

Metrics and Clinical Relevance of Percutaneous Penetration and Lateral Spreading

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Key Words

Percutaneous penetration · Urea · Skin relief · Tape-stripping method · Spectroscopy · Stratum corneum · Lateral spreading

Abstract

Background: Percutaneous penetration of urea in vivo in man has been documented. If urea can penetrate the skin, it may also move laterally. Lateral spreading of topical substances leads to unpredictable penetration dynamics and increased skin surface area exposure. **Methods:** The ability of urea, a low molecular-weight hydrophilic model, to penetrate the stratum corneum (SC) and spread outside the application site was investigated in vitro using tape stripping with spectroscopy. The parameters investigated were the following: time between urea application and tape stripping, formulations containing urea and use of a petrolatum-covered ring barrier around the marked application area. **Results:** The percentage of urea was determined in and around the application site. The spreading of topically applied urea to neighboring areas occurred and was time but not formulation dependent. A significant difference between protocols with and without the petrolatum ring was observed. **Conclusion:** These results suggest the clinical importance of lateral spreading, occurring predominately on the skin sur-

face. SC thickness varies between anatomical sites, predisposing areas such as the face and scalp margins to increased percutaneous penetration of topical products. The use of a protective petrolatum ring can inhibit lateral spreading of hair dye in individuals allergic to hair dye, limit systemic absorption and increase accuracy when assessing penetration dynamics.

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Introduction

When a drug is applied to the skin, the ability to penetrate the stratum corneum (SC) is an important part of its efficacy. The bioavailability of topically applied drugs is less than 10% [1], and the remaining drug is assumed to be metabolized or removed through epidermal exfoliation, washing or rubbing of the skin. Few studies have investigated the process of lateral spreading as a contributing factor to reduced drug recovery [2]. During lateral spreading, the flow of the applied drug can move laterally on the skin surface from the application area to the outside (fig. 1). This spreading is a competitive process to penetration and can modify the desirable effect expected in the treated area.

In this study, percutaneous penetration and the lateral spreading of urea were investigated in vitro with the tape-

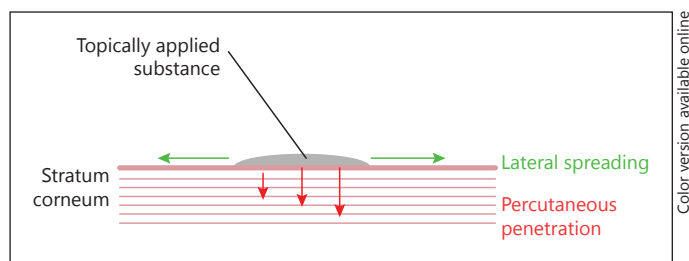


Fig. 1. Representation of the ways used by a drug once applied onto the skin surface.

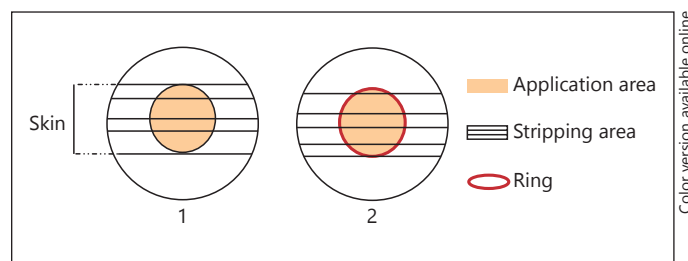


Fig. 2. Formulations A and B without the ring (circle 1) and with the ring (circle 2).

stripping method in association with spectroscopic measurements. Tape stripping, a widely used method in dermatological research, is commonly used to investigate SC physiology [3] as well as the bioavailability and bioequivalence of topical drugs [4]. A combination of both methods is often used to measure the homogeneity and the content of the SC recovered on the strips [5].

Urea is often used as a hydrating agent and is employed in the treatment of ichthyosis, psoriasis and other hyperkeratotic conditions (10%) [6], for dry skin (3–5%) [7], for avulsing dystrophic nails (40%) [8], and as a penetration enhancer. Its hygroscopic nature and keratolytic properties reversibly alter skin barrier function [8, 9]. Feldmann and Maibach [10] found that a cream containing urea (10%) increases hydrocortisone permeation 2-fold in vivo in man. Urea is also nontoxic and a component of the natural moisturizing factor present in the skin. This intercellular ingredient promotes the restoration of deficient hydration of the SC. The major components of the natural moisturizing factor are free fatty acids (40%), pyrrolidone carboxylic acid (12%) and urea (7%) [11, 12].

In conducting a more intensive study on lateral spreading, urea was chosen as a low molecular-weight hydrophilic model because it has been used in commercial preparations designed for normal and pathological skin. To obtain optimal treatment results, a predetermined amount of topical treatment (solution or cream) was used, so if spreading occurred its quantification would allow appropriate adjustment to the trans-SC drug delivery systems in other studies. Additionally, urea is a model compound because less than 1% of the applied dose penetrates in vivo in man, thus simplifying data interpretation [13].

The distribution of substances within the SC is influenced by the formulation used, thus the application of a solution and cream, both of which contained urea, was investigated. The time between application and tape stripping was changed and a barrier with a ring around the marked area was built for investigation. The percent-

age of urea recovered inside and outside the ring barrier compared to the applied dose was measured. This study was conducted with the hypothesis that formulation, time and a ring barrier all influence lateral spreading and cutaneous bioavailability.

Materials and Methods

Skin

This in vitro experiment was performed on abdominal human skin of cadavers (mean age 40 years) collected no later than 24 h after death. The skin was stored at -20°C for a maximum of 1 year before use. Mean pH (6.45) and moisture values ($62.8 \pm 3\%$) of the skin investigated were measured with a Skin-pH-Meter PH 900 PC and a Corneometer CM 825 PC (Courage & Khazaka Electronic GmbH, Köln, Germany and Acaderm, Menlo Park, Calif., USA).

Parameters not investigated in this experiment were constant during the study to avoid their influence on lateral spreading. The experiments were performed under standard conditions at a room temperature of $22 \pm 1^{\circ}\text{C}$.

Chemicals

Urea [$\text{CO}(\text{NH}_2)_2$] (Sigma-Aldrich, St. Louis, Mo., USA) was dissolved in water (formulation A) and in an oil-in-water emulsion as a base (Moisture Recovery Lotion; Walgreens, Springfield, Ill., USA), containing water, glycerin, petrolatum, stearic acid, glycol stearate, isopropyl isostearate, dimethicone, tapioca starch, cetyl alcohol, glyceryl stearate, magnesium aluminum silicate, carbomer, ethylene brassylate, triethanolamine, disodium EDTA, phenoxyethanol, methylparaben, propylparaben, and titanium dioxide (formulation B) to a concentration of 10%.

Topical Application

In detail, 100 μl of each formulation containing urea (10%) was applied onto marked in vitro skin areas of 5 cm^2 drawn with a ball-point pen. Formulations were applied with a syringe and then distributed homogeneously with a plastic gloved finger. The skin was not covered from the time of application to the beginning of tape stripping.

Both formulations (A and B) were applied to the marked areas of normal skin with and without the plastic ring coated with petrolatum to assess surface spread (fig. 2).

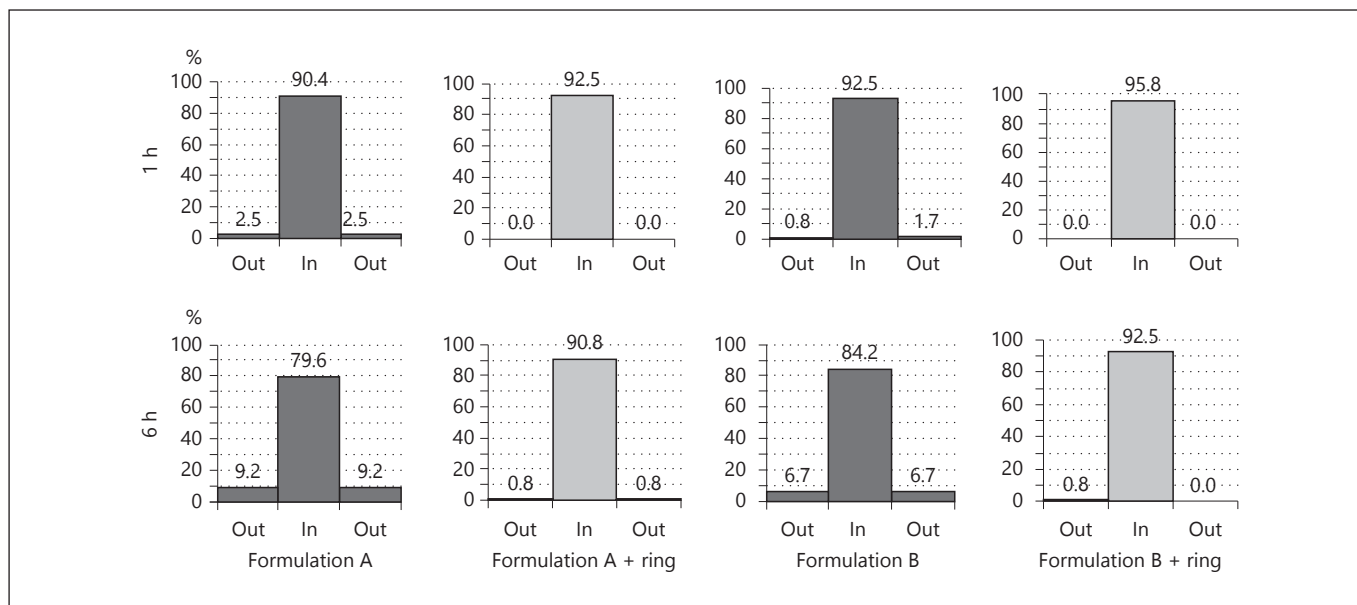


Fig. 3. Total recovery rates (%) of urea 1 and 6 h after topical application and tape-stripping protocols with and without the ring. Formulation A: urea + water; formulation B: urea + cream.

Tape Stripping

The application areas and the perimeter of the skin were stripped 10 times with adhesive tape (D-Squam[®], CuDerm, Dallas, Tex., USA) 1 and 6 h after topical application. Separate sites within the in vitro skin areas were used for 1- and 6-hour tape-stripping time points. Constant, uniform pressure with a gloved finger was applied to the tape strips prior to their removal in one fluid movement. Each strip was positioned on the same skin area. The strips were placed in a test tube filled with 5 ml of phosphate buffer saline solution (Sigma-Aldrich) and treated for 30 min in an ultrasonic bath (FS 60; Fisher Scientific, Hampton, N.H., USA) to recover urea in the successive layers.

The urea content of human skin approximates 1% [14]. The application of 10 tape strips on a piece of normal skin without treatment was performed so that physiological urea contained naturally in the natural moisturizing factor would not influence results obtained after the topical application of urea.

Spectroscopic Measurements

Urea present in the strips was analyzed by colorimetric assay; the condensation of urea with diacetyl monoxime reagent (2-3-butandione monoxime; Sigma-Aldrich) produces a yellow-colored compound after heating [15]. The reagent was dissolved in an acidic solution of hydrochloric acid (Fisher Scientific, Pittsburg, Pa., USA) and then added to the urea solution [16]. Spectroscopic measurements were performed with the Spectrophotometer SP-830, capable of detecting a wavelength range from 320–999 nm (Spectrophotometer SP-830 Plus, Barnstead International, Dubuque, Iowa, USA). The amount of urea was directly proportional to the intensity of the yellow color, measurable by its absorbance at a wavelength of 480 nm, using blank tape for the reference beam. The calibration curve describing the relationship between urea concentration and absorbance was prepared with different urea standard concentrations.

This method of color reaction with diacetyl monoxime in acidic solution has been used to determine the urea content in plasma or urine [16] and in the SC of patients suffering from atopic dermatitis compared to healthy volunteers and patients with psoriasis [14].

The relative recovery rate (%) was calculated as the ratio of the amount of substance found on the tape strips to the amount of substance applied in the application area, multiplied by 100.

Statistics

Urea values were calculated using the software program OpenOffice[®] Calc. Statistical analysis was done with the software R[®]. Wilcoxon test was utilized to compare the obtained recovery rate of urea between the different protocols and times.

A *p* value <0.05 was used as the reference for significant differences.

Results

Local distribution of urea in the SC was determined in and outside the application area in correlation with the horny layer profile. Urea recovered is given as the percentage of the applied dose. Formulation, time and presence/absence of a ring barrier were varied. The results are summarized as follows: formulation A (urea + water) and formulation B (urea + cream) with/without the ring (fig. 3).

Although urea was recovered from the application site for both protocols (fig. 3), the percent recovery was

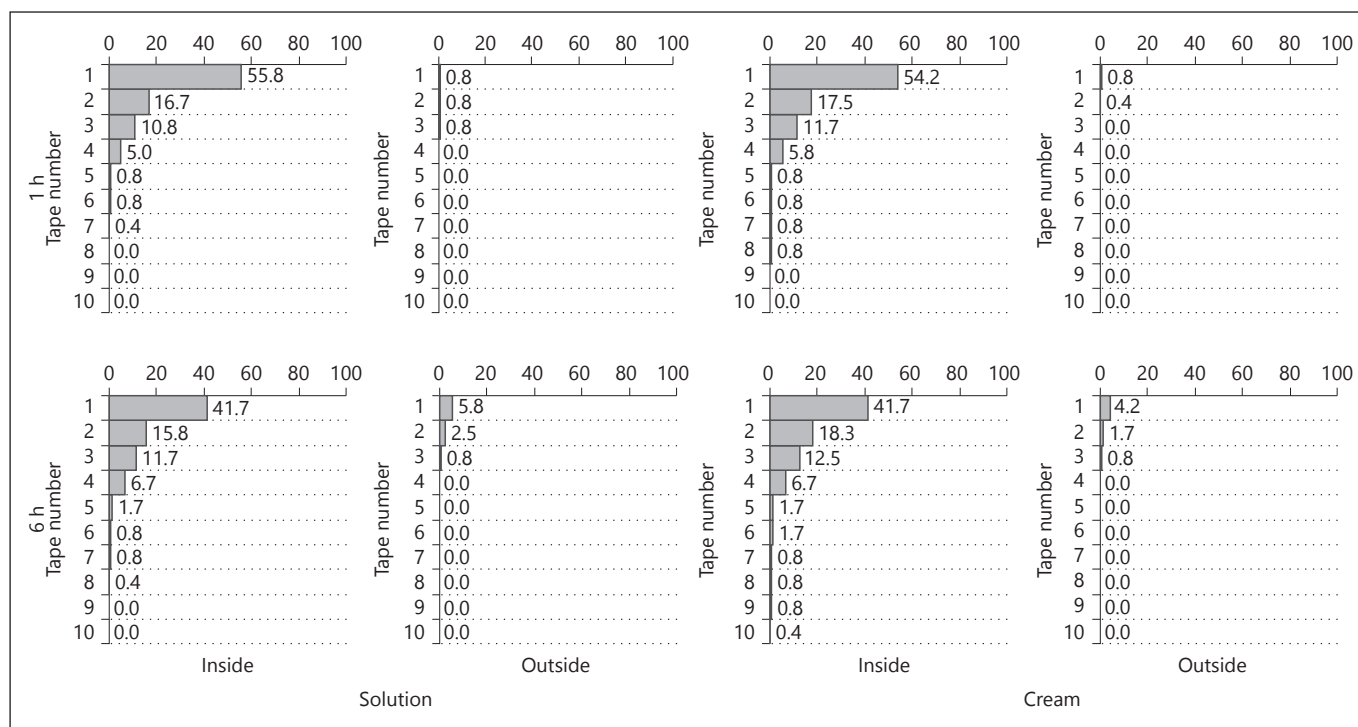


Fig. 4. Distribution of urea (%) in the SC 1 and 6 h after topical application inside/outside the treated area for the solution/cream without the ring.

greater inside the application area with the presence of the ring – 92.5 and 95.8% versus 90.4 and 92.5%, respectively, for formulations A and B 1 h after application, and 90.8 and 92.5% versus 79.6 and 84.2%, respectively, for formulations A and B 6 h after application. Additionally, the amount of urea recovered outside the marked application area was increased between 1 and 6 h without the ring for the solution (5–18.4%) and the cream (2.5–13.4%). These results suggest that the amount of urea recovered inside the application area increases with time and the presence of a ring barrier.

The petrolatum-covered ring barrier minimized lateral spreading at 1 and 6 h after application – 0% versus 5 and 2.5% for formulations A and B, respectively, at 1 h and 1.6 and 0.8% versus 18.4 and 13.4% for formulations A and B, respectively, at 6 h. Lateral spreading was symmetric for each protocol, with no differences observed between the left and right outside areas. Penetration profiles drawn below (fig. 4) represent the distribution of urea recovered in the different tape strips and the penetration of the compound into the SC in and around the application area. Urea recovery rates were measured in each strip and presented as the percentage of the total amount of urea applied.

Profiles in figure 4 represent the depth of the SC layers. The highest amount of urea was recovered in the first layer for all the protocols – cream/solution and in/outside the treated area.

The behaviors of the cream and solution in the layers of the SC were compared and no significant difference was observed between the centrally treated areas ($p = 0.2$ at 1 h and $p = 0.1$ at 6 h) and the outside areas ($p = 0.1$ at 1 and 6 h). Thus, no correlation was observed between the formulation, proportion of percutaneous penetration and proportion of spreading.

Time

Statistical analysis showed a significant difference between the behavior of urea at 1 and 6 h after topical application. The amount of urea recovered inside the application area was lower without the ring ($p = 0.03$ and $p = 0.04$ for formulations A and B, respectively) and higher on the outside ($p = 0.01$ and $p = 0.02$) where the lateral spreading took place. This demonstrates a correlation between time, the proportion of percutaneous penetration and the proportion of spreading observed.

Results obtained with the presence of the ring are summarized below (fig. 5).

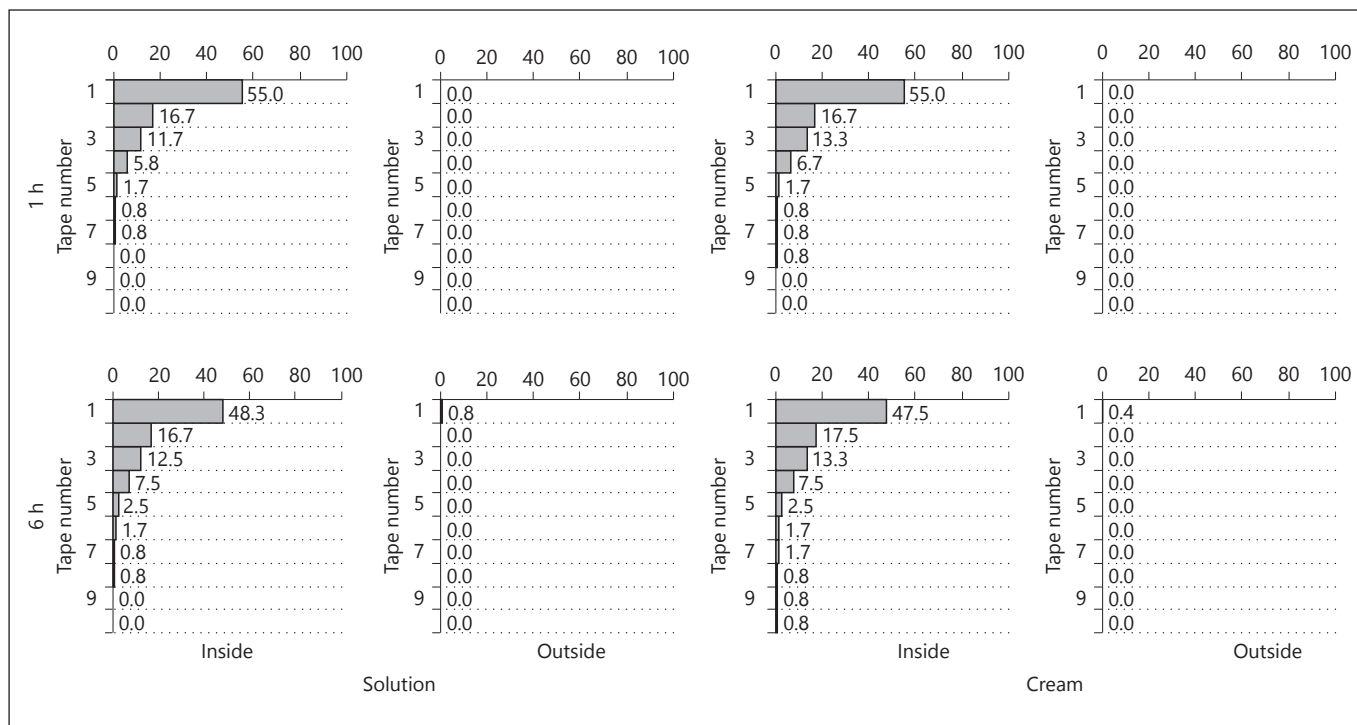


Fig. 5. Distribution of urea (%) in the SC 1 and 6 h after topical application inside/outside the treated area for formulation A (solution)/formulation B (cream) with the ring.

Ring

Statistical analysis demonstrated no significant difference between the solution and cream inside or outside the treatment area ($p > 0.05$).

Contrary to the first protocol without the ring, there was no significant difference in the recovery rate of urea with time inside and outside the application site ($p > 0.05$).

Although no significant difference occurred between protocols (with/without the ring) inside and outside the application site at 1 h ($p > 0.05$), there was a significant difference between protocols at 6 h ($p < 0.05$) when comparing figures 4 and 5. These results suggest that spreading takes place mainly on the skin surface and that the ring effectively minimizes lateral spreading by creating a barrier around the marked area (0.8 vs. 5.8% for the solution in the first SC layer and 0.4 vs. 4.2% for the cream at 6 h).

Discussion

Tape stripping in combination with spectroscopy as a model to quantify percutaneous absorption is often used to determine the horny layer profile, an important prerequisite for assessing the efficiency or safety of drugs,

cosmetics and UV filters in sunscreens [17]. Nevertheless, disturbances can appear with this procedure. The amount of urea fixed to the individual adhesive film and the thickness of the horny layer vary considerably for different individuals [18]. To determine SC thickness, the horny layer must be completely removed by repeating the tape-stripping procedure until the adhesive film is optically empty. As a result of this procedure, all components of the horny layer and the topically applied substance are transferred from the skin to the adhesive. If the movement of taking away the tape is not homogeneous, or if the skin structure is not homogeneous in the area used for tape stripping, it is expected that the particles fixed on the tape are not representative of SC thickness [18]. Therefore, this study considered the possibility that the concentration of topically applied urea could be determined quantitatively in relation to the number of tape strips and not to the real SC thickness.

Measurements of urea recovered in the different layers of the SC (fig. 3) show that 18.4% (solution) and 13.4% (cream) of topically applied urea can spread outwards from the application site after 6 h. In addition, the amount of substance found outside the application area seems dependent on the size of the area [19]. Relatively small ap-

plication areas are usually used so it is important to take lateral spreading into account.

Results show that applied urea was mainly located in the superficial layers of the SC 1 and 6 h after topical application. Similar behavior was observed *in vivo* for UV filters and clobetasol propionate applied topically in two different formulations [17, 20] and positioned in the upper part of the horny layer. Figures 4 and 5 show that the concentration of urea found in the uppermost part of the SC is not significantly different for both formulations.

Statistical analysis suggests that both formulations, the urea-containing solution and cream, behave similarly in terms of skin penetration and lateral spreading. Regarding the spreading of a substance on the skin surface, a qualitative study on the accuracy of the area adequately treated with 4 formulations (solution, cream, low-viscosity cream and ointment) was conducted by Ivens et al. [21]. Formulations were tagged with vitamin A acetate 1%, a fluorescence marker that fluoresces bright yellow when irradiated with a black Wood's light but remains invisible on the skin under normal illumination [22]. Results obtained *in vivo* showed that with the same amount of formulation applied (0.1 g), only the ointment with high viscosity was distributed equally in the center and periphery of the treated area. The formulations with lower viscosity were spread sparsely at the periphery of the treated area compared to the central portion. This indicates that while the solution and cream behave similarly, spreading is linked to the type of formulation used. In contrast, Weigmann et al. [23] used tape stripping in combination with high-performance liquid chromatography *in vivo* to demonstrate that the amount of clobetasol propionate contained in an emollient found on tape strips increased almost 4-fold with time compared to a cream when analyzing the application area. By showing a clear reduction of active substance inside this zone, it is suggested that emollient diffuses laterally on and through the uppermost part of the SC.

Finally, Ashworth et al. [20] used another procedure to quantify the lateral migration of radiolabelled clobetasol 17-propionate in two bases. They showed that successive skin surface biopsies taken from each application site and the immediately adjacent site yielded significant differences in behavior, with one spreading more laterally. When comparing the recovery amounts of urea at 1 and 6 h, there was a significant difference with increasing time for both formulations without the ring (fig. 4) inside and outside the treated area. The recovered amount of urea found 6 h after application of the formulation was re-

duced, especially on the surface of the treated area, while the amount found on the surface of the untreated skin sites was enhanced. Jacobi et al. [19] investigated the lateral spreading of UV filter substances using the same method seen in this present study – tape stripping and spectroscopy. The time between application and tape stripping varied in the protocols, and a time-dependent reduction of the recovered UV filter amount inside the application area was observed. The loss of substance inside the treated area corresponded to the increased concentration measured in the adjacent area, indicating that lateral spreading took place on the skin surface and increased with time.

Lateral spreading can be significant and has to be excluded if penetration processes of topical products are investigated. This can be realized with the application of a 'barrier' around the treated skin area. In the present study, the application of a petrolatum-covered ring onto the skin surface was investigated. The differences between protocols (with/without the ring) in and outside the application area at 6 h were significant. The ratio of urea on adjacent sites with the barrier (fig. 5) was less compared to the 'normal' protocol without the ring, suggesting a reduction in lateral spread. Meanwhile, the percentage of urea distributed outside the application area was symmetric in this study (fig. 3) – similar to results shown by Jacobi et al. [19]. The symmetric surface structure of the skin, consisting of a network of furrows, could be the reason for this result. Results obtained in other studies on lateral spreading are summarized in table 1.

In this specific study, the concentration of urea spread at 6 h. Thus, in calculating the applied dose, as related to penetration, mathematical correction factors were needed to compare experiments in which the applied dose was expressed in micrograms/centimeters squared. Parenthetically, the urea *in vivo* penetration experiment by Feldmann et al. [10] utilized the petrolatum barrier.

Skin surface topography varies by anatomical site. Primary furrow networks have been shown to function as pathways for lateral spreading, whereas follicles and wrinkles represent a reservoir for topically applied substances [24]. In senescence, skin topography can change and these structures can deepen, becoming larger. This change can disturb penetration and lateral spreading studies investigated by tape stripping. Lademann et al. [25] used optical methods such as laser-scanning microscopy, tomography and microscopic investigation of histological sections obtained by biopsies to evaluate a special protocol which could avoid these potential distur-

Table 1. Summary of the results obtained in other studies on lateral spreading in vivo in man using various methods and compounds

First author	Compound used	Method	In vivo/ in vitro	Results
Weigmann [17], 2001	Clobetasol propionate (cream and emollient)	Tape stripping + HPLC	In vivo in man	– Spreading is formulation dependent – Mainly recovered in the uppermost layer of the SC
Ashworth [20], 1988	Clobetasol propionate (2 different bases)	Biopsies + radioactivity	In vivo in man	– Spreading is formulation dependent – Mainly recovered in the uppermost layer of the SC
Jacobi [19], 2004	UV filters	Tape stripping + spectroscopy	In vivo in man	– Mainly recovered in the uppermost layer of the SC – Spreading is dependent on the size area – Spreading is dependent on time – Spreading is a symmetric process
Ivens [21], 2001	4 different bases	Fluorescence + black woods light	In vivo in man	– Not formulation dependent

HPLC = High-performance liquid chromatography.

bances. Patent blue dye was added to the formulation and tape stripping in combination with spectroscopic measurements was performed. Results demonstrated that using a roll to perform the tape-stripping procedure avoided the substance being left in furrows and wrinkles.

Typically, a small amount of active substance remains in the furrows and wrinkles. The rolling movement allows the skin to become stretched and flat during contact with the adhesive tape. This means that the SC can be completely removed only in the case of in vivo experiments when performing depth-penetration studies [26]. Living skin is more flexible and elastic, helping to minimize or avoid disturbances from the typical skin structure. Even a relatively large variation of skin properties in the volunteers measured does not proportionally correlate with the efficacy of lateral spreading [19].

Conclusion

The results of this study reflect the kinetics and intensity of lateral spreading. The extent of lateral migration on the skin surface appears to be time dependent, while the formulation used for topical application seems to play a less significant role.

Local distribution of urea was investigated in and around the application site, and lateral spreading was noted to take place predominately on the skin surface. Lateral migration of urea was clearly minimized in the presence of a ring barrier built around the application site.

This study demonstrates that spreading is not negligible and warrants consideration during percutaneous penetration and drug bioavailability studies. Exact knowledge of the amount of spread is a definite prerequisite to understanding and optimizing the efficacy of cosmetics and topically applied drugs. To ensure homogeneous treatment and to avoid potential side effects from topical spreading, patients should be instructed to apply these formulations several times or use higher quantities. Although transdermal-delivering drugs are package labelled as mass per patch, the data described here intimate that actual drug delivery surface data may be significantly greater than patch size. Studies remain to be conducted to ascertain if lateral spreading from patches differs from that utilized in this experiment.

Finally, this simple and noninvasive method is a reasonable procedure to assess lateral spreading. The combination of tape stripping and spectroscopy obtains equivalent results compared to the complex methods involving radiolabelled compounds or biopsies.

We do not wish to overgeneralize from this data until further quantitative information is available in vivo and until a broad range of varying physicochemical properties have been examined.

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