Transdermal drug delivery can offer significant advantages over the more conventional routes of delivery. These benefits include lower toxicity, the avoidance of the hepatic first-pass effects, less variability, better control, the immediate termination of drug delivery if required, and improved customer/patient compliance.

This promise, however, is not fully realised because, for many molecules, the skin constitutes a significant barrier. A variety of approaches have been proposed to reversibly breach this barrier, the most significant are the ‘passive’ approach involving the use of chemical penetration enhancers, and the more recent active approaches such as iontophoresis (use of a electrical potential to enhance penetration), electroporation (application of short, high-voltage pulses to induce temporary micropores in the skin tissue), sonophoresis (use of low-level ultrasound), and the use of microneedles.

While the most established approach is that involving chemical penetration enhancers, how such molecules increase the skin permeability is essentially still a mystery. Clearly, a greater understanding of the action mechanisms of penetration enhancers would be invaluable for the rational design of such molecules. In-silico methods are revealing the secrets of such molecules in modulating the permeability of lipid membranes, and there is potential in this technology for screening molecules with a view to identifying more effective and safer penetration enhancers.

In-silico methods

Our understanding of how permeation enhancers work is mainly based on empirical knowledge. While there are some pointers as to how a particular enhancer might exert its effect, the underlying molecular mechanisms remain obscure, which is a direct consequence of limitations associated with experimental methods.

An alternative, and a way forward, is to employ molecular modelling and simulation. These in-silico methods offer unprecedented molecular-level resolution with the ability to...
reproduce bulk and molecular level properties. They can yield important insights, inform experimental studies, and demonstrate a significant predictive potential.

The basis for molecular simulation is the molecular forces between atoms and molecules that are now sufficiently well characterised. This knowledge of the forces enables us to ‘simulate’ a collective behaviour of a specified system as a function of time (trajectory), for example, a lipid membrane in water containing molecules of a particular penetration enhancer. The method, known as molecular dynamics (MD) simulation, employs Newtonian mechanics to calculate the evolution of the molecular system, which is dictated by the interaction forces between the molecules (see Figure 1).

**Figure 1. Molecular dynamics simulation.**

1. The initial atomic positions are arbitrarily assigned; the atoms are also assigned velocities that are consistent with the temperature of interest. 2. The force on each atom due to the other atoms in its neighbourhood is calculated. 3. From knowledge of the force, initial positions, and initial velocities, the new set of positions of the atoms at some later time (typically 10⁻¹⁰ seconds) are calculated. The atoms are then displaced to their new positions. This process (the calculation of the forces and molecular displacements) is iterated millions of times to yield a collective trajectory of the dynamics of the molecules.

Molecular simulations can also yield estimates for thermodynamic properties, such as chemical potentials and permeability barriers. Simulations can be carried out at constant temperature and pressure, hence enabling direct comparison of the results with those obtained from experiments. The trajectories resulting from MD simulations on viewing reveal the dynamical behaviour of the molecules in the system. Thus, in effect we have a microscope with atomic resolution, albeit based on simulation. While individual molecular events may not be entirely accurate reflections of reality, it appears (as with real systems) that the average molecular behaviour in simulations can reflect real systems.

For membrane systems, simulations can routinely reproduce experimental quantities such as the bilayer thickness, area per lipid headgroup, volume per lipid, diffusion rate constants, lipid tail disorder parameters, electronic density profiles, and mechanical properties with an accuracy of a few per cent. Other than offering a window on the molecular level processes, molecular simulations can also yield estimates for thermodynamics properties, such as solubilities, chemical potentials and permeability barriers.

**Transdermal delivery**

The primary barrier to the delivery of drug molecules through the skin is the lipid matrix of the skin, the stratum corneum. The stratum corneum consists of dead top-most layer cells known as corneocytes, which are embedded in a matrix of lipids in a ‘bricks and mortar’-type arrangement. The lipids consist mainly of ceramides (+50%), cholesterol (+25%), cholesterol sulfate (+5%), fatty acids (10-15%) and a small amount of cholesterol esters, with little or no phospholipids.

As to how these lipids are structurally arranged, we are not entirely sure other than that they are in a crystalline lamellar form, comprising possibly as bilayer or three-layer ‘sandwich’ structures. Because of the complexity of the skin’s lipid matrix, it is common to carry out fundamental experiments on well-characterised phospholipid membranes, which qualitatively exhibit some of the features of the stratum corneum lipids. For this reason, both experimental studies and simulations have mostly focused on single-component bilayers of phospholipids, typically dipalmitoylphosphatidylcholine (DPPC). This approach follows the normal scientific practice of characterising simpler systems before introducing complexity.

Many compounds are known to compromise the barrier function of the skin and to facilitate the penetration of active molecules. These include urea and its derivatives, alkyl sulfoxides including dimethyl sulfoxide (DMSO), various surfactants, Azone® (1-dodecylazacycloheptan-2-one or laurocapram) and oleic acid. Of these, we have studied the effects of Azone, DMSO and oleic acid on DPPC lipid bilayers. Outlined below are the effects of DMSO to illustrate the powerful insights that can be obtained by molecular simulation.

**DMSO and molecular simulation**

DMSO is a small amphiphilic molecule with a hydrophilic sulfoxide group and two hydrophobic methyl groups (Figure 2). Its amphiphilic (dual) nature appears to be an important defining characteristic for its action on membranes. Other than being an effective penetration enhancer, DMSO is widely employed in cell biology (to induce cell fusion and cell differentiation) and as a cryoprotectant. The penetration-enhancing effects of DMSO are concentration-dependant and it is reported that concentrations in the region of 60% in the formulation are required to achieve significant penetration enhancement through skin.

The effects of DMSO on the DPPC membrane in the liquid-crystalline phase were studied systematically using molecular dynamics simulations. The DMSO concentrations varied from 0 mol % (solvent phase being pure water) to 100 mol % (solvent being pure DMSO). DMSO appears to exhibit three distinct modes of action on the phospholipid membrane, the specific mode being dependent on its concentration. Depending on concentration, DMSO is able to decrease the
membrane thickness (2.5–7.5 mol %), induce formation of transient water pores (10–20 mol %), or destroy the bilayer structure of phospholipid membranes (25–100 mol %). These modes of action are summarised visually in Figure 3.

The observed DMSO-induced thinning and expansion of a phospholipid bilayer coupled with the increased fluidity of its hydrophobic core are able to explain why DMSO promotes the permeation of solutes, in particular hydrophobic entities, across the membrane: the length of the path required for solute permeation is reduced as the membrane thickness drops, and diffusion itself is facilitated because of the enhanced fluidity of the membrane interior. The observations, however, do not entirely explain the DMSO-induced enhancement of hydrophilic molecules.

Probably the most remarkable finding of the study is the induction of transient water pores by DMSO. These water pores are essentially holes within the lipid membrane, which are filled with water and provide a continuous link between the separate regions of water on either side of the bilayer. The significance of the water pores is that they could serve as a pathway for hydrophilic molecules across the membrane since, in this case, a hydrophilic molecule can avoid direct contact with the membrane hydrophobic core. This mechanism is consistent with the experimentally observed concentration dependent effects of DMSO on membranes.

Experimentally, permeability enhancement only occurs at high DMSO concentrations, and high concentrations are also required to induce pore formation in the simulations. DMSO is known to enhance the penetration of both hydrophilic and hydrophobic molecules and the enhancement of hydrophilic compounds by DMSO has always been difficult to explain. Molecular mechanisms proposed in the past to explain permeability enhancement by DMSO include interaction with membrane proteins leading to structural defects at the protein-lipid interface, fluidisation of the membrane, and a 'solvent effect' that facilitates the partitioning of the drug molecules from the formulation into the skin.

These mechanisms can explain the enhancement of hydrophobic molecules but not (convincingly) of hydrophilic molecules. In fact it is difficult to conceive of a simple mechanism for the enhancement of a hydrophilic penetrant other than by the observed formation of water pores. Interestingly, the water pore formation links remarkably well with many of DMSO’s other pharmacological effects – including analgesia, protection against ischemic injury, and cryopreservation – that all involve modulation or disruption of ion or water transport across a cell membrane.

While phospholipids such as DPPC have served well as model membranes, there is clearly a need to move to the ceramide group of lipids, which are the main...
building blocks of the lipid matrix of the stratum corneum, and hence directly relevant to transdermal drug delivery. Until the recent studies from our group\(^6\), simulations of skin lipids have remained essentially an unexplored niche. The recent simulations have been carried out on bilayers of ceramide 2 (the predominant lipid in the stratum corneum), and have investigated the effects of DMSO on the ceramide bilayers in the gel phase structure, the phase that is thought to exist in the stratum corneum.

The gel phase is a rigid, densely packed structure in contrast to the more fluid, liquid crystalline structure typical of lipid domains of plasma membranes at physiological temperature. These studies reveal that DMSO at high concentrations induces a phase transition of the gel phase structure to the fluid-like, liquid-crystalline structure (Figure 4).

The liquid-crystalline phase of ceramides, being more fluid, is expected to be markedly more permeable to solutes than the gel-phase structure. These results suggest that DMSO at high concentrations fluidises the lipids of the stratum corneum and enhances its permeability. The possibility of water pore formation within the resulting liquid crystalline phase, as with phospholipids, exists but remains to be explored.

The step up from pure ceramide bilayers is to incorporate the next bit of complexity into these model membranes, that is towards developing heterogeneous systems containing the predominant components of the skin lipids, namely ceramide 2, cholesterol and a fatty acid. Such developments are underway, both by us and others, using a combined experimental and modelling approach. The research is attempting to correlate the permeability of these heterogeneous model membranes with that of real skin for a variety of molecules. The goal is to identify an appropriate composition and structure of the model membrane that could then serve as a test bed for molecular experiments (real and virtual) to investigate both the transport of molecules and the effects of membrane barrier modulators.

Although much of the above discussion emphasises the molecular insights that can be gained from computer simulations, it is also worthy to mention a special type of simulations that are able to provide a quantitative estimate of the permeability of a membrane to a particular molecule. Such simulations are based on free-energy calculations, essentially employing the full toolbox of statistical thermodynamics.

These calculations are still in their infancy, but clearly their wider application will have considerable impact on drug development. For example, a prototype study estimated the permeability of DPPC membrane at 323K for a number of small organic molecules\(^5\). The estimated permeability coefficients were found to be an order of magnitude larger than experimental data, but the relative permeabilities of the molecules were reproduced. Extension of this methodology to permeation of drug molecules across ceramide-based model membranes will set the foundations for an in-silico screen for identifying effective membrane permeability enhancers.

Outlook

It is acknowledged that transdermal delivery is unlikely to ever become a universal delivery route. However, this does not diminish its significance. For the appropriate molecule it is ideal, particularly from a patient’s perspective. Its significance is also reflected in the market value of transdermal drug delivery, which totalled $5.7 billion in 2006, and is predicted to increase to about $7.9 billion by 2010 (Kalorama Information).

A breakthrough in identifying one or two penetration enhancers that are effective and safe could significantly extend the molecular weights (currently <500 Daltons) of candidate molecules that can be delivered transdermally to include possibly biopharmaceuticals such as potent peptides. Such a breakthrough would also have a major impact on marketed drugs as companies are looking for innovative drug delivery systems to extend patent life and increase patient compliance.

The required molecular level resolution is now readily accessible by molecular simulation.

This issue of product life-cycle management is becoming increasingly important because of a lack of new chemical entities in the pipelines. The identification of suitable penetration-enhancing molecules could come about empirically, but a more rational approach based on molecular level understanding promises a higher chance of success. The required molecular level resolution is now readily accessible by molecular simulation, which is now emerging as a reliable, mature technology. Indeed, it is timely to initiate projects with the specific objective of identifying membrane penetration enhancers based on a combined approach involving molecular simulation in tandem with experiments such as high throughput screening\(^6\).

Such a project can also identify molecules that decrease the permeability of membranes. These molecules would be useful for their potential ability to block absorption of formulation excipients that may be irritants or which could induce an immune response, and for minimising toxic effects of chemicals such as insect repellents, pesticides and herbicides and agents that may be employed in chemical terrorism. \(^{\text{uv}}\)

References

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