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Dimethyl sulfoxide in topical pharmaceutical drug development: A fresh perspective

Nubul Albayati[a], Nigel Langley[b*], Hana Mohd[a], Bozena Michniak-Kohn[a], David W. Osborne[c]

[a]Ernest Mario School of Pharmacy, Center for Dermal Research (CDR) & Laboratory for Drug Delivery (LDD) Life Sciences Building, Rutgers-The State University of New Jersey, 145, Bevier Road, Piscataway, NJ 08854
[b]Gaylord Chemical LLC, 1404 Greengate Drive, Suite 100, Covington, NJ 07661
[c]Arcutis Biotherapeutics, Inc., 3027 Townsgate Road, Suite 300, Westlake Village CA 91361

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ABSTRACT

Dimethyl Sulfoxide (DMSO) has been approved as a pharmaceutical active ingredient, is used as the industry standard biological tissue cryoprotectant and is a pharmaceutical excipient that enhances the transdermal permeation of various drugs. The utility of pharmaceutical-grade DMSO as a skin penetration enhancer will be examined based on its physical properties, mode of action and concentration dependent characteristics. Since DMSO has been shown to have anti-inflammatory and analgesic activity, when used as a topical pharmaceutical excipient, DMSO cannot be viewed as a clinically inert, inactive ingredient for certain localized skin conditions. The toxicology and elimination kinetics of DMSO will be discussed in relation to using higher purity DMSO to diminish the incidence of garlic-like or oyster-like breath in patients after applying DMSO to the skin. The use of pharmaceutical grade DMSO having > 99.99% purity provides a fresh perspective in developing topical formulations. With its impurity profile being from 1000 PPM in standard USP-NF grades (99.9% purity) to <100 PPM (99.99% purity).

KEY WORDS: Dimethyl sulfoxide, DMSO, skin penetration enhancer, anti-inflammatory, analgesic

INTRODUCTION

Dimethyl Sulfoxide (DMSO) is a polar aprotic, non-mutagenic solvent, with a boiling point of 189°C. It is miscible with water and is an excellent solvent and solubilizer for both hydrophilic and hydrophobic (lipophilic) active pharmaceutical ingredients (APIs). DMSO is an API for interstitial cystitis (RIMSO-50 – 50% DMSO; 50% water; FDA approved in 2002), the industry-standard biological tissue cryoprotectant

*Corresponding address: Gaylord Chemical LLC, 1404 Greengate Drive, Suite 100, Covington, NJ 07661, Tel:, E-Mail: nlangley@gaylordchem.com

(sterile grade) and a topical pharmaceutical excipient used in FDA approved products (45.5 w/w% maximum potency per unit dose and an 8,008 mg maximum daily exposure for topical application). DMSO is both an anti-inflammatory and analgesic agent that is a highly effective skin penetration enhancer when applied topically.

This paper will review the utility of pharmaceutical-grade DMSO in topical drug development and will examine its physical properties, characteristics and mode of action as a skin penetration enhancer. Since DMSO has been shown to have anti-inflammatory and analgesic activity, a product development scientist must be aware of how using DMSO as an excipient in a topical formulation may increase the efficacy in the vehicle arm of blinded, vehicle-controlled clinical trials. Formulations containing DMSO for the treatment of inflammatory skin conditions or localized joint pain may require carefully designed Phase 2 clinical studies to adequately power pivotal trials that statistically separate active versus vehicle for the primary endpoints. For this reason, the first section of this paper will briefly review the literature from the perspective of DMSO as an anti-inflammatory and analgesic agent. Next, the mechanism by which DMSO enhances skin penetration will be summarized in terms of the lipid-protein partitioning concept of drug delivery across the intercellular path of the stratum corneum. This mechanistic overview will be followed by a comprehensive review of the literature describing DMSO as a skin penetration enhancer.

Finally, the toxicology and pharmacokinetics/pharmacodynamics of topically applied DMSO will be reviewed. Animal toxicology studies of DMSO have been conducted over the last six decades using highly varying purities of DMSO. Only one commercially available pharmaceutical grade of DMSO (Procipient®(Dimethyl Sulfoxide USP, PhEur) is sold as both an API and pharmaceutical excipient) is manufactured under ICH Q7 Current Good Manufacturing Practices (cGMP) with a Type 2 Drug Master File. The minimal purity of Procipient(r) at 99.99% exceeds the USP-NF monograph and is anhydrous, which may chemically stabilize actives that readily hydrolyze. Other USP-NF, non-GMP DMSO grades have a distinctive odor that is due to trace amounts of thiochemical impurities, namely dimethyl sulfide, dimethyl disulfide and bis-(methythio)methane. In addition, there may also be trace amounts of formaldehyde, oxidation products and water. If a non-pharmaceutical grade of DMSO is used for topical application, then the impurities in the DMSO will also penetrate the dosed skin. When present in the test articles of earlier reported studies, these readily absorbed impurities possibly contributed to the incidence and severity of halitosis after dosing with DMSO. It is proposed that use of DMSO having >99.99% purity may diminish the incidence of garlic-like or oyster-like breath in patients after using topical products containing DMSO.

DMSO AS AN ANTI-INFLAMMATORY AGENT

Dimethyl Sulfoxide (DMSO) exhibits notable anti-inflammatory properties and is often used in biological research due to its capacity to easily cross cellular membranes (1, 2). Numerous publications underscore its efficacy in reducing inflammation in several conditions, such as improving skin flap necrosis, reconstructive skin surgery, cancer, arthritis and wound healing. Furthermore, DMSO demonstrates utility in local cooling post-chemotherapy and in preventing tissue necrosis and ulceration (3, 4, 9, 10). Elisia *et al.*, conducted a study and examined the effects of DMSO *in vivo* through topical application to the models of mice having rheumatoid arthritis and human melanoma. Specifically, they observed that the use of concentrations of DMSO from 0.5% to 2% significantly reduced the expression of numerous pro-inflammatory cytokines, prostaglandin E2 (PGE2) and chemokines. This finding substantiates the anti-inflammatory properties of DMSO, through reduced levels of pro-inflammatory cytokines in the joints mainly represented by suppressed E. coli-induced ERK1/2, p38, JNK, and Akt phosphorylation, and reduction in the white blood cell levels as well (5).

According to a study carried out in 2020 by Guo *et al.,* DMSO can effectively work in wound and tissue repair. The study showed that a low dose of DMSO can have

a wound-healing effect on diabetic mice. The results of the study demonstrated that low DSMO concentration particularly 5 mM resulted in accelerated healing through cell proliferation and migration mediated by Akt/mTOR. Higher concentrations (20 mM) resulted in wound delay (6).

Another study that supports the role of DMSO in wound healing is by Kant *et al.*, where they investigated the impact of the topical application of 10% DMSO on wound healing in male rats. DMSO was found to accelerate wound closure on days seven and twelve but delayed closure on the 21st day compared to the control group. Histopathological analysis revealed differences in epithelialization, collagen deposition, and inflammatory cell infiltration between the groups, suggesting that while DMSO facilitates early-stage wound closure, it hinders later stages of wound healing (7).

In a study by Parra-Marquez *et al.*, the role of topically administered DMSO gel (90%) in countering skin flap necrosis was examined in rats. The authors noted from their data, that there was a difference between oral as well as injected and topical DMSO applications. The systemic impact of the oral and injectable led to a notable reduction in pro-inflammatory factors such as PGE2, IL-2, IL-8, NF-KB, TNF-α, and IFNγ. The study suggests that the topical DMSO application has comparatively fewer anti-inflammatory effects compared to oral or injectable administration (8).

The effect of DMSO used with a topical ascorbic acid solution for treating basal cell carcinoma (BCC) was investigated by Burke and Bailie in 2022. The research demonstrated that there were indirect anti-inflammatory effects of DMSO that reduce the lesion resolution time in comparison to when there is no DMSO present. The authors also showed that DMSO was safe in a patient that was exposed to small amounts of DMSO 400–600 ul per day (2).

The use of topical DMSO, when combined with local cooling post-chemotherapy, turns out to be a safe and effective solution, preventing tissue necrosis and ulceration (11). When applied topically to rabbits, a DMSO solution did not affect limb swelling, although it reduced ankle stiffness by 41% compared to the control group. The proposed mechanism for decreased joint stiffness included oxygen free-radical scavenging and inhibition of fibroblast proliferation (12). Research in plastic and reconstructive surgery shows that DMSO can lessen postoperative pain and inflammation, highlighting its potential to enhance patient comfort and recovery after skin surgeries (13, 10).

Another article on the use of DMSO topically for wound healing and its anti-inflammatory properties delves into how dermatological formulations containing DMSO can mitigate pain and expedite recovery (9). The findings in the literature suggest that the use of DMSO in concentrations below 50 percent, whether applied through rubbing or spraying, appeared to be safe. However, concentrations more than 50 percent are reported to have more risk of the known complications which are a garlic breath odor, skin irritation, burning sensation of the skin, urticarial reaction-based blister. Furthermore, in concentrations less than 50 percent, DMSO shows positive effects on wound healing and inflammation, along with providing an analgesic effect.

Overall, DMSO looks to be a promising inflammatory disease treatment in numerous investigations. Using Dimethyl Sulfoxide with additional medications may give a synergistic effect as an anti-inflammatory.

DMSO AS AN ANALGESIC AGENT

Papers discussing the analgesic effect of topically applied dimethyl sulfoxide indicate the potential relief of a variety of painful skin diseases as well as deeper tissue pain. These references describe the mechanisms of DMSO as an analgesic in dermatology and discuss the mechanism of clinical action, especially for rheumatic diseases such as osteoarthritis (1), and neuralgia as well as musculoskeletal pain (4).

Hanna, Fraunfelder, and Meyer (14) examined topical DMSO's effects on ocular inflammation. Their findings, show that DMSO reduces eye pain and

inflammation, indirectly supporting its potential use as a dermatological analgesic, leveraging similar anti-inflammatory properties to alleviate skin inflammation. A study by Hollebeeck S. *et al.*, concluded the activation of the Reduced universal transcription factor NF-κB, which administers immune response gene expression and apoptosis in combination with reduced expression and secretion of mRNA of proinflammatory mediators such as IL-1, has been exhibited due to the anti-inflammatory properties of the DMSO (15).

In the paper by Huang S. *et al.*, "Immunomodulatory effects and potential clinical applications of Dimethyl Sulfoxide" (16), the link between DMSO's immunomodulatory and analgesic properties is explored. DMSO's ability to modulate immune responses can be particularly beneficial in alleviating pain associated with immune-mediated skin disorders, such as psoriasis and eczema.

The study on DMSO's combined use with antimicrobials suggests potential benefits beyond targeting bacterial infections, including relief from discomfort caused by wound infection. By reducing infection-induced inflammation, DMSO may contribute to alleviating associated pain (17).

Overall, these findings position DMSO as a multifunctional agent capable of providing pain relief.

DMSO AS A SKIN PENETRATION ENHANCER

DMSO's applications extend to pharmaceutical drug formulations, where it enhances the solubility of both polar and nonpolar molecules (18). DMSO is miscible with the topical excipients water, ethanol, isopropyl alcohol and ethyl acetate. Its remarkable ability to penetrate skin and cell membranes has led to its adoption as a carrier for the delivery of pharmaceuticals into biological systems (19). Without the use of chemical penetration enhancers (CPEs) such as DMSO, only a limited number of drug actives have a sufficient rate of penetration across the stratum corneum (SC) to reach therapeutical concentrations in the viable epidermis.

There are multiple steps required for a topically applied, molecularly dispersed (dissolved) drug to be delivered into the viable epidermis. First, the active partitions between the corneocytes of the SC to diffuse through the lipid-rich intercellular spaces and then partitions out of the SC and into the viable epidermis. Success in partitioning into the SC is dependent on the octanol-water partition coefficient (log P) being 2 or higher. Success in adequately diffusing through the SC is dependent on the molecular dimensions of the active (traditionally approximated by molecular weight) being as small as possible. A molecular weight (MW) of less than 500 (known as the "500 Dalton rule" (20)) is typical for a drug to cross the SC of normal healthy skin. Success in partitioning out of the SC requires reasonable water solubility which can also be predicted by the log P. Once a molecule has a log P > 6 (solubility of the drug being one million times greater in octanol than in water), the drug tends to remain in the SC and never partitions out into the viable epidermis. In general, an active ingredient having a log P = 2-4 is good for percutaneous absorption with a log P = 3 being the ideal balance of a molecule being able to partition into and out of the SC.

The lipid-protein partitioning (LPP) concept (21, 22) mechanistically describes how chemical penetration enhancers (CPEs) facilitate delivery through the intercellular pathway by categorizing enhancers as lipid modifiers, protein modifiers and partitioning promoters. The LPP concept considers the stratum corneum (SC) to be the primary barrier to percutaneous absorption and that the intercellular pathway (the bilayer structured lipid located between the corneocytes) is the main penetration pathway for drugs. In healthy skin, the blend of ceramides, cholesterol and free fatty acids tightly pack to form a stiff bilayer configuration having an orthorhombic lateral arrangement of the polar headgroups (23, 24).

Alkyl chain fluidization

Long chained CPEs that are less polar than DMSO (such as oleic acid and isopropyl myristate) insert between the hydrophobic alkyl chains of the intercellular lipids, disrupting the packing to fluidize the bilayers and increase drug diffusion through the

SC. DMSO has not been shown to influence epidermal lipid alkyl chain organization (25).

Polar head group disorganization

DMSO modifies lipid order by interacting with the polar head groups of intercellular lipids and modifying the hydrogen bonds of ceramides (25). These interactions compete with water-mediated intermolecular hydrogen bonding and ionic forces (26) to disturb the head group domain, disordering the bilayer structure and increasing drug diffusion through the SC. Molecular simulation studies showed that DMSO accumulates in the head group region and weakens the lateral forces between ceramide molecules to shift the intercellular lipids to a more fluid configuration (27).

Keratinocyte protein modification

DMSO has also been shown to be a protein modifier that binds with the keratin filaments in the corneocyte to cause conformational changes in the proteins and form vacuoles (28, 29). Thus, intracellular (transport through the corneocyte) permeation of the drug is increased. Since most drugs are transported by the intercellular pathway, DMSO's disordering of the headgroup region of intercellular lipids presumably contributes more to increasing drug diffusion than DMSO's ability to disrupt corneocyte proteins.

Epidermal lipid partition promotion

The third LPP enhancement mechanism describes solvents that interact with the aqueous domain of the lipid bilayers to increase the solubility of the drug within the intercellular path (30, 31). Perhaps the best-characterized partition promoter solvents are ethanol and propylene glycol. These solvents penetrate into the SC and change the solubility parameter of the SC to more closely match the solubility parameter of the drug. This leads to an enhanced partitioning of the drug from the vehicle into the SC. Partitioning promoters inevitably are water-miscible solvents that readily dissolve lipids and have low molecular weights (MW ethanol=46 and propylene glycol (PG)=76). These physical chemical properties result in partition promoters rapidly 1) penetrating into, 2) diffusing through and 3) partitioning out of the intercellular pathway into the viable epidermis. Significant and lasting compromise of the skin barrier can be maintained when partitioning promoters are synergistically used with alkyl chain lipid modifiers such as combining PG and oleic acid. DMSO has a low molecular weight (MW=78), is water miscible, can readily dissolve lipids and has been described as a partitioning promoter (32).

It should be noted that unlike other hydrophilic enhancers, the DMSO-water system is well-known for exhibiting a strongly nonideal mixing behavior (33). DMSO-water mixtures exhibit a maximum in "excess" properties that have been found in viscosity, density, ΔH_{mix}, surface tension, dielectric constant and ESR splitting constant at a 1:2 mole ratio (34). The formation of two waters with one DMSO molecule to form molecular aggregates is considered the reason DMSO is such a good cryoprotectant (35). Over the water:DMSO mole fraction ratio of 2:1 to 1:1, DMSO-water hydrogen bonding is stronger than water-water hydrogen bonding (36).

For completeness, two often repeated disadvantages of formulating with DMSO need to be discussed in terms of clinically relevant dosing of human patients being treated topically with formulations containing not more than 45.5 w/w % DMSO (FDA IID maximum use limit for approved topical products). First is that DMSO is known to extract lipids from the SC (37) and that this CPE mechanism causes dry, irritated skin. For finite doses (approximately 3-5 mg product per square cm of skin surface area (38)) of topical products that do not contain more than 45.5% DMSO, measurable lipid extraction will not occur, and compromise of the skin barrier will not take place by this mechanism. The second often stated disadvantage is that concentrations of DMSO greater than 60 % in a topical product are required to generate enhancement activity and that these high concentrations of DMSO cause skin irritation (39). The skin permeation-enhancing effect of DMSO is concentration dependent with the greatest enhancement factors being obtained when DMSO concentrations are 60% or higher. However, the indirect penetration enhancer benefits

of increasing solubility of the active in the formulation from less than 0.1% up to 2-3% by formulating with 20-40% DMSO combined with around 2-fold direct enhancement by lipid modification can generate meaningful enhancement activity without causing skin irritation. The ability of DMSO concentrations near or below the Inactive Ingredients Database (IID) limit to disorganize intercellular lipids was quantified using a model SC lipid bilayer system. Unilamellar liposomes consisting of a 1:0.7:1 mixture of ceramide AP, cholesterol and stearic acid were loaded with 6-Carboxyfluorescein (6-CF) and exposed to 0%, 10%, 30% and 50% DMSO. The 5% 6-CF leakage found for 10% DMSO increased to 13.6% leakage for 30% DMSO and 60% leakage for 50% DMSO (40). Although, dramatic enhancement does not occur until reaching the IID maximum DMSO concentrations, a measurable enhancement was obtained with 10% DMSO.

Finally, when the intercellular pathway is blocked by preventing the drug from being molecularly dispersed (liposomal, solid lipid nanoparticles or other nanoparticulate topical drug delivery system), the portion of drug not lost to the environment will be targeted to the pilosebaceous unit (41). The addition of DMSO to the continuous phase of a dermal nanoparticulate delivery system will not alter how DMSO diffuses into the intercellular pathway to interact with the polar headgroups of ceramides or bind with keratin filaments in the corneocyte. A certain portion of the topically applied DMSO will partition into the pilosebaceous unit and interact with the stratum corneum-lined upper third of the hair follicle. Just as with drug-in-solution topical formulations described above, the DMSO may enhance percutaneous absorption for drug released in the hair follicle, but DMSO is not expected to measurably enhance the delivery of nanotechnology formulations designed to take advantage of transfollicular delivery.

DMSO AS A SKIN PENETRATION ENHANCER

A main aspect of DMSO application in the skin is its role in enhancing the transdermal permeation of various drugs. This includes compounds such as β-blockers, ephedrine hydrochloride, and papaverine hydrochloride (42,43).

FDA approved DMSO as a therapeutic agent in 1963. Since that time, there have been several FDA-approved formulations that include DMSO. Herpid®, a topical solution that contains 5% idoxuridine, has DMSO among its ingredients. Another important example is the FDA-approved diclofenac sodium topical solution, TDiclo, containing DMSO (Procipient®) with a concentration of 45.5% (w/w).

The absorption of diclofenac from TDiclo into viable human skin was compared with an aqueous topical solution. The results revealed that TDiclo exhibited significantly higher absorption of diclofenac after repeated dosing when compared to the aqueous solution. At a dose of 2 µL/cm2, the mean absorption was 10.2 µg for TDiclo, compared with 8.3 µg for the aqueous solution (44).

Sun *et al.* investigated the topical drug delivery of concentrated cabazitaxel in an α-Tocopherol and DMSO Solution. The absence of DMSO resulted in negligible amounts of cabazitaxel penetrating through the skin within 24 hours. In contrast, both the 30% DMSO/α-tocopherol mixture and DMSO alone demonstrated effective delivery of cabazitaxel. Also, their finding supports that Trans Epidermal Water Loss (TEWL) in 30% DMSO in α-tocopherol formulation exhibited a reversible effect within 8 hours. Sun *et al.*, recommended using α-tocopherol in topical formulations instead of DMSO alone (45).

DMSO was employed to enhance the permeation of 5% (w/w) acyclovir in two different formulations containing 5% and 10% (w/w) DMSO. The performance of these DMSO formulations was compared to that of acyclovir dissolved in distilled water. The results demonstrated that the formulation with an increased DMSO concentration of 10% in the aqueous solution significantly enhanced the transdermal flux, exhibiting a 2.36-fold improvement. Importantly, this enhanced formulation was found to be safe for application on the skin (46). These results can be compared to the 0.5% acyclovir topical

solution of neat DMSO compared to the commercial 5% acyclovir polyethylene glycol (PEG) ointment (ZOVIRAX®, Burrows Welcome). *In vitro* Permeation Testing (IVPT) lag time and flux values were generated using excised Guinea pig skin and were compared to treatment efficacy in an experimental cutaneous Herpes simplex virus (HSV) infection model using the dorsum of the Guinea pig. The 4- to 6-fold reduction in lag time and approximately 4-fold increase in flux for delivery of acyclovir from a dilute neat DMSO (far from saturation) solution showed (Table 1) a dramatic reduction in HSV lesions on the backs of the Guinea pig (47). The HSV lesion reduction caused by DMSO alone (modest but comparable to commercial acyclovir ointment) was attributed to the anti-inflammatory properties of DMSO since antiherpetic activity would not be associated with DMSO until two decades later. Wagner *et al.*, showed DMSO reduces virion infectivity, inhibits viral deoxyribonucleic acid (DNA) replication, and reduces the transcript level of many HSV-1 genes (48).

Another example that illustrates the concentration-dependent mode of action of DMSO is the Malik *et al.*, study on photodynamic therapy of superficial malignant tumors. They incorporated DMSO in the range of 2 – 20% (w/w) as a penetration enhancer. The results showed that higher concentrations of DMSO produced a more pronounced penetration effect and, hence, garnered widespread acceptance for the topical application of 5-aminolevulinic acid (5-ALA) in dermatology (49). One notable observation involved the percutaneous absorption of naphazoline by Stoughton *et al.*, where DMSO concentrations of 50%, 25%, and 10% (w/w) exhibited significant enhancement, with the highest concentration showing a 25-fold increase compared to formulations without DMSO (50). In the context of iododeoxyuridine (IDU) treatment for cutaneous herpes simplex virus (HSV) infections, topical formulations containing 1-20% IDU in vehicles composed of 90% to 100% DMSO were successful in reducing lesion severity in HSV infections (51).

The topical application of hyaluronan with a molecular weight ranging from 500 to 1200 kDa, has been reported to be used in combination with DMSO (52). In these studies, various percentages of DMSO were tested in conjunction with hyaluronan. It was determined that a concentration of DMSO exceeding 20% was necessary to attain anti-hyperalgesia of a similar magnitude to that achieved by the intradermal injection of hyaluronan in a saline vehicle (52). Furthermore, DMSO was observed to enhance the thermodynamic activity of fluocinonide penetrating across human skin (53).

Despite DMSO being an effective skin drug permeation enhancer, the ability of molecules to permeate through the skin is dependent on their molecular weight and it is unlikely that DMSO would enhance the permeation of large molecules such as proteins or polymers (54).

Although DMSO is compatible with a wide range of pharmaceutical ingredients, it may show some side effects, such as dermal irritation. However, this side effect can be decreased, or its appearance can be delayed using the appropriate formulation (55). In a

Table 1 IVPT Parameters and Efficacy of Topical ACV Treatments for HSV Lesions in Guinea Pigs with or without DMSO

TOPICAL TREATMENT	IVPT LAG TIME (hr)	IVPT FLUX (μg/cm2/hr)	MEDIAN NUMBER OF LESIONS ON DAY 4
Untreated	--	--	29
PEG Ointment	--	--	28
DMSO	--	--	25
5% ACV in PEG (250 mg/2.0cm2)	65, 77, 37	0.182, 0.165, 0.069	23
0.5% ACV in DMSO (100μ/2.0cm2)	14,12	0.676, 0.432	--
5.0% ACV in DMSO	--	--	5

set of semisolid preparations aimed at preventing local complications of extravasation, 50% DMSO was utilized to compound various formulations containing 2.5% α-tocopherol, a highly lipophilic molecule (log P = 11.862). Three distinct semisolid formulations were developed and compared against DMSO, a hydrogel, an O/W emulsion, and an oleogel. The hydrogel and the O/W emulsion exhibited superior controlled drug release, considering the intricate interactions between the formulation and the stratum corneum membrane, as well as the partitioning of drugs between the vehicle and stratum corneum. The findings of Casiraghi *et al.*, suggested that hydrogel and O/W emulsion formulations significantly decreased DMSO side effects such as pain, burning sensations, erythema, and pruritus (55).

Numerous studies have investigated the impact of DMSO in comparison to other solvents and permeation enhancers. For instance, in an *in vitro* study involving C14-labeled fluocinolone acetonide, triamcinolone acetonide, and hydrocortisone, DMSO exhibited superior penetration efficacy compared to 95% alcohol (50, 56). In a study by Maibach *et al.*, radio-labeled C14 hydrocortisone and testosterone were formulated using acetone or DMSO for transdermal delivery (57). The quantification of penetration across human skin was determined by analyzing the C14 levels present in urine over five days. In the absence of DMSO, the penetration of testosterone was twelve times greater than that of hydrocortisone (57). The addition of DMSO resulted in a 3.5-fold increase in the penetration of both steroids. In a comparative study involving ethanol, dimethylformamide (DMF), and DMSO as penetration enhancers for bepridil, it was found that DMSO (50%) showed superior skin penetration enhancement (58). DMSO, compared to oleic acid, oleyl alcohol, and sodium lauryl ether sulfate, exhibited the highest enhancement in N-acetylcysteine (NAC) delivery (59). In studies involving healthy adult male and female subjects. Stoughton *et al.*, applied 0.01 cc of each hexopyrronium bromide concentration, prepared in 20% DMSO, or 95% alcohol. The presence of DMSO increased the absorption of hexopyrronium bromide by a remarkable 25 times compared to without DMSO (56). Additionally, Stoughton *et al.*, compared the penetration-enhancing effect of water in comparison to DMSO under continuous exposure, water outperformed DMSO in promoting hydrocortisone penetration, but with transient exposure, DMSO demonstrated superiority over water (50). On the contrary, McKim *et al.,* reported that the inclusion of water in the formulation may impair DMSO efficacy as a penetration enhancer (60).

The impact of DMSO and its effectiveness in enhancing the flux of timolol maleate through human skin was found to be lower than that of lauryl chloride (61). In a separate study aimed at delivering lidocaine, a formulation incorporating 3% Aloe Vera as a permeation enhancer exhibited a drug release of 79.18%. Similarly, when 3% DMSO served as the permeation enhancer in the same formulation, the drug release increased to 84.52% (62).

Using Fourier Transform Raman spectroscopy researchers have uncovered further insights into the impact of DMSO on the skin. At concentrations exceeding 60% v/v, where DMSO improves flux, there is observable interaction with stratum corneum lipids. This interaction extended to alterations in the shape of keratin in the stratum corneum, transitioning from an alpha-helical to a β-sheet structure (63). The transformative effects of DMSO were not limited to protein structure changes but may have also involved modifications in stratum corneum configuration, in addition to any increased drug-partitioning effects (29).
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THE TOXICOLOGY OF TOPICALLY APPLIED DMSO

While DMSO has shown significant potential in anti-inflammatory treatments and pain management, however, it also presents certain challenges post-treatment. Garlic smell from the breath, burning or tingling, skin irritation, diarrhea and itching are notable concerns (18, 32, 64).

A review by Madsen et al., (64) highlights the common side effects to DMSO, mainly gastrointestinal adverse reactions, headaches, dizziness, vomiting and nausea. As well as skin reactions such as erythema , itching , urticaria, rash and skin dryness. The occurrence of

nausea, ranging from 2% to 14% in most studies, appeared less frequent with transdermal compared to intravenous administration. Skin reactions were frequently transient, lasting only minutes; however, some reported serious cases leading to treatment discontinuation. Two studies suggested that continuous days of treatment could resolve skin reactions. Hospitalization due to exfoliative erythroderma was reported in one case among 18 psoriasis patients treated with DMSO (64).

DMSO can interact with general anaesthetics, caustic revulsives, or anticholinesterase drugs (65). These concerns regarding the impact of DMSO on the efficacy and safety of administered drugs highlights the importance of thorough consideration when combining DMSO with other medications. (18, 64).

It is noteworthy that the appropriate concentration and application site of DMSO play a significant role in achieving the expected biological effects with minimal side effects. A study conducted by Hanna *et al*., (14) has reported that higher concentrations of DMSO, 90-100% can be irritating to the skin at the site of application when a standard model of ocular inflammation is used, whereas lower concentrations around 30% exhibit anti-inflammatory properties. In another study, Silvestri *et al.,* (66), reported that the patients undergoing topical idoxuridine in dimethyl sulfoxide therapy for genital herpes experienced some side effects, such as local burning and generalized contact dermatitis.

DMSO stands out as a low-toxicity solvent for treating various topical conditions (18, 64). Dermatological studies on DMSO indicate minimum occurrences of complications like burning sensation, skin irritation and itching. (64). These side effects are mainly due to the DMSO concentration, the application site on the skin, and the specific drug being formulated.

DMSO METABOLISM AND DMS IN EXPIRED AIR

Hucker *et al.* carried out a study to investigate the absorption, excretion, and metabolism of DMSO following a single topical dose or 14 days of oral dosing. Human subjects were treated with a 70% DMSO (99.5% minimum purity spiked with approximately 125 microcuries radiolabeled DMSO-S35per subject) solution in water using gauze, which was applied topically to the entire body surface while standing on aluminum foil (67). After applying the solution, the gauze was washed with water, squeezed out, and reapplied to the skin. Two subjects received a dermal dose of 1 g/kg DMSO.

Serum and urinary levels of radioactivity, along with concentrations of DMSO and the only sulfur-containing metabolite dimethyl sulfone ($DMSO_2$), were monitored for up to 400 hours. $DMSO_2$ was detected in the serum around 48 hours and persisted for up to 312 hours with a half-life of approximately 60-70 hours. Plasma C_{max} of DMSO was detected after 4 to 8 hours and then declined with a half-life of approximately 11 to 14 hours. Minimal traces of DMSO were detected after 48 hours. DMSO was detected in urine shortly after drug administration and continued for 48 hours. The lower renal clearance of $DMSO_2$ or potentially more extensive tissue binding of $DMSO_2$ might explain this observation. Additionally, DMSO could be irreversibly bound at a site, gradually converting to $DMSO_2$, and then being excreted. Total DMSO excretion averaged 13.0% of the dose for both subjects. Urinary excretion of DMSO became significant approximately 8 hours post-dosing and continued for 456 hours, with the average total amount excreted equivalent to 17.8% of the DMSO dose. Negligible DMSO-S^{35} was found in the feces and "Excretion of DMSO and/or metabolites in the expired air given DMSO dermally was also studied (unpublished results of the authors) and accounted for only a very minor fraction of the dose". The authors concluded that the fraction of DMSO excreted is entirely accounted for by unchanged material and $DMSO_2$ (67).

In the six decades since the Hucker *et al.* publication, $DMSO_2$ has been recognized as a stable metabolic end-product suitable for gaining insight into microbial-mammalian co-metabolism (68). Bioconversion between DMS and DMSO and $DMSO_2$ occurs in humans when DMSO is dosed to a patient or absorbed after intestinal microbial catabolism of methionine.

Once present systemically, DMSO can be oxidized to $DMSO_2$ by hepatic microsomes in the presence of oxygen and either NADPH or NADH (69) or reduced to DMS by MsrA which is expressed in kidney and liver tissues (70). The low urinary elimination rate of DMSO and $DMSO_2$ is attributed to slow metabolic conversion among DMS, DMSO and $DMSO_2$, and/or binding of DMSO with tissues. DMSO has been shown to bind to plasma protein, skin, liver, diaphragm tissues and cornea (69). $DMSO_2$ is volatile, odorless, metabolically stable, and found in urine, blood, brain, skin, sweat and earwax as summarized in He and Slupsky (68). DMS is excreted in the breath to produce a characteristic odor. Normal DMS levels in the breath are low at 0.34 +/- 0.03 nM compared to blood (3-6 nM) and urine (400 nM) (71). However, halitosis patients are characterized as overproducers of DMS (72) and MAT I/III deficient patients who have abnormal methionine production have DMS levels in the breath of 5.86 +/- 0.11 nM (71). About 3% of the administered DMSO dose can be detected as DMS in exhaled breath (73,74). The work of Hucker viewed through the modern lens of the He and Slupsky review (68) provides insight into the source of the characteristic garlic- or oyster-like odor to the breath of patients after topical dosing of DMSO. Most of the DMSO that becomes systemic after topical application binds to plasma proteins and body tissues including the skin and cornea. Slow metabolic conversion establishes an equilibrium between levels of DMSO and DMS with low levels of DMS being exhaled. Concurrent with DMSO being reduced to DMS and DMS being oxidized back into DMSO, hepatic microsomes are oxidizing DMSO into the stable metabolic end-product $DMSO_2$. Quantifiable urinary excretion of DMSO occurs within 2 hours of dosing compared to quantifiable urinary excretion of $DMSO_2$ not occurring until 8 hours after DMSO topical application.

The DMSO clinically used in the 1960s through the 1990s contained significant levels of thiochemical impurities which are carried directly into the bloodstream and begin being excreted through the lungs less than an hour after topical application. These thiochemical impurities may produce a more pungent oyster-like odor within an hour of dosing compared to less pungent odor of exhaled DMS. For stem cell transplant patients that receive significant amounts of DMSO after IV-infusion (high purity DMSO is used as a cryoprotectant for stem cell storage) the exhaled DMS is described as having a smell similar to garlic or creamed corn (75). When high purity (>99.99%) DMSO is topically applied, a few hours are required for the slow metabolic conversion to DMS to cause a breath odor similar to creamed corn.

The impact of DMSO purity on the severity and incidence of expired air having a pungent odor after topical dosing can be inferred after comparing a 1965 arthritis study with a 2009 arthritis study. In 1965 (76) six arthritis inpatients at Douglas Count Hospital and ten outpatients at the University of Nebraska Hospital Arthritis Clinic were given 30-60 mL bottles of a 90% DMSO in water solution. Although not stated, the DMSO used in this study was likely similar in purity to the Crown Zellerbach Corporation 99.5% pure DMSO used by Hucker (67). For the inpatients, an attendant used a cotton tip applicator to apply 4-6 ml topically to the arthritic joint three times a day for two weeks. Outpatients were given the same test article and instructed to self-apply a similar amount of solution using the same schedule of topical administration. A typical amount of DMSO applied to the skin per day in this 1966 study was 13.5 mL. The Adverse Side Reactions and Effects section of this MD thesis, it states "…patients have discerned, within several minutes of application, a sweet, sulphur-like, or oyster-like taste in the mouth. The air of expiration takes on a garlic like odor. This odor is thought to be due to the excretion of dimethyl sulfide, a DMSO metabolite, through the lungs." Five of the six inpatients and nine of the 10 outpatients recorded halitosis as an adverse effect after topical DMSO application. It is proposed that significant levels of thiochemical impurities contained in the 13.5 mL of the 99.5% pure DMSO (up to 67.5 mg of non-DMSO impurities dosed daily) were carried directly into the blood stream and were excreted through the lungs less than an hour after topical application. In 2009 the FDA approved a 1.5% diclofenac solution containing 45.5 % DMSO (Procipient® Gaylord Chemical >99.99% purity) for topical application to the arthritic joint up to four times

a day. Typical amount of DMSO applied to the skin per day was up to 4 mL. Using the same assumptions as applied to the 1965 arthritis study, not more than 0.04 mg of non-DMSO impurities were dosed daily in the Pennsaid study. According to the package insert, of the 1,243 patients dosed, only 12 noted halitosis as an adverse reaction (77).

Various techniques are available for detecting DMSO in both serum and urine. These methods encompass the use of water-soluble amphiphilic probes designed for sensitive detection of DMSO/DMF, gas chromatography, an electrochemical sensor, an iridium (III)-based luminescent probe, and fluorescent probes (78-80).

CONCLUSIONS

Dimethyl Sulfoxide is a polar aprotic solvent that dissolves both hydrophilic and lipophilic APIs. DMSO is the Active Pharmaceutical Ingredient in RIMSO-50 (50% DMSO:50% water) an anti-inflammatory and bladder irrigating drug that reduces swelling and pain due to interstitial cystitis. DMSO is the industry standard biological tissue cryoprotectant when added to the cells (final concentration of 10% DMSO). DMSO is an excellent pharmaceutical solvent that can increase the solubility of both polar and nonpolar molecules. DMSO is miscible with the topical excipients water, ethanol, isopropyl alcohol and ethyl acetate. When used as a topical pharmaceutical excipient up to 45.5 w/w % (maximum IID use level in a topical gel), DMSO is a highly effective skin penetration enhancer. The ability of DMSO to enhance skin permeation is concentration dependent, with measurable enhancement occurring with the addition of 5-10% DMSO and the most dramatic skin permeation enhancement occurring when greater than 40% DMSO is formulated in a topical product.

DMSO modifies epidermal lipid order by interacting with the polar head groups of intercellular lipids and modifying the hydrogen bonds of ceramides. These interactions disturb the head group domain, disordering bilayer structure and increasing drug diffusion through the SC. DMSO has also been shown to be a protein modifier that binds with the keratin filaments in the corneocyte to cause conformational changes in the proteins and possibly increase intracellular (transport through the corneocyte) permeation. Since most drugs are transported by the intercellular pathway, DMSO's disordering of the headgroup region of intercellular lipids presumably contributes more to increasing drug diffusion than DMSO's ability to disrupt corneocyte proteins.

Since the mid-1960s numerous studies have shown DMSO has anti-inflammatory properties. The *in vivo* topical application of DMSO concentrations as low as 0.5% to mice having rheumatoid arthritis and human melanoma significantly reduced the expression of numerous pro-inflammatory cytokines, prostaglandin E2 and chemokines. DMSO's ability to modulate immune responses is considered particularly beneficial in alleviating pain associated with immune-mediated skin disorders, such as psoriasis and eczema. DMSO has also been shown to reduce virion infectivity, inhibit viral DNA replication, and reduce the transcript level of many HSV-1 genes. Since DMSO has wide-ranging pharmacological activity, formulations containing DMSO as a topical excipient require carefully designed Phase 2 clinical studies to adequately power pivotal trials that statistically separate active versus vehicle for the primary endpoints.

While rapidly absorbed after topical application, DMSO generally exhibits low dermal toxicity, with prolonged exposure to high doses (>60 wt/wt %) causing minimal effects like mild skin irritation, itching, and a burning sensation. Once present systemically, DMSO can be oxidized into the stable metabolic end-product $DMSO_2$ which is odorless and excreted in the urine, or reduced to DMS which is excreted in the breath to produce a characteristic odor. DMSO having purity levels less than 99.99% can contain significant levels of thiochemical impurities which are carried directly into the blood stream and begin being excreted through the lungs less than an hour after topical application. These thiochemical impurities may produce a more pungent oyster-like odor within an hour of dosing compared to exhaled DMS which smells like garlic or creamed corn. Only 12 of 1,243 patients noted halitosis as an

adverse reaction after being dosed with up to 4 mL of a diclofenac topical solution containing 45.5 % DMSO (Procipient® Gaylord Chemical >99.99% purity).

When Marion Sulzberger published results from the first clinical studies of a corticosteroid ointment in 1952 (81), topically applied semisolids advanced from cerates and unguents into pharmaceutical dosage forms capable of delivering pharmacological actives to target sites within the skin. With the approval of a transdermal scopolamine patch in 1979, pharmaceutics researchers viewed the skin as a route of administration to deliver actives systemically and that the number of drugs suitable for topical and transdermal delivery could be expanded using skin penetration enhancers. Four years later Diprolene Augmented Ointment was approved by the FDA with "augmented" meaning this topical betamethasone dipropionate contained propylene glycol as a skin penetration enhancer and raising the potency of this ointment to the Class I category. Three topical creams (JAK inhibitor, AhR modulating agent and a PDE4 inhibitor) were approved to treat inflammatory skin conditions between September 2021 (ruxolitinib-Opzelura®) and July 2022 (roflumilast-Zoryve®) with tapinarof-Vtama® being approved on May 24, 2022. None of these products contain DMSO, despite JAK inhibitors being difficult to dissolve (82) and difficult to deliver across the stratum corneum. The fresh perspective from this review is that use of high purity (>99.99%) DMSO may minimize the incidence of halitosis and thus remove the longstanding perception that limited the topical use of this skin penetration enhancing, anti-inflammatory, well-tolerated solvent. It is anticipated that DMSO will become a preferred excipient in topical pharmaceutical products developed in the future.

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