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Pharmaceutical evaluation of steroidal ointments by ATR-IR chemical imaging: Distribution of active and inactive pharmaceutical ingredients

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ABSTRACT

We recently used micro attenuated total reflection infrared (ATR-IR) spectroscopy to conduct imaging analysis of ointments and evaluate the distributions of the active pharmaceutical ingredient (API) and excipients. An alclometasone dipropionate (ALC) ointment was used as a model product. Almeta®, a brand-name product, had a domain with absorbance at 1656 cm^{-1} attributable to the carbonyl group of ALC, the API. Absorbances at 1040 and 3300 cm^{-1} were also noted in this domain, indicating the presence of the solubilizer, propylene glycol. Data also suggested the presence of benzyl alcohol in this domain. More detailed analysis showed the distribution of surfactants and other excipients in the base. Similar results were obtained for Vitra®, a generic version of Almeta®. Imaging analysis with micro ATR-IR confirmed that both ointments are liquid droplet dispersions with ALC dissolved in propylene glycol and dispersed in a base. However, minor differences in the ingredient distributions of the two ointments were detected and reflect differences in excipient concentrations and type, or manufacturing differences. In summary, we used micro ATR-IR for imaging analysis of an original ointment, Almeta®, and its generic form Vitra®, and established a method for visually evaluating the distributions of the API and excipients in these ointments.

1. Introduction

Microscopic imaging systems that employ X-ray fluorescence, infrared, near infrared, terahertz, Raman, and a variety of other spectroscopic techniques have recently become very useful analytical tools in the fields of pharmaceutical design and quality control. Microspectroscopic imaging systems consecutively determine the spectra of ultra-small pixels in a plane of the sample, analyze the spectral data, and produce images with the results combined to obtain a two-dimensional chemoinformatic image. When such a system is used to image the surface of a pharmaceutical tablet, for example, information previously unavailable by high-performance liquid chromatography (HPLC) and other analytical technologies, such as the distribution and blend uniformity of the active pharmaceutical ingredient (API) and excipients, is obtained. This information has been used to evaluate generic products and drug products purchased on the Internet (Veronin and Youan, 2004; Westenberger et al., 2005). Many studies have been conducted to investigate the distribution and blend uniformity of the API and excipients in powders (Abhay et al., 2004; Bellamy et al., 2008; Li et al., 2008; Ma and Anderson, 2008; Shi et al., 2008).

The types and compositions of bases, and the techniques used to prepare ointments and other external preparations, significantly impact transdermal absorption of the drug (Stoughton, 1987; Jackson et al., 1989; Castanedo-Cazares et al., 2001; Curdy et al., 2004; Trottet et al., 2005). Using the above-mentioned microspectroscopic imaging systems to analyze ointment products would provide important information on the distribution of the API and excipients in these products. This type of information is currently available for tablets and powders. Spectroscopic evaluations of ointments often include infrared (IR) spectroscopy studies of drug release from ointment bases and the diffusion of drugs into artificial skin models (Wurstar et al., 1993; Hanh et al., 2000a,b,c; Remane et al., 2006; Guenther et al., 2008). However, we have been unable to identify any mention of the evaluation of an API or excipient distribution in an ointment using a microspectroscopic imaging system. We attribute this to the difficulty of imaging using near IR and IR diffuse reflectance, since diffuse reflectance is absent in liquid and semi-solid drug products (e.g., ointments).

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Table 1
Pharmaceutical ingredients used in the alclometasone ointments.

Pharmaceutical ingredients	Drug products	ALM	VIT
Base	White petrolatum	+	+
	White beeswax		+
	Liquid paraffin	+	+
Surfactant	Sorbitan esters of fatty acids	+	
	Glycerol stearate		+
Solubilizing agent	Propylene glycol	+	+
	Benzyl alcohol	+	+
pH modulator	Phosphoric acid		+

Steroid ointments are used primarily as antiinflammatory preparations. Many original products and their generic versions are currently available on the market. Alclometasone dipropionate (ALC) ointments are classified as mild-type ointments with relatively mild pharmacological actions for steroid products. These ointments are widely used to treat atopic dermatitis in children (USP, 2011). It is thought, however, that long-term application to the affected skin surface could emphasize differences in the pharmacological characteristics of the original product and its generic versions. Procedures to pharmaceutically evaluate ALC ointments should therefore be established, but the concentration of the API in steroid ointments is generally low. The API in ALC ointments is generally between 0.05% and 0.1% (w/w). To address this difficulty, we conducted imaging with micro ATR-IR, which combines IR microscopy with attenuated total reflectance (ATR). ATR analysis is more suited to spatial resolution than to measuring diffuse reflectance. IR analysis produces clearer spectra than near IR analysis, and the resulting spectra better facilitate the identification of a chemical structure (Andrew Chan et al., 2003). We conducted imaging analysis on the results and compared the distributions of the API and excipients in two ointment products. Finally, we evaluated the pharmaceutical characteristics of these ointments based on rheological characteristics, polarization microscopy findings, and analyses of the ingredients.

2. Experimental

2.1. Reagents

The ALC ointments Almeta® Ointment 0.1% (ALM), an original drug product by Shionogi & Co., Ltd. (Osaka, Japan), and Vitra® Ointment 0.1% (VIT), a generic version by Iwaki Seiyaku Co., Ltd. (Tokyo, Japan), were analyzed. The ALC used was a USP reference standard, and all other reagents used were reagent-grade chemicals by Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Polarization microscopy

The microscopic features of the ointments were characterized by applying a small amount of the sample to a microscope slide, covering with a cover slip, and observing under an E-600-Pol polarizing

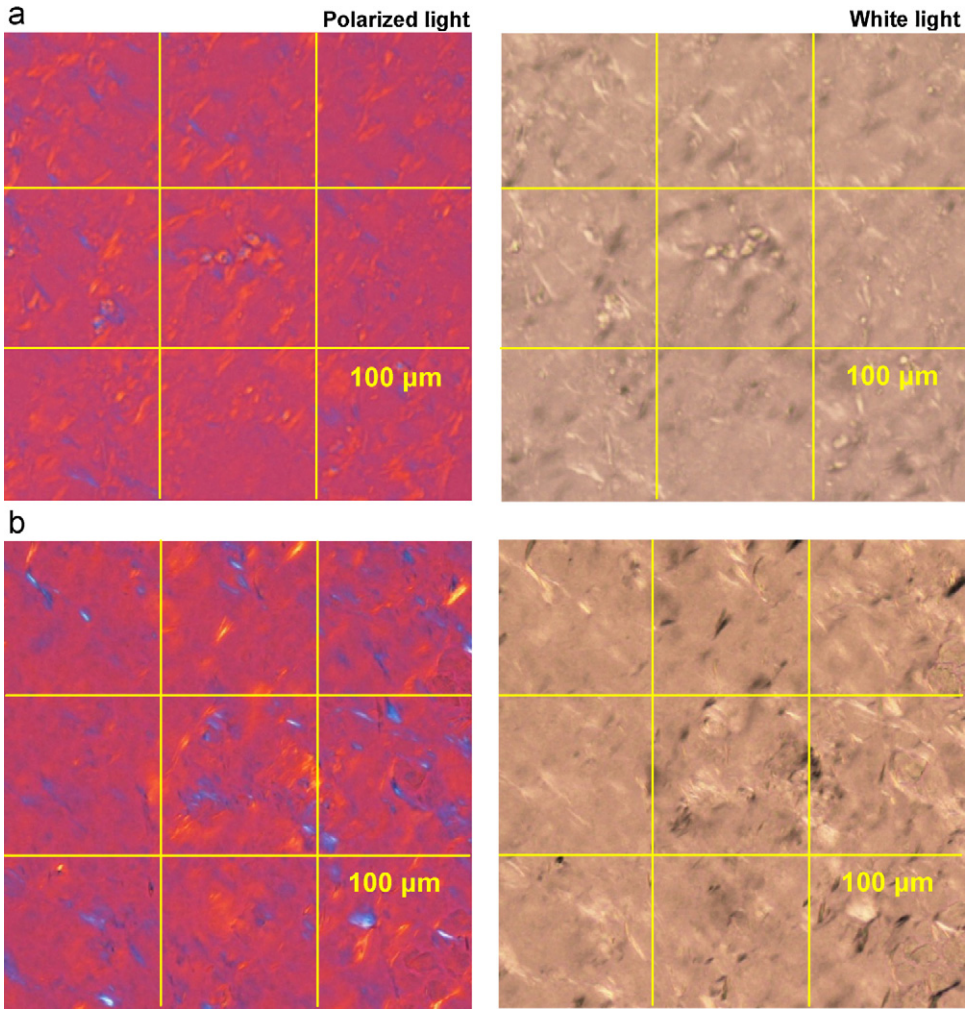

Fig. 1. Microscopic images of ALC ointments for (a) ALM and (b) VIT; magnification: 200×.

microscope (Nikon Corporation, Tokyo, Japan) in reflection mode at 200× magnification.

2.3. Determination of standard IR spectra

The pharmaceutical ingredients of ALM and VIT are shown in Table 1. The IR spectrum of each ingredient was determined with an FT/IR-4100 (JASCO Inc., Tokyo, Japan) equipped with an ATR unit (Dura-samplIR II, Smiths Detection Group Ltd., Watford, UK) at a wavenumber resolution of 4 cm^{-1} with 16 scans across the wavelength range of 4000–650 cm^{-1}.

2.4. Determining and imaging micro IR spectra

Spotlight400 (Perkin Elmer Japan, Yokohama, Japan), a line chemical imaging system equipped with a MCT linear array detector and Ge ATR accessory, was used to collect the IR spectra of ointments. Each spectrum came from a 1.56 μm × 1.56 μm square pixel. The background was measured in air, and the sample scans were recorded at 8 cm^{-1} spectral resolution with 2 scans across the range of 4000–750 cm^{-1}. Data were analyzed using Isys chemical imaging software (version 5.0, Malvern Instruments Ltd., Worcestershire, UK). The absorbance data of the IR spectra were subtracted by the absorbance at 1688 cm^{-1} to remove the baseline effect. The offset data from the ointments were used to generate chemical images using a peak height method based on the characteristic peaks of the pure components.

2.5. Determination of rheological characteristics

A spread meter (Rigo Co., Ltd., Tokyo, Japan) was used to evaluate ointment spread. The diameter D of a 0.5 (cm^3) sample of ointment was visually measured after 5–200 s. The yield value S_0 (dyne/cm^2) was calculated using the formula of Ichikawa (1977) using D_∞ (cm) at 200 s, the final measurement point (Scheme 1).

In the formula, G is gravitational acceleration (980 cm/s^2), P is the glass plate mass (g), and V is the volume of the sample (cm^3). A schematic diagram and photograph of the equipment used is provided in the supporting information (Fig. S1).

$$S_0 = \frac{48PVG}{\pi^2 D^5{}_\infty} \cdots (1)$$

Scheme 1.

2.6. Determining the content of propylene glycol (PG) and benzyl alcohol (BA) in the ointment

First, approximately 100 mg of the ointment was dispersed in 3 mL of hexane. Then, 5 mL of acetonitrile was added, and the resulting mixture was shaken for 10 min. One milliliter of the acetonitrile bottom layer was collected and diluted with acetonitrile to a volume of 20 mL. The resulting liquid was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a JMS-AM II15 mass spectrometer (JEOL Ltd., Tokyo, Japan). GC/MS was conducted according to the following conditions:

GC condition; column: HP-INNOWAX polar capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness, cross-linked polyethylene glycol), column temperature: 60 °C (2 min) → (15 °C/min) → 240 °C (6 min), injection temperature: 220 °C, injection mode: splitless, gas flow rate: 1.0 mL/min (He, constant flow).

MS condition; electric ionization (70 eV, 300 μA), interface temperature: 240 °C, ion source temperature: 200 °C.

3. Results and discussion

ALM, the original ALC ointment product, contains the API dissolved in an almost saturated state in propylene glycol (PG) and other solubilizers. In the interview form (Shionogi & Co., Ltd., 2011) of ALM, special technology is reportedly used to disperse this as microscopic droplets into a petroleum jelly base to form a liquid droplet dispersion. ALM and VIT (Iwaki Seiyaku, 2008), a generic version, contain multiple excipients (Table 1) and are thus appropriate drug products for evaluating API and excipient distribution. We began by microscopically observing these ALC ointments and conducting IR analyses of their ingredients. Then, the microscopic

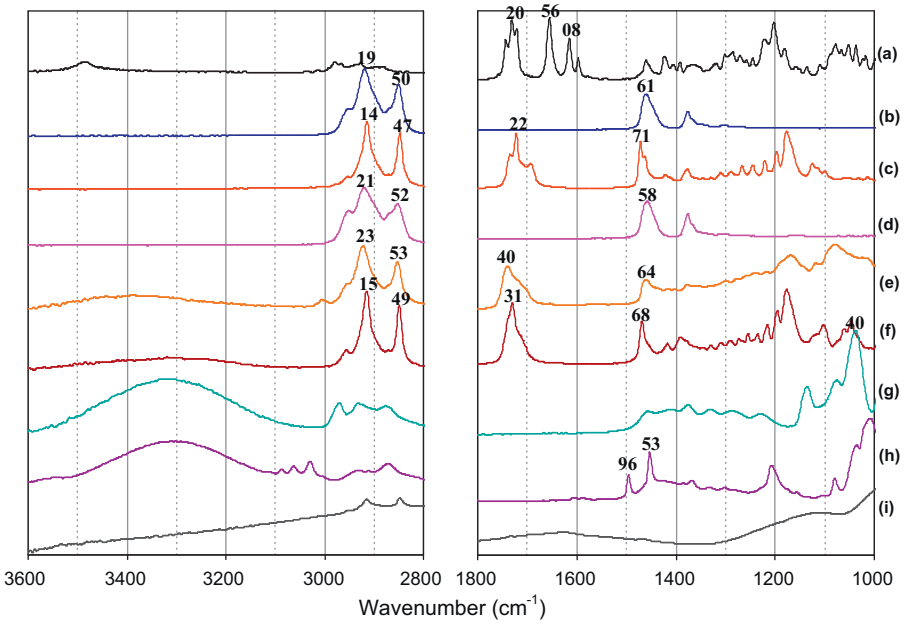

Fig. 2. IR spectra for pharmaceutical ingredients used in the ALC ointments; (a) ALC, (b) white petrolatum, (c) white beeswax, (d) liquid paraffin, (e) sorbitan esters of fatty acid (sorbitan monooleate), (f) glycerol stearate (glycerol monostearate), (g) propylene glycol, (h) benzyl alcohol and (i) phosphoric acid.

structures of these ointment products, as characterized by IR imaging, were investigated.

3.1. Evaluation of basic pharmaceutical characteristics

Fig. 1 shows the results of polarization microscopy of the ALC ointment products. Neither of the two ALC ointment products investigated exhibited a distinct droplet structure. Refractive light attributable to the partial crystalline structure of white petrolatum, the base, was evident. The lack of any crystals of ALC, the API, in the overall samples outside the fields shown in Fig. 1 suggests that the ALC in each ointment product is either dissolved in the base or dispersed as microparticles so small as to be not visually apparent. In either case, it is difficult if not impossible to distinguish ALM, the original product, from VIT, the generic version, based on these images.

Reference IR spectra of the API and major excipients (Table 1) of the two ointment products are shown in Fig. 2. Peaks near 1608, 1656, and 1720 cm^{-1} in the IR spectrum of ALC are attributable to ester and ketone groups. White petrolatum (WP) and liquid paraffin (LP), which form the base of the ointments, exhibit multiple peaks around 1461, 2850, and 2919 cm^{-1} that are indicative of C—H bending vibrations and stretching vibrations, respectively. The base white beeswax (WB, found only in VIT), the surfactant glyceryl monostearate (found only in VIT as glyceryl stearate (GS)), and sorbitan monooleate (found only in ALM as sorbitan esters of fatty acids (SE)), each displayed a single peak near 1720 cm^{-1}. The solubilizers propylene glycol (PG) and benzyl alcohol (BA), which serve as a solubilizer as well as a preservative, showed a broad peak around 3300 cm^{-1} and a sharp peak near 1040 cm^{-1}. A distinctive peak due to BA was observed near 1496 cm^{-1}.

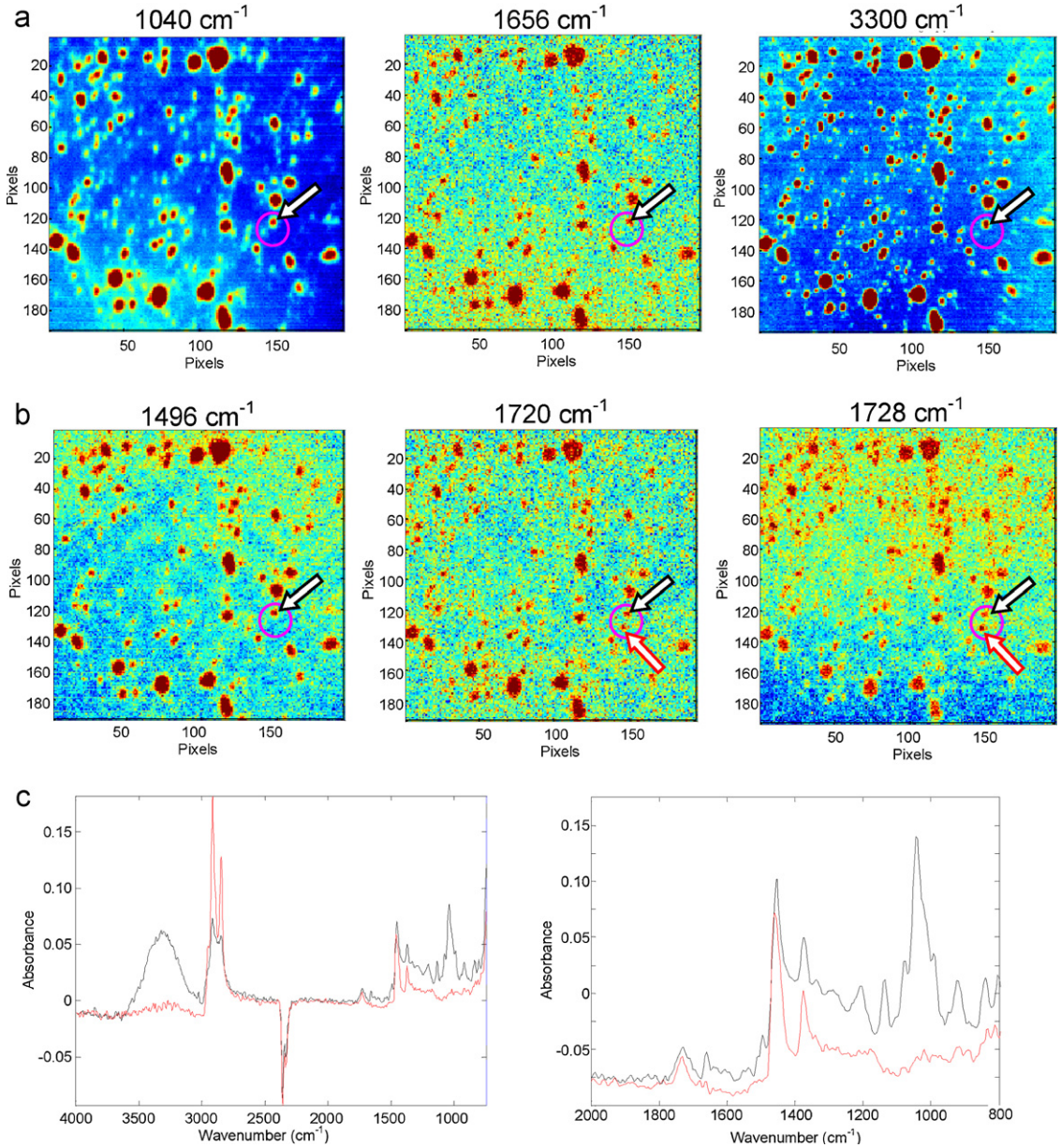

Fig. 3. (a) ATR imaging in the mid-IR of ALM ointments at 1040, 1656, and 3300 cm^{-1} and (b) 1496, 1720 and 1728 cm^{-1}, respectively. Red indicates high density and blue indicates low density. Units on the *x* and *y* axes are number of pixels. One pixel corresponds to 1.56 μm^2. (c) IR spectra obtained from the domains indicated by red allow (red spectra) and black allow (black spectra) in the ATR imaging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.2. Micro ATR-IR imaging

3.2.1. Imaging of ALM and VIT

Micro ATR-IR spectral imaging analysis (Figs. 3 and 4) at the wave numbers believed to be specific to each ingredient was conducted in reference to the reference IR spectra of the ointment ingredients (Fig. 2). The IR spectra shown by black lines in Figs. 3c and 4c were obtained from the domains indicated with black arrows in these figures. These spectra were compared with the reference spectra (Fig. 2). The presence of a broad peak near 3300 cm^{-1} and a sharp peak near 1040 cm^{-1} suggests the presence of PG. The presence of peaks near 2900 and 1400 cm^{-1} is indicative of the hydrocarbons WP and LP. Finally, the peak near 1656 cm^{-1} is attributable to the ester and ketone groups of the API, ALC. Figs. 3a and 4a show the distribution of the domains with absorbances at 1040 cm^{-1} and 3300 cm^{-1} (PG) as well as 1656 cm^{-1} (ALC) in the images obtained from the imaging analysis of ALM and VIT with micro ATR-IR. This domain with absorbances at the 1040, 1656 and 3300 cm^{-1} suggests that ALC is dissolved in PG and distributed as droplets in the base.

3.2.2. Surfactant distribution in the two ointment products

As ALC shows absorbance not only at 1656 cm^{-1} but also near 1720 cm^{-1} (Fig. 1), the domains of the images at 1656, 1720, and 1728 cm^{-1} in the ATR-IR images of ALM and VIT were expected to overlap. Analysis, however, revealed many domains with absorbance only at 1728 cm^{-1} in VIT (e.g., the domain indicated by the red arrow in Fig. 4b). Such domains were also observed for ALM, albeit only slightly (Fig. 3b). Images at the three wave numbers 1040, 1656, and 1728 cm^{-1} were superimposed (Fig. 5). The superimposed image shows that most of the domains observed for ALM are on top of one another (the domains shown in white to yellow in the figure). In contrast, for VIT, although some of the domains were duplicated, many appeared as dispersed. This shows that ALC and PG are distributed together in ALM while in VIT, multiple ingredients are independently dispersed. This finding is indicative of differences in the formulations and manufacturing processes of the two products.

The source of the many independently observed domains at 1728 cm^{-1} in VIT (shown in blue in Fig. 5b) appears to be the surfactant or WB, judging from a comparison to the reference spectra

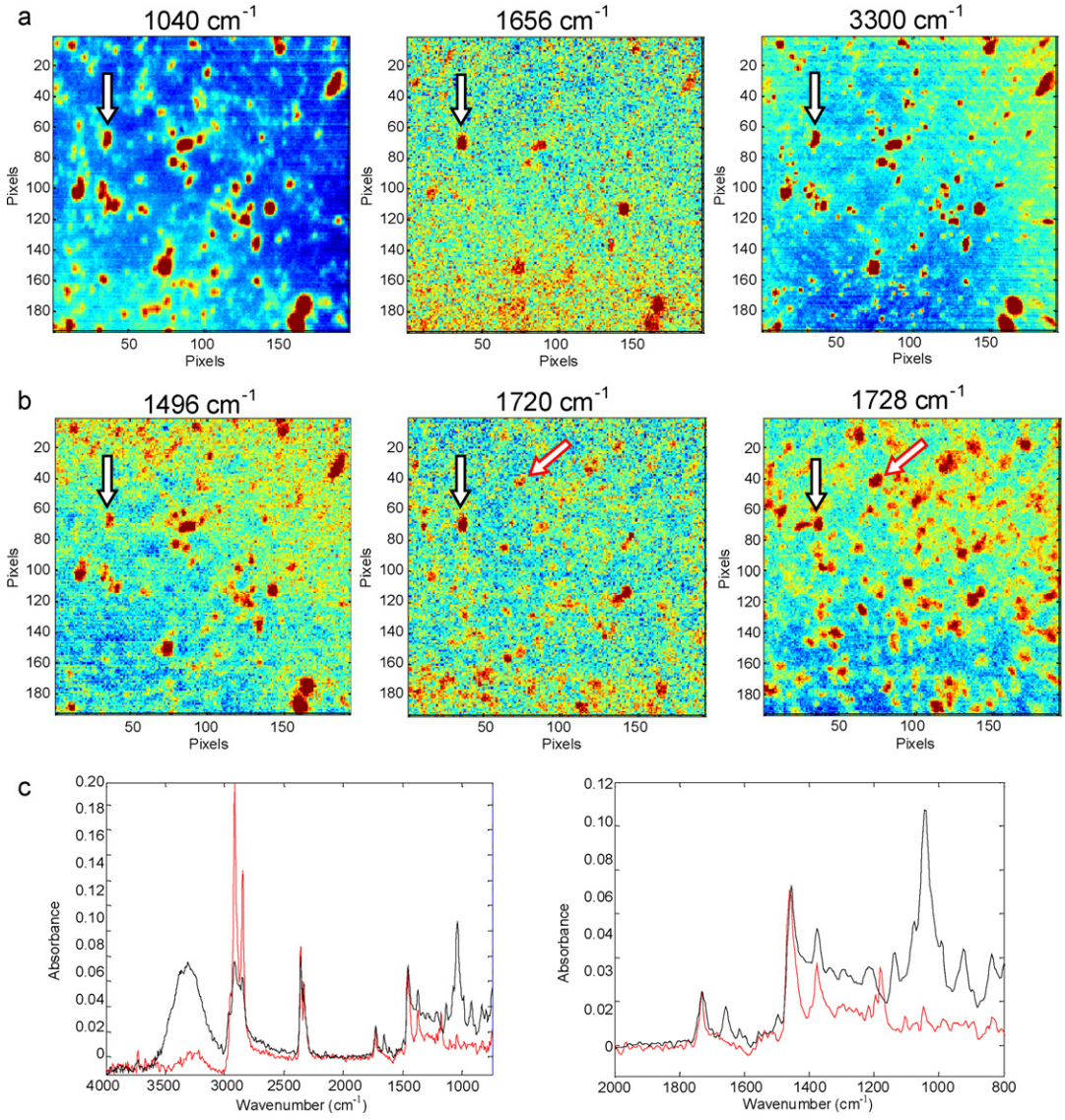

Fig. 4. (a) ATR imaging in the mid-IR of VIT ointments at 1040, 1656, 3300 cm^{-1} and (b) 1496, 1720 and 1728 cm^{-1}, respectively. Red indicates high density and blue indicates low density. Units on the *x* and *y* axes are number of pixels. One pixel corresponds to 1.56 μm^2. (c) IR spectra obtained from the domains indicated by red allow (red spectra) and black allow (black spectra) in the ATR imaging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

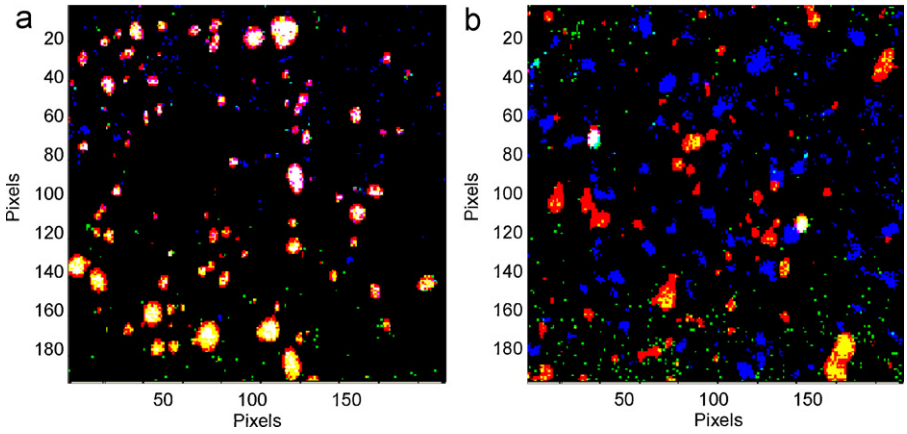

Fig. 5. Overlapped ATR imaging indicating a speculated distribution of pharmaceutical ingredients in ointment; (a) ALM and (b) VIT. Red, green and blue region indicates the domains where absorption is observed at 1040, 1656 and 1728 cm^{-1}, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(Fig. 2). Thus, the images at 1720 and 1728 cm^{-1} may indicate the presence not only of ALC, but also of the surfactant or other excipients. However, ALC and sorbitan esters of fatty acids (SE) are the only ingredients in ALM with absorbance in this area (Fig. 2 and Table 1). In the images at 1656, 1720, and 1728 cm^{-1}, the domains in which all repeats are indicative of ALC, and the domains with absorbances only at 1720 and 1728 cm^{-1} are indicative of the presence of SE. The spectra indicated with black and red lines in Fig. 3c (IR spectra of domains indicated by the black and red arrows in Fig. 3, respectively) show a distinct difference between the two domains. The peak attributable to ALC near 1656 cm^{-1} in the former is absent from the latter.

SE and glyceryl stearate (GS), which are included in ALM and VIT as surfactants, are difficult to accurately quantitatively determine because they are mixtures of multiple fatty acids and fatty acid esters of varying degrees of substitution, but HPLC analysis suggests their content to be on the order of several percent (see Supp. inf., Figs. S2 and S3). The evaluation of rheological characteristics using a spread meter indicated both ointments to have plastic fluidity. The yield values for ALM and VIT, which are 1621.2 ± 75.4 and 2986.0 ± 508.1 (dyne/cm^2), respectively, show VIT to be the harder ointment (Fig. 6). Since WB is a solid at room temperature, its inclusion in a formulation is expected to result in a harder ointment. The many domains at 1728 cm^{-1} in the VIT images (Fig. 5b) thus suggest the presence of WB and GS in relatively large amounts.

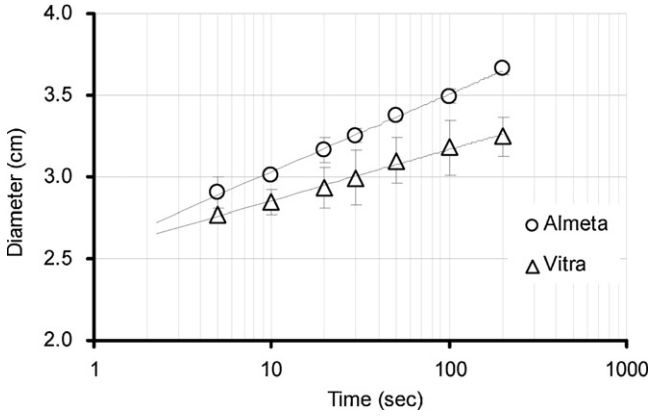

Fig. 6. Changes in sample diameter of ALC ointments in spread meter.

Table 2
Propylene glycol and benzyl alcohol content of alclometasone ointments.

Drug	Propylene glycol	Benzyl alcohol
ALM	1.1 ± 0.1	1.9 ± 0.0
VIT	3.0 ± 0.0	3.0 ± 0.0

3.2.3. Distribution and determination of PG and BA

Detailed investigation of the ALM and VIT images lead to the identification of small peaks near 1496 cm^{-1} in spectra obtained from domains with absorbances at 1040, 1720, and 3300 cm^{-1} (see Supp. inf., Fig. S4). Only BA shows the presence of absorbance peaks at this wavenumber (Fig. 2), showing that the droplets are constituted of BA and PG. The excipient BA is widely used as a preservative (Iwaki Seiyaku, 2008) but does not dissolve in hydrocarbons such as petrolatum. BA has complete compatibility with PG, so it follows that BA with PG helps solubilize ALC in the ointment. The determination of PG and BA content in ALM and VIT using GC/MS showed the PG content in VIT to be approximately 3-fold higher than that in ALM (Table 2). Data on the total PG and BA content indicate that VIT has about twice the solubilizer content as ALM (although BA do not appear to have been added for the purpose of solubilization). As mentioned above, VIT is the harder ointment. The use of WB as an excipient in VIT, despite the large solvent volumes, appears to increase ointment hardness.

The permeation of the API in external preparations through the skin is affected by the amount of the API dissolved in PG and other ointment ingredients (Ishii et al., 2005; Remane et al., 2006) and the rheological characteristics of the preparation, which influence the ease of spreading the ointment (American Pharmaceutical Association and Pharmaceutical Society of Great Britain, 1986). Although no critical difference in the distributions of the API in ALM and VIT was noted in this study, the dilution, blending, and other actions sometimes taken in the clinic according to patient symptoms could result in quality differences between the two products. We focused on evaluating the physicochemical quality of the two ointments in this study, but the skin permeability of ALC warrants further investigation and is the subject of a manuscript in preparation.

4. Conclusions

We used imaging analysis with micro ATR-IR to evaluate ALM, an original 0.1% ALC ointment, and VIT, a generic version. Differences in the distributions of the API and excipients were identified. ALC, dissolved in PG and BA, was found to be dispersed in the petrolatum

base. Differences in the degree of dissolution of the API and its concentration in droplets, as well as the dispersion of these droplets, may lead to differences in API permeation in the microstructures of the skin (Fig. S5). Consequently, pharmaceutical evaluation of original products and their generic versions could provide information to facilitate appropriate use. Imaging technology with micro ATR-IR has been previously used to evaluate tablet and powder product quality, but we believe our study to be the first of its kind on ointment products. Findings obtained from this study are therefore significant not only for their visual evaluation of the distribution of the API and excipients in ointments, but also for the contribution made to scientific progress.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2012.01.017.

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