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Magnetic Resonance Imaging Study on the Physical Stability of Menthol and Diphenhydramine Cream for the Treatment of Chronic Kidney Disease-Associated Pruritus

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A cream that contains menthol and diphenhydramine is widely prepared in hospital pharmacies and prescribed to patients for the treatment of pruritus associated with chronic kidney disease. However, there is a serious concern regarding its physical stability; therefore, we investigated this issue using magnetic resonance imaging (MRI). For a sample preparation, a menthol-containing ethanol solution was mixed with a commercial diphenhydramine cream. After storage for 7 d at 40°C, substantial phase separation into two distinct layers (upper and lower layers) was observed in the sample. This study further examined the components of the phase-separated layers using magnetic resonance (MR) spectroscopy and chemical shift selective images, and it was verified that the upper layer consisted of packed oil droplet layers, whereas the lower was an aqueous phase. Subsequently, the time-dependent phase separation of the sample at different temperatures was investigated. From the MR images, including a T_2 relaxation time map and apparent diffusion coefficient maps, it was obvious that the phase separation developed further with increasing temperature; the most substantial phase separation was observed from the sample stored at 40°C, while no phase separation was detected at 25°C. In the final phase of this study, we conducted a formulation study and succeeded in improving the cream's physical stability by adding a hydrophilic surfactant to the preparation.

Key words magnetic resonance imaging; T_2 map; chemical shift selective image; apparent diffusion coefficient map; magnetic resonance (MR) spectroscopy

Chronic kidney disease (CKD)-associated pruritus (also known as uremic pruritus) is a significant problem for many patients receiving long-term hemodialysis.[1–3] Pruritus is an unpleasant symptom that negatively impacts a patient's quality of life; for example, moderate/severe pruritus seriously affects sleeping and appetite regulation of patients.[4] In hospitals in Japan, a cream that contains menthol and diphenhydramine is widely prescribed to patients for the treatment of the symptoms. This preparation is made in hospital pharmacies by mixing a menthol-containing ethanol solution with a commercial diphenhydramine cream. Owing to the pleasant cooling sensation from the menthol, as well as the antihistaminic action of diphenhydramine, the preparation has received favorable responses from patients. However, there is a serious concern about the physical stability of the preparation based on accumulated on-the-job experience in hospitals. Substantial phase separation in the preparation is occasionally observed during storage. From this perspective, this study focused on the physical stability of the preparation.

To date, various methods have been proposed to evaluate the physical stability of emulsions. These include droplet-size analysis,[5,6] light scattering,[5,7,8] and turbidity measurements.[9] However, the sensitivity of these techniques is insufficient to detect the subtle destabilization processes of pharmaceutical emulsions. In addition, these sampling techniques are destructive by nature, and samples need to be transferred to an appropriate sample container (*e.g.*, a transparent cuvette or tube) for the measurements to take place. Thus, we cannot elucidate the natural destabilization processes of pharmaceutical emulsions in a commercial container (*e.g.*, a tube or jar).

Recently, we developed a nondestructive method to evaluate the physical stability of pharmaceutical emulsions by using magnetic resonance imaging (MRI).[10,11] MRI is one of the most popular molecular imaging methods, and enables nondestructive monitoring of a sample using the principle of NMR. In addition, it can visualize the molecular mobility of a sample using magnetic resonance (MR) parameters. Our method takes advantage of the visualization technique of the state of water to detect phase separation and creaming in pharmaceutical emulsions.[10] In addition, it is worth noting that the sensitivity of the detection limit of our method is superior to that of visible observations; for example, slight creaming, which is not visible to the naked eye, can be visualized clearly.[10] Nowadays, many research institutes and hospitals introduce MRI; thus, our method is thought to be applicable for evaluation of preparations made in hospital pharmacies.

To investigate the physical stability of the preparation in more detail, this study fully utilized MR techniques. As well as nondestructive monitoring of the phase separation, the distribution of specific compounds in the phase-separated sample

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was visualized using chemical shift selective (CSS) images. In addition, we attempted to improve the physical stability to make the preparation more useful.

Experimental

Materials The diphenhydramine cream (Restamin Kowa Cream®) was purchased from Kowa Pharmaceutical (Tokyo, Japan). *l*-Menthol and thymol were purchased from Pfizer (Tokyo, Japan). Mentha oil was purchased from Yoshida Pharmaceutical (Tokyo, Japan). *d*-Camphor was purchased from Kozakai Pharmaceutical (Tokyo, Japan). Polyethylene glycol (PEG)-60-hydrogenated castor oil (Nikkol HCO-60) was kindly donated by Nikko Chemicals (Tokyo, Japan). All other chemicals used were of analytical grade.

Sample Preparation The formulation of the menthol and diphenhydramine cream is shown in Table 1. After preparing the menthol-containing ethanol solution, it was mixed with diphenhydramine cream at a weight ratio of 14 : 86 for 1 min using a revolution/rotation-type hybrid mixer (Model HM-500, Keyence, Tokyo, Japan). The revolution and rotation speeds of the mixer were 2000 and 800 rpm, respectively. The sample was packed in a tube (1.5 mL) and stored at 25, 30, or 40°C for designated intervals. Samples were then continuously monitored using MRI.

MRI Study The MRI experiments were performed at room temperature using a 9.4 T vertical MRI scanner (Varian, Palo Alto, CA, U.S.A.). The ^{1}H-NMR spectrum of the entire sample was acquired using an S-PLUS sequence, while the spectrum of a region of interest (ROI) was acquired using a stimulated echo acquisition mode (STEAM) sequence (acquisition bandwidth=5 kHz). The ROIs were positioned in the center of each layer. The voxel size of the ROIs was fixed at $2\times2\times2$ mm^3.

The CSS images were acquired using a CSS spin-echo pulse sequence comprising 90° and 180° Gaussian radiofrequency (RF) pulses. The 90° pulse was band selective and the 180° pulse was a slice-selective refocusing pulse. We set the center frequency to the water and hydrocarbon protons from the ^{1}H-NMR spectra acquired using the S-PLUS sequence. The bandwidth of the RF pulses was 540 Hz. All of the CSS images were acquired using the following parameters: repetition time (TR)=2000 ms, echo time (TE)=15 ms, field of view (FOV)=35×30 mm^2, matrix size=128×128, number of excitations=4, and slice thickness=1 mm. T_2-Weighted images were acquired using a spin-echo pulse sequence with TR=2000 ms, TEs=25, 40, and 60 ms, FOV=30×30 mm^2, matrix size=128×128, number of excitations=2, and slice thickness=1 mm. The decay of the echo amplitude is given by the equation:

Table 1. Formulation of Menthol and Diphenhydramine Cream

Components	Weight (g)
Menthol-containing ethanol solution	14
l-Menthol	7
Thymol	0.4
Mentha oil	0.9
10% Camphor in ethanol	5.25
Polyoxyethylene castor oil 60 (Nikkol HCO-60)	0, 0.3, 0.7, 1.0, 2.0
Ethanol	ad 14
Restamin Kowa Cream	86

$$E = \frac{S}{S_0} = \exp\left(-\frac{\tau}{T_2}\right) \quad (1)$$

where S is the echo amplitude at time τ, T_2 is the relaxation time (spin–spin relaxation time), and S_0 is the amplitude at time $\tau=0$. Equation 1 was used to fit the experimental points of the echo amplitude decay in the acquired images pixel-by-pixel, resulting in the quantitative T_2 map.

Diffusion-weighted images (DWIs) were acquired using a pulsed-field gradient spin-echo (PGSE) sequence.[12–14] This pulse sequence superimposes a pair of square-shaped gradient field pulses (so-called motion-probing gradients) on the spin–echo pulse sequence. With regard to DWIs, the attenuation of the image intensity, E, is described by the Stejskal–Tanner equation:

$$E = \frac{S}{S_0} = \exp(-\mathrm{ADC}\times b) \quad (2)$$

where S is the amplitude of the image intensity in the presence of a gradient pulse, S_0 is the amplitude in the absence of a gradient pulse, and ADC is the apparent diffusion coefficient of water molecules. The b is the gradient factor expressed as:

$$b = \gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) \quad (3)$$

where γ is the gyromagnetic ratio of the proton, g and δ are the strength and duration of the gradient pulse, respectively, and Δ is the interval between two gradient pulses. In this study, the b-values were set at 0, 1000, 2000, and 3000 s/mm^2; the δ of 7 ms and Δ of 20 ms were fixed, while g was changed, ranging from 0 to 22.0 G/cm. The other acquisition parameters for DWIs were as follows: TR of 2000 ms, TE of 45 ms, FOV of 30 mm×30 mm, 128×128 matrix size, and a slice thickness of 1 mm. The ADC values were calculated pixel-by-pixel for the DWIs according to Eqs. 2 and 3, resulting in the ADC map.

Results and Discussion

We first evaluated the menthol and diphenhydramine cream after storage for 7 d at 40°C. As shown in Fig. 1a, substantial phase separation into two layers was observed. The lower layer was relatively clear, while the upper layer seemed to be in a state of creaming. The ^{1}H-NMR spectrum of the entire sample was also acquired (Fig. 1b). Two main peaks occurring at 1.0 ppm and 4.7 ppm were observed in the spectrum. They were assigned to the hydrocarbon groups of the oils and to water, respectively. A small peak was also observed at 3.5 ppm, which was assigned to the oxygenated proton mostly derived from ethanol. Because there was a sufficient difference in the chemical shifts of the substances to differentiate them, we acquired their CSS images (Fig. 1c). The CSS images were constructed by exciting protons in a very narrow frequency range, which enabled us to visualize the spatial distribution of specific compounds in the samples. In general, MR images are constructed by summing the NMR signals from all liquid state protons irrespective of their chemical structure, and so MRI data usually lack the specific spectroscopic information contained within the NMR signals.[15] On the other hand, CSS images can be referred to as an imaging technique that integrates MR spectroscopy (MRS) data into MRI data. We previ-

ously investigated the physical stability of a mixed preparation consisting of a moisturizing cream and a steroid ointment for use in the treatment of atopic dermatitis.[16] In the course of the previous study, we applied this technique and successfully visualized the heterogeneous distributions of water and oil in the phase-separated samples. Based on that study, we tried to visualize the distribution of specific compounds in the sample using the CSS image. The peaks at 1.0, 3.5, and 4.7 ppm were employed for the acquisition of the CSS images of oil, ethanol, and water, respectively. From the images, we successfully characterized their distribution (Fig. 1c). The oils seemed to be located only in the upper layer. The distributions of water and ethanol were completely consistent with each other; they were mostly distributed in the lower layers, and some fraction of them was also contained in the upper layer.

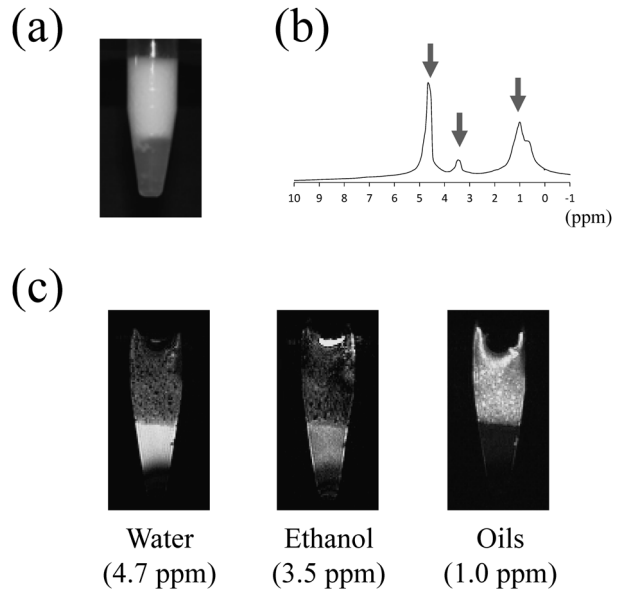

Fig. 1. Visualization of the Phase Separation of the Menthol/Diphenhydramine Cream

The cream was stored for 7 d at 40°C. (a) Optical photography. (b) ^{1}H-NMR spectrum of the entire sample acquired using S-PLUS. The acquisition parameters were TR=500 ms, flip angle of 4°, and an average of 32 scans. (c) CSS images. The images of water, ethanol, and oils were created by exciting the peaks at 4.7, 3.5, and 1.0 ppm, respectively.

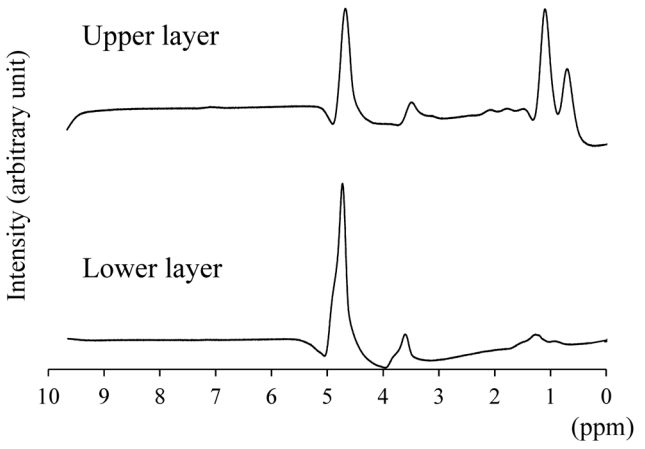

Fig. 2. ^{1}H-NMR Spectra of Each Phase-Separated Layer Acquired Using STEAM

The menthol/diphenhydramine cream was stored for 1 d at 40°C. ROIs (2×2×2 mm^3) were positioned in the center of the upper and lower layers. The acquisition parameters were TR=2000 ms and TE=10.20 ms from an average of 32 scans. The *y*-axis of each spectrum has arbitrary units.

Figure 2 shows the ^{1}H-NMR spectra of the upper and lower layers acquired by MRS. MRS is a technique that enables the acquisition of the NMR spectrum of a selected ROI. The spectral information was in good agreement with the CSS images and provided further comprehensive knowledge about the composition of the lower and upper layers (Fig. 2). The peaks at 3.5 and 4.7 ppm, oxygenated methylene proton of ethanol and the water proton, respectively, were detected in both the upper and lower layers. By contrast, peaks of hydrocarbon oils at around 1.0 ppm were mostly observed in the upper layer. These results indicate that the upper layer was a packed oil droplet layer with a considerable amount of water and ethanol, while the lower layer was an aqueous phase mostly composed of water and ethanol.

In the next phase of the study, we investigated the time-dependent change in phase separation following storage at different temperatures. To monitor the phase separation in the samples nondestructively, this study employed the ADC and T_2 maps as the visualization technique of the state of water. As far as oil-in-water (o/w)-type emulsion is concerned, the oil droplets dispersed in the continuous phase substantially affect the states of water.[11] Because surfactants are concentrated in the surface of oil droplets, the molecular mobility of water adjacent to oil droplets is more restricted than that of bulk water. By taking advantage of the difference in the state of water, these maps can visualize the phase separation in emulsion nondestructively. As shown in Figs. 3 and 4, T_2 and ADC values of the upper layer were much lower than those of the lower layer. This is a reasonable result because the upper layer is thought to be the packed oil droplet layer. With regard to the phase-separation behavior, higher temperature was accompanied by more substantial phase separation. The most substantial phase separation was observed from the samples stored at 40°C; obvious phase separation occurred within 24 h. Gradual but steady development of phase separation was observed from the samples stored at 35°C. By contrast, samples stored at 25°C were completely stable and no phase separation was observed from the sample during the experimental period. For further information, in a preliminary experiment, we evaluated the diphenhydramine cream alone for 3 weeks at 40°C, and confirmed that no phase separation was observed even after 3 weeks storage (data not shown). This indicates that the original diphenhydramine cream is quite stable by nature. Taken together, it is obvious that the substantial phase separation of the preparation is mainly attributed to the mixing of the menthol-containing ethanol solution with the diphenhydramine cream.

Figures 5a and b show histograms of T_2 and ADC for the samples stored at 40°C. They were generated by extracting the data of the MR parameters from the maps shown in Figs. 3 and 4. Two distinct peaks are evident; the peak with smaller MR parameters represents the upper layer, while the peak with larger values represents the lower layer. The area of the lower layer gradually expanded with prolonged incubation period. Furthermore, we found a relationship between the MR parameters and the incubation period (Figs. 5c, d). In the case of the upper layer, T_2 and ADC declined with prolonged incubation period: median for T_2 of 31.8 ms and 27.9 ms; median for ADC of 1.27×10^{-10} m^2/s and 0.73×10^{-10} m^2/s for 1- and

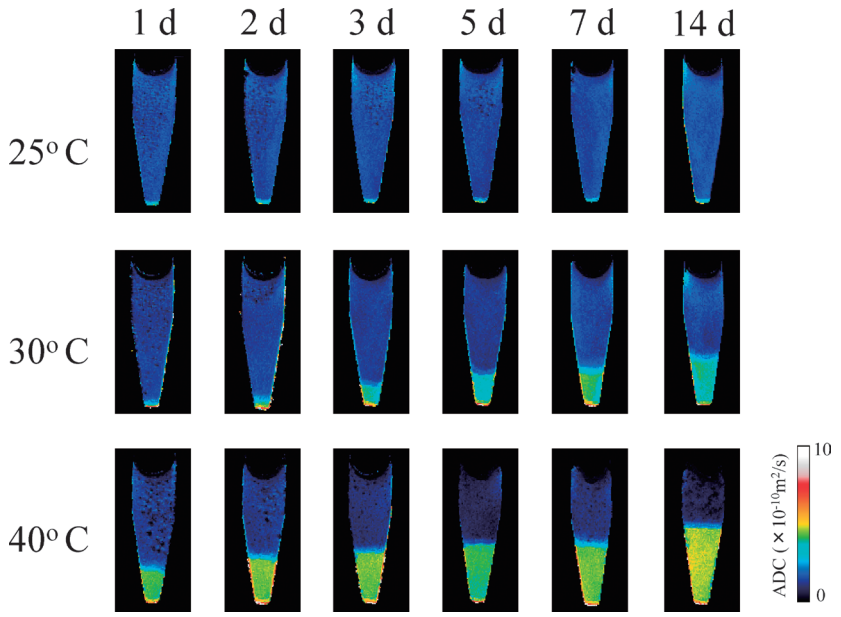

Fig. 3. ADC Maps to Visualize the Phase-Separation Behavior of the Menthol/Diphenhydramine Cream

The creams were stored at 25, 30, and 40°C for up to 14 d and then continuously monitored using MRI.

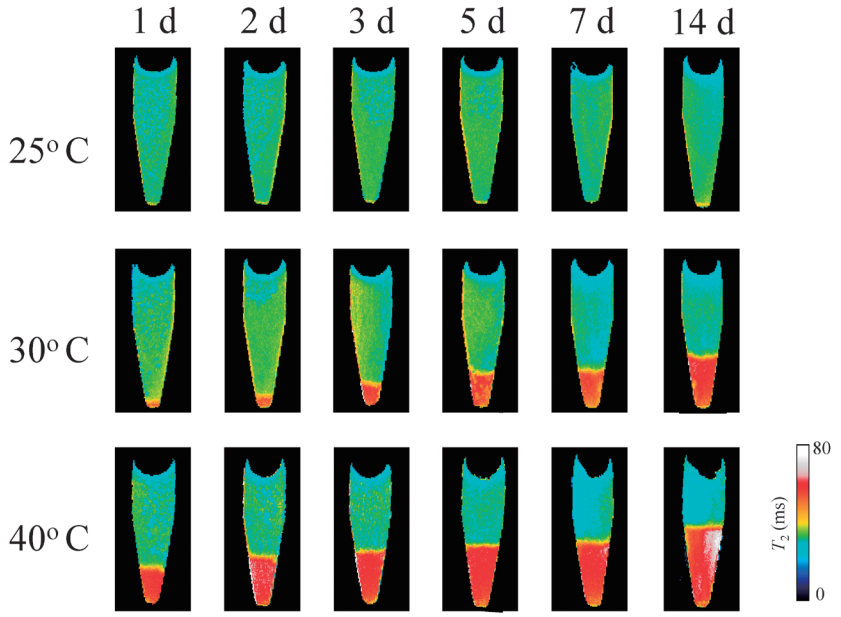

Fig. 4. T_2 Maps to Visualize the Phase-Separation Behavior of the Menthol/Diphenhydramine Cream

The creams were stored at 25, 30, and 40°C for up to 14 d and then continuously monitored using MRI.

14-d storage, respectively. In contrast, those of the lower layer increased: T_2 of 56.7 ms and 58.7 ms; ADC of 4.53×10^{-10} m^2/s and 4.66×10^{-10} m^2/s for 1- and 14-d storage, respectively. From these results, we assume that the compositions of the layers slowly changed with prolonged incubation period; the lower layer became a purer aqueous phase by excluding more oil droplets, while the upper layer was packed more tightly with oil droplets.

In the final phase of this study, we attempted to improve the physical stability of the preparation to make it more useful. As noted, the destabilization of the preparation is largely attributed to mixing the menthol-containing ethanol solution with diphenhydramine cream. Commercial pharmaceutical emulsions are not designed under the assumption that they would be mixed with other components. The surfactant content required to maintain a stable dispersion state may be changed by mixing these formulations with other components. Taking this issue into account, we added surfactant to the preparation and then examined the physical stability. Nikkol HCO-60 tested in this study is a popular hydrophilic surfactant for use in pharmaceutical emulsions. For example, it is incorporated into the hydrophilic cream listed in JP XVI. For the sample preparation, the surfactant was added to the menthol and diphenhydramine cream at concentrations ranging from 0.0% to 2.0% of total weight. These samples were stored for 1 d at 40°C, and then their ADC maps were acquired. From our

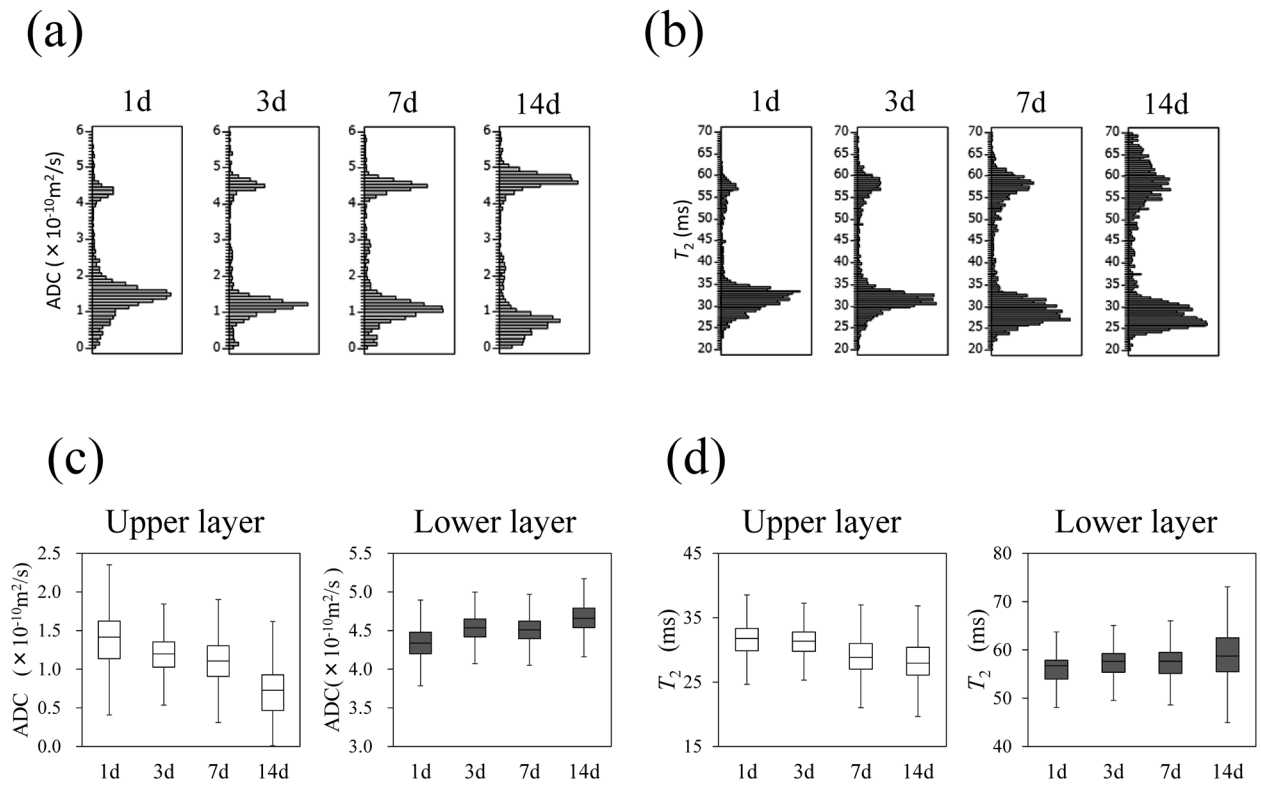

Fig. 5. Time-Dependent Change in ADC and T_2 Values of the Menthol/Diphenhydramine Cream Stored at 40°C

The data were extracted from the ADC and T_2 maps shown in Figs. 3 and 4. Histograms of (a) ADC and (b) T_2 were generated by random sampling of 2000 data from the maps. Box plots of (c) ADC and (d) T_2 were generated from the histogram data (a, b). ADC of 3×10^{-10} m^2/s and T_2 of 45 ms were used as thresholds to distinguish the upper and lower layers.

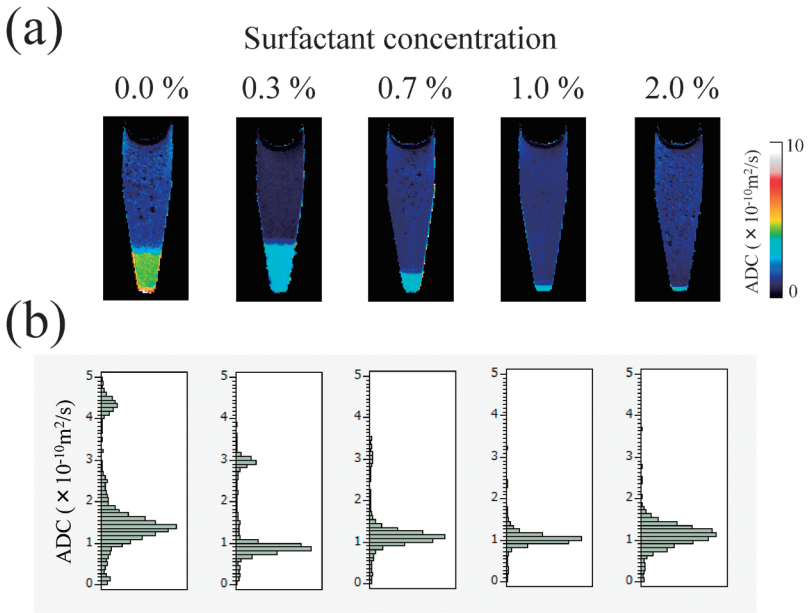

Fig. 6. Effect of the Addition of Surfactant to the Cream on the Phase Separation

For sample preparation, Nikkol HCO-60 was added to the cream at concentrations ranging from 0.0 to 2.0%, and then these samples were stored for 1 d at 40°C. (a) ADC maps of the samples. (b) Histograms of ADC. The histograms were generated by random sampling of 2000 data from the maps.

experiments, it was obvious that the addition of the surfactant had a great impact on the physical stability (Fig. 6a). According to the histograms generated from the maps (Fig. 6b), the proportion of the pixels of the lower layer to those of the entire sample was reduced steadily with increase in the surfactant concentrations: 15.8, 22.0, 8.6, 2.6, and 1.6% for surfactant concentrations of 0.0, 0.3, 0.7, 1.0, and 2.0%, respectively. Therefore, we succeeded in improving the physical stability of the preparation.

Conclusion

MRI is a powerful tool to evaluate the physical stability

of pharmaceutical emulsions. This study provided a comprehensive understanding of the physical stability of menthol and diphenhydramine cream. In the end of the study, was successfully improved the physical stability by adding hydrophilic surfactant to the preparation. We believe this study will provide benefit for the treatment of CKD-associated pruritus.

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Conflict of Interest The authors declare no conflict of interest.

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