Analysis of Human and Porcine Skin in vivo/ex vivo for Penetration of Selected Oils by Confocal Raman Microscopy

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Key Words
Vegetable oil · Petrolatum · Lipid · Keratin · Deconvolution · Swelling effect · Stratum corneum

Abstract
Background: The subject of oil penetration into the skin is controversially discussed in the scientific literature. Methods: Confocal Raman microscopy was used for analyzing oil penetration into the skin. The following methods were applied in the study: methods based on tracking specific peaks (method 1), the nonrestricted multiple least square fit (method 2), analyzing the lipid-to-keratin peak ratio using the perpendicular drop-down cutoff procedure (method 3), and the Gaussian function-based deconvolution procedure (method 4). Results: The results obtained using methods 1, 2 and 4 show that the investigated oils do not penetrate deeper than 11 μm into human and porcine skin. Petrolatum has a prominent swelling effect on the stratum corneum (32% in vivo, 28% ex vivo), while the other oils exhibit no significant swelling effect. By using method 3, the penetration profile of oils, and especially of petrolatum, into the skin was interpreted incorrectly for various reasons that are addressed herein below. Conclusion: Predominantly remaining in the uppermost corneocyte layers of the stratum corneum, topically applied oils do not reach the viable cells of the stratum spinosum. To exclude any possible mistakes when using the lipid-keratin Raman peak (2,820–3,030 cm\textsuperscript{-1}), the penetration analysis should be performed using the Gaussian function-based deconvolution procedure.

Introduction
The barrier function of the skin is mostly provided by the lipids of the stratum corneum [1–4]. External stressors are able to influence the physiology of the human skin’s stratum corneum. Among them are mechanical influence [5], solar irradiation [6–8], environmental pollutants [9, 10], and topically applied formulations [11, 12]. Oils have been topically used as the basis of many skin care products and ointments for a long time in the cosmetic industry and medicine [13–16]. Even though there have been some efforts to understand how the oils interact with the skin tissue, some aspects of oil penetration into the intact human skin are still controversially discussed [17–20].
Some authors used vegetable oils or oil-in-water emulsifiers as skin permeation enhancers and showed that some oil components (e.g. oleic acid and glyceryl trioleate) disrupt the stratum corneum lipids, thus enhancing the penetration [21–24]. Leite-Silva et al. [22] reported that the formulation containing paraffin oil acts as a penetration enhancer for zinc oxide nanoparticles which, along with titanium dioxide nanoparticles, normally do not penetrate through the barrier of the stratum corneum [25–28].

The generally accepted opinion that most oils are non-penetrating substances is, therefore, plausible [19]. However, some oils, such as petrolatum and olive oils, seem to be capable of penetrating through the stratum corneum and are considered penetration enhancers [21, 23]. The exact penetration depth of the oils into the skin and their influence on the ingredients of the stratum corneum, such as water, lipids and keratin, have not yet been definitively explored.

According to the widely accepted opinion, petrolatum is incapable of passing through the stratum corneum and its strong occlusion effect is due to the fact that it covers the skin surface, thus hampering the evaporation of water [29, 30]. However, using confocal Raman microscopy (CRM) in vivo, Stamatas et al. [20] reported that at a maximum penetration depth of <10 μm, neither vegetable oils nor paraffin oil penetrate into the stratum corneum, whereas petrolatum exhibits an enhanced penetration capability extending to 30 μm in depth, thus exceeding the thickness of the stratum corneum. Patzelt et al. [19] demonstrated in another in vivo study, using laser scanning microscopy, that vegetable oils, paraffin and petrolatum remain in the upper layers of the stratum corneum and cannot reach the deeper layers of the viable epidermis.

The oils are Raman-active substances [20, 31]. Therefore, the study reported herein aimed at investigating the penetration profiles of different oils in the human and porcine skin (in vivo and ex vivo) and evaluating their influences on the stratum corneum by means of CRM. The porcine skin was selected for the measurements due to its high similarity with human skin and appropriate-ness for ex vivo measurements [32, 33]. CRM was chosen due its sensitivity and measurement advantages in comparison to alternative methods [34, 35].

Based on CRM, the results obtained using the following four different methods for Raman spectra analysis will be compared: tracking specific peak in the fingerprint region (400–2,000 cm⁻¹; method 1), nonrestricted multiple least square fit (NMF) method in the fingerprint region (method 2) and the methods based on an analysis of the lipid-to-keratin ratio in the high wavenumber region (2,000–4,000 cm⁻¹) using the perpendicular drop-down cutoff procedure (method 3) as well as the Gaussian function-based deconvolution procedure (method 4). The advantages, shortcomings and limitations of these methods will be discussed.

Recently, the subject of oil penetration into the human skin analyzed in vivo using CRM was published in a short paper by Choe et al. [36]. In this paper, the results are extended to ex vivo measurements on porcine skin and are presented in more detail.

### Materials and Methods

**Applied Substances**

The following eight refined high-quality pharmaceutical oils were used in the ex vivo investigations performed on porcine skin:

- jojoba oil (cold-pressed; Henry Lamotte Oils GmbH, Bremen, Germany);
- soybean oil (Textron Technica SL, Barcelona, Spain);
- avocado oil (Cropure Avocado; Croda Chemicals Ltd., North Humberside, UK);
- almond oil (Afruse SL, Tarragona, Spain);
- olive oil (Henry Lamotte Oils GmbH, Bremen, Germany);
- white mineral oil (Crompton Cooperation, Amsterdam, The Netherlands);
- paraffin oil (Marcol 82tm; Esso SAF, Rueil-Malmaison, France);
- petrolatum (Fagron GmbH & Co. KG, Barsbüttel, Germany).

The oils showed specific fatty acid profiles with some fatty acids below 1%.

For in vivo measurements on human skin, the penetration ability of four oils (jojoba oil, almond oil, paraffin oil, and petrolatum) was investigated.

All oils under investigations can be subdivided into two groups:

- mineral oils (petrolatum, paraffin oil, white mineral oil) and vegetable oils (almond oil, soybean oil, olive oil, jojoba oil, avocado oil).

**Volunteers**

The investigations were performed on the inner forearm of 6 healthy volunteers (3 female, 3 male) aged between 23 and 62 years (average 37 years). The volunteers were instructed not to utilize any skin care products on the forearms for at least 72 h and not to take a bath or shower for at least 4 h before the beginning of the experiments. After an acclimation time of 20 min, 4 skin areas (size 2 × 2 cm) were marked on the inner forearms (2 areas on each arm) using a rubber barrier in order to avoid lateral spreading of the topically applied oils. Then four oils were topically homogeneously applied at an amount of 2 mg/cm² on the marked areas. After 60 min of passive penetration at normal conditions (room temperature 21°C, relative humidity 35% on average), the remaining oil was taken away by filter paper and CRM was carried out at more than 10 measuring points per one skin area.

The volunteers gave informed written consent to the experiments. Approval for the measurements was obtained from the Ethics Committee of Charité – Universitätsmedizin Berlin. All procedures were in accordance with the Helsinki Declaration.

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Porcine Ear Skin Samples

The porcine ears were obtained on the day of slaughter from a local butcher. The bristles were carefully cut out without any influence on the stratum corneum. To exclude any possible influence of the storage time on the skin samples, all measurements were performed only on fresh porcine ears whose storage time in the refrigerator did not exceed 1 day.

For ex vivo measurements, the oils (2 mg/cm²) were homogeneously distributed on the marked area (size 2 × 2 cm) of the porcine ear skin and left there for passive penetration for 60 min at normal conditions (room temperature 21 °C, relative humidity 35% on average). Then the skin surface was wiped off with the filter paper and the skin sample excised for CRM measurements. At least 10 measuring points per one skin area were analyzed.

Confocal Raman Microscopy

The Raman microscopic measurements were performed using a skin composition analyzer for in vivo/ex vivo skin measurements.

Fig. 1. Raman spectra of vegetable oils (almond oil, soybean oil, olive oil, jojoba oil, and avocado oil; a) and mineral oils (petrolatum, paraffin oil and white mineral oil; b) in comparison to intact human stratum corneum Raman spectrum measured in vivo at a depth of 4 μm. Raman intensities of all oils are divided by 3.
Two different lasers were used to analyze the samples in the fingerprint region (400–2,000 cm⁻¹; 785 nm, 25 mW on the skin) and in the high wavenumber region (2,000–4,000 cm⁻¹; 671 nm, 19 mW on the skin). The Raman spectra were recorded from the skin surface down to a depth of 40 μm at increments of 2 μm. The exposure time for one spectrum was 5 s for the fingerprint region and 1 s for the high wavenumber region. The skin areas without furrows and hairs were chosen to exclude their influence on the penetration measurements [37]. For each skin area, the spectra of both the fingerprint and high wavenumber regions were obtained at the same position. The spatial axial and spectral resolutions were 5 μm and 2 cm⁻¹, respectively. The Raman nonactive immersion oil, the measurement window and the skin all have the same refractive index of around 1.45 and therefore all depths measured using this method are real geometrical depths. The utilized CRM system has been described in detail in the literature [38, 39] and used for the determination of penetration profiles of topically applied substances into the skin [40, 41].

**Raman Spectra Analysis**

Tracking Specific Peak in the Fingerprint Region (Method 1)

If the oils exhibit Raman peaks that are not superimposed by the Raman spectrum of the intact skin, these peaks will serve as ‘marker’ peaks to indicate the oils in the skin. Figure 1 shows the Raman spectra of the human skin stratum corneum and investigated oils representing two oil groups – vegetable oils (almond oil, soybean oil, olive oil, jojoba oil, and avocado oil; fig. 1a) and mineral oils (petrolatum, paraffin oil and white mineral oil; fig. 1b) – in the fingerprint region. As can be seen, the vegetable oils have a specific Raman peak at 1,740 cm⁻¹, which does not exist in the intact skin and corresponds to the carbonyl (C=O) double-bond vibration mode (fig. 1a). This peak can be used as a marker peak for detecting the vegetable oils in the skin. In the case of mineral oils (petrolatum, paraffin oil and white mineral oil), there is no marker peak and nearly all Raman peaks overlap with the spectrum of the intact skin (fig. 1b). This method has been used for the determination of chemotherapeutics in the skin by tracking their specific peaks [42].

The NMF Method in the Fingerprint Region (Method 2)

In the fingerprint region, the Raman spectrum can be analyzed by the ‘Skin Tools 2.0’ software developed by River Diagnostics. The ‘Skin Tools 2.0’ adapts the NMF method mathematically for analyzing the fingerprint spectra of the skin. The NMF method approximates the spectra by the set of the known spectra of skin components such as the spectra of keratin, ceramide, cholesterol, urea, natural moisturizing factor, etc. This method calculates the fitting coefficients to minimize the residual errors and these coefficients give information on the semiquantitative concentration of the corresponding components. In the case of oil application, the Raman spectrum of the respective oil is taken into consideration by the software and the obtained coefficient is interpreted as oil concentration in the skin. The depth-dependent signal attenuation is compensated by the normalization on keratin concentration, which is homogeneously distributed in the stratum corneum. The NMF method has been previously described in detail elsewhere [41] and used for the determination of penetration profiles of topically applied substances into the skin [43].

Analysis of the Lipid-to-Keratin Ratio in the High Wavenumber Region Using Perpendicular Drop-Down Cutoff Procedure (Method 3)

Stamatas et al. [20] proposed this method, which calculates the lipid uptake in the skin. The main idea of this method is that the peak area ratio of lipids (2,820–2,900 cm⁻¹) to keratin (2,910–2,965 cm⁻¹) is altered by the penetration of oils. The authors calculated the areas under the curve in the range between 2,820 and 2,900 cm⁻¹ for lipids and between 2,910 and 2,965 cm⁻¹ for keratin using the perpendicular drop-down cutoff procedure and determined the corresponding lipid-to-keratin ratio for both intact and oil-treated skin. Subsequently, the subtraction of these two ratios was considered as the lipid uptake caused by oil penetration. Figure 2a shows the design of this method.

**Gaussian Function-Based Deconvolution Procedure of the Lipid-Keratin Peak in the High Wavenumber Region** (Method 4)

The main idea of this method is to separate the lipid-keratin peak (2,820–3,030 cm⁻¹) of the skin into 4 Gaussian functions by means of deconvolution (maxima at 2,850, 2,880, 2,935, and 2,980 cm⁻¹) and to evaluate each peak change in the oil-treated skin (fig. 2a). Next, Raman spectra of the oils are separated into 5 Gaussian peaks (e.g. for jojoba oil, maxima at 2,852, 2,880, 2,907, 2,935, and 3,009 cm⁻¹; fig. 2b). In order to gain reproducible results, the constrained nonlinear optimization method is used in the deconvolution process.

Supposing that the chemical composition of the oils remains unchanged during their penetration into the skin, the spectrum of oil-treated skin should represent the superposition of both the intact skin spectrum and the oil spectrum. Finally, the least square fitting method is applied to obtain the oil coefficients, which can be considered to be proportional to the concentration of the oil in the skin. This method has been previously described in detail by our group [44].

**Stratum Corneum Thickness Measurements**

The water in the stratum corneum originates from the viable cells of the stratum spinosum and evaporates on the surface of the skin. According to Fick’s first law, the water concentration increases linearly from the surface to deeper sites of the skin, reaching the maxima at the stratum granulosum. The boundary between the stratum corneum and stratum granulosum is determined to quantitatively assess the stratum corneum thickness. Based on this principle, many researchers determined the stratum corneum thickness by using CRM [45–48]. The thickness of the stratum corneum was calculated based on the water distribution profile measured with CRM in the range 3,350–3,550 cm⁻¹. The boundary points of the stratum corneum are set by the respective researchers according to different criteria. For example, according to Crowther et al. [45] the boundary point is at a water gradient value of 0.5, whereas Egawa and Tagami [46] set the boundary point where this value is almost 0. In this study we set the boundary of the stratum corneum at a water gradient value equal to 0.5.

Subsequent to the topical application of the oil, the skin surface is covered homogeneously and water is prevented from evaporating on the skin [19]. If the water concentration increases in the stratum corneum, the corneocytes thicken by absorbing water, thus increasing the stratum corneum thickness. This response is called a swelling effect [20, 45, 48]. The swelling effect can be measured by calculating the ratio of the stratum corneum thickness prior to and subsequent to oil treatment.
Results

In vivo and ex vivo Oil Penetration Measurements by Tracking Raman Peak of 1,740 cm⁻¹ in the Fingerprint Region (Method 1)

Figure 1 shows the Raman spectra of two oil groups – vegetable oils (almond oil, soybean oil, olive oil, jojoba oil, and avocado oil; fig. 1a) and mineral oils (petrolatum, paraffin oil, and white mineral oil; fig. 1b). The Raman spectra within each oil group vary only minimally. As shown in figure 1a, vegetable oils have a clear Raman peak at 1,740 cm⁻¹, which is not observed in the spectrum of...
the intact skin. Here the ratio of the carbonyl (1,740 cm\(^{-1}\))/keratin (1,655 cm\(^{-1}\)) peaks in the vegetable oil-treated skin spectrum represent the normalized concentration of vegetable oils, corrected for depth-dependent signal attenuation. For around 30% of the samples, the peak at 1,740 cm\(^{-1}\) did not even appear at all. The main reason seems to be that if the concentration of oil is low, this peak signal is overlapped by the intact skin Raman signal. The advantage of this method is that the researcher can directly see the presence of vegetable oil at high concentrations in the skin tissue. The drawback of this method is that the Raman spectra of mineral oils (e.g. petrolatum, paraffin, and white mineral oil) have no marker peaks, so that it is impossible to apply this method and very difficult to quantify the results. Table 1 summarizes the results obtained using this method and figure 3a shows the in vivo penetration profiles evaluated for vegetable oils.

### Table 1. Oil penetration depths into human skin in vivo and porcine skin ex vivo determined by the 4 CRM-based methods and the respective values of posttreated swelling of the stratum corneum

<table>
<thead>
<tr>
<th>Oil</th>
<th>Method 1, μm</th>
<th>Method 2, μm</th>
<th>Method 3, μm</th>
<th>Method 4, μm</th>
<th>Swelling of stratum corneum, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>5.1±2.4</td>
<td>6.7±0.75</td>
<td>8.8±0.75</td>
<td>6.8±0.4</td>
<td>9.2±7.9</td>
</tr>
<tr>
<td>Ex vivo</td>
<td>5.7±1.3</td>
<td>8.2±0.4</td>
<td>10.0±1.0</td>
<td>9.5±0.5</td>
<td>2.8±6.8</td>
</tr>
<tr>
<td>Avocado oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td></td>
</tr>
<tr>
<td>Ex vivo</td>
<td>8.3±2.2</td>
<td>7.6±0.7</td>
<td>7.0±1.0</td>
<td>9.3±1.2</td>
<td>19.1±8.3</td>
</tr>
<tr>
<td>Jojoba oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>4.9±1.4</td>
<td>7.3±1.6</td>
<td>10.7±2.7</td>
<td>8.0±3.1</td>
<td>3.7±6.1</td>
</tr>
<tr>
<td>Ex vivo</td>
<td>6.0±1.3</td>
<td>6.6±0.4</td>
<td>14.5±1.5</td>
<td>11.0±2.0</td>
<td>0.0±3.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td></td>
</tr>
<tr>
<td>Ex vivo</td>
<td>8.0±2.0</td>
<td>7.7±1.7</td>
<td>10.1±2.6</td>
<td>10.0±2.1</td>
<td>7.1±8.1</td>
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<tr>
<td>White mineral oil</td>
<td></td>
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</tr>
<tr>
<td>In vivo</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td></td>
</tr>
<tr>
<td>Ex vivo</td>
<td>6.9±1.5</td>
<td>14.0±4.0</td>
<td>10.5±1.5</td>
<td>4.6±1.0</td>
<td></td>
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<tr>
<td>Paraffin oil</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>In vivo</td>
<td>n.a.</td>
<td>6.8±0.9</td>
<td>15.3±5.3</td>
<td>6.5±1.6</td>
<td>10.6±7.3</td>
</tr>
<tr>
<td>Ex vivo</td>
<td>n.a.</td>
<td>6.4±0.6</td>
<td>12.7±3.4</td>
<td>10.0±2.0</td>
<td>11.7±10.0</td>
</tr>
<tr>
<td>Petrolatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>n.a.</td>
<td>7.0±0.8</td>
<td>20.7±4.9</td>
<td>6.3±1.2</td>
<td>31.5±5.2</td>
</tr>
<tr>
<td>Ex vivo</td>
<td>n.a.</td>
<td>7.8±0.9</td>
<td>21.5±5.9</td>
<td>9.4±2.0</td>
<td>27.9±13.1</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td></td>
</tr>
<tr>
<td>Ex vivo</td>
<td>7.4±1.8</td>
<td>5.6±1.9</td>
<td>9.0±2.0</td>
<td>8.6±2.4</td>
<td>3.8±6.7</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard deviations. Method 1: tracking specific peak in the fingerprint region. Method 2: NMF in the fingerprint region. Method 3: analysis of the lipid-to-keratin ratio in the high wavenumber region using perpendicular drop-down cutoff procedure. Method 4: analysis of the lipid-to-keratin ratio in the high wavenumber region using deconvolution procedure. n.a. = Not applicable; n.m. = not measured.

In vivo and ex vivo Oil Concentration Measurements Using the NMF Method in the Fingerprint Region (Method 2)

As can be seen from figure 1, the pretreatment of the skin surface with oils can influence the shape of the Raman peaks of the intact skin in the whole fingerprint range. The NMF method provides the advantage that the penetration profiles of the oils in the skin can be analyzed in the whole fingerprint range. As shown in figure 3b, the concentration of oils decreases exponentially from the surface into the skin. The maximum concentrations of the oils are observed on the skin surface. Then the oil concentration is drastically decreased, reaching its minimum at depths of 6–7 μm in vivo and 6–8 μm ex vivo (table 1). These results correlate with previous results observed by laser scanning microscopy, demonstrating that the oils predominantly remained in the upper parts of the stra-
The penetration depth of the oils, excepting petrolatum, coincided with that in previous publications [19, 20].

**In vivo and ex vivo Oil Penetration Measurements by Analysis of the Lipid-to-Keratin Ratio in High Wavenumber Region Using Perpendicular Drop-Down Cutoff Procedure (Method 3)**

Figure 3c shows the oil penetration profiles measured in vivo on human skin and determined by this method. Compared to the above-mentioned methods, all oils exhibit an extended penetration depth (table 1). Particularly for the petrolatum-treated skin, the lipid uptake is higher than for any other oils and the petrolatum penetration depth is determined, on average, to be 20.7 ± 4.9 μm in vivo and 21.5 ± 5.9 μm ex vivo. The tendency towards an enhanced penetration capability of petrolatum coincides well with the results of previous work [20], but the obtained penetration depth value is smaller (20.7 vs. 30.0 μm).
In vivo and ex vivo Oil Penetration Measurements by Gaussian Function-Based Deconvolution Method in the High Wavenumber Region (Method 4)

Figure 3d shows the semiquantitative concentration profiles of the oils measured in vivo on human skin and determined by the Gaussian function-based deconvolution method. The determined penetration depths are around 6–11 μm and comparable to the results obtained by the NMF method. The obtained results are summarized in Table 1.

Swelling Effect and Stratum Corneum Thickness

By using CRM, the stratum corneum thickness of healthy human forearm skin in vivo and porcine ear skin

![Graph showing oil penetration profiles](image-url)
ex vivo was determined to be 19.2 ± 2.5 and 18.2 ± 1.1 μm, respectively. As can be seen in figure 4, in the case of petrolatum, the boundary between the stratum corneum and the stratum granulosum is located in deeper sites of the skin (18 vs. 26 μm). Consequently, the stratum corneum is swelled by absorbing water after petrolatum application. The other investigated oils showed no significant differences in stratum corneum thickness between the intact and the oil-treated skin. The obtained values are summarized in the table 1. The petrolatum has an apparent swelling effect on the skin: the stratum corneum thickness increases on average by 32% in vivo and on 28% ex vivo. The obtained results are in accordance with results obtained in previous works [19, 20, 29].

Discussion

The results obtained using four different methods answer the question of how deep the oils can penetrate into the skin. The penetration depth of all oils determined by tracking specific peak, NMF and the Gaussian function-based deconvolution method ranges between 5 and 11 μm, on average, for both in vivo and ex vivo measurements. The obtained variations are explained by the different sensitivities of the applied methods, which is lowest for method 1 and highest for method 4. Taking into consideration that the stratum corneum thickness of healthy human forearm skin (in vivo) and porcine ear skin (ex vivo) was determined, on average, to be 19.2 and 18.2 μm, respectively, topically applied oils do not reach the viable cells of the epidermis, saturating only the superficial layers of the stratum corneum. There is no significant difference between the in vivo and ex vivo results in terms of the penetration capability of the oils. The results obtained for the investigated oils and especially for petrolatum using analysis of the lipid-to-keratin ratio by the perpendicular drop-down cutoff procedure (method 3) show a significantly higher penetration depth in comparison to the other methods.

Petrolatum, due to its high occlusion ability, gives rise to an increase of water in the stratum corneum and, as a result, causes a swelling effect, increasing the thickness of the stratum corneum by 32% on average. The topical application of the other oils, excepting petrolatum, shows no significant swelling effect.

The results obtained in the high wavenumber region for petrolatum-treated skin using analysis of the lipid-to-keratin ratio by the perpendicular drop-down cutoff procedure (method 3) and by other methods are extremely different. This difference can be explained as follows.

Showing the maximum Raman sensitivity of any of the other oils tested, petrolatum is strongly excited in the wavelength range between 2,800 and 3,000 cm⁻¹ [20]. Fig.
Figure 5 shows as an example the high wavenumber Raman spectra of petrolatum and avocado oil. As can be seen, both oils have their contribution not only in the lipid range (2,820–2,900 cm\(^{-1}\)) but also in the keratin range (2,910–2,965 cm\(^{-1}\)). The contribution of petrolatum is superior in comparison to other oils due to the high Raman sensitivity. Method 3 [20] uses the perpendicular drop-down cutoff procedure (fig. 2a) to determine the lipid-to-keratin ratio. However, the existence of petrolatum in the keratin range (2,910–2,965 cm\(^{-1}\)) of the Raman spectra may falsify the results. Also, the skin surface may be incorrectly determined as it is based on the analysis of the keratin peak gradient in the high wavenumber region (2,910–2,965 cm\(^{-1}\)). Our calculations, which compare the skin surface positions determined in both the fingerprint and the high wavenumber regions, show that after the application of 2 mg/cm\(^2\) petrolatum on the skin, the skin surface position is erroneously determined at a value of 4.5 ± 2.0 μm.

During the petrolatum-induced swelling effect corneocytes absorb additional water, which gives rise to the relative reduction of keratin concentration. The relative reduction of keratin results in an increase of the lipid-to-keratin ratio, which can be erroneously interpreted as the presence of petrolatum in the stratum corneum. The final results might also be affected by changes of lipid structure. The changes of the lipid structure of the stratum corneum subsequent to interaction with water have already been described by different work groups [29, 49, 50]. Such lipid changes could also occur when the petrolatum is topically applied to the skin as this induces a swelling effect in the stratum corneum. The increase of the peak intensity at 2,850 cm\(^{-1}\) caused by the lipid changes could be misinterpreted as the presence of petrolatum in the specific skin layer. All these limitations could also be related to other oils, although to a lesser extent, as their Raman sensitivities are low and they lack the strong swelling effect in comparison to petrolatum.

Thus, method 3 lends itself suitable for determining the oil penetration only restrictively. By using the Gaussian function-based deconvolution procedure of lipid-keratin peak (method 4) the above-mentioned limitations can be eliminated. Moreover, the results obtained using method 4 correlate with the results obtained using methods 1 and 2, as well as other techniques [19].

Although olive oil and other vegetable oils are known to act as penetration enhancers [21, 51], this action could so far only be proved when the oil was mixed with water or other substances. In this study it has been demonstrated that pure oils do not penetrate through the stratum corneum either in vivo or ex vivo. Their penetration ability rapidly decreases with depth so that they saturate only the superficial corneocyte layers.

Optical clearing is widely used in dermatology for visualization of the deep-located skin areas at higher qual-
ity [52–54]. Oils topically applied on the skin can potentially induce the optical clearing effect by reducing the diffuse reflection [55] and scattering [56] of the superficial layers of the stratum corneum. As a result, the detection limit in depth is enhanced [57]. The oil-induced alteration of the optical properties of the stratum corneum has no influence on the obtained results for oil penetration due to the normalization on keratin.

A comparison of the applied methods shows that the NMF method in the fingerprint region (method 2) and the method based on analysis of the lipid-to-keratin ratio in the high wavenumber region using the deconvolution procedure (method 4) are better suited for analysis of the penetration of oils into the skin both in vivo and ex vivo.

**Conclusion**

The maximum penetration depth and subsequent penetration profiles of eight different oils applied topically onto human/porcine skin were evaluated analyzing the Raman spectra obtained in vivo/ex vivo using CRM. Four different methods were applied, i.e. tracking specific peak in the fingerprint region (method 1), NMF method in the fingerprint region (method 2) and the methods which are based on analysis of the ratio of the lipid-to-keratin in high wavenumber region using the perpendicular drop-down cutoff procedure (method 3) as well as the Gaussian function-based deconvolution procedure (method 4). It was shown using methods 1, 2 and 4 that none of the investigated oils penetrates deeper than 11 μm into the skin, saturating only the superficial layers of the stratum corneum. Consequently, all the investigated oils did not reach the viable cells of the stratum spinosum. The maximum penetration depths of oils obtained in vivo on human skin and ex vivo on porcine skin are highly similar. The application of method 3 was shown to have strong limitations for investigating oil penetration into the skin. Method 1 is suitable for the investigation of vegetable oils only. Taking this into consideration, it could be concluded that methods 2 and 4 are most applicable for analyzing oil penetration into the skin. Analysis of the stratum corneum thickness measured in the high wavenumber region by water distribution shows that the topical application of oils gives rise to a swelling of the stratum corneum. This effect is highly pronounced for petrolatum in comparison to other oils.

**References**


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**Disclosure Statement**

The authors have no conflicts of interest to declare.
Oil Penetration into the Skin


