

#### Sweat resistance of sunscreens

Development of a perspiring skin simulator, evaluation of sunscreen failure mechanisms and enhancement of sunscreen substantivity

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Publication date: 2020

Document Version Publisher's PDF, also known as Version of record

#### Link back to DTU Orbit

Citation (APA):

Keshavarzi, F. (2020). Sweat resistance of sunscreens: Development of a perspiring skin simulator, evaluation of sunscreen failure mechanisms and enhancement of sunscreen substantivity. Technical University of Denmark.

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# [RIEMANN]

## Sweat resistance of sunscreens

Development of a perspiring skin simulator, evaluation of sunscreen failure mechanisms and enhancement of sunscreen substantivity



**DTU Chemistry** Department of Chemistry

## Abstract

Sunscreen application is one of the most well-known solutions for the protection of human skin against harmful ultraviolet (UV) radiation from the sun. The substantivity of sunscreen (that is, the ability of the sunscreen to bind to the skin and resist removal), as an indicator of long-lasting protection against UV radiation, can be affected by a variety of activities, such as wearing clothes, swimming, and sweating, that may occur after the application of sunscreen on the skin. Sweating occurs in many situations in which people use sunscreen. However, the interaction of sunscreen and sweat is largely overlooked, likely owing to the inevitable challenges associated with performing in vivo sweat resistance tests and the lack of suitable instrumentation for in vitro studies. Besides active sweating, passive diffusion of water through the skin, known as transepidermal water loss (TEWL) can be affected by the application of topical films, such as sunscreen and polymeric film-forming systems. The aim of this PhD project was to develop specific in vitro setups to mimic human skin perspiration, and then to obtain general information on the impact of sweating on sunscreen substantivity, to evaluate the effect of different parameters on sunscreen failure mechanisms during sweating, and to explore methods to develop sunscreen formulations with greater sweat resistance. Moreover, a systematic evaluation of the breathability of film-forming systems applied on the skin was performed by using an in vitro TEWL simulator.

The TEWL simulator was used to characterize the effect of the application of selected topical polymeric film formers on skin occlusion and TEWL. Subsequently, the in vivo TEWL studies were performed on two selected film formers. The comparison between the in vitro and in vivo TEWL studies confirmed that the TEWL simulator was able to predict the breathability of the polymeric film-forming systems.

I used the perspiring skin simulator to perform both qualitative and quantitative evaluation of sunscreen film performance in response to sweating. The results showed that sweating negatively affected sunscreen substantivity and UV protection through wash-off and redistribution of the sunscreen film. Further, two approaches for increasing the sweat resistance of sunscreen were investigated: manipulation of the concentration of hydrophobic film-formers and incorporation of water absorbing particles in the sunscreen formulation. The results indicated that the combination of moderate concentrations of a hydrophobic film-former and water-absorbing particles capable of forming a gellike structure in contact with water could successfully increase the film integrity of sunscreen and control the sunscreen wash-off and redistribution by localizing the water pressure.

## Resume

Påføring af solcreme er en af de mest kendte metoder til beskyttelse af menneskelig hud mod skadelig ultraviolet (UV) stråling fra solen. Solcremens substantivitet (dvs. dens evne til at binde sig til huden og modstå fjernelse), som en indikator for langvarig beskyttelse mod UV-lys, kan påvirkes af forskellige aktiviteter efter påføring af solcreme på huden, såsom påføring af tøj, svømning og svedning. Sved falder sammen med mange situationer, hvor folk bruger solcreme. Dog overses interaktionen mellem solcreme og sved ofte, muligvis på grund af uundgåelige udfordringer for at udføre in vivo svedmodstandstest og manglen på passende instrumentering til in vitro-studier. Udover aktiv svedning kan passiv diffusion af vand gennem huden, kendt som transepidermalt vandtab (TEWL), blive påvirket af anvendelse af topiske film såsom solcreme og filmdannende polymer systemer. Dette ph.d.-projekt havde til formål at udvikle specifikke in vitro-opstillinger til at efterligne menneskelig hudsved for at opnå generel viden om virkningen af svedtendens på solcreme substantivitet, evaluere effekten af forskellige parametre på solcreme svigtmekanismer under svedning og udforske nogle ruter til introduktion af solcreme sammensætninger med højere svedmodstand.

TEWL-simulatoren blev brugt til at karakterisere effekten af anvendelsen af udvalgte topiske polymer filmdannere på hud okklusion og TEWL. Derefter blev der udført in vivo TEWL-undersøgelser på to udvalgte filmdannere. Sammenligningen mellem in vitro og in vivo TEWL-undersøgelser bekræftede, at TEWL-simulatoren er i stand til at forudsige åndbarheden af de filmdannende polymer systemer.

Jeg brugte den svedende hudsimulator til at evaluere solcreme-filmens ydeevne ved svedning både kvalitativt og kvantitativt. Resultaterne viste, at svedning påvirker solcreme-materialiteten og UV-beskyttelsen negativt gennem vask af solcreme og omfordeling i filmen. Yderligere blev to fremgangsmåder til forøgelse af solcremens svedmodstand, dvs. manipulering af koncentrationen af en hydrofob filmdanner og inkorporering af vandabsorberende partikler i solcremeformuleringen, undersøgt. Resultaterne viste, at en kombination af en hydrofob filmdanner ved mellemkoncentration og vandabsorberende partikler, der er i stand til at danne en gellignende struktur i kontakt med vand, med succes kunne øge filmintegriteten af solcreme og styre solcremeudskylning og omfordeling ved at lokalisere vand under tryk.

## Acknowledgments

The work presented in this thesis was conducted at Department of Chemistry at the Technical University of Denmark (DTU) in collaboration with Riemann A/S and was financially supported by Innovation Fund Denmark. This work was performed under supervision of Professor Esben Thormann from DTU and Dr. Shadi Jafarzadeh from Riemann A/S. During my PhD project, I was blessed to work with many wonderful people, to whom I would like to express my deepest gratitude.

*Professor Esben Thormann*, thank you so much for giving me the opportunity to pursue my scientific dreams. Working under your supervision was one of the most incredible experiences of my life. I truly thank you for all the scientific discussions we had, your inspiring ideas, constructive comments, and your unique outlook that helped to shape and refine my research. You supported me with your continued dedication, help, and guidance. Besides your extensive scientific background and a constant passion for exploring new fields, your friendly attitude toward your students and your positivity, kindness, and optimism created a very pleasant working atmosphere for all of us. Not every PhD student is lucky enough to experience that and I am deeply grateful for everything you have done for me.

*Dr. Shadi Jafarzadeh*, a very special thanks to you for all your support, both during my scientific research and in my personal life. Despite your incredibly packed schedule at Riemann and all the challenges you faced, I had your dedicated attention and encouragement throughout the process. You put so much effort into making new collaborations for the project development, which advanced different aspects of my research. During our scientific discussions, you always amazed me with your attention to important details and your sharp memory which remembers, literally, EVERYTHING. From the minute I arrived in Denmark, you welcomed me with your heartwarming sweet greetings that took away all my tensions and worries. You, Esben, and your two lovely daughters made me feel at home and I cannot thank you enough for that. You are not only my supervisor, but also a great friend who owns a special place in my heart.

*Dr. Malene Barre Pedersen*, thank you so much for accepting me as your industrial PhD student at Riemann and all the support and encouragement I received from you during the last three years, even after you left Riemann and started a new career. You are one of the nicest and most thoughtful people I have ever met, and I enjoyed every minute of the time we spent together discussing the project or just talking about us. A big (Corona-free) **koala** hug to you.

*Dr. Saeed Zajforoushan Moghaddam*, I thank you for all the scientific discussions, collaborations, and "brainstormings" we had, and for your constructive comments and constant support during my project. You were always ready to help with instruments in the lab; in addition, your patience for explaining everything, when I forgot it for the 50<sup>th</sup> time, is really appreciated.

*Tao, Gokce, Daniel, Johannes, Rob, Junhao, Koosha, Runtian, Junjie, Roberto,* and *Frederik,* I truly thank you all for being such great, helpful, and amazing friends/colleagues for me at GPFI. I enjoyed working with you, not only for the memorable time we have spent together, but for your caring and encouraging attitude whenever I lacked inspiration.

*Bong, Heidi, Nina, Niloufar, Lidia, Sonja,* and *Valentina*, my sincere thanks to you for all the scientific discussions, instrumentation training, teamwork, and valuable feedback, and finally (yet importantly) for all the fun we had as the R&D team at Riemann, which will be a lifelong memory.

I would also like to acknowledge the great support of all my colleagues at DTU Kemi, especially Ishaq Khaliqdad, Andreas Graff Pedersen, and Lars Egede Bruhn for their technical and instrumental support and their constant smiles. I would like to thank Rune Kenneth Dybdal Christiansen at DTU Nanolab for his training and support regarding the laser drilling process. I am deeply grateful to Associated Professor Jonathan R. Brewer and Dr. Morten Frendø Ebbesen at the Department of Biochemistry and Molecular Biology at the University of Southern Denmark for their extensive knowledge about CARS microscopy, their innovative ideas and great support, which resulted in a wonderful collaboration. My deepest gratitude to my beloved family, Maman, Baba, Parisa, Mehdi, Mohammad, Maliheh, and Matin, who raised me with endless devotion, love, and support. The ones who, no matter what, always stand beside me to help my dreams come true and whenever I suffer I rely on their open arms and heart-healing love. I also thank my friends from home and here, Marzieh, Sara, Shahrzad, Azadeh, Mahboobehs (1 and 2), Noushin, Nasim, Eli, Farnoosh, and Sol for their support and love, and enduring all my complaining.

*Passengers of Ukraine Flight PS752*, it has been eight months and the pain of your heartbreaking loss grows more and more every day. All of you are deeply missed by your family, friends, and compatriots. Not even one day has passed without me thinking about you, especially the ones who were no different than me: a PhD student, studying abroad, hoping for a better future despite all the challenges, struggles and obstacles. You and your unfulfilled dreams will be always remembered by me. May your souls rest in peace.

*My dearest Deniz*, sen hayatımdaki en iyi şeysin. Thank you for everything, for the love, the joy, the peace, and the shine you bring to my life. Every second of last three years, you were the only one who stood so close to my heart, believed in me and made me believe in myself, and gave me the courage to survive the darkest times and fight disappointments. You are my best friend, my soulmate, and my love.

## List of abbreviations

Acr-DiMet	Acrylates/dimethicone copolymer		
Acr-OcAA	Acrylates/octylacrylamide copolymer		
AFM	Atomic force microscopy		
CARS	Coherent anti-Stokes Raman scattering		
COLIPA	European cosmetic toiletry and perfumery association		
Core-Shell	Core-shell particle		
D	Diffusion		
FDA	Food and drug administration		
HMHEC	Cetyl hydroxyethyl cellulose		
MED	Minimal erythema		
MPF	Monochromatic protection factor		
NaPA	Cross-linked sodium polyacrylate		
Natrosol	Hydroxyethyl cellulose		
O/W	Oil-in-water		
Р	Permeation		
PAC-6	Polyacrylate crosspolymer-6		
PMMA	Polymethylmethacrylate		
PPD	Persistent pigment darkening		
PU	polyurethane-64		
RH	Relative humidity		
RMS roughness	Average root-mean-squared roughness		
S	Solubility		
SC	Stratum corneum		
SPF	Sun protection factor		
TEWL	Transepidermal water loss		
Tg	Glass transition temperature		
UV	Ultraviolet		
UVAPF	UVA protection factor		
UVT	Ultraviolet Transmittance		
VP-Acr-LaMeAcr	Vinylpyrrolidone/acrylates/lauryl methacrylate copolymer		
W/O	Water-in-oil		
WVPR	Water vapor permeation rate		

## List of publications

- <u>Fatemeh Keshavarzi</u>, Saeed Zajforoushan Moghaddam, Malene Barré Pedersen, Nina Østergaard Knudsen, Shadi Jafarzadeh, and Esben Thormann. Water vapor permeation through topical films on a moisture-releasing skin Model. Skin research and technology, doi:10.1111/srt.12926.
- <u>Fatemeh Keshavarzi</u>, Nina Østergaard Knudsen, Jonathan R. Brewer, Morten F. Ebbesen, Niloufarsadat Mirmahdi Komjani, Saeed Zajforoushan Moghaddam, Shadi Jafarzadeh and Esben Thormann. In vitro skin model for characterization of sunscreen substantivity upon perspiration, (Submitted).
- <u>Fatemeh Keshavarzi</u>, Nina Østergaard Knudsen, Niloufarsadat Mirmahdi Komjani, Morten
  F. Ebbesen, Jonathan R. Brewer, Shadi Jafarzadeh and Esben Thormann. Enhancing the
  sweat resistance of sunscreens, (Ready to submit).

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## 1. Introduction

Whether exercising outdoors or spending a day on the beach, the skin is exposed to ultraviolet (UV) radiation from the sun. Even though sunlight has benefits for the human body, such as inducing vitamin D<sub>3</sub> synthesis and moderating depression, overexposure to UV radiation can cause various types of damage to the skin, including sunburn, photoaging, immunosuppression, and skin cancer. Sunscreen application is a common practical solution for the protection of skin against UV radiation. It contains active components, i.e., photostable UV filters, which are uniformly spread on the skin to reflect, absorb, or scatter UV radiation (UVB radiation, with a wavelength of 290–320 nm, and UVA radiation, with a wavelength of 320–400 nm) before it reaches the skin components and cause damage. It is essential that a sunscreen is photostable, nontoxic, and nonirritating. Moreover, to provide long-lasting UV protection after application, the sunscreen should properly bind to the skin and resist removal by different real-life activities, such as wearing clothes, bathing, toweling, swimming, and sweating.

Based on the required characteristics, three of the main factors used for the evaluation of sunscreen performance are the sun protection factor (SPF), the UVA protection factor (UVA-PF), and the substantivity.

There are universally accepted and well-established methods for the in vivo and in vitro measurement of SPF and UVA-PF values. For substantivity, one of the documented in vivo methods involves obtaining the water resistance of the sunscreen by comparing the measured SPF values before and after immersion of the sunscreen-treated skin in water. However, while all the other post-application activities affect the applied sunscreen film from the outside, sweat is secreted to the skin/sunscreen interface from beneath the film and may have a different effect on sunscreen performance. The impact of sweating on sunscreen has been mostly overlooked, both with regard to establishing suitable protocols to assess sunscreen substantivity and in the literature. Further, in vivo studies of resistance to sweat are complicated by biological variations and less-than-accurate analyses, stemming largely from difficulties in controlling the sweat rate and measuring the amount of sweat secreted. Unsurprisingly, the water resistance test seems to be an easier option for the evaluation of sunscreen substantivity.

With this project, I aim to build a better understanding of (1) how the impact of sweating on sunscreen substantivity can be investigated in a systematic and well-defined manner, (2) how sweating actually affects the substantivity of sunscreen at a mechanistic level, and to (3) explore some routes for formulating sunscreen with improved sweat resistance.

The human skin is a complex system with a variety of properties and functions and one of the most multifaceted organs in the body. The stratum corneum (SC), the outermost layer of the skin, is a flexible barrier against infection, chemical substances, and dehydration. The SC is responsible for "tuning" the amount of water passing through it and thereby modulating water evaporation to the environment based on differences in water activity on both sides of the skin. Transepidermal water loss (TEWL) is a vital indicator of skin barrier function and is affected by both environmental and individual parameters. Moreover, covering the skin with a sunscreen (or any other cosmetic or personal care product, transdermal drug delivery vehicle, or wound dressing) may partially occlude the skin and alter the TEWL. As many sunscreens contain polymeric film-forming systems with the ability to form a uniform and robust topical film on the skin, and owing to the various functionalities that film-forming systems can deliver, the development and evaluation of these systems and the assessment of their effect on skin properties, particularly skin hydration and TEWL, have received much attention.

The in vivo evaluation of film-forming systems faces many challenges. First, TEWL cannot be measured directly. The known methods all measure water evaporation from the skin surface, and if TEWL is the only source of water on the skin, the measured value represents the amount of water permeated through the skin. Second, in vivo TEWL measurements, besides being labor intensive, time consuming, costly, and ethically unfavorable, may result in scattered data due to different biological responses. Therefore, the viability of a systematic study to evaluate the effect of parameters of interest on skin hydration and TEWL is difficult. Understandably, the focus has shifted greatly from in vivo experiments to the development and introduction of artificial skin alternatives for in vitro studies.

The other important water-related function of human skin is thermoregulation. If the core body or skin temperature increases to a certain threshold, which may be due to either internal and/or external stimuli, such as emotional state, body circadian rhythm, temperature, humidity, or physical activities, heat stress compensation can occur through perspiration. The sweat is secreted

to the skin surface through numerous sweat glands spread out across the body and evaporates to dissipate heat and lower the body temperature. Besides cooling the body and skin, sweating can affect other skin characteristics, including skin hydration and skin surface pH. Moreover, sweating co-occurs with many situations in which people make use of one of the most well-known topical films, namely, sunscreen.

For the first part of my study, I developed an in vitro setup with a skin-mimicking gelatin-based substrate, which is referred to as the TEWL simulator. The surface chemistry and hydration of the substrate and the water vapor permeation rates through the substrate were adjusted to accurately mimic the human skin and the subsequent setup could successfully provide equivalent TEWL values close to that of real skin. Next, the water vapor permeation through five different polymeric film-formers with different characteristics, applied on the skin-like substrate, was investigated. For two of the selected film-forming polymers with high and low breathability, in vivo TEWL measurements were performed and the data were compared with the results obtained from the TEWL simulator.

For the second part of this study, I developed an in vitro setup with a multilayer skin-mimicking substrate that was able to mimic skin perspiration in terms of sweat pore size and density and sweat rate, and showed a skin-like affinity for sunscreen. This setup, the perspiring skin simulator, was used to obtain general information about sunscreen film performance in response to sweating. Moreover, the substantivity and sweat resistance of the sunscreen film was assessed qualitatively and quantitatively by employing different imaging techniques and in vitro SPF measurements before and after exposure to perspiration. I tried to draw a connection between factors influencing the performance of the sunscreen and the mechanisms involved in sunscreen failure during and after perspiration. Finally, as data obtained from the previous study highlighted the importance of sunscreen formulation in the retention of sunscreen substantivity upon sweating, to improve the sunscreen formulation: changing the concentration of hydrophobic film-former and incorporating water-absorbing particles.

## 2. Background

#### 2.1. Polymeric film-forming systems

#### 2.1.1. Applications of film-forming polymers

Polymeric film-forming systems are widely used for protective, decorative, and functional purposes.[1]–[4] These systems have been developed and evaluated in numerous fields, such as drug delivery systems, topical skin treatments, cosmetics, and personal care products.[5]–[10] In drug delivery, film-forming systems slow drug release to maintain steady levels of the drug in the body. Moreover, thin polymeric patches with cosmetic attributes increase patient conformity.[8] In topical treatments, the drug is applied directly at the affected site, which leads to reduced side effects and tuned drug levels on the affected site close to oral administration.[9] These topically applied films can be evaluated by several parameters, such as drying time, viscosity, stickiness, flexibility, transparency, and abrasion resistance on the skin.[11], [12] In cosmetic and personal care products, film-forming polymers have a great range of functionalities, acting as film-formers, rheology modifiers, and emollients to achieve optimal adhesion to the skin, water resistance, flexibility, and uniform film formation in the product.[13]

#### 2.1.2. Mechanisms of film formation

Film formation by polymeric solutions on a surface occurs by solvent evaporation of the polymeric solution or dispersion. The mechanism by which films are formed depends on whether the polymer is in a dissolved or dispersed state. In solution, while the solvent evaporates, polymer chains come more closely into contact and enter a gel state, eventually forming a polymeric film. In contrast, the dispersion form requires the coalescence and interpenetration of polymer chains during solvent evaporation. In other words, polymer chains reform to fill free spaces created by solvent evaporation.[1]

Various factors control the film formation of polymers on the substrate. Solution characteristics (viscosity, polymer molecular weight, and solvent type/evaporation rate), application methods (spraying and solvent casting), the interfacial tension between surfaces (wettability), and roughness of the substrate play key roles in polymer film formation.[14]–[17]

#### 2.2. Water vapor permeation through polymeric films

The permeation of water vapor through polymeric films follows the same mechanisms as the mass transfer of gases and vapors through polymers: capillary flow and activated diffusion.[18] In capillary flow, the permeant diffuses through pores, pinholes, and cavities in the porous film. In dense materials, the permeation of water vapor can be explained through a four-step process. First, the molecules adsorb on the surface of the polymer and dissolve into the film. This process is followed by the permeant diffusion through the polymer film driven by concentration gradients on both sides of the film. Diffusion happens through the occupation of the free volume between the polymer chains.[19] Finally, the molecules desorb from the film on the side with a lower water vapor concentration.[20]

The permeation (P) of water vapor through a polymeric film depends on the solubility (S) of the water vapor molecules in the polymer (polymer hydration), as well as water vapor mobility and diffusion (D) within the polymer matrix. Solubility is defined by the enthalpy change upon dissolution of the molecule in the polymer and the volume available for occupation.[19] The ideal sorption of gases (including vapors) in a polymer follows Henry's law, in which the concentration of adsorbed gas is proportional to the gas pressure. However, many gas/polymer combinations do not follow this law as there is more than one sorption mechanism involved in the sorption process. For example, gas molecules can adsorb onto the nanoporous surface of the polymer. The diffusion of water through polymer films is related to the free volume available for the water molecules to find their path to the other side of the film. Free volume, either voids and pores created by disorganized chain packing or spaces generated by polymer chain reconformation, allows the transport of molecules.[21]

The polymer characteristics and medium conditions on both sides of the film influence permeation. The chemical structure, molecular weight, glass transition temperature ( $T_g$ ), polymer polarity, degree of crystallinity, presence of additives such as plasticizer or filler, crosslinking density, and film thickness can manipulate the polymer hydration and the potential access to the free volumes for water vapor molecules. Diffusion is also dependent on water vapor pressure, water activity gradient, and temperature.[22]–[24]

Some standard test methods are available for measuring the water vapor permeability of polymers. Measurements of water vapor permeation rate (WVPR) are often based on two basic methods, the desiccant method and the water method, described by ASTM E 96/E 96M.[25] A test dish with an

wide neck is filled with either water or desiccant and covered by the test specimen (polymeric film). The whole assembly is then weighed and placed in a test chamber with controlled temperature and humidity. Airflow inside the chamber is needed to maintain uniform conditions. The test dish is reweighed after a certain period of time has passed and water vapor permeability is obtained from the following equation:

WVPR = 
$$\frac{(\frac{M}{t})}{a}$$
 2-1

where M is the cumulative weight change (g), t is the time over which the weight change happens (hours), and a is the test area (cup neck area,  $m^2$ ).

#### 2.3. Human skin characteristics

Skin is the most extensive organ of the human body; it is a multilayer complex system that possesses different properties and functionalities, such as protection against external invasion, thermoregulation, sensory reception, and control of fluid loss.[26]-[28] The skin has three distinguishable layers, as shown in Figure 2-1: the hypodermis (consisting of fat cells and responsible for maintaining core body temperature), the dermis (composed of collagen and elastin fibers, and containing nerves, proteins, sweat glands, blood vessels, and hair follicles), and the epidermis (protective barrier against intrusion of external substances and loss of moisture and nutrients).[26] Epidermal cells (keratinocytes) undergo progressive differentiation, which divides the epidermis into four layers.[27] The outermost layer of the epidermis, and consequently the skin, is the stratum corneum (SC), which has a thickness of 20–40 µm. The SC is composed of corneocytes embedded in a lipid matrix [29] and plays a crucial role as a mechanical, chemical, and thermal barrier against external factors and efficiently controls transepidermal water loss (TEWL).[28], [30] The SC is the first layer that comes in contact with materials covering the skin, such as cosmetic products, transdermal adhesive patches, wound dressings, and topical films. In this research, we have focused on the key functions of skin relevant to moisture, i.e., TEWL and skin perspiration.



Figure 2-1. Schematic illustration of human skin. The hypodermis mainly consists of the fat cells with collagen split. The dermis is composed of collagen and elastin fibers and contains nerves, proteins, sweat glands, blood vessels, and hair follicles. The epidermis is mainly made of keratinocytes which are differentiating progressively to the surface in a period of 21-30 days which divides the epidermis into four layers. The outermost layer of the epidermis, SC, is made of corneocytes embedded in a lipid matrix.

#### 2.3.1. TEWL

The hydration level of the SC and the amount of water passing through it influences various characteristics of the skin, such as barrier function, mechanical strength, and visual appearance.[31], [32] TEWL is the amount of condensed water that diffuses through the SC and evaporates to the outside owing to the difference in water activity on each side.[33] Variations in TEWL are due to individual variables such as the local thickness of SC, the temperature of skin surface, sex, and age, or environmental variables such as temperature and humidity.[33]–[37] TEWL is a crucial factor in the characterization of skin barrier function. However, there is no direct method for the measurement of TEWL. If TEWL is the only source of water on the skin surface, the measured value represents the water vapor flux through the SC.[38] There are two known methods for measuring TEWL: open chamber and closed chamber. In the open chamber method, the water vapor gradient between two fixed heights in an open cylinder held against the skin is estimated; in contrast, in the closed chamber method, the gradual build-up of relative humidity (RH) inside a chamber is measured.[39] Both methods have their strengths and limitations. Numerous in vivo and in vitro studies have been published on the application and comparison between two methods.[38]–[44]

#### 2.3.2. Perspiration

Human skin perspiration is a thermoregulatory reaction to the temperature increase caused by internal or external stimuli.[45] Once perspiration is activated, the sweat (mostly made of water) is secreted to the skin surface through the active sweat glands located across nearly all of the body. Sweating depends on individual variables, such as body location, level of physical activity, sex, and age, as well as environmental factors such as temperature and humidity.[46], [47] Sweating alters the skin conditions, such as skin surface pH and hydration of the SC, and if stimulated by moderate exercise or a hot environment, reduces the resistance of unprotected skin to sunburn.[48]–[50] Different techniques and methods in diverse fields have been used to visualize, measure, mimic, and find the correlation between various phenomena and sweating.[51]–[59]

#### 2.4. Skin models

Traditionally, in vivo studies have been used to find the reciprocal effect of moisture-releasing skin elements and external elements. However, in vivo skin-related studies are often plagued by ethical issues, high costs, performance complications, and intra- and inter-subject variability; consequently, research efforts have been focused on skin models. Various materials (e.g., polyurethane, epoxy resin, and poly(vinyl) alcohol) and methods have been used to make skin replicas that mimic one or more of the skin characteristics, based on research requirements (Figure 2-2).[60]



Figure 2-2. Materials used to simulate skin properties and functions (reprinted with permission from [60]).

#### 2.5. Sunscreen

The radiation emitted from the sun is composed of UV (100–400 nm), visible (400–780 nm), and infrared (780-5000 nm) radiation.[61] With regard to human health, UV radiation is of the most concern. UV radiation consists of UVC (100-290 nm), UVB (290-320 nm), and UVA (320-400 nm) radiation. Radiation with wavelengths below 290 nm is absorbed by atmospheric ozone, with UVB and UVA reaching the earth's surface.[62] The exposure of the human body to sunlight yields certain biological benefits, such as inducing the synthesis of vitamin D<sub>3</sub> and reducing the impact of depression.[63] However, overexposure to UV radiation may have devastating effects. UVB radiation is highly energetic and responsible for skin sunburn (blisters and redness; erythema), directly inducing DNA damage in the epidermis, and, consequently, increasing the risk of skin cancer.[61], [64], [65] UVA radiation, which has lower energy and longer wavelengths, penetrates through the epidermis to reach the dermal layer. UVA radiation is responsible for skin pigmentation, photoaging, wrinkling, and indirectly, skin cancer.[64] Both forms of radiation can destroy vitamin A in the skin and impair collagen, elastin, and lipid structures, to finally induce immunosuppression.[50], [66] As such, most skin cancers are avoidable by the reduction of the natural and artificial exposure to UV radiation.[65] Nowadays, awareness regarding the importance of preventing UV overexposure is well known. The sun protection strategies consist of avoiding exposure to the sun in the hours of peak UV radiation, seeking shade, covering skin with clothes/hat/sunglasses, and using sunscreen.[67], [68]

Sunscreens contain active ingredients (UV filters) to weaken the potentially harmful UV rays effect by absorbing, reflecting, or scattering UV radiation before it can penetrate the skin and damage its components.[66] Initially, sunscreen was used to stop the sunburn reaction. However, later studies highlighted sunscreen as an important element in preventing the long-lasting effects of sun exposure, such as skin cancer.[69], [70] An effective sunscreen provides complete protection against UV radiation while maintaining the required characteristics, including photostability, non-toxicity, durability, and substantivity, in conjunction with acceptable sensory properties and appearance. In addition, sunscreen application can enhance the biomechanical barrier function of the skin.[71], [72]

#### 2.5.1. Sunscreen formulation

The essential sunscreen components are UV filters, emollients, and, in emulsion-based sunscreens, emulsifiers; the secondary components are film-formers, thickeners, boosters, and sensory enhancers.[67] UV filters are the core active ingredients in sunscreen providing the required absorbance profile and extinction properties. There are two main types of UV filters: organic and inorganic. Organic UV filters, such as avobenzone, diethylamino hydroxybenzoyl hexyl benzoate, and octocrylene, contain aromatic compounds that absorb radiation in the UV spectrum and use it in a photochemical reaction or re-emit it at a longer wavelength in the form of heat or light.[61] Based on the portion of the UV spectrum they can absorb, organic UV filters can be divided into three groups: UVA filters, UVB filters, and broad spectrum filters. A combination of UV filters, considering their photo-compatibility with each other, enables the achievement of a highperformance UV protection. In Figure 2-3, an example of the UV extinction ability of two UV filters, diethylamino hydroxybenzoyl hexyl benzoate and ethylhexyl salicylate, and their combination if each used at 10 wt.% in a sunscreen formulation, is shown.[73] Inorganic UV filters, such as TiO<sub>2</sub> or ZnO particles, resist UV mainly by absorption, some scattering, and reflection based on their particle size. [74] Particles with scattering properties can also increase the optical path length of UV radiation and reduce the probability of UV radiation reaching the skin surface.[75] The different UV attenuation mechanisms for organic and inorganic UV filters are shown in Figure 2-4.

Emollients with different polarities are key multifunctional components in sunscreen formulations. They are responsible for the solubility and photo-stability of UV filters while enhancing the sensorial properties of sunscreen.[67] Moreover, the polarity of the emollient can contribute to the optimization of UV protection.[76] Emulsifiers are essential for emulsion-based sunscreen, and define the type of emulsion, i.e., oil-in-water (O/W) or water-in-oil (W/O).



Figure 2-3. Extinction ability of two UV filters and their combination if 10% of each is used in a sunscreen formulation. The chemical structures of the UV filters are also shown.

One of the most important properties of sunscreen is the film-forming ability on human skin. Filmformers in sunscreen have a crucial role, boosting the performance of UV filters by uniformly distributing the UV filters and increasing the sunscreen film integrity.[77] Based on their polarity, different types of film-formers are available, and can be divided into vinylpyrrolidone derivatives, acrylic polymer derivatives, polyester, polyurethanes, maleic derivatives, and silicones (Table 2-1).[75]



Figure 2-4. Reaction to the UV radiation of organic (left) and inorganic (right) UV filters. (reprinted with permission from [74])

Thickeners are used to tune the rheological profile of sunscreen; boosters to enhance the spreadability of the film and the performance of UV filters; and finally, sensory enhancers to prevent the sunscreen film from feeling greasy and sticky on the skin. A good sensory perception is crucial; otherwise, especially in sunscreens with a high concentration of UV filters, consumers apply a smaller amount of sunscreen, reducing the UV protection.[78]

Main categories of film formers	Compounds (INCI)	Typical use (%)	Remarks
	VP/hexadecane copolymer		
Vinylpyrrolidone	VP/eicosene copolymer	1-3	First three, most popular film formers, oil-soluble, in waxy or liquid form
derivatives	Tricontanyl PVP		
	Aqua (and) hydrolyzed wheat protein/PVP crosspolymer	0.5-2.5	Water dispersion (≈45 % solids)
	Acrylates copolymer	1-2	Water dispersion ( $\approx$ 30 % solids), neutralization required
	Acrylates/octylacrylamide copolymer	2	Soluble in ethanol, neutralization required when using water
Acrylic based	Polyacrylate 15 (and) polyacrylate 17	1.5-3.5	Water dispersion (≈30 % solids), no neutralization required
	C8-22 alkyl acrylate/methacrylic acid crosspolymer	0.5-3	Water dispersion (≈47 % solids), no neutralization required
Maleic derivatives C30-38 olefin/Isopropy maleate/MA copolymer		1-2	Neutralization required when using water, act also as an anionic emulsifier
	Silicone acrylate copolymers	2	≈30–40 % solids
	Alkylmethylsiloxanes	2	Substantive to skin
	Silicone elastomers	4	
Silicone based	Trifluoropropyldimethylsilo xy/trimethylsiloxy silsesquioxane (and) dimethicone	1-2	≈50 % solids, fluoro-modified silicone resin
Polyamides derivatives Polyamide-8		1-3	Oil structuring polymers forming Polyamide-8 cohesive non-water- soluble film

Table 2-1.	Different types	s of film-formers	in sunscreen.	[75]
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Polyester based	Polyester-5	1-2	Water dispersible
Polyurethane based	Polyurethane-34	1-3	Water dispersion (≈38–42 % solids) suitable in low viscosity products, no neutralization required



Figure 2-5. Sunscreens categories based on the product's polarity and viscosity. (reprinted with permission from [66])

Considering the polarity of sunscreen formulation and product viscosity, sunscreen vehicles can be categorized as oil-, ethanol-, emulsion-, or water-based (Figure 2-5). Each sunscreen formulation exhibits different rheological profiles, spreadability, and sensorial properties.[79]

# 2.6. Sun protection factor (SPF) and UVA protection factor (UVAPF) measurements

The judicious choice of various available ingredients for sunscreen formulations is part of the first level to achieve full protection against UV radiation. The second requirement is the ability to form a homogenous film with an identical thickness over the covered area of the skin. The performance of sunscreen can be assessed by its UVB and UVA protection. UVB radiation is mainly responsible for skin erythema (sunburn). The redness of the skin due to sunburn results from the increased blood content of the superficial vessels in the dermis layer of the skin.[80] The comparison of the amount of energy per unit area (J cm<sup>-2</sup>) required to cause minimal erythema (MED) in the absence and presence of sunscreen, i.e., the sun protection factor (SPF) can be defined as the level of

protection against UVB. The protection against persistent pigment darkening (PPD) is used for the measurement of UVA protection. The UVA protection factor (UVAPF) is defined as the ratio of the UVA dose required to produce darkened pigments in the skin in the presence and absence of the sunscreen.

#### 2.6.1. In vivo SPF measurements

The in vivo measurement of SPF is a validated procedure used worldwide to assess the effectiveness of sunscreen. It is commonly interpreted as the difference in time taken to get sunburn on protected skin compared with unprotected skin. However, the more accurate definition is the ratio of the least required energy to cause minimum perceptible redness on protected skin to the amount of required energy to cause the same redness on unprotected skin. There are standardized and reproducible methods from different references, such as the United States Food and Drug Administration (FDA), European cosmetic toiletry and perfumery association (COLIPA), and international organization for standardization (ISO24444) for in vivo SPF assessments. These measurements are performed on the back of a healthy human volunteer. The test site is divided into unprotected and protected areas and a xenon arc lamp solar simulator (or equivalent) with a defined spectral distribution is used to irradiate a number of small sites on the covered area, as well as on the unprotected area, with incremental erythemal doses. To validate the experiment for the sunscreen under test, the same procedure follows for a reference formulation applied on the back of the volunteer. Varying degrees of erythema are generated on the test area in response to increasing the UV dose, with the erythemal responses to UV radiation visually evaluated after 16– 24 hours. The MED is determined for unprotected and protected skin based on a trained assessor's observation. Consequently, in vivo SPF (with application 2 mg cm<sup>-2</sup> of sunscreen) is defined according to the following equation:

$$SPF = \frac{MED \ (protected \ skin)}{MED \ (unprotected \ skin)}$$
2-2

#### 2.6.2. In vitro SPF measurements

Other than the ethical considerations of the erythemal endpoint to determine skin protection, in vivo SPF measurement is time-consuming, expensive, subject-dependent, and not suitable for

routine sunscreen evaluation, which explains the interest toward refining a method to assess SPF by in vitro tests. There are different approaches to the measurement of SPF in vitro. Initially, in the solution-dilution method, the sunscreen is dissolved and diluted in a (UV-transparent) solvent and the transmission through it (measured by a spectrophotometer) is compared with the pure solvent.[81] The other approach is sandwiching a thin film of sunscreen between two quartz slides.[82]

Other methods for in vitro SPF measurements are based on spectrophotometric measurement such as the absorbance (calculated from transmittance) in which a defined amount of the sunscreen is smeared on a UV-transparent substrate. The sample is placed in a UV transmittance analyzer and exposed to UV radiation (290–400 nm) to measure the factor by which the intensity of the radiation is reduced compared with the bare substrate. The methods vary in terms of the substrate material, the amount of sunscreen applied, and the application procedure. The inverse of UV transmittance of the sunscreen film, 1/T, defines the intensity reduction. At a certain wavelength  $\lambda$ , 1/T( $\lambda$ ) is assigned as a monochromatic protection factor (MPF) that should be averaged over the relevant spectral range for erythemal reactions (290-400 nm). The other parameters involved in the in vitro SPF measurements are the intensity of the light source and the erythemal action spectrum over the targeted UV range. For the light source, different reference curves are used to introduce spectral irradiance. For example, the "Albuquerque" or "Melbourne" curves match the "standard Sun." COLIPA also introduced a UV solar simulator radiation curve, which matches the spectral irradiance of the simulator used for in vivo SPF measurements. An action spectrum describes the  $(\lambda$ -dependent) response to radiation of a certain biological effect. The "erythema action spectrum" represents the susceptibility of the skin to sunburn.[83] The product of the spectral irradiance of the solar simulator,  $S(\lambda)$  (W.m<sup>-2</sup>nm<sup>-1</sup>), and the erythemal action spectrum,  $E(\lambda)$ , equals the erythemal effectiveness spectrum (W.m<sup>-2</sup>nm<sup>-1</sup>), as shown in Figure 2-6. The erythemal effectiveness spectrum and the transmittance  $T(\lambda)$  data from the in vitro experiment define the in vitro SPF of the sunscreen, as shown below:

$$SPF = \frac{\sum_{290}^{400} E(\lambda)S(\lambda)}{\sum_{290}^{400} E(\lambda)S(\lambda)T(\lambda)}$$
2-3



Figure 2-6. Erythemal action spectrum, UV-SSR source from COLIPA, and Erythemal effectiveness.

#### 2.6.3. In silico SPF calculations

The calculation of SPF based on an applicable model, i.e., in silico SPF, has been the subject of many investigations. This method uses the same algorithms as for the in vitro SPF measurement, except that the transmission through the sunscreen would be calculated based on the concentration and characteristics of the UV filters and sunscreen film properties (film irregularities and photodegradation kinetics).[84] This method is also applicable for the calculation of UVA protection parameters.[85] The challenging part of this method is the proposal of a suitable mathematical description of the film irregularity.[85]–[88] Herzog summarized the models for film irregularities, e.g., step film model, quasi-continuous step film model, and the continuous height distribution model based on the gamma distribution.[89] Application of the latter model results in more realistic predictions of the SPF.[88] The calculated in silico SPF shows a good correlation with in vivo/in vitro studies.[90], [91] Consequently, it can be used for the stimulation, design, and development of new sunscreen formulations.[92] An actual function of in silico SPF calculations is available for sunscreen producers, such as BASF sun simulator, in which the SPF and UVAPF metrics for a combination of UV filters are available.[73]

#### 2.6.4. In vivo and in vitro SPF correlation

The concept of replacing in vivo SPF studies with in vitro tests has attracted much attention. However, in vitro studies do not possess sufficient data to support reproducibility or connection to in vivo SPF.[93] One reason for the discrepancy between in vivo and in vitro methods is the lack of skin-like properties in the substrate used for in vitro studies, i.e., roughness and affinity to the sunscreen. The other major influential factor is the sunscreen application procedure that can cause significant discrepancies between different laboratories.[94] Moreover, SPF values are timedependent owing to solvent evaporation and dewetting of the sunscreen film. Therefore, the single in vitro SPF measurements are unable to achieve accurate estimation of the time-integrated UV protection.[14]

Currently, the substrate material varies from surgical tape (Transpore<sup>TM</sup> tape) to roughened polymethylmethacrylate (PMMA) plate, based on the method used.[93] A lower quantity of sunscreen is applied in in vitro studies than in standard in vivo SPF measurements (~1.3 versus 2 mg cm<sup>-2</sup>) to compensate for the difference in roughness between human skin and the substrate (the skin has higher roughness compared to artificial substrates that causes non-uniformity in sunscreen film and consequently lower measured SPF).[95]

Several studies are centered on introducing new materials to overcome the lack of good spreadability or inadequate sunscreen/substrate affinity. Different substrates, including hairless mouse skin, human SC, and porcine ear skin, have been tested as a substitute for human skin.[81], [96], [97] Recently, Sohn et al. studied 14 different polymers to find an alternative to PMMA plates.[95] In a unique study by Paula et al., a combination of an artificial skin substrate and a UV dosimeter (made of iron-complex dye/nanocrystalline titanium dioxide) was used as a substrate to match the in vivo SPF studies by tracking the minimum perceptible bleaching of the substrate.[98] Miura et al. introduced a skin mimicking substrate with roughness tuned close to human skin.[99] In this case, in vitro SPF values for the applied sunscreen amount of 2 mg cm<sup>-2</sup> were comparable with in vivo SPF values.

In addition, some studies focused on substrate modifications, such as substrate plasma treatment or pretreatment of PMMA plates with an amphoteric surfactant, to increase the spreadability of the sunscreen.[100], [101] As outlined by Ferrero et al., the surface roughness of the substrate plays an important role in SPF measurements owing to its effect on sunscreen film geometry. They correlated the optimal sunscreen amounts with the surface roughness for achieving SPF values close to in vivo measurements.[102] Miksa et al. detailed a method in which a combination of using a sun UV light source and careful adherence to application and process specifications achieved results close to in vivo SPF.[103]

However, despite the enormous effort dedicated to in vitro sun protection factor measurements, none has been successfully validated to be used instead of the in vivo SPF test.[104], [105] Possible explanations for this include the anti-inflammatory effect and the anti-oxidative properties of the sunscreen on the biological response of skin, as well as changes in the optical properties of the skin due to sunscreen application.[106] Understandably, these effects cannot be considered in in vitro measurements.[107]

#### 2.6.5. In vivo and in vitro UVAPF measurements

Even though the SPF is a well-established method for skin sunburn protection, it is not a sufficient indicator for protection against photoaging and skin cancer.[67] The functionality of sunscreen should also be assessed against the UVA wavelengths.[92] A sunscreen with only UVB blocking agents could only reach a maximum SPF of 11, which illustrates the contribution of UVA radiation to sunburn, even if not significant.[108]

For the in vivo assessment, persistent pigment darkening is used as a clinical endpoint. Similar to SPF measurement, the minimum UVA radiation dose to create the first perceptible pigmentation with and without sunscreen is used to define in vivo UVA-PF. The required exposure doses, similar to in vivo SPF, are relatively high (10–20 J m<sup>-2</sup>), which adds up to other disadvantages of in vivo study.[109] Therefore, the available in vitro UVA methods are widely accepted, instead of extra in vivo UVA measurements.[67] Indeed, as the SPF is an absolute value for the quantitative evaluation of sunscreen, the in vitro UVA method measures the quality of protection.[84] The in vitro method (Equation 2-4) is also based on transmission through sunscreen film but with different weighting functions:[110]

$$UVAPF = \frac{\sum_{320}^{400} P(\lambda)I(\lambda)}{\sum_{320}^{400} P(\lambda)I(\lambda)T(\lambda)}$$
2-4

where  $P(\lambda)$  is the PPD action spectrum and  $I(\lambda)$  is the spectral irradiance of the UV source (W m<sup>-2</sup> nm<sup>-1</sup>) in the range of 320–400 nm for PPD testing.  $P(\lambda)$  and  $I(\lambda)$  are illustrated in Figure 2-7.



Figure 2-7. PPD action spectrum  $P(\lambda)$  and spectral irradiance of the UV source  $I(\lambda)$ .

In vitro SPF and UVAPF measurements provide more detailed information than in vivo studies, such as UV transmittance value at any wavelength, the ratio of UVA/UVB protection, and broad-spectrum protection data.[111] The ratio of the UVA attenuation to UVB attenuation is defined according to the following equation:

$$\frac{UVA}{UVB} = \frac{\frac{\int_{320}^{400} A(\lambda) d\lambda}{\int_{320}^{400} d\lambda}}{\frac{\int_{290}^{320} A(\lambda) d\lambda}{\int_{290}^{320} d\lambda}}$$
2-5

The UVA/UVB ratio varies from 0 (in case that there is no protection against UVA radiation) to 1 (for the sunscreens providing equal absorption through the whole UV spectrum). The closer the ratio is to 1, the more similar are the integral UVA- and UVB-attenuation values, meaning more uniform protection over the UV radiation range.

The wavelength for the area under absorption curve (A = -log T) from 290 nm to this wavelength is 90% of the whole area (from 290–400 nm), and is defined as the critical wavelength,  $\lambda_c$ :[110]

$$\int_{290}^{\lambda_c} A(\lambda) d\lambda = 0.9 \int_{290}^{400} A(\lambda) d\lambda$$
 2-6

 $\lambda_c$  can be used as a balance between UVA and UVB protection. To achieve the ideal sunscreen,  $\lambda_c$  should be increased to higher wavelengths such as 375 or 380 nm.[112]

#### 2.7. Sunscreen efficacy

SPF and UVAPF are considered as two of the most important indicators for sunscreen performance. As mentioned earlier, the optimal concentrations of the UV filters with proper absorbance and photostability properties are the prerequisites for sunscreen efficiency. However, sunscreens with the same composition of the UV filters can have different SPF values.[113], [114] A uniform distribution of UV filters, created by the formation of a homogenous sunscreen film with an identical thickness over the covered area, is the next important parameter. With this, the sunscreen film protection would be similar to that obtained by wearing clothes.[115] In reality, the deviation from the ideal functionality occurs owing to primary factors such as the sunscreen vehicle, the applied amount of sunscreen, skin condition, and secondary factors such as wearing clothes, bathing, exercising, swimming, and sweating.[116]–[122] These parameters mainly affect the key connecting parameters, i.e., film thickness and film thickness distribution of sunscreen that determine the level of UV protection.[113]

#### 2.7.1. Sunscreen film thickness and thickness distribution

In this section, the mathematical relationship between UV transmittance and thickness of an ideal sunscreen film is first presented based on Lambert-Beer law. Second, the effect of the irregularity of the sunscreen film using a simple model is explained, and finally, a study designed to exclusively investigate the effect of film homogeneity on SPF is presented.

#### 2.7.1.1. UV transmission through sunscreen film described by the Lambert-Beer law

According to Lambert-Beer law, the extinction (absorption) of light through a mass at a specific wavelength is calculated as follows:

$$A(\lambda) = \varepsilon(\lambda) c d \qquad 2-7$$

where  $\varepsilon(\lambda)$  is molar extinction coefficient (L mol<sup>-1</sup>cm<sup>-1</sup>), c is the molar concentration (mol L<sup>-1</sup>), and d is the optical path length of the cuvette (cm) containing the mass. A( $\lambda$ ) is derived from transmittance, T( $\lambda$ ):

$$T(\lambda) = \frac{I(\lambda)}{I_0(\lambda)}$$
 2-8

where  $I_0(\lambda)$  is the intensity of transmitted light through the pure solvent and  $I(\lambda)$  is the intensity of the transmitted light through absorbing mass contained solution (with the same solvent). A( $\lambda$ ) and T( $\lambda$ ) are related through the following equation:

$$T(\lambda) = 10^{-A(\lambda)} = 10^{-\varepsilon(\lambda) c d}$$
 2-9

This equation represents the thickness-transmission relationship for a UV filter. However, in most cases, sunscreens have a combination of UV filters and average  $\overline{\epsilon}(\lambda)$  and  $\overline{c}$  quantities are used:[92]

$$\overline{M} = \frac{\sum_{i=1}^{n} \beta_i}{\sum_{i=1}^{n} \frac{\beta_i}{M_i}}$$
2-10

$$\bar{c} = 10 \frac{\sum_{i=1}^{n} \beta_i}{\bar{M}}$$
 2-11

$$\bar{\varepsilon}(\lambda) = \frac{\sum_{i=1}^{n} \frac{\varepsilon_i(\lambda).\beta_i}{M_i}}{\sum_{i=1}^{n} \frac{\beta_i}{M_i}}$$
2-12

where  $\overline{M}$  is the average molecular weight,  $\beta i$  is the concentration as a percentage (w/v), Mi is the molar mass, and  $\epsilon i$  ( $\lambda$ ) is the molar extinction coefficient of a UV filter. The transmission calculation for a sunscreen as described by the Lambert-Beer law then becomes:

$$T(\lambda) = 10^{-\overline{\varepsilon}(\lambda)\,\overline{c}\,d}$$
 2-13

#### 2.7.1.2. Film irregularities

The effect of irregularities in sunscreen film was first modeled by O'Neill presented as a step film model.[87] The original uniform film, turning into an irregular film with the same average thickness, is illustrated in Figure 2-8. The side view of the homogenous film is a rectangle with a horizontal extension of 1 and a certain thickness, d. A fraction of the original film given by the thickness f.d and the width g is removed from its place and is put on top of the other side. The original rectangle becomes two film fractions with new dimensions: (1-f)d.g and d'.(1-g). In this

way, the amount of absorbing sunscreen stays constant and the transmission varies only due to changes in thickness:

$$d' = d(\frac{fg}{1-g} + 1)$$
 2-14

$$T_{SF} = g 10^{-\overline{\varepsilon}(\lambda) \, \overline{c} \, d(1-f)} + (1-g) 10^{-\overline{\varepsilon}(\lambda) \, \overline{c} \, d\left[\frac{fg}{1-g}+1\right]}$$
2-15

To clearly show the impact of induced irregularity, we assumed  $\bar{\epsilon}(\lambda)\bar{c} = 1$ , d=1 cm, g=0.5, and f=0.5. T( $\lambda$ ) and T<sub>SF</sub>( $\lambda$ ) (transmission of step film) will be calculated as 0.1 and 0.174, respectively, with the corresponding MPF of 10 and 5.75.



Figure 2-8. Side view of the uniform sunscreen film and the corresponding irregular film.

The result confirmed that with the same average thickness of sunscreen film, the transmission of a uniform film was lower than that of the corresponding irregular film.

In an in vivo study, the effect of the inhomogeneous distribution of UV filters on SPF measurements was investigated by defining two routes for the application of UV filters, as shown in Figure 2-9.[106] In the first case, an O/W emulsion sunscreen (containing 8.5% UV filters) was applied directly onto the skin, whereas the same emulsion without UV filters was placed inside an optical cell above the skin. In the second case, the emulsion without UV filters was applied onto
the skin while the mixture of emulsion/sunscreen (1:1) was placed inside an optical cell above the skin. Another protocol similar to the previous protocol was defined in which the dissolved emulsion and sunscreen were used inside the optical cells, respectively. The presence of UV filters inside the optical cell represents the uniform distribution of UV filters in both protocols. The in vivo SPF measurements for four cases illustrated the direct influence of UV filter distribution. The more uniform the distribution, the higher SPF was achieved. In the next sections, we describe the primary and secondary factors affecting the thickness and thickness distribution of sunscreen film.



Figure 2-9. (a) and (b) Arrangement for the determination of SPF for two protocols. (c) Measured SPF for different protocols (reprinted with permission from [106]).

## 2.7.2. Skin surface characteristics

The first inevitable obstacle in achieving uniformity of the sunscreen film on the skin is the skin itself. Owing to the combination of furrows, hair follicles, sweat glands, and wrinkles on the skin, its topography resembles a pattern of peaks and valleys. Understandably, this roughness will influence the uniformity of the shaped film on the skin. The density of the furrows and, consequently, the skin topography varies between different sun-exposed body sites.[123] The skin topography is also dependent on age. Korn et al. investigated the skin texture on different body

locations using a skin replica and confocal chromatic imaging in four age groups.[124] As shown in Figure 2-10, both anatomical sites and increasing age have a significant impact on skin roughness and therefore on the uniformity of topically applied sunscreen. Moreover, Gebauer et al. showed that for furrows with a larger volume, the distribution of UV filters was more inhomogeneous.[123]



Figure 2-10. Color-coded skin surface topography of different body sites and age group (reprinted with permission from [124]).



Figure 2-11. In vitro SPF vs PMMA plate roughness (application dose: 1 mg cm<sup>-2</sup>) (reprinted with permission from [102]).

Similar to the roughness of human skin, the characteristics of the substrate in skin-like replicas in in vitro studies affect assessing sunscreen performance.[125], [126] As briefly mentioned in Section 2.6.4, the roughness of the substrate surface and the affinity to the sunscreen film are crucial owing to their influence on spreading protocol.[127] As shown in Figure 2-11, the in vitro SPF has a non-linear relation with surface roughness.[102]

In real-world conditions, the best possible photoprotection is achieved by application of the sunscreen product as uniformly as possible, which raises the second major obstacle in reaching optimum UV attenuation: the inadequate applied amount of sunscreen.

## 2.7.3. Amount of sunscreen applied

Even though the skin surface topography influences the uniformity of the sunscreen film, if a sufficient amount of the sunscreen with suitable rheological properties is applied, the expected skin protection will be delivered. The standard quantity of applied sunscreen in in vivo studies (resulting in reproducible SPF values[116]) and the recommended amount for the consumers is 2 mg cm<sup>-2</sup>. As outlined by Parr and Diffey, despite broad variation in skin characteristics, an average volume of a sunscreen required to fill all the furrows presented on the area of 1 cm<sup>2</sup> of skin would be between 1 to 2 µl. Thus, sunscreen applied at a concentration of 2 mg cm<sup>-2</sup> forms a film with a thickness of 0.02 mm which covers the peaks (with a maximum thickness of 0.01 mm over the peaks) and the valleys in between would be protected by a sunscreen layer equivalent to at least their depth.[128] Recently, Korn et al. calculated the void volume of skin based on void volume fraction, v<sub>f</sub>, and surface texture parameter (arithmetic mean height, S<sub>a</sub>). Their results showed that the smallest and largest roughness (S<sub>a</sub> = 10.63 µm and 46.53 µm, respectively) resulted in a void volume of 0.77 mm<sup>3</sup> cm<sup>-2</sup> and 3.35 mm<sup>3</sup> cm<sup>-2</sup>, respectively. The results clarify that for a sunscreen with an approximate density of 1 mg mm<sup>-3</sup>, the void volume is smaller than the recommended application dose, except for the cases of largest roughness.

However, consumers apply considerably less than the recommended amount of sunscreen (0.39– 1.3 mg cm<sup>-2</sup>).[129]–[133] As a result, the actual SPF would be lower than that expected from the product label.[134] Lademann et al. used a sunscreen containing a fluorescent dye to qualitatively visualize the sunscreen distribution on the skin of beachgoers.[135] As shown in Figure 2-12, there were areas of the body that were almost uncovered. On the parts of the body covered, the application of sunscreen was inadequate to obtain the SPF assigned to the product label (10% or less of the recommended amount).



Figure 2-12. Fluorescent dye-containing sunscreen applied inadequately on different sites of the body (reprinted with permission from [135]).

Inadequate application of sunscreen also impairs the uniform film distribution. As shown in Figure 2-13, the underapplication of sunscreen led to the formation of an irregular film, leaving peaks and larger areas of the skin susceptible to sunburn.[136]



Figure 2-13. Effect of application amount of sunscreen on film uniformity. The quantities of applied sunscreen are 0, 0.5, 1, 1.5, and 2 mg cm<sup>-2</sup> (reprinted with permission from [136]).

The use of sunscreens with extremely high SPF or the reapplication of sunscreen has been suggested to compensate for the common underapplication of sunscreen.[68], [116], [122], [137], [138] Moreover, if the consumer spends more time on the application and reapplication of sunscreen, the probability of applying a higher amount of sunscreen is increased.[139]

## 2.7.3.1. Sunscreen application thickness and SPF relationship

Given the importance of the applied quantity of sunscreen on skin protection, several studies focused on the correlation of the sunscreen film thickness (resulting from the amount applied) and SPF. The reported data are contradictory, as shown in Table 2-2, and cannot be fully determined. A common point confirmed in all studies was that SPF value decreased with inadequate application. Ferrero et al. demonstrated that the relationship between the in vitro SPF and the amount of sunscreen applied was dependent on the roughness of the substrate surface (Figure 2-14).[102] In contrast, in silico SPF calculations have shown that the relationship between SPF and the applied quantity of sunscreen may vary based on sunscreen's UVA/UVB ratio (Figure 2-15).[84]

Study	volunteers	Labeled SPF	Applied quantities (mg cm <sup>-2</sup> )	Proposed relationship	
Schalka et al. ([140])	40 (divided in 2 groups)	15, 30	0.5, 1, 1.5, 2	Exponential	
Faurschou and Wulf ([141])	20	4	0.5, 1, 2, 4	Exponential	
Ou-Yang et al. ([138])	251 (study A), 76 (study B)	30, 50, 70, 100	0.5, 1, 1.5, 2	Linear	
Bimczok et al. ([142])	At least 30 (divided in 3 tests center)	20 (both lotion & spray), 25	0.5, 1, 2	Linear	
Teramura et al. ([137])	23	50	0.5, 1, 2	Logarithmic	

Table 2-2. A summary of different studies determining the sunscreen application quantity and in vivo SPF relationship.

Liu et al. ([143])	40	4, 15 (low)	051152	Linear
	40	30, 55 (high)	0.5, 1, 1.5, 2	Exponential



Figure 2-14. Effect of the application dose of sunscreen on in vitro SPF using PMMA plates with different roughness (reprinted with permission from [102]).



Figure 2-15. Effect of application dose of sunscreen on calculated SPF for two sunscreens with labeled SPF 10 and 30. The performance of sunscreens depends on the UVA/UVB ratio ranging from 0.13 to 0.98 (reprinted with permission from [84]).

## 2.7.4. Sunscreen vehicle

Another important influential parameter in the achievement of optimal skin protection is the sunscreen vehicle. It is responsible for providing the required rheological characteristics for

suitable film formation and a pleasant feeling for the consumer.[84] A good spreadability and a pleasant sensory perception encourage the consumers to apply greater amounts of sunscreen (Figure 2-16).[78], [112] The type of sunscreen impacts the amount applied by consumers; for example, the geometric means of the application thickness for the lotion, spray, and stick sunscreens are 1.1, 1.6, and 0.35 mg cm<sup>-2</sup>, respectively.[144] The viscosity and spreadability of the sunscreen vehicle influence the thickness of the film and, consequently, the SPF. Sohn et al. compared five vehicle formulations (O/W cream, O/W spray, W/O emulsion, gel, and lipo-alcoholic spray) with an identical concentration of UV filter. Sunscreens with a low viscosity showed a lower mean thickness of film and lower in vitro SPF than those with highly viscous vehicles.[113]



Figure 2-16. Effect of spreadability and sensory properties of suncreen on realistic UV protection (reprinted with permission from [78]).

## 2.7.5. Application method

Light or firm application, low or high pressure, and long or short spreading time can affect the surface thickness of the sunscreen and consequently the SPF.[113], [145], [146] An example of the effect of light or firm application of sunscreen is presented in Figure 2-17.



Figure 2-17. Effect of light (open columns) and firm (hatched columns) sunscreen application on the thickness of the sunscreen film after application. from ref (reprinted with permission from [145]).

### 2.7.6. Secondary parameters

Skin (substrate) roughness, the amount of sunscreen applied, the sunscreen vehicle, and the application method are the primary factors influencing sunscreen performance. After the application of sunscreen, other conditions can affect the UV protection supplied. The required properties to maintain the sunscreen film efficiency after application are sunscreen durability (resistance to removal by wear) and substantivity (resistance to removal by water). Both definitions can be used as a general term for resistance to removal.

Real-life activities, such as dressing, exercising, bathing, swimming, toweling, and sweating, are the secondary parameters influencing the sunscreen efficacy.[116]–[122] In addition to changing the film thickness and film distribution, these activities can affect the substantivity. As SPF measurements do not provide an evaluation of sunscreen in aqueous conditions or in relation to "rubbing off,"[147] another test is required to examine the substantivity of sunscreen. Therefore, the water-resistance test was introduced as a method to assess the third most important performance attribute of sunscreens (after UVB and UVA protection), i.e., substantivity of sunscreen.[148]

### 2.7.7. Sunscreen water resistance

To measure the capacity of a sunscreen to resist removal by water, different in vivo and in vitro methods have been introduced; most compare the SPF value before and after immersing the sunscreen-treated area in water. The standard in vivo water resistance test consists of a first application of sunscreen (2 mg cm<sup>-2</sup>) on the back of volunteers followed by initial SPF measurement after 15–30 minutes of sunscreen self-leveling. The test comprised cycles of immersion in water (20-minute immersion of the sunscreen-covered area in temperature-controlled water in a jacuzzi/spa with controlled water circulation) followed by air drying for 20 minutes. If the measured SPF value after two cycles of immersion is equal to or greater than 50% of the initial SPF, the product is labeled "water resistant"; if this criteria is met after four cycles of immersion, the sunscreen is labelled "very water resistant."[149], [150]

Besides the standard in vivo test, other techniques can be used to visualize the difference in water resistance of sunscreen on the skin or as an indirect assessment of the substantivity of the sunscreen in contact with water. For example, after exposure to water, many sunscreens lose their clarity and develop a white appearance on the skin; this is likely due to the diffusion of water into the sunscreen.[151] The reaction of different sunscreen formulations to water immersion is shown in Figure 2-18a; two formulations did not have a white appearance after immersion in water, implying higher water resistance.



Figure 2-18. Techniques for assessing the water resistance of the sunscreen. (a) Sunscreen whitening in contact with water. Sunscreen c2 and F1 showed no white appearance after immersion in water indicating great water resistance (reprinted with permission from [151]). (b) UVA images of the forearm before and after application of three sunscreens and after immersing the treated skin into the water for 10 minutes. The dark areas represent the UV absorption of sunscreens. Sunscreen B shows high water resistance than sunscreen A and C (reprinted with permission from [151]). (c) Relationship between contact angle measurements and water resistance of sunscreen. Data for 66 commercial and developed sunscreens are shown. The water resistance data is obtained by the SPF measurement/immersion/SPF measurement method (reprinted with permission from [152]). (d) In vitro SPF measured on three regions of interest of a tape strip (upper dry/unexposed sunscreen region, interfacial region, lower immersed region) following the water exposure. (reprinted with permission from [153]).

It is also possible to follow changes in the distribution of UV filters before and after immersion. In Figure 2-18b, the distribution of UV filters on the skin are illustrated for sunscreens with different formulations and water-resistance properties. The darker the application area after immersion represents the higher concentration of UV filter remaining on the skin.[151] The other suggested in vivo method for the evaluation of water resistance is the measurement of the wettability of the sunscreen-covered skin, based on the contact angle.[152] The results obtained from the standard in vivo water resistance test for 66 commercial sunscreens (Figure 2-18c) showed that if the water contact angle on the skin was greater than 30°, the sunscreen could be categorized as water resistant without further testing. It is also possible to follow the changes in sunscreen film thickness after cycles of immersion based on changes in the intrinsic fluorescence intensity of the sunscreen.[145]

The alternative in vitro procedures to evaluate the sunscreen's water resistance are based on in vitro SPF measurements before and after water immersion. The substrates vary from excised

human epidermis to roughened PMMA plates (the most common synthetic substrate for in vitro skin measurements).[154]–[157] Furthermore, the comparison of the UVA/UVB ratio before and after immersion can illustrate which of the ingredients are more prone to being washed off.[155], [156] If the ratio is constant and the water resistance is decreased, it means the formulation is removed as a whole from the skin, rather than the specific UV filters. If the ratio is significantly higher or lower than the original value, it indicates the preferential wash-off of the UVB or UVA filters, respectively.

Although the in vitro methods help to avoid the negative aspects of the in vivo tests, owing to either lack of correlation with the in vivo results or the failure to accurately predict the water resistance of sunscreen, there is still no universally accepted in vitro method to test the water resistance of sunscreen.[158] However, new techniques and substrates have been introduced to bridge the gap between the in vitro and in vivo water resistance tests.[148]

When sunscreen-treated skin is exposed to water, different processes occur, such as sunscreen wash-off, water diffusion into the bulk sunscreen, or leaching of UV filters from the film, damaging the integrity and substantivity of the film.[153] Puccetti's protocol revealed that the reduction in the concentration of UV filters on the skin was more significant at the air/water/skin interface. In this method, the sunscreen-covered area of the skin was vertically divided into three parts. The upper part stays outside the water, the middle part stays at the air/water interface, and the lower part is immersed in water. The results, shown in Figure 2-18d, illustrated a significant reduction in the concentration of UV filters at the interface. Hence, the waves and splashing at the interface can intensify the washing off process. Skin pretreatment with cosmetic products could also impair the sunscreen's substantivity after water exposure (possibly due to reducing the adhesion between skin and sunscreen).[117]

The substantivity of the sunscreen can be enhanced in two ways: increasing the adhesion between skin and sunscreen (sunscreen interaction with the SC by chemical bonding); or by creating a hydrophobic environment that repels water.[159], [160] Achieving these conditions is dependent on the type of sunscreen vehicle and the active ingredients of the sunscreen. For example, a comparison between O/W and W/O emulsion sunscreens showed that the O/W systems were more prone to wash-off due to the presence of a hydrophilic emulsifier.[67], [159] Moreover, stick products provide high resistance to removal because the waxy vehicle does not contain a hydrophilic component.[149] Although inactive ingredients such as emollients or solvents ensure

the proper distribution of the UV filters on the skin, hydrophobic film-formers plays a crucial role in maintaining the integrity of sunscreen films and increasing the water resistance of sunscreen.[8], [77]

#### 2.7.8. Sunscreen sweat resistance

There are no specific standards used for the evaluation of the sweat resistance of the sunscreen. Some supplementary performance protocols are available, such as the measurement of in vivo SPF or the analysis of tape strips of the skin before and after sweating induced by exercise or hot/humid environments.[149] The in vivo water resistance test is a standard method for the assessment of the sunscreen's substantivity. However, the water resistance test does not represent the influence of sweat on the UV protection of sunscreen because sweating affects the substantivity differently compared to all the secondary parameters. Sweating is the only condition in which the water comes into contact with the sunscreen from the beneath film and impairs the substantivity. Few studies have focused on the interaction between sweating and the sunscreen film, as outlined here.

In 1946, Giese and Wells tested 100 different sunscreen formulations to determine the required properties for an ideal sunscreen, including sweat and water resistance.[72] For all tested formulations, the SPF was reduced after sweating and washing the skin. However, the water resistance was more affected than sweat resistance (Figure 2-19a). Aziz et al. evaluated the performance of more than 10 sunscreens after exercise-induced sweating and swimming.[161] The mean reduction of SPF after sweating and swimming was 47% and 60%, respectively (Figure 2-19b). Leroy and Dompmartin compared the water and sweat resistance of two types of formulations (O/W and W/O emulsions) and showed that, even though the sweat resistance and water resistance for both formulations were decreased by the same proportion, the W/O sunscreen had higher substantivity in both water and sweat resistance test (Figure 2-19c).[121] Bodekær et al. tested the effect of wearing clothes, exercising, bathing, and toweling on the performance of two sunscreens with the same SPF: one containing organic UV filters and one containing an inorganic UV filter (TiO<sub>2</sub>).[118] The SPF values of the sunscreen with inorganic and organic UV filters were reduced by 38% and 41% after 4 hours and by 55% and 58% after 8 h, respectively; the timeline of each activity, along with the SPF measurements after 4 and 8 hours, is shown in Figure 2-19d. Korting and Schöllmann presented the liposomal sunscreen formulations with high water and sweat resistance against water immersion or profuse sweating, respectively (Figure

2-19e).[162] Moehrle used UV-detecting spore films to investigate the effect of sunscreen application on erythema prevention in male triathletes during the competition (swimming, biking, running). The result demonstrated that despite the use of water-resistant sunscreen (SPF +25), visible sunburn occurred, possibly due to sunscreen wash-off by water or sweating.[163] Bauer et al. investigated the usability and acceptance of two forms of sunscreens (gel and milk) among outdoor workers by performing a randomized, single-blind, crossover study.[79] The results regarding sweat resistance showed that even though both sunscreens were rated as "good" or "very good" by the participants, eye burning or redness due to sunscreen wash-off was more often reported for the milk sunscreen than for gel sunscreen (7.5% vs. 2.5%). Recently, Ruvolo et al. compared the effect of sunscreen reapplication on two active and non-active groups of volunteers. The results shown in Figure 2-20 illustrate the effect of sweating on SPF reduction after a single application of sunscreen.

In contrast, some studies have investigated the impact of sunscreen on skin perspiration. Connolly and Wilcox examined the impact of sunscreen application on physiological variables, including average skin temperature, ventilation, heart rate, and sweat loss, and found that sunscreen application did not impair sweat loss or heat dissipation from the body.[164] Ouyang and Schmalenberg applied two lotion sunscreens and two spray sunscreens to the forearm of the selected subjects to check the effect of sunscreen application on sweating and skin temperature,[165] and found that the skin temperature was decreased instantly owing to vehicle evaporation but that the sweat rate after exercise was not affected by the sunscreen film. Aburto-Corona and Luis Aragon-Vargas used two water-resistant sunscreens, one containing organic UV filters and one containing an inorganic UV filter (TiO<sub>2</sub>), to investigate changes in exercise-induced sweating in the presence of sunscreens and compared the results with the effect of antiperspirants on sweat production.[166] The effect of sunscreen containing the inorganic UV filter on sweat reduction was similar to that of an antiperspirant. However, sunscreens with organic UV filters did not affect sweat production.

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a	0	Dintment				Before	After	After				C	OPE	D			DE I	Dest	dia opp
						treatment	sweating	washin	ıg		Numbe	er of Stati	C SPF	Po	st-imme	Preduce	SPF	Post-swe	ating SPF
	4	1A bentonite				18	12	4		O/W emul	eion 10	25.9	n SPF	Mean	SPF	Reduc	47	13 1+5 4	And
	5	0A vanishing o	rean	n and stea	rate mixture	22	13	9		W/O emu	sion 10	23.0	±4.9	13.74	±6.4		35	14.1±7.9	33
	5	0C vanishing o	rean	n and stea	irate mixture	29	19	10											
	5	8 stearate mixt	ure			23	15	13										Exercise bike	
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	3	C lanolin-petrolatum			15	3	-		u	Activity									
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	1	01 tincture of	benz	oin		28	16	-				0	2	4		6	8	Hours after	
	С	Commercial No	. 1			24	4	1										application	
<b>1</b> _	С	Commercial No	. 2			19	6	8			Time after application	Inorganic mean SPF	Rang	e	Organi mean S	ic SPF	Range	P-value	
D										(h)	(SD)			(SD)		6 4 17 1	-0.0001	_	
		Following sweating			Following swimming					0.5	6.5 (3.1)	3.0-1	5.6	11.2 (3	6.2)	6.4-17.1	<0.0001	_	
	Preparation	"Passive" SPF	:	SPF	No. of subject	Reduction	SPF	No. of subject	Reduction	_	8	2.6 (0.85)	2.0-5	0	4.5 (1.0	6) 6)	2.4-12.3	<0.0001	-
	Modoratow	of a second seco			(70)		lested	(74)			Percent		-	Percen	ıt .			-	
	products					10	I	<b>N</b> / <b>T</b>	1		4	61.5 (30.1)	18-13	2	58.9 (1	8.0)	30-91	0.72	
	Опуна-Desci Тап	rt 4.3±1.0		5.5±0.7	,	19		N1			8	45.0 (21.9)	26-10	4	41.6 (1	7.1)	20-84	0.55	
	Uvex Controlux	4.5±0.8		3.0±0.5 2.7±0.9	9	33	18-10	NT 5	67										
	Piz-Buin Protective	4.8±1.0		-	NT	32	3.8+0.8	13	20	-		P2 (SPF 12/15) Sunscreen 2 (SPF 30)							
	Fannning Cream PAB	A 4.9±0.6	:	3.7±1.2	9	24		NT		e		In-hou	ise control reen 1 (SPF	(SPF 15) 50+)		<ul> <li>Sunscreen 3 (SPF 25)</li> <li>Sunscreen 4 (SPF 15)</li> </ul>			
-	Piz-Buin Ex	trem 5.0±1.2		4.5±0.7	12	10	4.5±0.6	13	10	_	100							98.8	
1	Creme Bron	nzage 5.4±1.2	:	3.9±0.8	11	29	3.5±1.0	21	35		90		95.3 89.4	87.6	95.7	953	95.3	91.4 95.3 91.2 91.2	
	Extra sun pi	rotection produc	ts								80				8	3.6			
	Golden Bear Breast Crea	uty 6.0±1.3	1	2.7±0.8	13	56	2.4±0.5	7	60		70	19.7							
	Anti Solaire 101	no. 6.5±1.9	-	3.0±0.6	14	55		NT		-	ê 60						·····		
	Contralum	6.6±2.0		4.0±0.9	12	39	2.2±0.4	7	66		50								
	Maximal su products	n protection								6 40									
	Spirit PABA	5%	:	5.4±1.0	10	51	2.2±0.7	8	80		30								
	Original Ecl	lipse Range 8-1	4	6.3±0.5	8	42	4.8±1.0	8	56							-			
	rotar Ecups	c	-	5.0±0.9	°	33	3.1±0.8	•	14	_	20								
	Ultra sun pr	rotection product	8							4	10					-			
-	Ilrido		Ľ	7.2±1.6	14	57	3.8±0.8	7	78	-	, †								_
	Contralum   Bain da 8-1-	Ultra Range 5->	19	6.5±1.7	14	61	3.4±0.5	7	80	-	Af	ter plain water i	mmersion	After salt	water im	mersion	After	perspiration	1
	oam de Sôle	eu	ľ	0.7XU.3	10	47	0.420.9	l'	0.5	1	0			and a set of the					

Figure 2-19. (a) Sweat and water resistance of various ointments (150 mg per 52 cm<sup>2</sup>). The values are the ratios of the time for appearance of minimal erythema with the ointment to the time for appearance of minimal erythema without the ointment (Reprinted from [72]). (b) Sweat and water resistance of a variety of sunscreens with different levels of sun protection, (Reprint from [161]). (c) Influence of sunscreen formulation on water and sweat resistance, (Reprint from [121]). (d) Activity schedule after sunscreen application and the change in mean SPF over time, (reprinted with permission from [118]). (e) Change in mean SPF in relation to the initial static SPF (set at 100%) under the influence of plain water and salt water immersion and perspiration, (reprinted with permission from [162]).



Figure 2-20. (a) Measured SPF protection for the non-active group at the testing time points over the 6-hour study. (b) Measured SPF protection for the active group at the testing time points over the 6-hour study. (c) UV reflection images of single and repeated sunscreen applications for non-active subjects. A, 15 minutes after product application, B, 2 hours after product application, C, 4 hours after product application, and D, 6 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application, B, 2 hours after product application, C, 4 hours after product application. (reprinted with permission from [167])

# 3. Materials and methods

## 3.1. Materials

## 3.1.1. Artificial skin materials

Two artificial skins have been prepared in this project, one each for TEWL and perspiration simulators. The first is a single layer substrate composed of gelatin, hydroxyethyl cellulose, glycerol, and formaldehyde. The second is a four-layered substrate that comprised a gelatin-based layer, PMMA film, double-sided adhesive, and polycarbonate membrane.

Gelatin is a non-toxic, water soluble, flexible material with a chemical structure similar to collagen (the main component of skin, tendon, and bone).[168], [169] By regulating its properties, gelatin can mimic the chemical and physical properties of human skin as a water-responsive skin replica.[168], [170]–[174] The chemical structure of gelatin obtained from collagen hydrolysis is presented in Figure 3-1. Gelatin from porcine skin (gel strength ~175 g Bloom, Type A) from Sigma Aldrich (Denmark) was used for both artificial skins.





Glycerol is a non-toxic polyol compound used as a plasticizer in artificial skin formulations. Glycerol incorporation eases the simulation of human skin in terms of flexibility and mechanical properties.[175] Glycerol (>99.5%) was obtained from Sigma Aldrich (Denmark).

To achieve a water-responsive artificial skin replica, gelatin was cross-linked by formaldehyde. Cross-linking enhances the hydrolytic stability of gelatin and the final film acts as a waterswellable but insoluble matrix.[173], [176], [177] One of the mechanisms for gelatin cross-linking with formaldehyde is shown in Figure 3-2.[178] Formaldehyde (ACS Reagent, 37 wt.% in H<sub>2</sub>O, containing 10%–15% methanol as the stabilizer) was obtained from Sigma Aldrich (Denmark).



Figure 3-2. An example of gelatin cross-linking by formaldehyde. The reaction of the amino group of lysine and an aldehyde results in the formation of an imine. Subsequently, an aminal, the amine form of an acetal is formed (reprinted with permission from [178]).

Hydroxyethyl cellulose is a nonionic water-soluble polymer used for enhancing the mechanical properties of the artificial skin.[173] Ashland kindly provided hydroxyethyl cellulose (Natrosol<sup>™</sup> 250 HHR, PC grade) for use in this study.

The PMMA film was used as a support for the gelatin-based layer of the artificial skin used in the perspiring skin simulator. PMMA film (PLEXIGLAS® Film 0F058, thickness: 200  $\mu$ m) used in this study was provided by Evonik (Germany), which has high UV transmittance and high mechanical strength.

A double-sided adhesive was used for attaching the PMMA film to the membrane. I selected an acrylic-based adhesive with hydrophobic properties and high transparency to UV light (tesa® 4900, thickness: 50 µm) from Tesa (Germany).

A polycarbonate membrane was chosen to regulate the water flow through the substrate used in the perspiring skin simulator. The track-etched hydrophilic membrane (PCT0220030, pore size:  $0.2 \mu m$ , pore density:  $3 \times 10^8 \text{ cm}^{-2}$ , thickness:  $10 \mu m$ ) was purchased from Sterlitech (USA).

## 3.1.2. Film-forming polymers

Acrylate/octylacrylamide copolymer is a hydrophobic film-former used for many applications in topical films and water-resistant sunscreens.[8], [11], [77], [179], [180] This copolymer was used as a film-forming polymer in paper I and as a film-former in the sunscreen formulation in papers II and III (Figure 3-5). Acrylate/octylacrylamide copolymer was obtained from Nouryon Surface Chemistry LLC.

Polyurethane is a versatile elastomer used in various industries for numerous applications, including polyurethane foams, high-performance adhesives, and surface coatings. Polyurethane can also be used as a film-forming agent in sunscreen formulation and other cosmetic products. For paper I, polyurethane-64 from Covestro was used. Polyurethane-64 is formed by a multi-step reaction in which isophorone diisocyanate and a mixture of polybutylene glycols are used as the monomers and 4,4'-methylenebis(cyclohexylamine) is used as the chain extender. This polymer is soluble in ethanol.

The polymer shellac is composed of polyesters and single esters that contain a large proportion of hydroxyl and carboxylic acid (Figure 3-3). Shellac is used in various applications, such as surface coating, drug delivery, and as a cosmetic ingredient.[181]–[183] For this study, shellac was obtained from Mantrose-Haeuser Co.



Figure 3-3. Chemical structure of shellac (reprinted with permission from [182]).

Vinylpyrrolidone/acrylates/lauryl methacrylate copolymer has three distinguishable functional groups: an oil-soluble group; an anchoring group; and a water-soluble group. Thus, the final formulation will be a water-dispersible polymer with hydrophobic character. Ashland kindly provided this copolymer.



Figure 3-4. Chemical structure of vinylpyrrolidone/acrylates/lauryl methacrylate copolymer.

Silicone acrylate is a graft copolymer of acrylic polymer and dimethylpolysiloxane used as a filmforming agent and as a dispersant for pigments in sunscreen and color cosmetics, respectively. The silicone ingredients are known to cause substantivity to the skin and to form soft and flexible films. The sample of silicone acrylate used in this study was obtained from Shin-Etsu Silicones.

# **3.1.3.** Model sunscreen ingredients

The materials used for the sunscreen formulations are listed in Table 3-1 and their chemical structures are presented in Figure 3-5.

Ingredient category	Chemical component	Manufacturer		
UVA filter	Diethylamino hydroxybenzoyl hexyl benzoate	BASF		
UVA filter	Butyl Methoxydibenzoylmethane	DSM Nutritional Products Europe Ltd		
UVB filter	Octocrylene	BASF		
UVB filter	Ethylhexyl salicylate	DSM Nutritional Products Europe Ltd		

Table 3-1. Composition of the investigated sunscreen formulation.

Emollient	Dibutyl adipate	BASF
Emollient	Phenoxyethyl caprylate	Evonik Nutrition & Care GmbH
Emollient	C15-19 Alkane	SEPPIC
Thickener	Ethylcellulose	Ashland
Film former	Acrylate/octylacrylamide copolymer	Nouryon Surface Chemistry LLC
Solvent	Ethanol	



Figure 3-5. Chemical structure of sunscreen ingredients.

# 3.1.4. Water-absorbing particles

In paper III, four water-absorbing particles were used to investigate their effect on sunscreen sweat resistance: Polyacrylate crosspolymer-6 (SEPPIC), cross-linked sodium polyacrylate (Stewart Superabsorbents LLC), cetyl hydroxyethylcellulose (Ashland), and a core-shell particle (shell: polysilicone-34, core: isononyl isononanoate and water; Momentive Performance Materials Inc.).

# **3.2.** Experimental techniques and methods

# **3.2.1.** Summary of techniques

Technique/ Instrument	Model	Application	Condition
Casting knife film applicator	Elcometer 3580/4 and Elcometer 3580/2, Elcometer Ltd., UK	Film application from polymeric solutions on the substrate	-
Vacuum oven	VT 6025, Thermo Electron LED GmbH, Germany	Drying film forming polymers	60 °C, 12 hours
Closed chamber evaporimeter	Aqua Flux <sup>™</sup> AF200, Biox Ltd., UK	In vivo TEWL measurements	24-25 °C, 55-59% RH
Contact angle measurements	Theta Lite optical tensiometer, Biolin Scientific, Sweden	Wettability of the skin-mimicking substrates and film forming polymers	Defined in section 3.2.2.1
Atomic force microscopy (AFM)	NanoWizard 3, JPK Instruments AG, Germany	Assessment of the topographical and mechanical properties of the skin-mimicking substrates and film forming polymers	Defined in section 3.2.2.2
Profilometer	SJ-410 surface roughness tester, Mitutoyo, Germany	Surface roughness measurement of skin-mimicking substrate used for perspiration experiment	-
Vacuum plate	Elcometer 4900, Elcometer Ltd., UK	Fixing the substrate	-
Plasma cleaner	PDC-32G plasma cleaner, Harrick Plasma, USA	Plasma cleaning and increasing the wettaility of the PMMA film	30 minutes in the air under the constant pressure of 1000 mTorr.
Laser micromachining tool	microSTRUCT™ C, 3D-Micromac AG, Germany	Creating sweat pores in the skin- mimicking substrates	laser irradiation wavelength: 355nm laser power: 10.5W

Table 3-2. Summary of some techniques used in this study.

			marking speed: 400 mm/s	
Flow sensor	Flow sensor3 digital, FLOW-03D, Elveflow, France	Measuring the flow		
Flow reader	Sensor readerV2, MSR2, Elveflow, France	skin model	-	
Homogenizer	Silverson L5T, Silverson Machines Ltd., UK	Preparing sunscreen formulations	Mixing speed: 3500 rpm	
Density meter	DA100M, Mettler Toledo™, Japan	Measuring the density of the sunscreens	-	
Viscometer	Brookfield DV- II+Pro, Brookfield engineering laboratories Inc., USA	Measuring the viscosity of the sunscreens	60 rpm, 23°C	
UV transmittance analyzer	Labsphere UV-2000S, Labsphere Inc., North Sutton, NH, USA	In vitro SPF measurements	Defined in section 4.4	
Area scan camera equipped with a $\rightarrow$	acA4024-29um, Basler AG, Germany			
UV band-pass filter and $a \rightarrow$	365nm, F/BP365- CMOUNT	visualizing the distribution of UVA filters	Will be referred to as UV camera	
High-resolution lens	Fujinon HF-1218- 12M, Fujifilm, Japan			
Coherent Anti-Stokes Raman Scattering (CARS) microscopy	TCS SP8 CARS microscope, Leica Microsystems, Germany	Assessing real-time information about sweat/sunscreen interaction	Defined in section 3.2.2.4	
Optical microscope	Nikon Eclipse LV100ND optical microscope, Nikon, Japan	visualizing the sunscreen film distribution on the skin-mimicking substrate	-	

## **3.2.2.** Details of some experimental techniques

#### **3.2.2.1.** Contact angle

To measure the wettability of the skin-mimicking substrate and polymeric films, water contact angle measurements were conducted by performing the sessile drop method at ambient conditions (25°C, 30% RH). A water droplet (2  $\mu$ l) was placed on the film surface and the measurements (Theta Lite optical tensiometer, Biolin Scientific, Sweden) were recorded within 10 s after deposition. The averaged values obtained from five measurements performed on different surface positions were reported as the contact angle value.

#### 3.2.2.2. Atomic force microscopy (AFM)

In this project, an atomic force microscope (NanoWizard 3, JPK Instruments AG, Germany) was employed to assess the topographical and mechanical properties of the skin-mimicking substrate and film-forming polymers. AFM height images were obtained by tapping mode imaging in air using a cantilever with a spring constant of 40 N/m (HQ: NSC15/AI BS, MikroMasch). Nanoindentation measurements for film-forming polymers were performed using the same cantilever with an approach and retraction speed of 0.5  $\mu$ m/s and a maximum set point of 2.64  $\mu$ N. Prior to the indentation measurements, the accurate normal spring constant of the cantilever was obtained according to the thermal noise method.[184] The sensitivity of the cantilever was also measured by acquiring 50 force curves on a silicon wafer surface. The indentation measurements were conducted over a 5 × 5  $\mu$ m<sup>2</sup> area at 25 points. For each sample, at least two different areas were analyzed. Subsequently, standard Hertzian and DMT models were fitted to the measured force curves to estimate the Young's modulus of the samples. Analysis and processing of the AFM data were performed using the instrument software (JPK data processing).

#### **3.2.2.3.** Ultraviolet Transmittance (UVT) analyzer

The Ultraviolet Transmittance (UVT) analyzer measures the spectral UV transmittance (in the 250–450 nm wavelength spectrum). The instrument consists of two optical chambers and two identical spectrometers. As shown in Figure 3-6, the optical components are placed in upper and lower chambers, i.e., the optics head and input optics, respectively. The optics head contains the integrating sphere (containing an UV pulsed flashlamp to generate the beam), and a spectrometer

No. 1 fiber optic sensor (embedded in the integrating sphere, transmitting the sampled light to spectrometer No. 1). There is an outlet for the UV sample beam at the bottom of the sphere. The input optical chamber includes a lens, mirror, and a spectrometer No. 2 fiber optic sensor. The sunscreen or the reference sample is placed in the gap between the sapphire window at the sphere outlet and the lens is embedded in the input optical chamber. The light beam is generated in the integrating sphere by the flashlamp and the fiber optic cable that feeds spectrometer No. 1 collects the radiation. The beam exits the outlet and meets the sample, where it is either reflected, absorbed, or transmitted. The transmitted light in the lower chamber is focused onto the fiber optic sensor and transmitted to spectrometer No. 2.



Figure 3-6. UV-2000S analyzer with the sample stage (left) and optics head and input optical chamber components (right).

For each measurement, a blank or empty sample is required for the UV transmittance analyzer to use as the reference for 100% transmittance. The processed data collected from both spectrometers generate transmittance spectra according to the equation:

$$T(\lambda) = \frac{S_2}{B_2} \times \frac{B_1}{S_1}$$
 3-1

where  $S_1$  and  $S_2$  are the sample scan recordings, and  $B_1$  and  $B_2$  are the blank scan recordings for spectrometers No. 1 and 2, respectively. Finally, according to Equation 2-3 and by choosing the spectral irradiance source, i.e., "Albuquerque" or "Melbourne" or COLIPA, in vitro SPF is measured. Moreover, in vitro UVAPF, the UVA/UVB ratio, and  $\lambda_c$  could be calculated from Equations 2-4, 2-5, and 2-6, respectively.

#### 3.2.2.4. Coherent Anti-Stokes Raman Scattering (CARS) microscopy

Coherent Anti-Stokes Raman Scattering (CARS) microscopy is a label-free imaging technique based on vibrational contrast, used in the material, biological science, and chemical industries.[51], [185]–[187] In this technique, by using a Stokes and pump laser beams, the molecular vibrations are excited. Through a complex procedure, three laser beams, i.e., Stokes ( $\omega_s$ ), pump ( $\omega_p$ ), and probe ( $\omega_{pr}$ ), interact with the sample to generate anti-Stokes emission.[186] The electrons located in the cloud around the molecular bond attain the virtual excited vibrational state when the difference between the Stokes and pump frequencies of the laser beams approaches the vibrational frequency of a molecular bond ( $\omega_v = \omega_p - \omega_s$ ). This vibrational excitation happens in the focal volume, in which the beams of pump and Stokes are coherently in phase and  $\omega_{pr}$ , as a third beam, is applied and is scattered to generate anti-Stokes radiation ( $\omega_{as} = \omega_{pr} + (\omega_p - \omega_s)$ ) to probe the excitations.[188] The emitted photon is blue-shifted and separated from the incident laser beams.[186]



Figure 3-7. Diagram of an SRS microscope with epi and forward channels (reprinted with permission from [189]).

In this project, CARS microscopy was used to investigate the real-time changes in the distribution of UV filters during and after perspiration. Initially, the CARS signals of the UV filter, i.e., octocrylene, the sunscreen ingredients, and the skin-mimicking substrate were acquired. The pump laser (PicoEmerald, APE, Germany) wavelength was varied from 787 to 877 nm in 1-nm increments (corresponding to wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>) with a Stokes laser fixed at 1064 nm. The chemical vibrations of the sunscreen formulation ingredients and the skinmimicking substrate are illustrated in Figure 3-8a. Octocrylene showed three distinct peaks at 3063, 2864, and 2210 cm<sup>-1</sup>. However, only the distinct peak at wavenumber 2210 cm<sup>-1</sup> attributed to the nitrile group (see Figure 3-8a) did not overlap other ingredients and skin-mimicking substrate. To perform CARS microscopy, we selected this wavenumber to solely follow the changes in the distribution of the UV filter during and after perspiration. Outlined in Figure 3-8b and c the highest chemical contrasts between the sunscreen and the skin-mimicking substrate that are visible at wavenumbers of 2210 cm<sup>-1</sup> compared with 2234 cm<sup>-1</sup> and 2194 cm<sup>-1</sup>. The distribution of octocrylene on the substrate at this wavenumber is shown in Figure 3-8d. For real-time studies, the sunscreen was applied onto the skin-mimicking substrate (2 mg cm<sup>-2</sup>) and after self-leveling for 15 minutes in darkness, the substrate was mounted on the chamber and the chamber was inversely placed on the sample stage of the microscope. All images were obtained with a field view of  $1550 \times 1550 \ \mu\text{m}^2$  at room temperature and image sequences were acquired at 2210 cm<sup>-1</sup> (pump laser: 861.8 nm) at a pixel size of  $3.033 \times 3.033 \ \mu\text{m}^2$ .



Figure 3-8. (a) CARS signals for sunscreen ingredients and skin-mimicking substrate from 3313 to 2009 cm<sup>-1</sup>. The highlighted peak at 2210 cm<sup>-1</sup> is attributed to the octocrylene nitrile group. (b) CARS signal of the skin-mimicking substrate for two regions of interest (ROI): bare substrate (ROI 1) and area with sunscreen (ROI 2). (c) Selected CARS images of ROI 1 and ROI 2 at 2234, 2210, and 2194 cm<sup>-1</sup>. The highest contrast between the regions happens at wavenumber 2210 cm<sup>-1</sup>. (d) Sunscreen distribution on the skin-mimicking substrate at wavenumber 2210 cm<sup>-1</sup>. This figure is a modified version of Figure 2 and S2 in paper II.

### 3.2.3. Methods

#### 3.2.3.1. Preparation of topical films

The optimal concentrations of film-forming polymers for film application were: 20 wt.% for wt.% acrylates/octylacrylamide copolymer, 25 for polyurethane. 15 wt.% for vinylpyrrolidone/acrylates/lauryl methacrylate copolymer, 40 wt.% for shellac (all in ethanol), and 40 wt.% for acrylates/dimethicone copolymer (in isopropanol). For the permeation test, the polymer solution was applied on the skin-mimicking substrate by the casting knife film applicator and left to dry for 18 hours before the permeation experiment. The wet thickness of applied films was adjusted based on a calibration curve (dry thickness vs. wet thickness) for each film-forming polymer, to obtain a dry thickness of 50 µm.

For some experiments, freestanding films of polyurethane and the acrylate/octylacrylamide copolymer were needed. However, it was not possible to make a freestanding film of the pure the acrylate/octylacrylamide copolymer owing to the brittleness of the dried film. Therefore, an appropriate amount of a plasticizer, dibutyl sebacate (Sigma Aldrich), was added to the solution before the film application.

#### 3.2.3.2. Water vapor uptake test

To measure the water vapor uptake of the skin-mimicking substrate and the film-forming polymers, a gravimetric method was used. Before the test, the samples were dried in the vacuum oven at 60°C for 12 hours. The initial weight of samples was recorded and the samples were placed in the chamber with controlled RH. The samples were weighed again after 72 hours and the normalized water vapor uptake was calculated. For the experiments on the skin-mimicking substrate, the RH inside the chamber was set to 11%, 33%, 53%, 75%, 84%, and 97% (by using LiCl, MgCl<sub>2</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaCl, KCl, and K<sub>2</sub>SO<sub>4</sub> saturated salt solutions, respectively).[190], [191] For the film-forming polymers, the humidity was set at 97%.

#### 3.2.3.3. In vivo TEWL studies

For in vivo TEWL studies, 10 healthy volunteers (six men and four women, 24–34 years of age, different ethnicities) were selected for application of the freestanding films of two film-forming polymers on their forehead. The participants had no history of dermatological diseases or visible areas of injury on their foreheads. Prior to the experiment, the participants provided written informed consent and were asked not to apply any topical products on their foreheads for at least 24 hours before the study. The participants were also asked not to drink caffeine-containing beverages in the 3 hours before the study and throughout the study period.

The participants stayed in the room for 30 minutes without physical activities to familiarize themselves with the conditions (24°C–25°C, 55%–59% RH). The participants' foreheads were marked in the middle, and the freestanding films of two film-forming polymers, i.e., acrylates/octylacrylamide copolymer-plasticizer and polyurethane (thickness: 50  $\mu$ m, size: 2×3 cm<sup>2</sup>) were placed on the left and right sides, respectively. Each film was fixed with surgical tape on the edges. The in vivo TEWL measurement was conducted at the same position on bare skin,

on top of the polymeric film, 1 and 2 hours after covering the skin, and finally repeated on the bare skin immediately after film removal.

### 3.2.3.4. Sunscreen formulations

Different sunscreen formulations were prepared for paper II and paper III. The ingredients, i.e., thickener, film-former (0.75 wt.%), emollients, and UV filters, were added into ethanol, respectively, and mixed using a homogenizer. Diethylamino hydroxybenzoyl hexyl benzoate, octocrylene, and ethylhexyl salicylate were used as the UV filters in the formulation; based on their concentrations (30 wt.%), an SPF of 28 was calculated.[73] This formulation was used as the main sunscreen in paper II and the base sunscreen in paper III. Moreover, in paper III, four sunscreens, containing 1% of each of the water-absorbing particles and two sunscreens containing 0 and 3 wt.% of the film-former were also prepared (the concentration of the film-former in the base sunscreen and sunscreens containing water-absorbing particles was fixed at 0.75 wt.%). For CARS microscopy studies (Section 3.2.2.4), two simplified sunscreens containing only one UV filter, i.e., octocrylene (20 wt.%), and either 0 or 1 wt.% of polyacrylate crosspolymer-6 were prepared. For some of the experiments, a commercial sunscreen (SPF30) was used. For in vitro water resistance studies, sunscreens containing 0, 0.75, and 3 wt.% of film-former were used.

#### **3.2.3.5.** In vivo SPF studies and sweat collection

We performed a pilot in vivo study in collaboration with proDERM (at the Institute for Applied Dermatological Research, Germany) to test the performance of a known commercial alcohol-based sunscreen upon physical activity-induced sweating. The efficiency was assessed by performing in vivo SPF measurements before and after sweating, gravimetric measurements of sweat amount during the perspiration, and the evaluation of the sunscreen film homogeneity on the sunscreen-treated foreheads of subjects using Visia-CR camera. In vivo SPF measurements and sweat collection were performed on the back of six subjects and UV camera images were taken of the sunscreen-treated forehead of two subjects before and after sweating, as illustrated in Figure 3-9.



Figure 3-9. Illustration of the test areas in in vivo experiments for assessment of the sunscreen efficiency upon sweating. (a) Areas on the back of the subject for In vivo SPF measurements and sweat collection. (b) Sunscreen-treated area on the forehead for UV camera imaging.

Prior to the experiment, the participants provided written informed consent for participation in the study. The experiments were performed in an air-conditioned room (temperature:  $18^{\circ}C-26^{\circ}C$ ). The sunscreen was applied at a quantity of 2 mg cm<sup>-2</sup> to the back of the subjects (area:  $30-60 \text{ cm}^2$ ) for SPF measurements. Four areas were also marked and covered by pads for collection of sweat over different time intervals. For UV camera imaging, the upper forehead was marked as close as possible to the hairline (width: 4-5 cm and height: 2-4 cm) and the sunscreen was applied at different quantities (1 and 2 mg cm<sup>-2</sup>). The procedure for all experiments is illustrated in Figure 3-10.



Figure 3-10. In vivo sweat resistance test setup. This figure is a modified version of Figure S4 in paper II.

# 4. **Results**

# 4.1. TEWL and perspiring skin simulators

### 4.1.1. TEWL simulator

The TEWL simulator consists of a skin model placed in a homemade chamber with controlled RH. The skin model comprises a water-responsive skin-mimicking substrate mounted on a cup containing a saturated salt solution that provides a constant RH below the substrate. The difference in RH between the two sides of the substrate results in water vapor permeation through the substrate, similar to the TEWL phenomenon occurring in human skin. In the following sections, the preparation of the skin-mimicking substrate and tuning of the skin model characteristics to achieve in vivo simulation of TEWL are explained.

#### 4.1.1.1. Preparing skin-mimicking substrate

Gelatin (10 g) and hydroxyethyl cellulose (2 g) were added to water (100 ml). The pH of water was adjusted to pH 9 by the addition of 1 M NaOH. The solution was stirred for 30 minutes at 50°C. Subsequently, glycerol (2 ml) and formaldehyde (2 ml) were added to the solution, and stirred for 1 minute. The final solution was applied on a PMMA substrate by the casting knife film applicator with different wet thicknesses. The applied solution was left overnight to dry and crosslink.

### 4.1.1.2. Characterization of moisture-releasing substrate

The appearance and relevant physical properties of the skin-mimicking substrate are outlined in Figure 4-1. Each component in the skin-mimicking substrate, i.e., gelatin, hydroxyethyl cellulose (Natrosol), glycerol, and formaldehyde, provides a specific functionality.[173] As mentioned in Section 3.1.1, gelatin can mimic both the chemical and physical properties of dry and hydrated human skin.[171], [192] Natrosol helps to achieve more skin-like mechanical properties, conferring less brittleness and a larger elongation capacity.[173] As shown in Figure 4-1a and b, the addition of Natrosol results in a visible roughness and texture and enhanced surface roughness of the dried film, which can then better mimic the surface texture of the skin. Glycerol serves as the plasticizer and enhances the flexibility of the composite. We also observed that the plasticizer eases the film formation of polymers on top of the gelatinous film. Finally, formaldehyde plays a role as the chemical crosslinker for gelatin, improving the mechanical strength and the hydrolytic

stability of the film.[173] When crosslinked, the gelatinous film can reversibly swell/shrink in response to moisture adsorption/desorption, with least changes in the structure.



Figure 4-1. Summary of the physicochemical properties of the skin-mimicking substrate. (a) The skin-mimicking substrate. (b) Microscopic image of the skin-mimicking surface. The texture made by Natrosol is visible. (c) AFM height image of the skin-mimicking substrate. (d) Cross-section profile taken at the line marked on the AFM height image. (e) Water droplet on the dry skin-mimicking substrate used in the contact angle measurement. (f) Normalized amount of water vapor uptake by the skin-mimicking substrate versus RH. This figure is a modified version of Figure 1 in paper I.

We assessed the surface roughness and the water-responsive behavior of the gelatin-based substrate, i.e., water vapor uptake and wettability, and compared it with human skin. The surface topography and corresponding cross-section height profile of the substrate surface are presented in Figure 4-1c and d. The average root-mean-squared (RMS) roughness of the substrate was approximately  $0.87 \pm 0.4 \,\mu\text{m}$  (for an area of  $100 \,\mu\text{m} \times 100 \,\mu\text{m}$ ). The average contact angle value for the substrate was measured as  $75^{\circ}\pm5^{\circ}$ . Both values were in the same range as the reported values of roughness and contact angle for human skin (Figure 4-1e). [29], [30], [173] As illustrated in Figure 4-1f, the normalized amount of water vapor uptake of the substrate as a function of the RH displays a nonlinear behavior. At up to 50% RH, the substrate absorbs the water vapor slightly. However, above 75% RH, more significant moisture uptake appears to occur. The obtained vapor uptake isotherm is similar to that of the human skin and the SC.[32]

#### 4.1.1.3. Adjustment of affecting parameters

The gelatin-based substrate showed similarities to human skin in terms of roughness, water vapor absorption, and hydrophilicity. We employed the substrate in the skin model to simulate the water vapor permeation corresponding to the in vivo TEWL. The water vapor permeation through the SC in human skin occurs as a result of the difference in water activity on both sides. To simulate these conditions, as shown in Figure 4-2a, the substrate was mounted on the cup (containing a saturated salt solution that provided a specific RH underneath the substrate) and the cup was placed in a chamber with controlled RH. The gelatin-based substrate acts as the barrier separating two mediums with different humidities. The humidity gradient produces a steady flux of water permeation through the film. In the equilibrium state, in vivo TEWL depends on the water activities on both sides of the SC and the thickness of SC.[193], [194] Similarly, we chose four thicknesses (30, 70, 100, and 140  $\mu$ m) and tested three RH gradients between inside the chamber and inside the cup should be 75% to hydrate the gelatin-based film. Thus, the three relative humidities for inside the cup (RH<sub>in</sub>) were chosen as 75%, 84%, and 97%. For inside the chamber, the relative humidity (RH<sub>out</sub>) was chosen as 33%, to represent the ambient humidity.

To measure the permeation of water vapor through the skin-mimicking substrate for each condition, we used the procedure of the ASTM-E96 standard. The samples were mounted on a cup containing 3 ml of the saturated salt solution and fixed with an exposed area of  $\sim$ 7 cm<sup>2</sup> on both sides of the sample. The cup was placed in the chamber (ambient temperature, equipped with a humidity sensor and a fan), where the RH<sub>out</sub> was adjusted to 33%±2% by a saturated MgCl<sub>2</sub> solution. The mass loss that resulted from the humidity gradient on the two sides of the film was calculated by removing and weighing the cups at intervals of 2 hours. The measurements was stopped after 8 hours. The mass loss rate was lower for the first 2 hours than that measured over the next 6 hours. This was due to the water vapor buildup in the film during the initial humidification step, implying an induction period, typical for water-absorbing films.[195] Accordingly, the fitted slope of the linear region observed in 2–8 hours of testing, for each case, was reported as the WVPR.

The normalized WVPRs (the simulated TEWL values) obtained using this setup are shown in Figure 4-2b. The results showed that for a given thickness of the substrate, an increase in the RH<sub>in</sub> results in higher permeation rates, and for each RH gradient, an increase in the thickness of the

substrate led to lower values of simulated TEWL. By adjusting these two parameters, the approximate simulated TEWL values in the range of 10–45 gm<sup>-2</sup> h<sup>-1</sup> could be obtained. This range represents the TEWL value in moderate to stressed conditions.



Figure 4-2. Summary of the TEWL simulator. (a) The TEWL simulator setup. (b) The water vapor permeation through the skinminicking substrates with four different thicknesses (30, 70, 100, and 140  $\mu$ m) and with a 75%, 84%, and 97% RH inside the cup. The humidity outside the cup was fixed at 33%. (c) Schematic representation of topical film application on the skin-minicking substrate and placement on the cup. (d) Schematic illustration of the TEWL simulator with an applied polymeric film. This figure is a modified version of Figure 1 in paper I.

To perform the in vitro TEWL studies for the film-forming polymers and test their breathability, the substrate thickness, RH<sub>in</sub>, and RH<sub>out</sub> were chosen as 90  $\mu$ m, 97%, and 33%, respectively. This condition provides a simulated TEWL value of approximately 37 g/m<sup>2</sup>h, which is a relatively high TEWL value for human skin.[47] By doing so, the breathability can be tested for a relatively large TEWL range and the contributions from the experimental error will be less considerable. We did not consider very low thicknesses (e.g., 30 or 50  $\mu$ m) to achieve the desired TEWL value to avoid possible substrate failure during the application of a polymeric film solution and/or damage during long permeation experiments.

To measure the breathability of the film-forming polymers, we used the procedure previously described for the skin-mimicking substrate. After application of the polymeric films on the skin-

mimicking substrate (Figure 4-2c), the sample was mounted on the cup containing 3 ml of a saturated  $K_2SO_4$  salt solution (providing 97%±1% RH) and fixed. The cup was placed in the humidity chamber, the humidity was adjusted to 33%±2%, and the experiment was started. A schematic side view of the TEWL simulator with an applied polymeric film is presented in Figure 4-2d.

We also investigated the effect of gelatin substrate on the WVPR of topical films to make sure that the permeation could be decoupled from the substrate. Three samples, i.e., a freestanding film consisting of acrylate/octylacrylamide-plasticizer, the same composition applied on the substrate, and the bare substrate, were prepared and the permeation test was performed as outlined in Figure 4-3. The topical film controls the permeation of water vapor because the WVPR through the freestanding film and the film applied on gelatin were almost identical. This demonstrated that, on condition that the permeation of the skin-mimicking substrate was much greater than the permeation of applied film, the permeation of the polymer is not affected.



Figure 4-3. The effect of the skin-mimicking substrate on permeation through the film forming polymers. Water vapor permeation through the bare gelatin-based substrate, acrylate/octylacrylamide - plasticizer film applied on the gelatin-based substrate and the acrylate/octylacrylamide - plasticizer free-standing film. This figure is a modified version of Figure 2 in paper I.
#### 4.1.2. Perspiring skin simulator

To assess the sunscreen behavior upon perspiration, perform the in vitro sweat resistance test, and investigate the impact of different parameters on sunscreen efficacy, it is necessary to have an operational perspiring skin simulator that provides a uniform flow rate associated with human sweating and represents skin-like affinity to the sunscreen formulations. Artificial substitutes for sweating are used in diverse areas, such as textiles, medicine, and cosmetics.[53], [196]–[198] However, there is a lack of suitable devices for sunscreen applications.

The perspiring skin simulator consists of a sweating substrate, a water reservoir, and a source providing the driving force for perspiration. The principal component of the perspiring skin simulator is the sweating skin-like substrate. A single-layer substrate cannot maintain the uniform flow.[52] In contrast, a double-layer skin substrate can mimic human sweating, in which the uniform distribution of flow is governed by one layer and the skin-like properties are provided by the other layer. The bottom layer is a hydrophilic membrane with considerably smaller pores and higher pore density than the pores on the top layer. This membrane dominates the pressure drop and therefore a uniform flow is supplied to the upper layer. When there is a consistent flow through the skin-like substrate, the upper layer can be tuned to have skin-like properties, such as sweat pore density, hydrophobicity, and skin texture.

We developed a multi-layer perspiring skin-like substrate and used it in the setup which we will refer to as the perspiring skin simulator. In Section 4.1.2.2, we assessed the setup for its ability to activate the sweat pores and attain controlled uniform perspiration, and examined the reproducibility of the perspiration rate.

#### 4.1.2.1. Preparation of multi-layer skin-mimicking substrate

The skin-mimicking substrate consists of the gelatin-based film, the PMMA film, the adhesive layer, and the track-etched membrane; each component provides a specific functionality. The procedure for making the gelatin-based layer is similar to that explained in Section 4.1.1.1. We also prepared a formulation without Natrosol by the following method:

Gelatin (7.5 g) was added to 50 ml water (pH adjusted to 9) followed by stirring 30 minutes at 50°C. Then, 1.5 ml glycerol and 1.5 ml formaldehyde were added and the solution was stirred for 1 minute. The solution was applied by the casting knife film applicator with a wet thickness of 250

 $\mu$ m (to achieve a dry thickness of 20–30  $\mu$ m) on the PMMA film, which was fixed on the vacuum plate and left overnight to dry and crosslink. Before gelatin-based film application, the PMMA film was plasma cleaned for 30 minutes in air under a constant pressure of 1000 mTorr to increase the wettability of the surface and improve the adhesion between the PMMA film and the gelatin layer.[199] Subsequently, the double-sided acrylic tape was applied from one side to the backside of the PMMA film while a release liner protected the other side of the tape. Before the membrane was attached, the top three layers were drilled to create the sweat pores. Initially, the samples were prepared using a drilling machine with two pore densities: 100 cm<sup>-2</sup> and 200 cm<sup>-2</sup>. The minimum pore size was 200  $\mu$ m, as attempts to achieve a smaller pore size failed. However, this method did not work, because the post-drilling residue of the substrate. Second, the pore size of 200  $\mu$ m was too large for sunscreen application. A sunscreen with low viscosity, e.g., 15 cP, passes through the holes and blocks all the pores. A pore size of approximately 100  $\mu$ m is suitable for sunscreen application. Moreover, the use of the drilling machine was a time-consuming process.



Figure 4-4. A summary of the perspiring skin simulator. (a) Preparation of the perspiring skin-like substrate. (b) The side view of the setup and layered skin-mimicking substrate. (c) Sweating substrate. This figure is a modified version of Figure 1 and 3 in paper II.

The laser drilling technique was therefore employed instead to create the pores. Unlike conventional drilling, it is a non-contact process in which the holes (pores), with a variety of sizes and at a variety of densities can be created in the material of interest using a fiber laser beam. The beam intensity, duration, and heat output can be controlled precisely by the user. This technique is highly accurate, fast, and repeatable compared with the previous method. Prior to the process, a pattern for placement of the pores, as well as pore size and density, was needed. We examined different patterns to find a pattern with a pore density of 200 cm<sup>-2</sup> (the diameter of the drilled area was 3 cm), which resembles the sweat pore density of human skin.[47] The pattern shown in Figure 4-4a was an appropriate match for the chosen pore density. This pattern also supports uniform perspiration compared with other patterns, e.g., a spiral pattern. Using the laser machine, we decreased the pore size in the pattern down to 100 µm. The actual pores in the substrate were slightly different because the laser beam forms funnel-like pores through the layers. For each set of samples, at the beginning of the process, the parameters (i.e., the laser power, laser marking speed, and the number of drilling repetitions) were adjusted to create reproducible pore size and pore structure and avoid burning the gelatin-based layer around the pores. We obtained drilled samples with a pore size of  $110-120 \mu m$  on the gelatin side and  $40-60 \mu m$  on the adhesive side. However, the small variation in the substrate thickness resulted in sweat pore size distribution. The pore size distribution was wider for the samples containing Natrosol, which was probably a result of the higher surface roughness and material discrepancy in the gelatin-based layer.

Finally, the release liner of the adhesive was removed and the film was applied to the track-etched membrane fixed on the vacuum plate. A deadweight (pressure: 500 Pa) was placed on top of the film for 2 hours to enhance the adhesion of the membrane to the film.

The selection of the membrane (type, pore size, pore density, and thickness) plays an important role in attaining the desired sweat rates. A theoretical model for a bilayer perspiring substrate, based on a fluid (water) resistance network, was presented by Hou et al.[52] In this model, in the equilibrium state, the driving pressure drops across the top layer ( $P_t$ ) and the bottom membrane layer ( $P_m$ ):

$$P_0 = P_m + P_t \tag{4-1}$$

On the assumption that the water resistance in the other parts of the perspiring skin simulator was negligible, the pressure drop in a hydrophilic membrane was therefore only due to the water resistance of the membrane,  $R_m$ :

$$P_m = R_m \times Q_n \tag{4-2}$$

where  $Q_n$  is the water flow rate. The water resistance in the membrane (neglecting the Laplace pressure due to the hydrophilicity of the membrane) for one sweat pore in the top layer,  $D_t$ , was defined as:

$$R_m = \frac{128\mu L_m}{\pi D_m^4} \times \frac{1}{\frac{\pi \alpha D_t^2}{4}}$$

$$4-3$$

where  $\mu$  is the water viscosity, and  $\alpha$ ,  $L_m$ , and  $D_m$  were the membrane pore density, thickness, and pore diameter, respectively.

 $P_t$  was calculated as the combination of the water resistance of the top layer,  $R_t$ , and the Laplace pressure,  $P_L$ :

$$P_t = R_t \times Q_n + P_L \tag{4-4}$$

$$R_t = \frac{128\mu L_t}{\pi D_t^4} \tag{4-5}$$

$$P_L = \frac{4\gamma \cos(\theta_a)}{D_t}$$
 4-6

where  $L_t$  is the thickness of the top later,  $\gamma$  is the water surface tension, and  $\theta$  is the advancing contact angle for the water in the sweat pore. To control the pressure drop almost solely by the membrane, the water resistance of the membrane must be much greater than water resistance of the top layer:

$$D_m \ll \sqrt[4]{\frac{4L_m D_t^2}{\pi \alpha L_t}}$$
 4-7

Therefore, a hydrophilic membrane with a low pore size and thickness and a high pore density is needed. The theoretical sweat rate (L h<sup>-1</sup> m<sup>-2</sup>) for a perspiring skin substrate with a pore density of  $\omega$  will be:

$$V = \frac{\omega \pi^2 D_m^4 D_t \,\alpha [\rho g h D_t - 4\gamma \cos(\theta)]}{512 \mu L_m}$$

$$4-8$$

The flow rate strongly depends on the pore size of the membrane, as seen in Equation 4-8. We compared the theoretical sweat rate associated with two hydrophilic polycarbonate membranes with different values for  $\alpha$ , L<sub>m</sub>, and D<sub>m</sub> (3×10<sup>8</sup> vs. 4×10<sup>8</sup> cm<sup>-2</sup>, 10 vs. 6 µm, and 0.2 vs. 0.1 µm, respectively). The data for the top layer were provided based on the characterization of our substrate. As shown in Figure 4-5, to obtain a sweat rate corresponding to the moderate perspiration of human (~0.9 L h<sup>-1</sup> m<sup>-2</sup>), significantly higher pressure is needed for the membrane with a pore size of 0.1 µm than for the membrane with a pore size of 0.2 µm. Therefore, for subsequent experiments, the membrane with the pore size of 0.2 µm was selected.



Figure 4-5. Comparison between theoretical sweat rates of two substrates with different pore sizes, pore density, and thickness.

In parallel, we checked the UV transmittance of the different layers of the skin-mimicking substrate for performing in vitro SPF measurements. As shown in Figure 4-6, even though the transmittance of the membrane was not as high as other layers, the overall UV transmittance of the substrate was suitable for the comparison of in vitro SPF values before and after sweating.



Figure 4-6. UV transmittance of the skin-mimicking substrate and its layers. This figure is taken form paper II (Figure S1).

For the substrate coated with the gelatin-based layer without Natrosol, the roughness line scan of the hydrated substrate was measured and surface height, z, was plotted versus distance, x, in two conditions: where the stylus goes through the pores, as shown in Figure 4-7a, and where the stylus does not go through the pores, as shown in Figure 4-7b. The RMS roughness of the film was  $13.90\pm1.75 \mu m$  for the measured distance with holes and  $0.16\pm0.04 \mu m$  for the measured distance without holes. Moreover, to measure the surface roughness close to the sweat pores, a representative AFM height image of the skin-mimicking substrate (in the dry state) and the corresponding cross-sectional height profiles are presented in Figure 4-7c and d, respectively. The surface roughness increased close to the sweat pores (Figure 4-7d).



Figure 4-7. Roughness line scan of the skin-mimicking substrate obtained with a profilometer (a) through a line with sweat pores and (b) through a line with no holes. (c) AFM height image of the skin-mimicking substrate close to a sweat pore and (d) cross-section profile taken at the line marked on the AFM height image. This figure is taken form paper II (Figure S7).

#### 4.1.2.2. Characterization of perspiring skin simulator

The preparation of the perspiring skin-mimicking substrate was followed by testing of the samples in the sweating simulator. The entire setup comprises a water tank with an adjustable height, a custom-made chamber (water reservoir), a flow sensor and flow reader, connecting tubes, and the skin-mimicking substrate.

Initially, the chamber used for the perspiration experiment was made from PMMA, which is a clear material with acceptable mechanical strength. However, after few attempts to activate the sweat pores and perform the perspiration experiments, propagated cracks on the walls and the lid were visible to the naked eye. Moreover, the method for mounting the substrate (sandwiching the substrate between two silicone gaskets and using a screw lid) did not prevent water leakage once the pressure was applied. We changed the material to polycarbonate and prepared a chamber with a different design (thicker walls, embedded O-ring in the lid, and more efficient substrate mounting

method), as shown in Figure 4-8, and used it for the perspiration experiments. The chamber has an inlet close to the bottom for feeding water into the chamber and an outlet close to the top for removing the trapped air before the onset of perspiration.



Figure 4-8. Chambers used in perspiration experiments.

In the next stage, the substrate was placed on the chamber and the lid with an O-ring beneath it was screwed to the chamber to fix the substrate. The water tank was connected to the chamber to provide a pressure-controlled flow of water through the substrate. The flow sensor, placed between the water tank and the chamber, measured the flow rate and sent the data to the flow reader connected to the software. A schematic view of the perspiring skin substrate and the setup is shown in Figure 4-4b.

To activate the sweat pores before the perspiration experiment, the skin substrate was mounted on the chamber and the water tank height was adjusted to provide high pressure ( $\Delta P \sim 8$  kPa). This pressure is high enough to make sure nearly all the sweat pores are activated and, regardless of a small variation in their size, will contribute to sweating at different pressures. In Figure 4-4c, a sweating skin model is illustrated in which most of the sweat pores are activated.

The difference between the water level in the tank and the chamber determines the applied pressure and, consequently, the perspiration rate. To attain the sweat rates corresponding to that of the human skin, different pressure levels were applied to the perspiring substrate. The height of the water tank was varied in 5-cm increments and the flow rate was measured. The experimentally measured sweat rates are shown in Figure 4-9 for skin substrates both with and without Natrosol. For each skin substrate, the steps were repeated three times to make sure that the sweating rates were reproducible at the same height and that the possible reduction in pore size due to hydration of the gelatin-based layer was negligible. However, the samples in which the gelatinous layer contained Natrosol failed to provide uniform and reproducible flow rates. The presence of Natrosol impairs the formation of uniform sweat pores in the laser drilling process (resulting in high Laplace pressure in some of the pores and lower overall flow rates) and its hydration affects the dements of the sweat pores during perspiration (resulting in non-reproducible flow rates). Moreover, we observed that samples containing Natrosol were more prone to burning during the laser drilling process. Therefore, for the rest of the experiments, we used the gelatin-based layer without Natrosol.



Figure 4-9. Checking reproducibility of the flow rate in skin-mimicking substrate. Plots of the average sweat rates at different applied pressures. (a) Sweat rates through skin-mimicking substrate without Natrosol. (b) Sweat rates through skin-mimicking substrate containing Natrosol. ( $\blacksquare$ ) represents the first pressure ramp, ( $\bullet$ ) shows the first repetition, and ( $\blacktriangle$ ) shows the second repetition. This figure is a modified version of Figure 3 in paper II.

## 4.2. Film-forming polymers on the TEWL simulator (paper I)

Using the TEWL simulator, the breathability of five film-forming polymers with different characteristics was investigated. We have abbreviated the acrylates/octylacrylamide copolymer as Acr-OcAA, the acrylates/dimethicone copolymer Acr-DiMet, as the vinylpyrrolidone/acrylates/lauryl methacrylate copolymer as VP-Acr-LaMeAcr, and polyurethane-64 as PU. In Figure 4-10, an overview of the WVPR for polymeric film-formers compared with the data for the bare skin-mimicking substrate is presented.



Figure 4-10. Permeation data for the film forming polymers applied on the skin-mimicking substrate compared with that of the gelatin-based substrate. This figure is a modified version of Figure 2 in paper I.

The VP-Acr-LaMeAcr and PU films showed the highest breathability (WVPR of 22.73±0.37 and 18.28±1.18 g m<sup>-2</sup>h<sup>-1</sup>, respectively). In contrast, the shellac, Acr-OcAA, and Acr-DiMet films displayed strong barrier properties, as shown by their low WVPRs (2.67±0.07, 2.86±0.11, and 7.55±0.16 g m<sup>-2</sup>h<sup>-1</sup>, respectively). The data obtained from TEWL simulator can be used to categorize the film-forming polymers based on their resistance to water vapor permeation. Note that these results were obtained for the films with an identical dry thickness of 50 µm, which is relatively high; a lower thickness may affect the obtained results.[179]

To compare the obtained in vitro data with in vivo TEWL values, we selected two of the examined film-formers, i.e., PU and Acr-OcAA, which resulted in high and low breathability, respectively. Freestanding films were prepared and affixed to the forehead of 10 subjects. Using a closed chamber evaporimeter, the TEWL values before film application and the apparent TEWL values after film removal were measured. Moreover, the WVPR of the films was measured 1 and 2 hours after the skin was covered.



Figure 4-11. 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). This figure is taken from paper I (Figure 4).

The results shown in Figure 4-11 demonstrated that the WVPR through the Acr-OcAA-plasticizer films after the skin was covered was lower than the TEWL of bare skin (both at 1 and 2 hours after the skin was covered). However, the apparent TEWL value (measured after film removal) was much greater (on average 53%) than the TEWL value of uncovered skin. This observation contradicted with the results obtained from PU films. After the skin was covered by the PU films, the WVPR was increased compared with the TEWL of the uncovered skin. In contrast, the apparent TEWL value did not change considerably (only 14% higher than the TEWL value before the film application).

It was expected that, similar to in vitro studies, the WVPR after covering the skin would be reduced and the apparent TEWL value after film removal would be higher than that of uncovered skin. The in vivo results for Acr-OcAA-plasticizer films were qualitatively in agreement with those from the in vitro study, proposing that the film was occlusive. Nevertheless, the reduction in permeation after the coverage was not significant, despite its high resistance to water vapor permeation. In contrast, the skin coverage of the PU films increased with an increase in water vapor permeation. This discrepancy could be attributed to the inconsistency in the driving force for in vivo TEWL before and after skin coverage. In the covered area, the local temperature of the skin may increase and change the gradient of water vapor or activate the sweat pores; consequently, the TEWL value increases.[200]–[202] Further, when the film-forming polymers are topically applied, they affect the water permeation through the SC, as well as skin hydration.[194], [203], [204] In the equilibrium state, the difference in water activity on both sides results in water vapor permeation. Covering the skin introduces a high RH to the outer layer of the skin, increasing the skin hydration and, consequently, the water permeation rate. However, the barrier properties of the layer against the increased permeation affect the measured value on the side of the film that is exposed to the environment.[11], [205]-[212] Sparr et al. studied the effect of the topical films with different barrier properties on skin hydration, WVPR, and TEWL value.[194] They demonstrated that, at a low RH level, an occlusive film significantly elevates the water activity on the outer layer of the skin. As a result, the water content in the SC increases, leading to a higher WVPR. However, the measured permeation rate on top of the film was lower owing to the resistance of the film toward water vapor. Therefore, we speculated that both film-forming polymers likewise induced a high concentration of water vapor on the skin surface. Hence, the Acr-OcAA-plasticizer film strongly prevented the permeation, despite the small apparent difference in the measured TEWL values before and after coverage. PU films, in contrast, could not act as a barrier against additional water vapor molecules, and led to increased water vapor permeation.

Considering both in vitro and in vivo studies, it was concluded that the TEWL simulator could predict the breathability of the different film-forming systems while avoiding scattered and subject-dependent data, as well as the common difficulties associated with in vivo studies. However, the absolute numbers obtained from in vivo and in vitro TEWL measurements were not comparable, because the biological responses such as temperature increase, sweat pore activation, and changes in SC structure do not happen during the measurements in the TEWL simulator, and the water vapor permeation is only governed by the film breathability.

The film-forming polymers were characterized further by comparing their resistance to water vapor and water. If a polymer has a high water contact angle, it repels water, but does not necessarily resist water vapor uptake. The measured contact angles for the shellac  $(64^{\circ}\pm3^{\circ})$  and VP-Acr-LaMeAcr  $(65^{\circ}\pm2^{\circ})$  films were lower than for the Acr-OcAA, Acr-DiMet, and PU films  $(95^{\circ}\pm2^{\circ}, 90^{\circ}\pm1^{\circ}, 75^{\circ}\pm5^{\circ},$  respectively), as shown in Figure 4-12b. The comparison between the contact angle measurements and water vapor uptake showed that only Acr-DiMet and PU

polymers exhibited both a low water vapor uptake and high water repellency (high water contact angle) and, in general, there was no direct correlation between water and water vapor affinity of the film-forming polymers. These results confirmed that some of the film-forming polymers prevented the water from penetrating through the surface, but did not stop water vapor uptake.[213] Thus, to determine the breathability of the polymeric films, the water vapor permeation tests cannot be replaced by contact angle measurement.



Figure 4-12. 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. This figure is taken from paper I (Figure 3).

In an attempt to expand the characterization of the film-forming polymers and determine a possible connection with permeation data, we measured the surface roughness and Young's modulus of the polymers using AFM. The surface topography and roughness of the polymeric film-formers are shown in Figure 4-13. The RMS roughness of the film-forming polymers was approximately a few nanometers (19, 6, 5, and 4 nm for VP-Acr-LaMeAcr, PU, shellac, and Acr-OcAA, respectively), except for the Acr-DiMet film, which had the lowest surface roughness (~410 pm). The Young's modulus of the polymeric film-formers, shown in Figure 4-14, demonstrated that the shellac, VP-Acr-LaMeAcr, and Acr-OcAA films had the highest modulus ( $22.16\pm3.55$ ,  $21.07\pm1.69$ , and  $11.17\pm0.76$  GPa, respectively) and the PU and Acr-DiMet films had a much lower modulus ( $33.8\pm2$  and  $89.6\pm1.56$  MPa, respectively).

As mentioned in Section 2.2, the water vapor permeation through a polymeric film depends on the solubility of water vapor molecules in the polymer (polymer hydration), as well as water vapor mobility and diffusion within the polymer matrix. The solubility is affected by the chemical affinity between the polymer and water vapor and the adsorption of molecules into the nanoporous surface of the polymer, available for occupation. The diffusion depends on the free volume available for the water molecules,  $T_g$ , and the crystallinity of the polymer.[19], [21], [214]

All the polymers (except VP-Acr-LaMeAcr) showed a low affinity to water vapor and the surface roughness of the films did not increase the chance of capillary condensation of water vapor molecules (Figure 4-12a and Figure 4-13). However, based on the permeation data (Figure 4-10) and mechanical properties of the film-formers (Figure 4-14), the diffusion behavior of water vapor through the polymeric films was different. For example, the high permeation rate through the PU films could be attributed to the presence of a large free volume and easier polymer chain reconformation (low modulus) or the very low permeation rate through Acr-OcAA could be due to high polymer chain packing (reflected in the high modulus), which allows very limited space for molecules to transport.



Figure 4-13. AFM height image of the film forming polymers and the cross-section profile taken at the line marked on the AFM height image.



Figure 4-14. The force curves for film forming polymers. The red curves represent the approach and the blue curves represent the retraction curves.

Among the tested film-forming polymers, VP-Acr-LaMeAcr showed the highest water vapor uptake and permeation, even though it had a high Young modulus. This could be related to its chemical structure and film-forming mechanism.[1] VP-Acr-LaMeAcr is an amphipathic polymer, in which the hydrophobic (lauryl methacrylate) and hydrophilic (vinylpyrrolidone) groups are connected by an anchoring group (acrylates). Therefore, the presence of hydrophilic segments close to the surface can facilitate the solubility of the water vapor molecules. In contrast, the other polymeric films are formed from polymeric solutions, whereas the VP-Acr-LaMeAcr films are cast from a polymeric dispersion. As mentioned before, the mechanism of film formation from polymeric solutions and from dispersions are not similar.[1] In solution form, while the solvent evaporates, polymer chains come more closely into contact and enter a gel state, eventually forming a polymeric film. The dispersion form requires the coalescence and interpenetration of polymer chains during solvent evaporation. That is, the polymer chains reform to fill free spaces created after evaporation of solvent. Therefore, the volume of microcracks and micro-voids present in the film increases, resulting in a porous macromolecular structure. This phenomenon, in conjunction with the chemical structure of VP-Acr-LaMeAcr, may facilitate the permeation of water vapor.

However, confirming the connection between the structural characteristics of the polymers and the permeation requires more data, including  $T_g$ , molecular weight, and crystallinity, which was beyond the scope of this study.

### 4.3. Sunscreen in the TEWL simulator

The TEWL simulator was used to evaluate the effect of sunscreen application on skin breathability. The sunscreen (containing 0.75 wt.% of Acr-OcAA as the film former) was applied on the skinmimicking substrate with a quantity of 2 mg cm<sup>-2</sup> and after 15 minutes, the substrate was mounted on the chamber for in vitro TEWL measurement. The same experiment was performed for the sunscreen without film-former to see if the presence of the film-former affected the WVPR through the sunscreen film. As illustrated in Figure 4-15a, the sunscreen was breathable with or without the film-former and did not occlude the skin-mimicking substrate.



Figure 4-15. In vitro and in vivo TEWL changes for sunscreen-treated skin-mimicking substrate and human skin. (a) Permeation data for the skin-mimicking substrate and the sunscreen (with and without Acr-OcAA) applied on the skin-mimicking substrate. (b) TEWL measurements performed on the forearm of five subjects before and after sunscreen application. (c) The average change in the TEWL values after sunscreen application compared to the TEWL values for the untreated skin.

Similar to film-forming polymers, we performed a pilot in vivo TEWL study on the forearm of five of our colleagues. Initially, the TEWL value of the untreated skin was measured. Then, 2 mg cm<sup>-2</sup> of sunscreen (containing 0.75 wt.% of Acr-OcAA as the film former) was applied on the marked area followed by homogenous distribution with a latex finger cot pre-saturated with sunscreen. The measurements were repeated on the sunscreen-treated area 1 and 3 hours after

application. As shown in Figure 4-15b and c, the application of sunscreen did not change the breathability of the skin, confirming the results obtained from in vitro studies. On average, the reduction in the in vivo TEWL value was 8%.

# 4.4. Sunscreen in the perspiring skin simulator (paper II and III)

In paper II, the performance of sunscreen in response to sweating was assessed qualitatively by different imaging techniques and quantitatively by in vitro SPF measurements. Subsequently, the effect of two parameters, i.e., sunscreen application dose and sweating rate, on sunscreen efficiency was investigated. In paper III, by employing the aforementioned techniques, we used two approaches to improve the sunscreen substantivity in response to sweating: incorporation of different concentrations of a hydrophobic film-former and the addition of four types of water-absorbing particles to the sunscreen formulation.

#### 4.4.1. Procedure for in vitro perspiration experiments

After activating the sweat pores of the skin-mimicking substrate, the initial sweating rate was achieved by adjusting the hydrostatic pressure. Subsequently, the substrate was detached from the chamber, the residue of water on the substrate was cleaned using a dust-free paper wipe, the substrate was placed on a molded PMMA plate (Helioplates HD2 or HD6; Helioscreen, Creil, France), and the blank transmittance spectrum using the UV transmittance analyzer was recorded. A defined amount of sunscreen (0.6 or 1.2 mg cm<sup>-2</sup> for studies in paper II and 0.6 mg cm<sup>-2</sup> for studies in paper III) was spotted onto the skin-mimicking substrate and distributed over the surface homogeneously. The sample was kept in darkness for 15 minutes to dry and self-level, after which the initial SPF was measured. The sample was then mounted onto the chamber and the perspiration was started at a rate of 1.46 or 3.31 µl min<sup>-1</sup>cm<sup>-2</sup>. The sweat rate after sunscreen application was lower, depending on the ratio of activated/inactivated pores. For paper III studies, we used a syringe pump to obtain a constant flow rate  $(1.5 \ \mu l \ min^{-1} \text{cm}^{-2})$  after sunscreen application. When the perspiration was stopped, the chamber was drained and the sample was allowed to dry. The post-perspiration in vitro SPF was then measured. During and after perspiration, the chamber was placed in a box, to which the UV camera was fixed above the sweat chamber and perpendicular to it. LED UV lamps were fixed on the wall of the box to emit UVA light suitable for the camera operation. In this way, distribution of the UV filters (with UV absorption at 365 nm, i.e., UVA filters) was monitored directly before, during, and after perspiration.

#### 4.4.2. Visualization of sunscreen film on the perspiring skin simulator

Initially, optical microscopy was used to visualize the morphology and performance of sunscreen on the perspiring skin simulator and to monitor any potential changes in the sunscreen film. The untreated skin-mimicking substrate is shown in Figure 4-16a. As shown in Figure 4-16b, after sunscreen application on the substrate and the drying time, the sunscreen forms a thin film that is uniformly distributed on the skin-mimicking substrate so that most of the sweat pores are covered. The sunscreen-treated substrate after 20 minutes of perspiration (with a sweat rate corresponding to 3 µl min<sup>-1</sup>cm<sup>-2</sup> for uncovered skin) is illustrated in Figure 4-16c. To achieve a better observation of the sweating effect, the same location as shown in Figure 4-16c was examined 20 minutes after drying, and is presented in Figure 4-16d. The sunscreen morphology was changed by sweat droplet formation and the uniformity of the sunscreen film was impaired. Some unprotected areas were also evident after the sweat droplets had dried. The formation and expansion of sweat droplets either completely removed the sunscreen film (sunscreen wash-off) or relocated the ingredients, including the UV filters, within the film (sunscreen redistribution) leading to change in film uniformity, average thickness, and a possible reduction in UV protection.



Figure 4-16. Microscopic images of (a) the bare skin-mimicking substrate, (b) the substrate with sunscreen applied before perspiration, (c) the substrate with sunscreen after 20 minutes of perspiration, and (d) the same spot as in (c), after 20 minutes of drying where the sweat droplet evaporates. This figure is taken from paper II (Figure 4).

As mentioned before, most of the sweat pores are covered by the sunscreen film. However, some of them are not covered and few sweat pores are partially occluded owing to sunscreen penetration into the pore. As a result, while sweating, different phenomena may occur; some examples are shown in Figure 4-17. For example, in the areas with uncovered sweat pores, the sweating starts right away. The water droplets emerge and spread on the surrounding sunscreen (Figure 4-17a, red circles). In case that the sweat pores are covered by the sunscreen, there are two subsequent possibilities: if the film thickness is low, the sweat droplet either overcomes film resistance and punctures it (Figure 4-17b) or if the droplet connects to other water droplets beneath the sunscreen film earlier, it will just thin the film (Figure 4-17c). As a result, the film initially bulges, to a certain extent, and when the water beneath it attaches to the others and spreads out, the film subsides. In contrast, if the thickness of the sunscreen film is high and the sweat droplet cannot overcome the film resistance, either sweating will cease or the droplet will propagate beneath the sunscreen film and find a way to join to other droplets in close proximity to form a larger sweat droplet. In the case that sunscreen has gone through the sweat pore, based on the amount of the sunscreen, either the sweat pore is occluded completely or the sweat droplet can push the sunscreen to the surface and sweating continues (Figure 4-17, blue semi-circle and green circle). The observations showed that, based on the sunscreen's affinity to the substrate and the film thickness, there was a constant competition between the sweat droplets trying to reach to the skin substrate surface and the sunscreen film holding its integrity and substantivity against sweating.



Figure 4-17. Behavior of sunscreen film on the skin mimicking simulator upon sweating. (a) Red circle indicates sweat droplet formation from uncovered sweat pores in which the perspiration starts effortlessly. Green circle and the blue semi-circle indicate sweat droplet formation from partially occluded sweat pores. In the sweat pore shown with the green circle, the sunscreen presented in the pore is pushed up by the sweat droplet. The sweat pore shown in the blue semi-circle is partially covered by sunscreen film and the sweat droplet overcomes the resistance of the film and perspiration starts. (b) The sunscreen film thins out resulted in film rupture. (c) The sweat droplet cannot overcome resistance of the sunscreen film and as a result the film initially bulges to some extent and when the water beneath in attaches to the other droplet and spreads out, the film subsides.

On the assumption of the uniform distribution of UV filters in the sunscreen film applied on the substrate, a similar redistribution could be attributed to the UV filters during sweating. Therefore, an area scan camera with UV band-pass filter (365 nm) was employed to follow solely the distribution of UV filters. In this approach, given the wavelength detectable by the camera filter, only UVA filters can be evaluated. The UV filter distribution on the skin-mimicking substrate in four stages is presented in Figure 4-18. The dark color on the substrate represents the UV absorption by the UV filters and the darker areas indicate a higher concentration of UV filters. The uniform distribution of the UV filters at the onset of perspiration is shown in Figure 4-18a. The activated sweat pores at the initiation of perspiration are shown in Figure 4-18b. After 20 minutes of sweating, sweat droplets were visibly scattered on the skin substrate (Figure 4-18c). After drying, relocation of the UV filters within the sunscreen film, similar to those shown in the microscopic images, was observed (Figure 4-18d). It is also clear that at some spots, the UV filters had been completely washed off, leaving unprotected areas on the skin-mimicking substrate.



Figure 4-18. (a)-(d) UV camera images illustrating the sunscreen distribution at different stages: (a) before perspiration, (b) at the beginning of perspiration, (c) after 20 minutes of perspiration, and (d) after 20 minutes of drying. Darker areas represent higher absorption of UV light. This figure is a modified version of Figure 5 in paper II.

For a better visualization of the mechanisms involved in sunscreen failure during sweating, we prepared skin-mimicking substrates in which only half of the surface (semi-circular area) was laser drilled to have sweat pores and the other half was left as a plain gelatin-based layer (Figure 4-19). The sunscreen was applied on the area with the sweat pores and after mounting on the chamber, the chamber was kept vertically, with the treated area on the upper side during perspiration and drying. Using the UV camera, the distribution of the UV filters was checked before perspiration onset, immediately after perspiration was stopped, and after the sample was dried.



Figure 4-19. Top: (left) schematic illustration of the skin-mimicking substrate in which only half of the surface (semi-circular area) was laser drilled to have sweat pores and the other half was left as a plain gelatin-based layer. (right) Substrate with applied sunscreen on the upper side before onset of perspiration. Bottom: The chamber is kept in a vertical position during perspiration and drying, enabling sweat to flow from the sunscreen-treated area to the untreated area. This figure is a modified version of Figure 2 in paper III.

The substrate under four conditions is shown in Figure 4-20a–d: before sunscreen application; after sunscreen application and before perspiration was started; 20 minutes after perspiration was started; and 20 minutes after perspiration was stopped. The washed-off sunscreen, carried by sweat droplets to the plain area, is shown in Figure 4-20d. This image also illustrates the redistribution of the UV filters on the treated area. Qualitatively, our observation was in agreement with the results obtained from a pilot in vivo study shown in Figure 4-20e–h. A commercial sunscreen was applied at a dose of 2 mg cm<sup>-2</sup> on the forehead of subjects, and images from the initial UV filter distribution in the sunscreen film were taken. After 15 minutes, the subjects were asked to perform physical activities to induce sweating. The exercise was continued for 20 minutes, followed by a drying period of 30 minutes, and post-perspiration images were then taken. The average measured sweat rate was 1.1 µl min<sup>-1</sup> cm<sup>-2</sup>, which is similar to the moderate sweat rate we used in our study. In Figure 4-20f, the uniform distribution of UV filters on the forehead of the subject before physical activity-induced sweating is shown; in contrast, Figure 4-20h shows that the UV filters were relocated and washed-off as a consequence of active perspiration.



Figure 4-20. Illustration of UV filters distribution before and after in vitro and in vivo sweating. The skin-mimicking substrate (a) with activated sweat pores (b) with sunscreen applied on the area with sweat pores. (c) After sweating for 20 minutes. (d) After drying. (e) The standard camera image of the subject forehead after sunscreen application before the beginning of the physical exercise. (f) The image of the same spot as (e) taken with the UV camera. (g) The standard camera image of the subject forehead after post-exercise drying. (h) The image of the same spot as (g) taken with the UV camera. This figure is a modified version of Figure 5 and S4 in paper II.

CARS microscopy, the third technique used for the visualization, allows the possibility of realtime probing of the interaction between a sweat droplet and the sunscreen film by providing a label-free chemical contrast. The sunscreen containing octocrylene as the only UV filter was applied on the skin-mimicking substrate with the application dose of 2 mg cm<sup>-2</sup>; then, artificial sweating was activated and changes in the distribution in the UV filter was followed in real time. Figure 4-21 shows how a sweat droplet, during its expansion and merging with other sweat droplets, is disturbing the homogeneity of the distribution of the UV filters. The figure shows two cross-sections, one at the surface of the substrate and one 60  $\mu$ m above the substrate. As the water droplets expand, the red halo observed at the borders of sweat droplets implied the movement of the UV filter parallel to the droplet expansion. Encased in a thin film of sunscreen, the sweat droplet relocates the surrounding UV filters as well.

The redistribution of the UV filters is not limited to the duration of perspiration. After perspiration was stopped, the chamber was drained and real-time sweat evaporation was followed. While the droplet is evaporating, the thinned layer of sunscreen (if not ruptured) returns to the surface of the

skin-mimicking substrate, altering the morphology compared with the initial conditions. The partial self-healing of the sunscreen layer is shown in Figure 4-22.



Figure 4-21. CARS images obtained during sweat droplet growth. (a) A schematic representation of cross-sections of a sweat droplet at the substrate surface,  $z=0 \ \mu m$  and at a height of  $z=60 \ \mu m$ . (b-g) Time evolution of sweat droplet growth and merging. As the sweat droplets expand, UV filters are noticeably pushed in the direction of expansion. This figure is taken from paper II (Figure 6).



Figure 4-22. CARS images obtained during the evaporation of a sweat droplet. (a) The initial distribution of UV filters. (b-f). Time evolution of sweat droplet evaporation (t=0, 182, 364, 546, 728 s, respectively). This figure is taken from paper II (Figure 7).

Based on the observation from the different imaging techniques employed (optical microscopy, UV camera imaging, and CARS microscopy), it could be concluded that the sweat droplet formation negatively affects the uniformity of sunscreen film, as well as the distribution of UV filters presented in the film. As the droplets grow, they can either relocate the sunscreen film and the UV filters, impairing the film uniformity, or remove the sunscreen film from the spots with poor sunscreen/substrate attachment, impairing the film thickness. It was also observed that the evaporation of sweat droplets during the drying process may cause a secondary redistribution of the UV filters.

#### 4.4.3. SPF measurements on the skin-mimicking substrate

To quantify the effect of sweating on the sunscreen film distribution and its UV protection properties, we measured the in vitro SPF before and after the perspiration experiment in accordance with the procedure explained in Section 4.4.1. As the spots of sweat after sunscreen application were scattered on the substrate of each sample, to examine the changes in UV protection over the whole substrate, we chose nine locations to measure the SPF before and after perspiration. The sunscreen-treated skin-mimicking substrate, which received a sunscreen

application dose of 0.6 mg cm<sup>-2</sup> is shown before (Figure 4-23a) and after (Figure 4-23b) exposure to perspiration (initial sweating rate of 3  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>) and the locations on the substrate in which the SPF was measured are shown in Figure 4-23c. The table in Figure 4-23 presents the corresponding pre- and post-perspiration SPF values for the sample. For this sample, in all locations, SPF was decreased by perspiration, confirming the visual observation of the impaired uniformity of the sunscreen film.



Figure 4-23. (a) and (b) UV camera images illustrating the UV filters distribution in different stages: (a) Before perspiration was started. (b) After 20 minutes of drying. Darker areas represent higher absorption of UV light. (c) Locations on the substrate surface used for SPF measurements. The table shows SPF measurements for each location before and after perspiration (stages a and d), for a sunscreen with an application dose of 0.6 mg cm<sup>-2</sup> and a sweating rate of 3  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>.

As discussed in Sections 2.7.1.1 and 2.7.1.2, the sunscreen film thickness and thickness distribution are two main parameters affecting sunscreen performance. The results obtained from visualizing the sunscreen behavior and measuring the UV protection changes due to perspiration illustrated that sweating could manipulate both parameters. The average thickness of the sunscreen film, i.e., the mass of active ingredients, will be reduced as a result of wash-off caused by sweat. Further, the film homogeneity and the thickness distribution will be disturbed owing to sweat droplet formation and expansion, resulting in relocation of the UV filters within the film and the introduction of irregularities to the sunscreen film.

# 4.4.4. Effect of the sweat rate and amount of sunscreen applied on sunscreen efficiency

The retention of sunscreen efficiency upon sweating depends on different parameters. We chose two key parameters, i.e., sweat rate and amount of sunscreen applied, and investigated their impact on UV protection by employing the perspiring skin simulator. The perspiring rates were chosen as  $1.46 \text{ and } 3.31 \,\mu\text{l} \,\text{min}^{-1}\text{cm}^{-2}$ , representing the moderate and heavy sweating on an untreated human forehead.[47] The application quantities were chosen as  $0.6 \text{ and } 1.2 \text{ mg cm}^{-2}$  to represent the amount of sunscreen commonly applied by consumers.[142] Accordingly, three sets of experiments were conducted: low sunscreen quantity and moderate sweating ( $0.6 \text{ mg cm}^{-2}$  and  $1.46 \,\mu\text{l} \,\text{min}^{-1}\text{cm}^{-2}$ ); low sunscreen quantity and heavy sweating ( $0.6 \text{ mg cm}^{-2} \text{ and } 3.31 \,\mu\text{l} \,\text{min}^{-1}\text{cm}^{-2}$ ). For each set of experiments,  $10 \,\text{skin-mimicking substrates}$  were examined to increase the data pool and SPF was measured at nine locations in each sample (corresponding to 90 SPF measurements for each set of experiments).



Figure 4-24. Initial versus final SPF values for a low amount of applied sunscreen (0.6 mg cm<sup>-2</sup>) with (a) moderate sweating (1.46  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>) and (b) heavy sweating (3.31  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>). Each color represents nine locations on an individual sample. The solid line represents the location of the data point if no effect of sweating was observed and the data point layer below the line thus represents a situation where the SPF values decreased. (c) Histogram of initial and final SPF for the samples tested with heavy sweating (3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>) and high amount of applied sunscreen (1.2 mg cm<sup>-2</sup>). (d) Statistical representation of the differences in measured SPF before and after perspiration. The colored area represents the interquartile range (IQR) showing the range of middle 50% of the data (25% to 75%). The middle line represents the median and the small square represents the mean value. The mean value for each set of experiments is shown beside each boxplot. The upper and lower lines represent the maximum and minimum SPF changes, respectively, excluding the outliers. The rhombuses outside the box indicate outliers. This figure is a modified version of Figure 8 in paper II.

The SPF values after sweating at moderate and heavy rates are presented in Figure 4-24a and b, respectively, versus their associated local initial SPF values before sweating, for a low application dose of sunscreen. At both sweating rates, there was a tendency toward SPF reduction due to perspiration. However, in some spots, the SPF was not changed or even increased. The measured values after perspiration were more scattered and there were more locations in which the SPF was

increased than with the samples with the high perspiration rate. To investigate the impact of sweating on failure mechanisms, the UV camera was used to image a selected sample of each set of experiments. The UV camera images of two sunscreen-treated samples, exposed to moderate and heavy sweating, 15 minutes after sweating was started and 20 minutes after sweating was stopped, are presented in Figure 4-25. The initial distribution of UV filters was similar for both samples. Moreover, the image histograms (grayscale value distribution) of the samples after drying are presented in Figure 4-25c and f. The comparison between the UV camera images indicated that with heavy sweating, the possibility of merging of droplets and forming larger droplets facilitates the sunscreen wash-off; in contrast, with moderate sweating, the formation of small and isolated droplets is more probable, which reduces the possibility of sunscreen wash-off (Figure 4-25a and d). The comparison between histograms (Figure 4-25c and f) shows that the intensity of UV absorption decreased more when the sample was exposed to the higher sweat rate, confirming the results obtained from SPF measurements.

Even though the low sweating rate did not amplify the sunscreen wash-off, the formation of sweat droplets disturbed the uniformity of the sunscreen film and increased irregularities, which reduced the SPF. When the reduction in SPF was compared with the number of active sweat pores, as presented in Figure 4-26, it was found that if more than 30% of sweat pores were active after sunscreen application (regardless of cumulative sweat amount), the SPF was mostly decreased.



Figure 4-25. The effect of sweat rate on the sunscreen distribution and wash-off. UV camera image for the sample exposed to low initial sweat rate (a) after sweating for 15 minutes (b) after drying. (c) Gray-scale value distribution of image (b). UV camera image for the sample exposed to high initial sweat rate (d) after sweating for 15 minutes (e) after drying. (f) Gray-scale value distribution of image (e). This figure is taken form paper II (Figure S5).



Figure 4-26. (a) SPF changes versus active pores after sunscreen application for the samples tested at low initial sweat rate (1.46  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>). Each color represents nine locations on an individual sample. (b) Statistical representation of the SPF change distribution based on the percentage of active sweat pores (0-30%, 30-60%, and 60-100%) for low initial sweat rate (1.46  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>). The colored area represents the interquartile range (IQR) showing the range of middle 50% of the data (25% to 75%). The middle line represents the median and the small square represents the mean value. The mean value for each set of experiments is shown beside each boxplot. The upper and lower lines represent the maximum and minimum SPF changes, respectively, excluding the outliers. The rhombuses outside the box indicate outliers.

The impact of sunscreen application dose upon heavy sweating was assessed by comparing the initial SPF values and their associated SPF values after sweating. As shown in Figure 4-24c, the initial SPF values for the samples treated with the high application dose of sunscreen (1.2 mg cm<sup>-</sup> <sup>2</sup>) were very high (>100) compared with treatment with the low application dose of the sunscreen. The high initial SPF value was attributed to the low roughness of the skin-mimicking substrate (Figure 4-7), which resulted in the formation of a uniform sunscreen film with high thickness.[95], [102] The results showed that, even though the average SPF reduction for the samples treated with the higher applicable dose of sunscreen was greater (Figure 4-24d), the post-perspiration SPF values associated with these samples were still relatively high (70% of the SPF values were >50), providing the required UV protection. Understandably, the application of a higher amount of sunscreen led to larger amounts of sunscreen being washed-off and redistributed, but the remaining sunscreen still retained strong UV protection. Owing to the high sweating rate, for both sets of experiments, wash-off of sunscreen is probable. As shown in the images from the UV camera (Figure 4-27), the application of a lower dose of sunscreen resulted in larger unprotected areas during similar perspiration conditions. Qualitatively, these results agreed with the findings of our pilot in vivo study, in which the sunscreen was applied on the back of subjects with two application doses, i.e., 1 and 2 mg cm<sup>-2</sup> and after dwelling for 15 minutes, the initial SPF was measured. After exercise-induced perspiration followed by skin drying, the SPF was measured again. As illustrated in Table 4-1, although the percentage of SPF reduction was similar for both application doses  $(\sim 35\%)$ , the higher initial SPF associated with higher application dose resulted in higher retention of UV protection after perspiration.



Figure 4-27. The effect of sunscreen application dose on the sunscreen distribution and wash-off. UV camera image for the sample treated with low sunscreen application dose  $(0.6 \text{ mg cm}^{-2})$  (a) after sweating for 15 minutes and (b) after drying. (c) Gray-scale value distribution of image (b). UV camera image for the sample treated with high sunscreen application dose  $(1.2 \text{ mg cm}^{-2})$  (d) after sweating for 15 minutes and (e) after drying. (f) Gray-scale value distribution of image (e). This figure is taken form paper II (Figure S8).

Table 4-1. SPF changes and sweat resistance of the commercial sunscreen with an application dose of 1 and 2 mg cm<sup>-2</sup>. (\*: confidence interval)

Product application dose (mg cm <sup>-2</sup> )	Initial SPF [Mean]	Initial SPF [Lower Cl*]	Final SPF [Mean]	Sweat resistance [Mean]	Sweat resistance [Lower Cl*]
2	30.7	20.0	18.5	64.1 %	46.7 %
1	15.4	13.6	10.4	65.7 %	57.7 %

As discussed in Section 2.6.5, more detailed information can be obtained from in vitro studies than in vivo studies, such as in vitro UVA protection factor (UVA-PF), the ratio of the UVA attenuation to UVB attenuation (UVA/UVB ratio), and the critical wavelength ( $\lambda_c$ ). The UVA/UVB ratio and  $\lambda_c$  illustrate the balance between UVA and UVB protection and the uniform protection over the UV radiation range. The comparison of these two factors before and after perspiration could potentially demonstrate which of the UVA and UVB filters are more prone to wash-off. If the sweat resistance decreases and the UVA/UVB ratio is constant, it means that the sweating has the same effect on both types of UV filters. If the ratio is higher or lower than the original value, this indicates the lower substantivity of the UVB or UVA filters upon sweating, respectively. As shown in Figure 4-28 and Table 4-2, the UV filters presented in our formulation underwent comparable changes in all three sets of experiments. However, the UVA/UVB ratio and  $\lambda_c$  may change differently, based on the selected sunscreen formulation.[155], [156]



Figure 4-28. UVA/UVB ratio comparison before and after perspiration. (a) Set 1: applied amount of 0.6 mg cm<sup>-2</sup> and perspiring rate of 1.46  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>. (b) Set 2: applied amount of 0.6 mg cm<sup>-2</sup> and perspiring rate of 3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>. (c) Set 3: applied amount of 1.2 mg cm<sup>-2</sup> and perspiring rate of 3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>. This figure is taken form paper II (Figure S9).

	Applied amount:		Applied amount:		Applied amount:	
	$0.6 \text{ mg cm}^{-2}$		$0.6 \text{ mg cm}^{-2}$		$1.2 \text{ mg cm}^{-2}$	
	Perspiring rate:		Perspiring rate:		Perspiring rate:	
Sample	Sample 1.46 $\mu$ l min <sup>-1</sup> cm <sup>-2</sup>		$3.31 \ \mu l \ min^{-1} cm^{-2}$		$3.31 \ \mu l \ min^{-1} cm^{-2}$	
	$\lambda_c$ Before perspiration	$\lambda_c$ After perspiration	$\lambda_c$ Before perspiration	$\lambda_c$ After perspiration	$\lambda_c$ Before perspiration	$\lambda_c$ After perspiration
1	371.4	376.1	371.6	374.6	371.4	371.2
2	371.0	376.8	371.1	374.0	371.0	370.9
3	371.0	372.1	371.7	374.8	370.9	374.2
4	371.1	374.9	371.0	374.6	371.3	374.9
5	371.8	371.7	371.4	373.2	370.4	373.2
6	372.0	377.1	371.3	373.3	369.8	370.9

Table 4-2.  $\lambda_c$  values before and after perspiration. This table is taken from paper II (Table S1).

7	370.8	371.4	370.8	374.3	370.4	370.7
8	371.0	374.1	371.8	373.6	371.8	375.0
9	372.2	373.5	371.9	374.3	369.9	370.8
10	371.2	371.8	372.1	374.6	371.2	376.9

#### 4.4.5. Effect of formulation on sweat resistance of sunscreen

Based on the results obtained from imaging techniques and in vitro SPF measurements, perspiration activated two mechanisms involved in sunscreen failure, i.e., sunscreen wash-off and sunscreen redistribution. Therefore, we used two approaches to see how these mechanisms may be altered. First, a hydrophobic film-former (acrylate/octylacrylamide copolymer) was used at different concentrations; second, a low concentration of water-absorbing particles was added to the formulation. As discussed before, the film-former plays an important role in the uniform distribution of the UV filters in the sunscreen film and increases the integrity of the film in contact with water.[75], [77], [215] Different water-repellent or water-dispersible materials, categorized as film-former to the sunscreen formulations.[75], [216] Moreover, the introduction of a hydrophobic film-former to the sunscreen formulation is a typical approach used to achieve high water resistance.[77] We aimed to see if this approach could also assist the retention of the UV protection of sunscreen upon sweating. Therefore, three sunscreen formulations containing 0, 0.75, and 3 wt.% of the acrylic-based film-former with hydrophobic properties were prepared.

The second approach used a different perspective, in which water-absorbing particles with different characteristics were added to the formulation to see if the localization of the water around such particles could reduce redistribution and wash-off. The information on the selected particle characteristics, i.e., chemistry, particle size, and water uptake capacity, and their abbreviated names are presented in Table 4-3. We selected two superabsorbent particles, NaPA and PAC-6, with a water uptake capacity of >300 g/g, but different particle sizes (approximately 10–40  $\mu$ m and 1–10  $\mu$ m, respectively) and two other particles, HMHEC and the Core-Shell, with more moderate water uptake capacities of ~20–30 g/g and particle size of 30–180 and <5  $\mu$ m, respectively. Therefore, NaPA and HMHEC will act as large water-absorbing domains in the sunscreen film, whereas PAC-6 and the Core-Shell will be embedded in the sunscreen film and, for the same weight fraction, will be more homogeneously distributed in the formulation.

Table 4-3. Water absorbing particles.

Particle	Particle size (µm)	Water absorbing capacity (g/g deionized water)	Abbreviation
Polyacrylate crosspolymer-6	1-10	>300	PAC-6
Cross-linked sodium polyacrylate	10-40	>300	NaPA
Cetyl hydroxyethyl cellulose	30-180	~20-30	HMHEC
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Core-shell particle Shell: polysilicone-34 Core: isononyl isononanoate and Water	<5	~20-30	Core-Shell

Similar to the previous studies, in vitro SPF measurements before and after perspiration, direct detection of the UV filter distribution (using the UV camera), and CARS microscopy were employed to investigate the effect of sunscreen formulation on the retention of UV protection. For all experiments in this section, the sweat rate and the application dose of sunscreen were fixed at  $1.5 \,\mu l \,min^{-1}cm^{-2}$  (provided by the syringe pump) and 0.6 mg cm<sup>-2</sup>, respectively, unless indicated otherwise. The perspiration duration and the subsequent drying time were set to 30 minutes and HD2 plates were used as the support for the skin-mimicking substrates in the in vitro SPF measurements. The initial in vitro SPF values for different sunscreens used in this study, measured by the COLIPA method for in vitro SPF measurements (application dose of 1.3 mg cm<sup>-2</sup>), are presented in Table 4-4.

	Sunscreens v	Sunscreens containing water absorbing					
	concentration			particles			
Sunscreen	0 wt.%	0.75 wt.%	3 wt.%	PAC-6	Core- Shell	NaPA	HMHEC
SPF	48	73	84	74	158	62	59

Table 4-4. In vitro SPF values for sunscreens used in this study. This table is taken from paper III (Table S1).

### 4.4.5.1. Effect of hydrophobic film former concentration

The in vitro SPF measurements before and after sweating and the average SPF values and their variations, respectively, are outlined in Figure 4-29a and b. The SPF reduction for the sunscreens containing 0, 0.75, and 3 wt.% of film-former was  $58\%\pm26\%$ ,  $42\%\pm24\%$ , and  $69\%\pm19\%$ , respectively. The results showed that incorporation of the film-former improved the sweat resistance of sunscreen through enhancing the sunscreen substantivity, most likely by stronger

entrapment of the UV filters in the film-former network, causing stronger bonding to the substrate, and repelling the water.[75], [77]. However, by increasing the concentration of the film-former up to 3%, the SPF reduction increased. The exact reason for this observation is unknown. However, the increase in the concentration of the film-former may alter other parameters which contradict to enhancing the substantivity. For example, sunscreen with 3 wt.% of film-former had a higher viscosity (39.5 cP) than those with 0 wt.% and 0.75 wt.% of film-former (22.7 and 24.8 cP, respectively), which could affect the rheological profile of sunscreen and thus its morphology on the skin-mimicking substrate. We observed that it was more difficult to achieve the initial uniform distribution of sunscreen film when a higher concentration of film-former was used.



Figure 4-29. *In vitro* SPF measurements for sunscreen formulations with different film former concentrations. (a) SPF after sweating versus initial SPF values measured on five individual samples and for nine positions on each sample. The dashed lines represent the location of the SPF values if there is no difference before and after sweating. (b) Statistical representation of the difference in measured SPF before and after sweating. The colored area represents the interquartile range (IQR) showing the middle 50% of the data (25% to 75%). The middle line represents the median and the small white dot is the mean value. The mean value for each set of experiments is also shown. The upper and lower lines represent the maximum and minimum SPF change excluding the outliers. The dots outside the colored region indicate the outliers. This figure is taken from paper III (Figure 3).

To examine the effect of film-former concentration on the mechanisms of sunscreen film failure during perspiration, the skin-mimicking substrate with only sweat pores over half of its area was used. For the experiments using this substrate, the sweat rate and the application dose of sunscreen were fixed at 3  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup> and 1.2 mg cm<sup>-2</sup>, respectively. The skin-mimicking substrate at four stages is shown in Figure 4-30a–d: before perspiration was started; 20 minutes after perspiration; and 20 minutes and approximately 2 hours after perspiration was stopped.



Figure 4-30. UV camera images of the skin-mimicking substrates. The images illustrate how sunscreens with different concentrations of hydrophobic film formers are washed off and redistributed during perspiration. (a) After sunscreen application on the area with sweat pores and at the beginning of sweating, (b) after sweating for 20 minutes, (c) 20 minutes after the sweating was stopped, and (d) after complete drying of the sample. This figure is taken from paper III (Figure 4).

The absence of film-former results in massive sunscreen wash-off and a drastic relocation of UV filters, as shown in Figure 4-30. This is because the film-former is responsible for holding the sunscreen film together and, as shown in the figure, the addition of 0.75 wt.% of film-former significantly reduced the effect of sweating on the redistribution of UV filters. However, sunscreen wash-off remained visible. An increase in the concentration of film-former up to 3 wt.% effectively prevented sunscreen wash-off; from an application perspective, this could reduce the wash-off induced discomfort, such as sunscreen running into the eyes or staining clothes, for consumers.

However, the retention of UV protection for the samples with 0.75 wt.% of film-former was higher than that of samples containing 3 wt.% of film-former. It appears that sunscreen redistribution has a more pronounced effect on SPF than sunscreen wash-off.

To test the effect of water-absorbing particles, we selected the sunscreen containing 0.75 wt.% of film-former as the base sunscreen, as it showed the optimal SPF retention upon sweating.

#### 4.4.5.2. Effect of water-absorbing particles

In vitro SPF measurements before and after sweating and the associated average SPF values and their variations, respectively, for the base sunscreen and sunscreens containing water absorbing particles are shown in Figure 4-31a and b. Sunscreens containing NaPA and HMHEC particles showed average SPF reductions of  $49\%\pm19\%$ , and  $61\%\pm21\%$  respectively, indicating that the addition of these particles had a negative effect or no impact on the retention of UV protection (the average SPF reduction for base sunscreen was  $42\%\pm24\%$ ). However, the SPF reduction for the sunscreen formulation containing PAC-6 particles was only  $13\%\pm20\%$ , illustrating that these particles could reduce the effect of sweating on the impairment of sunscreen efficiency.

Sunscreen containing Core-Shell exhibited a special property. This particle is made of a silicone copolymer shell covering a water-swellable polyacrylic core. The presence of Core-Shell in the sunscreen formulation increased the UV light scattering and boosts the initial SPF value (see Table 4-4). However, when the sunscreen was exposed to water, the core swelled moderately, altering the particle shape, which may affect its ability to scatter UV light; therefore, when testing the sweat resistance of this sunscreen, we investigated the impact of sweating on the boosted UV protection, which is different from the two previously mentioned failure mechanisms. As expected, the initial SPF value for the sunscreen containing Core-Shell was much greater than the other formulations (see in Figure 4-31a); however, after perspiration, the SPF was greatly reduced to the post-sweating values observed for the other sunscreen formulations, suggesting that the boosted UV protection could not be restored owing to irreversible changes in the shape of the Core-Shell particle. Hence, determining a connection between the water-absorbing characteristics of this particle and the sunscreen substantivity, based on the SPF data alone, would be complex.



Figure 4-31. *In vitro* SPF measurements for sunscreen formulations with and without water-absorbing additives before and after sweating. (a) SPF after sweating versus initial SPF values measured on five individual samples and for nine positions on each sample. The dashed lines represent the location of the SPF values if there is no difference before and after sweating. (b) Statistical

representation of the difference in measured SPF before and after sweating. The colored area represents the interquartile range (IQR) showing the middle 50% of the data (25% to 75%). The middle line represents the median and the white dot is the mean value. The mean value for each set of experiments is also shown. The upper and lower lines represent the maximum and minimum SPF changes excluding the outliers. The dots outside the colored region indicate the outliers. This figure is taken from paper III (Figure 5).

Similar to that in the previous section, we examined sunscreens containing the water-absorbing particles to assess their effects on the mechanisms of sunscreen failure (Figure 4-32).



Figure 4-32. UV camera images of the skin-mimicking substrates. The images illustrate how sunscreens with and without waterabsorbing particles are washed off and redistributed during perspiration. (a) After sunscreen application on the area with sweat

pores and at the beginning of sweating, (b) after sweating for 20 minutes, (c) 20 minutes after the sweating was stopped, and (d) after complete drying of the sample. This figure is taken from paper III (Figure 6).

The sunscreen with no water-absorbing particles showed considerable wash-off and moderate redistribution of the UV filters upon sweating. The presence of large particles, i.e., NaPA and HMHEC, resulted in considerable redistribution of UV filters and, in the case of NaPA, extensive sunscreen wash-off. These observations were in agreement with the conclusion based on in vitro SPF measurements where these particles showed a negative impact on the retention of UV protection. Redistribution of the UV filters was related to the size and swelling of the particles upon sweating. The volume expansion and movement of these particles within the sunscreen film resulted in pushing the UV filters aside, as well as disturbing the film's uniformity. In the case of superabsorbent NaPA, the water absorption increased the particle weight and, consequently, the particles were pulled downward, dragging the sunscreen along, which resulted in massive sunscreen wash-off. However, HMHEC particles can resist gravity owing to their low water-absorbing capacity.

The incorporation of small particles, i.e., PAC-6 and the Core-Shell, resulted in very limited sunscreen wash-off and for the sunscreen containing PAC-6 particles also a very limited redistribution of sunscreen. The observation regarding PAC-6 particles agreed with results obtained from SPF measurements. As mentioned before, the large SPF reduction for the formulation containing the Core-Shell particles originated from an irreversible change in the particle shape and, consequently, reduced the SPF boosting effect. However, the observation here was that the UV filter distribution can be altered by particle expansion.

The comparison between PAC-6 and NaPA particles illustrated that even though both had a high water uptake capacity, which should hypothetically favor the localization of sweat droplets, only the incorporation of PAC-6 increased the substantivity of the sunscreen formulation. To find the reason for this, we observed the water-absorbing behavior of pure particles and their corresponding sunscreens under an optical microscope. As shown in Figure 4-33a–d, when the NaPA particles absorbed water, their volume increased and the particles moved to reach their neighboring particles, but did not merge to form a network; while drying, they detached from each other to form small clusters. Therefore, the massive expansion of NaPA particles in the sunscreen film relocates the UV filters and intensifies the sunscreen wash-off. In contrast, Figure 4-33e–h shows

that when PAC-6 particles were exposed to water, they formed a gel-like structure that remains even after drying. This water-responsive behavior of PAC-6 particles in the sunscreen reinforces the integrity of the sunscreen film and, by localizing the pressurized water, resists or delays the relocation of UV filters (Figure 4-33j).



Figure 4-33. Microscopic images of NaPA and PAC-6 particles respectively in (a), (e) dry state, (b), (f) after exposure to water, (c), (g) during water evaporation (d), (h) after water evaporation. Sunscreen containing (i) NaPA and (j) PAC-6 particles during perspiration. This figure is taken from paper III (Figure S2).

Further, the drying of sweat droplets after perspiration was examined for both sunscreens (Figure 4-34). During drying, the NaPA particles in the sunscreen tended to move toward each other to form small clusters, leading to a redistribution of UV filters, whereas PAC-6 particles appeared to retain the gel-like structure during evaporation of the sweat droplets.



Figure 4-34. Drying of water droplets in sunscreens containing (a-b) NaPA and (c-d) PAC-6 particles. The red circle shows the NaPA particles which moved toward each other during the drying of sweat droplet. This figure is taken from paper III (Figure S3).

To determine the effect of PAC-6 particles on enhancing sweat resistance at a microscopic scale, two simplified sunscreens (containing a single UV filter, octocrylene) containing 0 and 1 wt.% of PAC-6 particles were prepared. As shown in Figure 4-35, similar to the sunscreen ingredients and the skin-mimicking substrate, PAC-6 did not show any distinguishable peaks that overlapped with octocrylene. Thus, we continued to monitor octocrylene at the same wavelength as in previous studies. The top view of the sunscreen-treated skin-mimicking substrate under sweating is shown in Figure 4-36. The droplets shown in Figure 4-36a formed a symmetric semi-spherical shape. However, the presence of PAC-6 particles and the formation of the gel-like structure in contact with water slightly changed the shape of the sweat droplet, making it less semi-spherical (Figure 4-36b).



Figure 4-35. CARS signal for the simplified sunscreen components and the skin-mimicking substrate in the wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>. The peak at 2210 cm<sup>-1</sup> is attributed to the nitrile group in octocrylene. This figure is taken from paper III (Figure S1).



Figure 4-36. Top view of sweat droplets formed on the substrate treated with simplified sunscreen (a) without and (b) with PAC-6 particles. The sweat droplets on the left image form a semi-spherical shape whereas the form of droplets on the right image are less symmetric. This figure is taken from paper III (Figure S4).

Furthermore, the growth of water droplets on the skin-mimicking substrate treated with sunscreen was monitored. As illustrated in Figure 4-37a, when sunscreen without PAC-6 particles was applied, the water droplet expanded in all directions and the sunscreen film around it thinned out in the z-direction, which probably led to the rupture of the sunscreen film. Moreover, the sweat droplet relocated the surrounding UV filters by forcing the sunscreen film around it to move as the droplet expands. As a result, when two droplets moved towards each other to merge, the concentration of UV filters in the area between two droplets increased, and finally, when the

droplets merged, a red line appeared at their common border, as shown in Figure 4-37a (t=289 s). A different sunscreen/sweat interaction was observed when the sunscreen containing 1 wt.% of PAC-6 was used. It appeared that the formation of the gel-like structure reinforced sunscreen film integrity and reduced the expansion of sweat droplets in the z-direction, consequently reducing the possibility of sunscreen film rupture. It could be seen that the incorporation of PAC-6 particles both localized (similar to the particle's water-responsive behavior shown in Figure 4-33f) and flattened the sweat droplets, to form a less concentrated ring with UV filters surrounding the expanding droplet. Therefore, when two droplets merge, the gel-like structure limits the relocation of the UV filters.

Our investigation on improving the water resistance of sunscreen illustrated that the incorporation of a hydrophobic film-former at a high concentration in sunscreen formulation could successfully reduce sunscreen wash-off, whereas intermediate film-former concentrations reduced sunscreen redistribution and resulted in better retention of UV protection. Thus, a sunscreen containing a hydrophobic film-former can provide higher efficiency and increase user-friendliness by reducing the discomfort caused by the sunscreen running into the eyes. The newer approach, incorporation of water-adsorbing particles in the sunscreen formulation, depending on the exact particle properties, resulted in both positive and negative effects on sunscreen performance in response to sweating. In general, the ability of the particles to localize the sweat droplets while introducing minimum disturbance to the sunscreen film uniformity appears to have protective effects on the sunscreen's integrity. We observed that PAC-6 particles could deliver the retention of sunscreen UV protection after sweating by changing the wettability of the sunscreen and the formation of a gel-like structure. In contrast, if the particles localize with too much water owing to swelling, a significant redistribution of sunscreen and a decrease in SPF will occur, as was observed when NaPA particles were incorporated in the sunscreen formulation. Overall, enhancing the sweat resistance of the sunscreen was achieved using a combination of hydrophobic film-formers to increase water resistance and small water-adsorbing particles to change the wetting behavior of the sunscreen film.



















t=343 s

t=412 s

Figure 4-37. CARS images showing the growth and merging of sweat droplets on the sunscreen-treated skin-mimicking substrate. (a) Sunscreen formulation with no water-absorbing particles. (b) Sunscreen formulation with 1% wt. PAC-6 particles. This figure is taken from paper III (Figure 7).

## 4.5. Comparison of sweat resistance and water resistance (unpublished study)

To differentiate the sunscreen interaction with water in two conditions, i.e., external water exposure and water exposure from underneath the applied sunscreen film (associated with water and sweat resistance of sunscreen, respectively), we performed an in vitro water resistance experiment and compared images from the UV camera and in vitro SPF measurements with the results already obtained from the in vitro sweat resistance test. To keep the substrate characteristics constant, we used the skin-mimicking substrates in which the sweat pores were laser drilled, only through the gelatin-based layer and PMMA film, and the adhesive remained untouched. Therefore, the surface roughness of the substrate was similar to that of the surface used for the perspiration test, and at the same time, the water beneath the substrate cannot penetrate through it. The skinmimicking substrate was hydrated before sunscreen application by exposing the gelatin-coated side of the substrate to water vapor for 10 minutes. Subsequently, the sunscreen was applied to the substrate at an application dose of 0.6 mg cm<sup>-2</sup> and the initial SPF was measured after 15 minutes. A water bath (distilled water, 33°C) equipped with a stirrer to provide a laminar flow (~30 rpm) was prepared. The substrate was mounted on the chamber, which was filled with water (temperature: 33°C) to avoid possible deformation of the substrate due to external water pressure. The chamber was placed in the water bath horizontally and left for 20 minutes (Figure 4-38a), after which it was removed and drained to let the substrate dry. After drying for 20 minutes, the water residue was carefully removed and the SPF was measured.



Figure 4-38. In vitro water resistance test. (a) Schematic illustration of the setup. UV camera images from skin-mimicking substrate treated with sunscreen (0.6 mg cm<sup>-2</sup>) (b) before and (c) after immersion in water. Skin-mimicking substrate treated with sunscreen (0.6 mg cm<sup>-2</sup>) (d) before and (e) after perspiration experiment. (f) Locations on the substrate surface used for SPF measurements. (g) SPF measurements for each location before and after immersion in water (stages b and c).

f

Images of the sunscreen-treated substrate before and after immersion in water are presented in Figure 4-38b and c. The intensity of UV absorption in some areas was decreased, possibly due to sunscreen wash-off. However, the redistribution of UV filters was not visible. This observation agreed with the in vivo water resistance test, as shown in Figure 2-18b, in which the intensity of UV absorption was decreased due to water exposure. A similar sunscreen-treated substrate exposed to moderate perspiration for 20 minutes is presented in Figure 4-38d. In Figure 4-38c and e, the different effects of water exposure from outside and beneath the sunscreen film on the film uniformity are shown. It appears that while sunscreen wash-off and redistribution are involved in the failure of sunscreen in response to sweating, exposure to water mainly triggers the sunscreen wash-off mechanism. However, if the conditions of the water resistance experiment (such as flow rate or water temperature) change, it may activate the other mechanism as well. In vitro SPF

measurements at different locations before and after water immersion, presented in Figure 4-38g, showed that in all locations, UV protection was decreased after water exposure.

Similar to the sweat resistance test, we investigated the effect of incorporating different concentrations of the hydrophobic film-former on the water resistance of sunscreen. The same sunscreen formulations used in Section 4.4.5.1 (containing 0, 0.75, and 3 wt.% of hydrophobic film-former) were applied on the skin-mimicking substrate at a quantity of 0.6 mg cm<sup>-2</sup> and subsequently assessed before and after water immersion by in vitro SPF measurements and UV camera imaging. The in vitro SPF measurements before and after water immersion are presented in Figure 4-39. For each set of experiments, five skin-mimicking substrates were examined and the SPF was measured at nine locations in each sample (corresponding to 45 SPF measurements for each set of experiments). On average, the SPF reduction was  $62\%\pm13\%$ ,  $44\%\pm23\%$ , and  $51\%\pm24\%$  for the sunscreens containing 0, 0.75, and 3 wt.% of film-former, respectively. The results demonstrated that absence of the film-former damaged the integrity of the sunscreen film and significantly impaired its UV protection ability. This effect was more obvious when intermediate film-former concentration was used and resulted in enhanced substantivity after water immersion. However, increasing the film-former concentration did not enhance SPF retention further.



Figure 4-39. In vitro SPF measurements for sunscreen formulations with different film former concentration before and after immersion in water. Final SPF versus initial SPF values measured on five individual samples and for 9 locations on each sample. The dashed lines represents the location of the data point if no effect of immersion in water was observed and the data point layer below the line thus represents a situation where the SPF values decreased.

To achieve a better understanding of the results, the UV camera images were obtained before immersing the samples in water and after the sample was dried. As shown in Figure 4-40, for the

sunscreen without film-former, the integrity of the film was greatly damaged because, in the absence of the protective network of the film-former, water can penetrate the sunscreen film effortlessly, resulting in massive sunscreen wash-off. In some spots, relocation of UV filters also occurred, probably due to poor sunscreen/substrate attachment. This observation is in agreement with the results obtained from in vitro SPF measurements. As illustrated in Figure 4-40, the sunscreen with 0.75 wt.% of film-former shows much greater substantivity than the sunscreen without the film-former, which was, again, in agreement with the in vitro SPF measurements. However, the reduction in UV absorption was visible at different locations. For the samples with a higher concentration of film-former, the water immersion did not affect the film integrity. However, these samples are more likely to lose uniformity of the film compared with the samples with a lower concentration of film-former. This could be due to the initial difficulty of achieving a uniform film, as the sunscreen containing 3 wt.% of film-former has much greater viscosity than the other two sunscreens.



Figure 4-40. UV camera images illustrating the UV filters distribution in two stages: (a) before and (b) after immersion in water for sunscreens containing different concentrations of hydrophobic film former.

## 5. Outlook and future work

During my PhD, I tried to study the sweat resistance of sunscreens by developing a new experimental tool (the perspiring skin simulator) and using different imaging techniques for qualitative and quantitative evaluation of the substantivity of the sunscreen. Moreover, I studied two approaches for increasing the sweat resistance of sunscreen in response to perspiration: manipulation of the concentration of hydrophobic film-formers and incorporation of water absorbing particles in the sunscreen formulation. Here I will briefly discuss how the work on these parts could be continued:

As a future direction, the perspiring skin model could be modified in terms of surface roughness of the skin-mimicking substrate to achieve a more real life-like topography of the sunscreen film on the skin. Moreover, even though the current substrate works well enough for comparing in vitro SPF values before and after the perspiration experiment, modifying the substrate to become a more UV transparent, could result in more accurate data and removing the track-etched membrane, could make the substrate a good candidate for normal in vitro SPF measurements. Additionally, the chamber could be modified to have a reservoir to collect the sweat for further analysis such as comparing the wash-off the UVA and UVB filters or the effect of different ingredients on the substantivity of the sunscreen.

Regarding the sunscreen formulation, I used ethanol-based sunscreens. However, due to a huge interest toward emulsion-based sunscreens, it would be stimulating to study the behavior of emulsion-based sunscreens and compare the efficacy of the O/W and W/O emulsions upon sweating. In addition, more water-absorbing particles with various chemical and physical characteristics could be tested in the sunscreen formulations to observe their effect on the sweat resistance of sunscreen. Finally, in this study, I used water as a representative for the sweat to avoid the salt residue after sweat drying and thus to have fewer complications using the different imaging techniques. However, it would be quite interesting to use liquids with a salt composition similar to human sweat for the experiments especially to get more accurate data for the experiment with sunscreen formulations containing water-absorbing particles.

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## Appendices

Appendix A

# Paper I: Water vapor permeation through topical films on a moisture-releasing skin model

DOI: 10.1111/srt.12926

#### **ORIGINAL ARTICLE**



### Water vapor permeation through topical films on a moisturereleasing skin Model

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**Funding information** Innovation Fund Denmark, Grant/Award Number: 615100007B

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#### 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 was 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

breathability, film-forming polymers, gelatin, permeation, skin model, transepidermal water loss (TEWL)

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#### <sup>2</sup> WILEY 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.<sup>1</sup> 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, that is, 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  $\mu$ m.<sup>2</sup> This layer acts as a flexible barrier against infections, chemical substances, and mechanical shock as well as dehvdration.<sup>3</sup> 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.<sup>4</sup> 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.<sup>5</sup> 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.<sup>5-10</sup> 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.<sup>11</sup>

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.<sup>12-15</sup> 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.<sup>15</sup> 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.<sup>16</sup> 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.<sup>17</sup> 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.<sup>18</sup> On the other hand, semipermeable wound dressings may reduce occlusion and thereby risk of skin maceration and the need for a daily dressing change.<sup>19</sup> 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.<sup>20</sup> 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.<sup>20</sup> 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.<sup>21</sup> Derler et al used silicone and polyurethane materials as mechanical skin equivalents to study the friction of skin against a reference textile.<sup>22</sup> Epoxy resin could be used as a thermal skin model, as its thermal diffusivity is close to that of the human skin.<sup>23</sup> Gelatin is a water-soluble protein compound obtained from collagen, the main fibrous protein in bones, cartilages, and skin.<sup>24</sup> Gelatin has diverse applications in skin models, wound dressings, and food and pharmaceutical industries.<sup>24-32</sup> 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  $H_2O$ ,

contains 10%-15% methanol as the stabilizer) were supplied by Sigma Aldrich. Hydroxyethyl cellulose (Natrosol<sup>™</sup> 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 skinmimicking substrate

10 g of gelatin and 2 g of hydroxyethyl cellulose were added to 100 ml of water (pH adjusted to 9 using 1 mol L<sup>-1</sup> 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  $\mu$ m 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  $\mu$ m 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 h prior to the permeation experiment.

#### 2.4 | Water vapor permeation test

The water vapor permeation rate through the gelatin-based skinmimicking 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  $K_2SO_4$  salt solution (providing 97 ± 1% relative humidity) and was sandwiched between two silicon gaskets and fixed by a cap with an exposed area of ~ 7 cm<sup>2</sup> 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  $33 \pm 2\%$  by a saturated MgCl<sub>2</sub> 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  $(\pm 10^{-5} \text{ g})$  with intervals of 2 h, and the experiment was stopped after 8 h. Six replicates were tested for each sample. For the first 2 h of each measurement, the mass loss rate was lower than that measured over the next 6 h 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.<sup>36</sup> Accordingly, the fitted slope of the linear region observed at 2-8 h 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 h 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 h, 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, MgCl<sub>2</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaCl, KCl, and K<sub>2</sub>SO<sub>4</sub> saturated salt solutions, respectively).<sup>37,38</sup>

#### 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/AI 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  $\mu$ l 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 h before the study. The subjects were also asked not to drink caffeine-containing beverages 3 h 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 min 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  $\mu$ m, size: 2 × 3 cm<sup>2</sup>) 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 (AquaFlux<sup>TM</sup> 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 h 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.<sup>34</sup> Gelatin, the main component of the artificial skin formulation, mimics both the chemical and physical properties of dry and hydrated human skin.<sup>33,39</sup> Natrosol provides less brittleness and a larger elongation capacity giving rise to more skin-like mechanical properties.<sup>34</sup> 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.<sup>34</sup> When cross-linked, the skinmimicking 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  $\mu$ m  $\times$  100  $\mu$ 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.<sup>2</sup> Figure 1(C) displays the representative water contact angle of the skin-mimicking substrate. Here, an average water contact angle of  $75 \pm 5^{\circ}$  was found, which is in the same range of the reported values for human skin.<sup>34,40</sup> 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.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, that is, the relative humidity inside the cup ( $RH_{in}$ ) and relative humidity outside the cup ( $RH_{out}$ ). The relatively high  $RH_{in}$  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  $\geq$  75%, RH<sub>in</sub> must be at least 75% to achieve a sufficiently hydrated substrate to mimic the behavior of skin.

Under 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.<sup>42,43</sup> 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

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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

140  $\mu$ m) were tested. In each case, three different relative humidity gradients were examined. The  $\mathrm{RH}_{\mathrm{out}}$  was fixed to 33% to resemble an average ambient humidity, while the RH<sub>in</sub> was adjusted roughly to 75, 84, and 97%. The normalized water vapor permeation rates (the simulated TEWL values) obtained using the setup (Figure 1E) 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<sub>in</sub> 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/( $m^2h$ ), 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  $\mu m,$  RH  $_{\rm in}$  of 97%, and RH  $_{\rm out}$  of 33% (Figure 1(F)). Using these conditions, a water vapor permeation rate of approximately 37 g/( $m^2h$ ) is obtained, which is considered to be a relatively high TEWL value for human skin.<sup>44</sup> 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 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  $\pm$  0.37 and 18.28  $\pm$  1.18 g/(m<sup>2</sup>h), respectively). In contrast, the shellac, Acr-OcAA, and Acr-DiMet films expressed high barrier properties, as given by their low permeation values (2.67  $\pm$  0.07, 2.86  $\pm$  0.11, and 7.55  $\pm$  0.16 g/(m<sup>2</sup>h), 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.<sup>45</sup>

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 ( $37.1 \pm 0.6$  wt.% uptake). Shellac and Acr-OcAA showed a similar low affinity to water vapor (4.4  $\pm$  0.3 and 4.0  $\pm$  0.1 wt% uptake, respectively), and PU and Acr-DiMet were the polymers with the lowest water vapor absorption (2.5  $\pm$  0.1 and 0.4  $\pm$  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°,  $75 \pm 5^{\circ}$ , respectively) than those of the shellac and VP-Acr-LaMeAcr  $(64 \pm 3^{\circ} \text{ and } 65 \pm 2^{\circ}, \text{ respectively})$ . 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.<sup>46</sup>

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.<sup>47-49</sup>

The polymeric film formers presented in this study, except VP-Acr-LaMeAcr, showed a low affinity to water vapor (Figure 3A).



FIGURE 3 Comparison of the water and water vapor affinity of the filmforming 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)



However, studies on the diffusion behavior of water vapor molecules through these polymers demonstrated diverse permeation rates (Figure 2B). VP-Acr-LaMeAcr showed the highest water vapor uptake and permeation, which could be attributed to its film-forming mechanism and chemical structure.<sup>50</sup> 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 free-standing 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 h 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 h 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.<sup>51-53</sup> Moreover, topical administration of film-forming systems on the skin affects skin hydration as well as water permeation through the stratum corneum.43,54,55 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.<sup>17,56-63</sup> 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.<sup>43</sup> Base on that background, we believe that both polymeric films similarly induced a high concentration of water vapor <sup>₿ |</sup>\_WILEY

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 (eg. 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 skinmimicking 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 skinmimicking 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.

#### 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.

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How to cite this article: Keshavarzi F, Zajforoushan Moghaddam S, Barré Pedersen M, Østergaard Knudsen N, Jafarzadeh S, Thormann E. Water vapor permeation through topical films on a moisture-releasing skin Model. *Skin Res Technol.* 2020;00:1–10. https://doi.org/10.1111/srt.12926 Appendix **B** 

# Paper II: *In vitro* skin model for characterization of sunscreen substantivity upon perspiration

# *In vitro* skin model for characterization of sunscreen substantivity upon perspiration

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#### Abstract

The resistance of sunscreens to the loss of ultraviolet (UV) protection upon perspiration is important for their practical efficacy. However, this topic is largely overlooked in evaluations of sunscreen substantivity due to the relatively few well-established protocols compared to those for water resistance and mechanical wear. In an attempt to achieve a better fundamental understanding of sunscreen behavior in response to sweat exposure, we have developed a perspiring skin simulator, containing a substrate surface that mimics sweating human skin. Using this perspiring skin simulator, we evaluated sunscreen performance upon perspiration by *in vitro* sun protection factor (SPF) measurements and various imaging techniques. Results indicated that perspiration reduced sunscreen efficiency through two mechanisms, namely sunscreen wash-off (impairing the film thickness) and sunscreen redistribution (impairing the film uniformity). Further, we investigated how the sweat rate affected these mechanisms and how sunscreen application dose influenced UV protection upon perspiration. As expected, higher sweat rates led to a large loss of UV-protection, while a larger application dose led to larger amounts of sunscreen being washed-off and redistributed but also provided higher UV protection before and after sweating.

Keywords: sunscreen, substantivity, SPF, perspiring skin simulator, gelatin, sweat resistance.

#### 1. Introduction

It is well known that sunscreen application is important for the prevention of harmful effects of ultraviolet (UV) radiation on human skin, such as sunburn, photoaging, immunosuppression, and skin cancer.<sup>1–3</sup> Sunscreen is a complex formulation consisting of many ingredients, including UV filters, emollients, and sensory enhancers, film formers, solvents, emulsifiers, and thickeners.<sup>4</sup> UV filters protect against UV irradiation by reflecting, absorbing, or scattering the UV light, and different filters provide the most efficient protection against specific range of light wavelengths

(UVB in the wavelength range of 290-320 nm and UVA in the wavelength range of 320-400 nm).<sup>5</sup> Emollients, emulsifiers, and solvents are included to ensure the solubility and photostability of the UV filters in the sunscreen.<sup>6</sup> Film formers help the sunscreen film to form a polymeric network on the skin surface that holds and distributes UV filters on the skin to boost the performance of UV filters.<sup>7,8</sup> Thickeners and emulsifiers adjust the viscosity to ensure that the product has good spreadability on the skin and enable the formulation of various forms of sunscreens, such as creams, lotions, sprays, or sticks.<sup>5</sup> An effective sunscreen is optimized with highly photostable UV filters that are uniformly spread on the skin to provide broad-spectrum UVB and UVA protection. In addition to optimal protection, substantivity of the product, the ability to bind to the skin and resist removal, is also important for a sunscreen to maintain long-term protection against UV irradiation under real-life conditions.<sup>9,10</sup>

The three main factors used to evaluate sunscreen performance on human skin are the sun protection factor (SPF), UVA protection factor (UVA-PF), and the substantivity.<sup>11</sup> Both SPF and UVA-PF can be measured *in vivo* as the minimum amount of UV energy required to cause a biological endpoint (minimal erythema and persistent pigment darkening, respectively) on sunscreen-treated skin divided by the amount of energy to cause the same effect on unprotected skin. One measure of substantivity is the water resistance test of sunscreen, which is measured by comparing the *in vivo* SPF values of the sunscreen-treated area before and after immersion in water. Moreover, as a means for a rapid, inexpensive, and subject-independent evaluation of formulations and to enable the design of new sunscreen products, *in vitro* SPF, UVA-PF, and water resistance measurements continue to gain more interest.<sup>10–19</sup>

The biological variation of human skin characteristics, such as skin surface topography, may differentially alter the effectiveness of applied sunscreen films. As such, sunscreens with the same

composition of UV filters may exhibit different SPF values after application, especially if the sunscreen does not have an optimal rheological profile or if an insufficient amount of sunscreen is applied.<sup>5,20-26</sup> Furthermore, one function of the human skin is thermoregulation in response to internal or external stimuli that change the body temperature.<sup>27</sup> If the skin or core body temperature increases to a threshold, heat dissipation can occur through perspiration where sweat is delivered to the skin surface through numerous active glands spread across the body.<sup>28</sup> In addition to cooling, sweating can alter skin conditions, such as skin surface pH and hydration of the stratum corneum (the outermost layer of the skin), or even reduce the tolerance of unprotected skin to sunburn.<sup>29,30</sup> Sweating co-occurs with many conditions in which people use sunscreen, such as exercising outdoors or sunbathing at the beach. This emphasizes that the functionality of sunscreen is important both during and after sweating given that sweating negatively affects sunscreen film homogeneity, substantivity, and consequently reduces the extent of UV protection. The effect of sweating on sunscreen efficacy is often considered to be similar to that of other post-application activities, such as swimming, bathing, and toweling.<sup>9,31,32</sup> However, during perspiration, sweat is released from the skin underneath the film and should be transported across the sunscreen film without impairing its protection, whereas during swimming or toweling the sunscreen film is exposed to water or friction externally and should resist film deterioration or removal. However, sunscreen substantivity is generally assessed by its ability to resist external water exposure or friction while the effect of sweating is commonly overlooked.<sup>21,32–38</sup>

There are few *in vivo* studies investigating the interaction between sweating and sunscreen application, most of which have focused on comparing the water and sweat resistance of sunscreens based on the product formulation.<sup>37,39,40</sup> The importance of sweat resistance in sunscreen was illustrated by studying professional triathletes, who, despite using water resistant

sunscreen, were sunburned after competition activities (including swimming, biking, and running), which was possibly due to sunscreen wash-off by sea water and sweating.<sup>41</sup> In another study, the effect of sweat-inducing physical activities on the UV protection of water resistant sunscreens was assessed.<sup>42</sup> While these studies assessed sunscreen film efficacy upon sweating, others investigated the effect of sunscreen film on perspiring conditions, such as skin occlusion or changes in sweat evaporation, skin temperature, and skin cooling.<sup>43–45</sup> *In vivo* evaluations of sunscreen behavior on perspiring human skin are costly, time- and labor-consuming, and complicated due to the intra-and inter-subject biological variability, along with the difficulties in controlling sweat rate and in collection of sweat for analysis. As such, a systematic study evaluating the impact of different parameters, such as sweat rate, sunscreen formulation, and sunscreen application dose, on UV protection properties would face many challenges.<sup>46</sup> To the best of our knowledge, no suitable protocol or *in vitro* method has been established to enable investigation of the sunscreen/sweat interaction to obtain an improved fundamental understanding of sunscreen behavior and substantivity upon perspiration.

In this study, we developed an *in vitro* setup including a skin-mimicking substrate capable of simulating the perspiration of human skin. Subsequently, this setup, hereafter referred to as the perspiring skin simulator, was used to investigate the performance of an ethanol-based sunscreen formulation both quantitatively and qualitatively by performing *in vitro* SPF measurements and by using an optical microscope and an area scan camera (hereafter referred to as a UV camera), respectively. Moreover, real-time information regarding the interaction between the sweat droplet and the sunscreen film during and after sweating was acquired by coherent anti-Stokes Raman scattering (CARS) microscopy. The *in vitro* SPF measurements and the combination of imaging techniques provided a general knowledge of sunscreen behavior on perspiring skin and enabled us

to map the mechanisms of perspiration-induced failure in UV protection. Finally, the effect of two parameters, namely sweat rate and sunscreen application dose, on sunscreen resistance against sweating was investigated.

#### 2. Materials and methods

#### 2.1. Materials

*Skin-mimicking substrate:* Gelatin from porcine skin (gel strength ~175 g Bloom, Type A), glycerol (>99.5%), and formaldehyde (ACS reagent, 37% wt. in H<sub>2</sub>O, with 10%-15% methanol as a stabilizer) were obtained from Sigma Aldrich (Denmark). Polymethylmethacrylate (PMMA) film (PLEXIGLAS Film 0F058, thickness: 200  $\mu$ m) was kindly provided by Evonik (Germany). Double-sided acrylic adhesive (Tesa 4900, thickness: 50  $\mu$ m) was obtained from Tesa (Germany) and track-etched hydrophilic membrane (PCT0220030, pore size: 0.2  $\mu$ m, pore density: 3×10<sup>8</sup> cm<sup>-2</sup>, thickness: 10  $\mu$ m) was obtained from Sterlitech (USA).

*Sunscreen formulation*: Diethylamino hydroxybenzoyl hexyl benzoate (obtained from BASF), octocrylene (obtained from BASF), ethylhexyl salicylate (obtained from DSM Nutritional Products Europe Ltd), C15-19 alkane (obtained from SEPPIC), phenoxyethyl caprylate (obtained from Evonik Nutrition & Care GmbH), acrylate/octylacrylamide copolymer (obtained from Nouryon Surface Chemistry LLC), ethylcellulose (obtained from Ashland), and dibutyl adipate (obtained from BASF) were used as ingredients in the sunscreen formulations evaluated in this study.

#### 2.2. Development of the perspiring skin simulator

To prepare the skin-mimicking substrate, 7.5 g gelatin was mixed with 50 mL water (pH adjusted to 9 with NaOH) and stirred for 30 minutes at 50°C. 1.5 mL glycerol, as a plasticizer, and 1.5 mL formaldehyde, as a cross-linker, were added and the solution, which was then stirred for one

additional minute. The solution was then applied using a casting knife film applicator (Elcometer 3580/4, Elcometer Ltd., UK) with a wet thickness of 250 µm on the PMMA film which was fixed on a vacuum plate (Elcometer 4900, Elcometer Ltd., UK) and left overnight to dry and cross-link. Prior to film application, the PMMA film was plasma-cleaned (PDC-32G plasma cleaner, Harrick Plasma, USA) for 30 minutes in the air under a constant pressure of 1000 mTorr. This process improves the wettability and adhesion of the PMMA film and consequently prevents delamination of the gelatin layer.<sup>47</sup> After forming a 20-30-µm thick gelatin film, the double-sided acrylic tape was applied from one side to the backside (non-coated side) of the PMMA film while a release liner protected the other side of the tape. Next, funnel-like pores, with a pore density of 200 cm<sup>-2</sup>, were laser-drilled through the samples using a laser micromachining tool (microSTRUCT<sup>TM</sup> C, 3D-Micromac AG, Germany). The laser irradiation wavelength, laser power, and marking speed were fixed to 355 nm, 10.5 W, and 400 mm s<sup>-1</sup>, respectively. This resulted in a pore size of 110-120 µm on the gelatin side and 40-60 µm on the adhesive side. Finally, the release liner of the adhesive was removed and was applied to the track-etched membrane fixed on the vacuum plate. A deadlift of 500 Pa was placed on top of the film for 2 hours to enhance the adhesion of the membrane to the layered system.

The perspiring setup comprises a water tank with adjustable height, a water reservoir under the skin-mimicking surface, a flow sensor (Flow sensor3 digital, FLOW-03D, Elveflow, France), a flow reader (Sensor readerV2, MSR2, Elveflow, France), connecting tubes, and the skin-mimicking substrate. The substrate is placed on the chamber with a lid and an O-ring beneath that fixes the substrate to prevent leakage. The water tank is connected to the chamber to provide pressure-controlled water flow through the substrate and a flow sensor is placed between the water

tank and the chamber to measure the flow rate. Figure 1 shows a schematic illustration of the perspiring skin simulator setup.



Figure 1. Schematic illustration of the perspiring skin simulator setup and the layered skin-mimicking substrate.

#### 2.3. Sunscreen formulations

Sunscreen was prepared by dissolving the ingredients in ethanol using a homogenizer (Silverson L5T, Silverson Machines Ltd., England). Based on the UV filter composition (30% wt.), an SPF of 28 was calculated using a sunscreen simulator software (BASF).<sup>48</sup> For CARS microscopy, a simple sunscreen formulation containing only one UV filter (20% wt. octocrylene) was prepared following the above-mentioned procedure.

#### 2.4. Parameters affecting sunscreen efficacy upon perspiration

Two key parameters, sweat rate and the amount of sunscreen applied, were assessed to investigate their impact on sunscreen substantivity. The sweat rates of 1.46 and 3.31  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup> were selected based on the sweat rates on untreated human foreheads in two different conditions of moderate and heavy sweating.<sup>46</sup> We selected 0.6 and 1.2 mg cm<sup>-2</sup> as the sunscreen application dose

based on the amounts of sunscreen commonly applied by consumers.<sup>49</sup> Accordingly, the following three sets of experiments were conducted: low sunscreen quantity and moderate sweating (0.6 mg cm<sup>-2</sup> and 1.46  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup>), low sunscreen quantity and heavy sweating (0.6 mg cm<sup>-2</sup> and 3.31  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup>), and high sunscreen quantity and heavy sweating (1.2 mg cm<sup>-2</sup> and 3.31  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup>).

#### 2.5. In vitro measurement of SPF

*In vitro* SPF measurements were obtained based on UV transmittance spectroscopy data as follows:

$$SPF = \frac{\sum_{290}^{400} E(\lambda)S(\lambda)}{\sum_{290}^{400} E(\lambda)S(\lambda)T(\lambda)}$$
1

where,  $E(\lambda)$  is the erythema action spectrum<sup>50</sup>,  $S(\lambda)$  is the spectral irradiance of the UV source, and  $T(\lambda)$  is the measured transmittance of the light through a sunscreen film applied on a UVtransparent substrate. For this, we used a bare skin-mimicking substrate placed on a molded PMMA plate (Helioplates HD6; Helioscreen, Creil, France) as the reference for all SPF measurements. All UV transmittance spectra of the different layers and the skin-mimicking substrate were obtained and have been provided in the supporting information (Figure S1).  $T(\lambda)$ was recorded from 290 to 400 nm through the substrate before and after sunscreen application, as well as after perspiration, using a UV transmittance analyzer (Labsphere UV-2000S, Labsphere Inc., North Sutton, NH, USA). Sunscreen was applied on the substrate using a pipette and distributed homogenously over the whole surface with a latex finger cot pre-saturated with sunscreen. The sample was allowed to settle for 15 minutes in the dark before the initial SPF measurement. After sweating at room temperature for 20 minutes, the chamber was drained and maintained vertically to off-load excess water on the substrate surface. The sample was then allowed to dry for 20 minutes, after which the skin-mimicking substrate was detached from the chamber and the post-perspiring *in vitro* SPF was measured. For each set of experiments, ten skin-mimicking substrates were examined, and for each substrate, the *in vitro* SPF was measured at nine locations to allow intra-sample comparison of data.

#### 2.6. Imaging

An optical microscope (Nikon Eclipse LV100ND optical microscope, Nikon, Japan) was used to visualize sunscreen film distribution on the skin-mimicking substrate before, during, and after perspiration.

An area scan camera (acA4024-29um, Basler AG, Germany) equipped with a UV band-pass filter (365 nm, F/BP365-CMOUNT) and a high-resolution lens (Fujinon HF-1218-12M, Fujifilm, Japan) was used to visualize the distribution of UV filters (specifically with UV absorption at a wavelength of 365 nm) in the sunscreen during perspiration. The chamber was placed in a box equipped with LED UV lamps emitting UVA radiation and the camera was fixed perpendicular to the chamber.

CARS imaging (TCS SP8 CARS microscope, Leica Microsystems, Germany) was initially performed to identify the CARS signals of octocrylene, the simplified sunscreen formulation, and the skin-mimicking substrate. The pump laser (PicoEmerald pump laser, APE, Germany) wavelength varied from 787 to 877 nm with 1-nm increments (wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>) with a Stokes laser fixed at 1064 nm. Figure 2a illustrates the chemical bond vibrations of octocrylene. The same wavelength scan was separately performed for the skin-mimicking substrate and the remaining component of the sunscreen (see supporting information, Figure S2). Among the distinguishable peaks of octocrylene (3063, 2864, and 2210 cm<sup>-1</sup>), the distinct peak at wavenumber 2210 cm<sup>-1</sup>, attributed to the nitrile group (see Figure 2a), was selected

for further studies because it did not overlap with peaks from other ingredients in the sunscreen formulation and the skin-mimicking substrate. As summarized in Figure 2b and 2c, the highest chemical contrast between the sunscreen and the skin-mimicking substrate was visible at wavenumber 2210 cm<sup>-1</sup>. Figure 2d presents the CARS image of the sunscreen distribution on the substrate at this wavenumber. Next, sunscreen was applied onto the skin-mimicking substrate at a quantity of 2 mg cm<sup>-2</sup> and was maintained in the dark at room temperature for 15 minutes to selflevel and dry. The substrate was then mounted on the chamber and the chamber was inversely placed on the sample stage of the microscope to probe the interaction between sweat droplets and the sunscreen film in real-time during and after perspiration. All images were obtained with a field of view of  $1550 \times 1550 \ \mu\text{m}^2$  at room temperature and image sequences were acquired at  $2210 \ \text{cm}^{-1}$ with a pixel size of  $3.033 \times 3.033 \ \mu\text{m}^2$ .



Figure 2. (a) CARS signal for octocrylene from 3300 to 2000 cm<sup>-1</sup>. The highlighted peak at 2210 cm<sup>-1</sup> is attributed to the octocrylene nitrile group. (b) CARS signal of the skin-mimicking substrate for two regions: bare substrate (green circle) and area with sunscreen (purple circle). (c) Selected CARS images of the substrate and substrate + sunscreen at 2234, 2210, and 2194 cm<sup>-1</sup>. The highest contrast between the regions occurred at wavenumber 2210 cm<sup>-1</sup>. (d) Sunscreen distribution on the skin-mimicking substrate at wavenumber 2210 cm<sup>-1</sup>.

#### 3. Results and Discussion

#### 3.1. Perspiring skin simulator

The skin-mimicking substrate consists of four layers, namely a gelatin-based film, the PMMA film, the adhesive layer, and the track-etched membrane (Figure 1), with each providing specific functionality. The outermost layer is the gelatin-based film which is responsible for skin-like properties, e.g., surface hydration, water-responsive behavior, and interaction with topical films.<sup>51–54</sup> The gelatin in this layer is crosslinked by formaldehyde to improve hydrolytic stability, and glycerol is added as a plasticizer to enhance flexibility.<sup>52</sup> The water contact angle of the gelatin-

based film was  $75^{\circ}\pm5^{\circ}$  (Figure S3), which is comparable to the data reported for human skin,<sup>52,55</sup> and the thickness of the film is comparable to that of the stratum corneum of human skin (20-30  $\mu$ m).<sup>56</sup> The PMMA film functions as a support to provide mechanical strength to the substrate. The third layer is a water resistant double-sided adhesive used to attach the above layers to the track-etched membrane, which connects the substrate to the water reservoir. The funnel-like holes through the first three layers of the substrate represent human sweat pores in terms of size and pore density.<sup>57</sup> The membrane, as the bottommost layer, is used to provide a uniform flow to mimic human sweating among all the pores.<sup>58–60</sup> Consequently, by applying specific hydrostatic pressure to the substrate, a uniform, controlled, and reproducible flow is supplied through the pores, as previously described by Hou et al.<sup>58</sup>

Figure 3b shows that experimentally measured sweat rates follow a linear relationship between the applied pressure ( $\Delta P = \rho gh$ ) and the flow rate (Q).<sup>58</sup> Repeated measurements confirmed that a possible reduction in pore size, due to swelling of the hydrated gelatin-based layer, is negligible. Minor variations in substrate thickness result in a distribution in sweat pore sizes. Therefore, to obtain equivalent sweat rates for various skin-mimicking substrates, the applied pressures were varied slightly between individual samples.



b

a

Figure 3. (a) Image of the skin-mimicking substrate during simulated perspiration. (b) Plots of the average sweat rates at different applied pressures. ( $\bullet$ ) represents the first pressure ramp, ( $\bullet$ ) shows the first repetition, and ( $\blacktriangle$ ) shows the second repetition.

#### 3.2. Application of sunscreen to the perspiring skin simulator

An optical microscope was used to visualize the behavior of the sunscreen on the perspiring skinmimicking substrate. Figure 4a shows the skin-mimicking substrate before sunscreen application. After the application and self-leveling of the sunscreen, a thin and evenly distributed film was formed on the skin-mimicking substrate that also covered most of the sweat pores (Figure 4b). Figure 4c shows the sunscreen-treated substrate after 20 minutes of sweating with a sweat rate corresponding to 3  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup> for uncovered skin (the sweat rate is reduced after sunscreen due to partial occlusion of the pores). Based on the affinity of the sunscreen to the substrate and the film thickness, there is a constant competition between the force of the sweat droplets emerging and the sunscreen film substantivity at different locations. Figure 4d shows the same location as that in Figure 4c, 20 minutes after drying. The sunscreen morphology around the sweat droplets changed and some unprotected areas were observed. Owing to sweat droplet formation and expansion, the sunscreen film is either completely washed-off from the substrate or rearranged on the skin-mimicking surface.



Figure 4. Microscopic images of (a) the bare skin-mimicking substrate, (b) the substrate with sunscreen applied before perspiration, (c) the substrate with sunscreen after 20 minutes of perspiration, and (d) the same spot as in (c), after 20 minutes of drying where the sweat droplet evaporates.

To better observe the potential changes in sunscreen distribution in response to sweating, we utilized a UV camera. Here, owing to the specific wavelength of the camera filter, only UVA filters were evaluated. Figure 5a-5d presents UV camera images of a sunscreen surface at four stages of sweating on the perspiring skin simulator. The dark areas on the image indicate regions where UV light is absorbed, and a higher intensity of darkness represents a higher concentration of UV filters in that area. Figure 5a shows the uniform distribution of the sunscreen before perspiration was initiated and Figure 5b shows the sweat pores with active perspiration as sweating began. Upon 20 minutes of perspiration, sweat droplets were distributed on the skin substrate (Figure 5c). After drying, the rearrangement of the sunscreen (showing depletion from one spot and accumulation in another) can be observed (Figure 5d). Moreover, some unprotected areas on the skin-mimicking

substrate may be attributed to sunscreen wash-off. To highlight the effect of perspiration on sunscreen wash-off, a set of skin-mimicking substrates were prepared in which only half of the substrate contained sweat pores (Figure 5e). Sunscreen was applied on the area with the sweat pores (Figure 5f) with a quantity of 1.2 mg cm<sup>-2</sup>. Subsequently, the skin-mimicking substrate was adjusted on the chamber and perspiration began while the chamber was maintained vertically, with the treated area on the upper side. In this manner, during perspiration (duration of 20 minutes), sunscreen could flow down to the untreated area (Figure 5g). Figure 5h shows the dried skin-mimicking substrate with the visible residue of the washed-off sunscreen on the untreated area. This image also illustrates the redistribution of the UV filters on the treated area. Further, we performed a pilot *in vivo* study (see supporting information section S4) that qualitatively demonstrated the same trends when sunscreen was applied on a human forehead and perspiration was induced.

#### 3.3. SPF measurements on the skin-mimicking substrate

The *in vitro* SPF measurements of sunscreen applied on the skin-mimicking substrate, before and after exposure to perspiration, were performed to quantify the effect of sweating on the sunscreen film distribution and its UV protection properties. The SPF values were measured at nine locations on each sample (Figure 5i). As an example, pre- and post-perspiration SPF values for a sample with a sunscreen application dose of 0.6 mg cm<sup>-2</sup> and a sweating rate of 3  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup> are presented in Figure 5j. Here, it can be observed that in all locations, the SPF value decreases in response to perspiration, which is in agreement with the visual observation of decreased film homogeneity.



Figure 5. (a)-(d) UV camera images illustrating the sunscreen distribution at different stages: (a) before perspiration, (b) at the beginning of perspiration, (c) after 20 minutes of perspiration, and (d) after 20 minutes of drying. Darker areas represent higher absorption of UV light. (e)-(h) The skin-mimicking substrate in which only half of the surface could perspire: (e) with activated sweat pores, (f) with sunscreen applied on the area with sweat pores, (g) after perspiring for 20 minutes, and (d) after drying. (i) Locations on the substrate surface used for SPF measurements. (j) SPF measurements for each location before and after perspiration (stages a and d), for a sunscreen with an application dose of 0.6 mg cm<sup>-2</sup> and a sweating rate of 3  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>.

The optical transmission through an absorbing sunscreen film depends on the film thickness and UV filter concentration, according to the Lambert-Beer law. However, if the film thickness is not uniform, the transmittance will increase regardless of the average thickness, as qualitatively illustrated by a simple step-film-model by O'neill<sup>61</sup> and by other more accurate descriptions of the sunscreen inhomogeneities.<sup>24,62</sup> Therefore, perspiration may differentially affect the sunscreen film; thus, in the present study, we suggest that the reduction in SPF following perspiration may be described by two main mechanisms, namely direct sunscreen wash-off due to running sweat and redistribution of the sunscreen over the skin surface. To this end, the first mechanism will lead to a decrease in the mass of active ingredients per unit area and thus result in a lower average

thickness of the sunscreen film, while the second mechanism will lead to a decreased film homogeneity and thus result in poorer protection.

#### 3.4. Imaging the interaction between sweat and sunscreen using CARS microscopy

Real-time probing of the interaction between a sweat droplet and the sunscreen film was characterized by CARS microscopy. Sunscreen containing octocrylene as the only UV filter was applied on the perspiring skin simulator and the UV filter distribution was followed in real-time during perspiration. It was observed that, as the water droplets expanded, the sunscreen film was pushed in the direction of their expansion. Figure 6 shows the octocrylene around an expanding sweat droplet that first expands and then merges with another droplet. The figure shows two crosssections, one at the surface of the substrate and the other at a location that is 60 µm higher than the substrate. The red halo at the borders of the sweat droplets implies that the UV filter is pushed away radially upon droplet expansion. Next, when active perspiration was stopped and the droplet subsequently evaporated, the thinned layer of sunscreen on the droplet appeared to return to the skin-mimicking surface and formed a different, less uniform morphology compared to the initial film structure. This partial self-healing of the sunscreen layer is shown in Figure 7.



Figure 6. CARS images obtained during sweat droplet growth. (a) A schematic representation of cross-sections of a sweat droplet at the substrate surface,  $z=0 \mu m$  and at a height of  $z=60 \mu m$ . (b-g) Time evolution of sweat droplet growth and merging. As the sweat droplets expand, UV filters are noticeably pushed in the direction of expansion.



Figure 7. CARS images obtained during the evaporation of a sweat droplet. (a) The initial distribution of UV filters. (b-f). Time evolution of sweat droplet evaporation (t=0, 182, 364, 546, 728 s, respectively).

The data obtained from the combination of different imaging techniques (optical microscopy, UV camera imaging, and CARS microscopy) indicate that the pressure from the initial growth of sweat droplets disturbs the homogeneity of the sunscreen film on the skin. As sweating continues, it can lead to the relocation of UV filters (impairing the film uniformity) or complete removal of those components of the sunscreen that are poorly attached to the skin substrate (impairing the film thickness). Finally, after perspiration, the evaporation of sweat droplets may cause a secondary relocation of UV filters (Figure 7). Taken together, all these actions will affect sunscreen film substantivity and reduce uniform UV protection of the skin in response to perspiration.

#### 3.5. Effect of sweat rate and sunscreen amount on sunscreen efficacy

We next investigated the effect of sweat rate and the applied amount of sunscreen on its sweat resistance properties. Figure 8a and 8b show the initial local SPF values and their associated SPF values after sweating at moderate and heavy rates (1.46 and 3.31  $\mu$ L min<sup>-1</sup> cm<sup>-2</sup>, respectively), for samples with a low application dose of sunscreen (0.6 mg cm<sup>-2</sup>). Each color represents an individual sample (n = 10) and there are nine measured values for each sample (corresponding to 90 SPF measurements for each set of experiments). For both moderate and heavy sweating, results indicated a general trend of decreasing SPF in response to perspiration. However, for some local spots, the SPF increased or did not change. At the lower sweat rate, the SPF value compared to the samples exposed to higher sweat rates. Based on the UV camera images (supporting information, Figure S5), at high sweat rates, the sweat droplets merge and form larger droplets, thereby increasing the possibility of sunscreen wash-off. In contrast, at the low sweat rate, the sweat droplets do not propagate extensively, resulting in less sunscreen wash-off from the surface.
Comparison of the initial SPF values and their associated SPF values after heavy sweating for low and high sunscreen application doses (0.6 and 1.2 mg  $cm^{-2}$ , respectively) revealed that the SPF reduction in response to perspiration was higher when a higher amount of sunscreen was applied (Figure 8c). However, it should be noted that the initial SPF values for the samples with a higher application dose of sunscreen were much higher (SPF>100) than those of the samples with a lower application dose of sunscreen. This may be attributed to the low surface roughness of our skinmimicking substrate, which results in a uniformly distributed sunscreen film with high thickness (experimental results are detailed in supporting information, Figure S6 and S7).<sup>64,65</sup> After heavy sweating, the SPF values of the samples with a high application dose remained relatively high (70% of the SPF values were >50), thereby demonstrating that UV protection was retained. For both sets of experiments, the sunscreen wash-off was likely due to the high sweat rate. Unsurprisingly, a higher application dose led to larger amounts of sunscreen wash-off and redistribution, but nonetheless provided higher UV protection even after heavy sweating. The images from the UV camera indicated that the low amount of applied sunscreen resulted in more unprotected areas during similar perspiration conditions (supporting information, Figure S8).



Figure 8. Initial versus final SPF values for a low amount of applied sunscreen (0.6 mg cm<sup>-2</sup>) with (a) moderate sweating (1.46  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>) and (b) heavy sweating (3.31  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup>). Each color represents nine locations on an individual sample. The solid line represents the location of the data point if no effect of sweating was observed and the data point layer below the line thus

represents a situation where the SPF values decreased. (c) Statistical representation of the differences in measured SPF before and after perspiration. The colored area represents the interquartile range (IQR) showing the range of middle 50% of the data (25% to 75%). The middle line represents the median and the small square represents the mean value. The mean value for each set of experiments is shown beside each boxplot. The upper and lower lines represent the maximum and minimum SPF changes, respectively, excluding the outliers. The rhombuses outside the box indicate outliers.

We also measured the UVA/UVB ratio and the critical wavelength ( $\lambda_c$ ) on the tested samples, as these parameters might provide additional information on the intensity of UV absorption over specific regions of the spectrum. Comparison of these two factors before and after perspiration may illustrate which of the UVA and UVB filters are more prone to wash-off. Results indicated negligible changes upon both moderate and heavy sweating (Supporting information, Figure S9), thereby implying that both UVA and UVB filters underwent comparable changes in our studied formulation.<sup>66</sup> Similarly,  $\lambda_c$  did not change upon perspiration (Supporting information, Table S1). However, depending on the selected UV filters in a given sunscreen formulation, results may vary. <sup>66,67</sup>

#### 4. Conclusion

We have developed a perspiring skin simulator that includes a multilayer skin-mimicking substrate and presents a new *in vitro* method to evaluate sunscreen substantivity upon perspiration. This setup mimics sweating human skin in terms of sweat pore size and density, sweat rate, and skinlike affinity for sunscreen products. Our results demonstrated that sweating diminished sunscreen efficacy by impairing the sunscreen thickness and uniformity. More specifically, sweating reduced the SPF via both sunscreen wash-off and sunscreen redistribution. Although this was observed for one specific sunscreen formulation, we expect this observation to be generalizable. Finally, the model was employed to demonstrate how different sweat rates and sunscreen application doses affect sunscreen performance. This study implies that higher sweat rates lead to lower substantivity, while a larger application dose can be used to achieve higher post-perspiration UV protection.

#### Acknowledgments

The authors gratefully acknowledge financial support from the Innovation Fund Denmark through the Grand Solutions Project #6151-00007B. The Danish Molecular Biomedical Imaging Center (DaMBIC, University of Southern Denmark) is acknowledged for the use of the CARS microscope.

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## Supporting information:

# *In vitro* skin model for characterization of sunscreen substantivity upon perspiration

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#### S1. UV transmittance data for layers of skin-mimicking substrate

The UV transmittance graphs for the skin-mimicking substrate and its different layers using a molded PMMA plate (Helioplates HD6) as the blank are presented in Figure S1. The results show that the UV transmittance through the multilayer substrate has been decreased mostly due to the membrane opacity. However, the substrate functions well for the *in vitro* SPF studies.



Figure S1. UV transmittance of the skin-mimicking substrate and its different layers; gelatin, PMMA substrate, adhesive, membrane.

#### S2. CARS wavelength scan

The wavelength scan of the skin-mimicking substrate and the sunscreen ingredients are presented in Figure S2. The pump laser wavelength was varied from 787 to 877 nm with 1 nm increments (wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>) with a Stokes laser fixed at 1064 nm. In octocrylene, the distinct peak at wavenumber 2210 cm<sup>-1</sup> attributed to the nitrile group does not overlap with the other sunscreen ingredients and the skin-mimicking substrate. Therefore, this peak was selected for further CARS studies.



Figure S2. CARS signal for the simplified sunscreen components and the skin-mimicking substrate in the wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>. The peak at 2210 cm<sup>-1</sup> is attributed to the nitrile group in octocrylene formulation.

#### S3. Contact angle measurement

The water contact angle measurements for skin-mimicking substrate were performed based on the sessile drop method (Theta Lite optical tensiometer, Biolin Scientific, Sweden) at ambient condition (25 °C, 30% RH) using a water droplet (2  $\mu$ l) placed on the film surface with a precision syringe. The measurements were recorded within 10 s after deposition. Five measurements were conducted on different surface locations and the averaged value was reported as the contact angle (Figure S3).



Figure S3. Water droplet on the dry skin-mimicking substrate used in the contact angle measurement.

#### S4. *in vivo* sweat resistance test

We performed a pilot *in vivo* study (at proDERM, institute for applied dermatological research, Germany) to investigate the performance of an ethanol-based commercial sunscreen (SPF 30) on perspiring human skin and compare the data obtained from the perspiration simulator with the *in vivo* results. The procedure is illustrated in Figure S4e. The forehead of two healthy male subjects was marked and the sunscreen was applied to test areas at a dose of 2 mg cm<sup>-2</sup>. After sunscreen drying for 15 minutes, the subjects were asked to perform the physical exercise (stepping) for 20 minutes. Afterward, the subjects were allowed to dry for 30 minutes. The areas with applied sunscreen were evaluated by pictures taken by standard and UV cameras both after sunscreen application (Figure S4f and g, respectively) and after skin drying (Figure S4h and i, respectively). Figure S4g shows a uniform distribution of UV filters. However, Figure S4i illustrates that after sweating both sunscreen redistribution and sunscreen wash-off happens confirming the

observation in the perspiring simulator. Figure S4a-d (similar to Figure 6e-h) are shown for better comparison between *in vivo* and *in vitro* studies.



Figure S4. Illustration of UV filters distribution before and after in vitro and *in vivo* sweating. The skin-mimicking substrate (a) with activated sweat pores (b) with sunscreen applied on the area with sweat pores. (c) After sweating for 20 minutes. (d) After drying. (e) The procedure of *in vivo* sweat resistance test. (f) The standard camera image of the subject forehead after sunscreen application before the beginning of the physical exercise. (g) The image of the same spot as (f) taken with the UV camera. (h) The standard camera image of the subject forehead after post-exercise drying. (i) The image of the same spot as (h) taken with the UV camera.

### S5. Effect of sweat rate on the UV filters redistribution and washoff

Figure S5 shows two samples treated with the same applied amount of the sunscreen (0.6 mg cm<sup>-2</sup>) but different initial sweat rates (1.46  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup> in Figure S5a-c and 3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup> in Figure S5d-f) 15 minutes after perspiration is started and after drying. The initial sunscreen distributions for both samples were identical. For heavy sweating, the water droplets merge and form big droplets which increase the risk of sunscreen wash-off. On the contrary, the formation of small

and isolated droplets is more probable at moderate sweating. Figure S5b and e illustrate the samples after drying for 20 minutes and Figure S5c and f represent the image histogram (gray-scale value distribution) for Figure S5b and e respectively which illustrates that at the high sweat rate, the sunscreen is more washed-off.



Figure S5. The effect of sweat rate on the sunscreen distribution and wash-off. Area scan camera image for the sample exposed to low initial sweat rate (a) after sweating for 15 minutes (b) after drying. (c) Gray-scale value distribution of image (b). Area scan camera image for the sample exposed to high initial sweat rate (d) after sweating for 15 minutes (e) after drying. (f) Gray-scale value distribution of image (e).

#### S6. The correlation between in vitro SPF and substrate roughness

To show the effect of substrate roughness on SPF *in vitro*, four different quantities of sunscreen  $(0.5, 1, 1.5 \text{ and } 2 \text{ mg cm}^{-2})$  were applied on three substrates with different roughness; two molded PMMA substrates HD6 and HD2 (Helioplates; Helioscreen, Creil, France) with ~ 6 and 2 µm roughness respectively and a PMMA film with a smooth surface and no distinguishable roughness

(PLEXIGLAS Film 0F058, Germany). The *in vitro* SPF increases with decreasing the roughness at all applied quantities of sunscreen (Figure S6).



Figure S6. The in vitro SPF versus applied amount of sunscreen and substrate roughness.

Figure S7 show the roughness line scan of the hydrated skin-mimicking substrate where surface height, z, is plotted versus distance, x using a profilometer (SJ-410 surface roughness tester, Mitutoyo, Germany) in two conditions; where the stylus goes through the holes (Figure S7a) and where the stylus does not go through the holes (Figure S7b). The average root-mean-squared (RMS) roughness of the film is  $13.90\pm1.75$  for the measured distance with holes and  $0.16\pm0.04$  µm for the measured distance without holes. Moreover, a representative AFM height image of the skin-mimicking substrate (in the dry state) and the corresponding cross-section height profile is shown in Figure S7c and d respectively, to illustrate the roughness close to the sweat pores. The results illustrate that the surface roughness increases close to the sweat pores (Figure S7d).

Figure S7 shows that the roughness of the skin-mimicking substrate is low and high application dose of the sunscreen will result in unrealistically high *in vitro* SPF values.



Figure S7. A roughness line scan of the skin-mimicking substrate obtained with a profilometer (a) through a line with sweat pores and (b) through a line with no holes. (c) AFM height image of the skin-mimicking substrate close to a sweat pore and (d) cross-section profile taken at the line marked on the AFM height image.

## S7. Effect of sunscreen application dose on the UV filters redistribution and wash-off

Figure S8 shows two samples exposed to the same initial sweat rate  $(3.31 \ \mu l \ min^{-1} \text{cm}^{-2})$  but treated with different amount of the sunscreen (0.6 mg cm<sup>-2</sup> in Figure S8a-c and 1.2 mg cm<sup>-2</sup> in Figure S8d-f), before (Figure S8a and d) and after perspiration (Figure S8b and e). The perspiration rate after sunscreen application for both samples are similar. Figure S8c and f represent the image histogram (gray-scale value distribution) for Figure S8b and e respectively which illustrates that

the low amount of applied sunscreen results in greater unprotected areas during similar perspiration conditions.



Figure S8. The effect of sunscreen application dose on the sunscreen distribution and wash-off. Area scan camera image for the sample treated with low sunscreen application dose (a) after sweating for 15 minutes, (b) after drying. (c) Gray-scale value distribution of image (b). Area scan camera image for the sample treated with high sunscreen application dose (d) after sweating for 15 minutes, (e) after drying. (f) Gray-scale value distribution of image (e).

# S8. Comparison of UVA/UVB ratio and $\lambda c$ before and after perspiration

Table S1 presents the calculated  $\lambda c$  before and after perspiration for all the samples tested in three sets of experiments. Comparison between  $\lambda c$  values showed little-to-no changes due to perspiration for both low and high perspiration rates as well as the low and high applied amount of sunscreen, implying that both UVA and UVB filters undergo comparable changes during perspiration.

Table S1.  $\lambda c$  values before and after perspiration

Sample	Applied amount: 0.6 mg cm <sup>-2</sup> Perspiring rate: 1.46 µl min <sup>-1</sup> cm <sup>-2</sup>		Applied amount: 0.6 mg cm <sup>-2</sup> Perspiring rate: 3.31 µl min <sup>-1</sup> cm <sup>-2</sup>		Applied amount: 1.2 mg cm <sup>-2</sup> Perspiring rate: 3.31 µl min <sup>-1</sup> cm <sup>-2</sup>	
	λc Before perspiration	λc After perspiration	λc Before perspiration	λc After perspiration	λc Before perspiration	λc After perspiration
1	371.4	376.1	371.6	374.6	371.4	371.2
2	371.0	376.8	371.1	374.0	371.0	370.9
3	371.0	372.1	371.7	374.8	370.9	374.2
4	371.1	374.9	371.0	374.6	371.3	374.9
5	371.8	371.7	371.4	373.2	370.4	373.2
6	372.0	377.1	371.3	373.3	369.8	370.9
7	370.8	371.4	370.8	374.3	370.4	370.7
8	371.0	374.1	371.8	373.6	371.8	375.0
9	372.2	373.5	371.9	374.3	369.9	370.8
10	371.2	371.8	372.1	374.6	371.2	376.9



Figure S9. UVA/UVB ratio comparison before and after perspiration. (a) Applied amount of 0.6 mg cm<sup>-2</sup> and perspiring rate of 1.46  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>. (b) Applied amount of 0.6 mg cm<sup>-2</sup> and perspiring rate of 3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>. (c) Applied amount of 1.2 mg cm<sup>-2</sup> and perspiring rate of 3.31  $\mu$ l min<sup>-1</sup>cm<sup>-2</sup>.

Appendix C

Paper III: Enhancing the sweat resistance of sunscreens

## Enhancing the sweat resistance of sunscreens

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#### Abstract

Perspiration negatively affects the performance of a sunscreen film by weakening its substantivity and uniformity through the activation of two mechanisms: sunscreen wash-off and redistribution. In this study, we used a perspiring skin simulator to investigate the effect of sunscreen formulation on its efficiency upon sweating. Specifically, we modified the sunscreen formulation by incorporating a hydrophobic film former and adding water-absorbing particles. Sunscreen performance before and after perspiration was assessed by *in vitro* sun protection factor (SPF) measurements, direct detection of changes in the sunscreen distribution using an ultraviolet (UV) camera, and coherent anti-Stokes Raman scattering (CARS) microscopy for the microscopic characterization of the UV filter potential relocation. The results showed that incorporating a hydrophobic film former can decrease sunscreen wash-off due to sweating, while an excessive amount of film former might negatively affect the sunscreen distribution. The addition of waterabsorbing particles, on the other hand, had either a negative or positive impact on the sunscreen substantivity, depending on the particle properties. While the addition of large water-absorbing particles appeared to increase sunscreen redistribution, smaller particles that could form a gel-like structure upon contact with water, appeared to change sunscreen wetting and sweat droplet spreading, thereby decreasing sunscreen wash-off and redistribution.

Keywords: sunscreen, sweat resistance, water-absorbing particles, film former, perspiring skin simulator, gelatin.

#### 1. Introduction

Overexposure to ultraviolet (UV) radiation from the sun can cause serious damage to the skin, such as sunburn, skin pigmentation, and DNA damage, which eventually increases the risk of skin cancer.<sup>1–3</sup> One of the well-known methods of avoiding the harmful effects of UV radiation is the application of sunscreen on the exposed skin. An effective sunscreen should form a continuous and uniform film that covers the skin to provide UV protection by reflecting, absorbing, or scattering the light over the broad UV spectrum.<sup>4</sup> A sunscreen should also be nontoxic, photostable, and durable, and have good spreadability and sensorial properties.<sup>4,5</sup>

Different factors and activities such as friction from toweling and wearing clothes, or water exposure due to swimming or sweating can affect sunscreen performance after application on the skin.<sup>6–12</sup> However, despite the fact that sweating is highly probable in many situations where sunscreen is used, such as during outdoor sports activities and sunbathing, the effect of sweating on the substantivity of sunscreens is rarely directly addressed. Our previous study, which involved an *in vitro* perspiring skin simulator, showed that the water released from the skin underneath the

applied sunscreen film will have a distinct negative impact on the efficiency of the sunscreen.<sup>13</sup> Specifically, our study showed that perspiration reduced the sun protection factor (SPF) by two mechanisms: 1) direct sunscreen wash-off, which leads to reduced film thickness and 2) redistribution of the sunscreen, which results in less uniformity. Both mechanisms negatively affect sunscreen substantivity and result in lower UV protection.

While the direct effects of sweating are widely neglected, the ability of a sunscreen to maintain high efficiency upon exposure to water (during swimming and bathing) is one of its documented and well-known properties.<sup>14–16</sup> Generally, water exposure can lead to the wash out of UV filters and the breaking down of sunscreen films, resulting in the reduction of UV protection.<sup>8,11,25,17–24</sup> However, retention of the sunscreen upon exposure to water naturally depends on the type of sunscreen and its ingredients.<sup>4,16,22,26</sup> Enhancing the sunscreen-skin adhesion (i.e., bonding between the sunscreen and the uppermost layer of the skin) or increasing the hydrophobicity of the formulation in order to repel water are known methods of improving the water resistance of a sunscreen.<sup>26,27</sup> However, it is not known whether the same approach can be used to maintain high sunscreen affecting the sunscreen-skin adhesion and where the water cannot just be repelled by a hydrophobic sunscreen formulation.

In the current study, we used our perspiring skin simulator to investigate how certain ingredients in a sunscreen formulation may affect its ability to offer high UV protection after sweating. As a first approach, we added different concentrations of a hydrophobic film former, typically used to induce high water resistance, to study the effect of this parameter on the efficiency of the sunscreen upon perspiration. Second, we added a low concentration of a number of selected water-absorbing polymeric microparticles to determine whether the localization of the water by these particles can reduce the wash-off and redistribution of the sunscreen. For both cases, the efficiency of the sunscreen formulations was evaluated by *in vitro* SPF measurements, direct detection of the UV filters distribution (using an area scan camera equipped with a UV band-pass filter, which will be referred to as UV camera), and coherent anti-Stokes Raman scattering (CARS) microscopy for real-time observation of UV filter potential relocation on a microscopic scale.

#### 2. Materials and methods

#### 2.1.Materials

To prepare the sunscreen formulations, diethylamino hydroxybenzoyl hexyl benzoate (from BASF), ethylhexyl salicylate (from DSM Nutritional Products Europe Ltd), octocrylene (from BASF), phenoxyethyl caprylate (from Evonik Nutrition & Care GmbH), C15-19 alkane (from SEPPIC), ethylcellulose (from Ashland), dibutyl adipate (from BASF), and a hydrophobic film former acrylate/octylacrylamide copolymer (from Nouryon Surface Chemistry LLC) were used as ingredients. Four water-absorbing polymers, polyacrylate crosspolymer-6, PAC-6 (from SEPPIC), cross-linked sodium polyacrylate, NaPA (from Stewart Superabsorbents LLC), hydrophobically modified (cetyl) hydroxyethylcellulose, HMHEC (from Ashland), and a core-shell particle (shell: polysilicone-34, core: isononyl isononanoate and water), which we will refer to as Core-Shell (from Momentive Performance Materials Inc.), were used as additives in the sunscreen formulation.

#### **2.2.** Perspiring skin simulator

In this study, we used a perspiring skin simulator, which is described in detail elsewhere.<sup>13</sup> Briefly, the setup comprises a multilayer skin-mimicking substrate, a chamber located underneath the substrate, a syringe pump, and connecting tubes, as illustrated in Figure 1. In the skin-mimicking substrate, the bottom layer is a track-etched hydrophilic membrane responsible for providing a

uniform flow for the upper layers, which consist of an adhesive layer and a polymethylmethacrylate (PMMA) film coated with a gelatin-based skin-like layer. These three layers were previously laser-drilled to make funnel-like holes that mimic sweat pores with a density of 200 cm<sup>-2</sup>, which is similar to the density of sweat pores in the human skin.<sup>28</sup> The pore size was adjusted to 110-120  $\mu$ m on the gelatin side and 40-60  $\mu$ m on the adhesive side. To perform the perspiration experiment, the skin-mimicking substrate was mounted and fixed on the chamber, and deionized water was pumped at a rate of 1.5  $\mu$ L min<sup>-1</sup>cm<sup>-2</sup> (corresponding to moderate sweating on the untreated human forehead).<sup>28</sup>



Skin-mimicking substrate during perspiration

Figure 1. Schematic illustration of the perspiring skin simulator setup and a picture of the skin-mimicking substrate during perspiration.

#### **2.3. Sunscreen formulations**

The sunscreens were prepared by dissolving the ingredients in ethanol using a homogenizer (Silverson L5T, Silverson Machines Ltd., England). Based on the UV filter composition (30% wt.), an SPF of 28 was calculated using a sunscreen simulator software (BASF).<sup>29</sup> To evaluate the

effect of the concentration of the film former, three formulations containing 0, 0.75, and 3% wt. of the acrylic-based film former were prepared. To investigate the effect of water-absorbing particles, the concentration of the film former was kept constant at 0.75% wt., and four formulations containing 1% wt. water-absorbing particles were prepared. For the purpose of CARS microscopy, two formulations containing only one UV filter, octocrylene (20% wt.) with 0 and 1% wt. of the PAC-6 particles were prepared following the same mixing procedure.

#### 2.4. In vitro SPF Measurements

The efficiencies of the sunscreens in protecting against UV radiation were measured using a UV transmittance analyzer (Labsphere UV-2000S, Labsphere Inc., North Sutton, NH, USA) and were subsequently converted to *in vitro* SPF values using the formula below:

$$SPF = \frac{\sum_{290}^{400} E(\lambda) S(\lambda)}{\sum_{290}^{400} E(\lambda) S(\lambda) T(\lambda)}$$
1

where  $E(\lambda)$  is the erythema action spectrum,  $S(\lambda)$  is the spectral irradiance of the UV source, and  $T(\lambda)$  is the measured transmittance of the light through a sunscreen film applied on a UVtransparent substrate. In this study,  $T(\lambda)$  was recorded through the bare skin-mimicking substrate placed on a molded PMMA plate (Helioplates HD2; Helioscreen, Creil, France) as the reference for SPF measurements. A quantity of 0.6 mg cm<sup>-2</sup> of sunscreen was applied on the skin-mimicking substrate using a pipette and was subsequently spread by light circular strokes over the whole surface with a latex finger cot, pre-saturated with the sunscreen. The sample was then kept in a dark place at room temperature for 15 minutes to self-level and dry before the initial SPF measurement. The final SPF value was measured after the perspiring procedure. For each sample, the SPF value was measured at nine locations.

#### **2.5.**Procedure for perspiration studies

The skin-mimicking substrate was placed on an HD2 plate to record the blank  $T(\lambda)$  spectrum using the UV transmittance analyzer. The sunscreen was then applied on the skin-mimicking substrate and after drying for 15 minutes, the initial SPF was measured. Afterwards, the sunscreen-treated skin-mimicking substrate was mounted on the chamber and the perspiration was started at a rate of 1.5 µL min<sup>-1</sup>cm<sup>-2</sup> continuing for 30 minutes using a syringe pump. Once the perspiration was stopped, the chamber was drained, the excess amount of water on the substrate surface was offloaded, and the sample was allowed to dry for 30 minutes. Subsequently, the skin substrate was removed from the chamber and post-perspiration *in vitro* SPF was measured. For each formulation, the perspiration experiment and the subsequent SPF measurement were performed five times to ensure good repeatability of the reported results.

In order to help understanding the active mechanisms that underlie sunscreen failure during perspiration, we prepared skin-mimicking substrates in which only half of the surface (semi-circle area) contained the sweat pores and the other half was the plain gelatin-based skin-like layer. Sunscreen was applied solely on the area with sweat pores. After mounting the substrate on the chamber, perspiration started and continued for 20 minutes while the chamber was kept in a vertical position with the treated area on the upper side. The distribution of UV filters was monitored before the onset of perspiration, immediately after stopping the perspiration, and after 20 minutes and 2 hours of drying, using the UV camera. Figure 2 shows a schematic view of the skin-mimicking substrate on the vertical chamber at different stages of the experiment.



Figure 2. Schematic illustration of the half-drilled skin-mimicking substrate in which only the upper side is able to sweat. (a) Untreated substrate, and (b-d) Substrate with applied sunscreen on the upper side: (b) before onset of perspiration, (c) during perspiration, and (d) after drying. The chamber is kept in a vertical position during perspiration and drying, enabling sweat to flow from the sunscreen-treated area to the untreated area.

#### 2.6.UV camera

During perspiration, the chamber was placed in a box in which an area scan camera (acA4024-29um, Basler AG, Germany) equipped with a UV band-pass filter (365 nm, F/BP365-CMOUNT) and a high-resolution lens (Fujinon HF-1218-12M, Fujifilm, Japan) was fixed perpendicular to the sweat chamber. The box was also equipped with LED UV lamps emitting UVA radiation to provide suitable light to allow the camera to detect the changes in the sunscreen distribution (with UV absorption at wavelength 365 nm i.e. UVA filters) during perspiration.

#### 2.7. Coherent Anti-Stokes Raman Scattering (CARS) microscopy

CARS spectra of the ingredients used in the simplified sunscreen formulation (containing only one UV filter, octocrylene, without and with 1% wt. of PAC-6 particles) and the skin-mimicking substrate were obtained in order to identify a unique vibration peak for CARS imaging (TCS SP8 CARS microscope, Leica Microsystems, Germany). The pump laser (PicoEmerald, APE, Germany) wavelength varied from 787 to 877 nm with 1-nm increments (wavenumbers ranged from 3313 to 2009 cm<sup>-1</sup>) with a Stokes laser fixed at 1064 nm. A distinct peak at wavenumber 2210 cm<sup>-1</sup>, attributed to the nitrile group in the UV filter, octocrylene, did not overlap with the

peaks of the other ingredients, PAC-6 particles, and the skin-mimicking substrate. Thus, it could be used as a unique peak to provide good chemical contrast between the active component of the sunscreen and the skin-mimicking substrate (see Supporting Information, Figure S1).<sup>13</sup> For realtime monitoring of the sunscreen/sweat interaction and redistribution of the octocrylene UV filters, the sunscreen was applied at a quantity of 2 mg cm<sup>-2</sup>. After the self-leveling and drying of the sunscreen, the substrate was mounted on the sweat chamber. The chamber was then placed inversely on the sample stage of the CARS microscope and perspiration was initiated. The field of view for the images was  $1550 \times 1550 \ \mu\text{m}^2$  with a pixel size of  $3.033 \times 3.033 \ \mu\text{m}^2$ , and the images were acquired at 2210 cm<sup>-1</sup> (pump laser: 861.8 nm).

#### 3. Results and Discussion

As previously demonstrated, perspiration can affect the ability of sunscreen films to provide protection from UV through different mechanisms (sunscreen wash-off or sunscreen redistribution), which lead to SPF reduction.<sup>13</sup> We investigated how these mechanisms could possibly be altered, first by changing the concentration of the hydrophobic film forming polymer in the formulation and second by introducing water-absorbing particles into the formulation.

#### **3.1.Effect of hydrophobic film former concentration**

The film former assists the uniform distribution of UV filters on the skin and increases the film integrity during contact with water.<sup>30–32</sup> Different categories of film formers are used in sunscreen formulations, including water-repellent or water-dispersible materials.<sup>32,33</sup> We selected an acrylic-based film former with hydrophobic properties and prepared three formulations containing 0, 0.75, and 3% wt. of this film forming polymer. These three formulations were tested on the perspiring skin simulator to assess the effect of the hydrophobic film former and its concentration on the sunscreen substantivity during perspiration. Figure 3a outlines the results obtained from *in vitro* 

SPF measurements before and after sweating. The data are based on five repetitions for each formulation and measurements at nine different locations on each sample (corresponding to 45 SPF measurements for each sample). Figure 3b shows the average SPF values and their variations. The SPF reduction for the sunscreens containing 0 and 3% wt. of the film former was higher than that of the sunscreen with 0.75% wt. film former ( $58\pm26\%$  and  $69\pm19\%$ , respectively, compared to  $42\pm24\%$  SPF reduction). The results illustrate that the hydrophobic film former enhances the sunscreen substantivity, probably by bonding effectively to the substrate, entrapping the UV filters in the film former up to 3% did not amplify the aforementioned characteristics and, in contrast, resulted in higher SPF reduction compared to the other two sunscreens. The exact reason for this observation is unknown. However, we noticed that adding 3% wt. hydrophobic film former led to a ~70 % increase in the viscosity of the sunscreen formulation, and the change in performance could be due to a complex interplay change in the initial film structure and sweat.



Figure 3. *In vitro* SPF measurements for sunscreen formulations with different film former concentrations. (a) SPF after sweating versus initial SPF values measured on five individual samples and for nine positions on each sample. The dashed lines represent the location of the SPF values if there is no difference before and after sweating. (b) Statistical representation of the difference in measured SPF before and after sweating. The colored area represents the interquartile range (IQR) showing the middle 50% of the data (25% to 75%). The middle line represents the median and the small white dot is the mean value. The mean value for each set of experiments is also shown. The upper and lower lines represent the maximum and minimum SPF change excluding the outliers. The dots outside the colored region indicate the outliers.

In an attempt to distinguish how the two SPF reducing mechanisms (sunscreen wash-off and sunscreen redistribution) are affected by altering the concentration of the film former, a skinmimicking substrate with only artificial sweat pores on half of its area was used. Here, the sunscreens were applied only on the area with the sweat pores and perspiration was started and continued for 20 minutes while the chamber was kept in a vertical position, with the treated area on the upper side (the schematic view is shown in Figure 2 and the treated substrates are shown in Figure 4a). For this experiment, the redistribution of sunscreen due to perspiration was observed as an uneven distribution of dark areas in the treated part of the skin-mimicking substrate, whereas the washed-off sunscreen was observed as black lines flowing down from the sunscreen treated area to the untreated area of the substrate. Figure 4b and Figure 4c show the substrates 20 minutes after perspiration started and 20 minutes after perspiration stopped, respectively. Figure 4d illustrates the completely dried samples (approximately 2 hours after perspiration was stopped).

As shown in Figure 4, when the sunscreen does not contain the film former, a drastic redistribution of UV filters and massive wash-off occur during perspiration. The film former acts as a network that holds the sunscreen film together, and in its absence, the water released underneath the film could significantly disturb the UV filter distribution. The addition of 0.75% wt. of the film former moderates the effect of perspiration on the redistribution of UV filters, while sunscreen wash-off is still visible. However, increasing the film former concentration up to 3% wt. effectively prevented sunscreen wash-off. Thus, regarding the *in vitro* SPF, a higher protection after sweating was observed for the formulation containing 0.75% wt. hydrophobic film former compared to the formulation containing 3% wt. hydrophobic film former. It appears that sunscreen redistribution has a more pronounced effect on SPF than sunscreen wash-off. We note, however, that in addition to SPF changes, running sunscreen might create other types of discomfort such as eye irritation or staining on clothes.

In the next experiment, where the effect of introducing water-absorbing particles into the formulation was studied, we fixed the concentration of the film former at an intermediate level (0.75% wt.).



Figure 4. UV camera images of the skin-mimicking substrates. The images illustrate how sunscreens with different concentrations of hydrophobic film formers are washed off and redistributed during perspiration. (a) After sunscreen application on the area with sweat pores and at the beginning of sweating, (b) after sweating for 20 minutes, (c) 20 minutes after the sweating was stopped, and (d) after complete drying of the sample.

#### **3.2.Effect of water-absorbing particles**

The selected particles possess different characteristics in terms of chemistry, particle size, polydispersity, water-absorbing kinetics, and water uptake capacity. Here, we will focus mainly on the particle size and water uptake capacity. NaPA and PAC-6 are both superabsorbent particles with water uptake capacities >300 g/g, whereas HMHEC and Core-Shell possess more moderate water uptake capacities of  $\sim 20-30 \text{ g/g}$ . With respect to size, NaPA and HMHEC are relatively large particles with diameters of approximately 10-40 and 30-180 µm, respectively, which means that

NaPA and HMHEC will act as large water-absorbing domains in the sunscreen film. On the contrary, PAC-6 and Core-Shell are smaller with diameters of approximately 1-10 µm. This means that PAC-6 and the Core-Shell will be embedded in the sunscreen film and that for the same weight fraction, the particles will be more homogenously distributed laterally in the sunscreen film compared to the larger particles. The Core-Shell, which is a translucent microgel particle comprising a water-swellable polyacrylic core covered by a protective silicone copolymer shell, has a special feature that allows it to scatter UV light and thus boosting the SPF when included in a sunscreen formulation (see Supporting Information, Table S1). However, upon exposure to water, the polyacrylate core will moderately swell and thus change the particle shape, leading to an expected alteration in its ability to scatter UV light. Thus, adding the Core-Shell to our sunscreen formulation will, therefore, enable us to explore an additional effect of perspiration on sunscreen efficiency, which is different from the two previously documented effects (sunscreen wash-off and sunscreen redistribution).


Figure 5. *In vitro* SPF measurements for sunscreen formulations with and without water-absorbing additives before and after sweating. (a) SPF after sweating versus initial SPF values measured on five individual samples and for nine positions on each sample. The dashed lines represent the location of the SPF values if there is no difference before and after sweating. (b) Statistical

representation of the difference in measured SPF before and after sweating. The colored area represents the interquartile range (IQR) showing the middle 50% of the data (25% to 75%). The middle line represents the median and the white dot is the mean value. The mean value for each set of experiments is also shown. The upper and lower lines represent the maximum and minimum SPF changes excluding the outliers. The dots outside the colored region indicate the outliers.

Figure 5a presents the *in vitro* SPF values measured after perspiration versus the initial SPF values for the sunscreens with and without 1% wt. of the water-absorbing particles, and Figure 5b shows the associated average SPF and distribution of measured values before and after perspiration. For the sunscreen formulation without water-absorbing particles, the average SPF reduction was 42±24%. For sunscreen formulations containing NaPA and HMHEC particles, the average SPF reductions were 49±19% and 61±21%, respectively, which means that the addition of these particles has either a negative or no impact on the sweat resistance of the sunscreen film. However, for the sunscreen formulation containing PAC-6 particles, the SPF reduction was only  $13\pm 20\%$ , indicating that these particles successfully increased the sweat resistance of the sunscreen. For the sunscreen formulation containing the Core-Shell particle, the initial SPF was as expected boosted to a considerably higher value than for the other formulations; however, after perspiration, the SPF was reduced drastically to a value that was similar to the post-perspiration values observed for the other sunscreen formulations. This clearly shows that the Core-Shell particles do not recover their SPF boosting ability after the water evaporates, indicating an irreversible change in their shape. However, based on these results alone, it is difficult to identify any connection between the influence of the Core-Shell particles and the sunscreen substantivity.



Figure 6. UV camera images of the skin-mimicking substrates. The images illustrate how sunscreens with and without waterabsorbing particles are washed off and redistributed during perspiration. (a) After sunscreen application on the area with sweat pores and at the beginning of sweating, (b) after sweating for 20 minutes, (c) 20 minutes after the sweating was stopped, and (d) after complete drying of the sample.

We also performed perspiring experiments using the substrate with sweat pores on half of its surface to investigate the contribution of failure mechanisms in SPF reduction. For the sunscreen without water-absorbing particles, moderate redistribution and considerable wash-off were observed. For the sunscreen formulations containing the large particles (NaPA and HMHEC), a significant sunscreen redistribution was observed. The sunscreen containing NaPA was also extensively washed off. These observations are in agreement with the negative impact of these particles on sweat resistance, which was observed through the SPF measurements. Here, we suggest that the significant redistribution is related to the large size and swelling of these particles. When water is absorbed by NaPA particles, their volumes increase significantly, and they start moving within the sunscreen film whereby they push the sunscreen aside or disturb its uniformity. Simultaneously, NaPA particles become so heavy that, as a result of gravity, these water-rich particles are pulled downward, dragging the sunscreen along. This phenomenon is referred to as sunscreen wash-off. In sunscreens containing HMHEC, the water-rich particles move within the sunscreen film, leading to the redistribution of UV filters. However, owing to their low water-absorbing capacity, the particles are less affected by gravity.

For sunscreen formulations containing smaller particles (PAC-6 and the Core-Shell), we observed very limited sunscreen wash-off, and for the formulation containing PAC-6 particles, a minor sunscreen redistribution was also observed. For the formulation containing PAC-6 particles, this observation is in agreement with the relatively low SPF reduction. For the formulation containing the Core-Shell particles, our observation confirms that the large reduction in SPF is rather a consequence of the lost SPF boosting effect than a disturbed sunscreen film. However, Figure 6d, which shows a sunscreen containing the Core-Shell particles, illustrates that UV filters redistribution could occur as a result of particle expansion. A provisional conclusion based on the size of water-absorbing particles is that small water-absorbing particles appear to better prevent sunscreen wash-off compared to larger particles, while the actual water uptake capacity seems to play a less significant role. Moreover, the water responsive behavior of large particles, that is, the

significant increase in the volume and the movement of these particles, could negatively affect the distribution of the sunscreen.

Even though both PAC-6 and NaPA particles have high water uptake capacity (which, according to our expectation, would result in the ability to localize the sweat droplets), only PAC-6 seems to increase the substantivity of the sunscreen formulation. Based on the water responsive behavior of these two particles and their corresponding sunscreens, observed using an optical microscope (see Supporting Information, Figure S2), PAC-6 particles appear to form a gel-like structure while exposed to water, which seems to strengthen the integrity of the sunscreen film and prevent or delay the movement of UV filters by localizing the pressurized water. On the other hand, when the large NaPA particles absorb water, they expand and move towards neighboring particles but do not merge to form a network. As seen in Figure 6, the massive expansion of the NaPA particles, however, disturbed the sunscreen distribution and intensified the sunscreen wash-off. Moreover, when the sweating was stopped and the drying process started, we observed that the NaPA particles moved toward each other to form small clusters, which affected the distribution of UV filters inside the sunscreen formulation (see Supporting Information, Figure S3). In contrast, PAC-6 particles seem to maintain the network structure upon evaporation of the sweat.

### **3.3.CARS Microscopy**

CARS microscopy was employed for the label-free visualization of any potential relocation of UV filters upon perspiration. Since the incorporation of PAC-6 particles enhanced the sweat resistance of the sunscreen, we prepared simplified sunscreens (containing only one UV filter, i.e., octocrylene, which is easy to trace using CARS microscopy) without and with 1% wt. of PAC-6 particles. Figure 7a shows the CARS image of the growth of water droplets on the skin-mimicking substrate treated with sunscreen with no water-absorbing particles. The water droplet expands in

all directions, and the sunscreen film surrounding the droplet thins out in the z-direction, which could finally lead to sunscreen film rupture. The water droplet on the substrate surface pushes the sunscreen film around it away in the direction of the sweat droplet expansion. Consequently, when two water droplets move toward each other, the concentration of UV filters on their common border increases transiently (Figure 7a, t=289 s). In contrast, the CARS images of the sunscreen containing 1% wt. of PAC-6 particles shows different droplet/sunscreen interactions. The waterabsorbing polymer readily absorbs the water flowing through the sweat pore in order to form a gel-like structure. It slightly changes the sweat droplet shape to a less semi-spherical one compared to sweat droplets formed on the substrate treated with the sunscreen without PAC-6 particles (Figure 7b, and also see Supporting Information, Figure S4). We believe that the gel-like structure strengthens the sunscreen film integrity and slows down the expansion of the sweat droplet in the z-direction, which reduces the possibility of sunscreen film rupture. The ability of PAC-6 particles to localize and flatten the sweat droplets can be observed by comparing the droplet heights in Figure 7a and b. The process by which two neighboring droplets merge is also different for sunscreens containing PAC-6 particles; the concentrated ring with UV filters surrounding the expanding droplets is less pronounced, and when the droplets encounter each other, the formation of the gel-like network structure appears to limit the UV filters redistribution.





t=0 s









t=361 s

















Figure 7. CARS images showing the growth and merging of sweat droplets on the sunscreen-treated skin-mimicking substrate. (a) Sunscreen formulation with no water-absorbing particles. (b) Sunscreen formulation with 1% wt. PAC-6 particles.

#### 4. Conclusion

The retention of sunscreen performance upon perspiration depends extensively on the ingredients used in the formulation. In this study, we evaluated the sweat resistance of sunscreens by manipulating the concentration of a hydrophobic film former and introducing water-absorbing particles. A perspiring skin simulator and three techniques (in vitro SPF measurements, direct imaging of sunscreen distribution using a UV camera, and coherent anti-Stokes Raman scattering (CARS) microscopy) were employed to investigate the potential mechanisms that underlie the failure of sunscreens upon perspiration. The results illustrated that the presence of a hydrophobic film former enhances the sweat resistance of sunscreens by reducing sunscreen wash-off for formulations with high film former concentrations and reducing sunscreen redistribution for formulations with intermediate film former concentrations. This means that a sunscreen formulation applied on sweating skin can potentially provide better protection against UV light and be less runny; therefore, it becomes more user-friendly when a hydrophobic film former is incorporated. The introduction of water-absorbing particles into sunscreen formulations was shown to have either a positive or negative impact on the sunscreen substantivity upon perspiration, depending on the properties of the particles. Generally, small particles with the ability to change the overall sunscreen wettability and to form a gel-like network appear to protect sunscreen integrity, while larger particles localize too much water upon swelling, leading to significant sunscreen redistribution and a decrease in SPF. Finally, as observed for the Core-Shell particles, perspiration can have other formulation specific effects on the UV-protecting performance of a sunscreen. Overall, a combination of hydrophobic film formers to increase water

resistance and small water-absorbing particles to change the wetting behavior appears to be a promising approach for the development of more sweat-resistant sunscreens.

### Acknowledgments

The authors are grateful for the financial support from the Innovation Fund Denmark through the Grand Solutions Project #6151-00007B. We also thank the Danish Molecular Biomedical Imaging Center (DaMBIC, University of Southern Denmark) for allowing us to use their CARS microscope.

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## Supporting information:

## Enhancing the sweat resistance of sunscreens

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## S1. In vitro SPF measurements of sunscreens

Following the COLIPA method for in vitro SPF measurements, we applied the sunscreens on a molded PMMA plate (Helioplates HD6; Helioscreen, Creil, France) with the quantity of 1.3 mg cm<sup>-2</sup> and measured their in vtiro SPF:

Table S1. In vitro SPF value	s for sunscreens used in this study.
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	Sunscreens with different film former			Sunscreens containing water absorbing			
	concentration			particles			
Sunscreen	0 wt.%	0.75 wt.%	3 wt.%	PAC-6	Core- Shell	NaPA	HMHEC
SPF	48	73	84	74	158	62	59

## S2. CARS wavelength scan



Figure S1. CARS signal for the simplified sunscreen components and the skin-mimicking substrate in the wavenumbers ranging from 3313 to 2009 cm<sup>-1</sup>. The peak at 2210 cm<sup>-1</sup> is attributed to the nitrile group in octocrylene.

## S3. Microscopic images of NaPA and PAC-6 particles and corresponding sunscreens



Figure S2. Microscopic images of NaPA and PAC-6 particles respectively in (a), (e) dry state, (b), (f) after exposure to water, (c), (g) during water evaporation (d), (h) after water evaporation. Sunscreen containing (i) NaPA and (j) PAC-6 particles during perspiration.



Figure S3. Drying of water droplets in sunscreens containing (a-b) NaPA and (c-d) PAC-6 particles. The red circle shows the NaPA particles which moved toward each other during the drying of sweat droplet.

# S4. CARS images of sweat droplet in absence and presence of PAC-6 particles



Figure S4. Top view of sweat droplets formed on the substrate treated with simplified sunscreen (a) without and (b) with PAC-6 particles. The sweat droplets on the left image form semi-spherical shape whereas the form of droplets on the right image are less symmetric.

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