Department of Clinical Pharmaceutics[1], School of Pharmaceutical Sciences, University of Shizuoka; Laboratory of Clinical Pharmacokinetics[2], Shizuoka General Hospital; Lemon Pharmacy[3], Shizuoka, Japan

The effect of storage time on the release profile of dexamethasone dipropionate from admixtures of steroid and heparinoid ointments

T. Suzuki[1,3], T. Uchino[1,2], Y. Miyazaki[1,2], Y. Kagawa[1,2]

Received June 20, 2013, accepted August 23, 2013

Dr. Tomonobu Uchino, Department of Clinical Pharmaceutics, School of Pharmaceutical Sciences, University of Shizuoka 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
uchinot@u-shizuoka-ken.ac.jp

Pharmazie 69: 104–108 (2014) *doi: 10.1691/ph.2014.3145*

We examined the stability and release profiles of dexamethasone dipropionate (DDP) from admixtures by using an innovator ointment (Methaderm [IM]), two generic ointments (Promethasone [GP] and Mainvate [GM]), and a heparinoid ointment. The admixtures were prepared using a spatula and an ointment slab and were stored at room temperature. Microscopic and Fourier transform-Raman spectrometric analyses showed that crystallization of DDP in admixtures of IM after 1 week of storage occured. And DDP crystals in all admixtures of GP and GM were observed. DDP was not decomposed in the admixtures after storage. Cumulative DDP permeation across a silicone membrane in a 1-week storage sample of the IM system decreased with DDP crystallization and reached a plateau after 2 weeks. In the GP and GM systems, DDP permeation decreased after 1 week of storage and increased again after 2 and 4 weeks. Each admixture was separated into 3 phases (liquid, lower, and upper solid phases) by ultracentrifugation to determine the apparent solubility of DDP. The DDP contents in the upper solid phase of the IM admixtures at 1, 2, and 4 weeks were lower than that in the 0-week sample. No significant differences were observed in the DDP content between the liquid phases throughout the storage period. Therefore, the amount of DDP dissolved in the upper solid phase may influence DDP release from the IM admixtures. The GP and GM systems showed no significant differences in the apparent DDP solubility. These results indicate that the dispersion state of DDP in the tested admixtures may be altered with storage.

1. Introduction

Topical corticosteroids are frequently prescribed for dermatological conditions, particularly for the treatment of psoriasis (Strowd et al. 2009) and atopic dermatitis (Luger et al. 2011). Moisturizers are also used for the treatment of psoriasis and atopic dermatitis. Although topical formulations typically consist of a single steroid compound, admixtures of commercial topical steroid formulations are often prepared upon a physician's request (Refai and Müller-Goymann 2002), and are used especially in dermatological practice. Patient compliance increases with the use of ointment admixtures (Etoh and Ohtani 2002). Furthermore, clinicians can prescribe large quantities of admixtures for up to 1 month of use. Therefore, it is important to evaluate the effect of storage on steroid release from admixtures. The stability of admixtures of topical steroid formulations and other components is important. Nagaya et al. (1987) reported that some admixtures of hydrocortisone butyrate ointment and another component showed syneresis and that the content of hydrocortisone butyrate in some admixtures decreased after 4 weeks of storage. In addition, Cornarakis-Lentzos and Cowin (1987) reported that decomposition products were detected in topical formulations of betamethasone 17-valerate or betamethasone dipropionate.

The pH of the admixture of a steroid ointment contributes to the dehydration or migration of the acyl group of the steroid compound. Bundgaard and Hansen (1981) reported that acyl group migration was subject to both specific acid and base catalysis. Minimum degradation of betamethasone 17-valerate was observed in an aqueous solution at 60 °C at pH of 3.5. Yamaoka et al. (1980) reported that dexamethasone in vanishing creams was unstable under basic conditions. However, when the pH of admixtures of steroid ointments was measured in an aqueous solution prepared by centrifugation of the topical product with water, degradation of hydrocortisone in an admixture of hydrocortisone and tocopherol ointments was observed at a pH of 3.6 (Ohishi et al. 1991). Therefore, measurement of pH of admixtures is required under *in situ* conditions.

The amount of steroid release is also an important factor for the evaluation the effectiveness of an admixture. Ohtani et al. (2002) reported that compared to a steroid ointment or cream alone, admixtures of steroid ointments and/or creams showed an increase in the cumulative amount of steroid permeation immediately after mixing. Since the thermodynamic activity of the drug and vehicle solubility affect the characteristics of drug release from topical formulations (Ishii et al. 2010; Refai and Müller-Goymann 1999), changes in formulations may cause changes in the release profiles of corticosteroids because of syneresis or steroid decomposition.

We previously reported that the different molecular states of three commercially available 0.1% dexamethasone dipropionate (DDP) ointments, namely, Methaderm ointment (IM),

Promethasone ointment (GP), and Mainvate ointment (GM), directly affected DDP release (Suzuki et al. 2012). In addition, the release of DDP from admixtures of three DDP ointments with Hirudoid soft (HS), a 0.3% heparinoid ointment, depended on the specific admixture combinations and their apparent DDP solubility.

The aim of this study was to investigate the effect of storage time on DDP stability and release from several admixtures of commercially available topical corticosteroid formulations. We used three different 0.1% DDP ointments—IM, GP, and GM—in this study. HS was used as an admixed ointment. Raman microscopy measurements were used to evaluate the admixtures. We investigated the amount of DDP permeation by using a silicone membrane and apparent DDP solubility as a function of storage time. A silicone membrane was used as an artificial membrane for the prediction of drug permeability across the skin (Ishii et al. 2010; Suzuki et al. 2012; Sugibayashi et al. 2010). Measurement of pH of the admixtures was performed using a pH spear, which is a handy pH tester widely used in food chemistry. Ohkawa et al. (2013) used this apparatus to evaluate pH changes in emulsion injections. Therefore, we used this apparatus to measure pH changes in our admixture samples.

2. Investigations, results, and discussion

Drug stability and changes in the physicochemical properties of admixtures of semisolid formulations are important factors for determining drug permeation. In this study, we selected three topical DDP ointments as model formulations and characterized their admixtures with HS as previously reported (Suzuki et al. 2012).

The microscopic images of the 3 admixtures after storage for 1 week are shown in Fig. 1. The IM, GP, and GM systems showed plate-like, centrosymmetric rod-like, and needle-like crystals, respectively. We previously reported that DDP crystals were present in GP and GM systems at 0 week; thus, these results indicate that the crystals observed in the GP and GM systems are attributable to DDP (Suzuki et al. 2012). However, the IM system showed different results. Although no crystals were formed in the admixtures of IM at 0 week, crystallization was observed in the sample stored for 1 week.

To identify crystals in the IM systems, a comparative study using Raman spectroscopy was performed using a DDP standard. The results of this experiment are shown in Fig. 2. The Raman spectrum of the DDP standard showed sharp characteristic peaks between 1600 and 1700 cm^{-1}, which can be attributed to the carbon-carbon double bond and carbonyl stretching vibration of the steroidal backbone (Fig. 2a) (Fini et al. 2008). These peaks coincided with the peaks of the crystals observed in IM systems. Therefore, the crystals observed in IM systems were identified as DDP.

To assess changes in the physicochemical properties of admixtures, we measured the potency of DDP and the pH of the admixtures. The residual ratio of DDP to the amount of DDP at 0 week of storage and the pH levels measured are shown in Fig. 3 Table 1, respectively. Since the residual amount of DDP ranged from 97% to 105%, DDP in the admixtures was considered chemically stable for 4 weeks. The pH of each intact ointment ranged from 5.3 to 5.7. Thus, the pH of the admixtures tested indicated a slightly acidic nature. Ohishi *et al.* (1993) reported that DDP in an admixture of IM and Hirudoid ointment was unstable at pH 8. Furthermore, hydrocortisone was unstable in an alkaline aqueous solution (Gupta 1978). Moreover, dexamethasone in vanishing creams was unstable in alkaline creams (Ohishi et al. 1991). The admixture prepared showed slightly acidic properties for the entire storage time tested, which indicated that DDP in the admixtures tested was chemically stable over the course of 1 month.

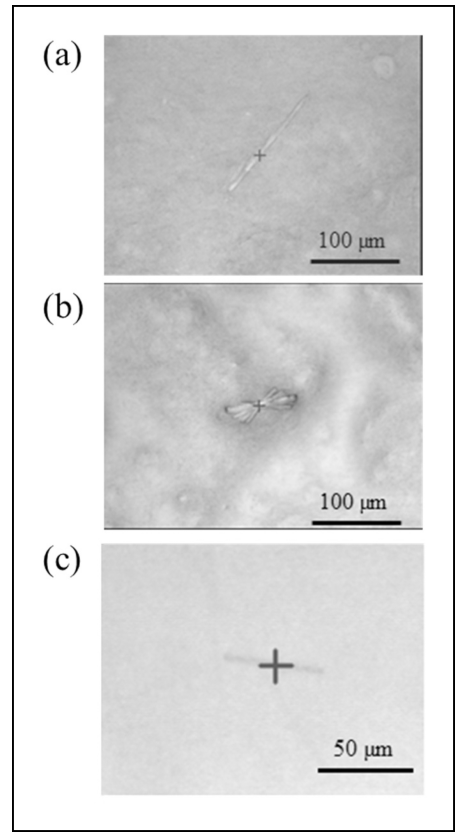

Fig. 1: Microscopic images of admixtures of Hirudoid soft (HS) with Methaderm (IM) (IMHs) (a), Promethasone (GP) (GPHs) (b), and Mainvate (GM) (GMHs) (c) after storage for 1 week. Dexamethasone dipropionate (DDP) crystals in GP or GM alone were observed in the admixtures. In the IMHs, a plate-like crystal was observed.

In our previous study, we showed that the molecular state of DDP in the admixtures affected DDP release (Suzuki et al. 2012). Our study showed that the molecular state of DDP in the IM system

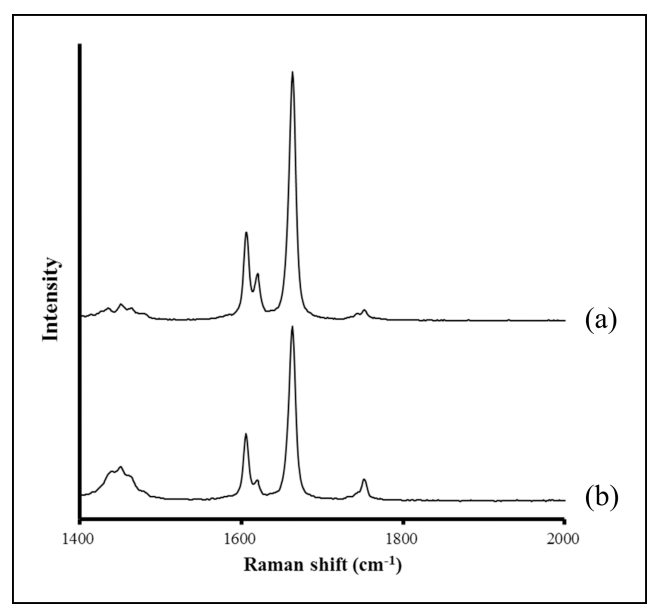

Fig. 2: Raman spectra of the dexamethasone dipropionate (DDP) standard (a) and an observed crystal in the admixtures of Hirudoid soft (HS) with Methaderm (IMHs) after storage for 1 week (b). Sharp bands from 1600 to 1700 cm^{-1} in the DDP standard coincided with those of the crystals observed in IMHs at 1 week after mixing.

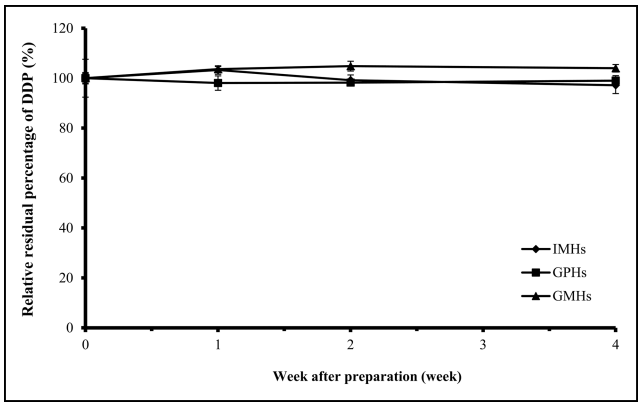

Fig. 3: Relative residual ratio of dexamethasone dipropionate (DDP) in admixture of DDP ointment and Hirudoid soft (HS). Admixtures of DDP ointment and HS were stored at 26 °C under the exposure to fluorescent light for 1, 2, and 4 weeks. All data represent the relative residual ratio of DDP to the corresponding admixture at 0 weeks. Results are presented as mean ± standard deviation (SD) (n = 3).

Table: Effect of storage time on pH of admixtures of DDP ointment and HS

Formulations	Week			
	0	1	2	4
IMHs	4.9 ± 0.1	5.0 ± 0.2	4.9 ± 0.3	5.0 ± 0.4
GPHs	5.3 ± 0.2	5.1 ± 0.5	4.9 ± 0.6	4.8 ± 0.5
GMHs	5.6 ± 0.3	4.9 ± 0.4	4.9 ± 0.3	5.1 ± 0.3

The pH values of intact ointments IM, GP, GM, and HS, were 5.3 ± 0.0, 5.3 ± 0.1, 5.5 ± 0.1, and 5.7 ± 0.1, respectively. Results are presented as mean ± SD (n = 3)

altered because of storage and thus this might have affected DDP permeation. The cumulative amount of DDP that crossed a silicone membrane from admixtures of DDP ointment and HS after storage is shown in Fig. 4. We observed a decrease in the cumulative amount of DDP in IM system samples stored for 1 week, and a plateau was observed after 2 weeks of storage, which indicated a decrease in the apparent solubility with DDP crystallization in the IM system. The cumulative amount of DDP decreased in the 1-week storage samples of GP and GM systems. However, evaluation of the same admixture systems showed that compared to the 1-week samples, the 2- and 4-week samples showed an increase in the cumulative amount of DDP.

Refai and Müller-Goymann (2002) reported that changes in steroid permeation are dependent on the concentration of the corticosteroid dissolved in the vehicle. To evaluate the apparent solubility of admixtures of DDP and HS, admixture samples were ultracentrifuged for 2 h at 60,000 rpm at 20 °C. We obtained three separate phases, and the amount of DDP in each phase was determined. The ratio of DDP in each phase of the admixture is shown in Fig. 5. The DDP ratio of the liquid phase of IM, GP, and GM and the solid phases of GP and GM showed no change during the storage period. However, the DDP ratio of solid phase 1 in the IM system at 1, 2, and 4 weeks was lower than that at week 0. The amount of DDP in solid phase 2 of the IM system increased with an increase in crystallization of DDP in the admixtures, which indicated that the DDP crystals in solid phase 1 moved to solid phase 2 on ultracentrifugation. These results might show that the DDP solubility in the solid phase 1 of IM decreased on mixing with HS, and time was required to form DDP crystals in the admixtures. No significant change was observed in the distribution of DDP in either the GP or GM systems in any phase as a function of storage time. However, DDP release from admixtures varied in each storage

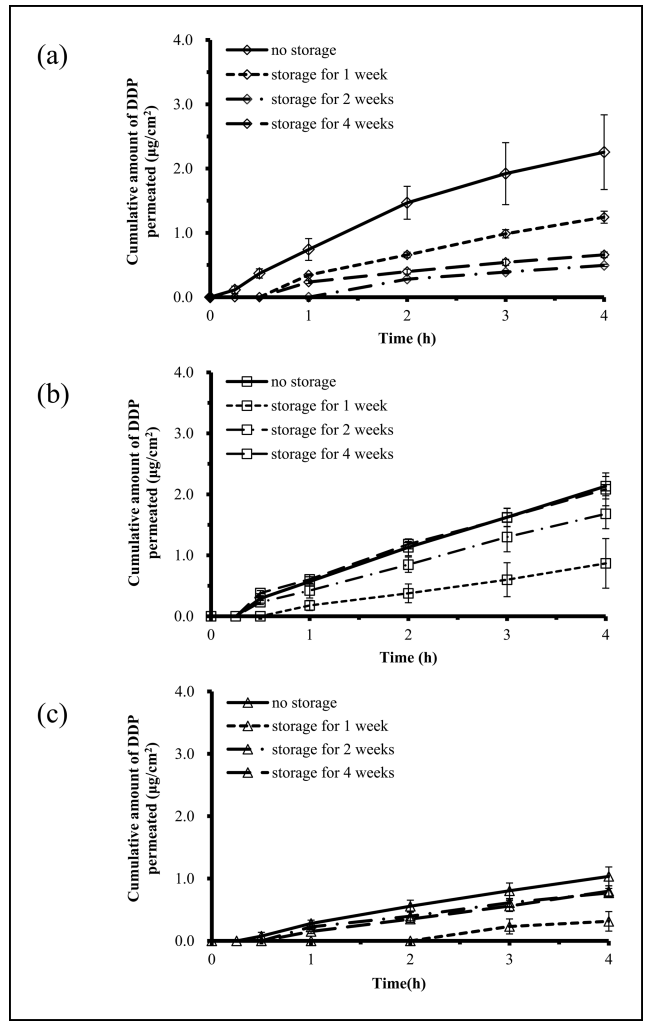

Fig. 4: Release profiles of the cumulative amount of dexamethasone dipropionate (DDP) permeation across a silicone membrane from admixtures of DDP ointment and Hirudoid soft (HS) as a function of storage time: Methaderm (IM) (IMHs) (a), Promethasone (GP) (GPHs) (b), and Mainvate (GM) (GMHs) (c). Results are presented as mean ± standard deviation (SD) (n = 3). Cumulative amount of DDP in 1-week storage sample of IM system decreased with DDP crystallization and reached a plateau after 2 weeks. In GP and GM systems, DDP permeation decreased after 1 week of storage and increased again after 2 and 4 weeks.

period. These results indicated that a dispersed state of DDP in the admixtures at each storage period altered DDP release.

In conclusion, we found that DDP crystals were formed in the IM system after 1 week of storage and were observed in the GP and GM systems throughout the storage period. Furthermore, although DDP was chemically stable in the admixtures, the release of DDP from the IM admixture decreased because of formation of DDP crystals and depended on the DDP content in solid phase 1. On the basis of these findings, we concluded that the molecular state of the steroid compound in the admixtures of the steroid ointment and other components can change during storage and the change can affect the release of steroid compounds.

3. Experimental

3.1. Materials

We used 3 DDP 0.1% ointments (innovator, IM [Taiho Pharmaceutical Co., Ltd., Tokyo. Japan]; generic GP [Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan] and GM [Sato Pharmaceutical Co., Ltd., Tokyo, Japan]); and HS (Maruho Co., Ltd., Osaka, Japan). DDP (molecular weight, 504.59; melting point, 200–206 °C; LogP, 3.66; solubility in water at 20 °C, 0.016 mg/mL), fluocinonide (internal standard), and butylparaben (inter-

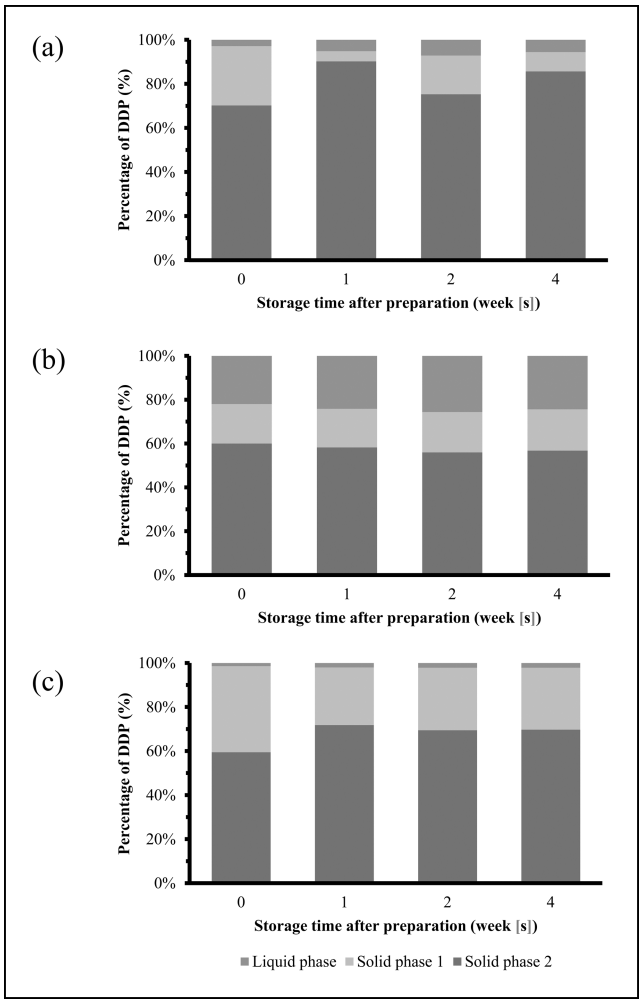

Fig. 5: Change in the relative dexamethasone dipropionate (DDP) amount in the 3 separated phases obtained from admixtures of DDP ointment and Hirudoid soft (HS) as a function of storage time: Methaderm (IM) (IMHs) (a), Promethasone (GP) (GPHs) (b), and Mainvate (GM) (GMHs) (c). The three separate phases were obtained by ultracentrifugation at 60,000 rpm at 20 °C for 2 h. In the IM system, the amount of DDP in Solid phase 1 decreased with an increase in storage time. No significant difference was observed in the amount of DDP among preservation periods in the GP or GM systems.

nal standard) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and solvents were of reagent or HPLC grade and used without further purification. The silicone membrane (TLC-S1-75; thickness, 75 μm) was a kind gift from Lintec Co., Ltd. (Tokyo, Japan).

3.2. Preparation of admixtures of DDP ointments with HS

Admixtures of DDP ointments with HS were prepared using an ointment slab and an ointment spatula as reported previously (Suzuki et al. 2012). DDP ointments and HS were weighed to 5 g on the spatula and equally mixed on the slab over a period of 5 min. Admixtures of HS with IM, GP, and GM were designated as IMHs, GPHs, and GMHs, respectively. Each sample was stored at 26 °C under exposure to fluorescent light over a period of 4 weeks.

3.3. Microscopy and Raman spectrometry

Microscopic images were obtained using an optical microscope (BH-2; Olympus Corporation, Tokyo, Japan). Raman spectra data were collected using a dispersive Raman spectrometer (Nicolet Almega XR with a 532-nm laser; Thermo Fisher Scientific K.K., Kanagawa, Japan). Spectra were obtained for 10 scans at 3 exposure times.

3.4. pH Measurements

A pH spear (Eutech Instruments Europe B. V., Nijkerk, Netherlands), which is a handy pH tester, was used to measure pH. The spear was directly inserted into admixtures at 1, 2, and 4 weeks. Measurements were performed by inserting the electrode into the intact ointments or prepared admixtures after calibration using standard solutions of pH 4.0 and 9.0.

3.5. Silicone membrane permeation experiments

The analysis of DDP permeation across the silicone membrane (thickness: 75 μm) was performed according to a previously described method (Suzuki et al. 2012) with a slight modification (Klein 2013). Briefly, the silicone membrane was set in a 5-mL vertical Franz diffusion cell (PermeGear, Inc., Hellertown, PA, USA) with an effective permeation area of 0.38 cm^2. The test ointments of 0.5 g were applied to the donor cell side. Because water solubility of DDP is independent of pH change, 5.0 mL degassed phosphate-buffered saline (pH 7.4) was applied in the receiver cell side to maintain sink conditions. The diffusion cell was maintained at 37 °C with a water jacket connected to a water bath. The receiver cell was agitated using a magnetic stirrer bar at approximately 600 rpm. At the times indicated, an aliquot (200 μL) was withdrawn from the receiver solution and the same volume of fresh phosphate-buffered saline was added to keep the volume constant. Samples were taken at the appropriate time points and an aliquot (50 μL) was injected into the HPLC system. The HPLC conditions for the measurement of permeated DDP are described in the HPLC analysis section.

3.6. Ultracentrifugal separation of admixtures and extraction of DDP

The admixtures were phase-separated using a centrifugal separation method (Suzuki et al. 2012). We placed 7 g of each admixture in a centrifuge tube and centrifuged at 60,000 rpm at 20 °C for 2 h. A liquid phase and 2 solid phases (the upper phase was labeled solid phase 1 and the lower phase was labeled solid phase 2) were obtained after ultracentrifugation. Liquid and solid phase fractions after ultracentrifugation were recovered using a pipette and spatula, respectively. Subsequent steps are described in the section on extraction of DDP.

3.7. Extraction of DDP from admixtures and 3 separated phases of each ointment with HS

The DDP in prepared admixtures and the 3 separated phases was extracted according to a previously reported procedure (Suzuki et al. 2012; Lombardi Borgia et al. 2008). An aliquot (700 μL) of the liquid phase and 100 mg of the solid phase or admixture was dispersed in 7 or 10 mL of chloroform, respectively. After addition of fluocinonide as an internal standard, 1 mL of the sample was withdrawn and exsiccated by vacuum rotation. In the IM system, butylparaben was used as an internal standard instead of fluocinonide. Residues were extracted 3 times with 500 μL of methanol. Ten microliters of each sample was then injected into the HPLC system.

3.8. HPLC Analysis

The HPLC system consisted of a pump (LC-10AT; Shimadzu, Kyoto, Japan), a column (COSMOSIL 5C18-MS-II Packed Column, 4.6 × 150 mm; Nacalai Tesque, Kyoto, Japan), an auto-injector (SIL-10AXL; Shimadzu, Kyoto, Japan), a UV detector (SPD-10A; Shimadzu, Kyoto, Japan), and an analysis system (CLASS LC-10; Shimadzu, Kyoto, Japan). The mobile phase was adjusted by changing the appropriate ratio of distilled water/acetonitrile from 35:65 to 60:40 to avoid the additional peaks originating from the additives in the formulations. The flow rate was 1.0 mL/min. Detection was performed at a UV wavelength of 254 nm. The limit of detection of DDP was 300 pg.

3.9. Statistical analysis

Permeation data obtained at 4 h after initiation of permeation experiments (given as mean ± standard deviation [SD]) were analyzed using Tukey's multiple comparison test with one-way analysis of variance (ANOVA). Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered to be significant.

Acknowledgments: The authors would like to thank Lintec Co., Ltd. for generously providing the silicone membrane. We also wish to thank Mr. Mamoru Komatsu of Thermo Fisher Scientific K.K. for his valuable help with the Raman measurements.

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