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Published in:
Skin Research and Technology

Link to article, DOI:
10.1111/srt.12926

Publication date:
2021

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Water Vapor Permeation Through Topical Films on a Moisture Releasing Skin Model

*Short running title: Water Vapor Permeation Through Topical Films*

Fatemeh Keshavarzi\(^1\), Saeed Zajforoushan Moghaddam\(^1\), Malene Barré Pedersen\(^2\), Nina Østergaard Knudsen\(^2\), Shadi Jafarzadeh\(^2\) and Esben Thormann\(^1\)*

\(^1\) Department of Chemistry, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

\(^2\) Riemann A/S, Orkla, Krakasvej 8, 3400 Hillerød, Denmark.

*Corresponding author: esth@kemi.dtu.dk*
Acknowledgments

Innovation Fund Denmark is gratefully acknowledged for financial support through the Grand Solutions Project #615100007B. The authors also thank Niloufarsadat Mirmahdi Komjani, Bong Øllgaard, and Heidi Christensen from Riemann A/S for assistance in performing in vivo experiments.

Abstract

Background: Covering the skin by topical films affects the skin hydration and transepidermal water loss (TEWL). In vivo studies to investigate the water vapor permeation through topical films are complicated, expensive, ethically not preferred, and time and labor-consuming. The objective of this study is to introduce an in vitro and subject-independent alternative evaluation method to predict the breathability of topical formulations.

Methods: In this study, we developed an in vitro setup to simulate the TEWL values of human skin and investigated the breathability of five polymeric film formers used in topical formulations. Furthermore, a comparative in vivo TEWL study was performed on ten human volunteers with defined areas of skin covered with films of two selected polymers possessing different barrier properties.

Results: By employing the in vitro setup, a vinylpyrrolidone/acrylates/lauryl methacrylate copolymer was determined to form the most breathable film, whereas acrylates/octylacrylamide copolymer and shellac films showed the highest barrier properties. The in vivo TEWL study demonstrated the same relative barrier properties for the acrylates/octylacrylamide and polyurethane-64 films, despite a more complex driving force for water vapor permeation due to moisture accumulation on the covered skin surfaces.
Conclusion: We obtained a good correlation between the *in vitro* and *in vivo* results, demonstrating that our model can categorize different polymeric film formers based on their breathability when applied to human skin. This information can aid in selecting suitable film forming polymers for topical formulations with either breathable or occluding functionalities.

Keywords: skin model, gelatin, transepidermal water loss (TEWL), permeation, film forming polymers, breathability.

1. Introduction

Human skin is a vital part of the body and has multiple important functions, including protection against intruding substances, self-healing and temperature regulation, all of which warrant specific properties.\(^1\) The skin is one of the most multifaceted organs of the body, with a huge inherent variability in terms of its thickness, topography, mechanical properties, and barrier function. The skin has three distinguishable layers, i.e., the hypodermis, dermis, and epidermis. The stratum corneum is the outermost layer of the epidermis, made of corneocytes embedded in a lipid matrix with a thickness of 20-40 µm.\(^2\) This layer acts as a flexible barrier against infections, chemical substances, and mechanical shock as well as dehydration.\(^3\) The evaluation of skin hydration has received substantial interest, as the water content of the stratum corneum influences various physical characteristics of the skin, such as barrier function, mechanical properties, and visual appearance.\(^4\) The amount of condensed water that diffuses through the stratum corneum and evaporates to the outside, due to water activity differences on both sides, is known as transepidermal water loss (TEWL), which is used as a measure of the skin barrier function.\(^5\) The TEWL values of human skin can vary widely due to the local stratum corneum thickness, skin surface temperature, age, and gender or environmental changes, such as the temperature and humidity.\(^5-10\) There are no direct methods for measuring TEWL values, and all the available methods measure water
evaporation from the skin surface; hence, if TEWL is the only source of water on the skin surface, then the measured value represents the water vapor flux through the skin.\textsuperscript{11}

In addition to the previously mentioned variables influencing the TEWL values, covering the skin by clothing, adhesive patches or topical films will also affect its hydration and water transport. Many skin products contain polymeric film forming systems, which can impact TEWL by occluding the skin when they form a polymeric network on the skin surface. The development and evaluation of these systems have received substantial attention due to the benefits derived from their ability to form a uniform and robust topical film on the skin in various applications.\textsuperscript{12–15} Local topical drug delivery gives the possibility to apply the drug directly on the affected site, reaching high drug levels at the target while limiting systemic exposure, which occurs with oral administration. The development of drug delivery systems with slow drug release is of great interest, as more prolonged levels of the drug can be attained at the target site.\textsuperscript{15} In cosmetics and personal care products, multifunctional film forming polymers are mostly used to deliver desired attributes to the skin as well as to the hair.\textsuperscript{16} Beside providing optimal adhesion to the skin and skin-like properties, film forming polymers may function as rheology modifiers, humectants, and emollients.

Understanding the effect of film forming systems on skin hydration, for example, is essential in designing topical formulations for specific applications, such as wound dressing, drug delivery, and skin moisturizing.\textsuperscript{17} A topical drug formulation designed to cause a skin occluding film will result in an increase in skin hydration, leading to elevated permeation and thus enhanced transdermal absorption of the drug component.\textsuperscript{18} On the other hand, semipermeable wound dressings may reduce occlusion and thereby risk of skin maceration and the need for a daily dressing change.\textsuperscript{19} Testing of these film forming systems to verify their specific performance on the skin is necessary, but also somehow complicated.
Generally, *in vivo* experiments on human and animal skin are unfavorable due to ethical issues and intrinsic variation in skin properties.\(^{20}\) Therefore, there is a high interest in developing artificial skin alternatives to be used in *in vitro* studies. Simulating the skin properties and functions, all within a single skin model, is challenging. However, a biomimicking skin replica, reproducing one or some of the looked-for properties, may be utilized. Dąbrowska et al. summarized some of the most important requirements as well as materials used for *in vitro* skin replicas.\(^{20}\) There are several alternatives for materials depending on the application, such as polyvinyl alcohol (PVA) gels, silicones, epoxy resins, and gelatin. Mazzoli et al. tuned the optical properties of PVA gels and added liquid ink to mimic the melanin of the skin.\(^{21}\) Derler et al. used silicone and polyurethane materials as mechanical skin equivalents to study the friction of skin against a reference textile.\(^{22}\) Epoxy resin could be used as a thermal skin model, as its thermal diffusivity is close to that of the human skin.\(^{23}\) Gelatin is a water-soluble protein compound obtained from collagen, the main fibrous protein in bones, cartilages, and skin.\(^{24}\) Gelatin has diverse applications in skin models, wound dressings, food and pharmaceutical industries.\(^{24-32}\) In addition to having a chemical structure similar to that of collagen, gelatin shows film forming and hydrogel-like properties. Accordingly, gelatin is a suitable candidate to fabricate water-responsive artificial skin models.\(^{33-35}\)

In this study, we introduce a setup including a gelatin-based skin-mimicking substrate, which can simulate the transepidermal water loss of human skin. The water vapor uptake, wettability and surface topography of the skin-mimicking substrate are assessed by using gravimetric analysis, water contact angle and atomic force microscopy (AFM) measurements, respectively. The moisture releasing skin model, which we will refer to as the TEWL simulator, was used to apply a humidity gradient across films of five selected polymeric film forming systems (commonly used in topical formulations) to examine the water vapor permeation (breathability) through the films. To better understand the water vapor
permeation data, the wettability of the film forming polymers was investigated using the water contact angle measurements to distinguish between the polymer affinity to water and water vapor. Finally, two of the polymers (one with low and one with high barrier properties) were chosen for in vivo TEWL studies to compare with the breathability data obtained from the in vitro TEWL simulator.

2. Materials and methods

2.1. Materials

Gelatin from porcine skin (gel strength ~175 g Bloom, Type A), glycerol (>99.5%) and formaldehyde (ACS reagent, 37 wt.% in H₂O, contains 10-15% methanol as the stabilizer) were supplied by Sigma Aldrich. Hydroxyethyl cellulose (Natrosol™ 250 HHR, PC grade) was kindly provided by Ashland. Dibutyl sebacate, used as a plasticizer for the acrylate/octylacrylamide copolymer, was purchased from Sigma Aldrich. The film forming polymers, the acrylates/octylacrylamide copolymer, abbreviated as Acr-OcAA (from Akzo Nobel Surface Chemistry), the acrylates/dimethicone copolymer, abbreviated as Acr-DiMet (from Shin-Etsu Silicones), the vinylpyrrolidone/acrylates/lauryl methacrylate copolymer, abbreviated as VP-Acr-LaMeAcr (from Ashland), polyurethane-64, abbreviated as PU (from Covestro), and shellac (from Mantrose-Haeuser Co.), were used in this study. Three of the polymers are copolymers containing acrylate as a common segment (Acr-OcAA, Acr-DiMet, and VP-Acr-LaMeAcr). The fourth film former is a polyurethane, and the last one is a shellac-based biopolymer, which is an unsaturated polyester resin composed of aliphatic polyhydroxy acids.

2.2. Preparation of a gelatin-based skin-mimicking substrate

10 g of gelatin and 2 g of hydroxyethyl cellulose were added to 100 ml of water (pH adjusted to 9 using 1 M NaOH), which was followed by 30 min of stirring at 50 °C. 2 ml of glycerol, used as a plasticizer,
and 2 ml of formaldehyde, used as a cross-linker, were added, and the solution was stirred for 1 more minute. The final solution was then applied by a casting knife film applicator (Elcometer 3580/4, Elcometer Ltd., UK) on a PMMA substrate with a wet thickness of 1500 µm and left overnight to dry and cross-link.

2.3. Preparation of topical films

Polymer solutions with an optimum concentration for film application, were prepared using Acr-OcAA (20 wt.%), PU (25 wt.%), VP-Acr-LaMeAcr (15 wt.%) and shellac (40 wt.%) all in ethanol and Acr-DiMet (40 wt.%) in isopropanol. For each polymer, a calibration curve (wet thickness vs. dry thickness) was plotted, and accordingly, polymer samples with a dry thickness of 50 µm were prepared. For the permeation measurements, the polymer solution was applied on the gelatin-based skin-mimicking substrate by a casting knife film applicator (Elcometer 3580/2, Elcometer Ltd., UK) and left to dry for 18 hours prior to the permeation experiment.

2.4. Water vapor permeation test

The water vapor permeation rate through the gelatin-based skin-mimicking substrate and film forming systems was measured according to the ASTM-E96 standard. The prepared film was placed on a cup containing 3 ml of a saturated K₂SO₄ salt solution (providing 97±1% relative humidity) and was sandwiched between two silicon gaskets and fixed by a cap with an exposed area of ~ 7cm² on both sides. The cup was placed in a homemade humidity chamber (ambient temperature, equipped with an air circulating fan and a humidity sensor), where the relative humidity was adjusted to 33±2% by a saturated MgCl₂ solution. The humidity gradient on the two sides of the film acts as a driving force for water vapor permeation through the films from the inside of the cups outwards, resulting in mass loss in the cup. This mass loss was measured by taking out and weighing the cups using an analytical balance (±10⁻⁵ g) with
intervals of 2 hours, and the experiment was stopped after 8 hours. Six replicates were tested for each sample. For the first 2 hours of each measurement, the mass loss rate was lower than that measured over the next 6 hours due to the accumulation of water vapor in the film during the initial humidification step, showing an induction period that is typical for water absorbing films. Accordingly, the fitted slope of the linear region observed at 2-8 hours of testing was, for each case, reported as the water vapor permeation rate.

To investigate the effect of any potential influence of the gelatin-based skin-mimicking substrate on the permeation of the topical films, three samples were prepared: a freestanding film consisting of Acr-OcAA and 30% plasticizer (dibutyl sebacate), the same composition applied on the gelatin-based substrate, and the bare substrate. Making a freestanding film of the pure film former was not possible because of the brittleness of the dried Acr-OcAA film. Therefore, a proper amount of plasticizer was added. The conditions were the same for the permeation experiments performed on the film forming systems.

2.5. Water vapor uptake test

The water vapor uptake of the skin-mimicking substrate and the polymeric film formers was examined using a gravimetric method. The samples were dried in a vacuum oven (VT 6025, Thermo Electron LED GmbH, Germany) at 60 °C for 12 hours to remove the residue of the solvent and initial moisture content. Afterward, the samples were weighed and placed in the chamber with a controlled relative humidity. After 72 hours, the samples were weighed again, and the normalized water vapor uptake was calculated. The relative humidity inside the chamber used to study the film forming polymers was set at 97% and, for the experiments on the skin-mimicking substrate, was set to 11%, 33%, 53%, 75%, 84% and 97% (by LiCl, MgCl₂, Mg(NO₃)₂, NaCl, KCl and K₂SO₄ saturated salt solutions, respectively).
2.6. Atomic Force Microscopy (AFM)

An atomic force microscope (NanoWizard 3, JPK Instruments AG, Germany) was employed to assess the topographical properties of the gelatin-based skin-mimicking substrate. The AFM height images were obtained by tapping mode imaging in the air using a cantilever with a spring constant of 40 N/m (HQ: NSC15/Al BS, MikroMasch). The AFM data were analyzed and processed by using the instrument software (JPK data processing).

2.7. Contact angle measurements

The water contact angle measurements (Theta Lite optical tensiometer, Biolin Scientific, Sweden) were carried out to assess the wettability of the gelatin-based skin-mimicking substrate and polymeric films. The experiment was performed based on the sessile drop method at ambient conditions (25 °C, 30% RH) using a 2 μl water droplet placed on the film surface with a precision syringe. The measurements were recorded within 10 s after deposition. The reported contact angles are, for each sample, the averaged values obtained from five measurements conducted on different surface positions.

2.8. In vivo studies

2.8.1. Study group

Ten healthy volunteers (six men and four women, 24-34 years old, mixed ethnicities) participated in this study. The participants handed in written informed consent prior to the experiment. None of the subjects had any dermatological diseases in their history or visible injured areas on their foreheads. All the subjects were asked not to apply any topical products, such as sunscreen and lotions, on their foreheads at least 24 hours before the study. The subjects were also asked not to drink caffeine-containing beverages 3 hours before the beginning and throughout the study period.
2.8.2. *In vivo* TEWL measurements

The subjects stayed physically inactive in the room for 30 minutes to familiarize themselves with the conditions. During the experiment, the room temperature and humidity were recorded for each measurement (24-25 °C, 55-59% RH). The foreheads of the subjects were marked in the middle, and the Acr-OcAA- plasticizer and PU films (thickness: 50 μm, size: 2×3 cm²) were placed on the left and right sides, respectively, and fixed with surgical tape on the edges. The TEWL values were measured using a closed chamber device (Aqua Flux™ AF200, Biox Ltd., UK). For each topical film, the TEWL measurement was conducted on the same position on bare skin, on top of the polymeric film, 1 and 2 hours after covering the skin, and finally again on the bare skin right after film removal. Each measurement was repeated three times, except for the measurements made after film removal.

3. Results and Discussion

3.1. Characterization of the moisture releasing skin model

The skin-mimicking substrate comprises gelatin, hydroxyethyl cellulose (Natrosol), glycerol, and formaldehyde. Gelatin, the main component of the artificial skin formulation, mimics both the chemical and physical properties of dry and hydrated human skin. Natrosol provides less brittleness and a larger elongation capacity giving rise to more skin-like mechanical properties. It was observed that the addition of Natrosol produces a visible texture and an enhanced roughness on the surface, thus better mimicking the surface texture of the skin. Glycerol serves as a plasticizer, enhancing the flexibility of the skin-mimicking substrate, and facilitates the film formation of polymers on top. Finally, formaldehyde serves as a chemical cross-linker for gelatin, thus enhancing the mechanical integrity and
the hydrolytic stability.\textsuperscript{34} When cross-linked, the skin-mimicking substrate can reversibly swell/shrink due to moisture adsorption/desorption, with minimum structural changes.

Figure 1 provides an outline of the relevant physical properties of the gelatin-based skin-mimicking substrate. Figure 1(a) and (b) present a representative AFM height image of the skin-mimicking substrate and the corresponding cross-section height profile. Accordingly, for an area of 100 \( \mu \text{m} \times 100 \mu\text{m} \), the average root-mean-squared (RMS) roughness of the film is approximately \( 0.87 \pm 0.40 \mu\text{m} \), which is comparable with the reported value for human skin.\textsuperscript{2} Figure 1(c) displays the representative water contact angle of the skin-mimicking substrate. Here, an average water contact angle of \( 75\pm5^\circ \) was found, which is in the same range of the reported values for human skin.\textsuperscript{34,40} Figure 1(d) represents the normalized amount of the water vapor uptake of the skin-mimicking substrate as a function of the relative humidity, demonstrating a nonmonotonous water vapor uptake behavior. Increasing the relative humidity up to 50\% had little-to-no effect on the film. However, a more significant moisture uptake seems to occur when the relative humidity is approximately \( \geq 75\% \). The observed vapor uptake isotherm resembles that of the human skin and stratum corneum.\textsuperscript{41}

The gelatin-based skin-mimicking substrate demonstrates a surface roughness, wettability, and hydration behavior that are similar to those of human skin. The TEWL simulator is composed of this skin-mimicking substrate, which is mounted on a cup, separating two compartments with different relative humidity values, i.e., the relative humidity inside the cup (\( \text{RH}_{\text{in}} \)) and relative humidity outside the cup (\( \text{RH}_{\text{out}} \)). The relatively high \( \text{RH}_{\text{in}} \) promotes the hydration of the skin-mimicking substrate, while the difference in the humidity levels inside and outside the cup will produce a steady flux of water permeation through the skin-mimicking substrate. As the gelatin-based skin-mimicking substrate shows a significant
water vapor uptake at relative humidity values ≥ 75%, RH\textsubscript{in} must be at least 75% to achieve a sufficiently hydrated substrate to mimic the behavior of skin.

Under \textit{in vivo} conditions, the water activity inside the body is constant, and for healthy skin, the water permeability through the skin depends on the water activity on the outside of the stratum corneum as well as the stratum corneum barrier properties and thickness.\textsuperscript{42,43} Likewise, in the TEWL simulator, the relative humidity gradient and thickness of the skin-mimicking substrate can control the water vapor permeation rate through the film. Skin-mimicking substrates with four different dry thicknesses (30, 70, 100, and 140 µm) were tested. In each case, three different relative humidity gradients were examined. The RH\textsubscript{out} was fixed to 33% to resemble an average ambient humidity, while the RH\textsubscript{in} was adjusted roughly to 75, 84, and 97%. The normalized water vapor permeation rates (the simulated TEWL values) obtained using the setup (Figure 1(e)) demonstrate that for a given humidity gradient, increasing the thickness of the gelatin-based substrate results in lower simulated TEWL values. Conversely, for a given thickness of the skin-mimicking substrate, increasing the RH\textsubscript{in} results in higher permeation values. Using a combination of these two parameters, one can obtain approximate simulated TEWL values in the range of 10-45 g/(m\textsuperscript{2}h), which covers the TEWL range of human skin in moderate to stressed conditions.

To study the breathability of the film forming polymers, we used a setup comprising a skin-mimicking substrate with a thickness of 90 µm, RH\textsubscript{in} of 97%, and RH\textsubscript{out} of 33% (Figure 1(f)). Using these conditions, a water vapor permeation rate of approximately 37 g/(m\textsuperscript{2}h) is obtained, which is considered to be a relatively high TEWL value for human skin.\textsuperscript{44} In this way, the difference in the breathability of the film forming systems applied on the skin-like substrate will be more distinguishable, and the contributions from experimental error will be less significant than if a lower water vapor permeation rate was used.
3.2. Water vapor permeation through topical films

To ensure reliable data for water vapor permeation through topical films, the effect of the skin-mimicking substrate on the permeation behavior of the topical films was investigated prior to the permeation test. Figure 2(a) shows the permeation rates measured for the skin-mimicking substrate, the freestanding film of the Acr-OcAA- plasticizer, and the Acr-OcAA- plasticizer film applied on the gelatin-based substrate. It is concluded that the topically applied film almost solely governs the permeation of water, since the permeation of the freestanding film is almost the same as that of the film applied on our skin-mimicking substrate. This illustrates that as long as the permeation of the skin-mimicking substrate is much higher than the permeation of the applied film, the substrate does not affect the permeation through the polymer film.

Using our TEWL simulator, the breathability of the polymeric topical films was investigated through permeation experiments. Figure 2(b) shows the permeation data for polymeric film formers compared to data obtained for the bare skin-mimicking substrate. For the same dry thickness of the polymer film, the highest permeation values are measured on the VP-Acr-LaMeAcr and PU films (22.73±0.37 and 18.28±1.18 g/(m²h), respectively). In contrast, the shellac, Acr-OcAA, and Acr-DiMet films expressed high barrier properties, as given by their low permeation values (2.67±0.07, 2.86±0.11, and 7.55±0.16 g/(m²h), respectively). By employing the TEWL simulator, it is thus possible to categorize film forming systems based on their barrier functionality against water vapor. The obtained results are based on relatively thick polymer films (50 μm), which is a key parameter in the permeation rate. For example, Zhai et al. showed that the topical administration of a 5% Acr-OcAA solution (resulted in a thinner layer than our polymeric film formed from the 20% solution) does not show an occlusive behavior, despite the hydrophobic chemical structure of the film.45
Note that the mechanisms of permeation for water and water vapor through polymer films are not equivalent. A polymer with a large water contact angle resists liquid water uptake, but does not necessarily repel water vapor. We measured both the water vapor uptake of the film forming polymers and water contact angles of the corresponding polymeric films (results presented in Figure 3). As seen in Figure 3(a), VP-Acr-LaMeAcr absorbed a large amount of water vapor (37.1±0.6 wt.% uptake). Shellac and Acr-OcAA showed a similar low affinity to water vapor (4.4±0.3 and 4.0±0.1 wt% uptake, respectively), and PU and Acr-DiMet were the polymers with the lowest water vapor absorption (2.5±0.1 and 0.4±0.0 wt.% uptake, respectively). As shown in Figure 3(b), the contact angles for the Acr-OcAA, Acr-DiMet and PU films are higher (95±2°, 90±1°, 75±5°, respectively) than those of the shellac and VP-Acr-LaMeAcr (64±3° and 65±2°, respectively). By comparing the results of the two tests, we see no direct correlation between the water contact angle and water vapor uptake data, as only a few of the polymers (Acr-DiMet and PU) exhibit both a high water repellency (high water contact angle) and low water vapor uptake. Therefore, the water vapor permeation of the polymer films, as a measure of the film forming systems’ breathability, cannot be predicted by the contact angle measurements. The results here confirm that although some film forming polymers may prevent the liquid water from penetrating through the surface, the film forming polymers might show significant water vapor uptake.46

Water vapor permeation across film forming systems is a result of the initial water vapor absorption of the polymer film (solubility) and the transmission of the molecules through the film (diffusion). The solubility and diffusion can be affected by various parameters, such as the chemical affinity between the polymer and water vapor, the capillary condensation of the vapor molecules on the film surface, and the glass transition temperature ($T_g$), free volume and crystallinity of the polymer.47–49
The polymeric film formers presented in this study, except VP-Acr-LaMeAcr, showed a low affinity to water vapor (Figure 3(a)). However, studies on the diffusion behavior of water vapor molecules through these polymers demonstrated diverse permeation rates (Figure 2(b)). VP-Acr-LaMeAcr showed the highest water vapor uptake and permeation, which could be attributed to its film forming mechanism and chemical structure. The VP-Acr-LaMeAcr films are cast from a polymeric dispersion, while the other films are formed from polymeric solutions. In solution form, during solvent evaporation, polymer chains come to closer to one another and enter a gel state, eventually making a polymeric film. This differs compared to dispersion, wherein polymer chains reform to fill the free spaces created by solvent evaporation, resulting in a more porous structure. Additionally, VP-Acr-LaMeAcr is an amphipathic polymer, in which an anchoring group (acrylates) connects the hydrophilic (vinylpyrrolidone) and hydrophobic (lauryl methacrylate) groups to one another. Hence, the presence of hydrophilic segments close to the surface in conjunction with a porous macromolecular structure can possibly facilitate the permeation of water vapor.

3.3. In vivo TEWL studies

To compare the data obtained from the TEWL simulator with the in vivo values, two of the film forming systems with high and low water vapor permeation rates (PU and Acr-OcAA) were chosen. A freestanding film of both polymers (in the case of Acr-OcAA with the aid of a plasticizer) was applied on the forehead of the ten healthy subjects. For each subject, the TEWL values before covering the skin by the polymeric films and the apparent TEWL values after the removal of the film were obtained. Moreover, the water vapor that permeated from the skin across the polymeric film was collected 1 and 2 hours after covering the skin. The results are summarized in Figure 4. For the areas covered by the Acr-OcAA-plasticizer films, the measured value of water vapor permeation across the polymeric film was
lower than the TEWL value of the bare skin before film application (both after 1 and 2 hours of covering the skin). The TEWL values of the bare skin increased significantly right after the film was removed from the forehead (on average 53% compared to the TEWL value before film application). In contrast, covering the skin with the PU freestanding films appeared to increase the water vapor permeation across the film compared to the TEWL values of the uncovered skin. Moreover, the TEWL values of the bare skin did not vary considerably after film removal (on average, only a 14% increase compared to the TEWL value before film application).

It was expected that covering the skin with a polymeric film would reduce the water vapor permeation compared to the TEWL value of bare skin, and, consequently, the TEWL value after film removal was postulated to be higher than that of the bare skin before film application. The results of the Acr-OcAA-plasticizer films agree qualitatively with the in vitro study, suggesting the film to be occlusive. However, the reduction in water vapor permeation after covering the skin by the Acr-OcAA-plasticizer film is approximately 20%, whereas it was found to be considerably higher in the in vitro study. Further, covering the skin with a PU film resulted in an increase in water vapor permeation. We attribute this observation to regulating the driving force for in vivo TEWL before and after covering the skin by the polymeric film. The temperature of the skin covered by the polymeric films may locally increase, leading to a change in the gradient of water vapor or sweat pore activation and, consequently, an increased TEWL value. Moreover, topical administration of film forming systems on the skin affects skin hydration as well as water permeation through the stratum corneum. As mentioned, the water permeation through bare skin is due to the water activity difference on both sides of the stratum corneum. A barrier layer introduces a high relative humidity to the outer side of the skin. Thus, the skin hydration increases, resulting in a high permeation rate. However, the measured permeation rate on top of the film is affected by its barrier properties. This agrees with results from Sparr et al., who compared the effect of the
top layers with different barrier properties on skin hydration, water vapor permeation rates, and TEWL values. Base on that background, we believe that both polymeric films similarly induced a high concentration of water vapor on the skin surface. However, while the Acr-OcAA-plasticizer film, to a high extent, prevents permeation of the extra released vapor from the skin interface, the PU film, with its limited barrier properties, experiences higher water vapor permeation rates than the TEWL values observed from the bare skin surface before covering the skin.

Comparing the in vitro and in vivo results, we conclude that the in vitro studies can predict the difference in resistance to water vapor of the Acr-OcAA and PU films on human skin. Both the TEWL simulator and the in vivo measurements showed a constant TEWL value after the induction time. Note that the biological responses to the topical administration of polymeric films (e.g., a local increase in temperature, sweat pore activation, and lipid structural changes) cannot be predicted using the TEWL simulator, where the temperature and RH gradient stay constant, and the water vapor permeation rate obtained different values solely due to the breathability of the different films. Consequently, the absolute numbers obtained from the in vivo and in vitro results are not comparable. Instead, the in vitro method benefits from avoiding scattered data, subject dependent biological responses and practical issues associated with in vivo studies. Thus, the in vitro method described in this study is a good tool for the prediction of the behavior of topical films on the skin by systematically measuring the resistance of polymeric film formers to water vapor permeation.

4. Conclusion

We have developed a TEWL simulator with a gelatin-based skin-mimicking substrate. The surface chemistry and hydration of the substrate were adjusted to simulate human skin, and its water vapor permeation rate successfully demonstrated values close to the ones observed for real skin. The water
vapor permeation through films of five different polymeric film formers was investigated using this TEWL simulator. The results demonstrate that the film of the vinylpyrrolidone/acrylates/lauryl methacrylate copolymer is the most breathable, while the acrylates/octylacrylamide copolymer and shellac showed the highest resistance to permeation. The comparison between the water contact angle and water vapor uptake measurements of the polymers confirmed that it is not possible to predict water vapor permeation solely from wettability data. *In vivo* TEWL studies were carried out to investigate the effect of two of the polymers, which demonstrated different water vapor permeation rates when covering the skin. The comparison between the *in vivo* and *in vitro* studies illustrated a difference in the driving force for water vapor permeation due to the absence of the biological responses to skin occlusion in the *in vitro* method. Despite this, the TEWL simulator was able to predict the breathability of the topical polymeric films on human skin, and this information can aid in selecting suitable film forming polymers for topical formulations with either breathable or occluding functionalities. Finally, the simple film forming polymers investigated in this study were not highly affected by the chemistry and structure of the gelatin-based skin-mimicking substrate. However, we suggest that our TEWL simulator, considering its highly skin-like properties, may also be relevant for studying water vapor permeation through films of complex topical formulations, where direct interactions between the specific ingredients and the skin interface might play an important role for the topical film structure and performance.

5. **References**


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Figures

Figure 1. Summary of the physicochemical skin-mimicking substrate properties. (a) AFM height image of the skin-mimicking substrate and (b) cross-section profile taken at the line marked on the AFM height image. (c) Water droplet on the dry skin-mimicking substrate used in the contact angle measurement. (d) Normalized amount of water vapor uptake by the skin-mimicking substrate versus RH. (e) The water vapor permeation through the skin-mimicking substrates with four different thicknesses (30, 70, 100, and 140 μm) and with a 75%, 84% and 97% RH inside the cup. The humidity outside the cup was fixed at 33%. (f) Schematic side view of the TEWL simulator with an applied polymeric film.
Figure 2. The effect of the skin-mimicking substrate on permeation through film forming polymers and the film forming polymer’s water vapor permeation data. (a) Water vapor permeation through the bare gelatin-based substrate, Acr-OcAA- plasticizer film applied on the gelatin-based substrate and the Acr-OcAA- plasticizer free-standing film. (b) Permeation data for the film forming polymers applied on the skin-mimicking substrate compared with that of the gelatin-based substrate.
Figure 3. Comparison of the water and water vapor affinity of the film forming polymers. (a) The water vapor uptake of the film forming polymers in a controlled relative humidity at 97%. (b) The water contact angle of the corresponding polymeric film.
Figure 4. Results for the in vivo TEWL measurements (a) Data of the TEWL measurements performed on ten subjects at different times after covering the skin by polymeric films (upper panel: Acr-OcAA-plasticizer, lower panel: PU). (b) The average change in the TEWL values after covering the skin by a polymeric film and after film removal compared to the TEWL values for the uncovered skin (Green color: Acr-OcAA-plasticizer, Blue color: PU).