Penetration studies of topically applied substances: optical determination of the amount of stratum corneum removed by tape stripping

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Abstract. Tape stripping is a standard measuring method for the investigation of the dermatopharmacokinetics of topically applied substances using adhesive films. These tape strips are successively applied and removed from the skin after application and penetration of topically applied substances. Thus, layers of corneocytes and some amount of topical applied substances are removed. The amount of substances and the amount of stratum corneum removed with a single tape strip has to be determined for the calculation of the penetration profile. The topically applied substances removed from the skin can be determined by classical analytical methods like high-pressure liquid chromatography, mass spectroscopy, and spectroscopic measurements. The amount of corneocytes on the tape strips can be easily detected by their pseudoabsorption. In the present paper, an easy and cheap corneocyte density analyzer is presented that is based on a slide projector. Comparing the results of the measurements obtained by the corneocyte density analyzer and by uv-visible spectrometry, identical results were obtained. [DOI: 10.1117/1.2359466]

Keywords: penetration; skin; corneocytes; pseudoabsorption; optical corneocytes density analyzer.

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1 Introduction

Tape stripping is a method often used for the investigation of the pharmacokinetics of topically applied substances.[1–5] After application and penetration of the substances, adhesive films are pressed and removed successively from the same area of treated skin. The removed tape strips contain amounts of the topically applied substances and amounts of corneocytes. During the tape stripping procedure, the stratum corneum is removed layer by layer. For the calculation of the penetration profile, two values have to be determined: first, the amount of topically applied substances on the tape strips and, second, the depths from where the single tape strips were removed.

The substances on the tape strips can be analyzed using classical analytical methods like high-pressure liquid chromatography (HPLC),[6] mass spectroscopy (MS),[7] spectroscopic measurements,[8] etc.

Much more difficult is the determination of the position in the stratum corneum from where tape strips were removed. Usually, the tape strip numbers are used as depth values, because tapes with increasing numbers were removed from deeper parts of the stratum corneum.[9–11] This method can be applied if an amount of stratum corneum removed by every tape strip is always identical. This assumption is untrue because the amount of stratum corneum on the tape strips decreases with increasing tape strip numbers.[12] Additionally, it is influenced by the topically applied formulation.[6] Fatty or oily formulations, for instance, decrease the amount of stratum corneum removed by the tape, because the adhesive forces of

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the tape are reduced by the formulation.[13] In contrast, an applied ethanolic solution increases the amount of stratum corneum on the tapes. To overcome this problem, different methods were developed to quantify the amount of stratum corneum on the removed tape strips. One method is the determination of the weight of the tape strips before and after application onto the skin.[14] The mass difference is determined, on the one hand, by the removed amount of stratum corneum, and on the other hand, by the amount of topically applied substances removed by the tape strips. In this way, the correlation between the weight and the amount of stratum corneum becomes disturbed.

Another method is based on the spectroscopic determination of the protein absorption of the corneocytes on the tape strips at 280 nm.[15] Unfortunately, the absorption is very weak and usually superposed by the absorption of the topically applied substances. Thus, this procedure can be applied only to the untreated skin.

By the use of selective staining methods, the protein absorption on the tape strips can be increased significantly, as described by Dreher et al.[16] During the staining procedure, the tape strips were destroyed so that they could not be used for the determination of the topically applied substances on the removed tape strips.

Kalia et al.[17] used transepidermal water lost measurements (TEWL) for the determination of the position in the stratum corneum from where the tape strips were removed. TEWL measurements are well suited to characterize the skin barrier, that is the stratum corneum. An intact barrier results in low TEWL values, while a damaged barrier leads to high TEWL values. During the tape stripping procedure, the stratum corneum is reduced continuously and the TEWL values increase. A good correlation between the amount of stratum corneum remained on the skin and the TEWL values were obtained. Unfortunately, the actual amount of stratum corneum on the first tape strips could not be determined exactly by this procedure. For the investigation of the dermatopharmacokinetics of topically applied substances, the first tape strips removed are of special interest, because they contain the highest amount of topically applied substances.

Weigmann et al.[12] proposed a method for the determination of the amount of stratum corneum on the removed tape strips based on the spectroscopical analysis of the pseudoabsorption of the corneocytes. The pseudoabsorption is influenced by the absorption, scattering, and reflection properties of the corneocytes.[18] It can be measured in the complete uv-visible (UV/VIS) spectral range, but the signal decreases with increasing wavelengths. It was proposed to measure the pseudoabsorption at a wavelength of 430 nm, which is outside the uv absorption of most molecules and where sufficiently high intensities of the pseudoabsorption can be obtained. The spectroscopic measurements must be carried out with a special spectrometer, which has a measuring area of ≥ 1 cm^2 to integrate the inhomogeneous distribution of the corneocytes on the removed tape strips.

In all cases, if the topically applied substances on the removed tape strips are determined by classical analytical methods like HPLC and MS, the tape strips will be measured spectroscopically only at 430 nm. Such measurements do not require a special expensive spectrometer. The measurements can be realized with simple measuring equipment, which is described in this paper.

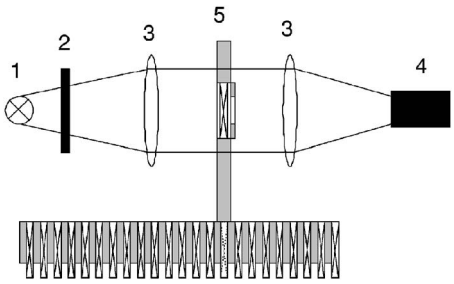

Fig. 1 Scheme of the corneocyte density analyzer.

On the basis of a slide projector, an optical device was developed, which automatically measures the pseudoabsorption of the corneocytes on the removed tape strips at 430 nm. The lamp of the slide projector was substituted by a halogen lamp and an interference filter ($\lambda = 430$ nm), a photodiode being positioned behind the lens system. The mechanical handling system of the slide projector was used to change the samples automatically. The measuring results of this small device were compared to the results obtained by the special spectrometer; identical results were obtained.

2 Materials and Methods

2.1 *Volunteers*

The experiments were carried out on the forearm of volunteers with skin types II and III between 28 and 35 years of age. Approval had been obtained from the Ethics Committee of the Charité.

2.2 *Topically Applied Substances*

A fatty water-in-oil emulsion, an ethanolic solution, and the Temovate cream (GlaxoWellcome Inc.) containing 0.05% of the steroid clobetasol propionate were topically applied onto the forearm of six volunteers on a skin area of 5×5 cm with a concentration of 2 mg/cm^2.

2.3 *Tape Stripping Procedure*

The tape stripping procedure was performed as described by Weigmann et al.[12] following the application of the formulations and a penetration time of 60 min, adhesive films (*tesa* film No. 5529, Beiersdorf, Hamburg, Germany) were pressed onto the skin with a roller and removed afterward. This procedure was repeated on the same skin area until the stratum corneum had been removed completely, which was checked by measuring the transmission of the removed tape strips. The tape stripping procedure was ceased when the transmission of the removed tape strips reached $\geq 98\%$ in comparison to an empty tape, which was used as a blank. The roller was used in this experiment to stretch the skin during the pressing of the tapes onto the skin. In this case, the influence of the furrows and wrinkles of the skin surface on the tape stripping procedure could be avoided.[19] The tape strips removed from the skin were immediately fixed to an empty slide frame for easy handling. The transmission of the tape strips was measured using a modified UV/VIS spectrometer Lambda 20 (Perkin Elmer, Germany) and the newly developed corneocyte density

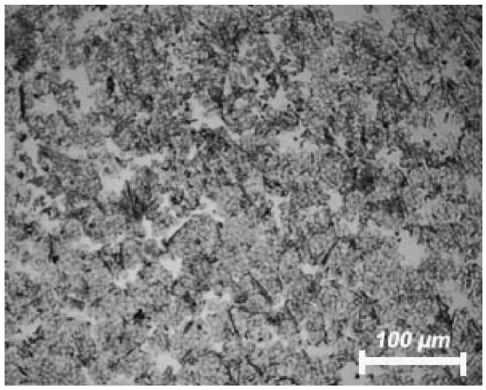

Fig. 2 Microscopic image of the removed tape strips.

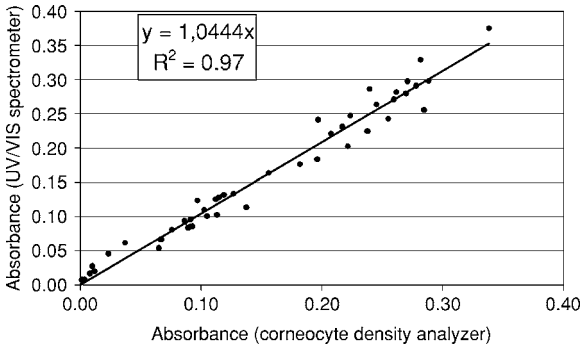

Fig. 4 Correlation between the pseudoabsorption determined by the modified UV/VIS spectrometer Lambda 20 and the corneocyte density analyzer.

analyzer (Universität der Bundeswehr, München).

2.3.1 *Determination of clobetasol propionate on the tape strips*

Clobetasol propionate was determined on the tape strips by HPLC measurements (Beckman System Gold 126 solvent module) and a reversed phase column (l=100 mm, i.d. =2 mm; Hypersil ODS 5 μm; Knauer, Berlin, Germany). Therefore the tape strips were extracted in acetonitrile.

2.4 *Determination of the Amount of the Stratum Corneum on the Removed Tape Strips*

Weigmann et al.[12] demonstrated that the pseudoabsorption at 430 nm of the corneocytes on the tape strips corresponds to their mass. Therefore, the amount of the stratum corneum removed by the single tape strips was determined using a modified spectrometer Lambda 20 with a measuring area of 1 cm^2 and the corneocyte density analyzer based on a modified slide projector.

2.5 *Corneocyte Density Analyzer*

A scheme of the corneocyte density analyzer is presented in Fig. 1. A slide projector was modified in such a way that the lamp (1) is powered by a regulated voltage source. An interference filter (2) had also been installed, which passes a very narrow light spectrum of 430 nm. The lenses (3) used are of standard equipment design. The light sensor (4) is composed of a large area silicon diode together with an instrumental amplifier.

The power supply for the lamp and for the electronics (for signal acquisition and processing to the computer) is installed in an additional module (not shown in the picture). The autofeed system (5) is controlled by a computer via two relays.

2.6 *Determination of the Horny Layer Profile*

The horny layer profile was determined by calculating the sum transmission at 430 nm of all removed tape strips. This value corresponds to 100% of the thickness of the removed stratum corneum.[20] Based on this relation, it was possible to determine the depth in the stratum corneum from where every single tape strip had been removed.[12]

2.7 *Determination of the Penetration Profile*

The penetration profiles are based on the horny layer profiles. For every tape strip, the amount of topically applied substances, removed together with the corneocytes, was determined. This value is presented in the penetration profile as a horizontal bar. The length of the bar corresponds to the concentration of the topically applied substances in the definite depth of the stratum corneum. The penetration profile represents the cut through the stratum corneum, where the distribution of the topically applied steroide in the horny layer is shown.[21]

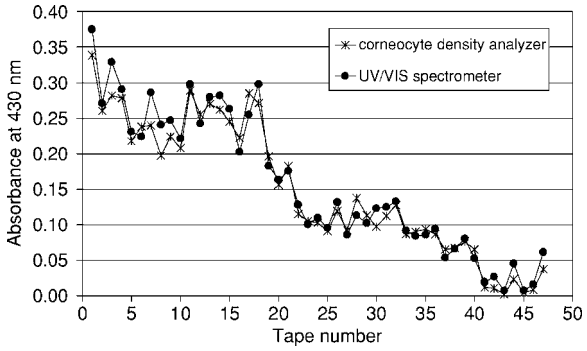

Fig. 3 Pseudoabsorption of the tape strips determined by the modified UV/VIS spectrometer Lambda 20 and the corneocyte density analyzer.

3 Results

In Fig. 2, a microscopic image of a removed tape strip is presented. The corneocytes on the tape strip can be well-recognized. They are distributed nonhomogeneously. The amount of stratum corneum removed decreases with the increasing tape strip numbers. The pseudoabsorption of the corneocytes on the single tape strips is presented in Fig. 3, for one series of tape stripping experiments carried out on the nontreated skin. All tape strips were measured twice with the spectrometer and the corneocyte density analyzer. Identical results were obtained.

In Fig. 4, the correlation between these two measured values is presented. A correlation factor of R^2=0.95 was obtained. The experiments were repeated on six volunteers. The corresponding correlation factors are shown in Table 1.

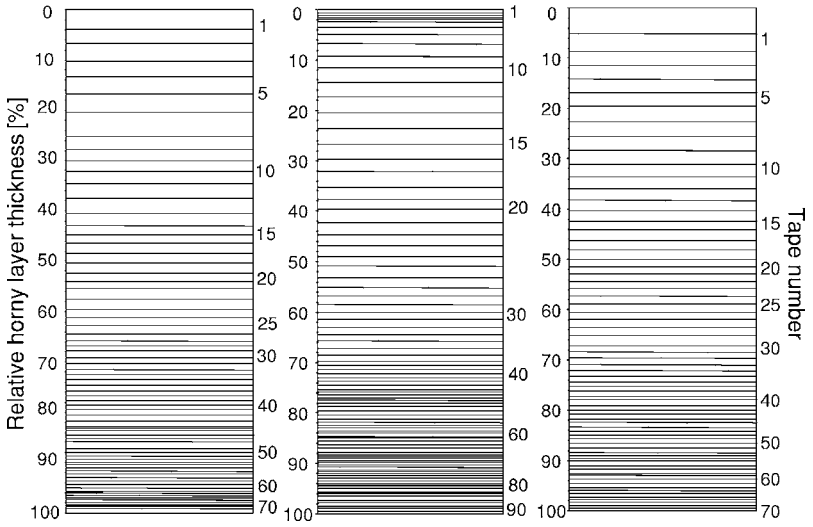

Fig. 5 Horny layer profile determined (a) for the nontreated skin, (b) after treatment with a fatty o/w emulsion, and (c) with an ethanolic solution.

Based on the determined amount of stratum corneum removed with every tape strip, the horny layer profile can be calculated. A typical example of a horny layer profile for the nontreated skin is demonstrated in Fig. 5(a). The distance between two horizontal lines corresponds to the amount of stratum corneum removed by the tape strips. The upper line represents the skin surface and the last line the boundary of the stratum corneum to the living epidermis. As can be seen from Fig. 5(a), the distance between the horizontal lines (i.e., amount of stratum corneum on the removed tape strips) decreases with the increasing tape strip numbers.

The amount of stratum corneum removed with a single tape depends on the applied formulation. The horny layer profiles, which were obtained from the same volunteer as the profile in Fig. 5(a), but after application of a fatty oil-in-water (o/w) emulsion and an ethanolic solution, are presented in Figs. 5(b) and 5(c). Significant differences can be observed in comparison to the results presented in Fig. 5(a). If the skin is treated with a fatty emulsion, the amount of stratum corneum removed by the first tape strips will be less than in the case of an ethanolic solution being applied. The horny layer profile is the basis for the determination of the penetration profile. A typical example of such a penetration profile obtained for the steroid clobetasol propionate is presented in Fig. 6.

Table 1 Correlation coefficients obtained for the correlation between the pseudoabsorption determined with an UV/VIS spectrometer Lambda 20 and the corneocyte density analyzer on six volunteers.

Number of the volunteer	Correlation coefficient between the amount of corneocytes determined by the spectrometer and by the corneocyte density analyzer
1	0.92
2	0.95
3	0.89
4	0.97
5	0.96
6	0.95
Main value	0.94

4 Discussion

The amount of stratum corneum removed by a single tape strip can be determined by the pseudoabsorption of the corneocytes. The optical measurements can be disturbed by the inhomogeneous distribution of the corneocytes on the tape strips. Therefore, a large measuring area of ≥ 1 cm^2 has to be used to average these nonhomogeneities. Commercial spectrometers have a small measuring spot of several square millimeters to analyze small samples. However, they are not suited for the determination of the amount of stratum corneum on the tape strips. In the present study, the optical system of an UV/VIS spectrometer Lambda 20 was changed to obtain a large measuring area (1.5 cm^2). For the determination of the pseudoabsorption, the tape strips must be measured only at

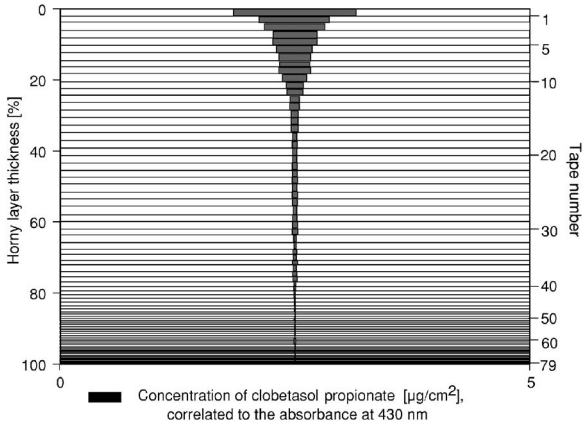

Fig. 6 Penetration profile of clobetasol propionate.

one wavelength. In our experiments, we used a wavelength at 430 nm, which is outside the absorption band of the topically applied substances and shows a high intensity of pseudoabsorption.[21] For this application, a spectrometer is not necessary. The measurements can be performed with a simple cheap device, as described in this paper. In all cases, identical results concerning the amount of corneocytes on the removed tapes strips were obtained (Fig. 3, Table 1). The corneocyte density analyzer has the advantage that the measurements were carried out automatically, using a mechanical autofeed system of the slide projector. This handling is very convenient, because for further treatment, the removed tape strips, in any case, have to be fixed to a frame. Therefore, sticking together or coiling up of the tape strips can be avoided. The measuring data are available as an EXCEL file, which can be used immediately for the calculation of the horny layer profile, as presented in Fig. 5.

The relation of the amount of topically applied substances to the horny layer profile (i.e., to the actual depth of the stratum corneum from where they were removed) and not the tape strip number is fundamental. Different formulations can significantly influence the amount of stratum corneum on the tape strips, as in Fig. 5. The differences in the horny layer profile obtained for a topically applied ethanolic solution and a fatty o/w emulsion appear mainly for the first tape strips, which usually contain high amounts of the topically applied formulations. In deeper parts of the stratum corneum, where the concentration of the formulations is small, nearly an identical amount of corneocytes was removed.

The penetration profile can be calculated (Fig. 6), based on the horny layer profile. It represents a cut through the stratum corneum, where the distribution of the topically applied substances in the corresponding skin layers can be clearly recognized. In the present case, the clobetasol propionate has penetrated through the skin barrier. The biological response in the form of a blanching effect could be detected.[22] In the stratum corneum, the highest amount of clobetasol propionate was determined close to the skin surface. Only small amounts were found in deeper parts of the stratum corneum on the boundary to the living cells.

Because of the noninvasive character of this procedure, the tape stripping method can be used for the analysis of the penetration kinetics.[14] The results obtained by this method are highly reproducible as can be demonstrated by standard experiment carried out at a different institution on different volunteers.[8]

Summarizing the results, it can be established that a combined method of tape stripping and spectroscopic measurements for the determination of the amount of stratum corneum on the removed tape strips, is well suited to determine the penetration of the topically applied substances into the stratum corneum. The determination of the actual amount of the stratum corneum on the tape strips can be easily carried out using the presented, cheap corneocyte density analyzer based on a slide projector, where the optical system had been changed and a sensor included. The mechanical autofeed system of this device is well suited for the handling of tape strips.

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