COMMENT

Response to comment by Puppels et al. on "A modification for the calculation of water depth profiles in oil-treated skin by in vivo Raman microscopy"

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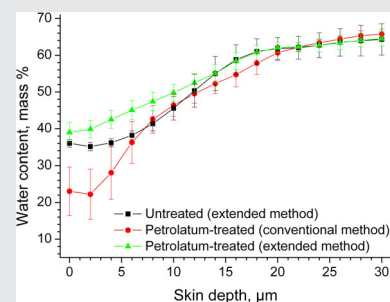
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Abstract

The presence of penetrated oils in the stratum corneum (SC), oil-induced occlusion of the SC and formation of occluding homogeneous film on the skin surface are discussed in relation to their influence on results of water profile calculations using conventional and newly proposed extended methods. It is shown that the conventional method does not determine the water profiles in treated skin correctly due to the superposition of Raman bands of SC's proteins and penetrated and remnant oils.

KEYWORDS

cosmetic oils, normalization, petrolatum, Raman microscopy, skin hydration, topical application

We have read with interest the comment on our recently published article [1], where we presented a new extended method for the calculation of water concentration profiles in the stratum corneum (SC) of oil-treated skin using intensity normalization and correct determination of the skin surface position based on the $1650\ cm^{-1}$ Amide I Raman band. Here, we would like to respond to the critical points which Puppels et al addressed in their comment.

The main critical point states that the newly proposed extended method considers only intrinsic skin constituents and ignores the presence of the extrinsic material, such as oils, that penetrate into the skin. Using the conventional method proposed by Caspers et al [2], to which we proposed a modification in our article [1], Puppels et al. explain the reduced amount of water in the SC of oil-treated skin (figure 3A, 3C in Ref. [1]) by the presence of oils and oil-induced SC swelling.

In response to this comment, it should be considered that petrolatum, which is known for its low penetration ability into the SC ($\approx 7\ \mu m$ or 35% SC depth), shows an obvious occlusion effect on the skin and a corresponding swelling of the SC ($\approx 32\%$) [3]. Although petrolatum was wiped off the skin after the treatment, it still formed a continuous film on the surface, which significantly decreased the trans-epidermal water loss, resulting in visual observation of water drop formation, that is water accumulation in the superficial SC as determined by laser scanning

microscopy [4]. However, these findings were not verified when analyzing water profiles of petrolatum-treated SC, if calculated by the conventional method (figure 3A in [1]): that is a 1.5-fold reduction of the superficial water concentration (≈13 mass %) was observed. A very similar water profile with lower water concentration in the entire SC for petrolatum-treated skin calculated using the conventional method was published elsewhere [5].

Thus, in the analysis using the conventional method, the main questions arise—despite the decrease of transepidermal water loss and subsequent water accumulation in the SC (swelling of the SC), why is the water concentration decreased throughout the SC in the petrolatum-treated skin and where is the water excess due to the skin occlusion with petrolatum? Based on the current explanations by Puppels et al, the water excess is distributed over a larger volume of the swelled SC and is additionally squeezed out from superficial SC depths due to the presence of oils.

We agree that the newly developed extended method does not take the amount of penetrated oils into the SC into account. However, let us first estimate how much oil penetrates into intact SC. According to Mack Correa et al [6], the amount of triglycerides (main substances of plant oils) penetrating the superficial SC is 6% to 8% of the mass of intercellular lipids, whose contribution to the dry mass of the SC is ≈15%. Thus, the amount of penetrated triglycerides is limited to ≈1%, which is much lower than the protein concentration in the SC (≈80%). Thus, the maximum amount of superficial extrinsic substances (oils) is approx. Two orders of magnitude lower than the concentration of intrinsic proteins (keratin), which correlates with recent results for the penetration of lipophilic retinol presented by Caspers et al [7] Considering this, the water substitution in the superficial SC depths (water content reduction ≈13 mass % obtained using the conventional method), which is expected to be comparable to the oil concentration (approximately 1%), can barely be a result of the penetrated oils. In support of this, it should be taken into consideration that water is mainly stored inside the corneocytes and the oil penetration stretches out the intercellular lipid lamellas, limiting the direct contact between oil and water. Thus, scenarios 1 and 2, proposed by Puppels et al in their comment regarding the oil-induced water squeezing from superficial SC depths and subsequent SC swelling, do not appear realistic. Moreover, swelling of the SC is always associated with an increase of water.

Swelling of the SC has also no influence on the obtained water decrease. For instance, 60 minutes of treatment with jojoba oil did not result in any significant SC swelling, and treatment with paraffin and almond oil resulted in a minor SC swelling (≈10%) [3]. However, a significantly decreased water concentration was observed in the superficial SC depths (figure 3C in [1]) calculated using the conventional method.

The thickness normalization of the SC to 100% is a unique possibility to compare untreated and treated SC, which did not influence the results. Although a swelling effect would not be recognizable in the thickness-normalized profiles, as correctly pointed out by Puppels et al, the SC thickness was determined independently in μm, which is shown in Figure 1. The water profiles are different and very similar to previously published (figure 3A,C in Ref. [1]).
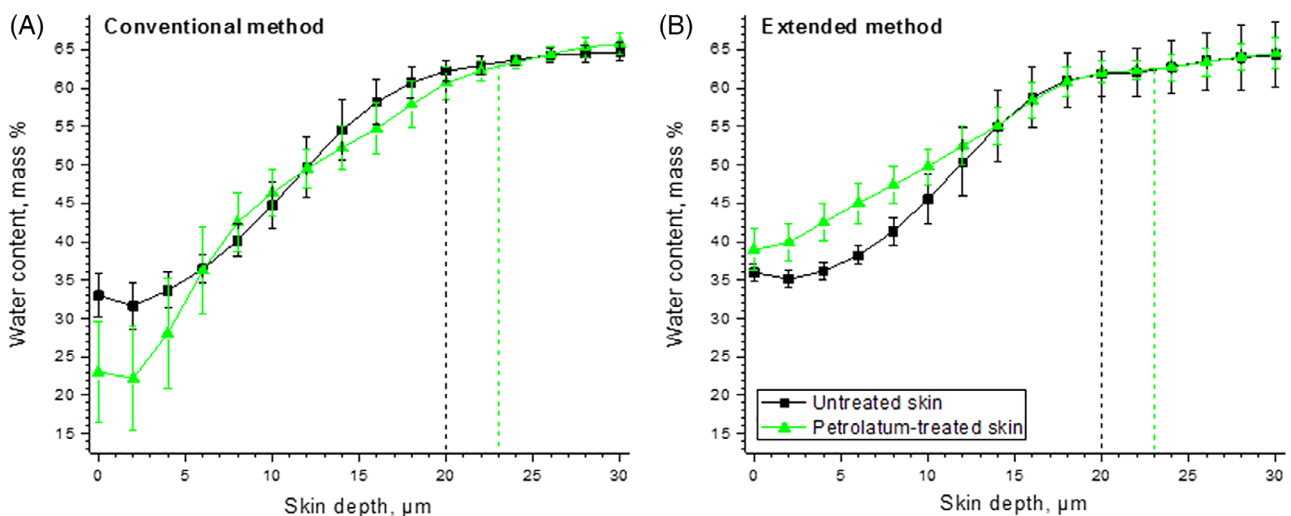

FIGURE 1 Depth profiles of water mass percentage in the SC shown in μm without normalization for its thickness for one exemplary volunteer determined by the conventional, A, and extended, B, methods. The dashed lines represent the SC thickness of untreated (20 μm) and petrolatum-treated (23 μm) skin. Petrolatum was applied in vivo on the volar forearm at 2 mg/cm² for 60 minutes

Thus, the observed reduction by ≈10–13 mass %, depending on the oil (figure 3A,C in Ref. [1]), of water concentration in the superficial SC depths in oil-treated skin is a result of the conventional calculation method, which does not take the superposition of oil- and keratin-based Raman bands into account [8]. This includes the normalization in the 2910 to 2965 cm^{-1} region, where the oil contribution is highly pronounced and cannot be ignored (figure 2B in Ref. [1]), additionally resulting in a miscalculation of the skin surface position due to the presence of remnant oil (figure 5 in Ref. [1]).

In opposition thereto, the newly proposed extended method has no such limitations, as it uses the intensity of the 1650 cm^{-1} Amide I Raman band for intensity normalization, to which the oil contribution is either absent or corrected. The results obtained using this method are well explained and do not contradict any published data. For instance, an increase of the water concentration was obtained in the intermediate SC depths in oil-treated skin, which is known to be related to the accumulation of water molecules inside the corneocytes during SC swelling [9, 10].

Moreover, the water mass percentage, calculated using either the conventional or the newly-developed extended method for untreated intact skin, correlate strongly ($R^2 = 0.96$). Practically, this comparison shows that both methods are well established for the determination of water depth profiles in the untreated skin. The SC thickness of untreated skin is similar, if determined using both methods.

The application of linear least squares regression [11] or multivariate curve resolution [12] could potentially solve a problem of intensity normalization for depth-dependent signal attenuation, but the problem of erroneous determination of the skin surface position, caused by the oil presence, should be additionally taken into consideration.

Although the newly developed extended method does not take the amount of penetrated oil into account, which is negligible in comparison to the amount of the main SC components (approximately 1:100), we conclude that the water profiles in the SC are correctly determined. This can be confirmed for all topically applied substances, not disrupting the SC barrier. We do not agree with the statement of Puppels et al saying that the conventional method would determine the water profiles more correctly. Moreover, due to the superposition of penetrated and remnant oil and protein-based Raman bands, the conventional method does not determine the water profiles in treated skin correctly.

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