Determination of penetration profiles of topically applied substances by means of tape stripping and optical spectroscopy: UV filter substance in sunscreens

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Abstract. Penetration profiles of topically applied drugs and cosmetic products provide important information on their efficacy. The application of tape stripping in combination with UV/VIS spectroscopy is checked to determine the local position of topically applied substances inside the stratum corneum, the penetration profile. The amount of corneocytes removed with each tape strip is quantified via the particle-dependent absorption, the pseudoabsorption, in the visible spectral range. The concentration of a typical UV filter substance, 4-methylbenzylidene camphor, is determined by optical spectroscopy using the tape strips removed originally. In this case, a time-dependent increase in the absorbance must be taken into account. Laser scanning microscopic investigations confirm that the nonhomogeneous distribution of the filter substance, on the strips, can explain this spectroscopic behavior. When reaching a homogeneous distribution, the UV spectroscopic signal reflects the correct concentration. These spectroscopic values are compared with high performance liquid chromatography (HPLC) data. The values obtained with both methods for the concentrations of 4-methylbenzylidene camphor are in good agreement. The data obtained are used to illustrate the determination of a penetration profile of a UV filter substance. The results demonstrate that the described protocol is well suited to characterize, in a simple manner, topically applied substances that have a characteristic UV/VIS absorption band. [DOI: 10.1117/1.1854683]

Keywords: UV filter; tape stripping; microscopy; laser scanning microscopy; optical spectroscopy; high performance liquid chromatography.

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1 Introduction

The exact knowledge of the local distribution of substances topically applied to the stratum corneum (SC) is a decisive prerequisite to understand and optimize the efficacy of cosmetics and drugs, particularly in dermatopharmacokinetics.[1]

The tape stripping procedure,[2–6] which removes the horny layer step by step with an adhesive tape, is well suited to investigate penetration processes. With this method, the corneocyte aggregates, as well as the substances topically applied, are definitely transferred to the tape strips. The quantitative determination of both amounts fixed to each tape strip can be used to calculate the horny layer profile, as well as the position of the substances under investigation inside the SC, thus providing the so-called penetration profile.

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The spectroscopic determination of the attenuation of the corneocytes quantitatively reflects, with a high sensitivity, the mass of the SC removed by each tape strip.[7,8] The data obtained provide the SC profile and the position inside the horny layer from where the strips were taken.

The topically applied substances fixed to the individual tapes are quantified by means of analytical methods, e.g., high performance liquid chromatography (HPLC) or optical spectroscopy if a characteristic UV/VIS absorption band exists, as in UV filter substances. It is an advantage of the spectroscopic measurements to record, in addition to the corneocyte-dependent pseudoabsorption, the characteristic bands of absorbing species simultaneously.[7,9] This allows the determination of the filter amount from the same tape used for the SC profile calculation.

In the present study, we have investigated time-dependent spectroscopic changes observed for a UV filter substance and

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the particle-dependent pseudoabsorption after removal of the tapes. The spectroscopic data of the UV filter substance were compared with the results obtained by HPLC measurements. Microscopic and laser scanning microscopic measurements were performed to obtain new information on the observed spectroscopic behavior. The described method was applied to determine the penetration behavior of a typical UV filter substance, 4-methylbenzylidene camphor.

2 Materials and Methods

2.1 *Volunteers*

The study was undertaken on nine healthy volunteers with skin phototypes 2 and 3,[10] aged between 24 and 45 years. Approval was obtained for these experiments from the Ethics Committee of the University Hospital Charité. The volunteers provided written informed consent prior to entry into the study.

2.2 *Topical Application of the Emulsions*

An oil/water (o/w) emulsion provided by Beiersdorf AG (Hamburg, Germany) was used containing 4% 4-methylbenzylidene camphor. The investigation of the fluorescence was performed using an o/w emulsion containing 0.2% of the dye curcumin (MERCK-Schuchardt, Hohenbrunn, Germany). This fluorescent dye was chosen because this fluorophore is permitted for use in foods. Furthermore, a comparable behavior of the time-dependent spectroscopic changes was found for curcumin and the UV filter substance.

The emulsions were topically applied to a skin area on the flexor forearms of the volunteers as described previously.[7] A skin area of 10×8 cm marked with a permanent marker was treated with 2 mg/cm^2 emulsion. The emulsion was distributed homogeneously inside the marked area using a gloved finger (pressure in the range of 60 g/cm^2) saturated with the emulsion.

2.3 *Tape Stripping*

The tape stripping procedure, using an adhesive film (tesa film number 5529, Beiersdorf, Hamburg, Germany, width 1.9 cm), started 1 h after the application of the emulsion. In agreement with the standard protocol described previously,[7,11] the tape strips with a length of 6 cm were applied centrally inside the treated skin area. The longer side of the *tesa* films were arranged parallel to the 10-cm borderline of the treated skin area. The tape strips were pressed ten times using a roller and removed with one quick move afterward.

2.4 *UV/VIS Spectroscopic Measurements*

After removal from the skin, the tape strips were fixed to a frame taken from a commercially available slide holder. With a razor blade, the edges of the strips were removed to a resultant thickness of 1.5 cm. This was done to avoid edge effects caused by a lack of perfect overlap of each tape strip take from the same area.[7]

2.4.1 *Experiments with intact tape strips*

As a first step, a modified UV/VIS spectrometer (Perkin-Elmer, Überlingen, Germany) with a quadratic beam of 10×10 mm was used to record the spectra between 240 and 500 nm. The removed tape strips were measured with a blank in the reference beam. This blank was taken from the direct neighborhood of the roll where the tape strip used for the experiment originates from. This was necessary because the adhesive layer has weak absorption maxima at 278 and 283 nm.

After stripping from the skin, the measurement was repeated on the same tape strip with different time delays up to 24 h. This was carried out because the absorbance increases drastically as a result of an increasing homogeneity in the distribution of the absorbing species on the tape strips. This special behavior of the investigated system is discussed in detail later on.

The influence of the corneocyte aggregates on the measured intensity, described by the term pseudoabsorption, is determined by the absorption, the reflection, and the diffraction of the horny layer particles fixed to the removed tape strips. This value was measured at 430 nm. Absorption bands resulting from other components contained in the investigated systems were not found in this spectral region. The pseudoabsorption characterizing the corneocytes was determined about 1 h after removal. This value remained constant up to 24 h and longer. The obtained data were used to calculate the horny layer profile.[7,12] This was done using the corneocyte-specific pseudoabsorption to determine the amount of horny layer particles removed with each tape strip. Adding the data of all strips obtained after complete removal of the stratum corneum provides the sum absorbance, which represents the complete horny layer thickness given in relative values. The penetration profile of the topically applied substance is obtained entering the amount of UV filter substance measured for each tape strip into the horny layer depth profile. To obtain a symmetrical position of the bars inside the graph, zero was set in the middle of the *x* axis, giving each half of the absolute *x* value to the right and left.

The maximum of the UVB filter 4-methylbenzylidene camphor was measured at 300 nm using the spectra of the tape strips. The determination of the filter concentration needs to be corrected for the influence of the horny layer particles on the absorbance. This was performed using the software UV WinLab Version 2.70.01 (PerkinElmer, Überlingen, Germany). The correction was realized using spectra obtained from tape strips at adjacent skin areas not treated with emulsions. These spectra were fitted to the spectra of the UV filter substance by a correction factor realizing an identical course of both spectra in the visible spectral range. This adapted spectrum from the adjacent tape strip site was used for the baseline correction.

2.4.2 *Experiments with dissolved tape strips*

The tape strips were cut to a length of 4.3 cm affording a size of 1.5×4.3 cm, and were then put into a testtube and filled with 6.45-ml ethanol (UVASOL, Merck, Darmstadt, Germany). Subsequently, the tubes were treated for 5 min in an ultrasonic bath (Sonorex Super RK 102H, Bandelin Electronic, Berlin, Germany). The solutions were purified by centrifugation (5 min with 4000 c/s, centrifuge MR 1812, Jouan GmbH, Unterfaching, Germany) to separate small horny layer particles. The uppermost part was decanted with a pipette into a quartz cell of 10 or 1 mm in length (Hellma, Jena, Germany).

The UV/VIS spectra of the extracts were measured between 240 and 500 nm using the pure solvent as a reference. The concentration was calculated from the determined absorption maximum on the basis of a calibration curve for the UV filter substance in ethanol.

2.5 *HPLC Measurements*

The concentrations of the UV filter substance in the extracts used for the UV/VIS spectroscopic measurements were determined by HPLC measurements.[13] The concentration was calculated based on the peak values. Standard solutions containing 4-methylbenzylidene camphor were measured for calibration. The HPLC system used consisted of a pump L-6200A, a detector L-4500 (Diode Array), an autosampler AS-2000A, and an interface D-6000 (all Merck-Hitachi, Darmstadt, Germany). The column LiChrospher 60 RP-select B (5 μm, 250×4 mm) was used (Merck, Darmstadt, Germany). A mixture of 82% acetonitrile (gradient grade), 17.9% water (for chromatography), and 0.1% phosphoric acid (pro analysi) (all Merck, Darmstadt, Germany) was applied as an isocratic eluent with a flow of 1 ml/min. A volume of 25 μl was injected. For the detection, a wavelength of 300 nm was used.

2.6 *Investigation of the Corneocyte Aggregates on the Tape Strips by Laser Scanning Microscopy*

Laser scanning microscopy was utilized to measure the distribution of the topically applied substances on the tape strips removed from the treated skin.[14] Therefore, 0.2% curcumin (MERCK-Schuchardt, Hohenbrunn, Germany) was added to the o/w emulsion containing the UV filter substance. The emulsion was applied onto the skin. After a penetration time of 1 h, tape strips were removed and investigated at different times by transmission and fluorescence measurements using a laser scanning microscope (LSM 2000, Carl Zeiss, Jena, Germany). The curcumin distribution was measured using the fluorescence signal at wavelengths above 600 nm after excitation with 488 nm radiation.

2.7 *Investigation of the Corneocyte Aggregates on the Tape Strips Using Light Microscopy*

Corneocyte aggregates on tape strips removed from treated skin were investigated by light microscopy. A light microscope (System microscope BX60, Olympus Optical Company, Hamburg, Germany) was used to take images of the tape strips at different storage times in the transmission mode. The microscope was equipped with a digital camera SIS ColorView 12 and the appropriate software analySIS AUTO 3.1 (both Soft Imaging System GmbH, Münster, Germany).

2.8 *Statistics*

The method of linear regression was used to determine the relationship between the spectroscopic data and the HPLC values. The coefficients of correlation R^2 were calculated using the software program Microsoft® Excel 97.

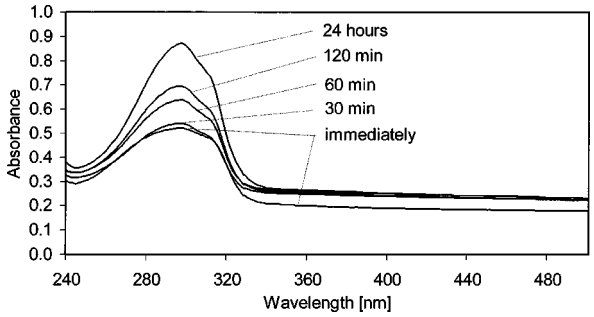


Fig. 1 UV/VIS spectra of the second tape strip, measured at different times after removal from the skin, which was treated 1 h before with an o/w emulsion containing 4% 4-methylbenzilidene camphor.

3 Results

3.1 *Time-Dependent Spectroscopic Changes of the Absorption of the UV Filter Substance*

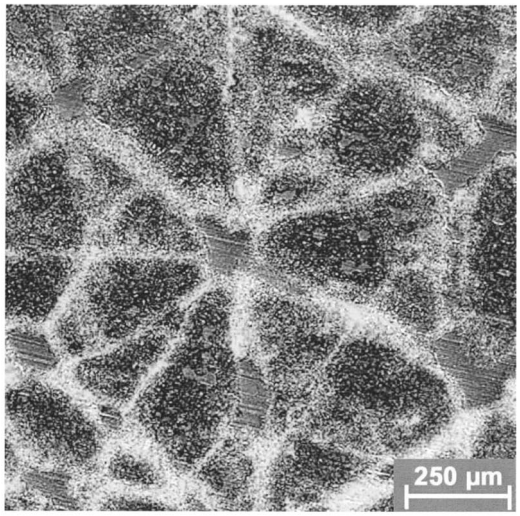
Figure 1 shows the typical spectra of a tape strip measured at different time intervals after removal from the skin treated with an o/w emulsion, containing the UVB filter 4-methylbenzylidene camphor. The absorbance in the visible range above 400 nm was characterized by a small change in the spectra, measured immediately and after 30 min. Later on, this value remained constant. In contrast, the typical absorption of the filter substance at the UV region increased in time. After a time period of 24 h, the absorption remained constant. A comparable result was obtained for curcumin. The absorbance was enhanced with increasing time after removal by a factor $f_{\text{curcumin}} = A_{6\text{h}}/A_{\text{immediately}} = 1.5$. The comparable factor for the UV filter substance was found at $f_{\text{UVB filter}} = A_{6\text{h}}/A_{\text{immediately}} = 1.7$.

Laser scanning microscopy was applied to check whether the spectral changes observed for the UV filter substance were caused by changes in the distribution of the filter substance on the removed tape strips. The experiments were carried out using the o/w emulsion containing the fluorescence dye curcumin.

Typical distributions of the dye on the same tape strip at different times are given in Fig. 2 as a superposition of fluorescent and transmission images. The fluorescent dye is characterized by the light structures on the background of the dark images of the corneocyte aggregates. The distribution was not homogeneous if the tape strips were measured immediately after removal [Fig. 2(a)]. After periods of 1 and 6 h, the areas covered with curcumin increased [Figs. 2(b) and 2(c)], however, 24 h after removal (figure not presented) the dye was distributed homogeneously on the tape.

3.2 *Absorbance of the UV Filter Substance Measured for the Removed Tape Strips and in Solution*

In the following experiments, we investigated whether the absorption of the UV filter substance measured on the removed tape strips could be used directly to determine the concentration. Therefore, the absorption spectra of the tape strips were measured 24 h after removal, when the absorbance remained constant. After the direct spectroscopic measurements, the tape strips were extracted and the absorbance of



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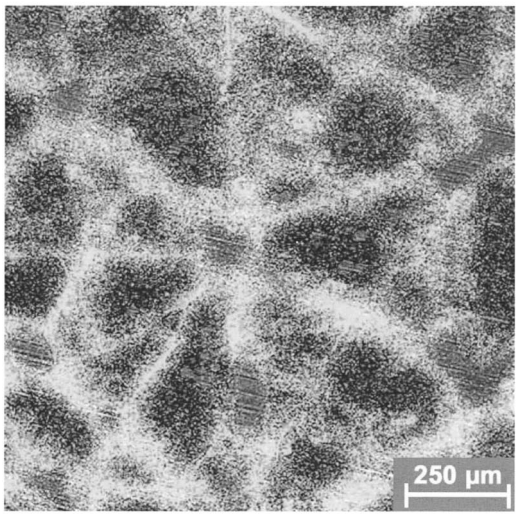

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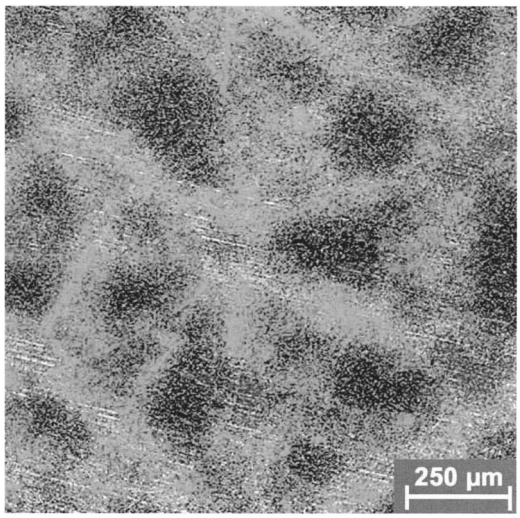


(c)

Fig. 2 Time dependence of the distribution of curcumin (light structures) on a tape strip determined by laser scanning microscopy: (a) Measured immediately, (b) measured 1 h after removal from the skin, and (c) measured 6 h after removal from the skin.

the UV filter substance in solution was determined. In Table 1, the results found for the absorbance of 4-methylbenzylidene camphor are summarized.

3.3 *Comparison of Spectroscopic Data and HPLC Measurements*

To estimate the accuracy of the spectroscopic method for the quantification of the concentration of 4-methylbenzylidene camphor, dependent on the concentration range, the results of the spectroscopic measurements were compared with HPLC measurements carried out on the same samples (Table 2). The data obtained are separated into two concentration ranges. In the upper part of the table, the tapes containing more than 1 μg/ml (corresponding to 1 μg/cm^2 tape) were summarized. The data of tapes with a lower or equal content are shown next.

The deviation of the concentrations of the UV filter substance determined by UV spectroscopy and HPLC was $\leqslant$5% for more than 95% of the total amount recovered, corresponding to 87.9 μg/ml. In less than 5% of the total amount recovered (corresponding to 3.7 μg/ml), the deviation was $\leqslant$25%. This result was confirmed correlating graphically the HPLC values with the data determined by UV/VIS spectroscopy. A linear regression line with a high coefficient of correlation was found ($R^2 \geqslant 0.99$).

3.4 *Spectral Changes in the Pseudoabsorption of the Corneocytes*

To obtain additional information on the time behavior of the pseudoabsorption at 430 nm, caused by the corneocyte aggregates, light microscopic measurements were performed. The results of the investigations of the tapes, stripped 1 h after application of the sunscreen, are given in Fig. 3. A pronounced edge structure of the corneocyte aggregates was observed, if the investigations were performed immediately after removal [Fig. 3(a)]. Later on, this structure disappeared [Fig. 3(b)]. Regarding the extension of the horny layer cells [Fig. 3(c)], no further changes were observed during an additional 23 h. The pseudoabsorption measured 1 h after removal of the tape strips was used to calculate the horny layer profile.

3.5 *Penetration Profile of the UV Filter Substance*

The results of the spectroscopic measurements characterizing the amount of the corneocytes removed by each tape and the corresponding concentration of the UV filter substance were used to calculate the penetration profile of a typical UVB filter. Figure 4 shows the local distribution of 4-methylbenzylidene camphor applied in the o/w emulsion inside the SC. The length of the black bars represents the amount of UVB filter substance found on the corresponding tape strips. The absorbing species are positioned in the uppermost part of the horny layer.

4 Discussion

4.1 *Time-Dependent Spectroscopic Changes*

It is a well-known spectroscopic fact that the absorbance of a system containing identical amounts of absorbers is reduced if the substance does not cover the measuring area homogeneously. Lambert-Beer's law, correlating absorbance and con-

Table 1 Absorbances of the UVB filter substance 4-methylbenzylidene camphor measured at 300 nm for the strips, removed from the skin 1 h after topical application and the corresponding extracts. (One volunteer, nine consecutive tape strips taken into account from 20 removed strips). Percentage in brackets in the last column correlated to the absorbance of the extracts. The value for tape 1 24 h after stripping is not measurable due to the absorbance outside the measuring range. The value for tape 1 24 h after stripping is normalized to a cell length of $d=1$ cm, with the solution measured with a cell length $d=1$ mm.

Tape number	Absorbance measuring the tapes 30 min after stripping	Absorbance measuring the tapes 24 h after stripping	Absorbance of the extracts	Difference between the absorbances measuring the tapes 24 h after stripping and the extracts
1	1.19	*	4.35	—
2	0.23	0.44	0.41	–0.03 (–7%)
3	0.15	0.22	0.24	+0.02 (+8%)
4	0.07	0.10	0.12	+0.02 (+17%)
5	0.05	0.08	0.07	–0.01 (–14%)
6	0.05	0.08	0.08	±0 (±0%)
7	0.04	0.06	0.07	+0.01 (+14%)
8	0.01	0.02	0.03	+0.01 (+33%)
9	0.01	0.02	0.02	±0 (±0%)

centration linearly, is valid only if the absorbing species are distributed homogeneously. The lower absorbance found for the tape strips measured directly after stripping (see Fig. 1) reflects such a nonhomogeneous distribution of the UV filter substance on the tapes. This is in agreement with a theoretical consideration given, especially taking into account the behavior of UV filter substances in sunscreens.[15]

Later on, the absorbance measured increased because the distribution became more homogeneous, reaching an endpoint after 24 h. These changes are reflected by the increasing absorbance of the characteristic curcumin band described by the factor $f_{\text{curcumin}}=A_{6\text{h}}/A_{\text{immediately}}$ (see Sec. 3.1). Both results obtained with laser scanning microscopy and optical spectroscopy confirm a diffusion of the filter substance into and inside the adhesive layer of the tape strips. This confirms the given interpretation for the time-dependent spectral changes observed for the absorption of the UV filter substance (see Fig. 1).

4.2 *Filter Absorbance Measured for the Removed Tape Strips and in Solution*

The identical absorbances of the UV filter substance measured on the tape strips 24 h after removal and in the corresponding extracts (see Table 1) demonstrate that the absorbing species on the tapes are distributed homogeneously to the same extent as in solution, in agreement with the results of the Laser Scanning Microscopic experiments. This means that the tape strips removed can be used after a sufficient time period, 24 h in the investigated system, to determine the amount of UV filter substance. This may be important for screening investigations, as the direct use of the tape strips is less time consuming than an extraction followed by HPLC. However, a general application of this protocol requires a UV/VIS absorption band and checking of each individual system (active substance, formulation, and tape) to ensure that sufficient diffusion into and inside the adhesive layer occurs.

4.3 *Spectral Changes of the Corneocyte Pseudoabsorption*

Referring to Fig. 3, it is obvious that the horny layer particles fixed to the tape strips have a pronounced edge structure immediately after removal, which disappears by 1 h. This surface phenomenon influences the scattering, reflection, and diffraction behavior responsible for the measured pseudoabsorption of the corneocytes.[7]

A simple explanation would be that the emulsion previously applied is concentrated on the surface of the corneocyte aggregates under *in vivo* conditions. This effect is transferred to the tape strips and can be detected immediately after removal. Diffusion processes then came into action, resulting in an increased homogeneous distribution of the emulsion and in a disappearance of the edge structures. This explains the changes in corneocyte-determined absorbance in the visible range, observed immediately after removal (see Fig. 1). As a result of matching the refractive index, the edge structure disappears and the corneocyte-determined absorbance remains constant.

To avoid disturbances arising from this effect, the spectroscopic determination of the amount of corneocytes on the removed tape strips does not start earlier than 30 min after the removal of the tape strips.[11]

Table 2 Concentration of 4-methylbenzylidene camphor on the removed tapes, determined by UV spectroscopy and HPLC after extraction.

Tape number	Concentration determined by UV spectroscopy [μg/ml]	Concentration determined by HPLC [μg/ml]	Difference between UV and HPLC*
	Concentration range >1 μg/ml		
1	75.7	77.3	−1.6 (−2%)
2	7.4	6.8	+0.6 (+9%)
3	3.1	3.0	+0.1 (+3%)
4	1.7	1.6	+0.1 (+6%)
	Sum amount: 87.9 μg/ml	Sum amount: 87.5 μg/ml	
	Mean value of deviation		−0.2 (4%)
	Concentration range <1 μg/ml		
5	1.0	0.9	+0.1 (+11%)
6	0.7	0.6	+0.1 (+17%)
7	0.6	0.5	+0.1 (+20%)
8	0.2	0.3	−0.1 (−33%)
9	0.3	0.4	−0.1 (−25%)
10	0.2	0.2	±0 (±0%)
11	0.1	0.2	−0.1 (−50%)
12	0.2	0.2	±0 (±0%)
13	0.2	0.2	±0 (±0%)
14	0.2	0.1	+0.1 (+50%)
	Sum amount: 3.7 μg/ml	Sum amount: 3.6 μg/ml	
	Mean value of deviation		0.10 (−10%)

*Percentage in brackets, correlated to the higher value

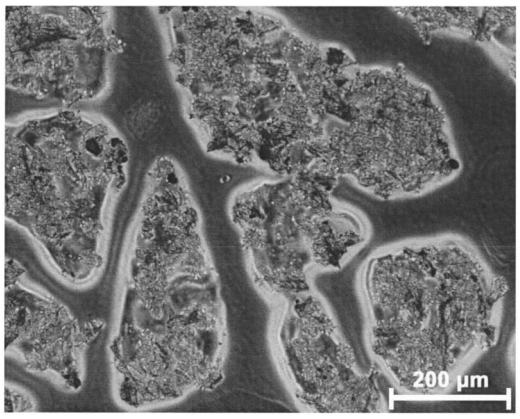
4.4 *Comparison of Spectroscopic Data and HPLC Measurements*

The comparison of the data obtained with spectroscopic measurements and HPLC exhibits that the differences are distributed statistically. Thus, a systematic error can be excluded.

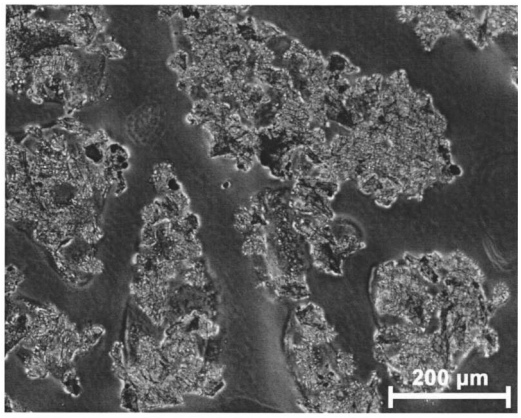
Considering the mean values of the deviation at the higher concentration range (see Table 2) and the correlation coefficients ($R^2 \geqslant 0.99$), it is obvious that the spectroscopic data are well suited to measure the concentration of the UV filter substance quantitatively at a concentration range of >1 μg/ml. The relative error increases with the decreasing absolute concentration of the UV filter substance. Therefore, the exact measurements in this concentration range require HPLC measurements.

4.5 *Penetration Behavior of UV Filter Substance*

The penetration profile typically obtained for 4-methylbenzylidene camphor is given to illustrate the information available from the described method (see Fig. 4). In agreement with the demand on an effective sunscreen, the filter substance is positioned in the uppermost part of the horny layer. This behavior is in agreement with the results of other studies also detecting the UV filter substances in the uppermost part of the stratum corneum.[7,9,16,17]



(a)



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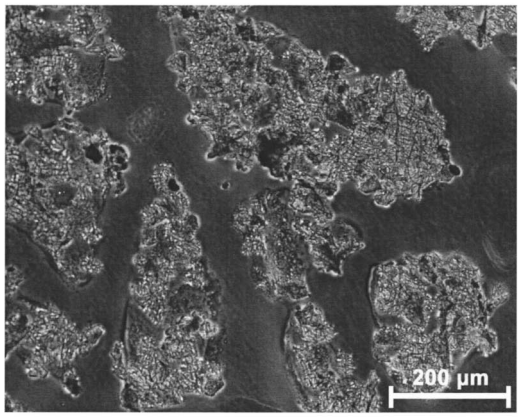

(c)

Fig. 3 Time-dependent changes in the pattern of the corneocyte aggregates after tape stripping determined by light microscopy: (a) Measured immediately, (b) measured 1 h after removal from the skin, and (c) measured 24 h after removal from the skin.

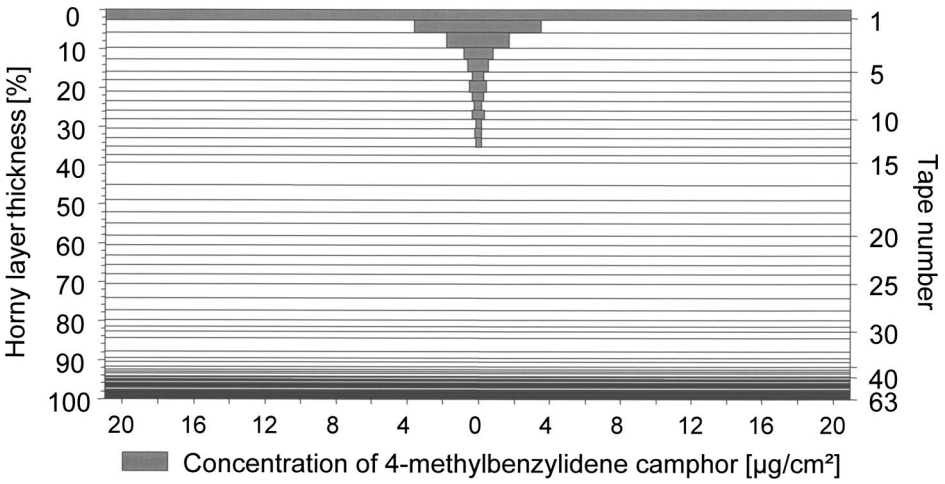

Fig. 4 Local distribution of the UVB filter substance 4-methylbenzylidene camphor, 1 h after application of an o/w emulsion.

5 Conclusions

The measured distribution of a UV filter substance on the tape strips removed is found to be nonhomogeneous. This reflects the nonhomogeneity of the skin surface as well as the characteristic changes arising as a result of tape stripping. UV/VIS spectroscopic measurements determine not only the amount of SC particles fixed to the individual tape strips, but at the same time the concentration of the UV filter substance in the area from which the SC strips are removed. This information can be used to determine penetration profiles of topically applied substances.

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