Optical investigations to avoid the disturbing influences of furrows and wrinkles quantifying penetration of drugs and cosmetics into the skin by tape stripping

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Abstract. Furrows and wrinkles, as typical structures of human skin, represent a reservoir for topically applied substances. This reservoir can influence penetration experiments of topically applied substances into the stratum corneum by tape stripping. Optical methods such as laser-scanning microscopy, optical coherent tomography, and the microscopical investigation of histological sections obtained by biopsies were used to check a special protocol, which avoids these potential disturbances. The use of a transparent adhesive film with high flexibility and the realization of an intense contact to the stretched skin by pressing the tape with a roll, moved laterally on the tape, are the prerequisites to obtain correct data. The application of this experimental technique and the performance of tape stripping allow the determination of the horny layer profile and the local distribution of topically applied substance, undisturbed by the characteristic structure of the natural skin. These results demonstrate that the presented tape stripping procedure is a valuable tool to determine, quantitatively, the penetration and the bioavailability of drugs and cosmetics inside the human stratum corneum, in relation to the horny layer profile.
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1 Introduction

Tape stripping using adhesive films is a well-known minimal-invasive method to investigate the percutaneous absorption of topically applied drugs or of active components in cosmetics.[1–6] The corneocyte aggregates of the horny layer were successively removed by tape stripping. This presents the possibility to determine the penetration of the applied substances into the stratum corneum. The tape stripping procedure was proposed by the U.S. Food and Drug Administration (FDA) as part of a standard method to evaluate the bioequivalent of topical dermatological dosage forms.[7] Usually, the number of the removed tape is the measuring value to which the concentration of the topically applied substances is related.[8–12]

The number of tape strips needed to remove the stratum corneum completely varies with the formulation and pressure applied: age, gender, anatomical site, and skin condition.[13] Fatty and oily formulations applied onto the skin reduce the adhesive forces of the tapes, so that fewer amounts of corneocytes are removed. This means that there is no direct correlation between tape number and penetration depth.

Different attempts to quantify the amount of stratum corneum removed by single tape strips and to determine the horny layer profile have been described in the literature. The methods of differential weighing of the tape strips,[14–16] determination of the characteristic protein absorption of the removed corneocytes,[17] staining of the horny layer cells,[16,18] and transepidermal water loss (TEWL) measurements[19,20] are used for this purpose. However, all these methods have limitations in practical application.

The method of differential weighing has the disadvantage that it was not possible to distinguish between the weight of the corneocytes and the weight of the amounts of topically applied substances removed by the tape strip.[14] Therefore, this method can only be applied to untreated skin. The same disadvantage occurs in the case of protein absorption measurements. As the protein absorption band at 280 nm is very weak and almost superposed by the strong absorption bands of the topically applied substances. After staining, the corneocytes on the removed tape strips and the samples are destroyed and

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cannot be used for the determination of the amount of topically applied substances on the tape strips.

TEWL measurements were successfully applied to characterize the thickness of the stratum corneum during the tape stripping procedure. Using this method, it is difficult to determine the amount of stratum corneum on the first tape strips removed, because the TEWL values are related to the remaining part of the stratum corneum and not to the stratum corneum removed.

Recently, a spectroscopic method was presented for the determination of the pseudoabsorption of the corneocytes, which allows quantification of the mass of the corneocyte aggregates fixed to the individual tape strips.[2,21] These measurements are not influenced by topically applied substances. The tape strips are not destroyed and can be used for the determination of topically applied substances on the individual tape strips.

The complete removal of the stratum corneum by tape stripping and the exact determination of the amount of removed stratum corneum on the tape strips is the prerequisite to calculate the horny layer profile. A meaningful penetration profile can be calculated by correlating the amount of the topically applied substances to the depth profile of the stratum corneum.

The quantitative application of the tape stripping method requires two prerequisites. First, it must be guaranteed that the horny layer particles are removed from a definite cell layer of the stratum corneum. Second, the uppermost part of the skin, the horny layer, must be transferred completely to the adhesive film; tape stripping is repeated until the tapes are optically empty. The wrinkles and furrows, characterizing the surface of the human skin, may disturb both demands.

A general overview on the cutaneous relief of the human skin is given by Leveque and Corcuff.[22] The wrinkles are described as determining structures in the uppermost part of the epidermis. Fiedler et al.[23] investigated the microrelief of the human skin by a texture analysis of replica taken from living skin. Characteristic changes were observed for the skin surface on different parts of the body and after cosmetic treatment. Schaefer and Redelmeier[24] have considered the typical structure of the skin in relation to the percutaneous absorption discussing, in particular, the dependence on the individual situation found in different volunteers.

The influence of furrows on the results obtained by tape stripping was considered in detail by van der Molen et al.[25] It was concluded that furrows and wrinkles disturb the tape stripping procedure when performing depth-penetration studies.

The influence of the hair follicle orifices, which are also a characteristic structure of the human skin surface, on the results of the tape stripping can be neglected because they represent only 0.1% to 1.0% of the skin surface, depending on the anatomical site.[26–29]

In the present paper, the influence of the tape stripping protocol on the potential disturbances arising from the characteristic wrinkles and furrows was checked applying optical methods. Optical coherent tomography was used to investigate the skin surface structure. Laser-scanning microscopy was applied to characterize the skin structure of the stratum corneum layers fixed to the individual tapes. The changes in the relief arising in the living skin after a complete removal of the horny layer by tape stripping were determined by the microscopic analysis of biopsies. As a result of the investigations, a tape stripping procedure is described, which avoids the influence of the furrows and wrinkles on the penetration profile.

2 Materials and Methods

2.1 *Skin Tissue*

The investigations were performed on six healthy volunteers (three male, three female), phototypes II and III,[30] aged between 24 and 36 years, on the flexor forearm and on the abdomen. Additionally, the tape stripping procedure was repeated on six samples of excised human skin obtained from the abdomen during cosmetic surgical treatment. Ethical approval had been obtained from the Ethics Committee of the Charité University Hospital.

2.2 *Sunscreen Application*

The investigations were carried out with an oil-in-water (o/w) emulsion containing 4% of the UV filter substance Eusolex®6300. Beiersdorf AG, Hamburg, Germany, provided the formulation. For visualization of the distribution of the formulation on the skin, 2% of the dye patent blue V was added to the formulation. 2 mg/cm^2 of the emulsion were applied to a marked skin area of 160 cm^2.[31]

2.3 *Tape Stripping*

A series of tape strips were taken from the treated areas of the flexor forearm and abdomen and from the excised abdominal skin, one hour after the application of the emulsion. An adhesive film (*tesa* Film 5529, Beiersdorf AG, Hamburg, Germany) with a width of 1.9 cm was used for tape stripping.

When the first tape was placed onto the skin its position was fixed by marks on the skin. These marks were transferred as cross hairs on every following tape for the recovery of definite furrows on the removed tape strips.

Two different methods were applied in the experiments to press the tapes onto the skin. In the first experiment, the tape was pressed onto the skin using a stamp. The tape on the skin was covered with a piece of paper, in order to avoid the back of the tape becoming contaminated by the stamp with the topically applied formulations from the neighboring skin areas not covered by the tape. The tape strips on the skin were pressed by a stamp for 15 sec at a pressure of 15 g/cm^2. Subsequently, the tape strips were removed from the skin. In a second experiment, the tape was pressed onto the skin with a roll[2] starting at one end of the film. The roll (pressure: 25 g/cm^2) was moved by hand ten times in different directions pressing the tape to the skin.

In both cases, after pressing, the tape was pulled off with one continuous quick movement. Tape stripping was repeated on the same skin area until the adhesive layer was optically empty. This was controlled by measuring the transmission at 430 nm, which correlates to the amount of corneocytes on the removed tape strips.[8] The tape stripping procedure was stopped when the transmission of the tape strips was $>98\%$. In Fig. 1, the stamp and the roll are presented. The tape stripping procedure is demonstrated in Fig. 2.

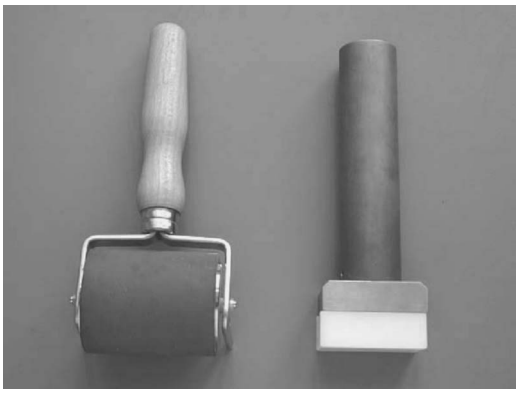

Fig. 1 The stamp or the roll were used to press the adhesive tape onto the skin.

2.4 *Spectroscopic Determination of the Penetration Profile*

The absorbance of the pseudoabsorption of the horny layer particles fixed to every removed tape strip was determined at 430 nm using the modified spectrometer (Lambda 20, Perking Elmer, Frankfurt/Main, Germany). These spectroscopic values correlate to the amount of removed stratum corneum.[8] Thus, it can be determined which amount of stratum corneum is removed by the individual tape strips.

The horny layer profile was calculated by adding the single amounts of the stratum corneum removed by every tape strip during the tape stripping procedure until the stratum corneum was removed completely. This sum absorbance correlates to 100% of the thickness of the stratum corneum. The horny layer profile represents a "cut" through the stratum corneum, which indicates the local position from where the individual tape strips were removed.[32]

The concentration of the UV filter substances on the removed tape strips was determined spectroscopically using the absorption band at 299 nm. The penetration profile was calculated by determining the amount of topically applied formulations (UV filter) on every removed tape strip and the position inside the stratum corneum from where the formulation was removed.

2.5 *Microscopic Analysis of Biopsies*

Biopsies were taken from natural skin of the flexor forearm and from a neighboring site after the horny layer had been completely removed by tape stripping. This was realized taking about 90 strips from the same skin area before the tapes were optically empty. The complete stripping procedure needs approximately 2 h. The biopsy was taken using a punch with a diameter of 3 mm. After extraction, the sample was frozen, cut into 10-μm-thin histological sections, and stained with hematoxylin and eosin for clear identification of the tissue

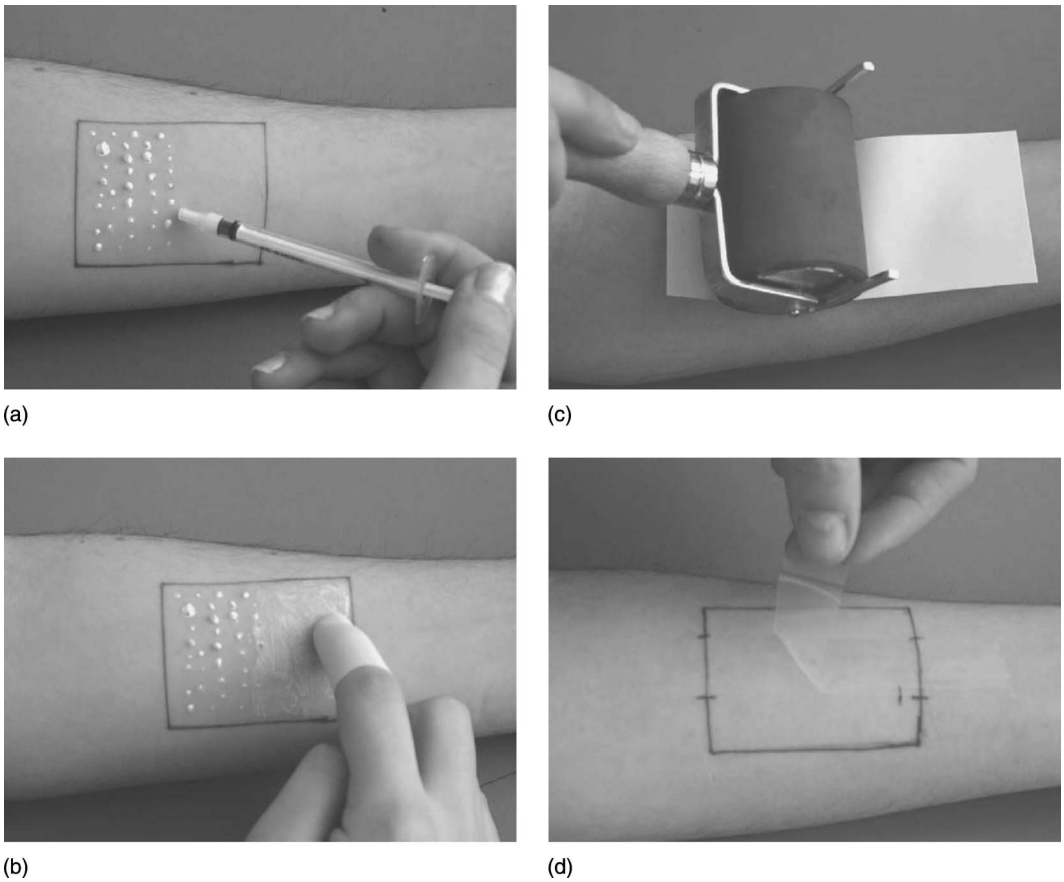

Fig. 2 Tape stripping procedure: (a) homogeneous topical application of the formulations, (b) homogeneous distribution of the formulations, (c) after penetration pressing the adhesive film on the skin using the roll, and (d) removal of the tape strip.

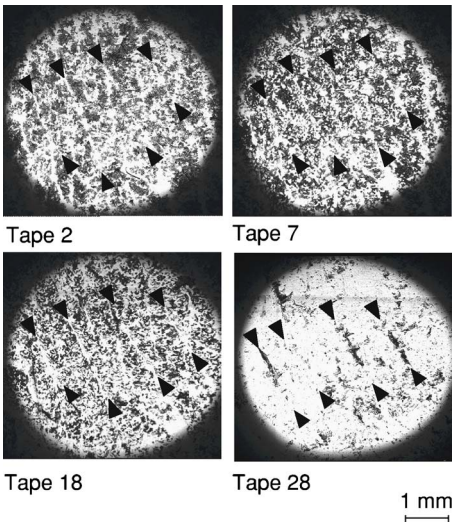

Fig. 3 Structure of the furrows and wrinkles transferred to the tape strip. The tape strips are pressed by a stamp. The black arrows describe the position of the wrinkles on identical parts of the skin site of the flexor forearm.

structure. The same experiments were repeated on the tape stripped abdomen skin.

The histological sections were analyzed by microscopy (Olympus BX60, Olympus Optical Co. Europe GmbH, Hamburg, Germany).

2.6 *Laser-Scanning Microscopy*

The local distribution of the corneocyte aggregates on the tapes was determined by transmission measurements using a LSM 2000 laser-scanning microscope (Carl Zeiss, Jena, Germany). These measurements were carried out at a wavelength of 488 nm with a fivefold magnification. The laser beam of the microscope was scanned over the surface of the tape strips.

2.7 *Optical Coherent Tomography (OCT)*

OCT measurements were performed using the commercial OCT system (SkinDex 300, ISIS Optronics, Mannheim, Germany). The skin surface structure was analyzed during the pressing of the stamp onto the skin surface and during the stretching of the skin surface by a rolling movement with the roll.[26]

2.8 *Optical Investigation of the Skin Surface*

A formulation containing 2% of the dye patent blue V was applied onto the skin with 2 mg/cm^2. Photographs documented the distribution of the dark dye on the skin before and after the removal of several tape strips. For this purpose, a camera (Canon EOS 50/50 with lens MP-E 65 mm F2.81-5x) with a ring flash was used. In the first series of experiments, the adhesive tapes were pressed onto the skin by a stamp without a rolling movement; in the second case a roll was used.

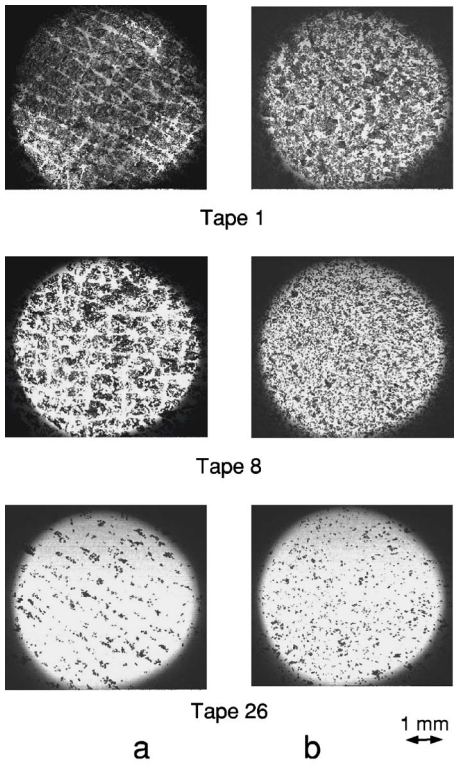

Fig. 4 Influence of different methods to press the tape strips onto the skin on the structure of the corneocyte aggregates fixed to the tapes. (a) Results obtained pressing the tape with a stamp and (b) results obtained pressing the tape with a roll.

3 Results

3.1 *Method to Avoid the Influence of Furrows and Wrinkles on the Tape Stripping Procedure*

A series of tape strips pressed with a stamp onto the skin were removed from the same marked area. These strips were investigated by laser-scanning microscopy in order to determine the influence of the furrows on the structures found on the tape. In Fig. 3, images of different tape strips are presented in the order of increasing tape numbers. The measurements were performed in the transmission mode. The corneocyte aggregates became visible as black areas, while the parts not covered with corneocytes are seen as bright areas. The areas marked with light arrows characterize the valleys on the first tapes (tapes 2, 7, and 18). At higher tape numbers, the situation changed. On tape 28, the corneocytes are concentrated mainly in the areas where the furrows were localized.

In the following experiments, it was investigated how different tape stripping protocols influence the distribution of the corneocytes on the removed tapes.

The pictures in Fig. 4(a) were obtained by transmission measurements of laser-scanning microscopy after pressing the tapes onto the skin using a stamp without lateral movement. In this case, the characteristic furrow and wrinkle structure is transferred to the tape strips. On the first tape strips, tapes 1 and 8 are given as examples; the furrows arise as white channels. The corneocytes (black area) were removed only from the flat skin surface, but not out of the furrows. In the last tape

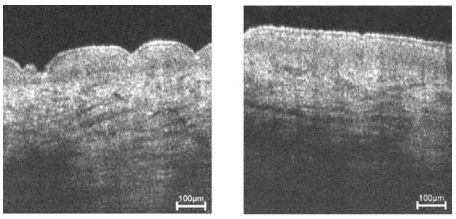

Fig. 5 OCT image of the changes in the surface structure of the skin during stretching of the skin surface by a roll. (a) Normal forearm skin before tape stripping and (b) forearm skin stretched by the roll during pressing of the adhesive tape onto the skin.

strip given, tape 26, the corneocytes were removed only out of the furrows.

In Fig. 4(b), the results are presented for the tape strips 1, 8, and 26, which were obtained by pressing the tapes onto the skin with a plastic roll. Under these experimental conditions, the removed corneocytes are distributed homogeneously. The characteristic structure of the furrows and wrinkles disappeared on the removed tape strips.

OCT measurements were used to investigate the changes of the skin surface structure during the application of the roll (Fig. 5). A typical furrow structure of the skin on the forearm before tape stripping is presented in Fig. 5(a). During the rolling movement, the skin surface became stretched. The furrow structure of the living skin disappeared and the skin surface became flat during contact with the adhesive film [Fig. 5(b)].

Biopsies were taken before and after tape stripping, in order to analyze the epidermis and the amount of removed corneocytes. In Fig. 6, the microscopic images of histological sections are given describing the changes observed in the skin after removing the stratum corneum by tape stripping. In Fig. 6(a), the wrinkles and furrows of the flexor forearm, as typical structures of the natural skin, are shown before tape stripping. After tape stripping, the skin surface became flat and the stratum corneum was removed completely [Fig. 6(b)]. The same results were obtained *in vivo* on abdominal skin.

In the case of the experiments carried out on excised abdominal skin using the same protocol, it was not possible to remove the stratum corneum completely. Small amounts of corneocytes always remained in the furrows and wrinkles [Fig. 6(c)].

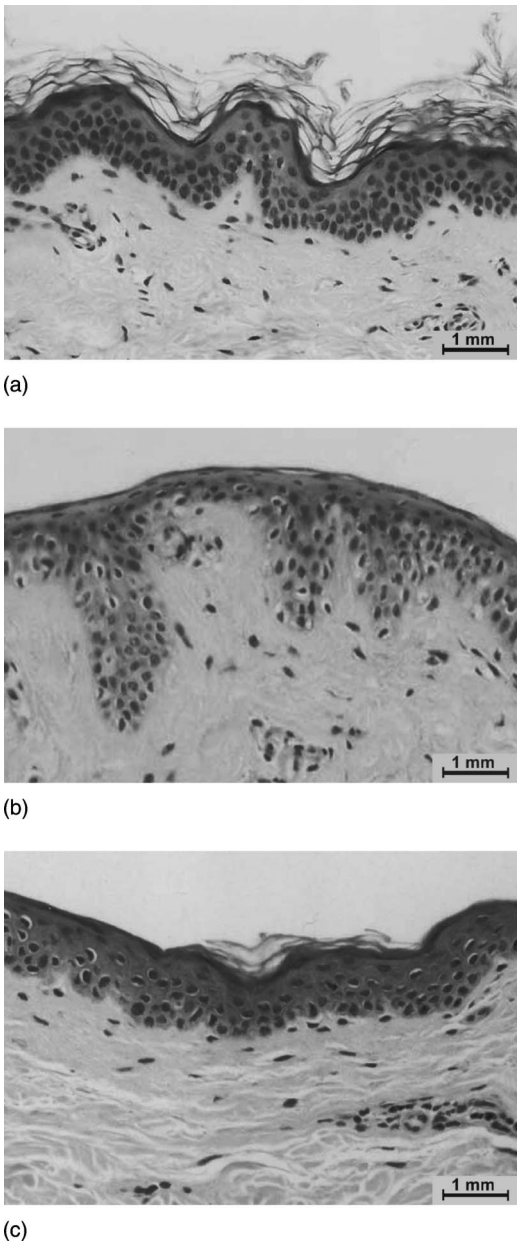

Fig. 6 Histological sections demonstrating the influence of the removal of the horny layer by tape stripping on the wrinkles and furrows characterizing the structure of the surface of the human skin. (a) Natural skin before tape stripping (*in vivo,* forearm). (b) Skin after repeating the tape stripping 84 times on the same skin site (*in vivo,* forearm). (c) Skin after repeating the tape stripping 92 times on excised abdomen skin.

3.2 *Influence of the Furrow Structure on the Results of Penetration Measurements*

After penetration of the topically applied emulsion, the tape strips were removed using the pressing and rolling procedure. In Fig. 7, results are given obtained after application of an emulsion containing the dye patent blue V. The photos were taken from the skin area before and after the removal of the strips varying the type of contact of the tapes to the skin, pressing without movement and rolling. Clear differences in the distribution of the applied emulsion on the skin surface could be detected during the application of both tape stripping procedures.

In the case of rolling and pressing of the adhesive tapes onto the skin, nearly identical amounts of stratum corneum were removed, which was checked by spectroscopic measurements.

Pressing the tape with the stamp onto the skin, the dye remained in the furrows after removal of the fifth tape strip [Fig. 7(a)]. If the roll was used, no dye was left in the furrows and wrinkles. In this case, it was only located in the orifices of the hair follicles [Fig. 7(b)].

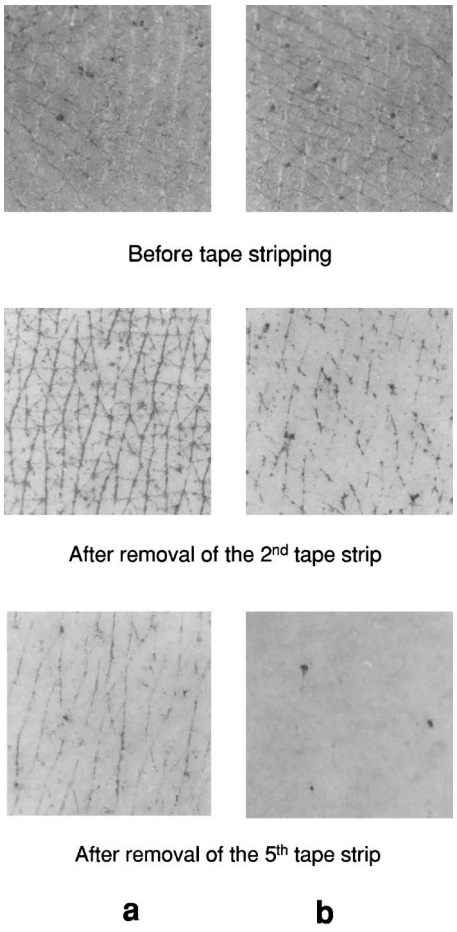

Fig. 7 Distribution of the dye patent blue V on the skin after tape stripping with different procedure of contacting the adhesive film: (a) under constant pressure with a stamp and (b) by pressing with a roll.

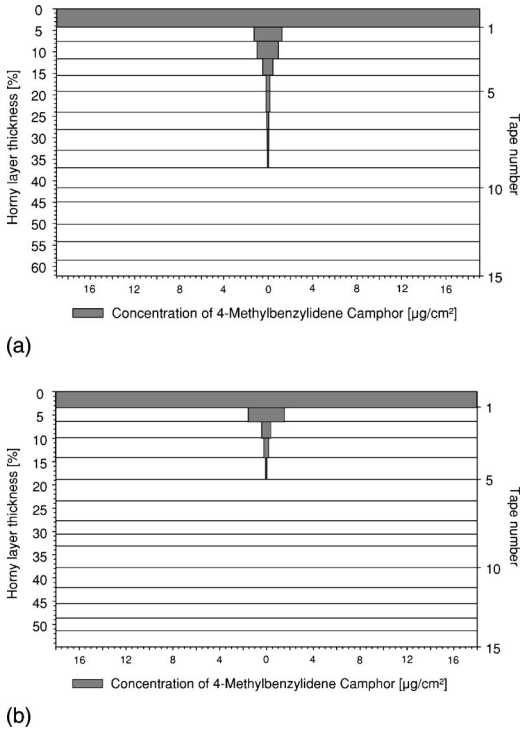

Fig. 8 Penetration profiles of a topically applied dye in the upper 50% of the stratum corneum obtained for different pressing procedures: (a) under constant pressure with a stamp and (b) by pressing with a roll.

In Fig. 8, the corresponding penetration profiles of the UV filter containing emulsion are shown. For a better comparison of the distribution of the UV filter in the upper layers of the stratum corneum only the upper 50% of stratum corneum is presented. The penetration profiles in Fig. 8 demonstrate that most of the topically applied UV filter containing emulsion was removed with the first tape strip. Using the stamp to press the tape strips onto the skin, small amounts of UV filter were detected up to a depth of 35% in the stratum corneum [Fig. 8(a)]. In the case of rolling, the UV filter could be found only up to a depth of 18% in the stratum corneum [Fig. 8(b)].

The experiments were repeated six times. Identical results were obtained.

4 Discussion

4.1 *Method to Avoid the Influence of Furrows and Wrinkles on the Tape Stripping Procedure*

The picture of the tape strips given in Figs. 3 and 4 clearly reflect the typical structure of the wrinkles and furrows. Identical structures were obtained investigating the skin surface, e.g., with replica[33] or by EDXA and scanning electron microscopy.[22]

From these results, it is obvious that the adhesive layer will not remove the components positioned in the valleys of the wrinkles at the beginning of the tape stripping procedure, if the tapes are pressed onto the skin with a stamp. Only at higher tape numbers, when most of the upper part of the horny layer has been removed, the corneocyte aggregates inside the valleys of the furrows began to stick to the adhesive film. The areas marked by the arrows in Fig. 3 demonstrate this effect. On tape 2, the furrows can still be recognized as bright lines, while on tape 28, corneocyte aggregates appeared in the same position. These corneocyte aggregates belonging to the valleys of the wrinkles are the dominating part of corneocytes on the 28th and the following removed tape strips. This is confirmed when comparing tapes 1 and 26 in Fig. 4.

This signifies that the quantitative determination of the concentration of a topically applied substance, in correlation to a definite part of the horny layer by tape stripping, is disturbed by the furrows and wrinkles while pressing the strips onto the skin with a stamp. For the quantitative application of the tape stripping method it is necessary to avoid these disturbances.

From the results obtained by laser-scanning microscopy (Fig. 4), it is evident that the influence of the furrows on the removed corneocyte aggregates can be reduced or even neglected, thus changing the protocol. A homogeneous distribution of the corneocytes on the tapes can be obtained pressing the adhesive film to the skin by a lateral movement with a roll. In this case, the furrow structure disappeared because, to a small extent, the roll moves the tape against the surface of the skin realizing a stretching of the skin, as shown by OCT measurements in Fig. 5, when the tapes come into contact

with the flat skin surface. Working with this technique, it is possible to exclude disturbances in penetration measurements arising from the furrows and winkles of the skin.

4.2 *Influence of the Furrow Structure on the Results of Penetration Measurements*

The results obtained, investigating the penetration of UV filter substances into the stratum corneum by tape stripping using a roll,[21] also demonstrated that this protocol drastically reduces the disturbances (Fig. 8).

The UV filter was found only in the upper layers of the stratum corneum, if the roll was used [Fig. 8(b)]. More than 95% of the UV filters were removed with tapes 1, 2, and 3 corresponding to about 15% of the horny layer thickness. If the tapes were pressed onto the skin by the stamp, small amounts of the UV filter were also detected on tape strips with higher numbers, which seem to be removed from deeper parts of the stratum corneum [Fig. 8(a)]. This is a misleading detection of the UV filters in deeper layers of the stratum corneum, because these small amounts were removed from the bottom of the furrows. In the present case, this small amount of wrongly detected UV filter represents only 3% of the total amount detected in the stratum corneum. This value can be significantly higher in other skin areas, for example, the face or if other formulations are applied.

For the determination of the horny layer profile, described by Weigmann et al.,[14] it is necessary to guarantee that all corneocytes are transferred to the tapes during the tape stripping procedure. Former studies assumed that corneocyte aggregates positioned in the valleys of the wrinkles and furrows cannot be removed.[25] This effect was tested by taking biopsies from the skin before and after tape stripping. The microscopic images presented in Figs. 6(a) and 6(b) demonstrate clearly that the stratum corneum can be removed completely by tape stripping under *in vivo* conditions. The swelling of the connective tissue, observed as a result of the stress, which is produced during tape stripping, supports the stretching process. The surface becomes smooth and all wrinkles and furrows disappear and do not disturb the complete removal of the horny layer. In the case of the experiments on excised abdomen skin, small amounts of corneocytes always remained in the furrows and wrinkles [Fig. 6(c)]. This is in agreement with the results obtained by van der Molen et al.[25]

This means that the stratum corneum can be completely removed only in the case of *in vivo* experiments. A sufficient flexibility of the skin, described by the cutaneous turgor and the elasticity, is only given in this case. Additionally, the swelling of the connective tissue needs natural living skin, not excised skin. Under these conditions, it can be guaranteed that the disturbing effects connected with the typical structure of the human skin can be neglected. Working with excised skin, the influence of the furrows cannot be suppressed by pressing the tape with a roll, because the cutaneous turgor is not active and the swelling cannot smooth the skin.

Summarizing the results, it could be demonstrated that the developed protocol, based on the application of a roll for stretching the skin in combination with a flexible tape, avoids the disturbance of the quantitative results obtained determining the penetration of drugs and cosmetics into the stratum corneum by tape stripping.

If the tape strips are pressed onto the skin by a stamp, without stretching the skin, the penetration profiles become adulterated by the furrows and wrinkles.

Tape stripping experiments on excised human skin are disturbed by the loss of the flexibility and elasticity of the skin, as a result of which, amounts of corneocytes remain in the furrows and wrinkles during the tape stripping procedure. This is of special importance with regard to the determination of the bioavailability of topically applied drugs and cosmetics by tape stripping.

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