Expert Opinion on Drug Delivery

ISSN: 1742-5247 (Print) 1744-7593 (Online) Journal homepage: http://www.tandfonline.com/loi/iedd20

Drug crystallisation – implications for topical and transdermal delivery

Jonathan Hadgraft & Majella E. Lane

To cite this article: Jonathan Hadgraft & Majella E. Lane (2016): Drug crystallisation – implications for topical and transdermal delivery, Expert Opinion on Drug Delivery, DOI: 10.1517/17425247.2016.1140146

To link to this article: http://dx.doi.org/10.1517/17425247.2016.1140146

Accepted author version posted online: 14 Jan 2016.

Publisher: Taylor & Francis

Journal: *Expert Opinion on Drug Delivery*

DOI: 10.1517/17425247.2016.1140146

Review

Drug crystallisation – implications for topical and transdermal delivery

Jonathan Hadgraft* and Majella E. Lane

Department of Pharmaceutics,
UCL School of Pharmacy,
29-39 Brunswick Square,
London,
WC1N 1AX
United Kingdom

*Corresponding author
Email:majella.lane@btinternet.com
Tel: +44 207 7535821
Fax:+44 870 1659275

Abstract

Introduction: Crystallization of actives in skin following topical application was suggested by studies in the 1950s and 1960s but is poorly understood. In contrast, the problem of crystallisation of actives on skin and in transdermal formulations has been known for many years.

Areas covered: With respect to crystallisation in skin, this review describes early reports of a skin "reservoir" and possible reasons underlying its genesis. Techniques to study crystallisation on and in skin and in transdermal patches are outlined. The role of the vehicle in skin delivery is emphasised. Studies which have investigated permeation from crystalline particles are described. Approaches to limit crystallisation of actives are discussed. Using supersaturation and antinuclean polymers, control of crystal size is possible; controlled release from crystals is also employed in transdermal patches.

Expert Opinion: Drug crystallisation has significant implications for topical and transdermal delivery. Approaches have been developed to counteract the issue for transdermal patches but crystallisation in and on the skin for other formulations remains unresolved. Greater knowledge of residence time of excipients and their interaction with skin at the molecular level is critical in order to address the problem. This will lay the foundations for better design of topical/transdermal formulations.

Keywords: Crystallisation, excipients, patches, reservoir, skin, supersaturation, topical, transdermal

Article Highlights

- The bioavailability of actives from topical and transdermal formulations is low when compared with other routes of drug delivery.
- Crystallisation of the active on and in the skin is proposed as one of the reasons underlying the limited amount of active which may be delivered (trans)dermally.
- Where the active crystallizes in the actual formulation, there are also significant implications for efficacy and safety.
- Drug crystallisation may be exploited for sustained release delivery.
- More insight is needed into excipient/vehicle interactions with, and disposition in, skin to develop more efficacious topical and transdermal formulations

1. Introduction

The Egyptians used powders and suspensions of solids in oils to treat the skin and were aware of the use of such to treat skin conditions and as cosmetics [1]. Powders have been used more recently to treat conditions such as Athlete's Foot and it is important to recognize that the solid material must dissolve to reach the site of action. Dissolution of the active in the skin lipids is therefore important. The influence of the formulation on delivery to and through the skin has been known for many centuries, for example in mediaeval times 'flying ointment' involved the extraction of psychoactive alkaloids from plant material, the conversion of the salt form to the free base (by treatment with an alkali) and the incorporation of the free base into a fatty ointment in order to promote skin penetration [2]. A more quantitative approach to formulation was achieved in the 1960s with the use of corticosteroids to treat skin conditions. It was discovered that, under occlusion, corticosteroids would permeate the skin and produce vasoconstriction [3]. The skin blanching effect was related to both the permeation of the steroid and its intrinsic potency. As the formulation was altered, the degree of vasoconstriction could be attributed to the efficacy of the formulation in delivering the active. For example, Barrett and co-workers investigated the permeation of fluocinolone acetonide (FA) from a simple formulation (white soft paraffin) but altered the physical state of the steroid by using ether coarse particles, micronised particles, or the addition of a small amount of propylene glycol (PG) that was just sufficient to dissolve the steroid [4]. The blanching results showed that the best formulation was when the corticosteroid was in solution and the worst was the formulation which contained the coarse particles. This was a clear indication that, under these conditions, the dissolution of the active was important in affecting delivery through

the skin. Moreover, this was one of the first examples which demonstrated that the presence of drug in solid or crystalline form versus drug in solution would influence the performance of topical formulations. The aim of this review is to provide an expert opinion on the relevance of crystallisation on and in skin for topical and transdermal preparations. Incidences where crystallisation is suggested in the early literature are reviewed. Examples of crystallisation or formation of a reservoir following application of transdermal and topical formulations are provided. Manipulation of crystal formation for drug delivery and systems based on solid drug particles are also considered. The use of antinucleant polymers to Inhibit crystal formation in both topical and transdermal preparations is described. Approaches to study this phenomenon on and in the skin as well as in transdermal patch formulations are outlined. For topical formulations and some transdermal systems the importance of the vehicle or excipients in maintaining the active in solution in the skin is emphasised.

2. Existence of a skin "reservoir" and early hints of drug crystallisation in skin

The existence of a depot or reservoir for actives applied to the skin was first suggested by Malkinson and Ferguson [5]. Following application of ^{14}C-hydrocortisone and unlabelled hydrocortisone in a petrolatum base to the forearm of two patients, (1.5 or 2.8 mg/cm^2) radioactivity was detected in the urine for up to 6 days. The application sites were covered for the 6 day period with a perforated aluminium patch and the authors postulated that small but steady amounts of hydrocortisone might be released from a depot of the active in the skin. Actual evidence for the existence and location of such a reservoir in the stratum corneum was first presented by Vickers [6]. In initial experiments FA was applied as a cream to the forearms of two subjects and occluded with Saran film. After 16 h the film was removed and vasoconstriction was observed for 6 – 8 h. Four days later a similar experiment was performed with the cream applied to a different site; normal washing took place during the experiments. Upon removal of the film two sites of vasoconstriction were observed, the first corresponding to the application area four days previously and the second corresponding to the area for the second experiment. If the arms were reoccluded with Saran film, even up to 12 or 14 days after initial application of the steroid, vasoconstriction reappeared without any reapplication of the steroid. This was suggested to reflect the presence of a depot of active in the skin from which steroid was released after

each re-occlusion. The studies were subsequently extended to investigate the phenomenon more fully. In the second set of experiments 19 healthy subjects were studied and a total dose of 0.2 mg of FA or triamcinolone acetonide (TA) was applied as an ethanolic solution to the forearms. Following drying, the application sites were occluded with Saran wrap for 16 h. Areas of vasoconstriction were recorded 1 h after removal of the occluding material and subjects resumed normal daily washing. Arms were re-occluded every two or three days until vasoconstriction was no longer evident following removal of the Saran wrap. Sustained vasoconstriction was seen in all volunteers but the duration varied from 3 to 15 days. In a third set of experiments, 12 of the subjects were given intradermal injections of TA in isotonic saline containing 1.8% ethanol. The amount of TA administered was the same as that which induced vasoconstriction when administered topically. Vasoconstriction was demonstrated in all subjects and faded by 6 to 8 h; two days later the areas were occluded but no vasoconstriction was evident. Finally, in one subject three further experiments were conducted which involved tape stripping (Scotch tape) the skin before topical application of FA, tape stripping the skin after application of FA and partial tape stripping of the skin. Where skin was tape stripped before application of FA vasoconstriction was evident 1 h after application of FA and lasted for 6 – 8 h; however no vasoconstriction was evident two days later. For sites tape stripped after application of FA, after two days vasoconstriction was only evident in control sites and sites which had been partially stripped; complete tape stripping resulted in no vasoconstriction. These results clearly indicated that the depot or reservoir of the active was sited in the outer epidermis rather than the dermis and specifically the stratum corneum. Further support for this was obtained in a later paper where biopsies of stripped and unstripped skin were assessed and the largest amount of active was observed to be in the stratum corneum [7]. It is interesting to note that the average turnover time of the stratum corneum is 14 days, comparable to the length of time of the perceived reservoir. This would indicate that any reservoir function of the skin resides in the stratum corneum and the reservoir itself may result from over-saturation of the of intercellular lipids with the active.

Stoughton and Fritsch had also observed that when FA was applied to the skin in an ethanol:dimethylsulphoxide (DMSO) vehicle, a reservoir was established compared with application of the active in ethanol alone [8]. Later, Stoughton also demonstrated the existence of a reservoir in human skin for both hydrocortisone and FA following application

in DMSO [9]. ^{14}C-hydrocortisone and ^{14}C-FA were applied to the forearms of adult subjects in each of the following vehicles (i) DMSO 40% in ethanol (ii) DMSO 20% in ethanol (iii) Ethanol. Preparations were left in place for 30, 120 and 240 min and application sites were then washed with soap and water. Radioactivity was subsequently monitored over 16 days with normal washing and bathing activities during this time. Table 1 shows the percent radioactivity in the stratum corneum for each formulation up to Day 12. Considering the data for the ethanol vehicle compared with the the DMSO:ethanol vehicles, these results are consistent with deposition of most of the active on the skin for the former, but penetration of the active deeper into the stratum corneum for the latter. The prolonged residence time of the active for the DMSO:ethanol vehicles is consistent with a "stranding" of the actives in the stratum corneum. These experiments also indicated that the reservoir was not easily removed by conventional washing or rinsing the skin surface with ethanol. In a separate set of experiments which involved tape stripping the skin, the location of the reservoir in the stratum corneum was confirmed.

Table 1: Average percent radioactivity retained in stratum corneum following application of ^{14}C-hydrocortisone and ^{14}C-fluocinolone acetonide to forearms of 3 human subjects in various vehicles (Adapted from reference 9)

	Day 0	Day 2	Day 4	Day 8	Day 12	Day 16
^{14}C-Hydrocortisone						
40% DMSO in ethanol	36	16	6	3	1.5	1.1
10% DMSO in ethanol	31	13	4	1	0.6	0.3
Ethanol	2.2	0	0	0	0	0
^{14}C-Fluocinolone acetonide						
40% DMSO in ethanol	34	15	7	3	0.8	0.1
10% DMSO in ethanol	22	9	4	1	0.2	0
Ethanol	1.8	0.08	0	0	0	0

Given the stratum corneum turnover is approximately 14 days, this induction of a reservoir is quite remarkable. This reservoir effect was later reported for other topically

applied substances which include acetylsalicylic acid, hexachlorophene and sodium fusidate [10,11]. No real explanation has been provided for the reservoir effect but it could be attributed to crystallisation of the actives in the intercellular spaces of the stratum corneum. The route of penetration of most materials through the skin is thought to be through the channels that surround the corneocytes [12]. These channels contain a complex mixture of lipids which are structured into lipid bilayers. There are therefore hydrophilic and lipophilic domains (Figure 1). The composition, organisation and role of the lipids in maintenance of a competent skin barrier are examined in a number of important recent publications [13-15]. Modulation of the lipids and thus the permeation pathway, because of vehicle effects is discussed further in section 6 Each corneocyte has a diameter of about 40 µm and a thickness of around 0.5 µm, separated from each other by a gap (inter-corneocyte space) of approximately 75 nm. The actual thickness of the stratum corneum is 15 -20 µm for the forearm or abdomen but there is regional variability with anatomic site [12].

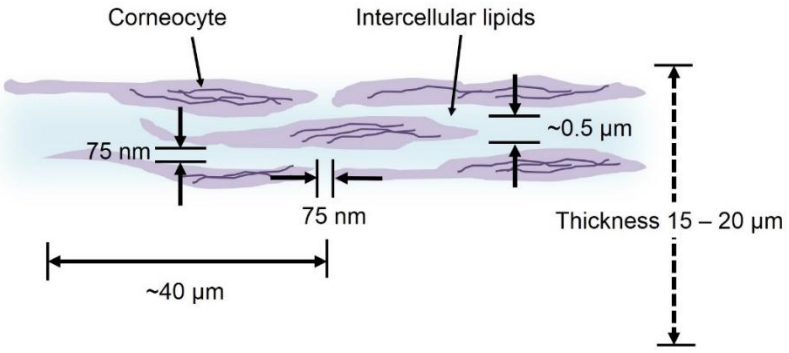


Figure 1. Schematic of corneocytes embedded in lipid matrix with average dimensions for stratum corneum, corneocyte thickness, and intercellular space

For an active to remain in solution, and therefore able to diffuse, it must have some solubility in both water and lipids. In the experiments by Stoughton, the ethanol would evaporate quickly (within a few minutes) and the DMSO would permeate into and through the skin rapidly [9]. Whilst the DMSO was in the stratum corneum, the steroid would remain in solution. However DMSO is a very good permeant and would rapidly be lost from the stratum corneum leaving the steroid 'stranded' and crystallized in the lipid bilayers. Under these conditions penetration into the deeper layers of the skin would depend on dissolution

and transfer sequentially, across hydrophilic and lipophilic domains; this would not be an efficient process and therefore not a rapid event. The poor bioavailability of topical steroids (~2 %), discussed further in Section 3, is consistent with limited solubilisation capacity in the stratum corneum.

3. Implications of crystallisation of active on and in the skin for topical and transdermal delivery

3.1 Topical delivery

A further publication from the 1960s which also examines a steroid, hydrocortisone, shows that its bioavailability is less than 2% [16]. It is surprising that the reasons for this have not been examined. The steroid was applied as a solution in acetone which would evaporate rapidly leaving a film of the active on the skin surface. Only steroid in direct contact with the intercellular spaces should diffuse deeper into the skin as it will be solubilised in the intercellular lipids. Steroid crystallized onto the corneocytes would be unable to diffuse into the dense keratinised cells and would therefore be unavailable. Even when applied to diseased skin the overall percentage of hydrocortisone which permeates is very low and crystallisation is a possibility. Wester and co-workers investigated the absorption of the molecule in patients with psoriasis [17]. ^{14}C-hydrocortisone was incorporated in a 0.5% cream formulation and 60 μL was applied to plaques on the forearm covering an area of 46.5 cm^2. For control subjects the same sites and application areas were used and all subjects were instructed not to wash the areas for 24 h. Percutaneous absorption was assessed by collecting urinary samples at intervals over 24 h and then at every 24 h interval up to 7 days. In subjects with healthy skin the amount absorbed was reported as 2.3 ± 1.4% at 24 h, while the corresponding value for psoriatic patients was 2.5±1.2%. From a practical perspective patients are often counselled to occlude plaques following application of topical steroids; this should theoretically create a more favourable environment for “stranded” drug to dissolve and thus achieve greater delivery of the steroid in a biologically active form. A cross-over study conducted by Melendres and colleagues which investigated percutaneous absorption of hydrocortisone following single topical dosing or multiple dosing underlined further the likelihood of formation of a depot of the active in the skin [18]. Radiolabelled drug was applied to the forearm of male subjects. The drug was applied in acetone to an the application site area of 2.5 cm^2 which was protected

by a non-occlusive polypropylene chamber. Four methods of application were investigated (i) single application of 13.33 μg of drug with removal by washing after 24 h (ii) single application of 40 μg of drug with removal by washing after 24 h (iii) three repeat applications of 13.33 μg of with dosing at 0, 5 and 12 h with removal by washing at 24 h (iv) three repeat applications of 13.33 ug as for the previous treatment but the application site was washed before application at 5 h and 12 h and a final washing step was conducted at 24 h. The percentage absorption of hydrocortisone was determined by collection and measurement of radioactivity in urine. Although application of a higher amount of hydrocortisone increased the amount absorbed it did not improve the percentage of active delivered. Unexpectedly, removal of the drug by washing did not result in any significant decrease in absorption compared with the multiple application protocol where no washing step was employed. This was suggested to reflect drug re-dilution at the skin surface and/or rehydration of the skin with consequent improvements in drug bioavailability. Comparing amounts absorbed for single versus multiple applications the authors also proposed a "solvent-vehicle" effect i.e. the greater amount of acetone administered for multiple applications promoted higher absorption of the active than expected because of enhanced dissolution of solid material at the skin surface or in the stratum corneum.

Crystallisation also has consequences for measurement of active in skin following topical application if bioequivalence (BE) needs to be shown between preparations. Tape stripping (TS) has been investigated extensively as a potential method to determine rate and extent of delivery of actives into the skin from topical preparations with a draft guidance published by the FDA in the 1990s [19]. The guidance was withdrawn 4 years later because of contradictory results between laboratories [20]. A number of sponsored studies have subsequently been conducted in efforts to refine and develop further TS for determination of quality and performance of topical products but only the Japanese Division of Drugs currently permits the use of TS in such determinations [21]. However the protocol as described does not account for changes in the physical state of the active. Caution needs to be exercised where claims are made for therapeutic equivalence of the active following application in different vehicles. For example, Umemura and co-workers applied lotion and ointment formulations of a vitamin D3 analogue, maxacalcatol, to volunteers and then used TS to determine amounts of active in the skin at different time points [22]. Because the

amounts of the active recovered were not significantly different for both formulations the authors noted that treatment with the lotion would be equally as effective as treatment with the ointment. In fact, this would need to be confirmed by a clinical study because the more volatile nature of the lotion formulation might reasonably be expected to deposit active on and in the skin to a greater extent than the ointment as we have noted in section 2 and earlier in this section.

3.2 Transdermal gels, solutions and sprays

Transdermal gels, solutions and sprays emerged in the 1990s and early 2000s for estradiol, oxybutynin and testosterone delivery. Typically, these formulations contain alcohol and may also contain water so that when applied onto the skin they dry quickly. It is clear that therapeutically relevant amounts of the various actives are deposited on the skin following application and safety communications have been issued by the US FDA in relation to accidental secondary exposure [23,24]. Interestingly, the prescribing information for Androgel™ 1% (testosterone gel) also refers to the skin serving as "a reservoir for the sustained release of testosterone into the systemic circulation" [25]. Similarly for an estradiol gel formulation it is noted that "the estradiol is stored in the subcutaneous tissue from where it is released gradually into systemic circulation" [26]. As these formulations are also re-applied to the same anatomic site it is possible to speculate that this method of use could facilitate increased dissolution of any crystallised active in the skin. This is also consistent with the "solvent-vehicle" hypothesis proposed by the authors of reference [18]. in the previous section where repeated application of the formulation promoted enhanced delivery.

3.3 Transdermal patches

Transdermal patches have evolved over the past decades to become thinner and smaller. For this to be realised the payload is often close to saturation which can result in stability issues, particularly on storage. Again, crystallisation is an important determinant and polymorphism of the active can be a contributory factor. Clearly, crystallisation has implications for release from patches as well as for efficacy and safety of the formulations. One of the first instances in which problems with storage of transdermal patches were demonstrated was the difference between estradiol patches that were in date compared

with out of date batches [27]. *In vitro* skin permeation studies were conducted on two different reservoir patch formulations from two batches; one batch had been recently manufactured while the other had been stored for 5 years. While release rates were similar for the patches which had been recently manufactured, the older patches delivered more of the active after 48 h. The authors suggested that this reflected a change in the formulation of the older patches, possibly resulting from accumulation of the active in adhesive or movement of ethanol out of the reservoir and, thus, a higher thermodynamic activity of the estradiol.

Fentanyl has been one of the most successful transdermal products and the original design contained a gelled reservoir of fentanyl crystals in an ethanolic solution. The design made use of the fact that zero order delivery of the potent drug would result from the thermodynamic activity of the reservoir remaining constant. Therefore diffusion across the rate controlling membrane in the device would be constant during the application time of the patch, 3 days [28]. During this time both fentanyl and ethanol diffused from the patch, which stopped working because the ethanol, not the fentanyl became exhausted. Clearly, this illustrates another case where crystalline material cannot diffuse into the skin. A number of deaths have been attributed to the failure of the reservoir patch because of claims that if the patch ruptures and the gel leaks, fatal doses of fentanyl are absorbed from the ethanolic suspension of fentanyl [29]. The delivery of fentanyl from the suspension has been examined *in vitro* and the results, in fact, indicated that fentanyl could not be delivered at a high rate even from a failed patch in which the gel leaked from the reservoir over the skin [29]. The increase of fentanyl levels in the blood was calculated to be, at most, 25%. It is also interesting to observe that the bioavailability of the fentanyl from the ethanolic suspension in this *in vitro* study is only 7.6%. This is another example of how crystallisation on the corneocyte plates gives rise to low doses of the active being absorbed.

A more recent problem was highlighted with rotigotine, which had regulatory approval for several years until some batches showed crystallisation on storage. Crystallisation occurred because of the formation of a new polymorph of the drug which was more stable, but less soluble, in the silicone matrix of the patch [30]. Approval was revoked and a new patch design and manufacturing process was able to solve the problem, with rotigotine patches now back on the market for the treatment of Parkinsonism and

restless leg syndrome [31]. Approaches to limit crystallisation in patch formulations are discussed further in section 7.2.

4. Skin penetration of the active from the crystalline/solid form

4.1 Topical formulations

As noted earlier, Barrett had demonstrated the importance of maintaining the active in solution for optimal permeation. Penetration of active from solid material *in vivo* was also evident [2], however the absorption of drug was limited by the dissolution rate into the white soft paraffin. Micronised FA (1 – 20 μm) incorporated in white soft paraffin produced a faster onset of vasoconstriction compared with coarse particles incorporated in the same vehicle. Lippold and Schneemann compared the penetration kinetics of betamethasone-17-benzoate for a range of solution and suspension preparations; skin penetration of the steroid ester reached a maximum for the suspension and was independent of the vehicle composition [32]. In a later study the same group evaluated the clinical efficacy of solution or suspension vehicles (liquid paraffin or triglycerides) of the same active in patients with dermatitis [33]. The suspension formulation was shown to improve symptoms to a significant extent compared with the solution formulation although *in vitro* tests confirmed more rapid drug release from the solution formulation. Barry and co-workers reported comparable vasoconstriction for 0.05 and 0.1% commercial desonide preparations where the active was suspended in a cream base [34]. Because the steroid is present at the same thermodynamic activity in both preparations there is clearly no advantage in using the higher strength preparation.

More recently, a series of mechanistic studies on skin penetration of a model active in powder form (~50 μm) and in solution was conducted by Romanchuk and Bunge [35]. Permeation of cyanophenol (CP) through skin and silicone membrane was evaluated in diffusion cell experiments. Solutions were in complete contact with the membrane of interest while this is not the case for the powder particles (Figure 2).

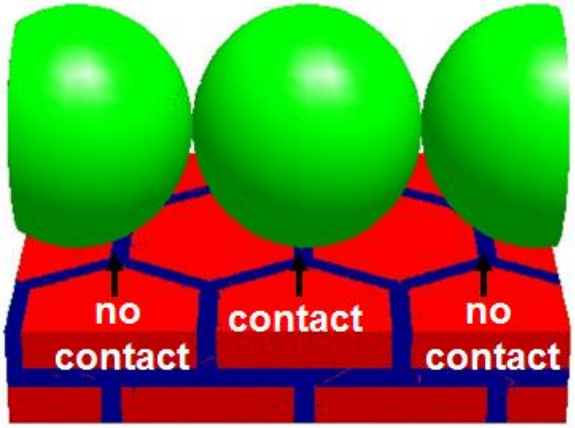


Figure 2. Powder particles and theoretical points of contact with corneocytes and intercellular lipids

Experiments were conducted over 6 h for silicone and 8 h for skin and powder was replaced with saturated solutions of the actives half way through the experiment. Steady state fluxes of CP were comparable in silicone for solution and powder forms however, results for skin indicated a significantly lower CP flux for the powder. Absorption from powders into both silicone and skin was possible in the absence of moisture and the data were consistent with a more limited surface area for active permeation in skin compared with silicone. The latter is entirely consistent with the intercellular route comprising the predominant pathway for permeation through the skin.

4.2 Transdermal formulations

In some circumstances, it can be of benefit to produce transdermal patches, which have a polymer matrix into which crystals of the active are dispersed. Where a drug has a narrow therapeutic window it may be necessary to have an upper limit to the rate at which the active is delivered. In the original reservoir devices this was achieved by the use of a rate controlling membrane. It is possible to produce controlled delivery by using a suspension of the active in a polymer adhesive matrix and relying on the dissolution of the particles to be the rate-controlling step. This is the basis of the Mylan Fentanyl Transdermal system where suspended crystals are incorporated into the matrix [36]. Benefits associated with

incorporating different crystal sizes of the active as well as solubilised drug in an adhesive patch matrix are also outlined in US patent application 2006/0078603 A1 [37].

4.3 Powderject technology

A more 'aggressive' approach to drug permeation from particles formed the basis of the Powderject™ system conceived in the 1990s. This is distinguished from the other "passive" formulations discussed in this review, as the technology will breach the stratum corneum in order to deliver its payload; it does however exemplify a system which delivers the active in crystalline or solid form and hence its inclusion here. Essentially, this comprised of a device which propelled the active in powder form (20 – 100 μm) through the outer layers of the skin and into the deeper epidermis using the energy of a supersonic jet of helium gas [38]. Despite the claimed advantages for such a drug delivery system - including being pain-free, needleless, better safety characteristics compared with injections, sustained or fast release modality and potential for targeting of the active - the technology has not progressed. This is surprising as the device was extensively tested for delivery of vaccine doses and studies appeared to yield very promising results in man. Dean and Chen investigated the delivery of a powdered trivalent influenza vaccine using a Powderject™ system compared with an intramuscular injection of the liquid form in human subjects [39]. Significant rises in the mean titers to all three viruses were observed for all subjects; values; increases in titer values, geometric mean titer values and seroconversions were comparable or better for the Powderject™ delivery system compared with the injection. Although skin reactions were observed (erythema, oedema, discoloration and flaking), they were reported to be self-limiting and reversible. Drape and co-workers employed this particle-mediated delivery approach in a phase 1 clinical trial to evaluate a monovalent DNA influenza vaccine [40]. Adult subjects received 1, 2 or 4 μg of the vaccine and serum haemagglutination-inhibition responses were elicited at all doses. This was the first successful demonstration of immunogenicity following administration of a DNA vaccine in humans and only mild to moderate reactions were reported at the skin site.

5. Supersaturation and generation of drug crystals

In 1960, Higuchi published a paper in which he showed the importance of thermodynamic activity in percutaneous absorption [41]. The delivery of the active would be maximum when it was administered as a saturated solution. Supersaturated states could be formed transiently, which could give rise to enhanced permeation, but these would be thermodynamically unstable. This hypothesis was tested by Coldman and co-workers in which FA was delivered to the skin, *in vitro*, in a simple formulation that contained a volatile and a non-volatile solvent [42]. As the volatile component evaporated, the steroid was left on the skin surface in a residual phase (the non-volatile component), and, depending on the conditions, it would be at various states of saturation, either sub- or super-saturated. The penetration rate was proportional to the degree of saturation and optimal amounts of the volatile solvent were identified for enhanced skin permeation. Importantly, the authors also noted that increasing the amounts of the volatile solvent beyond these values resulted in decreased permeation because of crystallisation of the steroid.

The pioneering studies on the effects of supersaturation were taken forward in the 1990s by several research groups who attempted to optimise formulations by deliberately creating supersaturated states using a solvent:co-solvent approach. Typically the active is preferentially soluble in one solvent and saturation or supersaturation is achieved by dilution of solutions of the active in this solvent with the co-solvent with lower solubilising capacity. Once the required degree of supersaturation is attained the systems may be stabilised on a temporary basis by the addition of polymers that stop the crystallisation process, so called anti-nucleant polymers [43-47]. The precise mechanism by which these polymers act is unclear and there do not appear to be any guidelines to determine which ones should be selected, either on the basis of the solvent or the active. The types of polymer that have been studied most extensively are the celluloses, e.g. hydroxypropylmethyl cellulose or the pyrrolidones, e.g. polyvinylpyrrollidone (PVP) [48-51]. Depending on the polymer employed in these systems, the time for nucleation may be altered and the crystal habit changed. Control of the size of the crystals generated is possible for some system with stable dispersions being achieved for up to 40 days [48]. It is easier to produce and stabilise steroids, such as hydrocortisone, than non-steroidal anti-inflammatory agents such as ibuprofen [52-53].

It will be evident that a number of commercial preparations exploit supersaturation itself as a delivery platform, and most recently the spray technology developed by Acrux

[54]. This combines supersaturation with a penetration enhancer, octyl salicylate (OS) in alcohol; on application to the skin, the alcohol will evaporate leaving a residual phase of supersaturated active in the enhancer. Because the residence time of OS is more favourable than other enhancers prolonged penetration of the active from such systems is possible [55] which is discussed further in the next section. Currently this technology is used in approved and marketed formulations for delivery of estradiol and testosterone. As noted in Section 3.2, while achieving therapeutic efficacy, clinically relevant amounts of the actives clearly do remain on the skin following application of these products.

6. Prevention of crystallisation

6.1 The role of the excipient

As noted by the authors of reference [18], in the optimisation of topical formulations it is important to recognise the role of the excipients in maintaining the active in solution in the skin. This will depend on both the solubilising power and the residence time of the excipient in the stratum corneum. It is interesting to note the paucity of data in the literature which deals with the disposition and penetration of commonly used excipients. We have previously reported the disposition of OS and PG when applied in volatile formulations to the skin [55]. Saturated and supersaturated solutions of oxybutynin were prepared in isopropanol with either PG or OS and permeation studies were conducted in Franz cells with mass balance studies for both the active and the two excipients. For all formulations OS always promoted superior penetration of the active compared with PG formulations because of the prolonged residence time of OS in the skin compared with PG. Once PG had been exhausted, as a result of penetration into the viable epidermis or loss through evaporation, drug permeation through the skin also ceased and significant amounts were recovered from the surface of the skin and following extraction of the skin. This indicated a "stranding" of the active in and on the skin because of depletion of PG. Because OS did not deplete it promoted longer permeation and more efficient delivery of the active. More recently we have begun to track permeation of other excipients including Transcutol™ (TC). Figure 3 shows that the rate and extent of permeation of TC is significantly higher compared with the glycols. Evaporation of the glycols however is also expected to contribute to the disparity in skin penetration observed [56]. Interestingly, TC is promoted for some skin applications by the manufacturer because it produces a "depot" effect for

certain actives [57] which is consistent with the permeation kinetics we have observed for the solvent.

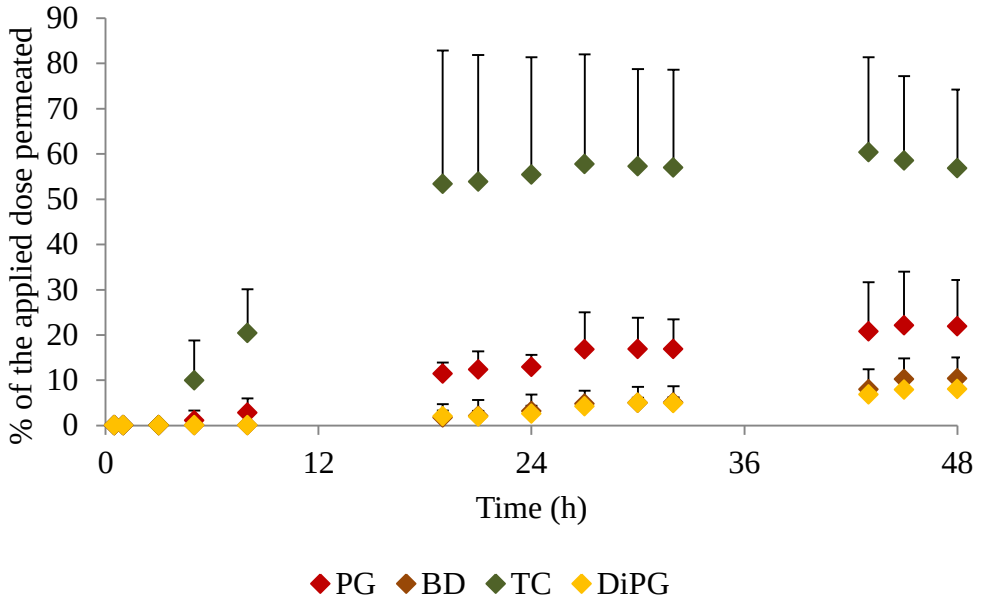


Figure 3. Percentage permeation of PG, butylene glycol (BD), Transcutol™ (TC) and dipropylene glycol (DiPG) in human skin *in vitro* following 10 µl/cm^2 dosing (n≥3, mean±SD)

A further problem is our relatively limited understanding of the mechanistic effects of specific excipients or vehicle components which also modulate the barrier properties of the stratum corneum [58]. PG has been suggested to integrate into the hydrophilic regions of intercellular lipids and to modify penetrant solubility in the stratum corneum [56-57]. The penetration of a number of actives appears to track PG permeation, both *in vitro* and *in vivo* [61-63]. As we have seen, however, PG is not the optimal vehicle or enhancer for other actives and its rapid clearance will compromise the ability of the active to move through the skin [55]. Evidence has also been reported which indicates an ability of PG to extract cholesterol from the stratum corneum and model lipid membranes [64]. With increasing PG content, the permeability of hydrocortisone was observed to increase significantly, possibly reflecting less densely packed lipid domains and thus explaining the enhanced penetration of the active. Most recently, the important role of cholesterol in appropriate lipid

organization of the lamellar phases of the SC barrier has been demonstrated by the same group [65], using a series of biophysical studies on models of lipid mixtures. Further investigations of this nature will be necessary for rational selection of excipients and penetration enhancers for specific actives.

6.2 Inhibition of crystallisation in transdermal and topical patch formulations

In Section 5 the important role of anti-nucleant polymers in stabilizing supersaturated systems was described. This approach has also been harnessed for prevention of crystallisation in transdermal patch formulations. Lipp investigated the use of silica, silica gel and PVP to inhibit crystallisation of steroids in matrix transdermal patches and noted that direct interaction of the inhibitor with the dissolved drug molecule was necessary to prevent the problem [66]. A screening model for suitable crystal inhibitors was also proposed in this paper based on the thermal behaviour of binary mixtures of drug and inhibitor. Significant reductions in heats of fusion were observed for the mixtures compared with drug alone and for specific PVP grades no heats of fusion could be detected; transdermal patches formulated with these grades demonstrated no crystallisation even after 5 years storage. US patent 5656286A also notes the use of PVP to ensure a combination of norethinderone acetate and estradiol remains substantially crystal free [67]. Most recently, Banga and co-workers have conducted a series of studies to identify the optimum inhibitors for transdermal patch formulations [68-70]. For captopril and levonorgestrel PVP was identified as the preferred crystallisation inhibitor; for desogestrel copovidone was effective in arresting crystal formation.

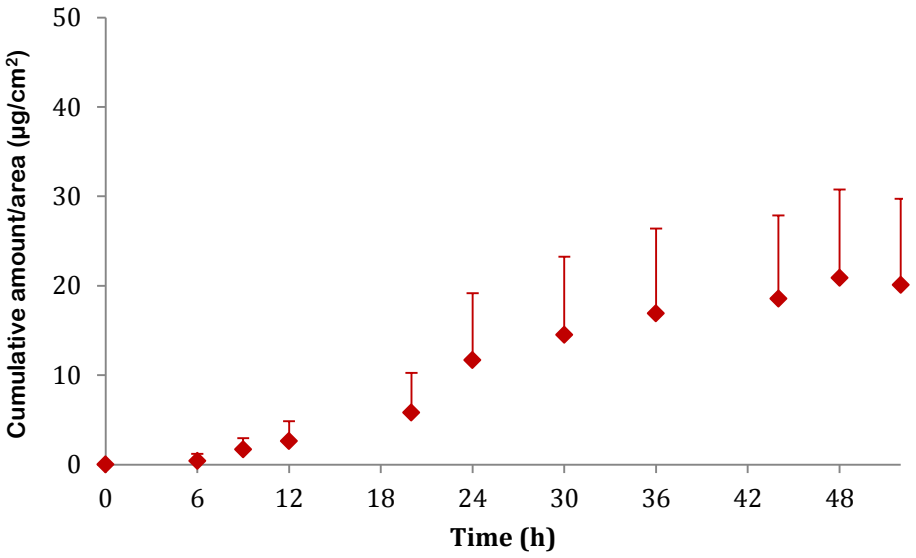
Cilurzo and co-workers have also used this approach to investigate prevention of crystal formation in a topical patch formulation of ibuprofen [71]. The patch consisted of a polydimethylsiloxane matrix. PG, two different grades of polyethoxylated castor oil and two co-polymers of methacrylic acid were individually assessed for their ability to inhibit drug crystallisation. PG was effective for up to 50 days without effects on skin permeation; the methacrylate co-polymers prevented crystallisation for up to 12 months but one of the co-polymers compromised skin permeation to a greater extent than PG. The efficacy of the various inhibitors was attributed to favourable solubility and interactions of the drug molecule and polymers.

7. Techniques to study crystallisation on/in the skin and in transdermal patches

7.1 Crystallisation on and in the skin

Crystallisation of active on the skin following topical application has been noted in a number of studies but researchers do not typically report visual illustrations of the problem. Figure 4a shows crystals of cilazepril deposited on porcine skin during an *in vitro* Franz cell experiment (unpublished data). The active was applied as a saturated solution in PG (10 $\mu l/cm^2$) and the corresponding permeation profile is shown in Figure 4b. After 6 h a white matrix is evident on the skin surface and from 24 h there is a plateau in permeation. This profile is consistent with depletion of the PG which results in deposition of the active on the skin; in addition the loss of vehicle is associated with no further significant permeation beyond 24 h.

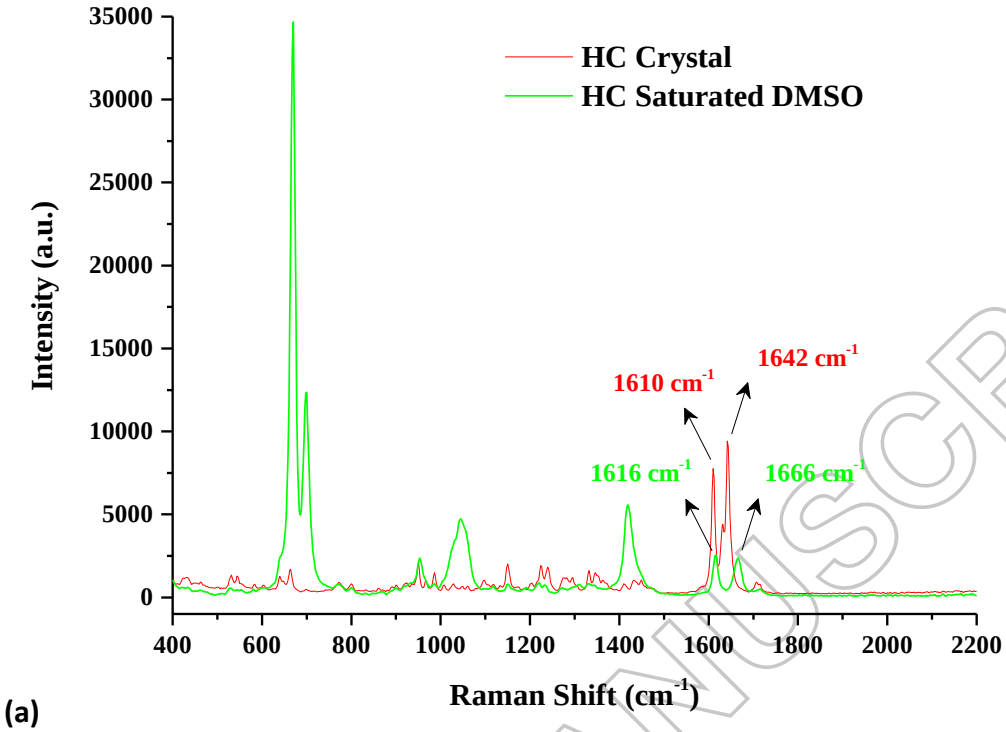
a



b

Figure 4 (a) Crystals of cilazepril formed on skin during an *in vitro* Franz cell experiment visualised with an Olympus SZX12 Stereomicroscope 50x (b) Permeation of cilazepril in porcine skin following application of a saturated solution in PG (n≥5; Mean±S.D.)

Other techniques which have been used to study drug crystallisation on the skin and in the skin include Confocal Raman spectroscopy and Stimulated Raman Scattering (SRS) microscopy. Spontaneous Raman spectroscopy collects vibrational, rotational and other low frequency modes from a sample following inelastic scatter after exposure of the sample to electromagnetic radiation [72]. Confocal Raman spectroscopy (CRS) combines a confocal signal collection approach with Raman spectroscopy and its application for skin interrogation was first reported by Caspers and co-workers [73,74]. These papers report the first applications of CRS in man for non-invasive depth profiling of water and other endogenous components of the skin such as natural moisturising factor. Subsequently CRS has been used to profile the disposition of a number of actives *in vivo* following their topical application to the skin including ibuprofen, niacinamide, retinol and salicylic acid [62, 75-78]. Figure 5a illustrates the Raman spectrum of crystalline hydrocortisone and a saturated solution of hydrocortisone in DMSO; Figure 5b shows the Raman spectrum collected from a depth of 1.9 μm into the volar forearm in one subject following application of a saturated solution of the active in DMSO (100 μl/cm^2) for 240 min. Data were collected using a River Diagnostics Skin Analyzer Model 3510 SCA. To our knowledge this is the first demonstration of detection of crystalline active in the skin *in vivo* and work is ongoing to investigate if this can be shown for other actives.



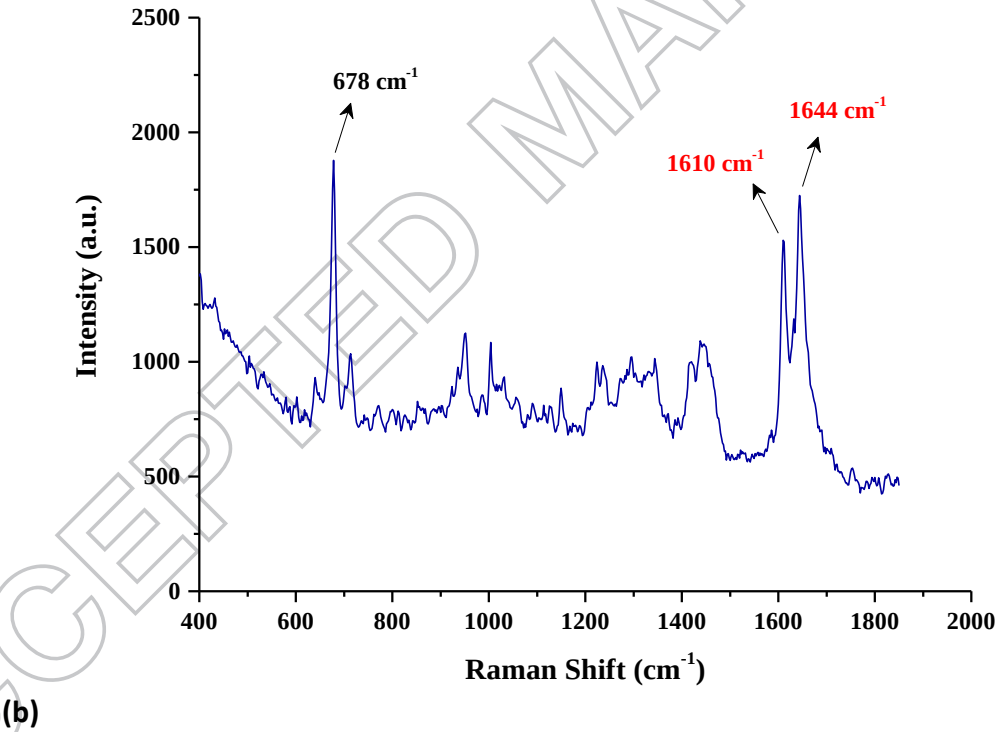


Figure 5: (a)Raman spectrum of crystalline hydrocortisone (red) and hydrortisone in DMSO (green) and (b) Raman spectrum at a depth of 1.9 µm in the volar forearm following application of a saturated solution of hydrocortisone in DMSO for 240 min (Spectra acquired at 2 µm steps, 5 s exposure time)

In spontaneous Raman scattering one laser light source is used to illuminate the sample but in SRS, two laser beams at different frequencies coincide on the sample. Where the frequency difference or Raman shift matches that of a molecular vibration then amplification of the emitted signal occurs because of stimulated excitation [79]. Belsey and co-workers used SRS to visualise deuterated ibuprofen crystals on excised porcine skin [80]. Within 30 min post-application of a solution of the molecule close to saturation in PG, large geometric crystals could be visualised on the skin. Limitations of SRS appear to be the need to deuterate the active of interest and the fact that its suitability for use in man has not yet been demonstrated.

7.2 Crystallisation in transdermal patches

Crystals which have formed *in situ* in transdermal matrix patches may be visualised using conventional optical or polarised microscopy [81,82]. However analysis of the crystals themselves is extremely challenging because of difficulty in ensuring complete separation of the matrix from the crystals. A customised X-ray diffraction sample holder was used by Lipp and Müller-Fahrnow for single crystal structure determination for gestodene or estradiol crystals formed in patches [81]. Both actives were observed to crystallise in their most common solid state forms, namely estradiol hemihydrate and gestodene polymorph I. Latsch and co-workers [83] used microcalorimetry to determine the crystallised fraction of active as a function of time for acrylate patches containing estradiol hemihydrate and norethindrone acetate. These workers also proposed the use of microcalorimetry as a tool to screen the effects of excipients on the rate of the crystallisation process.

8. Expert Opinion

In 1969 [84] Barrett noted that “There is much scope for developing vehicles which will enable the drug to reach the site of action rapidly and which will maintain a sufficient concentration at the site for the required length of time.” It is surprising that over 40 years on, formulations for topical delivery are still being produced in which the bioavailability is very low. One major cause of the low bioavailability appears to be the reservoir that the active can form on and in the stratum corneum as well as deposition on the skin. As we have noted in this review, there have only been a few attempts to document the extent of and physical state of the active in thereservoir and more importantly to try and determine

how the problem can be circumvented. Clearly, this is an area which has been under-researched to date, and there are conspicuous opportunities, particularly in relation to topical formulations, to improve delivery of the payload to the skin. The ultimate goal will be to achieve improved efficacy of targeting of the active with a consequent reduction in cost of goods over currently used topical and transdermal preparations. A major challenge will be to identify and harness a suite of tools to determine the physical state of the active in the skin following topical application. Although some progress is evident, particularly with reference to *in vivo* Raman spectroscopy, we have a comparatively limited arsenal at present to investigate the problem.

The importance of excipients in both topical and transdermal formulations cannot be over-stated. Considering topical formulations, common skin disorders such as eczema and psoriasis are significant determinants in sufferers' quality of life and their alleviation may be better addressed by a more complete understanding of how simple excipients interact with the stratum corneum. Little attempt has been made to show how excipients modify the solubility characteristics of the skin or how long they reside within the stratum corneum. With the increased sophistication and sensitivity of biophysical techniques, probing excipients *in vivo* in the skin has become a reality. However, it is important to realise that the experimental design of such attempts must be appropriate. Much has been written about the effects of formulations from infinite applications to the skin but it has to be recognised that a topical dose of a formulation for local effect is only of the order of 2 mg/cm^2. At such a low dose the formulation changes significantly over a short period of time, some of the components may well evaporate significantly, e.g. water. ethanol, PG. At the same time, both the active and some of the excipients will partition and diffuse into the skin. The time course over which the events occur will impact significantly on any crystallisation of the active on and in the skin. It is likely that some of the more lipophilic materials found in topical formulations, such as esters and fatty acids, will require extremely sensivite analytical methods to track their movement into the skin. These excipients already pose problems when monitored using conventional Franz cell studies where the focus is inevitably on tracking the active, not the vehicle. It is also important that our knowledge base on excipients improves so that we know how they affect the solubility characteristics of the complex lipids that make up the intercellular spaces. Recent studies with PG and cholesterol in model skin models are encouraging and it is anticipated that further insights

into other excipients should be forthcoming using this approach. Finally, we also need to know the residence time of excipients in the stratum corneum as this has been shown to correlate with that for the active.

The future may see the development of formulations which prevent crystallisation on and in the skin and permit rapid lateral diffusion so that the active is not 'stranded' on the corneocytes but can partition into the intercellular spaces. Anti-nucleant polymers have shown promise in the prevention and control of the growth of crystals in simple topical formulations, but have not been taken forward. Considering the more conventional transdermal patch formulations, crystallisation problems have, in some cases, been addressed by the application of anti-nucleant polymers, therefore it is surprising this approach has not been investigated more widely for topicals.

Transdermal gel formulations are restricted to a few actives at present but there are opportunties to improve delivery from these formulations where bioavailability is typically reported to be ~10%. This will require an understanding of how the vehicle and active interact in and on the skin and reformulation to optimise delivery of both; the optimum transdermal will minimise as much as possible the extent to which the active is deposited on skin or stranded in crystalline form. We believe that this will be area of continued interest to researchers as transdermals gels, rather than patches, are preferred by consumers and patients, as exemplified by the great success of Androgel™ in supplanting patches for testosterone delivery. This should also accelerate development of transdermal gels for other drugs which are currently delivered via transdermal patches.

Declaration of Interest:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

Papers of special note have been highlighted as:

*** of interest**

**** of considerable interest**

1. Ghalioungui P. The Ebers papyrus; a new English translation, commentaries and glossaries. Academy of Scientific Research and Technology, Cairo, 1987. 298 pages

2. Burton RJ. Witchcraft in the Middle Ages, Cornell University Press, New York (1972)

3. McKenzie AW, Stoughton RB. Method for comparing percutaneous absorption of steroids. Arch Dermatol 1962;86(5):608-610

4. Barrett CW, Hadgraft JW, Caron GA, et al. The effect of particle size and vehicle on the percutaneous absorption of fluocinolone acetonide. Br J Dermatol 1965;77(11):576-8

5. Malkinson FD, Ferguson EH. Percutaneous absorption of hydrocortisone-4-C14 in two human subjects. J Invest Dermatol 1955;25(5):281-3

6. Vickers CFH. Existence of reservoir in the stratum corneum. Experimental proof. Arch Dermatol 1963;88:20-3

**** First experimental evidence for existence of a depot of the active in the stratum corneum.**

7. Vickers CFH. Percutaneous absorption. In Modern Trends in Dermatology. Editor: RMB McKenna.London, Butterworth, 1966, pp. 84-106

8. Stoughton RB, Fritsch W. Influence of dimethylsulfoxide (dmso) on human percutaneous absorption. Arch Dermatol 1964;90:512-7

9. Stoughton RB. Dimethylsulfoxide (dmso) induction of a steroid reservoir in human skin. Arch Dermatol 1965;91:657-60

**** Demonstration of the influence of the vehicle on the fate of the active and generation of a depof in the skin which lasted for up to 16 days.**

10. Stoughton RB. Hexachlorophene deposition in human stratum corneum. Enhancement by dimethylacetamide, dimethylsulfoxide, and methylethylether. Arch Dermatol. 1966;94(5):646-8

11. Vickers CF.Percutaneous absorption of sodium fusidate and fusidic acid. Br J Dermatol 1969;81(12):902-8

12. Hadgraft J, Lane ME. Skin: the ultimate interface. Phys Chem Chem Phys 2011;13(12):5215-22

13. van Smeden J, Janssens M, Gooris GS, Bouwstra JA. The important role of stratum corneum lipids for the cutaneous barrier function. Biochim Biophys Acta. 2014;1841(3):295-313

**** Excellent review of recent advances in our understanding of the role of lipids in maintenance of a competent skin barrier.**

14. van Smeden J, Janssens M, Boiten WA, van Drongelen V, Furio L, Vreeken RJ, Hovnanian A, Bouwstra JA. Intercellular skin barrier lipid composition and organization in Netherton syndrome patients. J Invest Dermatol. 2014;134(5):1238-45

15. van Smeden J, Janssens M, Kaye EC, Caspers PJ, Lavrijsen AP, Vreeken RJ, Bouwstra JA. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. Exp Dermatol. 2014 Jan;23(1):45-52

16. Feldmann RJ, Maibach HI. Penetration of 14C hydrocortisone through normal skin: the effect of stripping and occlusion. Arch Dermatol 1965;91:661-6

17. Wester RC, Bucks DA, Maibach HI. Percutaneous absorption of hydrocortisone in psoriatic patients. J Am Acad Dermatol 1983;8:645-7

18. Melendres JL, Bucks DA, Camel E, et al. In vivo percutaneous absorption of hydrocortisone: multiple-application dosing in man. Pharm Res 1992;9(9):1164-7

*** Demonstrates how repeated application of a topical vehicle improves delivery of the active.**

19. US FDA. Guidance for Industry: Topical dermatological drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies. Draft Guidance, June 1998, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. 1998

20. US FDA. Draft guidance for industry on topical dermatological drug product NDAs and ANDAs – in vivo bioavailability, bioequivalence, in vitro release and associated studies; withdrawal. Fed Regist 2002;67:35122-3

21. Japanese Division of Drugs. Guidance for bioequivalence studies of generic products for topical use. July, 2003. National Institute of Health Sciences, Japan

22. Umemura K, Ikeda Y, Kondo K, et al. Cutaneous pharmacokinetics of topically applied maxacalcitol ointment and lotion. Int J Clin Pharmacol Ther 2008;46(6):289-94

23. FDA Drug Safety Communication: Ongoing safety review of Evamist (estradiol transdermal spray) and unintended exposure of children and pets to topical estrogen 07.29.2010

24. FDA Drug Safety Newsletter. Topical Testosterone Gel Products (Marketed as AndroGel 1% and Testim 1%): Secondary Exposure of Children to Topical Testosterone Products. Postmarket Reviews, Volume 2, Number 3, 2009

25. Androgel 1% prescribing information. Physician's Desk Reference 62nd edition. Thomson PDR, Montvale, New Jersey, 2007

26. Sandrena 0.5 mg gel. Summary of product characteristics. Electronic Medicines Compendium. Accessed December 2015

27. Brain KR, Hadgraft J, James VJ, et al. In vitro assessment of skin permeation from a transdermal system for the delivery of oestradiol. Int J Pharm 1993; 89:R13-R16

28. Calis KA, Kohler DR, Corso DM. Transdermally administered fentanyl for pain management. Clin Pharm 1992;11(1):22-36

29. Oliveira G, Hadgraft J, Lane ME. Toxicological implications of the delivery of fentanyl from gel extracted from a commercial transdermal reservoir patch. Toxicol In Vitro 2012;26(4):645-8
*** Despite claims that leaking patches are hazardous this study shows that the amounts of fentanyl likely to be absorbed should not result in fatalities**.

30. Chaudhuri KR. Crystallisation within_transdermal_rotigotine_patch: is there cause for concern? Expert Opin Drug Deliv 2008;5(11):1169-71

31. McAfee DA, Hadgraft J, Lane ME. Rotigotine: the first new chemical entity for transdermal drug delivery. Eur J Pharm Biopharm 2014;88(3):586-93

32. Lippold BC, Schneemann H. The influence of vehicles on the local bioavailability of betamethasone-17-benzoate from solution and suspension type ointments. Int J Pharm 1984;22(1):31-43

33. Malzfeldt E, Lehmann P, Goerz G, et al. Influence of drug solubility in the vehicle on clinical efficacy of ointments. Arch Dermatol Res 1989;281(3):193-7

34. Barry BW, Fyrand O, Woodford R, et al. Control of the bioavailability of a topical steroid; comparison of desonide creams 0.05% and 0.1% by vasoconstrictor studies and clinical trials. Clin Exp Dermatol 1987;12(6):406-9

35. Romonchuk WJ, Bunge AL. Permeation of 4-cyanophenol and methyl paraben from powde and saturated aqueous solution through silicone rubber membranes and human skin. J Pharm Sci 2006;95(11):2526-33

**** This article demonstrates that skin penetration occurs when the active is applied to the skin as a dry powder.**

36. Transdermal product formulation development. Kenneth J. Miller. In: Topical and Transdermal Drug Delivery. Principles and Practices. H.A.E. Benson, A.C. Watkinson (Eds). John Wiley & Sons, Inc., Hoboken, New Jersey. 2012. Pp. 287-308

37. Transdermal delivery of drugs based on crystal size. United States Patent Application Publication. US 2006/0078603 A1

38. Burkoth TL, Bellhouse BJ, Hewson G, et al. Transdermal and transmucosal powdered drug delivery. Crit Rev Ther Drug Carrier Syst 1999;16(4):331-84

39. Dean HJ, Chen D. Epidermal powder immunization against influenza. Vaccine 2004;16;23(5):681-6

40. Drape RJ, Macklin MD, Barr LJ, et al. Epidermal DNA vaccine for influenza is immunogenic in humans. Vaccine 2006;24(21):4475-81

41. Higuchi, T. Physical Chemical Analysis of Percutaneous Absorption Process from Creams and Ointments. J Soc Cosmet Chem 1960;11:,85-97

**** Seminal article which lays the foundation for the application of supersaturation in topical and transdermal drug delivery.**

42. Coldman MF, Poulsen BJ, Higuchi T.Enhancement of percutaneous absorption by the use of volatile: nonvolatile systems as vehicles. J Pharm Sci 1969;58(9):1098-102

***Provides evidence for crystallisation of active on the skin following evaporation of the vehicle.**

43. Davis AF, Hadgraft J. Effect of supersaturation on membrane transport: 1. Hydrocortisone acetate Int J Pharm 1991;76(1-2):1-8

**** Demonstrates the production of supersaturated systems and a linear relationship between degree of saturation and transport of the active.**

44. Pellett MA, Davis AF, Hadgraft J. Effect of supersaturation on membrane transport:2. Piroxicam. Int J Pharm 1994;111(1):1-6

45. Pellett MA, Castellano S, Hadgraft J, et al. The penetration of supersaturated solutions of piroxicam across silicone membrane and human skin in vitro. J Control Release 1997;46(3):205-214

46. Pellet MA, Roberts MS, Hadgraft J. Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique. Int J Pharm 1997;151(1):91-98

47. Megrab NA, Williams AC, Barry BW. Oestradiol permeation through human skin and silastic membrane: effects of PG and supersaturation. J Control Release 1995;36(3):277-29.

48. Raghavan SL, Schuessel K, Davis A, et al. Formation and stabilization of triclosan colloidal suspensions using supersaturated systems. Int J Pharm 2003;261(1-2):153-158

49. Raghavan SL, Trividic A, Davis AF, et al. Crystallisation of hydrocortisone acetate: influence of polymers. Int J Pharm 2001; 212(2):213-221

50. Raghavan SL, Kiepfer B, Davis AF, et al. Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers. Int J Pharm 2001; 221(1-2):95-105

51. Raghavan SL, Trividic A, Davis AF, et al. Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate. Int J Pharm 2000;193(2):231-237

52. Iervolino M, Raghavan SL, Hadgraft J. Membrane penetration enhancement of ibuprofen using supersaturation. Int J Pharm 2000;198(2):229-238

53. Iervolino M, Cappello B, Raghavan SL, et al. Penetration enhancement of ibuprofen from supersaturated solutions through human skin. Int J Pharm 2001;(1):131-141

54. Thomas BJ, Finnin BC. The transdermal revolution. Drug Discov Today 2004;9(16):697-703

55. Santos P, Watkinson AC, Hadgraft J, et al. Oxybutynin permeation in skin: the influence of drug and solvent activity. Int J Pharm 2010;384(1-2):67-72

*** This article demonstrates that the residence time of a penetration enhancer is directly correlated with the amount of active that may be delivered.**

56. Tsai JC, Cappel MJ, Flynn GL, et al. Drug and vehicle deposition from topical applications: use of in vitro mass balance technique with minoxidil solutions. J Pharm Sci 1992;81(8):736-43

57. Transcutol P. Technical Data Sheet. Gattefossé, France. July, 2011. Pp.1-3

58. Lane ME. Skin penetration enhancers. Int J Pharm 2013;447(1-2):12-21

59. Brinkmann I, Muller-Goymann CC. An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum. Pharmazie 2005;60:215-20

60. Kasting GB, Francis WR, Roberts GE. Skin penetration enhancement of triprolidine base by propylene glycol. J Pharm Sci 1993;82:551-2

61. Trottet L, Merly C, Mirza M, et al. Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride. Int J Pharm 2004; 274: 213-9

**First report to highlight the important role of finite doses in Franz cell studies and how the residence time of the penetration enhancer influences skin penetration of the active.

62. Pudney PG, Mélot M, Caspers PJ, et al. An in vivo confocal Raman study of the delivery of trans retinol to the skin. Appl Spectrosc 2007;61:804-11
**First demonstration in man of how the fate of the active is influenced by the penetration of the vehicle.

63. Bonnist EY, Gorce JP, Mackay RU, et al. Measuring the penetration of a skin sensitizer and its delivery vehicles simultaneously with confocal Raman spectroscopy. Skin Pharmacol Physiol 2011;24:274-83

64. Basse LH, Groen D, Bouwstra JA. Permability and lipid organization of a novel psoriasis stratum corneum substitute. Int J Pharm 2013;457(1):275-82

65. Mojumdar EH, Gooris GS, Bouwstra JA. Phase behavior of skin lipid mixtures: the effect of cholesterol on lipid organization. Soft Matter 2015;11(21):4326-36
** This paper provides important insights into the interactions of propylene glycol with skin lipids.

66. Lipp R. Selection and use of crystallization inhibitors for matrix type transdermal drug delivery systems containing sex steroids. J Pharm Pharmacol 1998;50(12):1343-9
* Outlines an approach based on calorimetry to identify suitable crystallisation inhibitors for transdermal patches.

67. Solubility parameter based drug delivery system and method for altering drug saturation concentration. US Patent 5,656, 286

68. Jain P, Banga AK. Inhibition of crystallization in drug-in-adhesive type transdermal patches. Int J Pharm 2010;394(1-2):68-74

69. Jain P, Banga AK. Induction and inhibition of crystallization in drug-in-adhesive type transdermal patches. Pharm Res 2013;30(2):562-71

70. Sachdeva V, Bai Y, Kydonieus A, et al. Formulation and optimization of desogestrel transdermal contraceptive patch using crystallization studies. Int J Pharm 2013;441(1-2):9-18

71. Cilurzo F, Minghetti P, Casiraghi A, et al. Polymethacrylates as crystallization inhibitors in monolayer transdermal patches containing ibuprofen. Eur J Pharm Biopharm 2005;60(1):61-6

72. Krafft C, Popp J. The many facets of Raman spectroscopy for biomedical analysis. Anal Bioanal Chem 2015;407(3):699-717

73. Caspers PJ, Lucassen GW, Wolthuis R, et al. In vitro and in vivo Raman spectroscopy of human skin. Biospectroscopy 1998;4:S31-S39
****First report of application of Raman spectroscopy for human *in vivo* studies.**

74. Caspers PJ, Lucassen GW, Bruining HA, et al. Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin. J Raman Spectrosc 2000;31:813-818

75. Stamatas GN, Boireau-Adamezyk, E. Development of a non-invasive optical method for assessment of skin barrier to external penetration. In: Biomedical Optics and 3-D Imaging. OSA Technical Digest, Miami, FL: Optical Society of America,2012. JM3A.42

76. Mateus R, Abdalghafor H, Oliveira G, et al. A new paradigm in dermatopharmacokinetics - Confocal Raman spectroscopy. Int J Pharm 2013;444(1-2):106-8

77. Mohammed D, Matts PJ, Hadgraft J, et al. In vitro-in vivo correlation in skin permeation. Pharm Res 2014;31(2):394-400

*** This paper demonstrates that it is possible to correlate results from in vitro Franz cell studies with studies in man.**

78. Mateus R, Moore DJ, Hadgraft J, et al. Percutaneous absorption of salicylic acid--in vitro and in vivo studies. Int J Pharm 2014;475(1-2):471-4

79. Freudiger CW, Min W, Saar BG, et al. Label-free biomedical imaging with high sensitivity by stimulated Raman scattering microscopy. Science 2008;322(5909):1857-61

80. Belsey NA, Garrett NL, Contreras-Rojas LR, et al. Evaluation of drug delivery to intact and porated skin by coherent Raman scattering and fluorescence microscopies. J Control Release 2014;174:37-42

81. Lipp R, Muller-Fahrnow A. Use of X-ray crystallography for the characterization of single crystals grown in steroid containing transdermal drug delivery systems. Eur J Pharm Biopharm 1999;47(2):133-8

****Successful application of X-ray diffraction to for characterization of crystals with no interference from patch matrix.**

82. Variankaval NE, Jacob KI, Dinh SM. Crystallization of beta-estradiol in an acrylic transdermal drug delivery system. J Biomed Mater Res 1999;44(4):397-406

83. Latsch S, Selzer T, Fink L, et al. Use of isothermal heat conduction microcalorimetry, X-ray diffraction, and optical microscopy for characterisation of crystals grown in steroid combination-containing transdermal drug delivery systems. Eur J Pharm Biopharm 2004 Mar;57(2):397-410

84. Barrett, CW. Skin Penetration. J Soc Cosmet Chem 1969;20:487-499