sciendo

10.2478/AMB-2024-0067

REVIEW

DEVELOPMENT STRATEGY OF DERMAL AND TRANSDERMAL FORMULATION: SYNERGISTIC EFFECT OF CHEMICAL PENETRATION ENHANCERS

M. Špaglová, D. Žigrayová, D. Krchňák

Faculty of Pharmacy, Department of Galenic Pharmacy, Comenius University – Bratislava, Slovakia

Abstract. *The skin is an attractive site for direct administration of drugs due to its easy access and patient compliance. The strategy in the development of a dermal pharmaceutical and a cosmetic product lies in a selection of suitable excipients capable of delivering the drug or active pharmaceutical ingredient at the site of its action. The key moment is overcoming the least permeable skin layer stratum corneum. Chemical penetration enhancers facilitate drug diffusion and accelerate drug delivery through the stratum corneum, possibly in combination with hydration of the skin or increasing temperature. The paper summarises basic information about the most common chemical enhancers and the studies investigating the synergistic action of suitable combinations of chemical enhancers, which may also include microemulsions.*

Key words: *chemical penetration enhancer, permeation, synergistic effect, SCOPE*

Corresponding author: *Miroslava Špaglová, Faculty of Pharmacy, Department of Galenic Pharmacy, Comenius University – Bratislava, Odbojárov 10, SK-832 32 Bratislava, Slovakia, e-mail: spaglova@fpharm.uniba.sk*

Received: *11 December 2023;* ***Accepted:*** *08 February 2024*

MECHANISM OF THE ACTION OF THE PENETRATION ENHANCER

Chemical penetration enhancers facilitate drug diffusion through the skin by several mechanisms. Most often they do that by disrupting the highly organized intercellular structure of the lipid bilayer, thereby reducing the resistance of the *stratum corneum* barrier layer, or by acting on desmosomes whose function is to maintain cohesion between corneocytes, by denaturing intracellular keratin, or by changing its conformation. The enhancers may also provide increased hydration of the stratum corneum, thus altering its properties and increasing the drug partition coefficient between the skin and the vehicle. Many of these substances are also characterized by the solubilizing capability. Drug solubilization modifies the thermodynamic activity of the drug or vehicle [1, 2]. The requirements for the characteristics of an ideal penetration enhancer are the following:

- pharmacologically inert
- non-allergenic
- fast action with a predictable duration
- temporary reduction of the skin barrier function without loss of endogenous material
- restoring the barrier function of the stratum corneum after removing the product from the skin
- cosmetically acceptable properties (color, odor)
- affordable [3].

GENERAL CLASSIFICATION OF CHEMICAL PENETRATION ENHANCERS

The classification of chemical penetration enhancers based mainly on chemical structure together with examples of common enhancers in dermal and transdermal applications are listed in Table 1.

Table 1. The Classification of Chemical Penetration Enhancers

Chemical Group	Examples
Alcohols	Ethanol, Propanol, Decanol, Octanol
Glycols	Propylene glycol
Amides	Azone (1-dodecylazacykloheptan-2-on/laurokapram)
Aliphatic acids	Lauric acid, Oleic acid, Linolenic acid
Esthers	Isopropyl myristate, Isopropyl palmitate
Ethers	Transcutol (dietylene glycol monoetylether)
Surfactants	Sodium lauryl sulphate, Benzalkonium chloride, Cetylpyridinium chloride, Cetyltrimethylammonium bromide, Polysorbates (Tween 20, Tween 60), Dodecylbetaine
Sulfoxides	Dimethylsulfoxid (DMSO), decylmethylsulfoxid (DCMS)
Pyrolidones	N-methyl-2-pyrrolidone, 2-pyrrolidone
Oxazolidines	4-decyloxazolidin-2-on
Phospholipids	Phosphatidylcholine, Phosphatidylethanolamine
Terpenes	D-limonene, 1-menthol, 1,8-cineole, Eugenol, Neridol, Farnesol
Essential oils	Cinnamon oil, Eucalyptus oil, Rosemary oil

The efficiency of the chemical penetration enhancers can be expressed as the so-called Enhancement Ratio (ER):

$$ER = \frac{Q_E}{Q_R} \quad (1)$$

wherein Q_E represents the drug amount that permeated trough/or in the skin from the enhancer-containing formulation and Q_R is the drug amount that permeated trough/or in the skin from the reference formulation [4].

SYNERGISTIC ACTION OF CHEMICAL PENETRATION ENHANCERS

The limiting factor for dermal drug penetration improvement by application of chemical penetration enhancers is the high molecular weight of many drugs and the possible irritative action of the penetration enhancers at higher concentrations. To avoid this problem, the enhancers began to be applied in synergistic combinations often called Synergistic Combination of Penetration Enhancers (SCOPE) [5, 6]. The enhancer mixtures are usually more effective than when used alone, due to various action mechanisms on the stratum corneum multiplying their action. A typical example is the solution of propylene glycol with amphiphilic surfactants. The most significant benefit of SCOPE application is the minimization of necessary enhancer concentration resulting in irritation and toxicity reduction [7]. Synergy can be objectively quantified using the parameter S, i.e., a degree of interaction between two enhancers given by Equation 1:

$$S = \frac{ER_{AB}^{XY}}{(1-X).ER_{B}^{Y}+X.ER_{A}^{Y}} \quad (2)$$

wherein ER_{AB}^{XY} is the penetration enhancement ratio of the mixture containing enhancers A and B at a total concentration of Y% (w/V) and X is the weight ratio of enhancer A. E_A^Y and E_B^Y are the enhancement ratio of enhancers A and B used separately at the same concentration of Y%. Interpretation of value: $S > 1$ indicates positive synergy, $S < 1$ negative synergy, $S = 1$ means no synergy [8, 9].

Karande and Mitragotri [8] present a simple way of classifying SCOPE as it is shown in Figure 1.

By combining properly selected penetration enhancers, it is possible to synergize their effect. Of course, the level of synergy depends on several aspects such as ratio, concentration, and physicochemical properties of the enhancer mixture [10]. Because Azone, Transcutol, and DMSO are among the most frequently used enhancers in topical application, we have focused in the paper preferentially on their characteristics and/or synergizing effect with other enhancers. However, Table 2 lists also other SCOPEs.

Dimethylsulfoxid (DMSO) is one of the oldest and best-studied chemical penetration enhancers. It is a strong, universal, aprotic solvent without color and odor. A large number of studies have demonstrated the supportive solubilizing effect of DMSO for both hydrophilic and lipophilic drugs. It changes the structure of intercellular keratin and interacts with lipids of the stratum corneum. DMSO acts as a cosolvent or drug solubilizer in many dermal products. It has its therapeutic effect [11]. Its topical administration treats systemic inflammation, but for many side effects, it is applied for this purpose only in veterinary medicine [12]. Although it acts as an excellent enhancer, its application is accompanied by many shortcomings.

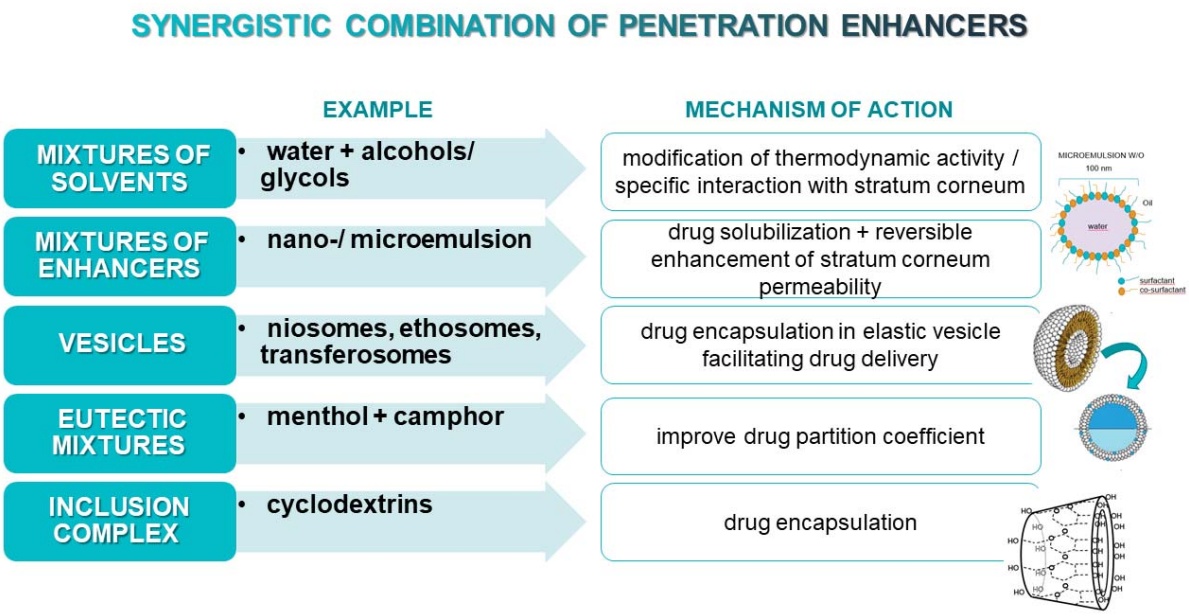

Fig. 1. The examples of synergistic combinations of penetration enhancers

The effect of DMSO is dose-dependent, with the concentration required to achieve optimal enhancer activity exceeding 60%. The dermal administration of such high concentrations can cause erythema, urticaria, flaking, or burning on the skin. Another limitation of DMSO application in dermal and transdermal products is the formation of a smelly dimethyl sulfide metabolite [13]. The mentioned limits have moved the attention to investigate other compounds structurally related to sulfoxide, e.g., dimethylacetamide (DMAC) and dimethylformamide (DMF). The application of DMSO, DMAC, as well as DMF to human skin, causes denaturation of intercellular protein, and interaction with the intercellular matrix of the stratum corneum, and thus facilitates the drug delivery from the preparation to the skin [12,14]. DMSO is controversial in clinical use because of possible side effects and potential toxicity [15]. Therefore, current studies focus on finding suitable substitutes rather than combining them with other enhancers.

The first compound specifically designed to improve drug penetration was Azone , chemically 1-dodecylazacycloheptan-2-one. It is highly lipophilic (logP octanol/water = 6.2), soluble in most organic solvents, and an excellent solvent for a wide range of drugs (steroids, antibiotics, antivirals). It is non-irritating, almost without its pharmacological activity. Only a slight antiviral effect has been reported. LD_{50} for rats is 9 g/kg. As in cases of other chemical enhancers, the effect of Azone is significantly dependent on concentration. The highest enhancer activity was found in low concentration, usually 0.1-5% [12,16]. It acts directly on the lipid bilayer of the stratum corneum improving drug penetration by increasing its fluidity. This mechanism of action was elucidated by monitoring the phase transition temperature using a turbidity test and the pyrene excimer fluorescence technique [17]. The comparison of the effect of Span 20 and Azon on the compression of a skin model representing *stratum corneum* fluidity showed that both enhancers have similar activity [18]. Azone was combined with borneol and menthol to promote in vitro transdermal delivery of ferulic acid through mouse skin [19]. A synergistic effect on the improvement of transdermal delivery of dl-praeruptorin A was confirmed when Azone was combined with propylene glycol [20]. The same combination of the enhancers in the simultaneous application of iontophoresis increased skin permeation of insulin significantly [21]. The other synergistic combinations with Azone are mentioned in Table 2.

Transcutol is a potent solubilizer of many drugs. It is non-toxic and biocompatible to the skin. It has found its application not only in the pharmaceutical industry but also in the cosmetics and food industries. In dermal preparations, it solubilizes active substances, which are insoluble in common solvents, such as ethanol or propylene glycol. In transdermal formulations, it prolongs depot release or improves systemic absorption [22]. Many studies have shown that it significantly improves the penetration of substances into the skin, especially when used in combination with another cosolvent [23,24]. Transcutol together with propylene glycol significantly increased the flux of clonazepam released from carbopol gel across the synthetic membrane, but this effect was not confirmed by an ex vivo permeation test through rabbit skin [23]. Another study showed a 2-fold higher ER when using Transcutol and Azone to promote

naproxen penetration from Pluronic F-127 gels compared to only Transcutol-containing gels [24].

The results of other studies investigating the synergy of the enhancers are listed in Table 2.

Chemical penetration enhancers are also popular in the improvement of the physical properties and penetration capabilities of vesicular and other drug nanocarriers. Mura et al. [43] investigated the properties of liposomes as minoxidil carriers compared to lecithin vesicles, in which a solution of Transcutol (5%, 10%, 20%, 30%) was used as a hydrophilic phase. The amount of drug entrapped in the vesicles and liposomes was approximately the same. Vesicles containing 5% and 10% Transcutol solution were more deformable. However, all Transcutol vesicles showed greater stability compared to liposomes over 3 months, considering changes in their size, degree of polydispersity, and zeta potential. The results of the diffusion test showed that Transcutol vesicles can deliver minoxidil deeper into the skin.

Table 2. The synergistic combination of penetration enhancers (SCOPE) studied in dermal or transdermal administration

Drug	Penetration enhancers	Enhancer ratio	Dosage form	Enhancement ratio (ER)	Reference
Diclofenac	1,8-cineole, ethanol, McIlvaine buffer	1:41:59	Gel ointment	17.1	[25]
Tetracaine	Menthol, ethanol	5:70	Gel	26.59	[26]
5-fluorouracil	Azone, propylene glycol	2:98	Solution	2.6	[27]
Insulin	Azone, propylene glycol	0.1:40	Cream	1.69	[28]
Metronidazole	Azone, propylene glycol	1:99	Gel	4.4	[29]
Lidocaine	Olive oil, α-linolenic acid, linoleic acid, vitamin E succinate, surfactants	3.23:0.45:1.81:0.91:13.60	Microemulsion	1.54	[30]
Lidocaine	Isopropyl myristate, n-methyl pyrrolidone	25:75	Solution	4.3	[31]
Formoterol	l-menthol, N-methyl-2-pyrrolidone	60:40	Solution	1.21	[32]
Tenoxicam	Oleic acid, propylene glycol	15:40	Gel	19.4	[33]
Nicardipine	Propylene glycol, oleic acid, dimethyl isosorbide	80:10:10	Solution	814/182/339	[34]
Nifedipine	Oleic acid, propylene glycol, dimethyl isosorbide	4:84:10	Solution	20	[35]
Estradiol	Lauric acid, propylene glycol, dimethyl isosorbide	3.3:60:33.7	Solution	2/ 24/ 122	[36]
17- β -estradiol	Lauroyl choline, oleic acid, propylene glycol	Non-specified	Gel	14	[37]
Acyclovir	Lauroyl choline, oleic acid, propylene glycol	Non- specified	Powder	5.08	[37]
Docetaxel	Sodium oleate, sodium lauryl ether sulfate, propylene glycol	64:16:20	Gel	Non-specified	[38]
Clebopride	Diethylene glycol monoethyl ether, isopropyl myristate	40:60	Gel	80 (compared to reference only with isopropyl myristate)	[39]
Naloxone	Propylene glycol, ethanol	33:67	Solution	10.9	[40]
Pentazocine	Isopropyl myristate, glyceryl monocaprylate	90:10	Solution	58.0	[41]
Tegafur	Ethanol, tricaprylin	40:60	Solution	69.19 (compared to aqueous solution)	[42]

As already mentioned, there are also pairs of enhancers that slow down percutaneous drug transport, e.g., Brij 30 together with Transcutol / or lauryl alcohol/ or Span 80/ or isopropyl palmitate caused retardation of in vitro donepezil release from transdermal patch (ER was 0.70/0.75/0.78/0.80) [44]. Further, the couple of 1,4-cyclohexanediol and 1,2-hexanediol caused a delay in the absorption of metronidazole (ER was 0.40-0.69), which may be perceived as effective if the potential systemic toxicity of the drug needs to be minimized while the therapeutic efficacy persists [45]. It is therefore not possible to argue that negative synergy is undesirable or unworkable in practice. Few studies have addressed negative outcomes, hence, there is a lack of documentation to help clarify the mechanism of action of a combination of enhancers and thus predict, for example, negative synergy.

MICRO- AND NANOEMULSIONS AS PENETRATION ENHANCERS IN DERMAL AND TRANSDERMAL ADMINISTRATION

Due to the composition, microemulsion, and nanoemulsion systems can be considered as composed penetration enhancer systems. Micro- and nanoemulsions as drug carrier systems are characterized by several advantages. Their preparation, especially in the case of microemulsions, is relatively simple. Microemulsions are formed spontaneously, by homogenization of the correct ratio of suitably selected constituents; oil, water, surfactant, and, if necessary, co-surfactant. However, the ratio of the components is crucial to ensure a very small droplet size of the inner phase, i.e., up to 100 nm in microemulsions, and up to 500 nm in nanoemulsions. To create nanoemulsions with an inner phase of 100 to 500 nm, it is necessary to reduce the droplet size of the initial coarse emulsion by ultrasonication or high-pressure homogenization. The paradoxical naming of these dispersion systems, which does not correspond to the size of the internal phase, stems from the fact that microemulsions were named before their droplet size characterization.

These dynamic systems with a continuously fluctuating interface are characterized by a high solubilization ability [46]. Due to the presence of hydrophilic and lipophilic regions, they can solubilize both hydrophilic and lipophilic drugs. As colloidal systems, they are used either as vehicles alone or as drug solubilizers in formulation, e.g., gels [47,48]. Microemulsions are sensitive to quantitative changes in composition because they exist only in a narrow region, usually defined by a ternary phase diagram. The characterization of the phase behavior of a microemulsion is an important point.

Many studies have confirmed their positive effect on drug penetration in dermal and transdermal delivery systems. Some of them are listed in Table 3. However, despite countless studies demonstrating the positive influence of micro-/nanoemulsions on drug penetration, their application in dermal and transdermal drug delivery is minimal. This may be due to possible skin irritation caused by the need for high tenside content for the formation of the microemulsion structure. The replacement of ionic tensides with non-ionic tensides presents one of the solutions. The other is the substitution of synthetic tensides with natural ones. As an example, nanodisperse systems containing natural surfactant, lecithin, appear to be more effective than liposomes because they can deliver the drug to the deeper layers of the skin [49]. Recently, they are of particular interest. [50-53] Lecithin microemulsions offer the ideal penetration enhancer properties due to that lecithin's function as a surfactant is synergized by the action of a suitably selected co-surfactant and the oil phase.

CONCLUSION

The efficiency of chemical penetration enhancers depends on their concentration and physicochemical properties. The effect is specific to the drug, or the active pharmaceutical ingredient. However, it can be noted that the application of traditional penetration enhancers (such as DMSO, DMF, DMAC, Azon, pyrrolidone, surfactants, and alcohols) causing significant destruction of the stratum corneum is declining. Many chemical enhancers may ensure effective penetration enhancement only when used in a high concentration that can irritate the skin. The combination of several penetration enhancers can lead to a synergy of their effect, and finally to the reduction of individual enhancer concentration needed for adequate enhancement effect on drug penetration. Synergistic penetration enhancers often used in the development of drug dosage forms include aqueous solutions with multiple solvents (cosolvents), surfactant vehicles, eutectic mixtures, inclusion complexes, and last but not least, microemulsions and transport vesicles.

Author contributions: *The authors have made equal contributions.*

Conflict of interest: *The authors declare no potential conflicts of interest.*

Table 3.

Drug	ME Composition	Dosage form	Enhancement/ (ER)	Reference
Clotrimazole	Isopropyl myristate Labrasol Cremophor EL Water	ME	5.1 (o/w) 2.8 (bicontinuous) 3.0 (w/o)	[54]
Penciclovir	Oleic acid Cremophor EL Ethanol Water	ME	2.5 times higher cumulative amount compared to commercial cream	[55]
Benzocaine	Isopropyl myristate Span 20 Cremophor EL Ethanol	ME	1.26	[56]
Acyclovir	Isopropyl myristate Tween 20 Span 20 Water Dimethyl sulfoxide + menthol + propylene glycol	ME	1.91 compared to ME without menthol and propylene glycol	[57]
5-fluorouracil	Isopropyl myristate Tween 80 Span 20 1-butyl-3-methylimidazolium bromide	ME	4-fold compared to aqueous solution; 2.3-fold compared to hydrophilic ointment	[58]
Finasteride	Water (5%) Oleic acid + Transcutol P (10%) Span 20 + Tween 80 (56.67%) Polyethylene glycol (28.33%)	ME	26.35	[59]
Fusidic acid	Ethyl oleate Tween 80 Ethanol Water	ME-gel	Non-specified	[60]
Naproxen	Isopropyl myristate Labrasol Span 80 Ethanol NaOH solution	ME	9.04	[61]
Pseudolaric acid	Isopropyl myristate Cremophor EL Transcutol P Carbopol gel	ME-gel	3.01	[62]

Continuation of Table 3

Clotrimazole	Clove oil Tween 80 Propylene glycol Water + Chitosan solution	ME	Sustained permeation due to the mucoadhesive properties of chitosan	[63]
Tazarotene	Jojoba oil Tween 80 Span 85 Water	ME	Non-specified	[64]
Tretinoin	Isopropyl myristate Tween 80 Isopropyl alcohol Water + Carbopol gel	ME-gel	1.68 (in vitro)	[65]
Minoxidil	Isopropyl myristate Tween 80 Isopropyl alcohol Water + Sodium alginate gel	ME-gel	1.52 (ex vivo)	[66]
Cephalexin	Oleic acid Span 20 Tween 80 Cremophor EL Ethanol Phosphate buffer	ME	Non-specified	[67]
Sylimarin	Isopropyl myristate Tween 20 Labrasol Span 20 HCO-40 Transcutol	ME	Non-specified	[68]
Curcumin	Grape seed oil Water Plurol Tween 80 Ethanol Sodiumhyaluronate salt	ME-gel	3.47	[69]
8-methoxypsoralen	Ethyl oleate Cremophor E35 Ethanol Water	ME	1.98	[70]
Imiquimod	Oleic acid Transcutol d-α-tocopheryl PEG 1000 succinate	ME-gel	Non-specified	[71]

Continuation of Table 3

Celecoxib	Isopropyl myristate PEG-6 Caprylic/Capric Glycerides PEG-7 glyceryl cocoate Water	ME	8.85	[72]
Diphenhydramine	Isopropyl palmitate Tween 80 Span 20 Water +Glycolipid	ME	Non-specified	[73]
Adapalene	Imwitor 742 Platacare 2000 UP Propylene glycol Water	ME	5.9	[74]
Cyclosporine	Oleic acid TPGS (D-α-Tocopheryl polyethylene glycol 1000 succinate) Transcutol Water	ME-gel	6.3	[75]
Griseofulvin	Oleic acid Tween 80 Ethanol	ME	9.38	[76]
5-aminolevulinic acid	Ethyl oleate PEG-8 caprylic/capric glycerides:polyglycerol-6 dioleate (3:1) Water	ME	17.5	[77]
Clobetasol	Isopropyl myristate Cremophor EL Isopropyl alcohol Water + Carbopol 934P	ME/ ME-gel	Non-specified	[78]
Progesterone	Polyoxyethylene-10-dodecyl ether Tributyrin Water	ME	Non-specified	[79]
Amphotericin B	Isopropyl myristate Tween 80 Propylene glycol Water	ME	3.08-fold increase in skin retention (ME7 against drug solution)	[80]
Dithranol	Isopropyl myristate Tween 80 Phospholipid Ethanol	ME	6.87-fold increase in skin retention (%; F42 against drug suspension)	[81]

REFERENCES

1. Lopez-Castellano A, Merino V. Chemical Enhancers. In Current Technologies To Increase The Transdermal Delivery Of Drugs; Juan Escobar-Chávez J, Merino V, Eds. BENTHAM SCIENCE PUBLISHERS, 2010; 23-40 ISBN 978-1-60805-191-5.
2. Dragicevic N., Maibach HI. Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Nanocarriers; Eds.; Springer: Berlin, Heidelberg, 2016; ISBN 978-3-662-47861-5.
3. Vitorino C. Sousa J. Pais A. Overcoming the Skin Permeation Barrier: Challenges and Opportunities. Curr. Pharm. Des. 2015, 21, 2698–2712, doi:10.2174/1381612821666150428124053.
4. Caserta F, Brown MB, McAuley WJ. The Use of Heat and Chemical Penetration Enhancers to Increase the Follicular Delivery of Erythromycin to the Skin. Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 2019, 132, 55-62, doi:10.1016/j.ejps.2019.02.030.
5. Parhi R, Suresh P, Mondal S, Kumar PM. Novel Penetration Enhancers for Skin Applications: A Review. Curr. Drug Deliv. 2012, 9, 219–230, doi:10.2174/156720112800234585.
6. Karande P, Jain A, Mitragotri S. Discovery of Transdermal Penetration Enhancers by High-Throughput Screening. Nat. Biotechnol. 2004, 22, 192-197, doi:10.1038/nbt928.
7. Kováčik A, Kopečná M, Vávrová K. Permeation Enhancers in Transdermal Drug Delivery: Benefits and Limitations. Expert Opin. Drug Deliv. 2020, 17, 145-155, doi:10.1080/17425247.2020.1713087.
8. Karande P, Mitragotri S. Enhancement of Transdermal Drug Delivery via Synergistic Action of Chemicals. Biochim. Biophys. Acta BBA – Biomembr. 2009, 1788, 2362-2373, doi:10.1016/j.bbamem.2009.08.015.
9. Ng KW, Lau WM, Williams AC. Synergy Between Chemical Penetration Enhancers. In Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum; Dragicevic, N., Maibach, H.I., Eds.; Springer: Berlin, Heidelberg, 2015; pp. 373–385 ISBN 978-3-662-47039-8.
10. Bozdaganyan ME, Orekhov PS. Synergistic Effect of Chemical Penetration Enhancers on Lidocaine Permeability Revealed by Coarse-Grained Molecular Dynamics Simulations. Membranes 2021, 11, 410, doi:10.3390/membranes11060410.
11. Anigbogu ANC, Williams AC, Barry BW, Edwards HGM. Fourier Transform Raman Spectroscopy of Interactions between the Penetration Enhancer Dimethyl Sulfoxide and Human Stratum Corneum. Int. J. Pharm. 1995, 125, 265-282, doi:10.1016/0378-5173(95)00141-5.
12. Williams AC, Barry, B.W. Penetration Enhancers. Adv. Drug Deliv. Rev. 2012, 64, 128–137, doi:10.1016/j.addr.2012.09.032.
13. Marren K. Dimethyl Sulfoxide: An Effective Penetration Enhancer for Topical Administration of NSAIDs. Phys. Sportsmed. 2011, 39, 75–82, doi:10.3810/psm.2011.09.1923.
14. Barry BW. Mode of Action of Penetration Enhancers in Human Skin. J. Controlled Release 1987, 6, 85-97, doi:10.1016/0168-3659(87)90066-6.
15. Jiang J, Wang RK. Comparing the Synergistic Effects of Oleic Acid and Dimethyl Sulfoxide as Vehicles for Optical Clearing of Skin Tissue in Vitro. Phys. Med. Biol. 2004, 49, 5283-5294.
16. Batheja P, Sheihet L, Kohn, J et al. Topical Drug Delivery by a Polymeric Nanosphere Gel: Formulation Optimization and in Vitro and in Vivo Skin Distribution Studies. J. Control. Release Off. J. Control. Release Soc. 2011, 149, 159-167, doi:10.1016/j.jconrel.2010.10.005.
17. Beastall JC, Hadgraft J, Washington C. Mechanism of Action of Azone as a Percutaneous Penetration Enhancer: Lipid Bilayer Fluidity and Transition Temperature Effects. Int. J. Pharm. 1988, 43, 207-213, doi:10.1016/0378-5173(88)90275-X.
18. López-Castellano A, Cortell-Ivars C, López-Carballo G, Herráez-Domínguez M. The Influence of Span20 on Stratum Corneum Lipids in Langmuir Monolayers: Comparison with Azone. Int. J. Pharm. 2000, 203, 245-253, doi:10.1016/s0378-5173(00)00463-4.
19. Ran X, Wang J, Liu X et alл Study on the Effect of Compound Penetration Enhancer on the Permeability of Angelica's Water-Soluble Component Ferulic Acid in Vitro. China Surfactant Deterg. Cosmet. 2022, 52, 134-139.
20. Zhang XH, Chuan XX, Hong XX et al. Influence of Penetration Enhancers on in Vitro Transdermal Permeation of Dl-Praeruptorin A. Chin. J. New Drugs 2012, 21, 926-930.
21. Hao JS, Zheng JM, Yang WZ. Transdermal Iontophoresis of Insulin: Effect of Penetration Enhancers on Blood Glucose Level in Diabetic Rats. Yao Xue Xue Bao 1995, 30, 776-780.
22. Osborne DW, Musakhanian J. Skin Penetration and Permeation Properties of Transcutol – Neat or Diluted Mixtures. AAPS PharmSciTech 2018, 19, 3512-3533, doi:10.1208/s12249-018-1196-8.
23. Mura P, Faucci MT, Bramanti G, Corti P. Evaluation of Transcutol as a Clonazepam Transdermal Permeation Enhancer from Hydrophilic Gel Formulations. Eur. J. Pharm. Sci. 2000, 9, 365-372, doi:10.1016/S0928-0987(99)00075-5.
24. Escobar-Chávez JJ, Quintanar-Guerrero D, Ganem-Quintanar A. In Vivo Skin Permeation of Sodium Naproxen Formulated in Pluronic F-127 Gels: Effect of Azone and Transcutol. Drug Dev. Ind. Pharm. 2005, 31, 447-454, doi:10.1080/03639040500214662.
25. 25. Obata Y, Takayama K, Machida Y, Nagai T. Combined Effect of Cyclic Monoterpenes and Ethanol on Percutaneous Absorption of Diclofenac Sodium. Drug Des. Discov. 1991, 8, 137-144.
26. Fang C, Liu Y, Ye X et al. Synergistically Enhanced Transdermal Permeation and Topical Analgesia of Tetracaine Gel Containing Menthol and Ethanol in Experimental and Clinical Studies. Eur. J. Pharm. Biopharm. 2008, 68, 735-740, doi:10.1016/j.ejpb.2007.02.007.
27. Goodman M, Barry BW. Action of Penetration Enhancers on Human Skin as Assessed by the Permeation of Model Drugs 5-Fluorouracil and Estradiol. I. Infinite Dose Technique. J. Invest. Dermatol. 1988, 91, 323-327, doi:10.1111/1523-1747.ep12475655.
28. Príborský J, Takayama K, Nagai T et al. Combination Effect of Penetration Enhancers and Propylene Glycol on in Vitro Transdermal Absorption of Insulin. Drug Des. Deliv. 1987, 2, 91–97.
29. Wotton PK, Møllgaard B, Hadgraft J, Hoelgaard A. Vehicle Effect on Topical Drug Delivery. III. Effect of Azone on the Cutaneous Permeation of Metronidazole and Propylene Glycol. Int. J. Pharm. 1985, 24, 19-26, doi:10.1016/0378-5173(85)90141-3.
30. Zhang D, Ye D, Jing P et al. Design, Optimization and Evaluation of Co-Surfactant Free Microemulsion-Based Hydrogel with Low Surfactant for Enhanced Transdermal Delivery of Lidocaine. Int. J. Pharm. 2020, 586, 119415, doi:10.1016/j.ijpharm.2020.119415.
31. Lee PJ, Langer R, Shastri VP. Role of N-Methyl Pyrrolidone in the Enhancement of Aqueous Phase Transdermal Transport. J. Pharm. Sci. 2005, 94, 912–917, doi:10.1002/jps.20291.
32. Kakubari I, Nakamura N, Takayasu T et al. Effects of Solvents on Skin Permeation of Formoterol Fumarate. Biol. Pharm. Bull. 2006, 29, 146-149, doi:10.1248/bpb.29.146.

33. Larrucea E, Arellano A, Santoyo S, Ygartua P. Combined Effect of Oleic Acid and Propylene Glycol on the Percutaneous Penetration of Tenoxicam and Its Retention in the Skin. Eur. J. Pharm. Biopharm. 2001, 52, 113-119, doi:10.1016/S0939-6411(01)00158-8.
34. Aboofazeli R, Zia H, Needham TE. Transdermal Delivery of Nicardipine: An Approach to In Vitro Permeation Enhancement. Drug Deliv. 2002, 9, 239-247, doi:10.1080/10717540260397855.
35. Squillante E, Maniar A, Needham T, Zia H. Optimization of in Vitro Nifedipine Penetration Enhancement through Hairless Mouse Skin. Int. J. Pharm. 1998, 169, 143–154, doi:10.1016/S0378-5173(98)00110-0.
36. Funke AP, Schiller R, Motzkus HW et al. Transdermal Delivery of Highly Lipophilic Drugs: In Vitro Fluxes of Antiestrogens, Permeation Enhancers, and Solvents from Liquid Formulations. Pharm. Res. 2002, 19, 661-668, doi:10.1023/A:1015314314796.
37. Loftsson T, Somogyi G, Bodor N. Effect of Choline Esters and Oleic Acid on the Penetration of Acyclovir, Estradiol, Hydrocortisone, Nitroglycerin, Retinoic Acid and Trifluorothymidine across Hairless Mouse Skin in Vitro. Acta Pharm. Nord. 1989, 1, 279-286.
38. Bathara M, Date T, Chaudhari D et al. Exploring the Promising Potential of High Permeation Vesicle-Mediated Localized Transdermal Delivery of Docetaxel in Breast Cancer To Overcome the Limitations of Systemic Chemotherapy. Mol. Pharm. 2020, 17, 2473-2486, doi:10.1021/acs.molpharmaceut.0c00211.
39. Rhee YS, Huh JY, Park CW et al. Effects of Vehicles and Enhancers on Transdermal Delivery of Clebopride. Arch. Pharm. Res. 2007, 30, 1155-1161, doi:10.1007/BF02980252.
40. Panchagnula R, Salve PS, Thomas NS et al. Transdermal Delivery of Naloxone: Effect of Water, Propylene Glycol, Ethanol and Their Binary Combinations on Permeation through Rat Skin. Int. J. Pharm. 2001, 219, 95–105, doi:10.1016/S0378-5173(01)00634-2.
41. Furuishi T, Oda, S, Saito H et al. Effect of Permeation Enhancers on the in Vitro Percutaneous Absorption of Pentazocine1). Biol. Pharm. Bull. 2007, 30, 1350–1353, doi:10.1248/bpb.30.1350.
42. Lee CK, Uchida T, Noguchi E et al. Skin Permeation Enhancement of Tegafur by Ethanol/Panasate 800 or Ethanol/Water Binary Vehicle and Combined Effect of Fatty Acids and Fatty Alcohols. J. Pharm. Sci. 1993, 82, 1155-1159, doi:10.1002/jps.2600821118.
43. Mura S, Manconi M, Valenti D et al. Transcutol Containing Vesicles for Topical Delivery of Minoxidil. J. Drug Target. 2011, 19, 189–196, doi:10.3109/1061186X.2010.483516.
44. Subedi RK, Ryoo J-P, Moon C et al. Formulation and in Vitro Evaluation of Transdermal Drug Delivery System for Donepezil. J. Pharm. Investig. 2012, 42, 1-7, doi:10.1007/s40005-012-0002-y.
45. Liu H, Li S, Wang Y et al. Effect of Vehicles and Enhancers on the Topical Delivery of Cyclosporin A. Int. J. Pharm. 2006, 311, 182-186, doi:10.1016/j.ijpharm.2005.12.029.
46. Mathur V, Satrawala Y, Rajput MS. Physical and Chemical Penetration Enhancers in Transdermal Drug Delivery System. Asian J. Pharm. AJP 2010, 4, doi:10.22377/ajp.v4i3.143.
47. Mehta D, Rathod H, Shah D, Shah C. A Review on Microemulsion Based Gel: A Recent Approach for Topical Drug Delivery System. Res. J. Pharm. Technol. 2015, 8, 118, doi:10.5958/0974-360X.2015.00021.9.
48. Heuschkel S, Goebel A, Neubert RHH. Microemulsions – Modern Colloidal Carrier for Dermal and Transdermal Drug Delivery. J. Pharm. Sci. 2008, 97, 603-631, doi:10.1002/jps.20995.
49. Klang V, Valenta CV, Klang C. Valenta, Lecithin-Based Nanoemulsions, J. Drug Delivery Sci. Technol. 21 (2011), 55-76. J. Drug Deliv. Sci. Technol. 2011, 21, 55-76, doi:10.1016/S1773-2247(11)50006-1.
50. Sahle FF, Metz H, Wohlrab J, Neubert RHH. Lecithin-Based Microemulsions for Targeted Delivery of Ceramide AP into the Stratum Corneum: Formulation, Characterizations, and in Vitro Release and Penetration Studies. Pharm. Res. 2013, 30, 538-551, doi:10.1007/s11095-012-0899-x.
51. Singh V, Sharma H, Veerma R et al. Topical Non Steroidal Anti Inflammatory Drug (NSAIDs) Microemulsions: Rationale, Review and Future Prospective. Asian J. Pharm. AJP 2013, 7, doi:10.22377/ajp.v7i1.33.
52. Santos P, Watkinson AC, Hadgraft J, Lane ME. Application of Microemulsions in Dermal and Transdermal Drug Delivery. Skin Pharmacol. Physiol. 2008, 21, 246-259, doi:10.1159/000140228.
53. Kumar KS, Dhachinamoorthi D, Saravanan R.et al. Microemulsions as carrier for novel drug delivery.
54. Zhang J, Michniak-Kohn BB. Investigation of Microemulsion and Microemulsion Gel Formulations for Dermal Delivery of Clotrimazole. Int. J. Pharm. 2018, 536, 345-352, doi:10.1016/j.ijpharm.2017.11.041.
55. Zhu W, Yu A, Wang W et al. Formulation Design of Microemulsion for Dermal Delivery of Penciclovir. Int. J. Pharm. 2008, 360, 184-190, doi:10.1016/j.ijpharm.2008.04.008.
56. Üstündağ Okur N, Çağlar E, Arpa M, Karasulu HY. Preparation and Evaluation of Novel Microemulsion-Based Hydrogels for Dermal Delivery of Benzocaine. Pharm. Dev. Technol. 2016, 22, 1-11, doi:10.3109/10837450.2015.1131716.
57. Kaur A, Sharma G, Gupta V et al. Enhanced Acyclovir Delivery Using w/o Type Microemulsion: Preclinical Assessment of Antiviral Activity Using Murine Model of Zosteriform Cutaneous HSV-1 Infection. Artif. Cells Nanomedicine Biotechnol. 2018, 46, 346–354, doi:10.1080/21691401.2017.1313262.
58. Goindi S, Arora P, Kumar N, Puri A. Development of Novel Ionic Liquid-Based Microemulsion Formulation for Dermal Delivery of 5-Fluorouracil. AAPS PharmSciTech 2014, 15, 810-821, doi:10.1208/s12249-014-0103-1.
59. Soleymani SM, Salimi A. Enhancement of Dermal Delivery of Finasteride Using Microemulsion Systems. Adv. Pharm. Bull. 2019, 9, 584-592, doi:10.15171/apb.2019.067.
60. Okur ME, Ayla Ş, Yozgatlı V et al. Evaluation of Burn Wound Healing Activity of Novel Fusidic Acid Loaded Microemulsion Based Gel in Male Wistar Albino Rats. Saudi Pharm. J. 2020, 28, 338-348, doi:10.1016/j.jsps.2020.01.015.
61. Üstündağ Okur N, Apaydın Ş, Karabay Yavaşoğlu NÜ et al. Evaluation of Skin Permeation and Anti-Inflammatory and Analgesic Effects of New Naproxen Microemulsion Formulations. Int. J. Pharm. 2011, 416, 136-144, doi:10.1016/j.ijpharm.2011.06.026.
62. Wan T, Xu T, Pan J et al. Microemulsion Based Gel for Topical Dermal Delivery of Pseudolaric Acid B: In Vitro and in Vivo Evaluation. Int. J. Pharm. 2015, 493, 111-120, doi:10.1016/j.ijpharm.2015.07.058.
63. Kumari B, Kesavan K. Effect of Chitosan Coating on Microemulsion for Effective Dermal Clotrimazole Delivery. Pharm. Dev. Technol. 2017, 22, 617-626.
64. Abdel-Hamid S, Nasr M. Optimizing the Dermal Accumulation of a Tazarotene Microemulsion Using Skin Deposition Modeling. Drug Dev. Ind. Pharm. 2015, 42, doi:10.3109/03639045.2015.1062512.
65. Špaglová M, Papadakos M, Cuchorova M, Matušová D. Release of Tretinoin Solubilized in Microemulsion from Carbopol

and Xanthan Gel: In Vitro versus Ex Vivo Permeation Study. Polymers 2023, 15, 329, doi:10.3390/polym15020329.
66. Špaglová M, Cuchorova M, Čierna M et al. Microemulsions as Solubilizers and Penetration Enhancers for Minoxidil Release from Gels. Gels 2021, 7, 1-17, doi:10.3390/gels7010026.
67. Okur NÜ, Er S, Çağlar EŞ et al. Formulation of Microemulsions for Dermal Delivery of Cephalexin. ACTA Pharm. Sci. 55, doi:10.23893/1307-2080.APS.05524.
68. Panapisal V, Charoensri S, Tantituvanont A. Formulation of Microemulsion Systems for Dermal Delivery of Silymarin. AAPS PharmSciTech 2012, 13, 389-399, doi:10.1208/s12249-012-9762-y.
69. Scomoroscenco C, Teodorescu M, Raducan A et al. Novel Gel Microemulsion as Topical Drug Delivery System for Curcumin in Dermatocosmetics. Pharmaceutics 2021, 13, 505, doi:10.3390/pharmaceutics13040505.
70. Wu JY, Li YJ, Liu TT et al. Microemulsions vs Chitosan Derivative-Coated Microemulsions for Dermal Delivery of 8-Methoxypsoralen. Int. J. Nanomedicine 2019, 14, 2327-2340, doi:10.2147/IJN.S191940.
71. Telò I, Favero ED, Cantù L et al. Gel-like TPGS-Based Microemulsions for Imiquimod Dermal Delivery: Role of Mesostructure on the Uptake and Distribution into the Skin. Mol. Pharm. 2017, 14, 3281-3289, doi:10.1021/acs.molpharmaceut.7b00348.
72. Subongkot T, Sirirak T. Development and Skin Penetration Pathway Evaluation of Microemulsions for Enhancing the Dermal Delivery of Celecoxib. Colloids Surf. B Biointerfaces 2020, 193, undefined-undefined, doi:10.1016/j.colsurfb.2020.111103.
73. Neubert RHH, Schmalfuß U, Wolf R, Wohlrab WA. Microemulsions as Colloidal Vehicle Systems for Dermal Drug Delivery. Part V: Microemulsions without and with Glycolipid as Penetration Enhancer. J. Pharm. Sci. 2005, 94, 821-827, doi:10.1002/jps.20233.
74. Bubić Pajić N, Ilić T, Nikolić I et al. Alkyl Polyglucoside-Based Adapalene-Loaded Microemulsions for Targeted Dermal Delivery: Structure, Stability and Comparative Biopharmaceutical Characterization with a Conventional Dosage Form. J. Drug Deliv. Sci. Technol. 2019, 54, 101245, doi:10.1016/j.jddst.2019.101245.
75. Benigni M, Pescina S, Grimaudo MA et al. Development of Microemulsions of Suitable Viscosity for Cyclosporine Skin Delivery. Int. J. Pharm. 2018, 545, 197-205, doi:10.1016/j.ijpharm.2018.04.049.
76. Aggarwal N, Goindi S, Khurana R. Formulation, Characterization and Evaluation of an Optimized Microemulsion Formulation of Griseofulvin for Topical Application. Colloids Surf. B Biointerfaces 2013, 105, 158–166, doi:10.1016/j.colsurfb.2013.01.004.
77. De Campos Araújo LMP, Thomazine JA, Lopez RFV. Development of Microemulsions to Topically Deliver 5-Aminolevulinic Acid in Photodynamic Therapy. Eur. J. Pharm. Biopharm. 2010, 75, 48-55, doi:10.1016/j.ejpb.2010.01.008.
78. Patel HK, Barot BS, Parejiya PB et al. Topical Delivery of Clobetasol Propionate Loaded Microemulsion Based Gel for Effective Treatment of Vitiligo: Ex Vivo Permeation and Skin Irritation Studies. Colloids Surf. B Biointerfaces 2013, 102, 86-94, doi:10.1016/j.colsurfb.2012.08.011.
79. Biruss B, Valenta C. The Advantage of Polymer Addition to a Non-Ionic Oil in Water Microemulsion for the Dermal Delivery of Progesterone. Int. J. Pharm. 2008, 349, 269-273, doi:10.1016/j.ijpharm.2007.08.003.
80. Butani D, Yewale C, Misra A. Amphotericin B Topical Microemulsion: Formulation, Characterization and Evaluation. Colloids Surf. B Biointerfaces 2014, 116, 351-358, doi:10.1016/j.colsurfb.2014.01.014.
81. Raza K, Negi P Takyar S et al. Novel Dithranol Phospholipid Microemulsion for Topical Application: Development, Characterization and Percutaneous Absorption Studies. J. Microencapsul. 2011, 28, 190-199, doi:10.3109/02652048210.546435.