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ORIGINAL RESEARCH

Passive Enhancement of Retinol Skin Penetration by Jojoba Oil Measured Using the Skin Parallel Artificial Membrane Permeation Assay (Skin-PAMPA): A Pilot Study

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Introduction: Retinol is known to have positive benefits on the skin including enhancements in barrier function, increased epidermal thickness, reductions in fine lines and wrinkles and reductions in hyperpigmentation. Improved methods to enhance the penetration of retinol are desirable.

Methods: A study was conducted to examine if addition of natural jojoba (*Simmondsia chinensis*) oil might help passively enhance the penetration of retinol through the skin's lipid barrier. The model used to examine the passive penetration of the retinol is the skin parallel artificial membrane permeation assay (Skin-PAMPA). In this study, three formulations were examined. The formulations included two control blends: a moisturizing emulsion without retinol and the same product containing 1.0% retinol without jojoba oil. The remaining formulation contained similar concentrations of retinol with 10% jojoba oil. The studies were conducted by applying the products to the Skin-PAMPA models at 37°C/5% CO_2 for 16 hours and then extraction of the acceptor reservoir with cyclohexane (ratio 1:5 acceptor fluid to cyclohexane). The resulting acceptor reservoir cyclohexane solutions were analyzed for retinol by High Performance Liquid Chromatography (HPLC).

Results: The formulations without retinol showed no indications of retinol penetration by HPLC. The control formulation with 1.0% retinol demonstrated that retinol had permeated the membrane in the 16-hour timeframe with a measured Area Under the Curve (AUC) of 7 units. Analysis of the formulation containing 1.0% retinol and 10% jojoba oil indicated retinol had permeated with a AUC of 285 units, a nearly 40-fold increase in active retinol permeation.

Discussion: The ability for jojoba oil to directly act to help skin permeation of a key skin care active like retinol has not been previously demonstrated. This potential for jojoba oil to enhance passive skin penetration of critical skin actives, like retinol, can help to improve the performance of skin care products employing active topical ingredients.

Keywords: skin permeation, retinoids, HPLC, lipid barrier, *Simmondsia chinensis* oil

Introduction

Jojoba (*Simmondsia chinensis*) seed oil is unique among the many naturally occurring plant oils. Most typical seed oils present themselves as fatty acid triglycerides which are formed in the seeds in small, individually packaged droplets called lipid bodies or oleosomes.[1] The stored oil is used to help provide nutrition during germination of the seeds. Jojoba oil, however, is comprised of liquid waxes, not triglyceride oils but is typically referred to as an oil due to the liquid nature of the waxy esters.[2] The oil is typically isolated on commercial scales by cold pressing to produce virgin oil often followed by a second pressing to produce non-virgin grades.[3] Jojoba oil is also unique among vegetable oils because of its stability against oxidation, a trait not shared by most triglyceride oils. The jojoba plant is particularly interesting as it grows under quite arid conditions which means the plant grows abundantly without the need for large quantities of water.

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Jojoba oil has been popularly used for many years in skin and hair care applications and has been shown more recently to have skin benefits including wound healing, emollient, hydrating, anti-inflammatory, and anti-microbial benefits, among others.[4–7] The linear wax esters that comprise jojoba oil share a very profound similarity to wax esters known to exist in human sebum.[8] This compatibility with human sebaceous lipids is likely one reason jojoba oil has been so popularly used in topical skin preparations. In addition, the oil lends itself to various natural chemical modifications to provide fatty acids and fatty alcohols as well as the hydrogenated esters, acids and alcohols which tend to be higher melting solid waxes.[3] Jojoba waxes and the various derivatives have been recognized as safe for use in cosmetics by the Cosmetic Ingredient Review Board since 2008.[9]

Within the pantheon of skin care actives that have been proven efficacious, retinoids form one of the most popular ingredients used among consumers. Retinoic acid is a very powerful exfoliant with demonstrated antiaging, antimicrobial, and other homeostatic effects.[10] However, it is also a known teratogen, so its use is tightly controlled. The retinoic acid derivatives like retinaldehyde, retinol and various retinol esters are popular and safer, but less effective substitutes for retinoic acid, that have also been shown to improve skin. However, the use of retinol has been demonstrated to have a powerful skin dehydrating effect which can lead to skin dryness and potentially to skin irritation.[11] Recent work has demonstrated that jojoba oil, formulated as microemulsions, has the potential to enhance the penetration of various drugs through the skin.[12] In addition, previous work demonstrated that retinoids formulated in a soft colloidal nanocarrier system composed of jojoba oil could enhance the benefits of the retinoid in psoriatic patients.[13] In this application, skin penetration is not as difficult as the drug being studied, tazarotene, is being delivered to skin which has a disrupted barrier.

The study of skin penetration has evolved with the development of new models to examine the ability of various ingredients to accelerate or improve passive skin permeation of active molecules of interest.[14] Ideally, examining skin penetration is done clinically using skin biopsies or extensive tape stripping methods which allow examination of a particular skin active directly from the skin in which it has come into contact. These methods are expensive and quite time consuming and do not readily lend themselves to more rapid examination of multiple formulations or ingredients. A very popular method for investigating skin permeation is the use of Franz Cells which typically incorporate either a synthetic skin equivalent or actual cadaver skin maintained in a medium that keeps the skin moist. Franz Cell studies are considered the benchmark of in vitro test methods for non-clinical work. In 1998, Kansy et al reported on a new method to examine skin penetration called the parallel artificial membrane permeability assay or PAMPA for short.[15] The method employs the use of a synthetic polymer membrane surface treated with a hydrophobic coating that closely mimics the skin lipid barrier.[16–23] More importantly, unlike the single chamber Franz Cell, the PAMPA method allows for studies to be conducted in 96-well plates, like those used to examine living cells and skin mimics. The method can be adapted depending on the selection of the synthetic membrane and the hydrophobic coating.[20] Most current commercially available assays employ polyvinylidenefluoride (PVDF) coated with phospholipids.[21] The method is designed to specifically measure passive transdermal delivery of molecules. Studies have examined closely how the PAMPA assay provides comparable data to the skin penetration data demonstrated from Franz Cell studies.[18,22] In the following studies, the Skin-PAMPA was used to examine the influence of jojoba oil to enhance the skin permeation of retinol from various moisturizing emulsion formulations. To the best of our knowledge, this is the first time that the Skin-PAMPA has been used to examine transdermal retinol penetration.

Materials and Methods

Retinol Formulations

A summary of the formulas examined in the study is shown below in Table 1. Formulation 1 is a control formulation that comprises 10% jojoba oil without added retinol. Formulation 2 is a retinol test formulation that contains retinol without added jojoba oil. The formulation also contains 9% soybean oil as well that was added with the retinol. Formulation 3 is the second retinol test formulation containing 10% jojoba oil and 9% soybean oil.

Skin-PAMPA

Transdermal penetration was approximated with the Parallel Artificial Membrane Permeation Assay (PAMPA) using MultiScreen Filter/Receiver Plates from Millipore-Sigma (cat. MAIPNTR10 and MATRNPS50). This assay is designed to

Table 1 Summary of Formulations

Ingredient	Formulation 1	Formulation 2	Formulation 3
DI Water	80.95	80.95	70.95
Disodium EDTA	0.05	0.05	0.05
Glycerin	0.90	0.90	0.90
Phospholipids	0.10	0.10	0.10
Potassium Cetyl Phosphate	2.50	2.50	2.50
Cetyl Alcohol	2.00	2.00	2.00
Glyceryl Stearate	2.50	2.50	2.50
Preservative	1.00	1.00	1.00
Simmondsia Chinensis (Jojoba) Seed Oil	10.00	–	10.00
Soybean Oil	–	9.00	9.00
Retinol	–	1.00	1.00
Totals	**100**	**100**	**100**

measure the permeability of a substance from a donor compartment through a phospholipid-coated PVDF filter into an acceptor compartment, Figure 1. Because the membrane has no transporters or efflux systems, only passive permeability is observed.

The method is based on the application note "Evaluation of the Reproducibility of Parallel Artificial Membrane Permeation Assays (PAMPA)" by Millipore-Sigma Corporation.[24] Briefly, it consists of applying 150µL of a test material on a filter immediately after preconditioning it with 5µL of 1% (w:v) L-α-phosphatidylcholine (a natural phospholipid, Sigma, cat.# 3644). The incubation with test materials is pursued in the tissue culture incubator at 37°C/5%CO_2 for 16h on top of a receiver chamber (acceptor compartment) filled with 300µL of 5% DMSO in PBS. Each sample was run in duplicate to establish significance.

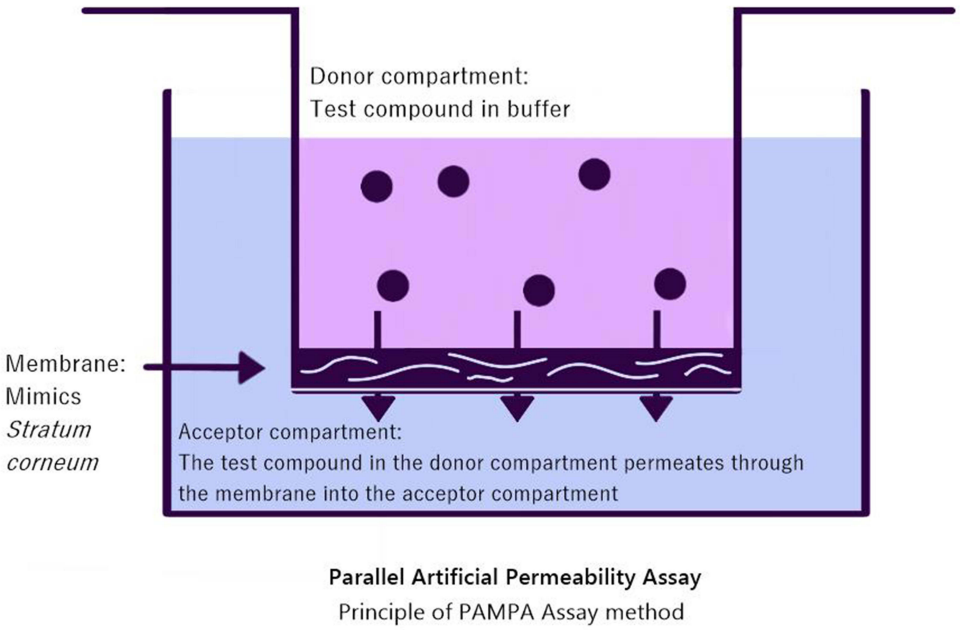

Figure 1 Schematic image of a single PAMPA permeation compartment showing the donor and acceptor sites and the lipophilic membrane designed to mimic the human stratum corneum.

Retinol HPLC Assay

At the end of the experiment, 50µL from each acceptor compartment are extracted in cyclohexane at a 1:5 ratio and one µL is injected to the HPLC system. The chromatographic profile of the injected sample is then compared to one of a 10µL retinol control formulation, Formulation 2, containing just retinol dissolved in 300µL of the receiver chamber solution and extracted with cyclohexane. All samples were analyzed using Agilent HPLC series 1100 (Agilent Technologies, Palo Alto, CA) station equipped with the series 1100 degasser, autosampler G1313A, Zorbax SB-C8 reverse phase column protected by Javelin direct-connection column filter, diode array UV/Vis detector and ChemStation software, at 325nm (A325nm). Ethanol:Methanol:Ethyl Acetate (2:2:1) was used as mobile phase.

Calculations

The amount of retinol that elutes into the acceptor compartment was determined by the area under the curve (AUC) calculated automatically by the Agilent's HPLC software (ChemStation) expressed in units. Statistical significance was calculated using double tail *t*-test and the threshold was established at $p \leq 0.05$.

Results

Chromatography Results: Non-Eluted Control Sample Formulation 3 with Retinol

The chromatographic spectrum for donor chamber control sample Formulation 2 containing retinol is shown below in Figure 2. The retinol elutes at approximately 9.6 minutes confirming that retinol can be examined under the chromatographic conditions after extraction with cyclohexane.

Chromatographic Results: Eluted Control Formulation 1 Samples Without Retinol

The chromatographic spectrum for control sample Formulation 1 which does not contain retinol after 16h permeation is shown below in Figure 3. The spectrum confirms that samples without retinol do not show the retinol peak at 9.6 minutes.

Chromatographic Results: Eluted Sample Formulation 2 with Retinol Without Jojoba Oil

Figure 4 demonstrates that the moisturizing formulation with 1.0% retinol, Formulation 2, without added jojoba oil after 16h permeation demonstrates approximately a 0.5% enrichment in retinol in the acceptor reservoir after cyclohexane extraction.

Chromatographic Results: Eluted Formulation 3 Sample with Retinol and Jojoba Oil

Figure 5 demonstrates the chromatographic spectrum of Formulation 3 after 16h permeation containing 1.0% retinol and jojoba oil at 10%.

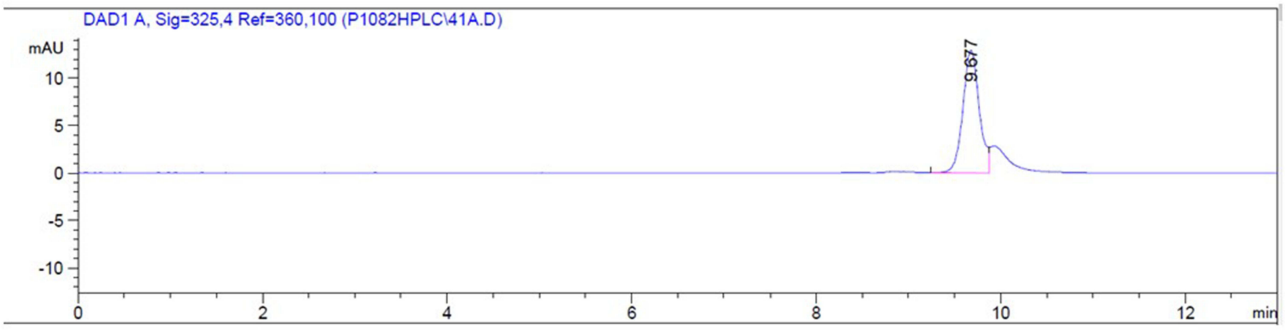

Figure 2 HPLC elution profile of sample, Formulation 2, (10µL) mixed with 300µL of 5% DMSO/PBS and extracted 1:5 with cyclohexane, measured at 325nm.

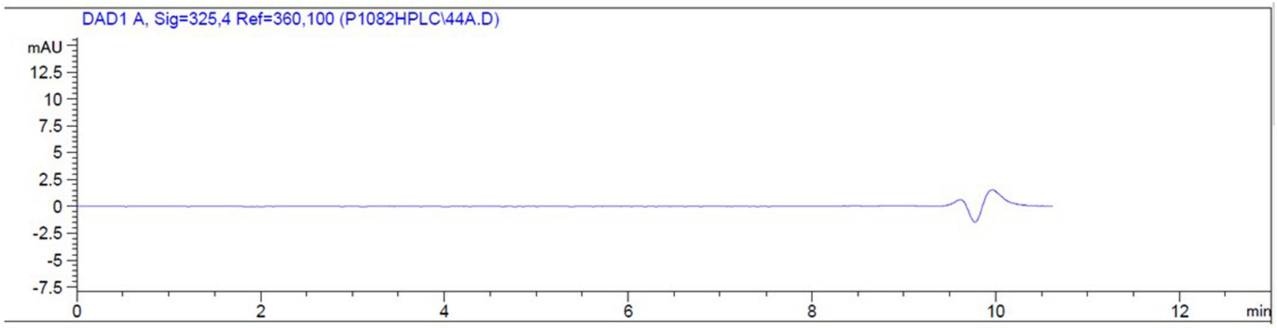

Figure 3 HPLC elution profile of Formulation 1 without retinol sample from the acceptor chamber extracted 1:5 with cyclohexane, measured at 325nm.

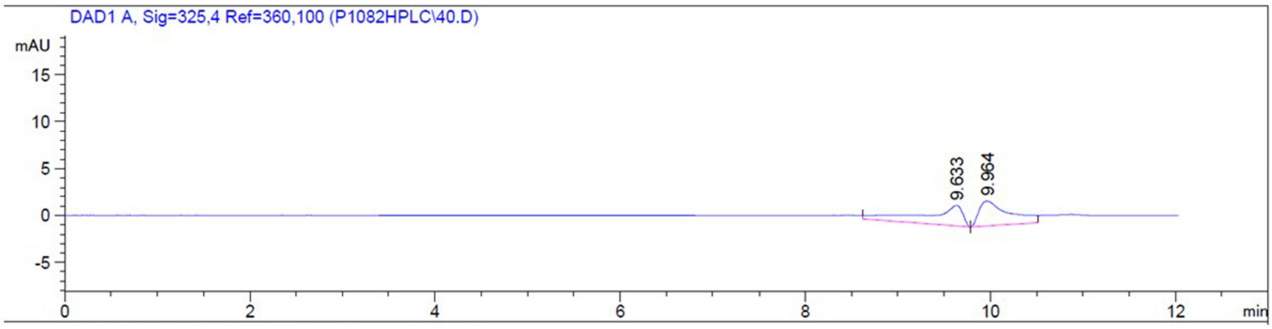

Figure 4 HPLC elution profile of the sample Formulation 2 from the acceptor chamber extracted 1:5 with cyclohexane, measured at 325nm.

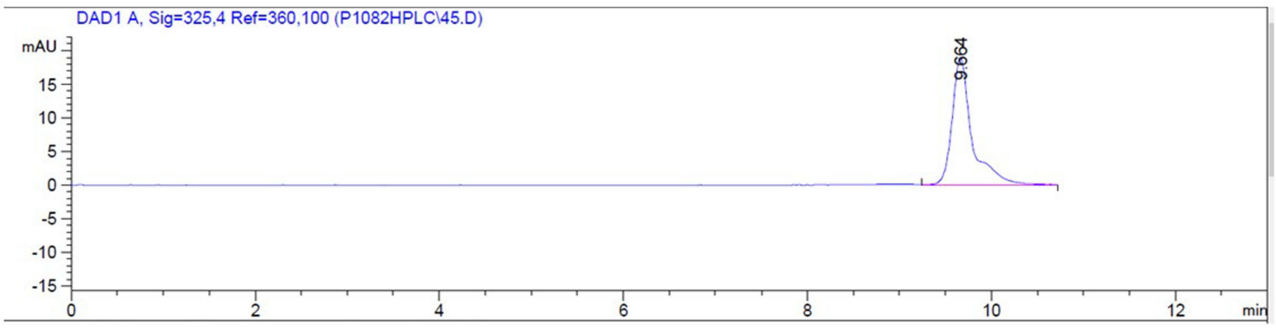

Figure 5 HPLC profile of Formulation 3 with 1.0% Retinol and 10% jojoba oil sample from the acceptor chamber extracted 1:5 with cyclohexane, measured at 325nm.

Comparison of All Samples Eluted with the PAMPA

Figure 6 shows the Area Under the Curve (AUC) data for the three samples that permeated through the PAMPA membrane were eluted in the PAMPA. The data, based on the chromatographic spectra (Figures 2–4), indicate that the addition of jojoba oil to the sample formulation containing retinol increased the AUC from 7 units (no jojoba oil, Figure 3) to 285 units (jojoba oil, Figure 4), a nearly 40-fold increase in permeation of the retinol because of inclusion of the jojoba oil at 10%. In addition, Formulation 2 contained added soybean oil which also was present in Formulation 3 at comparable levels. However, it was found that Formulation 2 did not provide the enhanced benefits in skin permeation noted for Formulation 3 that contained both jojoba oil and soybean oil. This suggests that the sebaceous lipid mimicking nature of jojoba oil may be a principal reason the jojoba oil works superior to triglyceride oils to enhance penetration of lipophilic active ingredients like retinol.

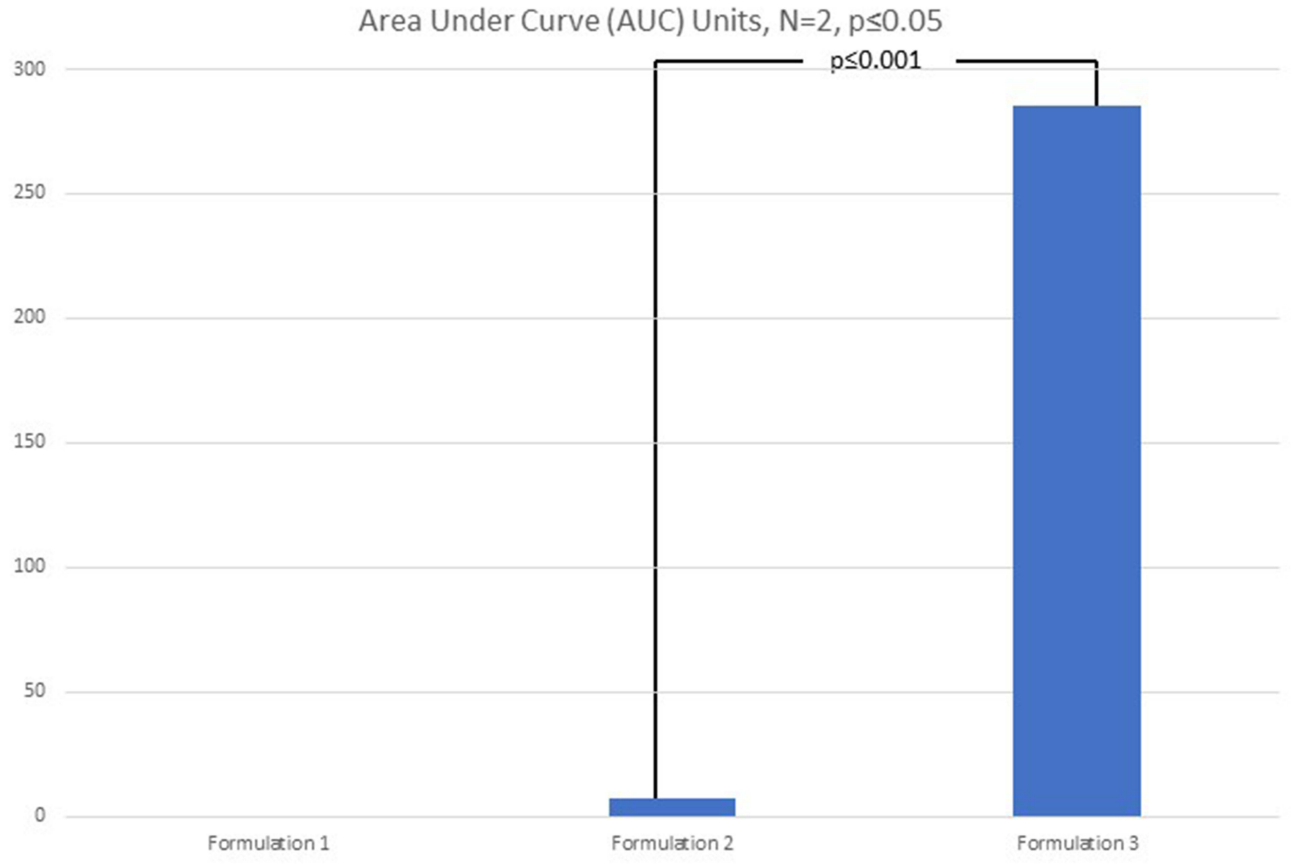

Figure 6 Graph of Area Under the Curve (AUC) units for Formulations 1–3 eluted through the PAMPA. Formulation 1 contains 10% jojoba oil without retinol and serves as a background control. Formulation 2 contains retinol without jojoba oil. Formulation 3 contains retinol and 10% jojoba oil. The data demonstrates that the addition of 10% jojoba oil to the formulation increases retinol permeation via the PAMPA by approximately 40-fold.

Discussion

Retinol is one of the most well-known topical skin care active ingredients. However, as was recently reported, retinol can have a dehydrating effect on the skin which, over time, may lead to increased skin erythema and flaking.[11] Technologies that enhance the skin permeability of retinol may allow formulators who are employing retinol to lower the active levels of the molecule which can help to mitigate the skin dehydrating effects. Methods to help improve the overall efficacy and safety of retinoid use can include, for example, encapsulation or incorporation of the retinoids into unique emulsion structures such as micro- or nanoemulsions.[25,26] In addition, retinol and retinol derivative permeation has been shown to improve when the molecule is delivered to the skin using occlusive barriers.[27]

There are various mechanisms to increase the passive diffusion of skin care actives through the skin.[28] The most effective is to disrupt the skin's lipid barrier to allow molecules to move through the stratum corneum more efficiently. One molecule that does this very well is dimethylsulfoxide (DMSO). DMSO can penetrate the skin's lipid barrier very quickly as DMSO itself has been promoted as a topical analgesic.[29] DMSO will carry anything dissolved into it essentially systemically to the blood stream when applied topically. Such aggressive skin penetration is not particularly desirable when ingredients are intended to only influence the stratum corneum and upper epidermal layers of the skin.

Jojoba oil wax esters share very common similarity to the skin's sebaceous lipids as was noted earlier.[8] Jojoba oil enhances the passive penetration of lipophilic active ingredients allowing for improved penetration of solubilized actives.[7] This likely occurs because the oil can increase the fluidity of the lipid barrier of the skin which can create small channels that allow lipophilic actives dissolved in the oil, like retinol, to passively pass through the skin's lipid barrier. The use of the Parallel Artificial Membrane Permeation Assay (PAMPA) to examine retinol penetration has not been reported previously. The

PAMPA is a particularly rapid and effective testing methodology for examining skin penetration enhancers that work by lipid barrier disruption. The PVDF membrane of the assay is coated with phosphatidylcholine prior to commencement of the assay. This creates a barrier that can closely mimic the functional properties of the skin's lipid barrier.[24] The testing method focuses on the influence of permeation enhancers to function through passive lipid barrier disruption mechanisms and may limit ingredients that do not function through such lipophilic disruption mechanisms. PAMPA would not be effective for skin permeation that might use active transport systems such as transmembrane transporters, channels, and pumps.

Conclusions

In the present study it was found that addition of 10% jojoba oil to a moisturizing formulation containing retinol was able to effectively increase the permeability of the retinol when examined using the skin-PAMPA. The increase in retinol permeation was nearly 40-fold compared to a similar formulation without jojoba oil. The findings demonstrate for retinol previously reported benefits of jojoba oil in transdermal delivery.[12,13] The demonstrated use of the PAMPA to examine retinol skin permeation suggests the test method may be useful for examining other lipophilic topical skin care actives to improve passive skin permeation rapidly and accurately.

Ethical Approvals

No humans were tested in the work reported here.

Acknowledgments

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article, gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sturtevant D, Lu S, Zhou ZW, et al. The genome of jojoba (*Simmondsia chinensis*): a taxonomically isolated species that directs wax ester accumulation in its seeds. *Sci Adv*. 2020;6:eaay3240. doi:10.1126/sciadv.aay3240
2. El-Mallah MH, El-Shami SM. Investigation of liquid wax components of Egyptian Jojoba seeds. *J Oleo Sci*. 2009;58:543–548. doi:10.5650/jos.58.543
3. Wisniak J. The chemistry and technology of Jojoba oil: state of the art. Sixth International Conference on Jojoba and Its Uses. 1984:311–322.
4. Ranzato E, Martinotti S, Burlando B. Wound healing properties of jojoba liquid wax: an in vitro study. *J Ethnopharmacol*. 2011;134:443–449. doi:10.1016/j.jep.2010.12.042
5. Tietel Z, Kahremany S, Cohen G, Ogen-Shtern N. Medicinal properties of jojoba (*Simmondsia chinensis*). *Israel J Plant Sci*. 2021;68:38–47. doi:10.1163/22238980-bja10023
6. Blaak J, Staib P. An updated review on efficacy and benefits of sweet almond, evening primrose and jojoba oils in skin care applications. *Int J Cosmet Sci*. 2022;44:1–9. doi:10.1111/ics.12758
7. Gad HA, Roberts A, Hamzi SH, et al. Jojoba oil: an updated comprehensive review on chemistry, pharmaceutical uses, and toxicity. *Polymers*. 2021;13:1711. doi:10.3390/polym13111711
8. Wertz PW. Human synthetic sebum formulation and stability under conditions of use and storage. *Int J Cosmet Sci*. 2009;31:21–25. doi:10.1111/j.1468-2494.2008.00468.x
9. Becker L. *Safety Assessment of Simmondsia Chinensis (Jojoba) Seed Oil, Simmondsia Chinensis (Jojoba) Seed Wax, Hydrogenated Jojoba Oil, Hydrolyzed Jojoba Esters, Isomerized Jojoba Oil, Jojoba Esters, Simmondsia Chinensis (Jojoba) Butter, Jojoba Alcohol, and Synthetic Jojoba Oil*. Washington, DC: Cosmetic Ingredient Review; 2008.
10. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy. *Clin Interv Aging*. 2006;1:327–348. doi:10.2147/ciia.2006.1.4.327
11. Gruber JV, Stojkoska V, Riemer J. Retinol has a skin dehydrating effect that can be improved by a mixture of water-soluble polysaccharides. *Cosmetics*. 2020;7:80. doi:10.3390/cosmetics7040080

12. Assaf SM, Maaroof KT, Altaani BM, Ghareeb MM, Alhayyal AAA. Jojoba oil-based microemulsion for transdermal drug delivery. *Res Pharma Sci*. 2021;16:326–340. doi:10.4103/1735-5362.319572
13. Nasr M, Abdel-Hamid S, Moftah NH, Fadel M, Alyoussef AA. Jojoba oil soft colloidal nanocarrier of a synthetic retinoid: preparation, characterization, and clinical efficacy in psoriatic patients. *Curr Drug Deliv*. 2017;14:426–432. doi:10.2174/1567201813666160513132321
14. Rapalli VK, Mahmood A, Waghule T, et al. Revisiting techniques to evaluate drug permeation through skin. *Expert Opin Drug Deliv*. 2021;18:1829–1842. doi:10.1080/17425247.2021.2010702
15. Kansy M, Senner F, Gubernator K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *J Med Chem*. 1998;41:1007–1010. doi:10.1021/jm970530e
16. Ottaviani G, Martel S, Carrupt PA. Parallel artificial membrane permeability assay: a new membrane for the fast prediction of passive human skin permeability. *J Med Chem*. 2006;49:3948–3954.
17. Masungi C, Mensch J, Dijck AV, et al. Parallel artificial membrane permeability assay (PAMPA) combined with a 10-day multiscreen Caco-2 cell culture as a tool for assessing new drug candidates. *Pharmazie*. 2008;63:194–199.
18. Balazs B, Vizeralek G, Berko S, et al. Investigation of the efficacy of transdermal penetration enhancers through the use of human skin and a skin mimic artificial membrane. *J Pharm Sci*. 2016;105:1134–1140. doi:10.1016/S0022-3549(15)00172-0
19. Sinko B, Garrigues TM, Balogh GT, et al. Skin-PAMPA: a new method for fast prediction of skin penetration. *Eur J Pharm Sci*. 2012;45:698–707. doi:10.1016/j.ejps.2012.01.011
20. Fontananova E, Bahattab MA, Aljilil SA, et al. From hydrophobic to hydrophilic polyvinylidenefluoride (PVDF) membranes by gaining new insight into material's property. *RSC Adv*. 2015;5:56219–56231. doi:10.1039/C5RA08388E
21. Yu H, Wang Q, Sun Y, Shen M, Li H, Duan Y. A new PAMPA model proposed on the basis of a synthetic phospholipid membrane. *PLoS One*. 2015;10:e0116502. doi:10.1371/journal.pone.0116502
22. Sinko B, Vizeralek G, Takacs-Novak K. Skin PAMPA: application to practice. *ADMET DMPK*. 2014;2:191–198.
23. Sinko B, Bardos V, Vesztergombi D, et al. Use of an in vitro skin parallel artificial membrane assay (Skin-PAMPA) as a screening tool to compare transdermal permeability of model compound 4-phenylethyl-resorcinol dissolved in different solvents. *Pharmaceutics*. 2021;13:1758. doi:10.3390/pharmaceutics13111758
24. Evaluation of the Reproducibility of Parallel Artifical Membrane Permeation Assays (PAMPA). Available from: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/pharmacology-and-drug-discovery-research/evaluation-of-the-reproducibility-of-pampa. Accessed January 30, 2023.
25. Jankowski A, Dyja R, Saarecka-Hujar B. Dermal and transdermal delivery of active substances from semisolid bases. *Ind J Pharm Sci*. 2017;79:488–500. doi:10.4172/pharmaceutical-sciences.1000255
26. Essendoubi M, Alsamad F, Noel P, et al. Combining Raman imaging and MCR-ALS analysis for monitoring retinol permeation in human skin. *Skin Res Tech*. 2021;27:1100–1109. doi:10.1111/srt.13069
27. Duell EA, Kang S, Voorhees JJ. Unoccluded retinol penetrates human skin in vivo more effectively than unoccluded retinyl palmitate or retinoic acid. *J Invest Dermatol*. 1997;109:301–305. doi:10.1111/1523-1747.ep12335788
28. Ita KB. Chemical penetration enhancers for transdermal drug delivery – success and challenges. *Curr Drug Deliv*. 2015;12:645–651. doi:10.2174/1567201812666150804104600
29. Wolff DG, Christophersen C, Brown SM, Mulcahey MK. Topical nonsteroidal anti-inflammatory drugs in the treatment of knee osteoarthritis: a systematic review and meta-analysis. *Phys Sportsmed*. 2021;49:381–391. doi:10.1080/00913847.2021.1886573