In Vitro Testing of Sunscreens for Dermal Absorption: A Platform for Product Selection for Maximal Usage Clinical Trials

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Sunscreen products contain UV filters as active ingredients for the protection of the skin against UVR. The US Food and Drug Administration (FDA) issued a new proposed rule in 2019 (84.FR.6204) for sunscreens and identified the need for additional safety data for certain UV filters including their dermal absorption data. Dermal absorption data reveal systemic exposure of UV filters in humans, which can be obtained from clinical maximal usage trials. FDA guidance recommends conducting in vitro skin permeation tests (IVPTs) to help select formulations for maximal usage clinical trials as IVPT results may be indicative of in vivo absorption. This case study reports in vitro methodologies used for the selection of sunscreen products for an FDA-sponsored proof-of-concept maximal usage clinical trial. An IVPT method was developed using human cadaver skin. Commercially available sunscreen products were tested to determine the skin absorption potential of common UV filters using the IVPT. All the studied sunscreen products demonstrated a certain degree of skin absorption of UV filters using IVPT, and a formulation rank order was obtained. These sunscreen products were also characterized for several formulation properties including the globule size in emulsions, which was found to be an indicator for the rank order.


INTRODUCTION

UV filters are active ingredients in sunscreen products. They function to protect the skin from sunburns and UV-related skin damage. These small molecules protect the skin by absorbing, scattering, or reflecting UVR. Ideally, UV filters are intended to work on the skin surface without penetrating the skin and thereby reaching the systemic circulation. However, UV filters such as oxybenzone have been detected in the systemic circulation (Calafat et al., 2008; Janjua et al., 2008; Matta et al., 2019). Sunscreen products are recommended for frequent, daily application in quantities that may result in coverage of up to 80% of the body surface (Heerfordt et al., 2018). Therefore, application of sunscreen ingredients may lead to systemic exposure in a single day (Hayden et al., 1997; Janjua et al., 2004; Matta et al., 2020, 2019; Michele, 2018) and substantial exposure over a lifetime.

Sunscreen products are regulated as cosmetics in some countries. However, in the United States, sunscreens are regulated as drug products, primarily under the over-the-counter drug monograph system (FDA, 2019a). Despite increasing use across a broad population, there are limited data on whether or to what extent UV filters are systemically absorbed from various sunscreen formulations and whether there are adverse effects from systemic exposure (Adamson and Shinkai, 2020). Therefore, evaluating the extent of absorption of common UV filters is important for public health. Different excipients in sunscreen formulations could enhance the absorption of UV filters to different degrees. Therefore, it is important to evaluate the absorption of active ingredients from a representative range of formulations. In 2019, the US Food and Drug Administration (FDA) issued a new proposed rule (monograph) (FDA, 2019c) on Sunscreen Drug Products for Over-the-counter Human Use. This rule requests additional data to determine whether certain active ingredients listed in the 1999 Final Monograph (FDA, 1999) are generally recognized as safe and effective in sunscreen products.

One of the approaches to determine systemic exposure is conducting clinical trials under maximal usage conditions (maximal usage trial or MUsT) (FDA, 2019b). Per the 2019 published MUsT Guidance for Industry (FDA, 2019b), in vitro skin permeation test (IVPT) is recommended to guide the selection of formulations to include in the MUsT. Formulations selected for evaluation by MUsT should be those with the highest potential for absorption of UV filters. In this case study, we aimed to use in vitro approaches to guide the...
selection of products for a proof-of-concept MUST study \cite{Matta2019}. Although general IVPT guidelines are available \cite{FDA2016, OECD2004}, studying in vitro skin absorption of UV filters is challenging because of the diverse physicochemical properties and combined presence of UV filters in sunscreen products (Supplementary Table S1). Existing studies in the literature have weaknesses in study designs or insufficient data, which limit utility \cite{FDA2019, Oh2019} as a definitive methodology for testing UV filters. Therefore, we created a pilot IVPT to explore parameters useful for the purposes of guiding formulation selection for further clinical absorption testing. In addition, formulation characteristics of sunscreen products such as emulsion types, distribution of UV filter, and globule sizes were evaluated for their influence on skin absorption.

RESULTS AND DISCUSSION
There is a complex interplay among the properties of the UV filters, the sunscreen formulations, and the IVPT outcomes. Physicochemical properties of the chemicals, such as molecular weight, melting point, partition coefficient (logP), and topological polar surface area, are known predictors for skin absorption potential \cite{Liu2016}. Skin absorption is expected if the chemical’s logP is between –1 and 4, molecular weight is less than 500 g/mol, melting point is less than 200 °C or topological polar surface area is less than 120 Å² \cite{Chandrashekar2008, Golla2012, Lee2010}. All the UV filters studied have at least one physicochemical property that meets these criteria for skin absorption (Supplementary Table S1). Avobenzone and octocrylene have logP values greater than 4, indicating that their skin permeability could be lower than that of oxybenzone, ecamsule, and parabens, whose logP values are lower. The IVPT results confirmed these predictions.

In this study, the skin absorption of UV filters was found to be influenced by the formulation. For example, the extent of absorption of oxybenzone following topical administration of cream, lotion, and spray is different. Various formulation characteristics, such as the presence or absence of permeation enhancers, emulsion type, drug distribution, and globule size \cite{Frelichowska2009}, may play a role in skin absorption. Globule size of emulsion formulations is thought to be relevant to drug release and skin absorption of active ingredients. Therefore, semisolid sunscreen formulations were characterized for their globule sizes.

Determination of emulsion type and distribution of UV filters in semisolid sunscreen products
Globule size may be critical for drug release only if globules contain the active ingredients. In this study, emulsion type and the distribution of UV filters in sunscreen emulsions (cream and lotions) were determined using Raman microscopy to see whether most of the UV filters were present in the globules. Raman spectra were obtained for the US Pharmacopeia reference standards of each analyte in the formulations (Figure 1a). Circular globules in the sunscreen matrix for all three products were observed from an optical montage (Figure 1b). The averaged spectra for each emulsion phase (matrix and globules) were overlaid and compared with the reference standards (Figure 1c). Although it was expected to see peaks related to the UV filters present in both phases, the cream showed a larger contribution for peaks matching octocrylene and avobenzone (e.g., 404, 1,297, 1,595, 1,607, and 2,218 cm⁻¹) in the globules, whereas ecamsule (e.g., 1,184 and 1,638 cm⁻¹) had a larger relative contribution to the Raman spectra that were obtained from the surrounding matrix. Ecamsule was the least hydrophobic among all the UV filters that were studied (logP value = 1.4, Supplementary Table S1), presenting majorly in the matrix indicates that the cream is an oil-in-water emulsion. Both lotions showed a stronger signal for the hydrophobic active ingredients (octocrylene, avobenzone, and oxybenzone) in the globules relative to the matrix (Figure 1c), indicating that the lotions are also oil-in-water emulsions. Distribution of the UV filters in the cream was further confirmed using confocal Raman mapping and multivariate image analysis (Figure 1d–f). Because all the emulsions that were studied are of the oil-in-water type and the UV filters are mostly present in the globules, these formulations are expected to exhibit similar release profiles for avobenzone, octocrylene, and oxybenzone after application to the skin.

Globule size determination using cryogenic scanning electron microscopy
The size of oil globules in emulsions may have an effect on drug release rate and skin permeation efficiency \cite{Doucet1998}. Therefore, globule size was characterized using cryogenic scanning electron microscopy. Two distinct magnifications (×500 and ×2,500) were selected for appropriate display of the globules (Figure 2a–c). Both lotions contained globules within the size range of 1–5 μm in diameter. A-lotion also contained a small fraction of larger globules that varied in size (10–25 μm, Figure 2b). Image analysis of the cream sample did not yield globules in the 1–5 μm size range but revealed a broader distribution of globules between 5–25 μm in diameter. Histograms were created (Figure 2d), and each histogram was fitted with a log-normal distribution, which was later used to calculate the mean and SD of globule diameters of each sunscreen product (Figure 2d, inset). The mean globule size was found to be 2.4 ± 0.8 μm for B-lotion, 3.1 ± 1.7 μm for A-lotion, and 9.4 ± 3.7 μm for the cream. Literature reports that microspheres smaller than 3 μm distribute randomly in the stratum corneum. In contrast, microspheres larger than 10 μm do not penetrate but remain on the skin surface \cite{Frelichowska2009}. The average globule sizes for the lotions are 3 μm or less and about 10 μm for the cream. The globule size appeared to be inversely related to the permeation of octocrylene from the lotions and the cream (Figure 3), suggesting that smaller globules with larger surface area for interaction with the skin may facilitate skin permeation of UV filters. However, owing to the differences in the excipients used in these formulations (Supplementary Table S2), it cannot be concluded that the difference in globule size is the only reason for the differences observed in the skin absorption of octocrylene. Metamorphosis \cite{Roberts2017} of the emulsions upon contact with the skin is a dynamic process that may also affect the release and permeation of active ingredients.
Skin permeation of various sunscreen ingredients

Sunscreen products evaluated in this study exhibited skin permeation of UV filters. Some sunscreen products differ in the quantity of UV filters and parabens (Supplementary Table S1). To facilitate the comparison of permeation potential of these ingredients among various sunscreen products, the permeation results of each ingredient were dose normalized according to the content of the corresponding ingredient in A-lotion. Summary results in Figure 3 and Supplementary Table S4 are cumulative permeation at each sampling time point from four donors. Cumulative permeation data of individual donors are shown in Supplementary Tables S5–S8. Table 1 presents the rank order of skin absorption (permeation and retention) for all products based on the total amount observed at 24 hours.

The results in Figure 3a and Table 1 show that the permeation of avobenzone exhibits the following rank order: A-lotion = B-spray ≥ cream ≥ A-spray ≥ B-lotion. At 3 hours, the permeation of avobenzone was observed from the lotions and sprays but not from the cream. A-lotion exhibited significantly higher permeation of avobenzone than B-lotion at 3 hours (P < 0.001), but the significance of the observation disappeared at 24 hours. Octocrylene had a logP value of 6.8 and was the most lipophilic UV filter among those that were tested. The permeation of octocrylene exhibited the following rank order: A-lotion ≥ B-spray ≥ B-lotion ≥ A-spray > cream (Table 1 and Figure 3b). The cream exhibited the lowest permeation of octocrylene at 3 and 6 hours, but the significance of the observation disappeared at 24 hours. This trend suggests that the cream (with larger globules of approximately 10 μm) may delay the permeation of octocrylene compared with the lotions (with smaller globules of approximately 3 μm). The lotions with similar oil globule sizes were observed to have similar levels of skin permeation.
of octocrylene. However, the permeation of octocrylene from the lotions compared with B-spray was not significantly different.

Across all products, the total permeation of oxybenzone was up to 200-fold greater than that of avobenzone and octocrylene. This observed high in vitro permeation confirmed the predictions deduced from the physicochemical properties of the UV filters (Supplementary Table S1) and agreed with the published reports on high in vivo absorption of oxybenzone (Jiang et al., 1999). Permeation of oxybenzone between the two lotions was not significantly different (Figure 3c). However, both lotions were found to exhibit significantly higher permeation of oxybenzone than B-spray. The results in Table 1 show that the permeation of oxybenzone from various products exhibited the following rank order: B-lotion ≈ A-lotion ≈ A-spray > B-spray. Interestingly, with an infinite dose applied to the skin, in vitro permeation of oxybenzone was higher from the water-based A-spray and lotions than from the alcohol-based B-spray.

Ecamsule was only present in the cream and was found to permeate the skin over the course of 24 hours in greater quantities than avobenzone and octocrylene (Figure 3d). Having a logP value of 1.4 and being mainly in the emulsion matrix (Figure 1) may facilitate skin permeation. Parabens were only found in the lotions and the cream and were used as positive controls for skin permeation. The results in Figure 3e and f show that the permeation of both parabens was similar among all the products.

**Comparative results of skin retention**

The summary results of all donors (Figure 4a and Supplementary Table S9) show that B-spray exhibited the highest skin retention of all the UV filters compared with other sunscreen products. Skin retention of avobenzone and octocrylene from B-spray was found to be significantly higher than the skin retention of these ingredients from the cream, probably owing to formulation differences. Although B-spray exhibited the highest skin retention of oxybenzone among all formulations, the differences were not statistically significant. The skin retention of methylparaben from A-lotion was found to be significantly higher than that from the B-lotion but not from the cream (Figure 4b). Skin retention data of individual donors are shown in Supplementary Tables S10–S12.

**Rank order of sunscreen products**

The rank order of skin permeation and retention of UV filters and parabens in the studied sunscreen products is described in Table 1. Among all products, A-lotion had the highest permeation of avobenzone and octocrylene, and B-spray had the highest skin retention of all the UV filters. Notably, the trend of oxybenzone detected in the permeation sample was in a reversed sequence to that observed in the skin. B-spray had the highest skin retention of oxybenzone but the lowest permeation of oxybenzone in the permeation samples compared with other products. However, because the majority of oxybenzone was retained in the skin (54.4 μg/cm²) rather than found in the skin permeation samples (7.1 μg/ cm²), skin retention of UV filters predominated the overall
results of the rank order. For emulsion sunscreens, A-lotion demonstrated the highest absorption of all ingredients. B-spray and A-lotion were selected for clinical MUsT study (Matta et al., 2019) owing to the withdrawal of B-lotion from the market.

As the first attempt of IVPT method optimization for sunscreen products, there are possible limitations in this study that warrant further investigation of the methodologies. For example, because this study evaluated only marketed products with different formulations and excipients, the contributions of various formulation variables to the observed in vitro characteristics of the products cannot be clearly ruled out (Benson et al., 2005). A design of experiment approach may be needed to closely evaluate the effect of an individual process or formulation variables on the performance of the final product (i.e., viscosity). Second, the IVPT study used split-thickness cadaver skin, including the entire epidermal layers and partial dermis with a fixed total thickness. Further studies may be done with the skin of different thicknesses for method optimization. It is worth noting that cadaver skin may not have viable cells, which is different from ex vivo skin cultures and freshly excised postoperative human skin. The status of the cornified envelope, stratum corneum, and epidermal tight junctions among these skin models may also be different from viable skin, and data variability may be higher when cadaver skin is used. Caution should be exercised when choosing a suitable skin model for IVPT. We have demonstrated in a previous study that cadaver skin obtained from the same source as for this study and freshly excised viable human skin have comparable barrier functions (Yang et al., 2015). Third, a single application using an infinite dose of the sunscreen product was employed in this study to match the total amount of sunscreen used in the clinical MUsT study. However, in-use conditions such as single or repeated finite dose application of sunscreen (i.e., 2–10 mg/cm²) may be employed to optimize the IVPT method, as finite dosing may produce different hydration and viscosity effects compared with infinite dosing. In addition, finite dosing may allow precise assessment of the absorption of UV filters by recovery analysis (the quantitation of actives in each compartment, including the actives in the skin and those
removed by wiping). Fourth, because the IVPT was exploratory for UV filters and the primary objective was to assist product selection for MUsT studies, the sampling time points may have been less than the ideal sampling plan for IVPT of topical products. More frequent sampling could be implemented to obtain the entire in vitro flux profile of the UV filters for comparison with the in vivo pharmacokinetic profile. Fifth, this IVPT study was conducted using an average skin surface temperature of 32 °C; further testing with elevated temperature or in the presence of UVR could better mimic outdoor conditions. Moreover, this study utilized commercial static Franz diffusion cells to achieve the
required sink conditions for all hydrophobic UV filters. The reason for choosing a static diffusion system over a dynamic flow-through system such as PermeGear in-line cells was to maintain a detectable concentration of UV filters in the receptor solution by minimizing the receptor volume (SCCP, 2006). The challenge of using the static diffusion system with manual sampling is the lack of freedom to take samples compared with the flow-through system (with automated sampling capability). There were also reports about unstirred aqueous diffusion layers present in the receiver chambers of the Franz cells. Unstirred layers may lead to an underestimation in the actual extent of drug permeation (Miller and Kasting, 2012; Yousef et al., 2017). Therefore, a suitable receiver solution should be carefully selected to avoid unstirred aqueous layers by maintaining sink conditions (i.e., including 4% BSA in the receiver solution) and efficient stirring (i.e., at 600 r.p.m.). Finally, this study was designed as a pilot to determine rank-order trends; therefore, the design included only four chemical UV filters in marketed sunscreen products. Other UV filters with different physicochemical properties may be included in future studies with more sophisticated bioanalytical methods to overcome analytical challenges.

In conclusion, fit-for-purpose methodologies were used to evaluate the absorption potential and in vitro biopharmaceutical characteristics of various sunscreen products to predict product performance. The use of discriminatory IVPT accompanied with in vitro formulation characterization collectively provided the basis for the selection of products for a US Food and Drug Administration-sponsored study.
MUST clinical pilot study (Matta et al., 2019). The IVPT method described in this study may also be used for product selection during early development stages for further in vivo safety evaluation (Adamson and Shinkai, 2020). Most importantly, the study provided a rank-order reporting platform for IVPT results that can be further optimized to better enhance its predictability for in vivo absorption of the product.

MATERIALS AND METHODS
Description of materials and additional methods are in the Supplementary Materials and Methods.

Preparation of skin samples for permeation studies
Dermatomed human cadaver skin (from four female donors, 60–80 years of age, 250 µm average thigh-skinn thickness) was obtained from Science Care (Phoenix, AZ) with written informed consent (IRB review was not needed as the definition of human subject pertains to living individuals). Circular skin samples were punched out using a die cutter (18.5 mm in diameter) and gently cleaned with water. The individual skin thickness was measured using a caliper and recorded. The barrier integrity of the skin was tested by measuring transepidermal water loss using a vapometer (Delfin Technologies, Kuopio, Finland). Skin samples that were free from any visual physical damage and have a transepidermal water loss <10 g/cm²·h were used in IVPT (Benech-Kieffer et al., 1997).

In vitro skin permeation test
IVPT of various sunscreen products was carried out using vertical Franz diffusion systems (PermeGear, Hellertown, PA). Each jacketed Franz diffusion cell (15 mm orifice diameter, 1.77 cm² exposure area, 12 ml receiver volume) was placed on the stirrer and the skin surface temperature was maintained at 32 °C. The receiver chamber was filled with PBS containing 4% BSA (w/v) (Freitas et al., 2015) and stirred constantly at 600 r.p.m. Prepared circular skin samples were sandwiched between donor and receiver chambers with the stratum corneum facing the donor side. To obtain the maximum possible skin absorption, an infinite dose of 100 mg of sunscreen product (lotion or cream) was applied to the stratum corneum side of the skin. For spray products, the solution was first sprayed into a glass scintillation vial and then 120 µl (equivalent to 100 mg) of the solution was immediately applied to the skin surface (Matta et al., 2019). The experiment was carried out under nonocclusive conditions, whereas the entire system was protected from light using aluminum foil. Aliquot skin permeation samples (500 µl) were collected from the receiver chamber at 0, 3, 6, and 24 hours for analysis, and 500 µl of fresh receiver solution was replenished into the receiver chamber. Permeation results obtained from individual donors were summarized in Supplementary Tables S3–S8. Sunscreen ingredients retained in the skin samples were extracted for analysis using methanol. Skin retention results obtained from individual donors were summarized in Supplementary Tables S10–S12.

Data availability statement
Data sets related to this article can be found at https://doi.org/10.17632/hgj8kcygj2.1, an open-source online data repository hosted at Mendeley data.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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ACKNOWLEDGMENTS
The study was funded by the US Food and Drug Administration. The authors are grateful to all the working group members and collaborators within the Food and Drug Administration who participated in the discussion of this study and the review of this manuscript. Special thanks to Ahmed Zidan and Caroline Strasinger for their expert opinions and Murali K. Matta, Luke Oh, Vikram Patel, Robbert Zusterzeel, Edward (Dennis) Bashaw, and David G. Strauss for their feedback in the context of the pilot maximal usage conditions studies.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.04.009.

REFERENCES
Y Yang et al.

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