Preparation of a polymer containing hexadecylpyridinium bromide groups and its utilization as a transdermal drug penetration enhancer

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A novel polymeric transdermal penetration system was investigated. The polymer was synthesized by the reaction of poly(4-vinylpyridine) and hexadecyl bromide, and its enhancing activity on drug penetration was evaluated by means of *in vitro* experiments. The amount of 5-fluorouracil (5-FU) that permeated through the skin was determined by using a two-chamber diffusion cell, in which isolated rabbit abdominal skin was mounted. Addition of the polymer effectively enhanced the penetration rate of 5-FU. The permeability coefficient was 2–8 times as much as that in the absence of the polymer. The enhancing activity increased with increase in the degree of quaternization. The mechanism was investigated by differential scanning calorimetry of whole skin and stratum corneum treated with the polymer. Through this investigation, it was shown that, although the polymer interacted with the lipids and proteins of the membranes of the stratum corneum, it may not penetrate into deeper layers of the skin. The absence of irritation of the polymer to the skin was confirmed by the Draize test.

(Keywords: poly(vinylpyridine); drug transport; synthesis; quaternization)

INTRODUCTION

Some surfactants are well known to enhance the rate of transdermal drug penetration (TDP). However, ionic surfactants, especially, sometimes irritate the skin, and their usage is limited[1]. Therefore, non-ionic surfactants have been added to the vehicle to enhance drug penetration into the skin because they are less irritant[1,2]. It is a basic requirement that a TDP enhancer is not toxic, is not irritant and is physiologically inactive[3]. Wong *et al.* have reported the preparation of non-toxic biodegradable transdermal penetration enhancers, namely unsaturated cyclic ureas, and have noted their enhancing effect[4,5].

In our previous paper, we have reported the polymerization of a cationic surfactant monomer, namely a benzalkonium chloride monomer, and noted the enhancing activity of this polymeric enhancer[6]. The objects of that report were the avoidance of irritation to the skin and the maintenance of high penetration enhancing activity. In this paper, a structurally alternative cationic surfactant polymer was studied to confirm whether the compound could enhance drug penetration or not. The polymeric enhancer was prepared by the reaction of poly(4-vinylpyridine) with hexadecyl bromide. Further, the enhancing mechanism of the resulting polymeric enhancer was investigated by differential scanning calorimetry of whole skin and stratum corneum treated with the polymer. The extent of irritation of the compound to the skin was determined by the Draize method.

EXPERIMENTAL

Materials

4-Vinylpyridine (4-VP) was purchased from Wako Pure Chemical Industries Ltd, and purified by distillation under reduced pressure. Anhydrous toluene was prepared by distillation over metallic sodium. Hexadecyl bromide was obtained from Tokyo Kasei Co. Ltd and used without further purification. 5-Fluorouracil (5-FU) was purchased from Sigma (St Louis, USA) and used as received.

Preparation of poly(4-VP)

First, 11.7 ml of 15% n-hexane solution of n-butyllithium was added to a solution of 20 ml of freshly distilled 4-VP in 100 ml of dry toluene at −78°C under an argon atmosphere. After stirring for 30 min, a small amount of methanol was added to the reaction mixture to terminate the polymerization. The minimum amount of methanol was added to dissolve the precipitated polymer, and the mixture was poured into a large amount of n-hexane to afford precipitation of poly(4-VP). This procedure was repeated twice to ensure complete removal of the unpolymerized monomer. The yield was 19.5 g of the product, as a slightly yellowish white powder. Another poly(4-VP) having a lower molecular weight was prepared by increasing the ratio of n-butyllithium to 4-VP.

Preparation of a polymer containing hexadecylpyridinium moiety

The appropriate amount of hexadecyl bromide was added to *N*,*N*-dimethylformamide (DMF) solution of

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poly(4-VP), and the mixture was stirred at 60°C for 4 h. After cooling to room temperature, the reaction mixture was poured into a large amount of ethyl acetate. The polymer was separated by filtration, washed with ethyl acetate, and dried *in vacuo* for two days. The content of hexadecylpyridinium bromide groups in the polymer was controlled by changing the ratio of hexadecyl bromide to poly(4-VP), as shown later in *Table 2*.

Characterizations

The number-average and weight-average molecular weights of poly(4-VP) were determined via gel permeation chromatography (pump, HLC-803A; detector, RI-8010; column, TSK-Gel G5000HXL, G4000HXL, G3000HXL, G2000HXL, Tosoh Corporation). DMF was used as the solvent, and polystyrenes were used as the standard for calibrating molecular weight.

A Perkin–Elmer 240 Elemental Analyzer was employed to calculate the content of hexadecylpyridinium bromide in the obtained polymer.

Measurement of amount of 5-FU permeated through the skin

The abdomen of rabbit (Japanese White, male, 2.5–2.7 kg) was carefully shaved, under anaesthesia using sodium pentobarbital. Next, the blood was gradually withdrawn from the femoral artery and the rabbit was dehaemotized completely. Then, the abdominal skin was excised and mounted between the two half-cells of a two-chamber diffusion cell whose cross-section was 0.95 cm^2. The schematic representation of the diffusion cell is shown in *Figure 1*[7].

An ethanol suspension (2 ml) containing 5-FU (1% w/v, suspended) and the polymer (5% w/v, perfectly dissolved) was placed into the donor compartment, while a phosphate-buffered solution (2 ml) adjusted to pH 7.4 was in the receiver compartment. Then this diffusion cell was immersed in a water bath regulated at 37°C. Both solutions in the donor and the receiver compartments were mechanically stirred. An aliquot (100 μl) was withdrawn from the receiver compartment at 2 h intervals during 12 h, and submitted to high-performance liquid chromatography (h.p.l.c.) (pump, HLC-803A; detector, UV-8 model 2; column, TSK-Gel ODS-80TM, Tosoh Corporation) to measure the amount of 5-FU permeated through the skin. The mobile phase in h.p.l.c. was a mixture of acetonitrile and 1×10^{-3} M aqueous KH_2PO_4 solution (1/9 by volume), and the flow rate was 1.0 ml min^{-1}. The sample was diluted with the same solution as that used for the mobile phase. The amount of 5-FU was determined from the integrated area of the chromatogram monitored at 266 nm.

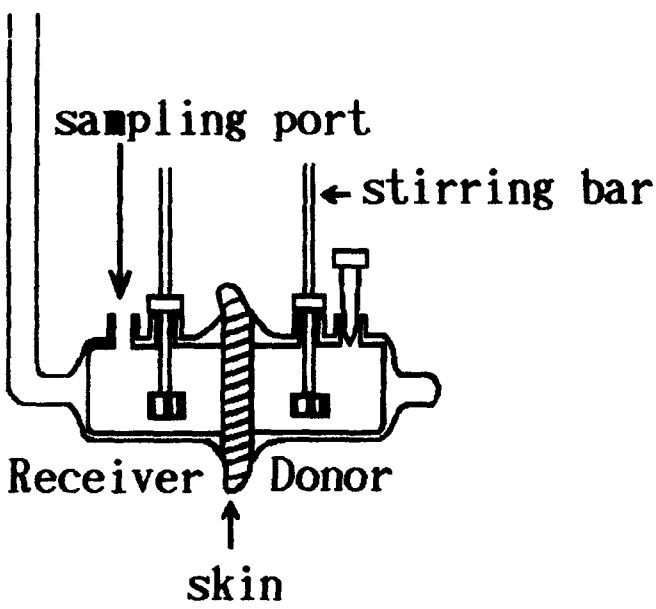


Figure 1 Schematic representation of two-chamber diffusion cell

Data from the diffusion experiment are presented as the mean ± s.e.m. of three or four experiments, and a statistically significant difference between results with and without enhancer was confirmed by the Student's *t*-test.

Determination of solubility of 5-FU before or after addition of the polymer

5-FU was suspended in EtOH (1% w/v) and stirred at 37°C over a day. A given volume of the sample was taken out from this suspension and the concentration of 5-FU dissolved was measured by h.p.l.c. after insoluble 5-FU was removed by filtration. Then, the polymer was added to the residual suspension (the concentration of the polymer was adjusted to 5% w/v), and stirring was continued again at 37°C for another day. The concentration of 5-FU further dissolved upon the addition of polymer was determined by exactly the same method.

Differential scanning calorimetry (d.s.c.) of rabbit skin

The thermal behaviour of rabbit whole skin and stratum corneum treated with the polymer was investigated by using a differential scanning calorimeter (Seiko I&E DSC 20 and SSC/580 thermal controller). The excised abdominal skin was mounted between the two half-cells of the two-chamber diffusion cell. Ethanol or an ethanol solution containing 5% of polymer was poured into the donor compartment and a phosphate-buffered solution, which was the same as that adopted in the 5-FU diffusion experiment, was poured into the receiver compartment. After stirring both sides for 16 h at 37°C, the treated skin was removed and submitted to d.s.c. measurement at a heating rate of 10°C min^{-1} from 10 to 190°C. Concerning the stratum corneum, the whole skin treated by the method mentioned above was immersed for 3 h in Tris buffer (pH 7.4) containing 0.5% trypsin[8,9]. Then, the stratum corneum was isolated from the epidermis and the sample was air-dried on a filter paper at room temperature for a day. The thermal behaviour was measured by d.s.c. under the same conditions as described above.

Draize test[10]

Any irritation to the skin due to the polymeric enhancer was evaluated by the Draize test using rabbits. The two groups of four rabbits each were prepared. One group (normal skin group) was made up of rabbits whose back skins were only shaved, and the other group (damaged skin group) was made up of rabbits in which abrasions through the stratum corneum were given in the same position. The gauzes (1 inch × 1 inch) were soaked in 1,3-butylene glycol solution containing 5 wt% of the polymer (P-9), and were applied on the shaved skins. Conditions after 24 and 72 h were decided on the basis of the following:

(1) erythema and incrustation

Condition	Points
Without erythema	0
Very slight erythema	1
Obvious erythema	2
Medium or strong erythema	3
Strong and crimson erythema and slight incrustation	4

(2) oedematization

Condition	Points
Without oedema	0
Very slight oedema	1
Obvious oedema	2
Medium oedema	3
Strong oedema	4

The points corresponding to the conditions after 24 and 72 h (erythema and oedema together) were summed up respectively, and the average value was defined as *PII* value.

RESULTS AND DISCUSSION

Preparation of the polymers

The results of the preparations of poly(4-VP) are summarized in *Table 1*. The polymers were synthesized by anionic polymerization according to the literature[11]. The molecular weights of both polymers obtained were larger than expected, because of heterogeneity in the polymerization. The synthetic scheme of the polymer containing hexadecylpyridinium bromide is shown in *Scheme 1* and results are summarized in *Table 2*. In general, quaternary ammonium salts are easily prepared by the reaction of the tertiary amines with the alkyl halides (Menschutkin reaction). In this reaction, use of an aprotic solvent, such as formamide, ethylene glycol and DMF, is effective[12]. In our study, DMF was suitable. The content of hexadecylpyridinium bromide moiety in the polymer was in the range of 11–62 mol%, which could be controlled by changing the molar ratio of hexadecyl bromide to poly(4-VP) from 1/5 to 3/1.

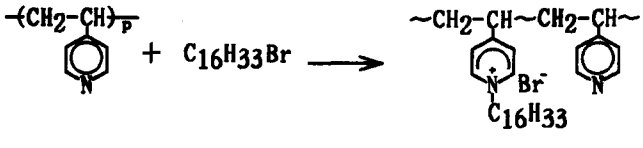
Skin penetration experiments

Figures 2 and *3* show the permeation profiles of 5-FU through the skin with the polymers used as a penetration enhancer. Polymers P-4, P-5, P-8 and P-9 effectively enhanced drug absorption. In these polymers, the contents of pyridinium salt were more than 45 mol%. On the other hand, the addition of P-1, P-2 and P-6 depressed the rate of drug penetration. These polymers have a relatively small amount of pyridine moiety.

Table 1 Preparations of 4-vinylpyridine homopolymers

Sample No.	Monomer/ n-butyllithium (molar ratio)	M_n[a] $\times 10^{-3}$	M_w[a] $\times 10^{-3}$	Yield (%)
4VP-1	10/1	11.5	22.5	99.5
4VP-2	3.3/1	9.8	15.2	79.8

[a]Determined by g.p.c.



Scheme 1 Preparation of polymeric enhancers

Table 2 Quarternization of 4-vinylpyridines with hexadecyl bromide

Sample No.	Poly(4-VP) (g)	Hexadecyl bromide/ poly(4-VP) (molar ratio)	Content of pyridinium group[a] (mol%)	Yield (g)
P-1	1.5[b]	1/5	11	1.85
P-2	1.5[b]	1/2	19	1.36
P-3	1.5[b]	1/1	28	1.06
P-4	2.5[b]	2/1	54	4.15
P-5	2.5[b]	3/1	62	5.43
P-6	1.5[c]	1/2	20	1.79
P-7	1.5[c]	1/1	40	2.44
P-8	1.5[c]	2/1	47	2.69
P-9	2.5[c]	3/1	56	5.20

[a]Determined from elemental analysis
[b]4VP-1
[c]4VP-2

In drug permeation through the skin, the permeation profile can be represented by Fick's first law of diffusion under a sink condition:

$$Q = (DK/h)AC_d t \quad (1)$$

where Q is the cumulative amount of drug permeated at time t, D is the diffusion coefficient, K is the partition coefficient of the drug between vehicle and skin, h is the thickness of skin, C_d is the concentration of drug in the vehicle and A is the effective area of drug permeation. In equation (1), the value of DK/h is the so-called permeability coefficient. The permeability coefficients through the skin for 5-FU with the use of each polymeric enhancer were calculated on the basis of the data in *Figures 2* and *3*. The relationship between the value of permeability coefficients for 5-FU and the content of hexadecylpyridinium salt in the polymer is shown in *Figure 4*. In the cases of both polymers delivered from 4VP-1 and 4VP-2, the enhancing activity was intensified steeply with increase in the content of hexadecylpyridinium bromide units. On the other hand, the polymers delivered from 4VP-2 with lower molecular weight showed a larger permeability coefficient than those from the larger 4VP-1. Therefore, this indicates that the activity of penetration enhancement is probably affected not only by the content of hexadecylpyridinium bromide in the polymer but also by the molecular weight of the polymer.

Solubility of 5-FU in ethanol in the presence or absence of the polymers is shown in *Table 3*. The solubility of 5-FU was almost unchanged by adding the polymer. Hence, the solubility of the drug remained almost constant in all the donor solutions of the diffusion experiment. Such a high enhancing activity of the polymers in drug penetration indicates that the polymers work at the surface of the skin, and consequently the diffusivity of the drug increases.

D.s.c. measurements

In our previous paper[6], it was revealed by means of d.s.c. measurement that benzalkonium chloride polymer

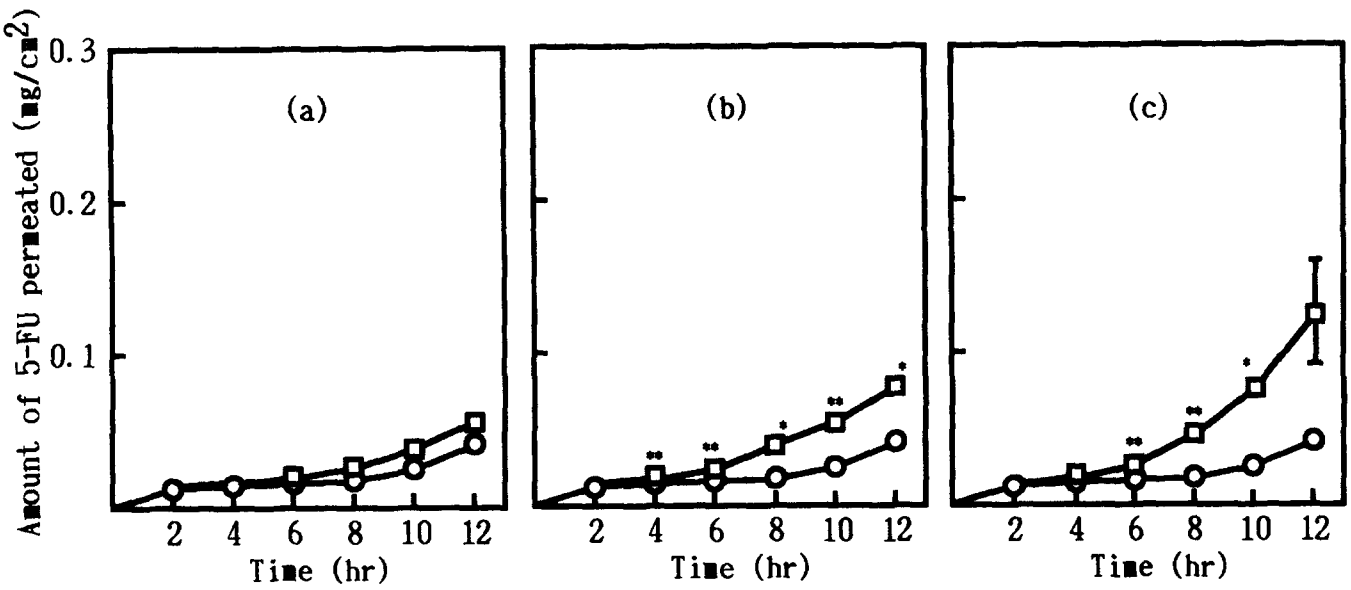


Figure 2 Permeation profiles of 5-FU through rabbit abdominal skin using polymeric enhancers: (a) P-3, (b) P-4, (c) P-5; (○) control (without enhancer), (□) with enhancer; * $p < 0.05$, ** $p < 0.02$ *versus* control

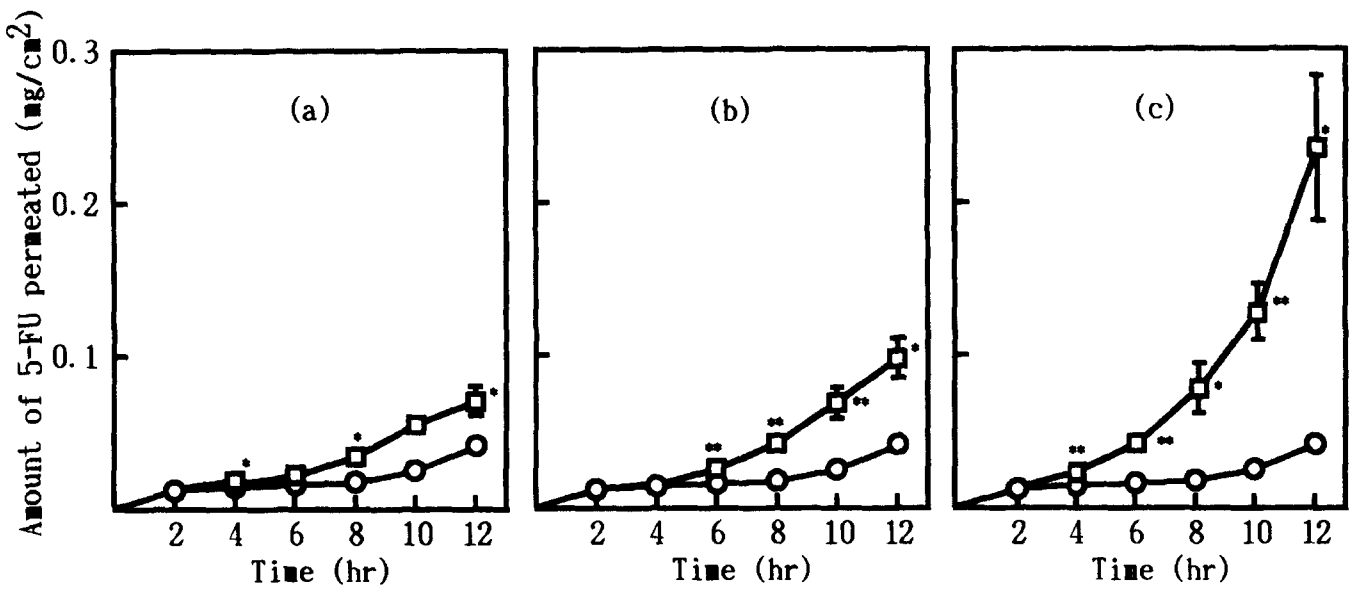


Figure 3 Permeation profiles of 5-FU through rabbit abdominal skin using polymeric enhancers: (a) P-7, (b) P-8, (c) P-9; (○) control (without enhancer), (□) with enhancer; * $p < 0.05$, ** $p < 0.02$ *versus* control

Table 3 Effect of the added polymer on the solubility of 5-FU in EtOH

	Solubility (mg ml^{-1})		
Sample No.	Without polymer	With polymer	With/without
P-5	4.48	5.84	1.30
P-9	4.48	6.38	1.42

permeated only the stratum corneum and did not permeate to the dermis layer. In order to confirm whether the present polymer containing hexadecylpyridinium bromide units acts similarly or not, the thermal behaviours of stratum corneum and whole skin treated with the polymer solution were investigated by the d.s.c. method.

D.s.c. curves of stratum corneum untreated and treated with the polymer are shown in *Figure 5*. Intact stratum corneum showed the four endothermic peaks, 47°C (t_1), 64°C (t_2), 79°C (t_3) and 110°C (t_4), respectively. Barry reported that the peaks at t_1, t_2 and t_3 were due to melting of lipids in the stratum corneum and the t_4 peak was attributed to protein denaturation[13]. In the polymer-treated case the t_1 and t_3 peaks disappeared and the t_4 peak shifted to higher temperature and became broader. Therefore, the enhancing effect with these polymers seemed to be due to the interaction with both lipid and protein portions. *Figure 6* shows the thermal profile of whole skin. In the case of polymer modification, the

endothermic peak at 110°C (based on protein denaturation) corresponded to that of intact skin. The peak at around 90°C is probably due to the residual ethanol remaining in the tissue.

From these d.s.c. investigations, it seemed that the polymeric enhancers changed the structure of lipid membranes and proteins in the stratum corneum, leading to enhancement of the rate of drug penetration. Moreover, the polymers themselves might not penetrate into the deep layers of the skin owing to their bulkiness.

Irritation of the skin

The results of the Draize test are summarized in *Table 4*. In the case of polymeric enhancer P-9, the *PII* value was smaller than 0.5. This indicates that the polymer does not irritate the skin and is as safe as transdermal enhancers in practical use[10]. This result is not inconsistent with that obtained in d.s.c. measurements. Consequently, it was demonstrated that the hexadecylpyridinium bromide moiety might be responsible for the high enhancing activity of drug penetration, and also that such a polymeric enhancer showed low irritation to the skin owing to its high molecular weight.

Table 4 Results of Draize test

Enhancer	Points		*PII*
	Normal skin	Damaged skin	
P-9	0.5 ± 0.4	0.0	0.3 ± 0.2
Control	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3

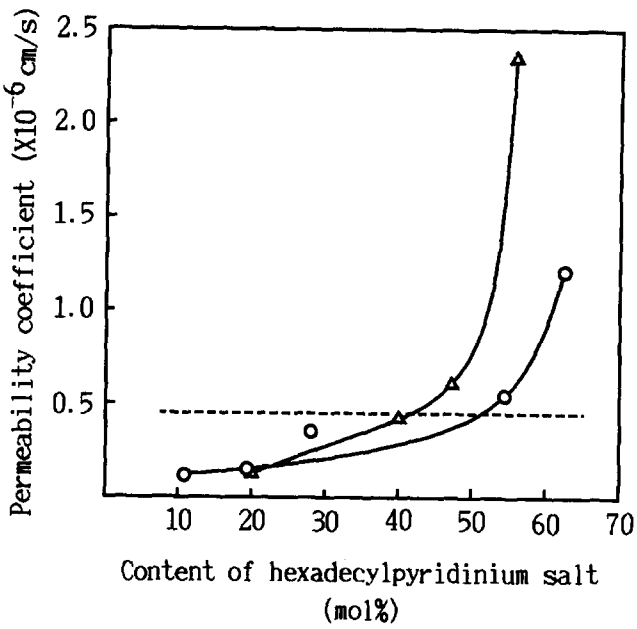


Figure 4 Effect of the content of hexadecylpyridinium salt in the polymer on the permeability coefficient of 5-FU through the skin: (○) using polymers P-1, P-2, P-3, P-4 and P-5; (△) using P-6, P-7, P-8 and P-9. The permeability coefficient without enhancer is 4.90×10^{-7} cm s^{-1}, as shown by the broken line

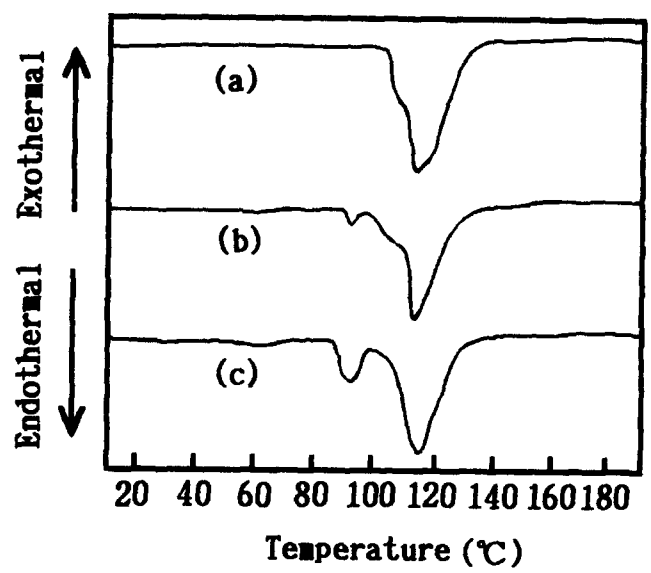


Figure 6 D.s.c. curves of whole skin: (a) intact, (b) treated with ethanol, (c) treated with an ethanolic solution of P-9

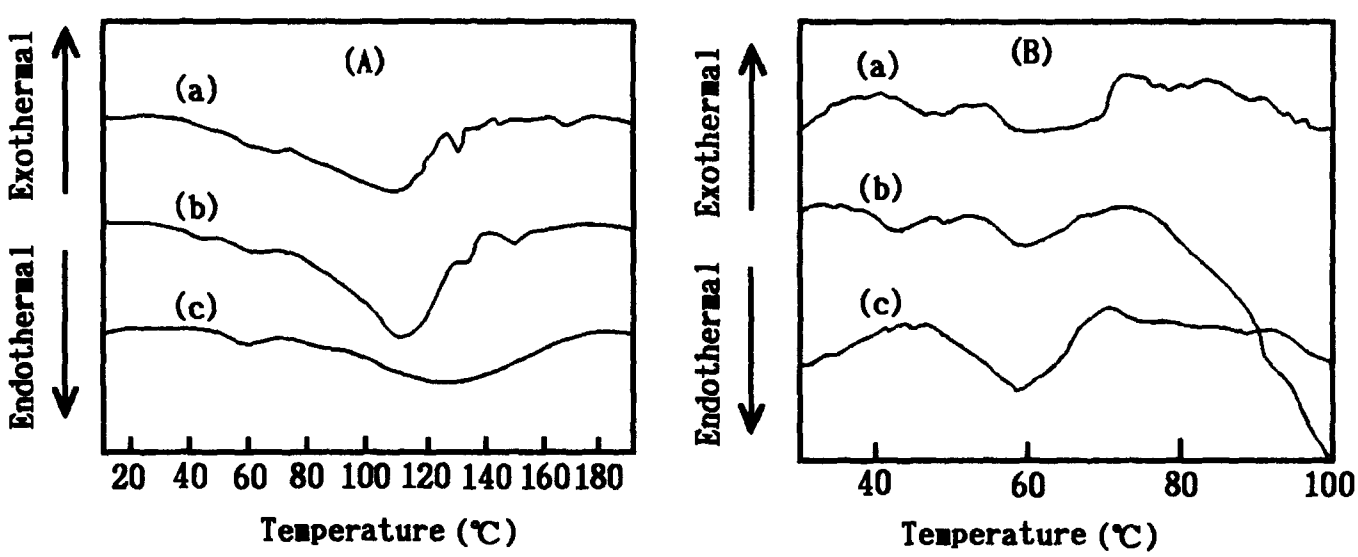


Figure 5 D.s.c. curves of stratum corneum: (A) temperature range from 10 to 190°C, (B) temperature range from 30 to 90°C (both axes are extended); (a) intact, (b) treated with ethanol, (c) treated with an ethanolic solution of P-9

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