Physicochemical Characterization of Tretinoin Tocoferil Emulsion and Povidone-Iodine Sugar Ointment Blend Developed for Improved Regulation of Wound Moisture

Yasuhiro Noda,[a,b] Mika Saito,[b] Kazuya Watanabe,[b] Akimasa Sanagawa,[b] Yu Sobajima,[b] and Satoshi Fujii*[,b]

[a] *Department of Pharmacy, College of Pharmacy, Kinjogakuin University; Nagoya 463–8521, Japan: and*
[b] *Department of Molecular and Cellular Pathobiology and Therapeutics, Nagoya City University Graduate School of Pharmaceutical Sciences; Nagoya 467–8603, Japan.*
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Maintenance of proper moisture and regulation of infection are simultaneously required to promote healing of pressure ulcers. Continuous use of water-rich ointment may often lead to excess moisture and induce edematous granulation tissue. Use of water soluble ointment may excessively absorb exudates and induce dry granulation tissue. Selection of appropriate topical ointment is desired to avoid worse clinical outcomes. For adjustment of wound moisture a novel blended ointment (tretinoin tocoferil-povidone-iodine (TR-PI)) was developed consisting of emulsion base, tretinoin tocoferil oil-in-water (o/w) emulsion (TR-cream), and sugar base, povidone-iodine and sugar (PI-sugar). For the characterization of TR-PI water absorption was tested using Franz diffusion cell with cellulose membrane. For rheological characteristics spreadability was tested using spread meter and yield value was calculated. Iodine permeation was tested using a permeation cell with silicon membrane. Water absorption rate constant of TR-PI with combination ratio of PI-sugar at 75% (TR-PI75, 18.5 mg cm^{-2} min$^{-0.5}$) was equivalent to that of TR-cream alone (16.4 mg cm^{-2} min$^{-0.5}$). The yield value of TR-PI75 (26.1 Pa) exhibited intermediate values as compared to those of TR-PI with combination ratio of PI-sugar at 50% (11.3 Pa) and TR-cream alone (46.8 Pa). The amount of released free-iodine from TR-PI75 was similar to that released from PI-sugar alone. TR-PI75 may have superior performance in keeping the moist environment in wounds and in preventing infection. TR-PI75 can be used to promote formation of favorable granulation tissue in pressure ulcers with moderate exudates.

Key words blended ointment; iodine; pressure ulcer; spreadability; water absorption

Excess pressure and shear stress on skin tissue will induce pressure ulcers. Maintenance of proper moisture is emphasized to promote healing of pressure ulcers. Water soluble base having water absorption property is used for wounds rich in exudates, and emulsion base having water retaining property is used for wounds poor in exudates.[1] We have previously reported that macrogol ointment and emulsion ointment blend is suitable for regulation of water absorption in wounds with moderate exudates.[2] Topical products often used in Japan for pressure ulcers to promote granulation include tretinoin tocoferil (TR)-cream (Olcenon™) and povidone-iodine (PI)-sugar (U-PASTA™). TR-cream is an oil-in-water (o/w) emulsion containing 0.25% TR[3] used for wounds poor in exudates. PI-sugar is a water soluble base containing 3% PI and 70% sugar.[4] PI-sugar is used for wounds rich in exudates. When PI-sugar is used for wounds intermediate in exudates, one cannot control the moist environment because of its water absorption property. For moderately-severe pressure ulcers it is desirable to provide new stable ointments with adequate coating properties that can maintain proper moisture and exhibit iodine release performance to prevent infection. For this purpose a novel blended ointment (TR-PI) consisting of TR-cream and PI-sugar with combination ratio of PI-sugar at 75% (TR-PI75) is used to improve clinical outcomes[5]. However, the pharmaceutical scientific basis for using this combination ratio remains unclear.

We have previously established the evaluation method of water absorption capacity of ointment base using Franz cell model with semi-permeable membranes[6] and reported that water absorption capacity of ointment base is classified based on the mode of absorption. High water-absorbing PI-sugar belongs to an active type, where base can absorb water by osmotic pressure. Low water-absorbing TR-cream belongs to a passive type, where base can absorb water into matrix by diffusion-control.[6] Water absorption property of water soluble ointment is regulated by the blend with TR-cream.[2] Using this method we aimed to determine the water absorption characteristics of TR-PI. For rheological characteristics the potential change of spreadability of TR-PI75 was examined using spread meter. Admixture of ointments and/or creams may affect release of medicinal properties or skin penetration activity.[7,8] TR-PI contains iodine and release of iodine can be affected by the characteristics of base.[9] As the characteristics of base may be altered by blend of TR-cream and PI-sugar ointment, the release of iodine from TR-PI was characterized.

Experimental

Materials TR-cream was obtained from Pola Pharma Inc. Co., Ltd. (Tokyo, Japan). PI-sugar was from Kowa Pharmaceutical Co., Ltd. (Tokyo, Japan). These ointments are available in Japan. Components of w/o emulsion base for TR-cream are liquid paraffin, cetanol, polyethylene glycol monostearate, isopropyl myristate, glycerin and D-sorbitol. Components of water soluble base for PI-sugar are macrogol, glycerol, polyoxyethylene-polyoxypropylene glycol, potassium iodine, pectin, hydrogenised bean-lecithin, citric acid and sodium hydroxide. The mixing ratio of other components is not disclosed. The simulated wound exudates supplemented

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* To whom correspondence should be addressed. e-mail: sfujii@phar.nagoya-cu.ac.jp

with 5% bovine serum albumin (BSA) was prepared by Hanks' prescription. The cellulose ester membranes with molecular weight cut-off (MWCO) 100 kDa were from Spectrum Laboratories Inc. (Rancho Dominguez, CA, U.S.A.). BSA and cellulose ester membrane were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Silicon membrane (thickness 0.10 mm) was from AS ONE Corporation (Osaka, Japan). The phosphate buffered saline (PBS) was prepared by Mg/Ca ion free Dulbecco's prescription.

Measurement of Water Absorption Rate Using Franz Diffusion Cell TR cream was tempered with appropriate quantity of PI-sugar on a plate to give blended ointment with contents of PI-sugar adjusted between 0–100% on the basis of the total weight. Uniform mixture of the components was achieved by ointment slab and spatula and was visually confirmed. Blended ointment (1.2 g) was applied to the cellulose ester membrane mounted on the Franz diffusion cell.[6,10] Twenty milliliters of simulated wound exudates was introduced to the bellower cell. A water jacket of the permeation cell maintained the system at 32°C. The temperature was maintained at 32°C from the respective of the Organization for Economic Co-operation and Development (OECD) guidance document for the conduct of skin absorption studies. After every 30 min the water level in the branch tube attached to the cell was checked and the simulated fluid was added to the cell from the edge of the branch tube by a syringe until the water level reached its original level. The reduction of syringe weight by adding the simulated fluid was considered equivalent to amount of water absorbed to the ointment sample. Measurements were performed at least 3 times and the means of amount of water absorbed were calculated.

Determination of Rheological Characteristics A spread meter (Rigo Co., Ltd., Tokyo, Japan) was used to evaluate spreadability of ointment. A 0.5 cm^3 sample of ointment was set on the spread meter and was allowed to stand in an incubator controlling ambient temperature. The diameter D of ointment was visually measured after 10–900 s. The yield value S_0 (Pa) was calculated using the formula of Ichikawa[11] using D_∞ (cm) at 900 s, the final measurement point (Eq. 1). In the formula, G is gravitational acceleration (980 cm/s^2), P is the mass of the glass plate (g), and V is the volume of the sample (cm^3) (Eq. 1).

$$S_0 = \frac{48PVG}{\pi^2 D_\infty^5} \quad (1)$$

Microscopic Observation of the Dispersed System of Blended Ointments Blended ointment was spread out on the glass slide with cover glass. The dispersed system was observed by phase contrast microscopy (IX41, Olympus, Tokyo, Japan).

Assessment of the Concentration of Free-Iodine in the Aqueous Solution Apparent permeability of iodine depends on the activity of iodine in aqueous solution.[12] For measurement of permeability of iodine through silicon membrane with a thickness of 0.1 mm, a permeation cell commercially available was employed. The permeation cell consisted of 2 compartments with a membrane between them. The area of membrane for permeation was 4.91 cm^2. Each compartment was agitated by a magnetic stirring bar. Fifty milliliters of a test solution and 10% NaI solution were placed in the donor and the receptor compartments, respectively. A water jacket of the permeation cell was maintained at 32°C. One milliliter of samples was pipetted from the receptor solution after 60 min, and assayed spectrophotometerically at 352 nm employing a spectrophotometer.

Assessment of the Stability of Iodine in Blended Ointments Blended ointments (10 g) were stored in an ointment container at 25°C under the dark. Samples were collected at 0, 2, 4 and 24-weeks for determination of the concentration of intact medicinal properties in blended ointments. For titration of available iodine 0.5 g of blended ointment was diluted with 20 mL of water and 10 mL of 1% potassium iodide–0.5% starch solution was added to the sample solution. As the addition of 0.5% starch solution according to the Japanese Pharmacopoeia 16th Edition was insufficient for indicating the endpoint of titration (the color change of indicator dye), 1% potassium iodide solution was added for iodine solubilization. The available iodine in the solution was titrated with 0.01 M thiosulfate solution. Every reaction was run for at least 3 times and means of amount of iodine consumption were calculated.

Data Analysis All experiments were performed at least in triplicate. Data are expressed as means±standard deviations (S.D.s). Water absorption rate constants were obtained from the slope of the regression line. The differences in the water absorption rate constants of blended ointments were evaluated using Tukey's multiple comparison tests. Probability values of less than 0.05 were considered statistically significant.

Results

Water Absorption Property of Blended Ointments Used to Absorb Simulated Wound Extracts TR-cream and PI-sugar were mixed uniformly at all compounding ratios. With PI-sugar amount of absorbed simulated wound extracts increased linearly over time and PI-sugar was dissolved completely at 60 min. Plots of the initial amount of water absorption *versus* time delivered a curved line and the rate of water absorption became null after 60 min (data not shown). A linear correlation was observed at any combination ratio when amount of water absorbed was plotted over square root of time (Fig. 1), indicating that Fickian diffusion is the predominant process of absorption.[13] The water absorption rate constants were obtained from the slope of the regression line[2] and plotted against the combination ratio of PI-sugar (Fig. 2). The water absorption rate constants (mg $cm^{-2} min^{-0.5}$) of PI-sugar and TR-cream were 59.9 and 16.4, respectively. With TR-cream the water absorption rate constant was significantly decreased when the combination ratio of PI-sugar was 90% ($p<0.01$). The water absorption rate constants for TR-PI75 was 18.5. No significant changes were observed when the combination ratio of PI-sugar was between 0–80%.

Spreadability of Blended Ointments The effects of PI-sugar on spreadability were evaluated with spread meter. At 4°C, 25°C and 40°C sample spread diameter was measured upon application of a known volume for a predetermined time. Yield values were plotted against the PI-sugar combination ratio (Fig. 3). At 25°C yield values of TR-cream and PI-sugar were 46.8 Pa and 446 Pa, respectively. With TR-PI yield value decreased when the combination ratio of PI-sugar was 90% and became similar to that of TR-cream. When the combination ratio of PI-sugar was within a range of 10–80%, yield value showed gradual changes and became smallest when the

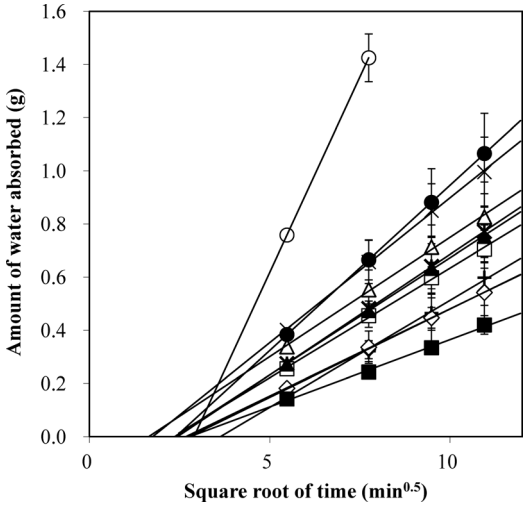

Fig. 1. Total Amount of Water Absorbed into Blended Ointments Evaluated by Using 100 kDa MWCO Membrane and Hanks' Buffer Supplemented with 5% BSA

The square root of time was plotted on the *X*-axis. The total amount of water absorbed was plotted on the *Y*-axis. The blended ointment of TR-cream and PI-sugar (TR-PI) was tested. The combination ratios of PI-sugar are expressed as+(0%), ◆ (10%), − (20%), ◇ (30%), ■ (40%), □ (50%), ▲ (60%), △ (70%), * (75%), × (80%), ● (90%), and ○ (100%). Results are expressed as means±S.D. (n=3–12).

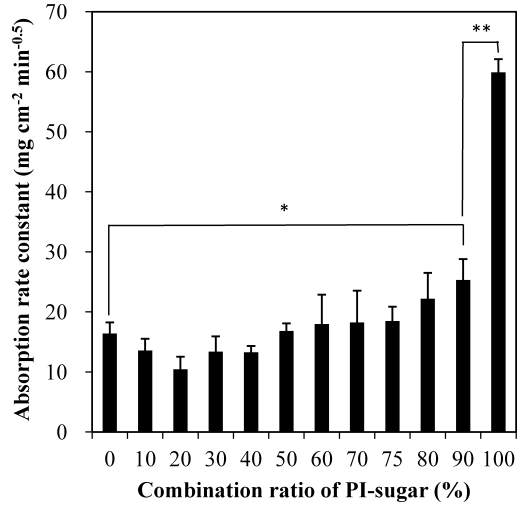

Fig. 2. Water Absorption Characteristics of Blended Ointments

The combination ratios of PI-sugar (%) in the blended ointment (TR-PI) were plotted on the *X*-axis. The water absorption rate constants per unit area obtained from the slopes on the lines in Fig. 1 were plotted on the *Y*-axis. Results are expressed as means±S.D. (n=3–12). Probability value of less than 0.05 is expressed as *, that of less than 0.01 is expressed as **.

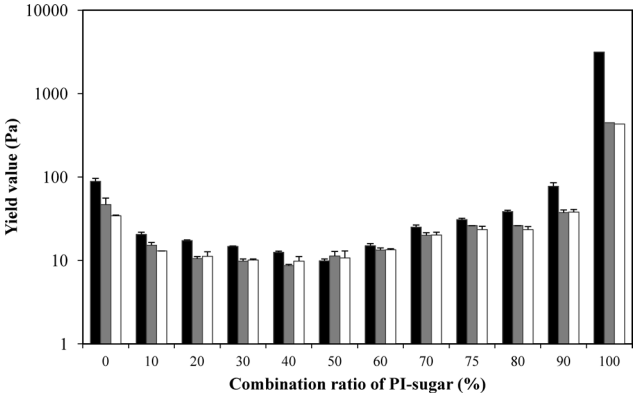

Fig. 3. Yield Values of Blended Ointments

Spreadability of ointments was assessed by a spread meter at 4°C ■, 25°C ■, and 40°C □. The combination ratio of PI-sugar (%) in blended ointments was plotted on the *X*-axis. The yield values of the ointment were plotted on the *Y*-axis. Results are expressed as means±S.D. (n=3).

Table 1. Influence of Storage Temperature on the Yield Values of TR-PI Blended Ointments with Combination Ratio of PI-Sugar at 75%

Sample	Temperature (°C)		Yield value (Pa)
	Storage	Spreading	
A	25	25	26.1±0.0
B	4	25	30.0±0.6**
C	4	4	30.9±1.1**

** $p<0.01$ *versus* the value of sample A.

combination ratio of PI-sugar was around 50% (11.3 Pa). The yield value of TR-PI75 was 26.1 Pa. The yield value of TR-cream decreased when the temperature was increased. The yield value of PI-sugar was unchanged when the temperature was increased from 25°C to 40°C. As to TR-PI, when the combination ratio of PI-sugar was within a range of 20–100%, the yield value did not change when the temperature was changed from 25°C to 40°C. When the temperature was changed from 25°C to 4°C, yield value of PI-sugar alone increased by 7-fold from 446 to 3150. With TR-PI with the combination ratio of PI-sugar between 0–80%, the increase in yield value remained modest within 2-fold. The effects of storage temperature and testing temperature on yield values of TR-PI75 are summarized in Table 1. The value was significantly increased when the sample was stored at 4°C and tested at 25°C as compared to the value in the sample stored at 25°C and tested at 25°C.

Microscopic Analysis of the Dispersed System of Blended Ointments TR-PI was subjected to microscopic analysis of the dispersed system of TR-cream. With TR-PI75 sucrose and/or macrogol crystal hindered the images (Fig. 4a). Dark round materials in the images represent sucrose which was dissolved during observation. Thus, observation was performed after dissolving sucrose with the addition of equal volume of water (Fig. 4b). Obtained image was compared with the image of TR-cream with the addition of equal volume of water (Fig. 4c). Uniform dispersion was observed without aggregation or degradation of dispersion media in Figs. 4b, c.

Permeation of Free-Iodine through Silicon Membrane TR-PI with the combination ratio of PI-sugar at 90%, 75%, and 50% was dissolved in PBS and used as the donor cell medium. Control medium consisted of water instead of TR-cream with the combination ratio of PI-sugar at 90%, 75% and 50%. The absorbance value at 352 nm in the receptor cell fluid increased over time.[6] Therefore, absorbance value after 60 min incubation was used as the apparent free-iodine (I_2) permeability rate, which is in proportion to the concentration of I_2 in the donor cell fluid. Assuming that one would apply a defined amount of ointment to wound, equal amount of blended ointment was dissolved in PBS in all experiments regardless of PI-sugar combination ratio. In controls I_2 permeability rate was negatively correlated with the combination ratio of PI-sugar. I_2 permeability rate became least when the combination ratio of PI-sugar was 100% (Fig. 5). In contrast I_2 permeability rate was positively correlated with the combination ratio of PI-sugar with TR-PI. I_2 permeability rate peaked when

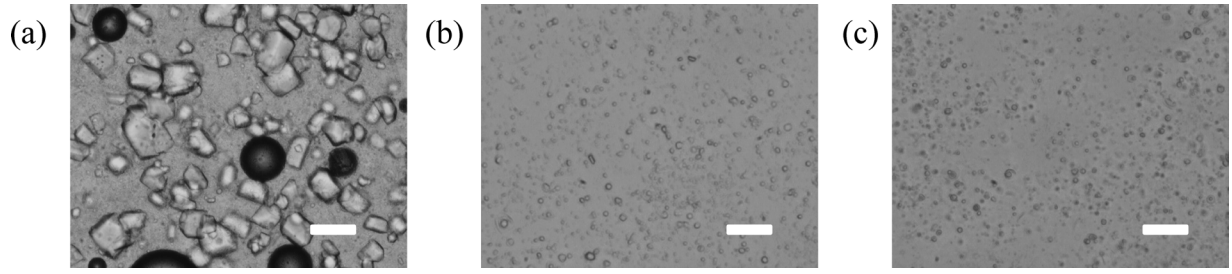

Fig. 4. Microscopic Analysis of the Dispersion System of Blended Ointments

Because sugar particles in TR-PI disturbed the observation of the dispersion system of TR-cream, TR-PI was diluted with water to dissolve sugar particles. Images of TR-PI75 without dilution (a), TR-PI75 diluted with water (b), and TR-cream diluted with water (c) are shown. The bars in images indicate 50 μm.

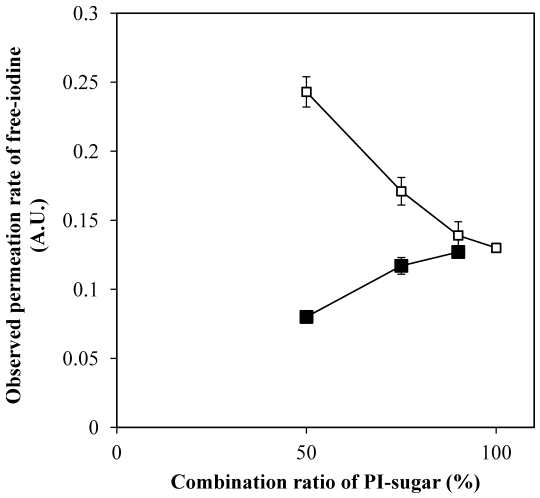

Fig. 5. Released I_2 Level of the PBS Containing Blended Ointments

The combination ratios of PI-sugar (%) in blended ointments were plotted on the *X*-axis. The observed permeation rates of I_2 were plotted on the *Y*-axis. PI-sugar blended with water □, PI-sugar blended with TR-cream ■. Results are expressed as means±S.D. (n=3). Released I_2 levels of the PBS in the donor cell were proportional to the observed permeation rates of I_2.

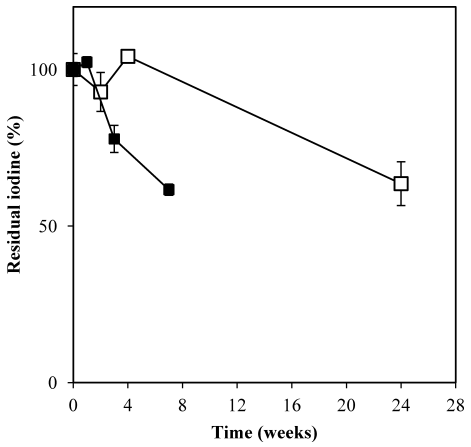

Fig. 6. Stability of the Iodine in the Blended Ointments TR-PI75 at 25°C (□) for Up to 24 Weeks and at 40°C (■) for Up to 7 Weeks

Time after the preparation (weeks) was plotted on the *X*-axis. The residual iodine in the blended ointment was plotted on the *Y*-axis. Values of the medicinal properties at 0 week were set as 100%.

Table 2. Permeation Rate of Free-Iodine through the Silicon Membrane

Combination ratio of PI-sugar (%)	Initial total iodine concentration of the medium in the donor cell (%)	Observed permeation rate of free-iodine (AU)[a)]		Free-iodine index[b)]		
		Control	Blend	Control	Blend	*t*-Test
100	0.100	0.130±0.002	—	1.0		
90	0.090	0.139±0.010	0.127±0.011	1.1	0.98	
75	0.075	0.171±0.010	0.117±0.001	1.3	0.90	*
50	0.050	0.243±0.011	0.080±0.006	1.9	0.62	**

Blend: TR-cream was blended with PI-sugar. Control: Water was blended with PI-sugar. The test sample (16 g) was dissolved in 50 mL of PBS. Results are expressed as means±S.D. (n=3). *a*) Value of the observed density at 352 nm of the medium in the receptor cell after 60 min incubation. *b*) Ratio of the value of blend *versus* that of control with combination ratio of PI-sugar at 100%. *p<0.05, blend *versus* control with combination ratio of PI-sugar at 100%. **p<0.01, blend *versus* control with combination ratio of PI-sugar at 100%.

the combination ratio of PI-sugar was 100%. I_2 permeability rate was arbitrarily set as 1.0 with TR-PI with the combination ratio of PI-sugar 100%. The value of I_2 index was 0.90 and 0.62 with combination ratio of PI-sugar at 75% and 50% (Table 2). These values were significantly different from the I_2 index value with the combination ratio of PI-sugar at 100%.

Stability of Medicinal Properties in Blended Ointments TR-PI75 was stored at 25°C. Remained amount of total iodine was unchanged over the 4 weeks of storage. However, remained amount was reduced to 63.5% over the 24 weeks of storage (Fig. 6). When these samples were stored at 40°C the remained amount of total iodine was unchanged over 1 week and was reduced to 61.6% over 7 weeks of storage. With the storage at 40°C TR-PI with the combination rate of PI-sugar at 20–50% base was condensed and dissociated after overnight incubation.

Discussion

Optimization of Combination Ratio for Blended Ointments Despite the magnificent contribution of TR-PI75 in improving clinical outcome, the pharmaceutical scientific basis for its use remained unsolved. In this study adequacy of

the combination ratio of PI-sugar at 75% was critically evaluated. No significant changes in the water absorption rate constants were observed when the combination ratio of PI-sugar was between 0–80%. When the combination ratio of PI-sugar was >60% base was stable and dissociation was not observed. When the combination ratio of PI-sugar was between 10–80%, yield value was largest when the combination ratio was between 70–80%. Iodine release is decreased with the decrease in the combination ratio of PI-sugar. Taken together, the combination ratio of PI-sugar between 70–80% appears most appropriate and TR-PI75 falls within this range.

Mechanisms of Water Absorption in Blended Ointments The water absorption rate constant was dramatically decreased when PI-sugar was blended with TR-cream at the ratio of 9 : 1, suggesting that transition from active to passive water absorption could take place when TR-cream is added to PI-sugar. No significant changes were observed when the combination ratio of PI-sugar was between 0–80%. The water absorption rate constant of TR-PI75 was equivalent to that of TR-cream alone and to that of TR-PI with combination ratio of PI-sugar at 80%. On-off transitional phenomenon was observed when a macrogol ointment containing 5% sulfadiazine (SL-pasta) was blended with TR-cream (TR-SL).[2] In contrast to TR-SL, intermediate water absorption rate between TR-cream and PI-sugar was not observed, suggesting that the mechanism of water absorption of TR-PI is distinct from that of TR-SL. Microscopic analysis revealed sucrose crystals in TR-cream base. With PI-sugar dissolution of sucrose produces osmotic pressure, which is the driving force of active water absorption. TR-cream exists as an o/w emulsion form. Under this condition continuous water phase could donate water to wound surface. When TR-cream was blended with PI-sugar, sugar might be dissolved in continuous water phase resulting in higher osmotic pressure to induce water absorption. As shown in Fig. 4a, when TR-cream was blended with PI-sugar, not all sucrose is dissolved in base and sucrose remained insoluble as a crystal form. It is highly possible that particles of oil phase of the emulsion can surround sucrose and prevent sucrose from easily contacting water, resulting in slower water absorption. Microscopic analysis also suggests that the dispersion system of TR-PI75 is relatively stable. PI-sugar does not interfere with the dispersion system of TR-cream, enabling the blend of PI-sugar and TR-cream. With the combination rate of PI-sugar at 20–50% base was condensed and dissociated after overnight incubation, suggesting that sucrose is practically dissolved. Therefore, it is likely that water absorption is decreased due to sucrose dissolution when the combination ratio of PI-sugar was <50%. When the combination ratio of PI-sugar was ≧50%, undissolved sucrose is surrounded by oil phase and water absorption is moderately decreased.

Spreadability of Blended Ointments Water absorption of TR-PI with the combination ratio of PI-sugar 50% was similar to that of TR-PI75. However, in TR-PI with the combination ratio of PI-sugar at 20–50% base is condensed and dissociated upon the storage at 40°C. To keep stability of the base the combination ratio of PI-sugar should be over 60%. Furthermore, TR-PI with the combination ratio of PI-sugar ≤50% is in liquid form and is inappropriate as an external medication for wound healing. Spreadability is one of the important attributes of topical dosage forms that may account for patient acceptability. The yield values of PI-sugar and TR-cream were 446 and 46.8 (Pa) at 25°C, respectively. Yield values show PI-sugar to be harder ointment and TR-cream to be softer ointment. Yield value of TR-PI75 was 26.1, suggesting that this blended ointment is too soft for topical use. However, the rheological characteristics obtained indicated that this blended ointment has plastic fluidity.

Upon storage at 4°C yield value of PI-sugar increased by 7 fold. Yield value of TR-PI75 increased only slightly upon storage at 4°C. The yield value is significantly increased when the sample was stored at 4°C and tested at 25°C as compared to the value in the sample stored at 25°C and tested at 25°C. These results suggest that recrystallization of sucrose led to form three-dimensional cross-linked structure and the dispersion system was stabilized at 4°C.

Release Patterns of Free-Iodine from Blended Ointments Blend of different kinds of bases is not recommended because it may affect stability and medicinal properties. For instance, with steroid cream water soluble base may interfere with emulsion system and increase steroid release, leading to unexpected adverse reactions.[7,8] A blend of PI-sugar and TR-cream may induce iodine toxicity. Thus, we dissolved blended ointments in PBS and evaluated the permeation of I_2 released to PBS through silicon membrane. This experiment simulated the clinical milieu where blended ointments applied to wounds are dissolved in exudates and I_2 is released. In controls I_2 permeability rate was negatively correlated with the combination ratio of PI-sugar. PI-sugar contains PI. Our results are consistent with the previous report showing that the concentration of PI is decreased when the concentration of I_2 is increased.[14] Concentration of I_2 in TR-PI was consistently smaller than that of PI-sugar. Bactericidal property of iodine is derived from the injury of cellular membrane by I_2 and is in proportion to I_2 concentration.[15] We did not observe an increase in the concentration of I_2 after PI-sugar is blended with TR-cream. In contrast, release of I_2 is inhibited when the combination ratio of PI-sugar is decreased. Indeed, I_2 concentration in TR-PI75 is 90% of that in PI-sugar. This falls within the reasonable range of Japanese content regulation of general drug formulation (90–100%). Thus, it is unlikely that the toxicity of TR-PI75 exceeds that of PI-sugar. The concentration of TR permeated through silicone membrane was below measurable limits, suggesting that release of TR from emulsion oil particle is not facilitated.

Stability of Medicinal Properties in Blended Ointments Because the same treatment could be used for 1–2 months for wounds with moderate exudates to promote granulation, stability was tested for up to 24 weeks at 25°C. In TR-PI75 stored at 25°C over 4 weeks the remained amount of total iodine was unchanged, suggesting that this blend formula can be stored for 1 month after preparation. As the remained amount of total iodine can be affected by storage temperature, storage at 4°C is recommended. With PI-sugar alone the remained amount of total iodine was unchanged. With TR-PI the remained amount of total iodine was decreased in association with the decrease in tretinoin tocoferil (TR), analyzed by HPLC (data not shown). TR is an ester compound of all-*trans* retinoic acid (tretinoin) and *alpha*-tocopherol. Tretinoin has tetraen side chain. The decrease in iodine content associated with the decrease in the remained amount of TR suggests that iodine diminished in reacting with unsaturated bond of TR. Sucrose in PI-sugar could be partially dissolved in water

of TR-cream and was considered not accurately measurable. Thus, the remained amount of iodine was used for stability assessment.

Strategic Use of the TR-PI Blended Ointments for Pressure Ulcers TR-cream, an o/w emulsion, is recommended for pressure ulcers with less wound exudates[1)] and promotes granulation. PI-sugar exhibits active water absorption and is recommended for pressure ulcers rich in exudates. PI-sugar has antibacterial activity and promotes granulation. In pressure ulcers with medium wound exudates TR-cream may increase water content of wound surface and induce edematous granulation tissue. PI-sugar may excessively absorb exudates and induce dry granulation tissue. For pressure ulcers with intermediate exudates keeping the moist environment is essential. If the characteristics of base are not suitable for wound exudate status, mixture of water-soluble base with emulsion base is useful.[16,17)] Blend of ointments at particular ratio is necessary to optimize water absorption. TR-PI75 is highly expected to induce moist environment by passive water absorption. Formulation design with moist environment and bactericidal activity is ideal. TR-PI75 can release I_2 sufficient to show bactericidal activity. It also contains TR as medicinal property, suitable for pressure ulcers with medium exudates, promoting granulation while preventing infection.

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References

1) Sanada H.,"Guideline for Local Treatment of Pressure Ulcers," Japanese Society of Pressure Ulcers, Syorinsha, Tokyo, 2012.
2) Noda Y., Watanabe K., Sanagawa A., Sobajima Y., Fujii S., *Int. J. Pharm.*, **419**, 131–136 (2011).
3) Kawabata H., Kamada T., Takatsuka Y., Takeuchi S., Suzuki S., Makino T., Utsunomiya A., *Haematologia* (Budap.), **31**, 369–372 (2002).
4) Knutson R. A., Merbitz L. A., Creekmore M. A., Snipes H. G., *South. Med. J.*, **74**, 1329–1335 (1981).
5) Mizokami F., Furuta K., Noda Y., Isogai Z., *J. Jpn. Soc. Hosp. Pharm.*, **46**, 1643–1646 (2010).
6) Noda Y., Fujii K., Fujii S., *Int. J. Pharm.*, **372**, 85–90 (2009).
7) Ohtani M., Nakai T., Ohsawa K., Kim S., Matsumoto M., Etoh T., Kariya S., Kanou S., Uchino K., *Yakugaku Zasshi*, **122**, 1153–1158 (2002).
8) Ohtani M., Yamada N., Takayama K., Kotaki H., Etoh T., Kariya S., Uchino K., Iga T., *Yakugaku Zasshi*, **122**, 107–112 (2002).
9) Noda Y., Fujii S., *Int. J. Pharm.*, **394**, 85–91 (2010).
10) Kawashima Y., Takeuchi H., Hino T., Niwa T., Lin T. L., Sekigawa F., Kawahara K., *Pharm. Res.*, **10**, 351–355 (1993).
11) Ichikawa I., "Wakariyasui Kami Inki Insatsu No Kagaku," Insatsu Choyokai Foundation, Tokyo, 1977, pp. 133–135.
12) Takikawa K., Nakano M., Arita T., *Chem. Pharm. Bull.*, **26**, 874–879 (1978).
13) Peppas N. A., *Pharm. Acta Helv.*, **60**, 110–111 (1985).
14) Berkelman R. L., Holland B. W., Anderson R. L., *J. Clin. Microbiol.*, **15**, 635–639 (1982).
15) Rodeheaver G., Bellamy W., Kody M., Spatafora G., Fitton L., Leyden K., Edlich R., *Arch. Surg.*, **117**, 181–186 (1982).
16) Shigeyama M., Ohgaya T., Kawashima Y., Takeuchi H., Hino T., *Chem. Pharm. Bull.*, **47**, 744–748 (1999).
17) Shigeyama M., Ohgaya O., Takeuchi H., Hino T., Kawashima Y., *Chem. Pharm. Bull.*, **49**, 129–133 (2001).