Effects of Mixing Procedure Itself on the Structure, Viscosity, and Spreadability of White Petrolatum and Salicylic Acid Ointment and the Skin Permeation of Salicylic Acid

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White petrolatum is a mixture of solid and liquid hydrocarbons and its structure can be affected by shear stress. Thus, it might also induce changes in its rheological properties. In this study, we used polarization microscopy to investigate how different mixing methods affect the structure of white petrolatum. We used two different mixing methods, mixing using a rotation/revolution mixer and mixing using an ointment slab and an ointment spatula. The extent of the fragmentation and dispersal of the solid portion of white petrolatum depended on the mixing conditions. Next, we examined the changes in the structure of a salicylic acid ointment, in which white petrolatum was used as a base, induced by mixing and found that the salicylic acid solids within the ointment were also dispersed. In addition to these structural changes, the viscosity and thixotropic behavior of both test substances also decreased in a mixing condition-dependent manner. The reductions in these parameters were most marked after mixing with a rotation/revolution mixer, and similar results were obtained for spreadability. We also investigated the effects of mixing procedure on the skin accumulation and permeation of salicylic acid. They were increased by approximately three-fold after mixing. Little difference in skin accumulation or permeation was detected between the two mixing methods. These findings indicate that mixing procedures themselves affect the utility and physiological effects of white petrolatum-based ointments. Therefore, these effects should be considered when mixing is required for the clinical use of petrolatum-based ointments.

Key words ointment; petrolatum; mixing procedure; viscosity; skin accumulation; salicylic acid

Results and Discussion

Using a polarization microscope, we first examined the structure of white petrolatum before and after it had been mixed using one of two mixing methods; i.e., with a rotation/revolution mixer or with a combination of an ointment slab and an ointment spatula. Before it was mixed, solid petrolatum components, which were presented as bright domains, were observed, as shown in Fig. 1a. After the petrolatum had been mixed with a rotation/revolution mixer, the solid components were fragmented and dispersed, as shown in Fig. 1b, which might indicate that the three-dimensional crystalline network found in the solid petrolatum had been destroyed. We also examined the effects of mixing white petrolatum with an ointment slab and an ointment spatula and found that this too resulted in the breaking down of the solid components, as shown in Fig. 1c.

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We next investigated the effects of each mixing procedure itself on the structure of a salicylic acid ointment in which petrolatum was used as a base. As shown in Figs. 1d–f, the salicylic acid solids in the ointment were fragmented and dispersed by mixing, with the extent of these processes depending on the mixing procedure employed. The solid petrolatum within the ointment also seemed to be broken down and dispersed by mixing, although it was not clear due to the presence of the salicylic acid solids.

The structural changes induced in white petrolatum and the salicylic acid ointment by mixing suggested that mixing might cause changes in their rheological properties. Since viscosity is an important rheological property with regard to the stability of white petrolatum-containing ointments during storage and the sensations they induce in patients during their use and might affect the skin permeation of any drugs contained within such ointments, we next examined the effects of each mixing procedure on the viscosity of white petrolatum and the salicylic acid ointment. As shown in Fig. 2a, white petrolatum exhibited non-Newtonian flow behavior. The viscosity of
white petrolatum changed markedly after it had been mixed. Before it was mixed, the white petrolatum exhibited high viscosity and a large hysteresis loop, which indicated that it was markedly thixotropic. In addition, it also displayed yield stress. The thixotropic behavior and yield stress demonstrated by the white petrolatum were assumed to be due to the network structure of its solid components. After the white petrolatum had been mixed with a rotation/revolution mixer, its viscosity decreased markedly, and it displayed less thixotropic behavior and reduced yield stress. A reduction in viscosity was observed even when the mixing was performed at a rate of 500 rpm for 20 s. When the white petrolatum was mixed with an ointment slab and an ointment spatula, its viscosity decreased in a repeat count dependent manner; however, it exhibited greater viscosity than the white petrolatum that had been mixed with a rotation/revolution mixer, and the same results were obtained when the above mixing procedure was carried out by different individuals. In addition, it continued to display thixotropic behavior. The yield stress of the white petrolatum also decreased in a mixing condition-dependent manner. Similar findings were obtained for four other white petrolatum products of similar quality and another four high quality products that are currently available in Japan. Further studies of these products are currently being conducted.

Furthermore, similar results were obtained for the salicylic acid ointment, as shown in Fig. 2b. Like white petrolatum, the salicylic acid ointment exhibited significant thixotropic behavior and yield stress. After the ointment had been mixed with a rotation/revolution mixer, its viscosity decreased, and its thixotropic behavior almost disappeared. On the other hand, after the ointment was mixed with an ointment slab and an ointment spatula its viscosity decreased in a mixing time-dependent manner, but it still displayed greater viscosity than was observed after it was mixed with a rotation/revolution mixer. In addition, it continued to exhibit significant thixotropic behavior. The yield stress demonstrated by the ointment decreased in a mixing method-dependent manner.

The differences between the rheograms for the white petrolatum (Fig. 2a) and salicylic acid ointment (Fig. 2b) seemed to be due to the effects of the ointment’s high concentration of salicylic acid. If this is true, then osmotic pressure or the presence of undissolved salicylic acid solids might affect the viscosity of such products. It is also possible that the viscosity of the white petrolatum used as a base in the salicylic acid ointment differed from that of the white petrolatum examined in this study because the viscosity of some of the high purity white petrolatum products differed from that of the white petrolatum used in the main part of this study.

After the white petrolatum and salicylic acid ointment had been mixed, their viscosities partly recovered. However, they did not recover completely, even after four weeks (Fig. 2c), suggesting that most of the changes were irreversible.

We next examined the effects of mixing on the spreadability of white petrolatum and the salicylic acid ointment. Spreadability is an important physical property of ointments because it is an indicator of their utility. As shown in Fig. 3a, the slopes of the regression lines corresponding to spreadability decreased in a mixing condition-dependent manner. The spreadability of white petrolatum was lowest after it had been mixed with a rotation/revolution mixer.

Similar results were obtained for the spreadability of the salicylic acid ointment, as shown in Fig. 3b; i.e., the spreadability of the ointment was lowest after it had been mixed with a rotation/revolution mixer. As was found for viscosity, the spreadability of the salicylic acid ointment differed from that of the white petrolatum, probably for similar reasons to those mentioned above. We will investigate the exact reasons for these differences in a future study.

To examine whether the method used to mix white petrolatum-based ointments itself affects the skin accumulation and permeation of the drugs they contain, we examined the effects of two mixing methods on the skin permeation of salicylic acid after the application of salicylic acid ointment in vitro to Yucatan micropig skin. As shown in Fig. 4, after being incorporated into the micropig skin the salicylic acid mainly remained in the dermis and gradually permeated into the receptor fluid from 6 h onwards. The dermal accumulation and permeation of salicylic acid were increased by nearly three-fold by both mixing procedures. Little difference in the skin permeation or accumulation of salicylic acid was observed between the mixing methods. Although mixing induced less marked increases in the epidermal accumulation of salicylic
acid, this might have been due to issues with the experimental method, such as the incomplete washing out of ointment that had adsorbed to the epidermal surface and/or anomalies in our data caused by the thinness of the epidermis.

The present findings indicate that the structure of white petrolatum is altered by mixing itself and that the extent of these changes depends on the mixing method employed. Intensive mixing resulted in the dispersion of the solid components of white petrolatum and the partial destruction of its three-dimensional network structure. These structural changes seemed to have induced reductions in the rheological properties of white petrolatum, e.g., its viscosity and elasticity. The presence of yield stress was also attributed to its three-dimensional network structure, which causes flow resistance. These properties seem to play important roles in the manufacturing of white petrolatum-based products and affect their sensory performance as well as their stability during storage, although the precise effects of different mixing procedures on the sensory performance of such products has not been clarified.

The salicylic acid solids was also found in salicylic acid ointment after mixing and saturated condition was retained. However, the solids were fragmented and dispersed. Furthermore, the mixing of the salicylic acid ointment resulted in an increase in the skin accumulation and permeation of any drugs they contain are affected by the method used to mix them. Fukami et al. reported that when a steroideal ointment (Kindavate® ointment) was diluted with white petrolatum (Propeto®) mixing with an autorotation/orbital mechanical mixer resulted in a lower viscosity than manual mixing by pharmacists. Our findings are consistent with their findings. Therefore, the method used to mix ointments can affect the activity of any drugs that they contain, as well as their storage stability and sensory features. This should be taken into account during the use of ointments, especially when they contain drugs that exhibit strong activity such as steroids. Pandey–Ewing reported that significant structural differences exist between petrolatum grades.

The effects of mixing procedure itself on the structure and rheological properties of white petrolatum-based products demonstrated in this study are common to all white petrolatum products including ointments in which they are used as bases. However, the rheograms of white petrolatum products might be affected by the composition and purity of the hydrocarbons contained within the products as well as the conditions in which the products are kept, e.g., the temperature at which they are kept and the period that they are stored for. Further studies are currently in progress to determine the effects of variations in purity on the structure and rheological properties (including hardness and stickiness) of white petrolatum-based products. Furthermore, it is necessary to determine the optimal mixing conditions for producing white petrolatum-based products without causing changes in their rheological properties. Therefore, we are studying the effects of different mixing conditions, such as the use of mixer- or slab and spatula-based mixing methods and variations in mixing strength and time, on the rheological properties of white petrolatum-based products with different purities. We are also studying the reversibility of the changes in the rheologi-
cal properties of such products induced by different mixing conditions.

**Conclusion**

Both of the mixing procedures examined in this study dispersed the solid components of white petrolatum, as well as the solid salicylic acid components found in a salicylic acid ointment in which white petrolatum was employed as a base. These changes induced reductions in the rheological properties, e.g., the viscosity and spreadability, of both white petrolatum and the salicylic acid ointment, which might have been due to the destruction of the three-dimensional network structure of white petrolatum. After the salicylic acid ointment had been mixed, the skin incorporation and permeation of salicylic acid also increased. These findings indicate that mixing procedures themselves affect the utility and physiological effects of such ointments. Therefore, such effects should be considered when mixing is required for the clinical use of petrolatum-based ointments.

**Experimental**

**Materials** White petrolatum was obtained from Yoshida Pharmaceutical Co. (Tokyo, Japan), and salicylic acid ointment (5%) was purchased from Toho Chemicals (Tokyo, Japan). White petrolatum was also obtained from Maruishi Pharmaceutical Co. (Osaka, Japan), Nichi-Iko Pharmaceutical Co. (Osaka, Japan), Sioe Pharmaceutical Co. (Osaka, Japan), and Kenei Pharmaceutical Co. (Osaka, Japan), and these samples were used to confirm our findings regarding the rheological properties of white petrolatum. All other reagents were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A Yucatan micropig skin set was purchased from Charles River Japan (Yokohama, Japan) and stored at −80°C until use. The fat and subdermal tissue were removed from the micropig skin according to the method of Fujii et al.14)

**Microscopic Analysis** The internal structures of the white petrolatum and salicylic acid ointment were examined using a BX53 microscope (Olympus, Tokyo, Japan) equipped with a polarization microscopy system.

**Measurement of Viscosity and Spreadability** The viscosity of the white petrolatum and salicylic acid ointment were measured before and after each mixing procedure. The mixing procedure was performed by either mixing the test substance twice with an NR-50 rotation/revolver mixer (Thinky, Tokyo, Japan) at 1000 rpm for 30 s or mixing it with a water jacket (37°C). The available diffusion area was approximately 0.78 cm², and the receptor cell had a capacity of about 4.6 mL. After 2 h pretreatment of the skin with NaCl solution, both the donor and receptor compartments were washed, and then about 100 mg of salicylic acid ointment were added to the donor compartment (thickness of the ointment layer: 2 mm), while phosphate-buffered saline (pH 7.4) was added to the receptor compartment. One hundred and fifty-microliter samples of receptor fluid were collected periodically over a 27 h period and used for the skin permeation experiments.

To prepare samples for the skin accumulation experiment, the skin tissue was removed from the diffusion cell as soon as the final receptor fluid sample had been collected (at 27 h), and then the treated area was punched out and washed with ice-cold methanol. Next, the epidermis was separated from the dermis using a heat separation technique,15) and then both layers were weighed (the mean weight of the epidermis and dermis was about 10 mg and 140 mg, respectively). Each skin sample was then minced and placed in 10 mL methanol, before being homogenized using a Polytron PT3100 tissue homogenizer (Kinematica, Lucerne, Switzerland) at 5000 rpm for 1 min.16)

After being prepared as described above, the samples for the skin permeation and accumulation experiments were then centrifuged, and the salicylate concentrations of their supernatants were assessed by high performance liquid chromatography using an L-2130 pump equipped with an L-2420 UV-Vis detector (Hitachi High Technologies Co., Tokyo, Japan). Separation was performed on a reversed-phase column (Mightysil RP-18 GP, 3.0 mm i.d., 150 mm) using a mobile phase (pH 2.0) consisting of methanol, water, and phosphoric acid (85%) at a volume ratio of 60:140:1. The detection wavelength was 303 nm, and ketotifen was used as the internal standard. The temperature of the column oven was set at 40°C. Accumulation amount of 0.01 µmol salicylate in the skin can be easily detected in this system.

**Statistical Analysis** Data were analyzed using the Kruskal–Wallis test. Individual differences between median values were examined using Dunn’s multiple comparisons test.

**Conflict of Interest** The authors declare no conflict of interest.

**References**