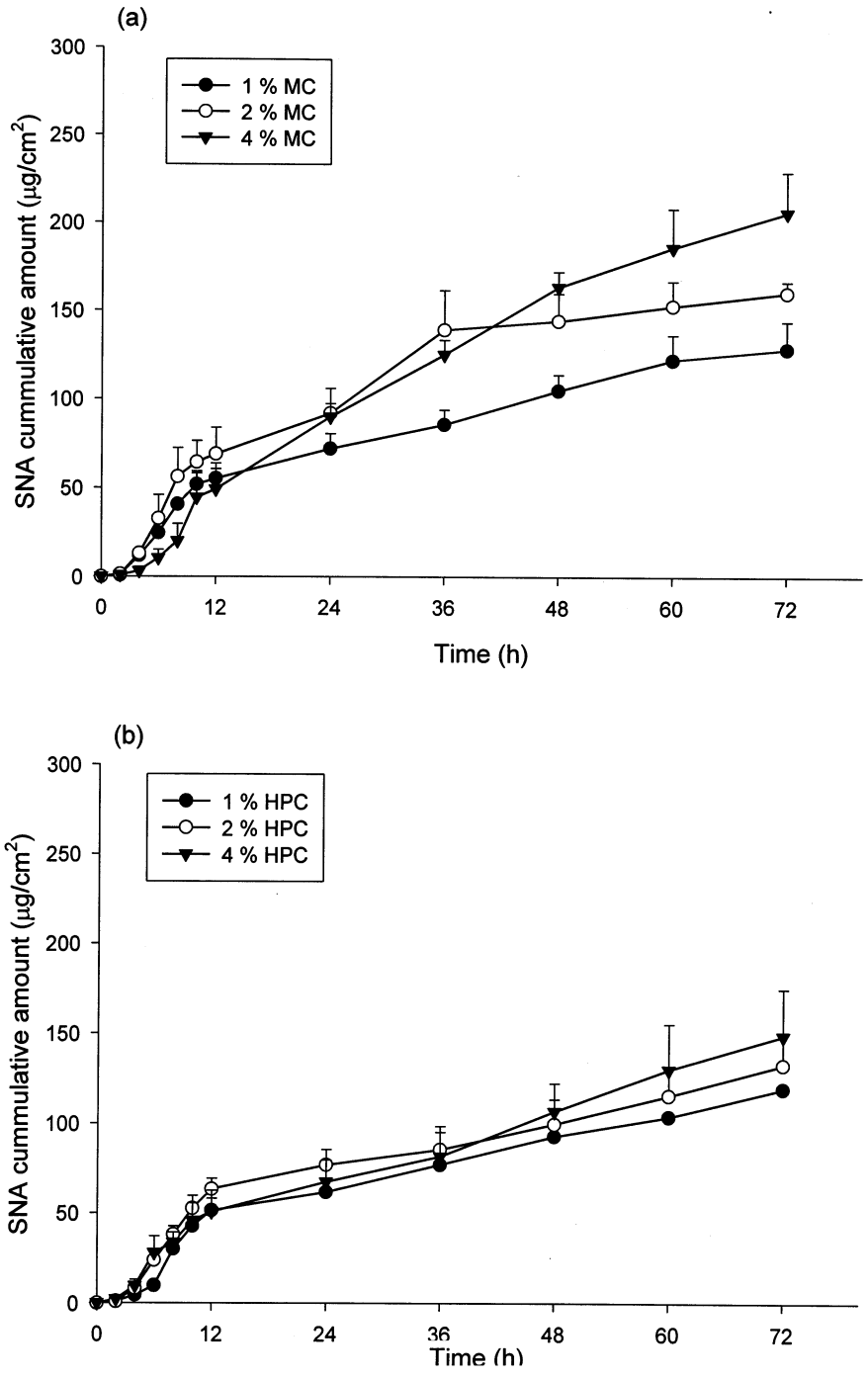

Fig. 3. In vitro permeation profiles of SNA from ethanol/pH 4.2 buffer volatile vehicle (25:75, v/v) in the presence of MC (a) and HPC (b) with different concentrations. All data represent the means of three experiments ± S.D.

Table 1
Solubility of SNA in ethanol/pH 4.2 buffer co-solvent system with different ratios at 37°C

Ethanol/pH 4.2 buffer ratio	Solubility (μg/ml)
0:100	77.17 ± 3.84
15:85	627.52 ± 30.35
25:75	1305.81 ± 63.49
40:60	2365.59 ± 83.50

Each data represents the mean $\pm$ S.D. ($n = 3$).

3.3. Effect of ethanol proportions on the permeation of SNA

In order to clarify the effect of ethanol on SNA permeation through the skin, influence of ethanol proportions in volatile vehicle on the solubility and permeation of SNA were investigated. Table 1 shows the solubility of SNA in different ethanol/pH 4.2 buffer solutions. SNA solubility increased following the increase of ethanol proportions as expected. Three volatile vehicles with 2% MC as well as 15, 25, and 40% ethanol concentrations respectively were used for in vitro permeation study in an open condition. The result shows that the permeation of SNA decreases in the order of $25 > 40 > 15\%$ during the first 12 h application (Fig. 4). A combination of two opposing factors may be considered to explain this result: (a) the lower ethanol proportion with lower SNA solubility may quickly attain the supersaturation after evaporation of vehicle; (b) the thermodynamic activity of SNA in the vehicle with higher ethanol proportion may be raised quickly due to the faster evaporation as shown in Fig. 5. This indicates that the effect of ethanol proportions on SNA permeation reaches a maximum at a certain proportion.

The slopes of SNA cumulative amount–time profile in Fig. 4 calculated from 12 to 72 h showed a trend of 15% (1.96 μg/cm^2/h) > 25% (1.53 μg/cm^2/h) > 40% (0.97 μg/cm^2/h) ethanol proportions. Since the volatile solvents of these three vehicles were completely evaporated during 14-h application, factors other than the vehicle effect may be expected to influence the permeation of SNA after 14 h. A part of ethanol in

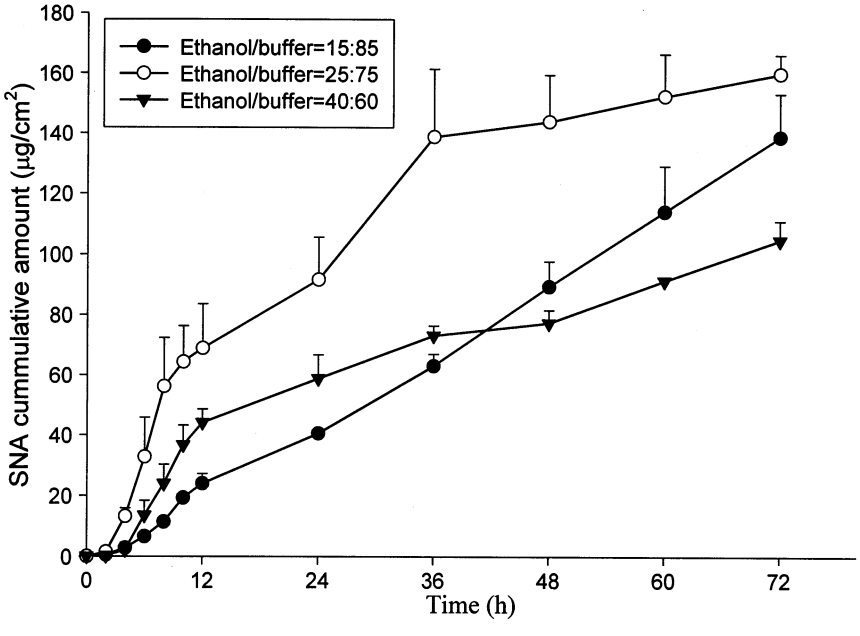

Fig. 4. In vitro permeation profiles of SNA from ethanol/pH 4.2 buffer volatile vehicle with different ratios in the presence of 2% MC. All data represent the means of three experiments $\pm$ S.D.

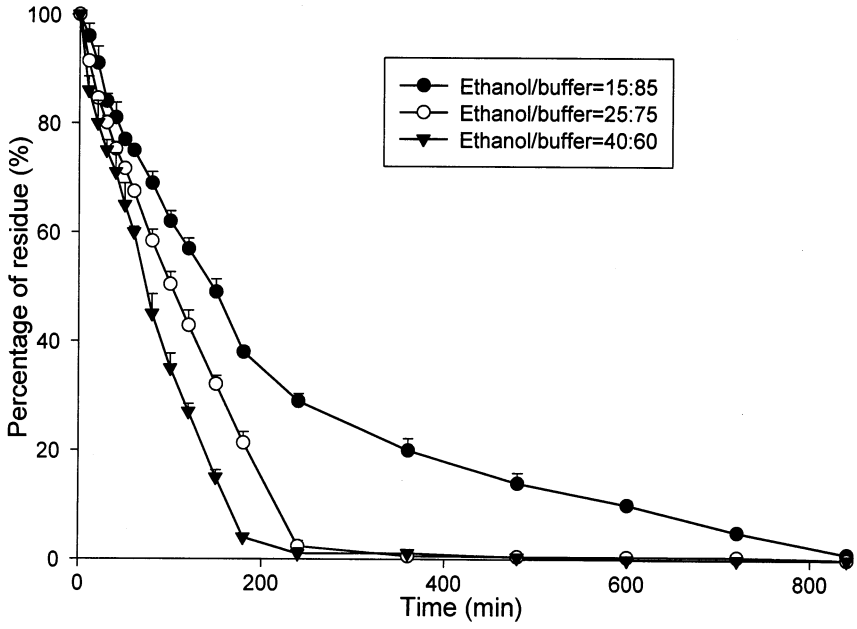

Fig. 5. Evaporation of ethanol/pH 4.2 buffer volatile vehicle with different ratios in the presence of 2% MC at 25°C and 65% relative humidity as a function of time. All data represent the means of three experiments ± S.D.

the volatile vehicle may penetrate into the stratum corneum after spreading over the skin. Increasing the ethanol concentration in stratum corneum leads to increased barrier properties of the skin because the outer portion of the stratum corneum is substantially dehydrated (Berner et al., 1989; Megrab et al., 1995b). In order to verify this mechanism on the permeation of SNA, different proportions of ethanol were added in the donor compartment of in vitro side-by-side diffusion cell in a closed condition to determine the SNA permeation. The SNA flux was calculated by the slope of the linear portion of the penetration curves and expressed as the mass of drug passing across 1 cm^2 of skin over time. As shown in Fig. 6, the SNA flux decreased following the increase of ethanol proportion no matter what the pH values of water phase were. There was even no SNA cumulative amount during 72-h application when the ethanol concentration was above 30%. It is well known that percutaneous absorption of most substances is increased by raising the water content of stratum corneum and its dehydration should therefore decrease the permeation of such substances (Megrab et al., 1995b).

3.4. Effect of *n*-propanol on the permeation of SNA

The permeation of SNA across the skin was also determined from *n*-propanol/pH 4.2 buffer (25:75 v/v) volatile vehicle (Fig. 7). The permeation of SNA from *n*-propanol/pH 4.2 buffer co-solvent system was slightly higher than that from ethanol/pH 4.2 buffer co-solvent system. The reason of the higher SNA permeation in the vehicle with *n*-propanol may be the higher solubility of SNA in *n*-propanol/pH 4.2 buffer solution (25:75, 1975.82 μg/ml) than that in ethanol/pH 4.2 buffer solution in the same ratio. Another possible reason was that the reduction of the diffusional barrier by extracting stratum corneum lipids and proteins increases with increasing chain length of alkanols (Kai et al., 1990; Goldberg-Cettina et al., 1995). However, the

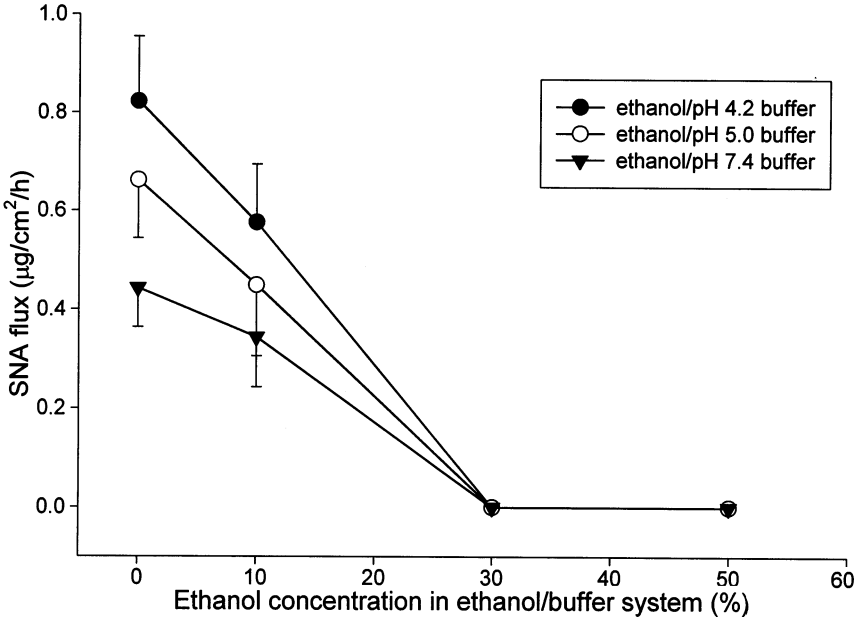

Fig. 6. Flux of SNA from ethanol/buffer co-solvent system with different ratios. All data represent the means of three experiments ± S.D.

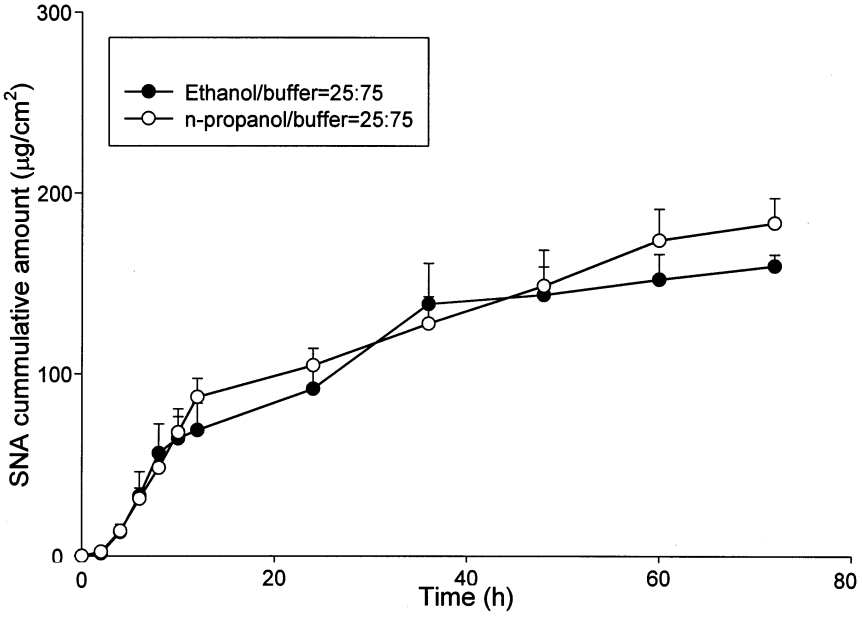

Fig. 7. In vitro permeation profiles of SNA from ethanol/pH 4.2 buffer and *n*-propanol/pH 4.2 buffer volatile vehicles. All data represent the means of three experiments ± S.D.

donor depletion effect limited the further increase of SNA permeated amount from *n*-propanol/pH 4.2 buffer solution.

4. Conclusion

This study has investigated the feasibility of using volatile vehicles of SNA as a means of enhancing permeation of SNA across skin. Evaporation of the vehicle precipitates the drug as a solid film and this is accompanied by a fall in the permeation. The thermodynamic activity and permeation of SNA were significantly modified in the presence of antinucleant polymers including MC and HPC. The permeation of SNA from volatile vehicles basically increased following the increase of polymer concentration. The effect of ethanol proportions in volatile vehicles on SNA permeation reached a maximum at a certain proportion and decreased with further increase in the proportion during the first 12 h of application. However, the permeation of SNA eventually decreased following the increase of ethanol proportions after 12-h application due to the fact that ethanol decreases the hydration level of stratum corneum and thus the permeation of SNA was retarded. In this paper, the molecular mechanisms affecting the SNA crystallinity and thermodynamic activity by polymers were not studied. Further investigation is in progress to elucidate these mechanisms.

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