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# FORMULATION INSIGHTS: INFLUENCE OF EXCIPIENTS AND PERMEATION ENHANCERS ON THE TRANSDERMAL DELIVERY OF ACECLOFENAC GEL

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**ABSTRACT**

Topical gels have emerged as effective alternatives to oral non-steroidal anti-inflammatory drugs (NSAIDs), offering localized drug delivery with reduced systemic side effects. Aceclofenac, a widely prescribed NSAID, demonstrates limited skin permeability, necessitating the incorporation of suitable excipients and permeation enhancers to optimize transdermal delivery. This study evaluates the effect of different excipients and permeation enhancers on the physicochemical properties, spreadability, and permeation efficiency of aceclofenac gel formulations. Various polymers, solvents, and surfactants were examined for their role in improving drug release and dermal penetration. Permeation enhancers such as propylene glycol, ethanol, and dimethyl sulfoxide (DMSO) were found to significantly influence drug flux across the skin barrier by altering lipid bilayer fluidity and solubility. Comparative analysis revealed that optimized combinations of excipients and enhancers not only enhanced drug permeation but also improved patient-friendly attributes such as non-greasiness and ease of application. These findings underline the critical role of formulation strategies in designing effective topical gels for targeted anti-inflammatory therapy.

## I. INTRODUCTION

Human skin provides an excellent barrier against the external harsh conditions, pathogens and other environmental threats. At the same time, it provides ample opportunities for transdermal drug administration owing to its large surface area and easy accessibility1 . Transdermal drug delivery methods have certain advantages over conventional methods such as intravenous injections, oral ingestion and intramuscular injections2,3 . For example, in case of oral administration, drugs have to pass through the gastro-intestinal (GI) tract, where they could be prone to degradation due to the harsh physicochemical environment4 . Transdermal delivery eliminates drug exposure to the GI tract and frst-pass metabolism, and ensures the sustained and controlled release of drugs2–4 . Realizing the full potential of transdermal delivery, however, is limited by the protective barrier provided by the outermost layer of the skin known as stratum corneum (SC). Te SC is about 15–20μm in thickness and is comprised of keratin-rich corneocytes surrounded by the lipids5,6 . Te SC layer is arranged in a brick and mortar like structure where corneocytes occupy the majority of SC volume and the space between the corneocytes is flled with a lipid matrix which provides pathways for percutaneous absorption6 . Te SC is highly selective and only few molecules (small and relatively lipophilic) can pass through it. Te SC is supported by viable epidermis, dermis and subcutaneous connective tissue, and these layers could potentially ofer additional barriers to drug transport. Efective breaching of the SC's protective barrier is a major challenge in transdermal drug delivery. Hence, only a small number of transdermal formulations are commercially available7 . Various ways have been proposed to breach the SC barrier and these methods are broadly classifed as active and passive methods. Te former class uses external energy sources such as iontophoresis8 ,

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sonophoresis9,microneedles10,
electroporation11 to temporarily breach the skin barrier whereas the latter class uses chemical permeation enhancers and ionic liquids, among others12,13. Although, active methods ofer quicker onset and have found applications in local anesthetic14, glucose monitoring15 and vaccination16, they pose certain limitations such as complexity and cost17. To date, several transdermal permeation enhancers have been studied18,19. More than 350 molecules have been shown to enhance the skin permeability via diferent mechanisms. Tese molecules include fatty acids and fatty alcohols, alcohols and glycols, terpenes, sulphoxides, laurocapram, pyrrolidones, surfactants, urea, among others13,18,19. However, very few of them have been successfully used in currently marketed transdermal products19. Hence, exploration of new chemicals that can safely improve skin permeability still remains an active area of transdermal research.

Several in-vivo and in-vitro studies have been carried out on animal or human skin to develop novel CPEs18–26. Eforts have also been made to use known CPEs to design their synergistic combinations. Specifcally, a novel tool termed in vitro skin impedance guided high-throughput (INSIGHT) screening was developed and used to obtain synergistic mixtures of CPEs which could deliver macromolecular drugs, including heparin, luteinizing hormone releasing hormone (LHRH) and oligonucleotides, across the skin20. Attempts have also been made to study the mechanisms of action of CPEs and classify them based on their action rather than chemical identity18,19,22. Design of CPEs based on frst principles, in spite of its clear appeal, has been limited by the complexity of the enhancer-skin interactions. In the last decade, advances in computing hardware and development of efcient algorithms have encouraged researchers to use computer simulations for many drug delivery applications27,28. Researchers have also developed simple in-silico models of skin lipid matrix29–31 and successfully coupled them with macroscopic models32. Tese models have been validated with available experimental release profles of drugs. In these reported studies, difusion of molecules through the skin lipid matrix is obtained from molecular dynamics simulation and used as an input to the macroscopic (having both corneocyte and lipid matrix) model to obtain the release profle through the skin SC32. Recently, researchers have also explored computer simulations for the design of formulation using fullerenes for cosmetic application33, design of nanoparticle for drug delivery application34, and co-delivery mechanism of gold nanoparticles for protein delivery35. In this study, we present the development of a multilayer in-silico multilayer skin lipid matrix model34 for testing the efect of CPEs on SC lipids. Long CG molecular dynamics (MD) simulations of multilayer skin lipid matrix model were carried out in the presence of CPEs at diferent concentrations (1, 3 and 5% w/v). Predictions of the simulations were compared to experimental measurements from the literature.

## II. SYSTEM, MODEL, PARAMETERS AND METHODS

**in-silico skin model.** The corneocytes and skin lipids are organized in brick and mortar assembly inside the SC6. The lipid matrix is composed of various types of ceramides (CERs), fatty acids (FFAs) and cholesterol (CHOL)36. The CERs are classified based on the number and position of -OH groups and the degree of unsatu- ration present in the structure37,38. To date, 18 classes of the CERs have been discovered with over 300 to 1000 distinct CER derivatives37,38. In spite of the tremendous advances in computational capabilities in the last decade, simulating a multilayer skin lipid matrix with accurate CER and FFA distribution is still beyond the current tech- nical feasibility. Here, we represent the ceramide family only by non-

hydroxy sphingosine ceramide (CER-NS). Although it is a simple approximation, it has been validated in previous studies33–35. The skin permeation process is very slow and generally occurs at a ms-μs time scale depending upon the size, shape and surface chemistry of the molecule39–41. In order to model the heterogeneous mixture of CERs, FFAs, CHOL multi-layer with various chemical penetration enhancers at the realistic time and length scale, CG models42,43 were used. The CG param- eters for ceramide (CER), free fatty acid (FFA) and CHOL were taken from our earlier work33–35. The parameters for the permeation enhancers molecules were derived from the atomistic models. In the MARTINI model, atoms were mapped to CG beads using mapping rules. Generally on an average four or three (for ring structures) heavy atoms were represented by a single bead. The parameterization of CPEs is discussed in section 2.2 in details.

The skin lipids are arranged in multiple lamellar domains. In order to simulate the realistic lamellar model, a multilayer skin lipid matrix model is used in this study. The CG structure of skin lipid bilayer (Fig. 1), equili- brated for 3 μs, was taken from our earlier work34. In the multilayer skin lipid matrix model, two lipid bilayers are kept next to each other and solvated with water across the top and bottom leaflet. In the experimental data used for comparison20,22 the formulation possessed 1.5% (w/v) CPE in 1:1 ethanol: phosphate buffered saline. The presence of phosphate ions in the buffer may potentially contribute to the experimental outcome which is not totally captured in the simulations. To mimic the experimental condition, half of the water in multilayer skin lipid matrix model was replaced with ethanol. The simulations were further run for 3 μs in NPT ensemble to obtain a structure (Fig. 1) that was used as an in-silico multilayer skin lipid matrix model for the testing. The simulation box possessed 936, 900, 936, 5760, 5760 CER, CHOL, FFA, water and ethanol molecules, respectively. The initial size of the lipid layer was 15.92 nm × 15.92 nm × 14.92 nm. Hence forward, in-silico multilayer skin lipid matrix model is referred as skin lipid layer.

## III. RESULTS AND DISCUSSION

**Interactions of CPEs with skin lipid layer.** The MD simulations of each permeation enhancer with skin lipid layer were carried out at three different concentrations (1% w/v, 3% w/v and 5% w/v). The permeation enhancers were randomly placed in the upper part of the multilayer model (near the headgroup of top layer). The system was energy-minimized using the steepest decent method. The minimized structure was subjected to a 200 ns NVT run by restricting the motion of the permeation enhancer molecules using position restraints. Later, the constraints were slowly released and the system was run for another 200 ns in NVT run followed by 250 ns in NPT equilibration. The final equilibrated structure was subjected to a 3 μs production run using the NPT ensemble. The configuration was sampled every 100 ps in the production run for the calculation of structural and thermodynamic properties.

The permeation enhancers belong to several functional groups such as fatty acid, alcohols, esters, and terpe- nes, among others (Table S1). Figure 2 shows the final snapshots (at the end of 3 μs run) of each fatty acid system interacting with the skin lipid layer at a concentration of 1%w/v. The snapshots at 3%w/v and 5%w/v are shown in Figs S6 and S7 respectively. The mechanisms of interactions of each fatty acid with skin lipid layer are similar, first they partition from the upper (1:1: water: ethanol) layer to the upper leaflet of the lipid layer. Subsequently, they translocate in both lateral and normal directions of the lipid layer. Many of the fatty acid molecules also cross the first layer of the skin lipid layer and reach to the bottom of the skin lipid layer. Each enhancer was found to

be well dispersed in the interior of the lipid layer (Top view Fig. 2).

Molecules such as Isopropyl palmiate (ISP), Octylpyrrolidone (OCP), Glyceryl monooleate (GMO) and Geraniol (GOL) partitioned completely from the upper solvent layer to the skin lipid layer (Fig. 2), whereas LEM partitioned partially and DMSO did not partition in any of the simulations. Permeation of ISP, LEM and GOL was similar to that of the fatty acids. While OCP and GMP partitioned into the lipids, they formed small clusters inside the skin lipid layer (Top view Fig. 2) hindering their motion in the normal direction, and very few mole- cules crossed the upper lipid layer. In our simulations, DMSO did not partition at any concentration. One reason could be the favorable condition provided by the solvent and second could be the concentration used here. In an earlier simulation study of DMSO with phospholipid bilayer, the partition and pore formation in the bilayer was achieved at a higher concentration (>26 mol %)56. Similarly, phase transition and partition of DMSO in CER bilayer occurred at very high concentration (>40 mol %)57. In current simulations, DMSO concentration is in the range of (<1 mol %) and hence could be a reason for lack of partitioning.

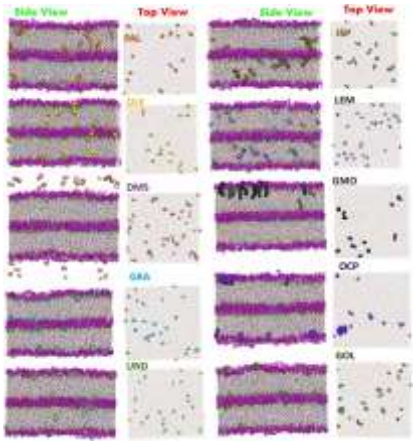



Figure 2. Interaction of CPEs with in-silico skin. Snapshots of the final configuration of the skin lipid layer interacting with chemical permeation enhancers (at 1%w/v concentration). Side view (shows permeation of CPEs) and top view (shows dispersion or agglomeration of CPEs inside the layers) are shown. All systems were run for 3 µs. The skin lipid constituent CER, CHOL and FFA are shown (in CPK form of VMD software) in red, green and blue colors, respectively. The solvent molecules (ethanol and water) are not shown in this figure. The permeation enhancers are shown in VDW form of VMD software. All snapshots were captured using VMD software60.

The density distributions of each penetration enhancer inside the lipid layer in different systems are shown in the Fig. 3. It is clear that each permeation enhancer, except DMSO, entered and crossed the skin lipid layer. The extent of permeation depends on the size, structure and interaction with the skin lipid layer constituents. Based on the hydrophobicity (as presented in terms of log P, Table 1), permeation enhancers partition from the solvent into the lipid layer and then move (both in lateral and normal directions) based on their mobility (diffusion coefficient). The interactions of CPEs with skin lipid constituents were quantified in terms of radial distribution function g(r) and are shown in Fig. 4 (at 1%w/v concentration). The g(r) profiles for higher concentration 3%w/v and 5%w/v systems are provided in supporting information (Fig. S8). The radial distribution function was calculated using the GROMACS function g_rdf. The g(r) was calculated between the center of mass of individual lipids con- stituents (CER, FFA and CHOL) and each CPE. In case of fatty acids, long chain fatty acids PAL and OLE inter- acted heavily with each FFA, as can been seen by a clear peak position in g(r) profiles. On the other hand, small chain fatty acids UND and GRA interacted mostly with FFA and CHOL, as suggested by the dual peak of CHOL. This could be due to the reduced hydrophobicity of small fatty acid and large differences in chain length (UND chain length = 10 C united atom, GRA chain length = 8 C united atom) with that of FFA (chain length = 24

C united atoms) present in skin lipid layer. Other permeation enhancers interact with each of the lipid constituents. DMS did not partition inside the skin lipid layer hence g(r) values are lesser than 1.

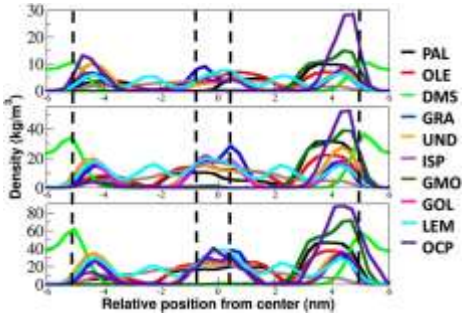



Figure 3. CPEs distribution in the skin lipid layer. The density distribution of each permeation enhancer along the lipid layer normal calculated in the last 1 μs of production run. The z = 0, represents the mid of the lipid layer. The dash black vertical lines indicate the average position of headgroups in each layer.

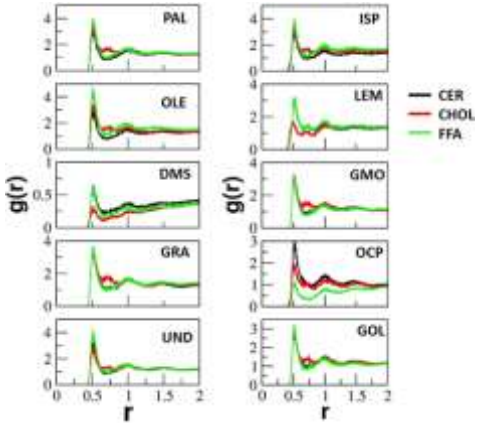



Figure 4. Interaction of CPEs with individual constituents of skin lipid layer. The radial distribution function g(r) of each permeation enhancer with the skin lipid constituents (at 1%w/v concentration), calculated in last 500 ns of production run. The peaks in g(r) profiles show the extent of the interaction between permeation enhancers and the particular lipid constituent.

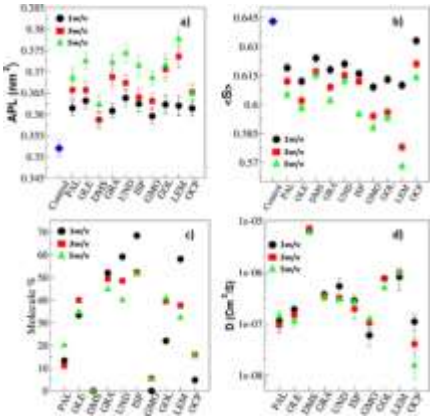


Figure 5. Effect of CPEs on structure of skin lipid layer. The structural properties: (a) area per lipid (APL), (b) overall order parameter (<S>) and dynamics properties, (c) percentage of molecule permeation from the top to the bottom layer (molecule %), and (d) diffusion coefficient of permeation enhancers in the skin lipid layer. The properties are calculated in the last 1 μs of production run. Control indicates the skin lipid layer system without permeation enhancer.

**Structural Properties of Skin layer in the presence of CPEs.** Each permeation enhancer molecule, other than DMSO, partitioned and moved to the top and subsequently bottom layer of the skin lipid layer (Fig. 2). These molecules significantly changed the lipid layer structural properties. We calculated two most important properties from the simulations namely, area per lipid (APL) and overall order parameter (<S>). The details of the calculation of these properties are given in section 2.4. The APL gives information about the extension of the lipid layer in the XY plane once the permeation enhancers are inside the lipid layer. The overall order parameter gives information regarding the orientation and disturbance in the packing of the lipid layer. Other two dynamics properties, percent permeated from one layer to another layer and diffusion coefficient of molecules inside the skin lipid layer were also calculated.

Figure 5a–d show the structural properties APL and <S> as well as the dynamics properties percent perme- ation and diffusion coefficients

of each system at different concentrations. The percent permeation of CPEs is the fraction of total CPE molecules placed initially in the upper reservoir that crossed the upper leaflet and reached to the bottom leaflet. Due to periodic boundary conditions, in some cases few molecules moved from the upper reservoir to the bottom reservoir, these molecules were excluded from the calculation of percent permeation. The trajectory of each molecule was visualized and molecules that crossed the upper leaflet were counted manually. The diffusion coefficient was calculated from the mean square displacement determined using g_msd utility of GROMACS (see Fig. S9). There is qualitative inverse correlation between APL and overall order parameter. It is clear that at higher concentrations, more molecules permeated into the lipid layer and they induced expansion of the lipid layer in the lateral direction. At the same time, increased CPE molecules present in the lipids reduced the order and the order parameter.

In case of fatty acids, the oleic acid system led to lowest <S> and UND led to highest APL at a given con- centration. The increased APL for UND system originates from the permeation of larger number of molecules (Fig. 5c). The OLE system has a lower order parameter because of its unsaturation, which provides a kink in the skin lipid layer. All fatty acid molecules remained dispersed in the skin lipid layer (Top view Fig. 2), as their dif- fusion coefficient did not change significantly with the concentration (Fig. 5d). The smaller sized UND and GRA exhibited a high diffusion coefficient as compared to PAL and OLE, which resulted in their higher molecular permeation. An interesting point to be noted is that PAL and OLE exhibited a similar order of diffusion coef- ficient, but OLE exhibited higher permeation. This is because oleic acid significantly disturbs the packing due to its structure. The packing disturbance could be related to the order parameter. Based on the simulations, the trend for disturbing the packing by fatty acids should be in the following order OLE > PAL > GRA > UND. Our observations of permeation enhancing mechanism of fatty acid are well in line with the experimental findings reported in the literature21–23.

**Kim et al.21** have performed permeation experiments of diclofenac in the presence of various fatty acids on rat-skin and showed that palmitic acid and oleic acid were the best permeation enhancers in the saturated and unsaturated category, respectively. Our simulations predicted that oleic acid worked as the best permeation enhancer among all fatty acids studied. Karande et al.22 have performed experiments on porcine skin and meas- ured skin conductivity before and after the application of fatty acid permeation enhancers. The enhancement in skin conductivity was related with the enhancement in molecular flux58. The experimental ER values reported for OLE, PAL and UND were 30.01, 18.87 and 3.66, respectively. Ibrahim et al.23 studied the effects of fatty acids that are commonly present in cosmetic and in topical formulations on permeation enhancement across the human epidermal membrane (HEM). The flux enhancement ratio was in the order of OLE >PAL > UND. Kezutyte et al.59 performed permeation experiment of lipophilic model drug tolnaftate in the presence of oleic, linoleic, lauric and capric acids into human skin using time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging. It was shown that the flux of the drug was maximum (1.87 times of control experiment) in the presence of oleic acid.

DMSO exhibited minimum APL values and diffusion coefficients were found to be an order of magnitude higher for a given concentration as it did not partition in the lipid layer. However, surprisingly, the overall order parameter was not the maximum for the DMSO. The OCP system exhibited maximum <S> for a given concen- tration, the reason could be the agglomeration of

OCP (Top view Fig. 2) in the skin lipid layer after partitioning. The cluster creates local changes in the tail order parameter, while if the molecules were well dispersed (like in the case of other enhancers) they disturbed the packing throughout the lipid layer. The diffusion coefficient values also decreased (due to agglomeration, Fig. 2) with an increase in the concentration of OCP. GMO also formed small clusters, but affected the order parameter significantly due to its structure. The GMO molecules did not cross the lipid layer significantly. Small molecules such as LEM and ISP crossed the lipid layer significantly (as can be seen from the percentage molecule permeate and diffusion coefficient) but did not create much disordering in the skin lipid layer as compared to GMO.

Overall, based upon the above four parameters and observation (Table 2), it could be concluded that small hydrophobic molecules partition well into the skin lipid layer and do not agglomerate. On the other hand, bigger hydrophobic molecules partition well and disturb the lipid layer packing significantly, but they sometime form small clusters and limit permeation by the diffusion rate.

**In-silico screening.** Based on the conclusions made in section 3.2, the overall order parameter could be used for quantifying the structural changes induced by CPEs in the skin lipid layer. Experimental evaluation of overall order parameter is extremely challenging. Hence, indirect comparisons of predicted order parameters with exper- imental measurements are necessary. Karande et al.22 have reported on the effect of several CPEs on porcine skin which was quantified as the conductivity enhancement ratio (ER) defined as skin conductivity at the end of 24 h normalized by that at time zero. Since conductivity is a measure of ion mobility in the skin which is limited by diffusion across the lipids, the enhancement ratio is a measure of CPE-induced lipid disruption.

In simulations, overall order parameter also reflects disruption of the lipids. Specifically, the order parameter is inversely proportional to the extent of lipid disruption. Figure 6 shows the comparison of ER and reciprocal of overall order parameter (1/<S>) for each CPE at 1%w/v. Comparisons of ER and 1/<S> for higher concentration 3%w/v and 5%w/v are provided in supporting information (Fig. S10). Experimental ER and simulated 1/<S> exhibited a good correlation. For example, ER values of fatty acids showed the trend of OLE > PLE > UND, and a similar trend was observed in simulations. Another significant trend was observed for OCP/GMO, which exhib- ited the lowest/highest ER and the lowest/highest 1/<S> value for each concentration. GMO exhibited highest perturbation of lipids and it interacted extensively with all three constituents of the skin lipids (as shown through radial distribution function in Fig. 4). OCP also exhibited affinity towards CER and CHOL, but due to agglom- eration (Fig. 2) and less diffusion (Fig. 5d), it did not significantly perturb the packing, hence exhibited a lower permeation enhancing effect.

The in-silico model presented here reasonably captures the experimentally observed trends and could be used for future testing purposes. Some challenges remain which need to be further explored. First, the model only used single type of CER, which is a simplistic representation of skin lipid layer. We are currently developing parameters for other CERs which will further enhance the model in future. Second, the model only has a lipid matrix, but, some CPEs first partition into corneocytes and then into the lipid layer. Studying such CPEs, with current model, may not show partitioning at all. For example, DMSO was not able to partition because of its hydrophilicity and low concentration. Simulations of corneocytes is a challenging task. However, a combination of a micro and macro scale model that incorporate corneocytes may pave the way. Also note that the toxicity of

CPEs could not be captured using current model since it does not incorporate biological elements required for assessment of toxicity.

| Name | Functional group | $EF^{a}$ | $\log P$ (conclusion) | Observation |
|---|---|---|---|---|
| Oleic Acid (OLE) | Acid | 30.83 | — | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and many molecules are crossing lipid layers |
| Palmitic Acid (PLA) | Acid | 18.81 | — | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and many molecules are crossing lipid layers |
| Linoleic Acid (LIOL) | Acid | — | 2.28 | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and many molecules are crossing lipid layers |
| Undecanoic acid (UND) | Acid | 11.66 | — | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and many molecules are crossing lipid layers |
| DMSO (DMS) | DMSO | 12.23 | — | • No Partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in solvent (water / ethanol) layer and no crossing of lipid layer |
| Geraniol (GOL) | Unsaturated alcohol | 22.74 | 3 | • Partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and crossing lipid layer |
| Glycerol monooleate (GMO) | Glycerol ester | 34.22 | 6.86 | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Small clusters are forming in the lipid bilayer and very few molecules crossing layer |
| Isopropyl palmitate (ISP) | ester | 27.15 | 10.04 | • Complete partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and crossing lipid bilayer |
| Limonene (LIM) | Unsaturated monoterpene | 22.82 | 4.57 | • Partitioning from solvent to the top layer of skin lipid layer<br>• Dispersed in lipid layer and crossing lipid bilayer |
| Octyl pyrrolidone (OCP) | Pyrrolidone | 7.81 | 5.87 | • Partitioning from solvent to the top layer of skin lipid layer<br>• Agglomerating in the skin lipid bilayer and very few molecules crossed the layer at high concentration |

Table 2. The structural and physical properties of each permeation enhancer used in simulation and the observation obtained after the production run.

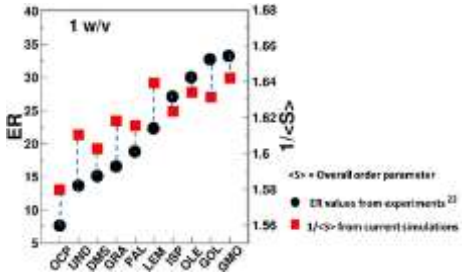



Figure 6. In-silico testing validation. Comparison of experimentally measured enhancement ratio (ER) and the calculated (1/<S>) from simulations. Both properties are plotted on different axes due to the differences in the magnitudes of each property.

## IV. CONCLUSIONS

The study establishes that the selection of excipients and permeation enhancers plays a pivotal role in determining the therapeutic performance of aceclofenac gel formulations. Properly optimized combinations enhance the drug's permeability, stability, and patient compliance, thereby increasing its clinical utility in localized pain and inflammation management. Permeation enhancers such as propylene glycol and ethanol demonstrated superior outcomes in improving drug flux without compromising formulation stability. These results suggest that rational excipient selection, combined with safe permeation enhancers, can transform aceclofenac gel into a more effective transdermal delivery system. Future research should focus on advanced permeation technologies, in vivo evaluation, and the development of novel carrier systems such as nanoemulsions or liposomal gels to further enhance therapeutic outcomes.

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