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Advanced Topical Formulations (ATF)

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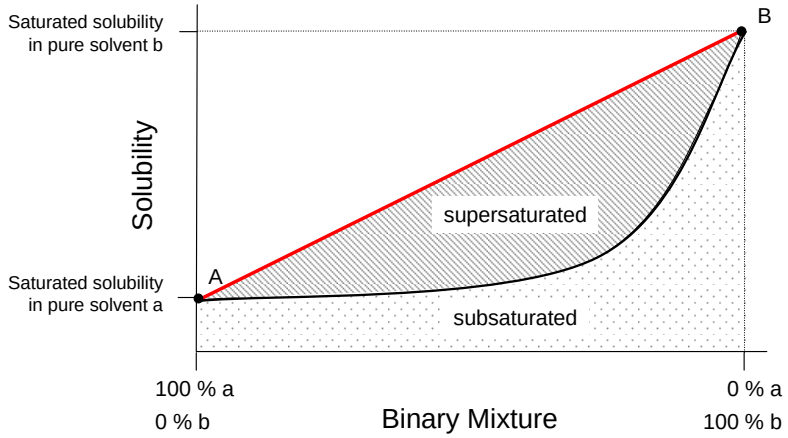
Graphical abstract



Abstract

Topical formulations aim to target the skin for a variety of cosmetic, protective or therapeutic needs. Despite the use of creams and ointments over the millennia, the bioavailability of actives from topical preparations remains quite low, often not exceeding 1-2% of the applied dose. In this review we examine the reasons underlying the poor performance of topical preparations. We also outline a rational approach, based on Fick's laws of diffusion, to develop advanced topical formulations. Methodologies which are currently used in research and development are critically examined and the importance of understanding the fate of the vehicle as well as the active is emphasised. Advanced topical formulation development will also be facilitated by emerging and sophisticated analytical techniques that are able to probe real time delivery of actives to the skin. A good understanding of the underlying physical chemistry of both the formulation and the skin is crucial in the development of optimised topical products.

Keywords: Advanced topical formulation, crystallisation, skin, supersaturation, penetration

Forenote

This is to acknowledge the contribution that Sandy Florence has made to the pharmaceutical sciences, in particular, the role of physical chemistry in developing optimised formulations.

1. Introduction - The Watershed

In the same way that our calendar runs from BC to AD, there was a marked watershed in our understanding of topical drug deliver before steroids (BS) and after steroids (AS). The major steroid in question was hydrocortisone which was developed some 60 years ago (Fourman et al., 1950). Delivery of agents to the skin has been documented by the Egyptians (BC), the Romans (AD) and in mediaeval times when 'flying ointment' was recorded (Hadgraft and Lane, 2016). It is interesting to note that, in the 13^{th} – 14^{th} centuries that it was known how to extract hallucinogenic materials from plants (e.g. mandrake). The extract had to be treated with lime to render the active more permeable through the skin (Burton, 1972). We now know that the base form was liberated and it was then compounded into a fat (i.e. a simple formulation was manufactured). Even though flying ointment was known and it was recognised that nitroglycerin induced headaches in armament workers by the late 1800s and also that nicotine could cause death as a result of skin penetration, the skin was largely regarded as an impermeable barrier (Laws, 1910; Faulkner, 1933; Rothman, 1943). Before steroids, very little was known about the role of the formulation and how delivery through the skin could be markedly affected by simple changes to the formulation.

2. Skin structure and route of penetration

For decades it has been appreciated that the stratum corneum, the outer layer of the skin is the rate controlling membrane to permeation. It is a unique barrier that is only some 15 micrometres thick (Hadgraft and Lane, 2011). It has evolved to prevent excessive water loss from mammals. How does it achieve this? It is comprised of dead keratinised cells that overlap, embedded in a lipid matrix, and the structure has been regarded as resembling a brick wall (Michaels et al., 1975). This is interspersed by hair follicles and sweat glands (Figure 1). There has been much debate concerning the route of penetration. Scheuplein and co-workers published a series of papers in the mid sixties to mid seventies which covered various aspects of percutaneous penetration. This included mechanisms and routes of penetration (Scheuplein 1965,1966,1967), the effect of temperature (Blank et al., 1967) and specific details on steroid permeation (Scheuplein et al., 1969). They also considered effects of surfactants (Scheuplein and Ross, 1970) and penetration from solvent deposited solids (Scheuplein and Ross, 1974).

Over the years, a number of biophysical techniques have been used to identify the major route. Experiments by Albery and Hadgraft (1979) using methyl nicotinate as the probe indicated that the primary route was intercellular, i.e. around the corneocytes. This was substantiated by a thermodynamic analysis on the penetration of water (Potts and Francoeur, 1990) and by the imaging of butanol (Nemanic and Elias, 1980), mercuric chloride (Bodde et al., 1989), norethindrone, oestradiol (Neelissen et al., 2000) and a lipophilic fluorescent dye Nile Red (Talreja et al., 2001). When the first studies were conducted to determine the route, little was known about the constituents of the intercellular spaces. However advanced analytical and scattering techniques have shown that there is a complex array of lipids (predominantly ceramides) that are structured into ordered bilayers (Garson et al., 1990; Bouwstra et al., 1991). Thus a diffusing permeant, whether it be water or a xenobiotic, encounters a tortuous path and has to cross, sequentially, hydrophilic and hydrophobic domains. The lipid chains of the ceramides close to the polar head groups are held tightly together which further slows progress of the permeant as it has to diffuse through a viscous region. The length of the tortuous path will be a function of the number of cell layers and their size; this is a simple geometric fact that has been investigated using trans epidermal water loss (TEWL) studies in humans (Hadgraft and Lane, 2009; Machado et al., 2010). TEWL varies from site to site on the body and is largest on areas such as the face where there are fewer layers of cells and the cell size is smaller. Both factors impact on skin permeability and the facial region is particularly permeable hence the caution advised when using steroids on the face (Eichenfield et al., 2014). It is also interesting to note that the penetration of methyl nicotinate varies from site to site along the back (Fountain et al. 1969b).

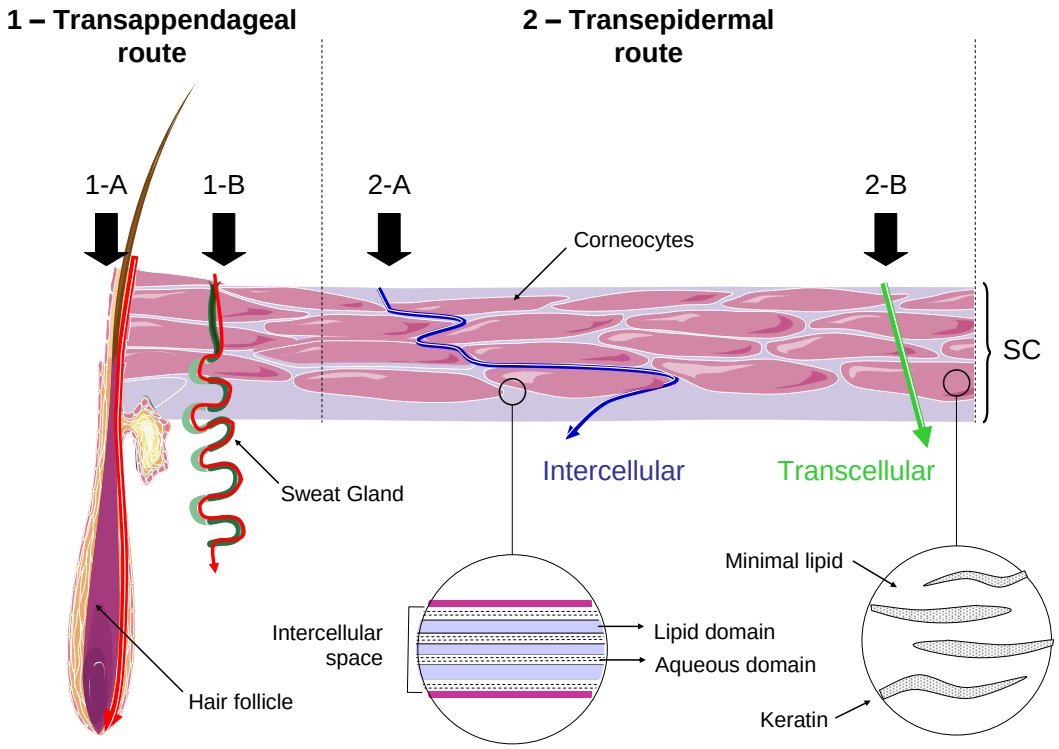


Figure 1. Potential pathways through the stratum corneum. 1 - Transappendageal route through (A) hair follicles and (B) sweat glands; 2 - Transepidermal route across intact SC via (A) intercellular lipids and (B) via keratinised cells (or transcellular).

One of the other implications of the intercellular route is the fact that when a formulation is spread over the skin surface only a small 'active' area for diffusion is available. The bulk of the area, the large surface of the corneocytes will not be accessible for a permeant. This is probably the major contributing factor to the low bioavailability (~1-3%) of steroids when applied to the skin as solution in acetone (Feldmann and Maibach, 1965; 1966; 1969). It is probable that the steroids crystallise on and in the upper intercellular channels of the skin and are therefore unavailable for continued diffusion; we have recently presented evidence for this phenomenon (Hadgraft and Lane, 2016). This would also explain the steroid reservoir mentioned above. It has been demonstrated that this is particularly pronounced when DMSO was used as a solvent. DMSO is a particularly good solvent and an excellent penetrant. It is does not stay on the skin or in the skin and when used as a vehicle for a steroid the active will crystallise essentially leaving it stranded and unable to dissolve.

It will then be sloughed off the skin, which is a particularly dynamic membrane, the stratum corneum having an average turnover time of 14 days.

3. Corticosteroids and nicotinic acid esters

Corticosteroids penetrate through the stratum corneum and constrict the blood vessels at the epidermal – dermal junction. This manifests itself as a pallor, the degree of which could be related to the potency of the steroid and the amount permeated (McKenzie and Stoughton, 1962). On the other hand, the nicotinates cause vasodilatation of the blood vessels and the onset of erythema could be related to the degree of penetration (Fountain et al., 1969a). The advent of the more potent corticosteroids led to more investigations on formulation effects. It was soon recognised that hydration of the skin, using occlusion, improved the permeation of the steroids and that the degree of penetration was related to the concentration applied (McKenzie and Stoughton, 1962). At the same time Cronin and Stoughton (1962) used ethyl nicotinate to show that there was regional variation in skin permeability. Barrett et al. (1964) used methyl nicotinate in various formulations to show that there were considerable differences in absorption when equal concentrations of the nicotinate were dissolved in aqueous cream, oily cream, white soft paraffin or macrogol ointment (Table 1). These authors also showed permeation was directly related to concentration.

Table 1: Penetration of methyl nicotinate from various topical vehicles. Adapted from Barrett et al. (1964)

Concentration of methyl nicotinate	Time of onset of erythema (min)			
	Aqueous Cream	**Oily Cream**	**White soft paraffin**	**Macrogol**
1	3.3	4.1	**5.3**	**8.7**
0.5	4.0	4.5	**6.6**	**10.8**
0.25	-	-	**-**	**17.1**
0.1	4.6	5.2	**8.0**	**22/3**
0.05	5.9	6.8	13.1	Not measured
0.01	7.4	10.5	19.7	Not measured
0.005	10.9	12.5	Not measured	Not measured
0.001	Not measured	Not measured	Not measured	Not measured

In the mid-60s there was an interest in enhancing skin penetration and one of the most researched solvents was dimethyl sulphoxide (DMSO). Stoughton (1965) showed that the penetration of fluocinolone acetonide was enhanced by DMSO and that a steroid

reservoir was induced. However, the reason for the formation of the reservoir was unclear. Other agents were also examined as potential penetration enhancers such as dimethyl formamide and dimethyl acetamide (Feldmann and Maibach, 1966). These vehicles also influenced transepidermal water loss (TEWL) as reported by Baker (1968). Sarkany et al. (1965) investigated formulation effects and conducted a clinical study on eczema and psoriasis treatment. Unequivocal evidence showing a link between vasoconstrictor effect and clinical efficacy could not be demonstrated.

4. Physicochemical determinants of topical delivery

When steroid treatment was coming to the fore in the late 50s, Higuchi (1960) presented a paper showing the importance of Fick's laws of diffusion in understanding percutaneous absorption (Equation 1).

$$J = -D\frac{dc}{dh} \qquad \textbf{Equation 1}$$

Most simply this analysis shows the significance of the driving force for diffusion, D across a membrane of thickness, h. At its most basic this is the concentration, c, of the active but strictly it is the chemical potential, which is related to the concentration and the activity coefficient. If an active is applied in a solvent (and the solvent does not interact with the skin) the flux of the active across the skin will be related to the degree of saturation. It is therefore possible to apply formulations which contain different concentrations of the drug and their penetration rates will be the same. This was shown by Hadgraft et al. (1973) in which methyl nicotinate, at different concentrations but at the same chemical potential was applied in simple water – glycerol mixtures. (Figure 2) The time of onset of erythema was the same thus demonstrating the importance of chemical potential. Similar results were found for the steroids (Lippold and Schneeman, 1984). These workers showed that the degree of blanching induced by a steroid was dose dependent up to the point at which the steroid was at its saturated solubility in the formulation. Concentrations above the solubility limit (i.e. suspensions) did not improve the vasoconstriction response; in simple terms a 1% suspension will be as effective as a 5%.

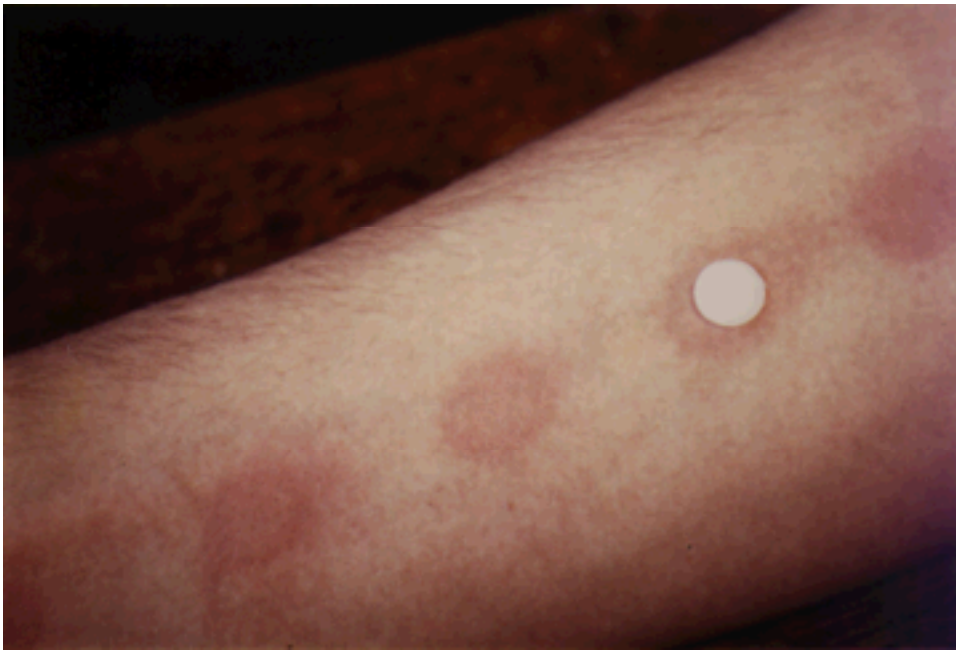


Figure 2. Appearance of erythema following application of filter paper impregnated with a solution of methyl nicotinate to the forearm

The fact that the chemical potential is important also means that if it can be 'artificially' raised above the solubility limit, there is an opportunity to improve delivery. Supersaturated states are thermodynamically unstable but if they can be transiently stabilised over the application time of the formulation, enhanced permeation is possible. Interestingly this hypothesis was first tested and proved using steroids (Coldman et al., 1969). They used the presence of a volatile solvent, which evaporated on application to the skin, to create a supersaturated state in the non-volatile solvent. After evaporation the active is delivered into the skin from the so-called residual phase. Supersaturation was taken further in a series of publications by Davis and co-workers (Davis and Hadgraft, 1991; Pellett et al., 1994; Pellett et al., 1997a) who used a mixing process to create supersaturation (Figure 3), which was stabilised using anti-nucleant polymers such as hydroxypropylmethyl cellulose (HPMC).

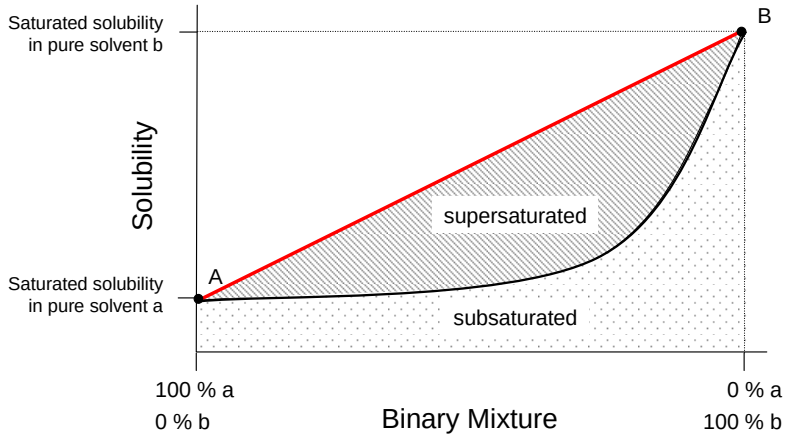
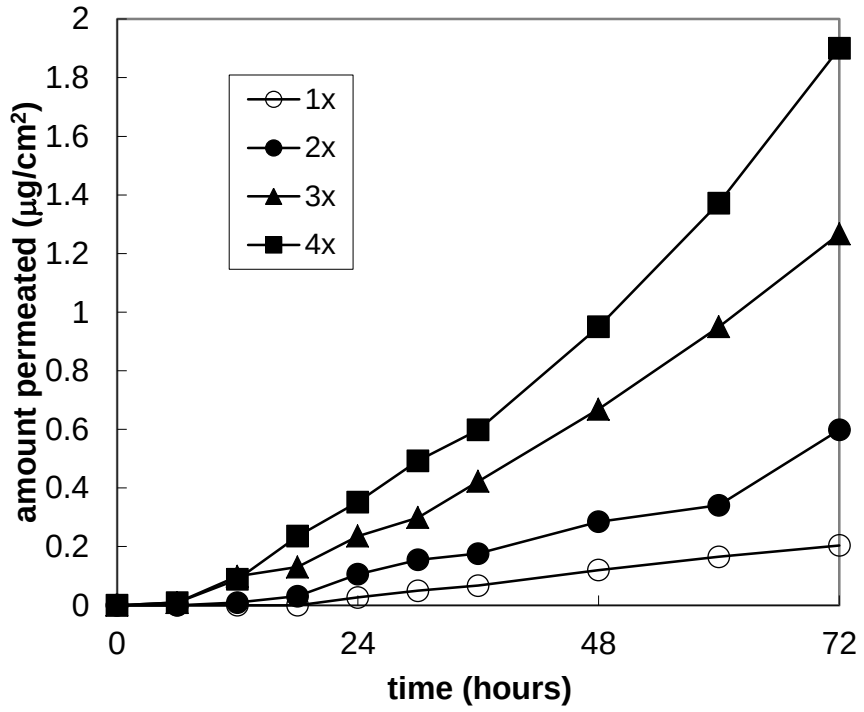


Figure 3. The black line represents the saturated solubility of a drug in the binary mixture of solvent "a" and solvent "b". By mixing a saturated solution in "system b" with pure "system a" supersaturated ($\overline{AB}$) solutions can be obtained. Adapted from Davis et al. (1991).

Initial studies with hydrocortisone were conducted on model membranes and skin but the work culminated in an *in vivo* skin blanching study on hydrocortisone acetate. By using supersaturation it was possible to make formulations that were bioequivalent with the commercially available 1% preparation (Davis and Hadgraft, 1993). These supersaturated preparations contained 0.02% of the active, underlining the potential of the approach for development of more efficacious and cost-effective topical formulations. For piroxicam, the amount of the active which penetrated human skin *in vitro* increased linearly with increasing degree of saturation (Pellet et al., 1997b). Moreover, an excellent correlation (r^2=0.97) was also obtained between amounts of active extracted from the stratum corneum and degree of saturation (Figure 4).



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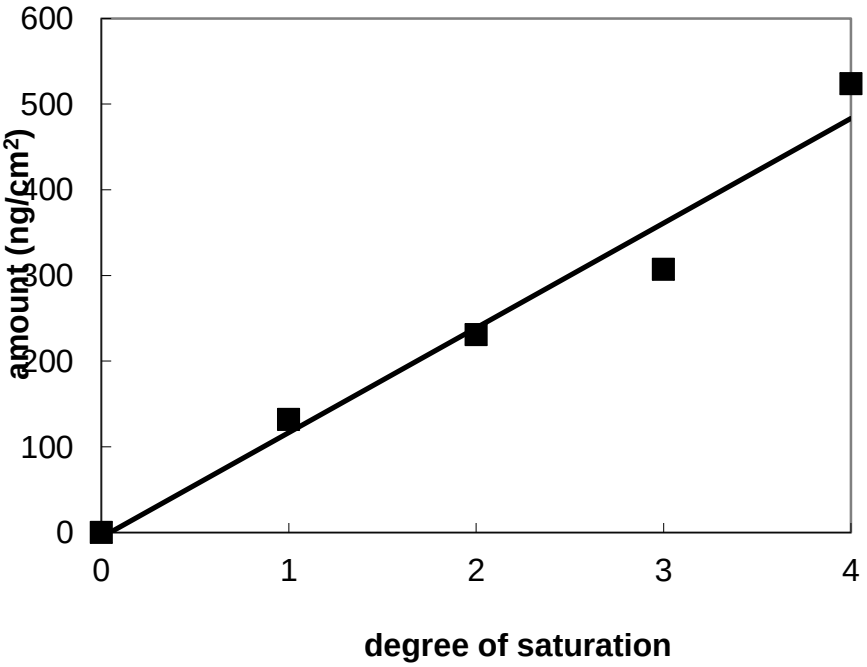

4b

Figure 4 (a) Cumulative amounts of piroxicam diffused across human skin following application in saturated and supersaturated solutions and **(b)** Corresponding amounts of piroxicam in the stratum corneum as determined by tape stripping at the end of the permeation experiments (Adapted from Pellet et al., 1997b).

Another way of considering chemical potential effects is to look at Fick's first law of diffusion in terms of concentration and partition coefficient (Equation 2).

$$J_{ss} = \frac{DK_c\Delta c}{h} \qquad \textbf{Equation 2}$$

Again if it is assumed that the vehicle does not interact with the skin, the concentration just inside the skin, at the interface of the skin and the vehicle, will be Kc,where K is the skin – vehicle partition coefficient and c the concentration of the active in the vehicle. In an interesting series of papers using fluocinolone acetonide and its acetate ester, Ostrenga et al (1971a,b) found that an optimum vehicle could be identified where the product of K and the solubility was at a maximum. This was demonstrated using a series of propylene glycol water mixtures and for the acetonide, the optimum concentration was about 30% PG / 70% water whereas for the acetate ester it was the reverse i.e. 70% PG /30% water (Figure 5). This is in line with the fact that the acetate ester is the more lipophilic of the two molecules.

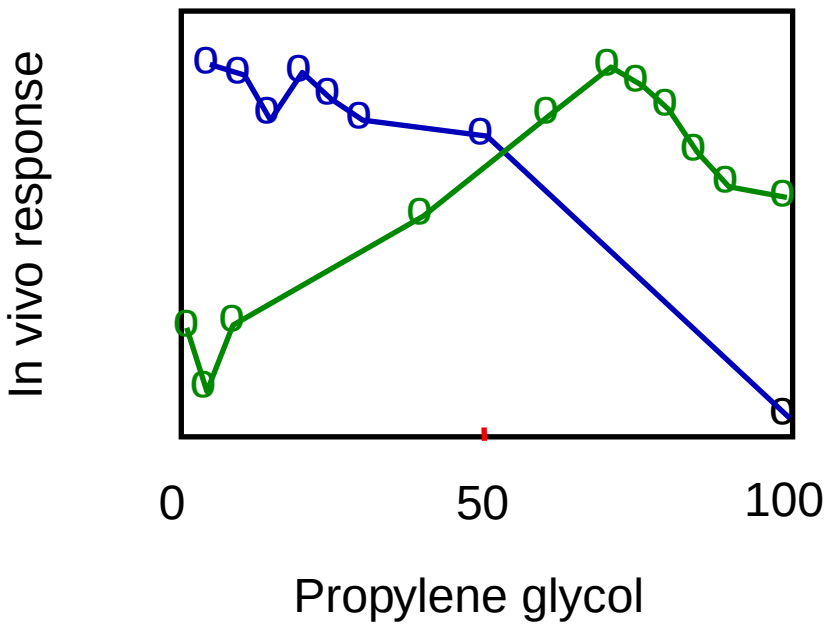

Figure 5. Blanching response following topical application of fluocinolone acetonide (blue circles) and fluocinolone acetonide acetate (green circles) in various PG:water mixtures (Adapted from Ostrenga et al., 1971a).

A further factor in Higuchi's paper (1960), in which the role of Fick's laws of diffusion is discussed, is the diffusion coefficient, D. In general terms the smaller the active, and thus the lower the molecular weight, the faster the active will diffuse through the skin. This means that the three major determinants in controlling skin penetration are (i) solubility of the active (ii) partition behaviour and (iii) diffusion coefficient. In general, those compounds that permeate well have a log octanol – water partition coefficient (Log $P_{o/w}$) of about 2, good solubility in both oils and water, and are relatively small. These are the characteristics of two of the drugs that are currently delivered via the transdermal route, nitroglycerin and nicotine. All of the above statements have the caveat that the formulation components do not interact with the skin, clearly some will and the implications of this can be profound. Before discussing the interplay between excipients and the skin, it is important to identify the rate controlling process in dermal penetration and how this is influenced by the structure of the skin and the route of penetration.

5. Penetration enhancers

Since the barrier properties of the stratum corneum are formidable, it has been a challenge to produce medicines which effectively treat skin disorders. Many actives do not have ideal physicochemical properties and therefore the formulation scientist has significant difficulties in optimising their delivery. These constraints led to extensive research into compounds that enhance penetration. The first major compound was DMSO but this was soon followed by many more. With the application of various biophysical techniques it proved possible to describe some of the possible mechanisms of action. Considering Fick's first law of diffusion the two factors that can be modified are the partition behaviour and the diffusion coefficient (Hadgraft, 2009; Lane, 2013). If a formulation component enters the structured lipids of the stratum corneum it may alter their solubility characteristics and will thereby alter the partition from the formulation into the skin. If this is favourable, enhanced permeation will result. Alternatively, the excipient may intercalate into the structured lipids and alter their fluidity. If it makes them more fluid diffusion through them will be more rapid and, again, enhanced permeation could result.

It is very difficult to deconvolve the two effects of partition and diffusion on membrane penetration of an active. However, using deuterated materials and Attenuated Total Reflectance - Fourier Transform Infra Red (ATR-FTIR) spectroscopy it has been possible

to show that oleic acid forms pools in the structured lipids and that penetration of an active is enhanced as a result of diffusion through these more fluid domains (Potts et al., 1991). On the other hand, Azone also fluidises the structured lipids but intercalates into them in a more homogenous way (Harrison et al., 1996b). The major structural difference is the cis double bond in oleic acid which is probably responsible for the pool formation. Considering the molecular structure of Azone, the seven membered ring interacts with the polar headgroups of the skin lipids. This forces the alkyl chains apart and they therefore become more fluid.

It has been suggested that small solvent molecules such as propylene glycol or diethyleneglycol monoethylether (Transcutol), improve the solubility of penetrants in the stratum corneum (Kasting et al., 1993; Watkinson et al., 2009; Osborne, 2011). This effect has also been studied using ATR-FTIR (Harrison et al., 1996a). The fact that both D and K can be influenced means that if excipients in a formulation can favourably alter both, then synergistic effects are found. This had previously been reported for mixtures of Azone and propylene glycol (Wotton et al., 1985). Supersaturation was described above and it is also possible to obtain synergistic effects when supersaturation is combined with chemical penetration enhancement (Davis et al., 2002).

6. Solvent effects

With the advent of *in vivo* confocal Raman spectroscopy it has been possible to examine the effects of excipients on the distribution of actives within the stratum corneum. Mohammed et al. (2014) examined the penetration of nicotinamide from different excipients, both singly, and in combinations (binary and ternary). One of the important findings of the work was to show that the amount of active in the stratum corneum was related to the uptake of the solvent in the stratum corneum (Figure 6). Very few studies have been conducted on the uptake of emollients or their penetration across the skin. Small molecules such as ethanol and propylene glycol have been examined and they permeate quite quickly (Berner et al., 1989; Liu et al., 1991; Trottet et al., 2004). Larger, more lipophilic emollients such as isopropyl myristate and isostearyl isostearate have not been researched in any detail. Unpublished work from our laboratory has shown that the uptake is small, and because of the high log P values of these materials they will not permeate across the skin (Oliveira, 2009). They will have a high residence time in the skin.

Santos et al. (2010) studied the permeation of oxybutynin from finite doses in either propylene glycol or octyl salicylate. They found that the propylene glycol permeated rapidly and suggested that the active was left crystallised on and in the skin because of the disappearance of the solvent. This contrasted with the formulations that contained octyl salicylate where delivery of oxybutynin was better and more sustained. Because the octyl salicylate had a longer residence time in the skin, it maintained the active in solution and therefore available for delivery to the deeper layers.

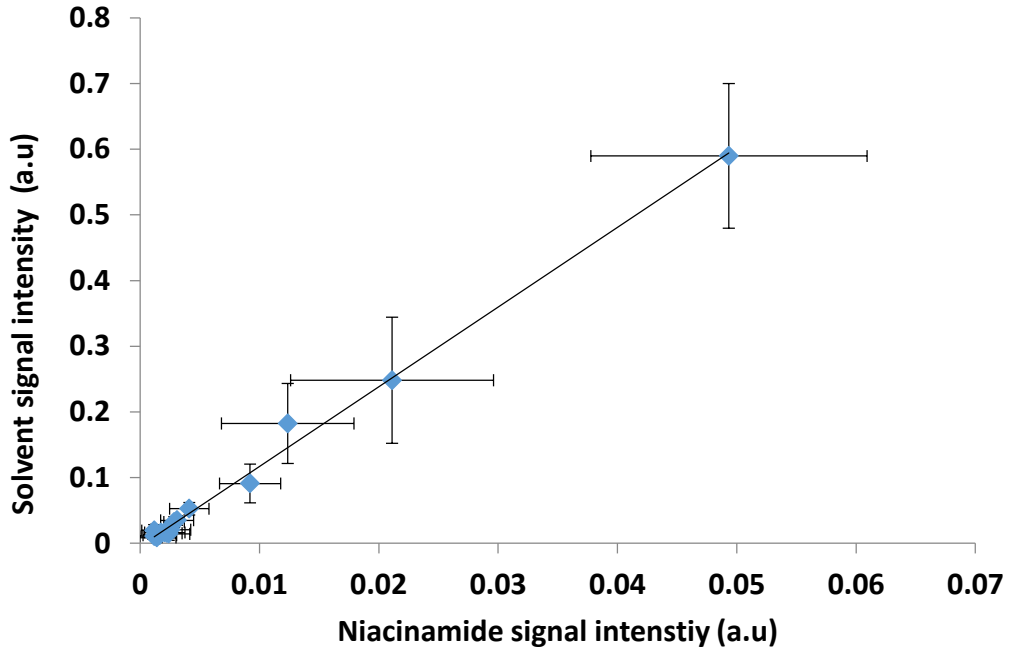

Figure 6: Correlation between signal intensity of niacinamide and signal intensities of PG or DMI which permeated into the SC *in vivo* with depth (0, 4, 8, 12, 16, 20 into the SC) for the mid ventral forearm (n=8; Mean ± S.D.). Adapted from Mohammed et al. (2014).

7. Finite versus infinite doses in skin permeation studies

One of the important points in the studies conducted by Santos et al. (2010) was the use of finite doses. In the clinic, a typical dose to the skin is 2 mg/cm^2. Many studies, *in vitro*, use infinite doses and often this does not give a true representation of how the excipients will work *in vivo*. For example, a representative clinical dose of propylene glycol will be subject to both evaporation, from the skin surface, and diffusion through the skin. The levels of PG will therefore deplete very rapidly. At infinite doses there will be no

depletion and the effects of PG in the skin may be significantly overestimated (Hadgraft and Lane, 2016). .

There are not only problems associated with the use of finite doses, it should also be noted that many experiments have been conducted on rodent skin, which also gives misleading results. For a true representation of topical delivery to man, human skin should be used with as close a clinical dose as possible. The closest one can get to this, in the absence of human tissue, is pig tissue. A number of studies have reported the similarity between pig and human skin tissue with reference to barrier properties (Dick and Scott, 1992; Singh et al., 2002; Barbero and Frasch, 2009; Luo et al., 2016).

8. Psychorheology

The cosmetics industry would not exist if it did not produce products that were acceptable to consumers. It is extremely good at producing products that 'feel good' and the public want to buy again and again. The pharmaceutical industry could learn from this and perhaps patient compliance would be better if topical products were more cosmetically acceptable. The subject has not been studied extensively but was recognised by Boyd (1976). It is clear that medicines for treating mild eczema have to have much better 'feel' than, for example, a product to treat melanoma. However, in modern product design there should be an element which considers the desirability of using the product on a regular and repeated basis. There are many publications, in the pharmaceutical literature, which consider the basic rheological properties of the product, but one should question their relevance in relation to their ability to deliver the active and how the patient perceives them.

9. Conclusions - Advanced Topical Formulations

Important principles underlying the basis for the design of advanced topical formulations have been known for a number of years but all the elements are rarely considered in an holistic approach. Firstly, the properties of the active should be taken into account. Ideally it will have a log $P_{o/w}$ around 2, and will have reasonable solubility in both oils and water, usually associated with a low melting point. If there are several candidates that are structurally related then there will be a balance between the ideal physicochemical properties and the potency.

The active needs to be delivered to the 'active' regions of the skin, i.e. the intercellular spaces at as high a thermodynamic activity as possible. This means that it will be close to saturation and therefore subject to the possibility of crystallisation. There needs to be the possibility of lateral diffusion to the intercellular spaces from the formulation deposited onto the corneocytes. It is possible that the enhanced penetration from some liposomal formulations is a result of the formation of a lipid layer on the skin surface through which lateral diffusion is facilitated. Rapid evaporation of a volatile solvent is likely to result in supersaturation but this will need to be stabilised with the judicious choice of anti-nucleant polymers.

It is important to recognise the evaporation of formulation components, such as water, and the residual phase that remains is of most importance in the formulation design. The residual phase should present the active at a high activity state but also deliver appropriate enhancers into the skin, most particularly solvents that will remain in the stratum corneum and maintain the active in solution. The solubility characteristics of the residual phase both in terms of the active and the skin lipids need to be considered. In order that the emollients also penetrate into the SC, it is important that they are also at a high thermodynamic activity.

These simple rules may be difficult to achieve, in reality, but will result in more effective topical delivery. Other factors that will also be built in to the final formulation are clearly the feel of the product, as discussed above, and also its stability. The increased sophistication of biophysical techniques, particularly those that can be used *in vivo*, will aid the formulation process and give much more insight into the future developments of topical formulation design.

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