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Chemical mapping of tulobuterol in transdermal tapes using Microscopic Laser Raman Spectroscopy

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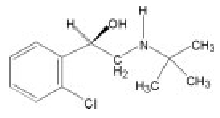
Microscopic Laser Raman Spectroscopy and Mapping (MLRSM) technique was used to investigate the distribution of tulobuterol (TBR) crystals in transdermal tapes. TBR is one of suitable compounds for the transdermal pharmaceuticals because it has high permeability into skin. In case of TBR transdermal tapes, some commercial products also contain TBR crystals in order to control a release rate from a matrix. Therefore, the presence of TBR crystals in the matrix is a critical factor for quality assurance of this type of TDDS tapes. The model tapes prepared here employed two kinds of matrices, i.e., rubber or acrylic, which are generally used for transdermal pharmaceuticals. TBR crystals in the matrix were observed by MLRSM. Accurate observation of the distribution of TBR in the tapes was achieved by creating a Raman chemical map based on detecting unique TBR peak in each pixel. Moreover, differences in the growth of TBR crystals in the two kinds of matrices were detected by microscopic observation. MLRSM also enabled the detection of TBR crystals in commercial products. The present findings suggest that Raman micro-spectroscopic analysis would be very useful for verifying and/or assessing the quality of transdermal pharmaceuticals in development, as well as for manufacturing process control.

1. Introduction

Tulobuterol (TBR) transdermal tapes are applied in cases of bronchial asthma as a bronchodilator (β_2-blocker). TBR suitable for use in transdermal drug delivery because it has high permeability into the keratin layer (Uematsu et al. 1993). TBR pharmaceutical products with a Transdermal Drug Delivery System (TDDS) have advantages such as eliminating the side effects including abdominal pain and appetite loss (Iikura et al. 1995), and maintaining effective blood TBR levels for approximately 24 h (Horiguchi et al. 2004). The release rate of TBR from the matrix is controlled by the formation of TBR crystals. The crystallization of TBR has the possibility of influencing the TBR blood level profile. Therefore, it is necessary to characterize not only the release rate of TBR from a matrix, but also to characterize its crystallinity in dosage form in the matrix. *In vitro* penetration testing using stripped animal skin and *in vitro* release testing have been used to evaluate transdermal pharmaceuticals in terms of penetration and release. Because these evaluation methods show only one of several alternative physicochemical parameters (e.g., release rate, rate of penetration rate of an active substance, etc.), it has remained difficult to clarify the chemical status and quality of transdermal pharmaceuticals. In case of transdermal tapes containing an active drug as crystals in a matrix, the active drug is slowly released from the matrix into the keratin layer, as crystals will gradually dissolve in a matrix. Therefore, the crystallization of TBR is an important quality parameter. However, evaluation of the correlation of release rates between animal skin and human skin using *in vitro* penetration testing of animal skin has also remained difficult. Therefore, it has become desirable to develop analytical methods of both microscopically and chemically detecting and observing crystals of active drugs in a matrix.



Tulobuterol (TBR)

Laser Raman spectroscopy is a method of spectroscopic analysis of spectra of Raman scattered light obtained by exposure of a sample to a laser. Raman spectroscopy has been used for the identification and quantification of polymorphs (Deely et al. 1991; Falcon et al. 2004; Ferrari et al. 2004; Findlay et al. 1998; Hu et al. 2005; Langkilde et al. 1997; Ono et al. 2004; Schöll et al. 2006; Starbuck et al. 2002; Wang et al. 2000; Murphy et al. 2005) and for monitoring the crystallization process (Taylor et al. 1998;

Murphy et al. 2005; Nørgaard et al. 2005) because it enables the detection of crystals, and in particular, differences between crystal forms. Therefore, Microscopic Laser Raman Spectroscopy/Mapping (MLRSM) was employed in the present study to microscopically and chemically detect TBR crystals in transdermal tapes. The applicability of this spectroscopic analytical method was examined both for the purpose of quality control (i.e., to confirm the crystals of TBR in the matrix), as well as with the aim of enhancing our understanding of relevant quality attributes of prototype pharmaceuticals in various stages of development.

2. Investigations and results

2.1. Determination of a unique wave number range in the Raman spectrum for TBR in model tapes

A typical Raman spectrum obtained from the TBR reference standard substance is shown in Fig. 1. Typical spectra of placebo tape (a) and model tape (b) of rubber matrices are shown in Figs. 2 and 3, respectively. To find characteristic wave numbers of TBR, these spectra were compared with the Raman spectrum obtained from a model tape. The peak bending vibration of C–C at 415cm^{-1} was used as the characteristic peak of TBR, and the integrated values obtained from the wave number range from 420 cm^{-1} to 400 cm^{-1} were used for making the Raman chemical maps.

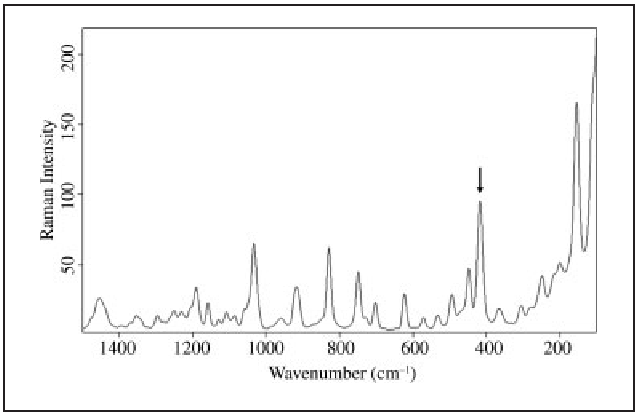


Fig. 1: Typical Raman spectrum of the TBR reference standard. The peak at 415 cm^{-1} was chosen as characteristic, because no interfering peak was observed in the vicinity of this peak

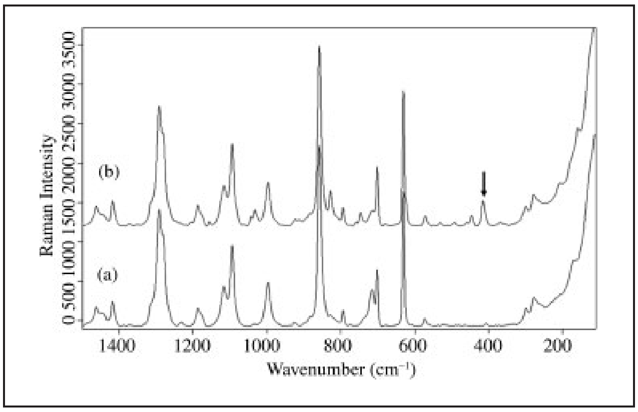


Fig. 2: Typical Raman spectra of placebo (a, rubber matrix) and model tape (b, rubber matrix). The arrow indicates the peak chosen for the specific detection of TBR. Comparatively strong intensity was observed

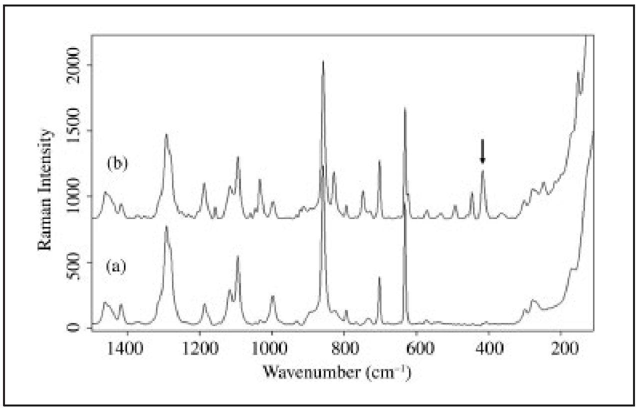


Fig. 3: Typical Raman spectra of placebo (a, acrylic matrix) and model tape (b, acrylic matrix). The very similar Raman spectrum of placebo tape was observed compared with that of the rubber matrix, because the absorption of supporting boards that were made from PET was also detected

2.2. Optical micrograph and Raman chemical mapping of the crystals of TBR in a rubber matrix

Figure 4a shows the micrograph of a 600 × 500 μm area in the R-10 sample. An enlarged micrograph (200 × 200 μm) is shown in Fig. 4b. Pillar-shaped crystals (short, 1 μm–2 μm; long, 10 μm–20 μm) that formed in lumps were observed. Figure 4c and d show the three-dimensional (3D) map and the Raman chemical map that corresponds with the area in Fig. 4b. In the Raman chemical map, the distribution of TBR in the matrix corresponded with the distribution of crystals in the optical micrograph. The Raman absorbance intensity corresponded with the distribution of optically observed TBR.

2.3. Optical micrograph and Raman chemical mapping of the crystals of TBR in an acrylic matrix

A micrograph of a 600 × 500 μm area and an enlarged micrograph of a 200 × 200 μm area of the A-20 sample are shown in Fig. 5a and b, respectively. In Figure 5c and d, the respective 3D chemical map and Raman chemical map are given that correspond to Fig. 5b. A lump of crystals with radiating branches was observed in the matrix. The Raman chemical map of TBR corresponding to the micrograph was obtained. The Raman chemical maps, which show the distribution of Raman chemical intensity, indicated trace amounts of crystal growth.

2.4. Shapes of crystals of TBR in two types of matrix

The micrograph of early-stage TBR crystallization in an acrylic matrix is shown in Fig. 6a. A lump of crystals with radiating branches was observed. Figure 6b shows the micrograph obtained approximately at the level of the top of branch of the crystal. The findings suggest that the pillar-shaped crystals were successively generated at the top of branches, and that the branches grew radially from the core. In case of the rubber matrix, pillar-shaped crystals that formed individual lumps were observed, as shown in Fig. 4a and b. No signs of a core were observed, and lumps of pillar-shaped crystals occurred individually in the matrix. These findings suggest that the TBR crystal growth mechanism differs in the two types of matrix analyzed here. Empirical evidence suggests that when crystallization was rapid, numerous crystalline lumps lacking a nucleus appeared in all areas of the ma-

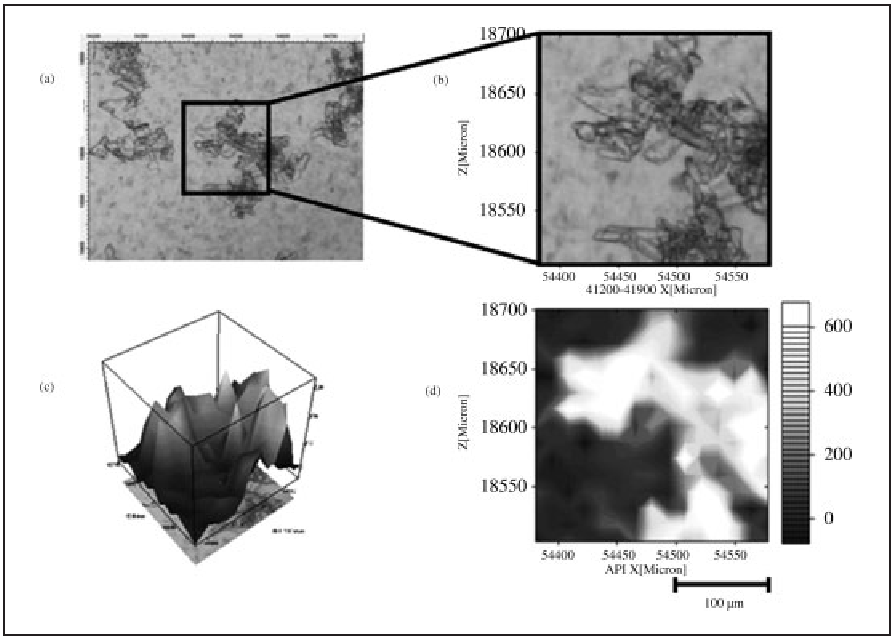


Fig. 4:
Micrographs and Raman chemical maps obtained from the model tape (R-10). a: Micrograph of a 600×500 µm area, b: Enlarged micrograph of a 200×200 µm area, c: 3D Raman chemical map that corresponds with that in d, d: Raman chemical map that corresponds with b. The distribution of the TBR crystals in the matrix was clearly detected by both methods

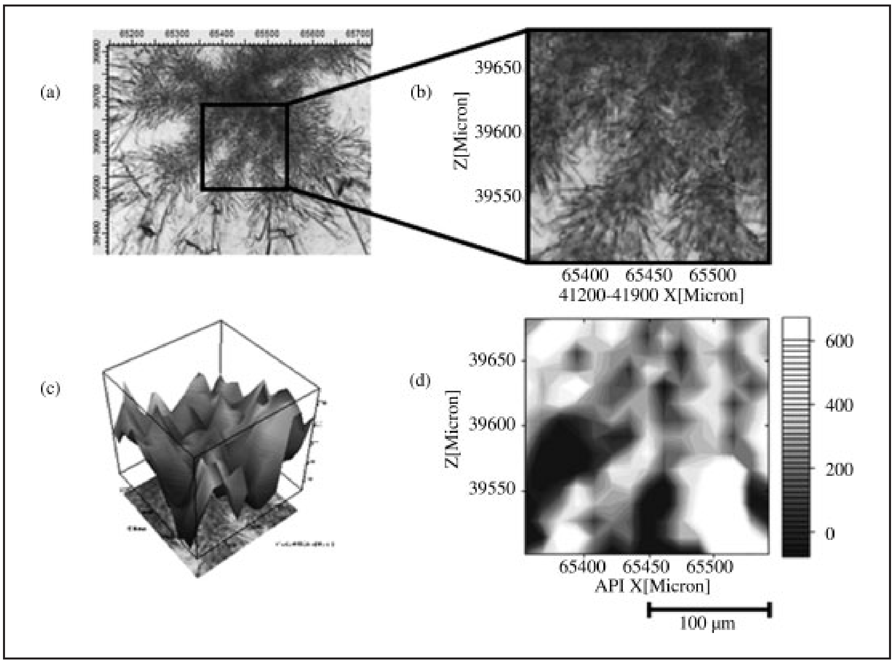


Fig. 5:
Micrographs and Raman chemical maps obtained from the model tape (A-20). a: Micrograph of a 600×500 µm area, b: Enlarged micrograph of a 200×200 µm area, c: 3D Raman chemical map that corresponds with d, d: Raman chemical map that corresponds with b. The mass of the crystals, with radiating branches, was observed in the matrix. The Raman chemical map of TBR corresponding to the micrograph was obtained

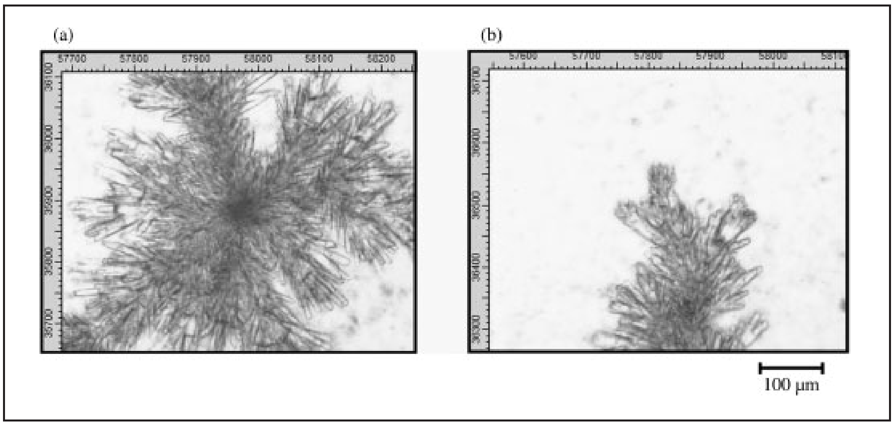


Fig. 6:
Micrographs of the mass of TBR crystals obtained from A-20. a: Core with radiating branches, b: Top of the branch. Pillar-shaped crystals generated successively at the top of branches were observed microscopically

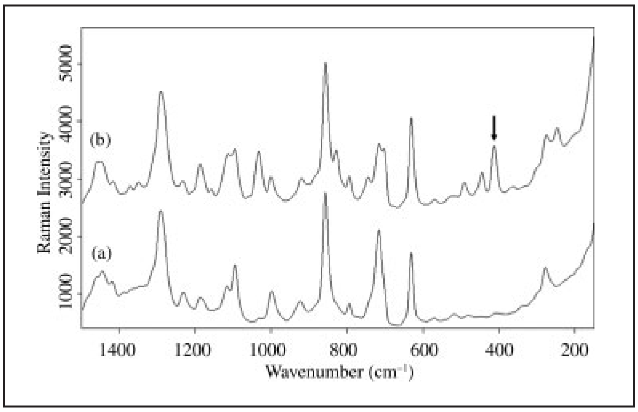


Fig. 7: Raman spectra of commercial tape. a: Background, b: The area where crystals were observed. The characteristic peak (415 cm^{-1}) of TBR was detected

trix, but in cases of slow crystallization, crystals formed around a nucleus. An empirical understanding of such processes would also suggest that the TBR crystals formed more rapidly in the rubber matrix than in the acrylic matrix. The processes of crystallization in these matrices agreed with the empirical evidence obtained here. It appears that new crystals will form around a nucleus, because surrounding molecules are stimulated to crystallize by a nucleus, as shown in Fig. 6. Raghavan et al. (2001) reported that the nucleation process depends in such cases on the hydrogen-bonding functional groups of not only the active drug, but also the polymer. Therefore, it appears likely that differences between the polymer structures of matrices contribute to differences in the process of crystal formation in those matrices. Although further study will still be needed to explain this phenomenon, it appears that the growth mechanism of TBR crystals in each matrix

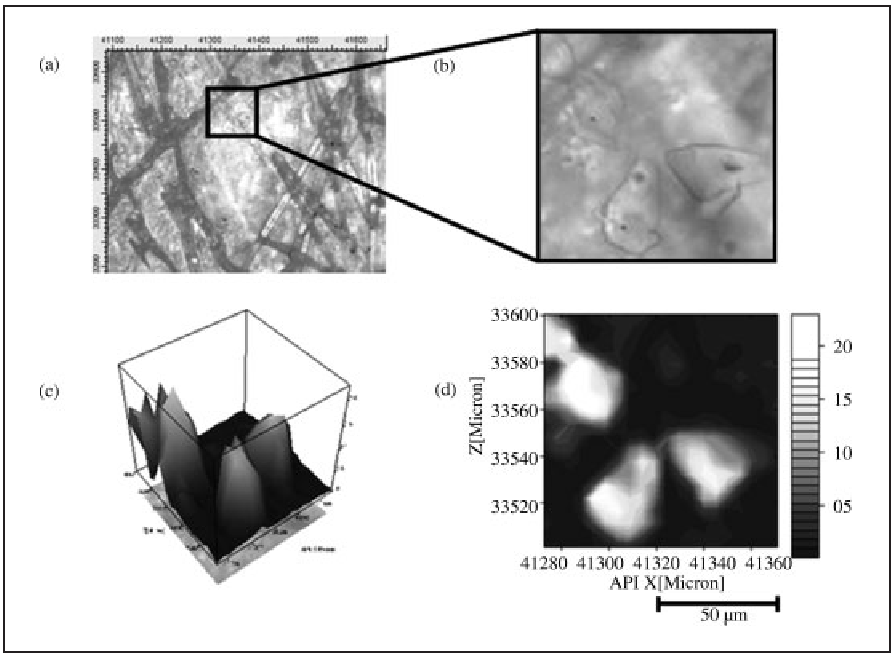


Fig. 8:
Micrographs and Raman chemical maps obtained from the commercial tape (1 mg TBR in the tape). a: Micrograph of a 570 × 450 µm area, b: Enlarged micrograph of a 100 × 100 µm area, c: 3D Raman chemical map that corresponds with d, d: Raman chemical map that corresponds with b. TBR crystals were clearly detected in the matrix

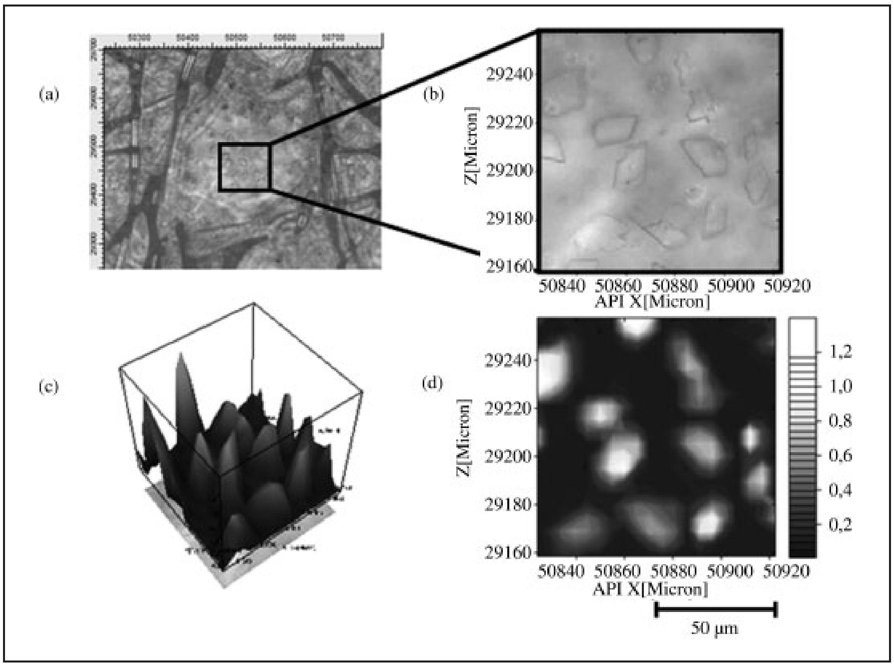


Fig. 9:
Micrographs and Raman chemical maps obtained from commercial tape (2 mg TBR in tape). a: Micrograph of a 570 × 450 µm area, b: Enlarged micrograph of a 100 × 100 µm area, c: 3D Raman chemical map that corresponds with d, d: Raman chemical map that corresponds with b. The crystal distribution suggested that rubber matrix was used for these products

may depend in part on hydrogen bonding between functional groups of TBR and the polymer.

2.5. Analysis of commercial products using MLRSM

MLRSM was used here to detect TBR crystals in commercial products. In these samples, there was a supporting board made of cloth, and a liner made of a white, plastic-like material. The tapes were measured after the liner was removed. Figure 7 shows the Raman spectra of commercial tape. Figure 7a or b were obtained from the area where crystals were not observed or the area where crystals were observed, respectively. The characteristic peak of TBR at 415 cm^{-1} was detected. Figures 8 and 9 show the micrographs (a and b) and the Raman chemical maps (c and d) obtained from the commercial product, Hokunalin® tape, examined in 1 mg and 2 mg sizes, respectively. Areas in which TBR crystals were observed were selected for obtaining the Raman chemical maps. In both micrographs, more crystals appeared to be present in the 2 mg tape than in the 1 mg tape. However, according to the documentation for this product, several sizes of tape, prepared by cutting sections from a larger sheet, can yield various products. Therefore, the TBR content in a particular unit area in several types of Hokunalin® tapes will remain equivalent. It has been hypothesized that the number of crystals in a measured area is affected by the area selected for mapping. Moreover, TBR crystals were also observed that did not assume the lump-shaped formation in this product. Round, pillar-shaped crystals ranging in size from 6×15 μm to 30×40 μm were also observed. The formation of TBR crystals in the product was similar to that observed in a model tape made of a rubber matrix. Helpful information was provided in the attached documentation regarding the medical additives (e.g., polyisobutylene, polybutene, and lipocyclic petroleum resin) used to prepare the rubber matrix. The results of the present study suggest that the crystal formation patterns in a matrix yield useful information about unique matrix characteristics.

3. Discussion

The application of MLRSM to detect the crystals of an active drug in transdermal tapes has been studied. In the case of these TDDS pharmaceutical products, microscopy and chemical mapping method were useful for evaluating the quality of these products as non-destructive spectroscopic technology. Moreover, MLRSM could be used to measure products equipped with a liner for the purpose of quality control during processing, as well as to assess the crystallization of an active drug during storage. Non-destructive spectroscopic methods may be used for analysis of the chemical state and distribution of an active drug, not only in the case of transdermal tapes, but also in film-form products in pharmaceutical development. Furthermore, these methods could be applied as analytical tools to evaluate various factors affecting product quality in the manufacturing process.

4. Experimental

4.1. Microscopic Laser Raman Spectroscopy and Mapping (MLRSM) instrument and measurement conditions

MLRSM measurement was performed using the SENTERRA Dispersive Raman Microscope (Bruker Optics K.K., Germany). Excitation wavelength, laser power, integration time, number of scans, spatial resolution and grating were set at 785 nm, 100 mW, 10 s, 1 scan, 2 μm and 1200 lines/mm, respectively.

4.2. Materials

Tulobuterol (TBR, purity > 99.0%) was provided by Hisamitsu Pharmaceutical Co., Inc. (Tokyo, Japan). 2-Ethylhexyl acrylate vinylpyrrolidone copolymer, isopropyl myristate, polyisobutylene, polybutene, and lipocyclic petroleum resin for matrices of model patches were used as Japanese Pharmaceutical Excipients (JPE)-quality products. Hokunalin® Tape (1 mg and 2 mg) (Maruho Co. Ltd., Osaka, Japan) were purchased from a commercial source.

4.3. Preparation of model tapes

Model tapes were prepared by the TDDS Laboratory, Hisamitsu Pharmaceutical Co., Inc. (Tsukuba, Japan). In order to identify crystalline lumps of TBR in the matrix, two types of matrix, rubber and acrylic, were prepared. TBR and other matrix adhesive solution ingredients were mixed and thoroughly stirred. The mixture was extended on a liner and residual solvents were removed by drying. The matrix was adjusted to a constant thickness (approximately 50 μm) and pasted onto a supporting board. A polyethylene terephthalate (PET) film was selected for the liner and the supporting board of the model tapes. Then, the sample was cut to a size of 36 mm diameter. TBR crystals in the model tapes were generated by leaving the sample to stand for one week (a rubber matrix) or one month (an acrylic matrix).
Model tapes were prepared that contained 0w/w% (R-0, placebo), and 10 w/w% (R-10) of TBR in a rubber matrix that consisted of polyisobutylene, polybutene, and lipocyclic petroleum resin. Small white crystals were seen in all areas of the matrix in the R-10 sample. Model tapes were prepared that contained 0 w/w% (A-0, placebo) and 20 w/w% (A-20) of TBR in an acrylic matrix composed of acrylic adhesive polymer and isopropyl myristate. Due to the solubility of TBR, higher TBR concentrations were necessary to generate crystals in the acrylic matrix than in the rubber matrix.

4.4. Measurement of model tapes and commercial products

The model tapes with the liner were placed on a measurement stand with the liner side facing up. For the measurement of the model tapes, micrographs were obtained and chemical mapping was performed by microscopically focusing on crystals of interest. For the MLRSM measurements of the commercial TBR transdermal tapes, the tapes were placed without the liner on the measurement stand, with the adhesive side facing up.

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