Topical Pharmacokinetics for a Rational and Effective Topical Drug Development Process

By

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I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

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Abstract

Topical drugs are not developed by the same process as oral drugs. The process is more uncertain and contains gaps. This leads to a poor discharge of risks before going to the clinical phases.

The topical drug development process is reviewed in the introduction of the thesis. In particular, past and current topical drug development practices are described and compared to the oral drug development process. The large risks taken during the topical drug development are pointed out. These risks are largely associated with a lack of pharmacokinetic's involvement prior to the drug candidate selection stage. Pharmacokinetics is considered after drug selection when it is often too late. Furthermore, the topical pharmacokinetic techniques available appear to be not suitable for three reasons: accessibility to the pharmacokinetic techniques, meaning of the data generated and reliability of these data. It concludes that the knowledge of target skin tissue concentration would be key for a more rational drug development process.

To this end, the primary objective of this thesis is to define a way of measuring drug concentration in skin tissue after topical application that is reliable, effective and practical. A secondary objective is then from the knowledge of the skin tissue concentration, to develop a topical PharmacoKinetic/PharmacoDynamic model to predict likely efficacy for a topical drug candidate.

First a direct skin tissue concentration approach is described that brings theoretical reliability into the pharmacokinetic data generated and improves throughput. However the pharmacokinetic data generated have limited use as total drug (bound + unbound) tissue concentration is measured while, pharmacodynamically, only the unbound fraction is of interest.

An indirect skin tissue concentration determination is then proposed. It consists in predicting the *in vivo* unbound drug concentration in diseased skin tissues. Three steps

are required: In the first step, the *in vitro* percutaneous flux is linked with the unbound drug concentration in the dermis. From there, the *in vivo* unbound drug concentration in all the skin tissues is defined using different physiological parameters. Finally, taking into account the effect of the skin disease on skin permeability and dermal capillary clearance, the *in vivo* unbound drug concentration in skin tissues in diseased skin is defined. The predicted concentration is therefore calculated from a constant (which is skin disease dependent) and from the *in vitro* percutaneous flux (which is an accessible and reliable experimental pharmacokinetic data).

A PharmacoKinetic/PharmacoDynamic model is then built. This model delivers two types of information: -1- The "efficacy index" which is a prediction of efficacy for a drug candidate based on percutaneous flux and drug potency and –2- the "systemic safety index" which is an assessment of systemic exposure based on total systemic clearance and plasma protein binding.
To check the validity of this new model, a validation exercise is run with the key eight topical drugs classes: NSAIDS, anaesthetics, retinoids, corticosteroids, vitamin D3 derivatives, antifungals, antibacterials for acne and immunomodulators. For seven out of the eight classes, the validation of the model is good. For the last class, the antibacterials for acne, the model underpredicts efficacy and it is suggested that the route of entry of antibacterial agents in acne occurs via the sebaceous duct as opposed to the more classic stratum corneum pathway.

Finally, three pilot studies are conducted with the aim to improve the quality and relevance of the data generated with *in vitro* percutaneous flux studies as well as the access to this technique and throughput of this technique.

To Angela, Elena and Silvia

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Chapter 1.

Introduction

1.1. General Introduction

The aim of the introduction to this thesis is to give a general overview of the past and current, practices and processes, used to develop a topical drug.

First, the general drug development process is described as well as the role played by pharmacokinetics to improve the effectiveness of the development process of drugs administered orally.

The way topical drug candidates are selected is then reviewed in an historical overview of the development of the main topical drug classes. This part therefore describes the different concepts and tools that have been used to select topical drug candidates.

The third part of the introduction compares the oral and the topical drug development process. It shows in particular that some of the key pharmacokinetic steps present in the oral drug development process are absent in the topical drug development process. It concludes that some of the principles applied for the oral drug development process could be used to improve the topical drug development process.

In the following part, a review of the skin diseases treated effectively topically as well as a review of some topical drug characteristics is attempted with the aim to define which key factors influence the successful development of a topical drug.

Finally the different topical pharmacokinetic sampling techniques are reviewed. The aim of this section is to see whether or not these techniques could offer a way to bridge the gap that exists in between the oral and topical drug development processes.

1.2. The Drug Development Process and the Role of Pharmacokinetics

1.2.1. The Drug Development Process

Developing a new pharmaceutical molecule is a long and demanding path, typically requiring 10 to 12 years and an investment of about £350 millions [1]. The entire process requires an immense amount of effort with a great deal of risk. According to the Pharmaceutical Research and Manufacturers of America, for every 5,000 potential medicines tested, on average, only 5 are tested in patients, and only 1 of the five is approved for patient use [1].

There are 5 key stages in the drug development process:

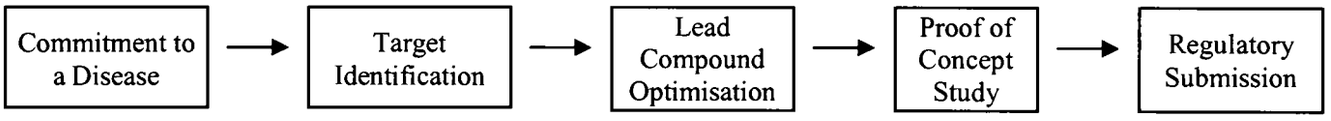

Figure 1. The 5 key stages of the drug development process

- **"Commitment to a Disease" to "Target Identification"**

The R&D process begins by determining the disease for which it is wished to find a treatment. This choice is largely governed by the market size and the patient unmet needs for diseases.

The disease being defined, potential targets in the body are then identified using past knowledge in the field of the disease, genomics coupled with computer aided techniques and serendipity.

• **"Target Identification" to "Lead Compound Optimisation":**

The target being identified, suitable *in vitro* assays are developed in order to screen compounds against this new target.

Lead compounds then need to be identified, and three approaches can be followed at the same time to achieve that. The first two are assimilated as "rational drug design" while the third one has mainly emerged in the 90's and could be called modern serendipity! 1-Modification of the structure of a known drug / 2-Synthesis of novel compounds based on knowledge of which chemical structures will most likely interact with the target of interest / 3-Screening randomly an already existing bank of compounds against a target of interest.

Synthesised compounds as per 1 and 2 as well as the use of the compound libraries of the pharmaceutical company as per 3 are then screened against the *in vitro* assays developed for that new target. Over the years the pharmaceutical industry compound libraries have grown and nowadays consist of several hundred thousands of compounds and more. High throughput screenings (HTS)(automated robotic systems) are therefore used in order to test this very large amount of compounds as such automated systems allow screening of thousands of compounds daily.

Hits are then identified and subsequent related compounds are synthesised in order to improve the characteristics of the potential leads. As leads are optimised they go through *in vitro* and *in vivo* tests to evaluate, potency, selectivity, pharmacokinetics, pharmacodynamics and toxicology potential.

The selection among the lead candidates is based on criteria such as the following:

1. Which compounds are likely to be the most effective?
2. What is the projected dose range and easiest method of delivery?
3. Which compounds are easy to manufacture on a large scale?
4. What return on investment will be generated: Can the compound meet or surpass the market standards?

The most promising compound becomes the lead candidate.

• **"Lead Compound Optimisation" to "Proof of Concept Study":**

When the lead candidate is identified, a substantial number of further studies are conducted. A comprehensive toxicological profile is done in at least two different animal species. Pharmacokinetic studies as well as pharmacodynamic studies are performed from which it is possible to project the required dose and plasma level in the human Phase 1 clinical trials.
The compound is put into a pharmaceutical dosage form, classically a tablet, so that it can be administered to man. The drug needs to be stable in this dosage form.

The compound moves then into human studies. This is the First Time In Human (FTIH) stage or Clinical Phase 1 studies. The aim of these studies is to escalate the dose in healthy volunteers such that it reaches at least the likely effective dose seen in animals with acceptable side effects.

The compound moves then into the Proof of Concept or Clinical Phase II studies, where the drug is given to a limited number of patients suffering from the disease of interest. These studies may also include surrogates of the disease. This is a key stage in the drug development program as this is when it will be known whether or not the drug has the potential to deliver the expected therapeutic benefit.

•**"Proof of Concept Study" to "Regulatory Submission":**

After the Proof of Concept stage, the compound moves into the very large and expensive clinical trials involving hundreds to thousands of patients (Phase III studies). The aim is to establish with certainty the efficacy and especially safety of the compound.

All the data gathered during the pre-clinical and clinical programs are then compiled in a regulatory dossier that is submitted to a regulatory body for approval.

It is noteworthy that developing new drug treatment is expensive. Over the years, pressure has been building up on the pharmaceutical industry to develop fast and economically, safe and efficacious drugs that provide "added value" to the healthcare marketplace. Fewer and fewer drug development failures are therefore affordable, and tackling the drug development process in a smarter and more effective way is a key issue that the industry faces.

1.2.2. Evolution of the Role of Pharmacokinetics in the Drug Development Process

Pharmacokinetics is one of the many disciplines that contributes to the discovery and development of drugs and helps to develop drugs more effectively. However, this contribution has not always been part of the drug discovery process…

Although the basic mathematics of pharmacokinetics were described in some detail almost 60 years ago [2;3], about 30 years went by before pharmacokinetics and the closely related discipline of drug metabolism started to make an appreciable contribution to the understanding and management of drug action. The rapid advances of pharmacokinetics and drug metabolism in the 60's and 70's was the results of combined

influences of improved analytical methodology and the increased awareness of the fundamental importance of pharmacokinetics in drug development and therapy. Today, pharmacokinetics and drug metabolism are among the most highly interactive disciplines in pharmaceutical R&D. They provide materials to support pharmacology, toxicology and clinical studies.

The disciplines of pharmacokinetics and drug metabolism now face many challenges as the 90's have seen the partial replacement of rational drug design by high throughput screening where the emphasis for the chemistry effort is on producing increasing numbers of molecules closely related to the initial lead compound. This has allowed companies not only to synthesise whole libraries of new compounds in a few weeks, but also to test them for up to 20 different activities at the same time. This enormous number of potential new molecules needs to be reduced to a reasonable number of candidates that can be taken into the costly development scheme. In the past, some basic pharmacology in a few animals together with some limited acute toxicity was normally sufficient, but today there is an increased reliance on kinetic and metabolic data to predict both efficacy and safety in man to make such a choice. This puts a lot of pressure on these disciplines, as some studies can be particularly expensive from a resource point of view.

Since the mid nineties, in order to improve the output from the pharmacokinetics discipline, new approaches for high throughput *in vivo* exposure screening have been put in place by the industry. This has largely been made possible thanks to recent advances in liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS)-based quantitative analytical techniques. These approaches include [4]:

- N-in-1 dosing. A cocktail of potential active molecules is given to the animals. The pharmacokinetic characteristics of 5 to 15 drugs can then be assessed in one study. This limits the number of animals used as well as the resources.
- Postdose pooling. As an alternative to N-in-1 dosing, plasma samples collected from different animals after dosing individual drugs can be combined for assay therefore

reducing the number of samples to analyse (this can be used when drug-drug interactions are expected but is more resource intensive and requires more animals)

- AUC estimation from one pooled sample. Plasma samples from the same animal at different time points are pooled in a weighed ratio that reflects the size of their respective time interval. AUC can be calculated from this single pooled sample analysis (but the entire concentration profile cannot be obtained).
- Continuous sampling method. Continuous blood withdrawal from animals at a suitable rate has been explored to obtain a single sample for each animal. The major advantage of this method over the sample pooling is the reduction in time required for sample collection and processing.

Such approaches are an improvement compared to previous ways of performing pre-clinical Drug Metabolism PharmacoKinetics (DMPK) studies. However, some authors argue that this is probably not enough for the pharmacokinetics and drug metabolism discipline to be used fully to reduce the enormous number of molecules coming from the high throughput screening/combinatorial chemistry. To meet such high demands at the discovery stage, Welling [5] and Campbell [6] push for greater emphasis on rapid *in vitro* metabolism screen and *in silico* predictions. For the development stage, they argue for greater emphasis on PK/PD for both toxicokinetics and pharmacology, for early metabolism studies, for absolute bioavailability studies, for early systemic availability of market-image formulation studies. Welling [5] argues against other types of studies such as multiple food effect studies or some interaction studies. For his argument, he reminds that "the objective of pharmacokinetic support for a drug development program should be to provide information that supports safety and efficacy claims. It is not, as appears often to have been assumed, to provide an exhaustively complete and in-depth pharmacokinetic and metabolism profile of a drug candidate under every conceivable situation".

The evolution and challenges of the pharmacokinetics discipline in relation to the drug development process are of interest especially if one wants to step into what the future of topical pharmacokinetics could be like in 10 or 20 years time. However, as will be shown

in sections 1.3 and 1.4, it cannot be said that so far pharmacokinetics has played the key role in the development process of a topical that it now plays for the development of an oral dosage form. As well, as will be seen in section 1.6, current topical pharmacokinetic sampling techniques are unable to offer the type of DMPK support that an oral drug in development can receive.

1.3. Topical Drug Development – Choosing the Topical Drug Candidate: An Historical Overview

The previous section has showed, how, nowadays, oral drugs are developed, the importance of having an effective drug development process, and the key role played by pharmacokinetics to improve the effectiveness of the process.

Switching now to the topical drug development arena, this section is going to review past and more current topical drug development processes.

The focus of this review will be related to the topical drug candidate selection.

1.3.1. The Pragmatic Topical Drug Development Approach: An Existing Oral/Systemic Drug is Further Developed as a Topical.

Historically most topical drug classes seem to have been originally developed following the pragmatic principle that, **if an existing drug with an interesting pharmacology is effective orally/systemically, it could well work topically without giving side effects and therefore would deserve to be tried topically**.

The next paragraphs review the development of the "first in their class" topical drugs. Most of the major classes of topical drugs are reviewed with the aim of understanding how the first molecule of each class was selected.

1.3.1.1. Local anaesthetics (< 1900)

Local anaesthetics can be considered as the oldest class of synthetic topical drugs as most of the molecules of this class were first synthesised in the first half of the 20th century [cocaine (plant extract 1860), benzocaine (1895), procaine (1906), butacaine (1920), amylocaine (1928), dibucaine (1931), tetracaine (1932), lidocaine (1948), prilocaine (1960)][7;8].

Natives of the Andes region of Peru were certainly the first known users of a local anaesthetic by chewing the leaves of the Coca shrub that produced both numbness of the tongue and intense central nervous system stimulation [9]. In 1860, Niemann reported the extraction of cocaine from the coca shrub [8]. The local anaesthetic properties of cocaine were first noted a decade later after its introduction by a Peruvian army surgeon, who also recorded the less attractive properties of the drug [9].

It is difficult for such an old drug like cocaine to grasp how its pharmacological/medical use as a local anaesthetic was generated. Indeed one could interpret in different ways the facts presented in the previous paragraph. The pragmatic approach described in the introduction to this section is therefore difficult to demonstrate for this class of drug.

It is noteworthy, that most of these drugs are not indicated for use on skin but on the eyes or on mucosal membranes. It is only recently, that topical treatments indicated for skin anaesthesia have been introduced: Ametop® (tetracaine), and EMLA® (lidocaine + prilocaine). This does suggest that the history of local anaesthetic development for a skin anaesthesia indication has not been straightforward.

1.3.1.2. Corticosteroids (1952)

Topical corticosteroids constitute the most important class of topical drugs available. They are considered as the most effective treatment and the most widely used treatment

of dermatoses. They form as well one of the oldest topical drug class (appearing in the 50's) and the largest one with more than 20 molecules marketed [10].

The history of corticosteroids begins after the demonstration in 1927 that crude extracts of adrenal tissue could maintain life in adrenalectomised animals. In 1936, adrenal cortex isolated Kendall's compound E (later to be known as cortisone) was proved to be effective in a non-specific test. Over the next decade, synthesis of this compound as well as other adrenal cortex isolated compounds (like hydrocortisone) took place. Eventually in 1949, Kendall's compound E was administered orally in two patients with rheumatoid arthritis, an inflammatory disease [11]. That year, compound E (cortisone) and compound F (hydrocortisone) of Kendall are first listed in the Index Medicus under the heading Adrenal Preparations [12]. In 1951, oral cortisone is reported to be of use for dermatology conditions [13]. At the same time, cortisone acetate ointment is tried but failed to deliver benefits [14-16]. The first effective topical corticosteroid trial comes a year later with topical hydrocortisone reported by Sulzberger and Witten [17].

Hydrocortisone, an existing molecule (an endogenous compound) is the first topical corticosteroid to be developed successfully. The pragmatic concept to try topically an effective existing molecule does apply for the first successful drug of this important topical drug class.

1.3.1.3. Retinoids (1962)

For dermatoses, the next important class of topical drugs developed after the corticosteroids is the retinoids. This class of compounds is largely used for the treatment of psoriasis and acne.

As for the corticosteroids, the history of retinoids starts in the 20's when in 1925, Wolbach and Howe demonstrate that deprivation of vitamin A in animals and man led to hyperkeratosis [18]. In the 40's, the oral administration of large doses of vitamin A is tried with various success to treat various dyskeratotic disorders like acne or ichthyosis

[19-21]. In the 50's, topical vitamin A palmitate is not found to be effective for psoriasis [22]. Eventually, the function of the acid metabolite form of vitamin A is elucidated [23;24], and led to the successful testing of topical vitamin A acid in dyskeratotic disorders like ichthyosis, acne or psoriasis [25-28].

As for the corticosteroids, vitamin A acid, an endogenous molecule shown to be active orally for dyskeratotic disorders was then later found to be effective topically on the same disorders. The pragmatic concept described earlier in this section does apply to the first molecule in the topical retinoid class.

1.3.1.4. Antifungals (1967)

Topical antifungals represent another important class of topical drugs not used to treat dermatoses but fungal infections. This is with the topical corticosteroids and NSAIDs one of the largest class (> 15 molecules marketed) [7].

If for the two previous classes a clear historical starting point could be set, "treatments" for fungal infections have however been around for a long time. For the purpose of this historical review, one could suggest that the family of currently available antifungals should be considered. There are nowadays two main classes of topical antifungals available: the imidazole type (fungistatic) and the allylamine type (fungicidal), the latter being the newer class which is slowly taking over the old imidazole class. The imidazole class of compounds appeared first in the mid 60's with Etonam [29;30], shortly followed in just a few years by clotrimazole, miconazole, econazole and isoconazole.

The literature reviewed on this class does suggest that contrary to the retinoids and corticosteroids, the drug development path followed has been to first test topically the effectiveness of the drug instead of testing it orally first. The pragmatic principle stated at the beginning of this section does not seem to apply for the development of the imidazole drug class.

1.3.1.5. NSAIDs (1971)

Non Steroidal Anti Inflammatory Drugs (NSAIDs) constitute another large class of topical drugs, Dromgoole in 1994 lists 18 different topical NSAID molecules [31]. Their topical efficacy remains still controversial despite successful controlled trials. Indeed, the study of pain is and has always been difficult due to the subjective nature of the measured end-point. The need of controlled trials is therefore even more important for such a class than it is for others.

The history of NSAIDs starts with aspirin one of the oldest synthesised molecule of the pharmacopoeia (1853). In the first part of the 20th century other NSAIDs were synthesised: fenbufen (1936), felbinac (1946), phenybutazone (1951). Eventually, the NSAIDs burst occurs in the 1960's (indomethacin (1963), benzydamine (1964), ibuprofen (1964), diclofenac (1966), ketoprofen (1968), piroxicam (1970)...). These drugs are primarily developed for oral use as analgesics but a lot of them will reach the market as well in a topical format and it is likely that quite a few of them are tried topically in uncontrolled trials before the 1970's.

Among this large list of drugs, the systematic review of topical NSAIDs clinical trials by Moore et al. [32] shows that the first NSAID with proven topical efficacy in a controlled trial is benzydamine, a molecule first synthesised in Italy in 1964. In 1965, benzydamine efficacy in traumatology after oral delivery was established by several controlled studies [33;34]. The topical use of benzydamine was justified in 1968 by experimental findings on its ability of penetrating skin and accumulating at high concentrations in the inflamed tissue [35]. The first controlled study with topical benzydamine used to treat patients presenting oedema and post traumatic pain was then published in 1971 [36].

In the NSAID family, the first in the class topical molecule with proven efficacy had established oral/systemic efficacy, showing the pragmatic approach followed once more.

1.3.1.6. Antivirals (1983)

As for antimicrobial agents, a clear historical starting point is difficult to set, as many treatments have been claimed to have antiviral properties. With iodoxuridine in the 60's the road towards effective treatments started. However in the late 70's the discovery of the nucleoside analogue acyclovir represent a key milestone for antiviral treatments. Its oral efficacy against herpes simplex virus was first proven in 1982 in the treatment of genital herpes [37]. This was followed the following year by two small successful trials with topical acyclovir for the management of herpes simplex labialis [38;39].

In the antiviral family, the first key molecule in the class with proven topical efficacy had established oral/systemic efficacy prior to topical efficacy, showing the pragmatic approach followed once more.

1.3.1.7. Vitamin D3 derivatives (late 1980's)

The third class of topical drugs relevant to psoriasis after the corticosteroids and retinoids is the vitamin D3 derivatives.

Dermatological interest in vitamin D3 and its active metabolites in the treatment of psoriasis started in 1985, when Morimoto et al [40] described a patient with senile osteoporosis and psoriasis who benefited from oral administration of alphacalcidiol (a vitamin D3 metabolite) [1α(OH)D3]. In the following years, Morimoto et al. performed further successful studies in larger group of psoriasis patients with alphacalcidiol and its hydroxylated metabolite calcitriol [1,25(OH)2D3] [41;42]. In 1989, the first successful topical use of a vitamin D3 derivative is showed by Morimoto et al. They described good clinical results in chronic plaque psoriasis after topical application of 0.1-0.5μg/g of calcitriol in an ointment [42].

Calcitriol, an endogenous compound that had showed oral efficacy was further tested successfully topically. The pragmatic approach described earlier applies for this class of drug.

1.3.1.8. Immunomodulators (1992)

The last class of topical drugs that has reached commercialisation is the immunomodulators (or immunosuppressors) that are indicated for the treatment of atopic dermatitis.

Their history is strictly linked with the development and use of the immunosuppressor drug cyclosporin, patented by Sandoz in 1978. Only a year later, the case for oral cyclosporin in psoriasis was made [43]. In order to avoid the immunosuppressive side effect of cyclosporin, topical cyclosporin was tested in 5 different trials on psoriasis but all of them failed to show any improvement [44-48]. The use of oral cyclosporin in non-psoriatic dermatoses was established in 1987 [49;50]. In two guinea pig allergic contact dermatitis model studies, topical cyclosporin delivered benefits. However these animal model results do not translate well to a human use of topical cyclosporin as its benefits is either small [51] or absent [52].
In 1986, tacrolimus a smaller and more potent immunosuppressor was synthesised by Fujisawa. Its immunosuppressive oral activity was demonstrated in transplant patients [53] and psoriasis patients [54]. The immunosuppressive activity being established, Lauerma et al. demonstrated clear topical efficacy of tacrolimus in contact allergic dermatitis [55].

In this last topical class, the first molecule to show topical efficacy had a proven record of oral efficacy, showing once more the pragmatic approach used for the first molecule of this class.

1.3.1.9. Summary

Table 1 below summarises the previous sections. Overall, it appears that for most of the topical drug classes, the first member of the class was developed pragmatically by applying topically a drug effective orally/systemically.

Table 1. First in their Class Topical Drugs by Year

Drug Class	Drug	Year (Oral/Systemic)	Year (Topical)	Pragmatic Approach
Anaesthetics	Cocaine	< 20th century	<20th century	?
Corticosteroids	Hydrocortisone (active form of cortisone)	Endogenous 1949	1952	✓
Retinoids	Retinoic Acid (Vitamin A metabolite)	Endogenous 1925	1962	✓
Antifungals	Etonam	1969?	1967	✗
NSAIDs	Benzydamine	1965	1971	✓
Antivirals	Acyclovir	1982	1983	✓
Vitamin D3 derivatives	Calcitriol (Vitamin D3 active metabolite)	Endogenous 1985-1989	1989	✓
Immuno-modulators	Tacrolimus	1990	1992	✓

Developing a new drug has always been a long and costly operation. However, deciding to "try topically" a drug already developed for which the toxicity (the systemic one at least) is well established, sounds like a quicker and less costly operation than developing a totally new drug for a topical administration. As well, as can be shown in the table above, such a simple approach appears to be successful: the beginning of most of the topical drug classes followed that development path.

It should however be noticed that if most of these "first" in their class drugs made it to a topical format via this approach, some failures or issues appeared for quite a few of these classes from day one:

* For the corticosteroids, in 1951 cortisone, the first corticosteroid effective orally ever tried topically did fail in the clinic [14-16].
* If retinoic acid can be considered as first in its class, the topical use of retinol its prodrug failed to work in acne or psoriasis in earlier studies [22].
* Calcitriol is indeed effective topically in psoriasis but leads to systemic exposure that has prevented its further commercialisation.
* Among the anaesthetics, benzocaine an older molecule than lidocaine or tetracaine is not indicated to be for used on uncompomised skin (indicated for mosquito bites or on mucosal membranes).
* In the family of immunomodulators, before tacrolimus was tried topically, cyclosporin A had been tried in several trials: all of psoriasis trials failed [44-48], and the two atopic dermatitis trials had either limited benefit [56] or no benefit demonstrated [52].

This simple process has proven its value but has showed as well its limits. The limits of this development approach are primarily an unpredictable efficacy and secondly some unpredictable potential for systemic exposure.

1.3.2. Moving Towards Improved Topical Drug Development Processes: Use of in-vivo Models

1.3.2.1. The Particular Case of Corticosteroids: Use of Human Models (early 1960's)

Soon after the first success of topical hydrocortisone in 1952, new corticosteroids have been synthesised and tried in inflamed skin conditions topically. The unpredictive outcome in patients as seen with the failure of topical cortisone, triggered the need to search for a model that would predict the efficacy of these new corticosteroids in the clinic. The vasoconstrictor nature of such compounds was soon discovered and used as a surrogate marker of topical efficacy for this class of drugs: The corticosteroid blanching assay was born [57;58].

The key advantages of this technique are:

- a one day experiment is long enough to assess efficacy of a new drug
- there is no need to use patients with inflamed skin disease as simple healthy human volunteers can be used
  several compounds/formulations can be used in the same volunteer
  there is no requirement for complicated method of assessment as a trained panel is able to assess the blanching score
  the small local area treated allows the development of new chemical entities with only a limited toxicological package

This technique for its simplicity, ease of use and reliability, therefore became the gold standard and key decision tool to develop the subsequent corticosteroids and their formulations.

In the following decade, other types of human models were used to test topical corticosteroids. One is the use of induced inflammation model like the crotton oil model [59] derived from the animal model, or, the UV erythema test [60]; another one is the use

of microplaque disease models like the microplaque assay for psoriasis [61] or the poison ivy test for contact dermatitis [62].

1.3.2.2. The Topical Rodent Models (1960's)

Although the blanching assay was very successful for the development of corticosteroids, it was not going to help to develop new classes of drugs, as vasoconstrictor properties are not common for other classes of compounds. However the principle of the blanching assay was recycled in an animal model. In the blanching assay, the end point measurement is a change of colour "pink to white". In the animal, a colour change was also used as the end point. This time, by causing an irritation to the skin of the animal, the skin colour would turn towards a reddish colour, then the topical application of an effective anti-inflammatory drug would return the animal skin colour towards normality [63-65]. As well as the induced inflamed models, the pharmacological antiproliferative effect of corticosteroids was used in various models [66-68].

The induced inflamed animal models as well as the antiproliferative animal models, offered a platform of models that could be used for further new classes of topical drugs. Indeed, for the two major dermatose skin conditions - atopic dermatitis and psoriasis inflammation (for both dermatoses) and keratinocyte proliferation (for psoriasis only) represent the two main pharmacological targets.

For practical reasons the animals used in these models would be small animals: rodents. This choice of the animal was helped by the fact that classically, rodents are the pharmacological animal models of choice used in the pharmaceutical industry.

1.3.2.3. The Combined Use of Topical Model and Systemic Rodent Models (1980's)

Efficacy has always been the primary end point for GO/NO GO decisions in topical drug development. With the development of very potent corticosteroids however, the issue of systemic exposure became more and more critical.

In the early 80's, new topical corticosteroids were developed (mainly designed for pulmonary delivery) with a lower potential to induce systemic exposure. The new synthesised drugs were called "soft drugs". The term "soft" conveys the principle that this new generation of drugs would be cleared more quickly in the body or would be less absorbed systemically than the previous generation. To design such new drugs, the corticosteroids were tested topically in a rodent/human model as well as systemically in a rodent model [69]. A good "soft" drug candidate would then be a drug that would be active topically at a low dose while a large systemic dose would be required to deliver the immunosuppressive effect.

This concept of designing drugs acting topically and not systemically was used to develop the latest corticosteroids as well as the new topical immunomodulators to overtake tacrolimus.

1.3.2.4. The Combined Use of a Topical Pig Model and a Systemic Rodent Model (1990's)

An important issue with the rodent inflamed skin model is the fact that it largely overpredicts the efficacy observed in human as shown in Table 2 [70]. This naturally leads to failures when the topical drug reaches the clinical stages. Little is published in the literature on that subject but it is believed that in the pharmaceutical industry a very large number of such drug development failures exist.

Table 2. Difference in Topical Dose Strength to Show Efficacy: Rat vs. Swine vs. Human

Compound	Rat ED50, %	Pig ED50, %	Human Clinical Dose, %	Rat/Human Potency Ratio	Pig/Human Potency Ratio
FK506	0.0037	0.27	0.3	0.01	0.9
Clobetasol-17-propionate	0.0001	0.033	0.05	0.002	0.7
Hydrocortisone	0.006	>1.0	2.5	0.002	<2.5
Cyclosporin	0.034	>3.0	>3.0	<0.01	Inact./Inact.

In order to improve the existing rodent inflamed skin model, different hypotheses have been suggested to explain this overprediction.

- The classic hypothesis is that the pharmacological mechanism within the animal model is quite different from the pharmacological mechanism of the human skin condition.
- A less classic hypothesis is that the difference observed could be due to a difference in the animal and human topical pharmacokinetics.

The classic hypothesis may well be relevant for some animal models, however the second hypothesis has gained ground in the last 10 years.

Indeed, the second hypothesis is now well established in the pharmaceutical industry in the toxicological area: ToxicoPharmacokinetics/ToxicoPharmacodynamics. Allometry was the first approach used to predict human toxicology from animal work. The trend in the industry now is to go a step further and to apply Physiological Based PharmacoKinetics.

Somewhat behind the toxicology area, the same approach is starting to be used for the choice of the dose to give in the Proof of Concept study (Phase II clinical trials).

In the topical kinetic literature, the knowledge that rodent skin is more permeable than human skin is well established.

Brain et al. [71] review the data available in the ranking of skin permeability among animal species vs. human skin and they conclude:

1. animal skin with high follicular density is poorly representative of human skin [72;73]
2. rat and rabbit do not give reliable estimation of human penetration [74-76]
3. pig and rhesus monkey reasonably approximate absorption of several compounds in human [74;77-81]
4. shaving or depilation of hairy skin may alter the barrier function [82;83]

The differences observed among species is not small and can be molecule size dependent as suggested by Table 3 [84]:

Table 3. Difference in Topical Pharmacokinetics in Between Species

Species	Type	Permeability coefficient ($cm^2/hr \times 10^{-5}$) Water	Permeability coefficient ($cm^2/hr \times 10^{-5}$) Paraquat	Animal/human ratio for Paraquat
Man		92.97	0.73	1
Rat	Wistar Alpk/AP	103.09	26.68	40
	Hairless	103.08	35.51	50
	Nude	151.72	35.34	50
Mouse	Alpk/AP	143.75	97.16	135
	Hairless	350.70	1066.39	1460
Guinea Pig	Dunkin-Hartley	442.09	195.63	270
Rabbit	NZ White	252.61	79.92	110

Some groups therefore started to investigate whether the actual drug delivery could be involved in this overprediction of topical efficacy. In 1992, Meingassner and Stutz [85] set up a new inflamed skin model, using a pig as the animal model. The concept behind this choice was that the pig has a skin permeability comparable to the human one.
In 1998, Mollison et al. [70] proved that the drug delivery hypothesis was correct by showing that the amount of drug to get efficacy in the pig model was equivalent to the human one while much lower doses were required in the rodent model (Table 2).

This same approach has been followed by at least two pharmaceutical companies in the last 10 years to develop new topical immunosuppressors: Novartis [86] and Abbot [70]. Such a development approach led eventually to the development of pimecrolimus, a novel immunomodulator drug that received FDA approval in Dec. 2001.

1.3.3. Topical Drug Candidate Selection Summary

The previous two sections suggest that topical drug classes have over the past 50 years largely been developed in the same way into two distinct stages:

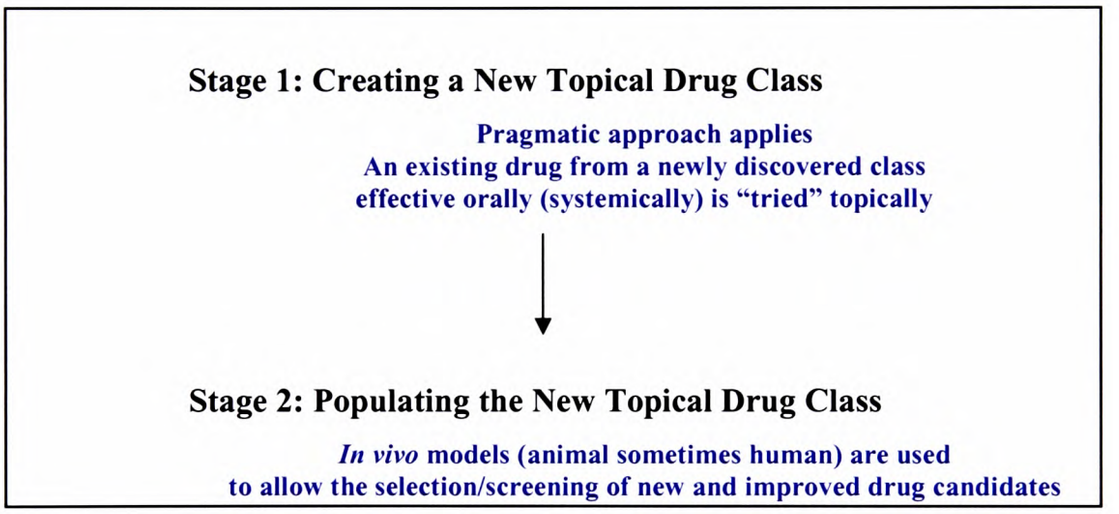

Figure 2. Building up a New Topical Drug Class

It is interesting to note that pharmacokinetics does not seem to play any role for the selection of a topical drug candidate whether for the first drug of the class or the consecutive ones.

The first stage is a quick and very opportunistic approach as it is very much a matter of putting an existing drug in a topical format and testing it in patients. One could say that a lot is left to chance and that seems quite true when it is realised how often the first tested drug in a class failed either for efficacy reasons (cortisone, retinol palmitate, cyclosporin)

or safety reasons (calcitriol) as seen in 1.3.1.9. The concept of speed and low cost involved in this very opportunistic approach does make the use of topical pharmacokinetics sound inappropriate.

The second stage of the process as opposed to the first stage does usually involve some pre-clinical tests. This is a natural way to approach that stage as the aim of the populating stage should very much be to design superior new drugs compared to the existing ones in the class.
The single use of a topical rodent model does not reliably allow improvement of the drug candidate selection because it does not predict human efficacy nor does it predict systemic exposure. However, the combined use of a pig model and a rodent systemic model (1.3.2.4) should however improve the drug candidate selection, as they are relatively good predictors of systemic exposure and human efficacy. These models therefore answer indirectly the same questions that topical pharmacokinetics would answer. This makes the use of topical pharmacokinetics redundant for the drug candidate selection at the populating stage as well.

These conclusions do not support the use of topical pharmacokinetics at the drug candidate selection stage of the drug development process.

The view on such conclusions would change however:

1- **If one considers that a pig model is a difficult model to set up and manage. The industry is used to small rodent models and few pharmaceutical companies investigating new topicals seem to have switched to the use of pig models.**

2- **If one notices that only few skin diseases have validated animal models (even rodents one).**

3- **If a topical pharmacokinetic tool did exist that proved to be more effective (for cost or time or reliability) than combined animal models.**

1.4. Topical Vs. Oral Drug Development

The drug development process has already been described conceptually in 1.2.1. and the topical drug candidate selection stage has been discussed in 1.3. The aim of this section is to investigate the drug development process more in detail and compare the oral and topical processes.

For that purpose the three central stages of the drug development process reviewed in 1.2.1 are divided into 5 steps.

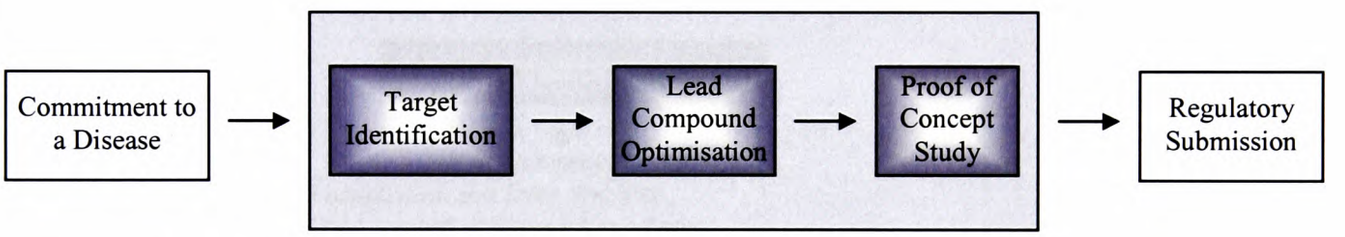

Figure 1. The 5 key stages of the drug development process

1.4.1. The Oral Drug Development Process

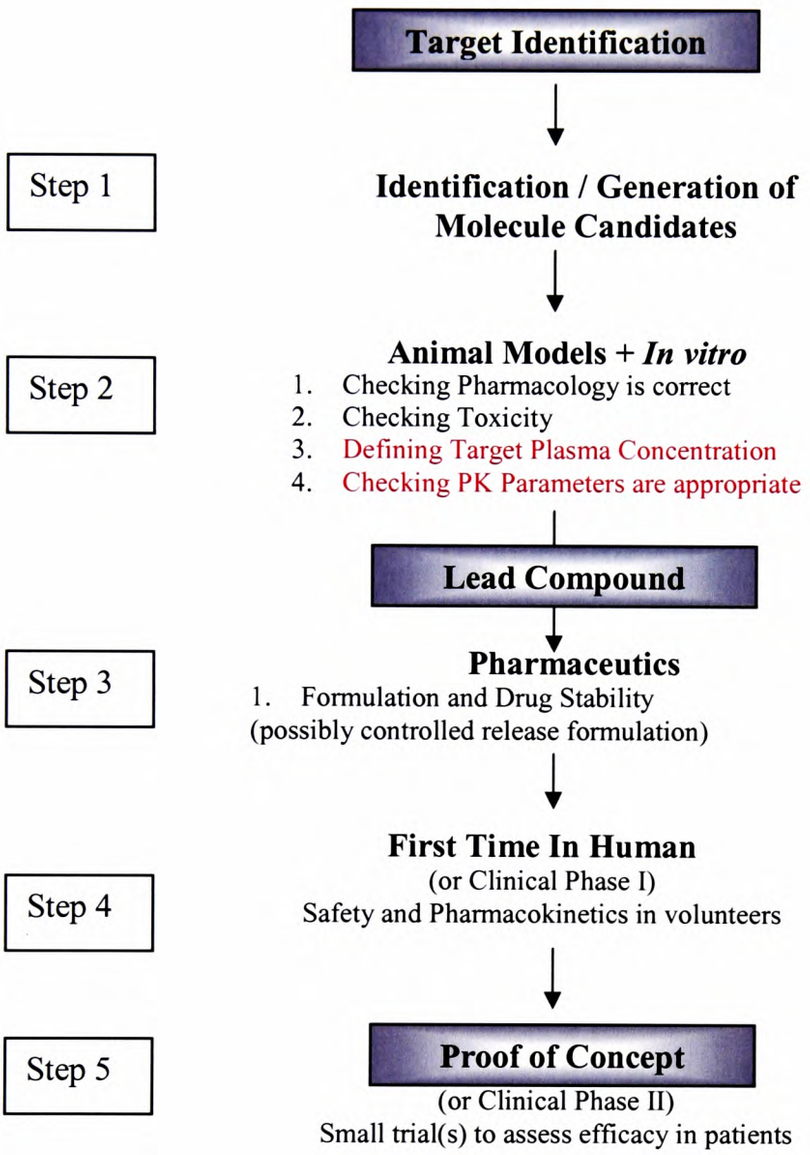

Figure 3. The Central Steps of the Oral Drug Development Process

1.4.2. The Topical Dug Development Process... or Maximising Drug Delivery

The topical drug development process follows in theory the same 5 steps. In fact as discussed in 1.3.3, very often, because a drug with an interesting pharmacology already exists, that drug is developed (for topical use) from Step 2 or even from Step 3.

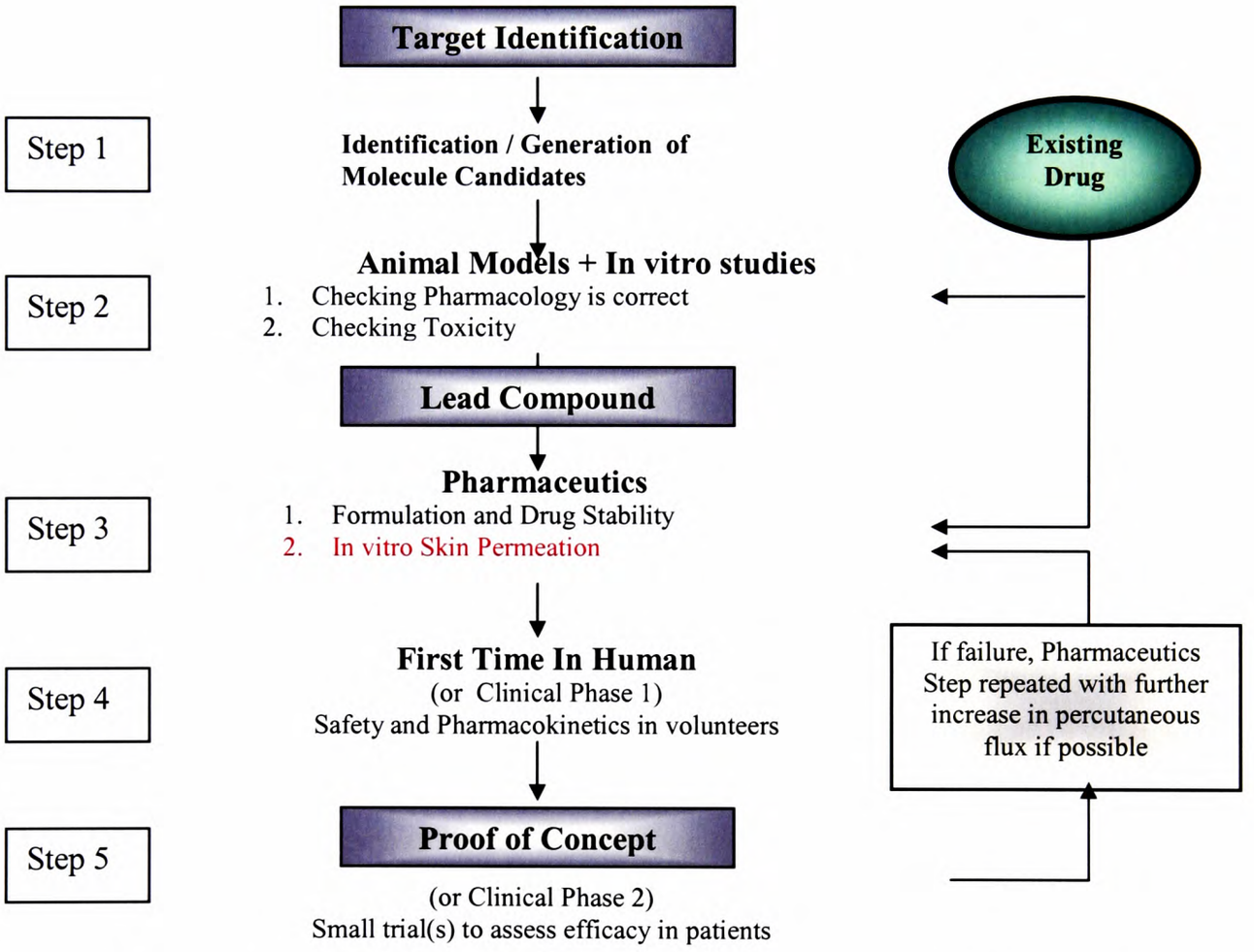

Figure 4. The Central Steps of the Topical Drug Development Process

1.4.2.1. The Two Key Differences in Between the Oral and Topical Drug Development Process

Both oral and topical processes are quite similar in their different steps apart from Steps 2 and 3.

1.4.2.1.1. Defining Target Tissue (Skin) Concentration

In the oral development process, one key role of Step 2 is to define the target plasma concentration required to get the desired pharmacological response in the animal model. This concentration will indeed become a target concentration to reach in the Clinical Phases 1 and 2. The aim for the Clinical Phase 1 stage is then to escalate the dose given to human volunteers up to a dose that will give similar plasma concentration (more precisely, similar unbound plasma concentration) as the one observed in the animal that showed the pharmacological response. If such doses can be administered safely to human volunteers, such doses can be given then to patients with reasonable confidence that at this dosage the drug administered should exhibit its pharmacological activity.

In the topical development process, this target concentration is absent as there are no established ways to measure reliably drug levels in the skin tissue or to link plasma drug levels with skin tissue levels (see 1.6). Also, plasma levels following topical delivery are exceedingly low. This induces a lot of uncertainty when moving on from Step 2. This means as well that the Proof of Concept study has to answer two questions: 1 - Is the pharmacology correct? and 2- Is there enough drug at the target site? A negative outcome in the Proof of Concept study is therefore difficult to interpret [87]. If the pharmacology is blamed while the true problem was a drug delivery one, this can have as a consequence the abandoning of a pharmacological target that could have been proven useful if the correct compound had been selected in the first place.

1.4.2.1.2. Checking that the PK Parameters are Appropriate

In the oral drug development process, a drug candidate must meet the two following criteria to pass pre-clinical PK selection:

1- Bioavailability + Rate of Delivery
2- Appropriate "Half Life" that will allow reasonable dosage regimen (e.g. once a day tablet ideally)

- **Bioavailability + Rate of Delivery : The use of the Lipinsky's rules to select good and discontinue bad drug candidates in the oral drug development process and the lack of such rules in the topical drug development process**

With most of the key biological membranes, there are established empirical rules or equations defining the rate (flux) at which a molecule can be transferred through that membrane. Most of the work has naturally been conducted on the gastrointestinal membrane, as it is the key membrane that a drug given orally will have to cross to enter the systemic circulation.

While selecting an oral drug candidate, there are two criteria that are examined regarding the gastrointestinal membrane passage across: 1- Will it be possible to get a sufficiently high bioavailability such that subject to subject plasma level variation is small? and 2- Will it be possible to deliver enough drug to reach the pharmacologically effective drug plasma level?

To answer to these questions researchers have sought to understand the physicochemical properties that favour intestinal absorption [88-91].

The so-called "rule-of-5" has proved very popular as a rapid screen for compounds likely to be poorly absorbed [92]. The rules states that if a compound satisfies any two of the following rules, it is likely to exhibit poor intestinal absorption:

-Molecular weight >500

-Number of hydrogen bond donors > 5 (a donor being any O-H or N-H group)

-Number of hydrogen bond acceptors > 10 (an acceptor being any O or N including those in donor groups)

-Calculated log P > 5.0 (with C Log p Daylight software, version 4.51)

[Compound classes that are substrates for biological transporters are exceptions to the rule]

However these rules are not used on their own. The "triad of potency, solubility and permeability" has to be taken into account. For example, solubility guidelines to the Pfizer's chemists suggest a minimum solubility of 50μg/ml for a compound that has a mid-range permeability and an average potency of 1.0 mg/kg [92].

This set of rules and guidelines indicate which drug candidates that would not deliver enough via the GI tract for their potency.

Interestingly, in topical drug development this approach is largely incomplete. Indeed, if there are tools to predict the rate at which a molecule can cross the skin membrane as reviewed by Pugh et al. [93], these tools do not define whether the rate (flux) for a particular molecule is sufficient or not to deliver the required concentration. There should be some link with the drug potency as shown in the Lipinsky paper [92], with the "triad of potency, solubility and permeability"; but the lack of the knowledge of the drug concentration in the skin tissue prevents such rules to be established. Bad drug candidates cannot therefore be discontinued from further development with that method. The only tool remains the use of existing animal models.
As reviewed in 1.3.2 and particularly in 1.3.2.4, such animal models are often rodent models for which predicting for human efficacy is very poor due to the large difference in skin permeability.

• **The concept of "half life" in topical therapy**

This concept just does not exist in topical therapy!

Classically when a clinical trial for a new topical candidate is designed, the clinical protocol states that the topical should be administered "twice a day". This is more an historical heritage than a rationale reason for "a twice a day" dosing.

It is interesting to see that with some topical preparations after having been registered as "twice a day", further clinical work has been sometimes conducted and has showed that reduced dosing regimen can give similar efficacy: This is the case for example of Temovate® (clobetasol propionate) or Cutivate® (fluticasone propionate) once a day being equivalent to twice a day. The case of the antifungal Lamisil® (terbinafine) is spectacular as a single dose has shown to be as effective as a once a day treatment for one week [94].

1.4.2.2. Consequences for the Pharmaceutics Stage: Maximising the Percutaneous Flux!

The knowledge that the animal models overpredict the topical efficacy in human, as well as, the knowledge that a poor drug candidate from a skin permeability point of view cannot be screened out, have a logical consequence on what should be done at the pharmaceutical development stage of a topical drug:

"The percutaneous flux of the drug candidate must be maximised in order to limit the risk of not delivering enough drug."

This requirement pushes the topical drug development project team to impose to the pharmaceutics team involved in the formulation of the drug to go for a percutaneous penetration enhancement program. An iterative process then follows that has for aim to maximise percutaneous permeation for the compound candidate. This process can be summarised in Figure 5 below:

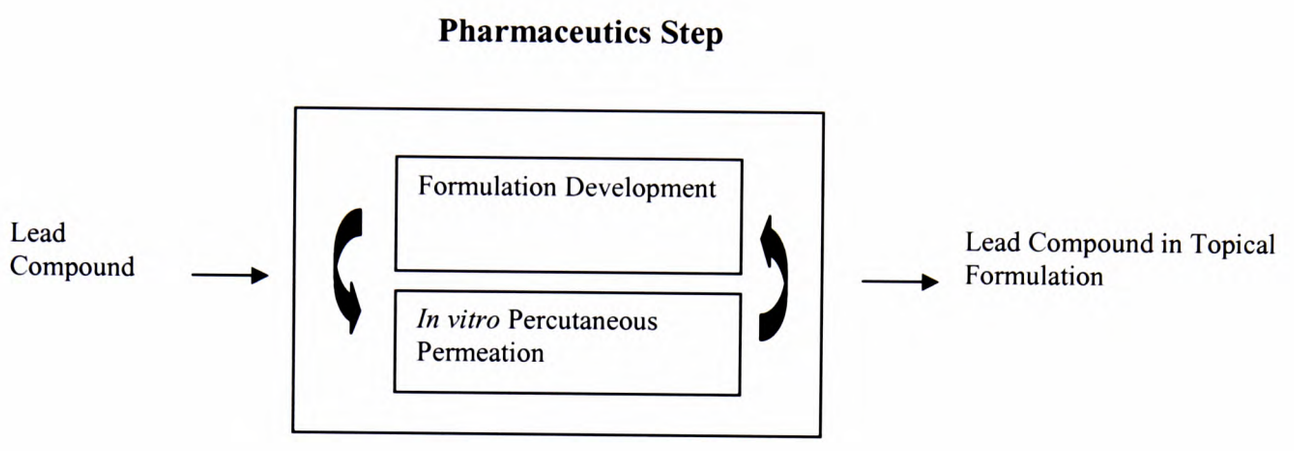

Figure 5. Classic Topical Drug Development Pharmaceutics Process

The consequences of this development approach are:

- Cost in resource
- Cost in development time
- Uncertainty on the irritancy outcome as the penetration enhancers used often have potential irritancy associated with them
- Uncertainty on the consumer acceptability of the topical formulation developed as the presence of penetration enhancer can have negative effect on the aesthetics of the formulation so will have the move from a cream to an ointment presentation.

1.4.3. What can be learned from the Oral Drug Development Process?

The previous section shows the consequences of lacking a skin concentration target in Step 2 of the topical drug development process. It has been shown that the best solution that the pharmaceutical industry has been able to use, is to push as much drug as possible through the skin.

It is however ***missing the point*** as such an approach will have 3 potential consequences:

1- the topical treatment is effective and does not cause systemic side effect
2- the topical treatment is effective but does cause systemic side effect (over dosing)
3- the topical treatment is not effective (still not enough drug at the target site)

Case N°1 is ideal as it delivers the required treatment.

Case N°2 is generally spotted in the human clinical phase 1 in volunteers and requires the percutaneous penetration to be reduced which can be done easily by for example reducing the concentration of the drug in the topical formulation.

Unfortunately however, case N° 3 will usually occur. In such circumstances the topical drug development project team considers that the percutaneous penetration of the drug candidate could have been further improved. The drug candidate goes back then to the Pharmaceutics Step as shown on Figure 4. Such an iterative approach is not necessarily successful, as the percutaneous penetration of a molecule can be increased up to a point but not further. This is illustrated for example in the iterative clinical trials that have investigated the use of topical cyclosporin in the treatment of psoriasis as discussed in 1.3.1.8.

In summary, the true solution to these problems is likely to lie in transforming the current topical drug development process in a process that would be equivalent to the oral drug development process: Modifying Step 2 as shown in Figure 6.

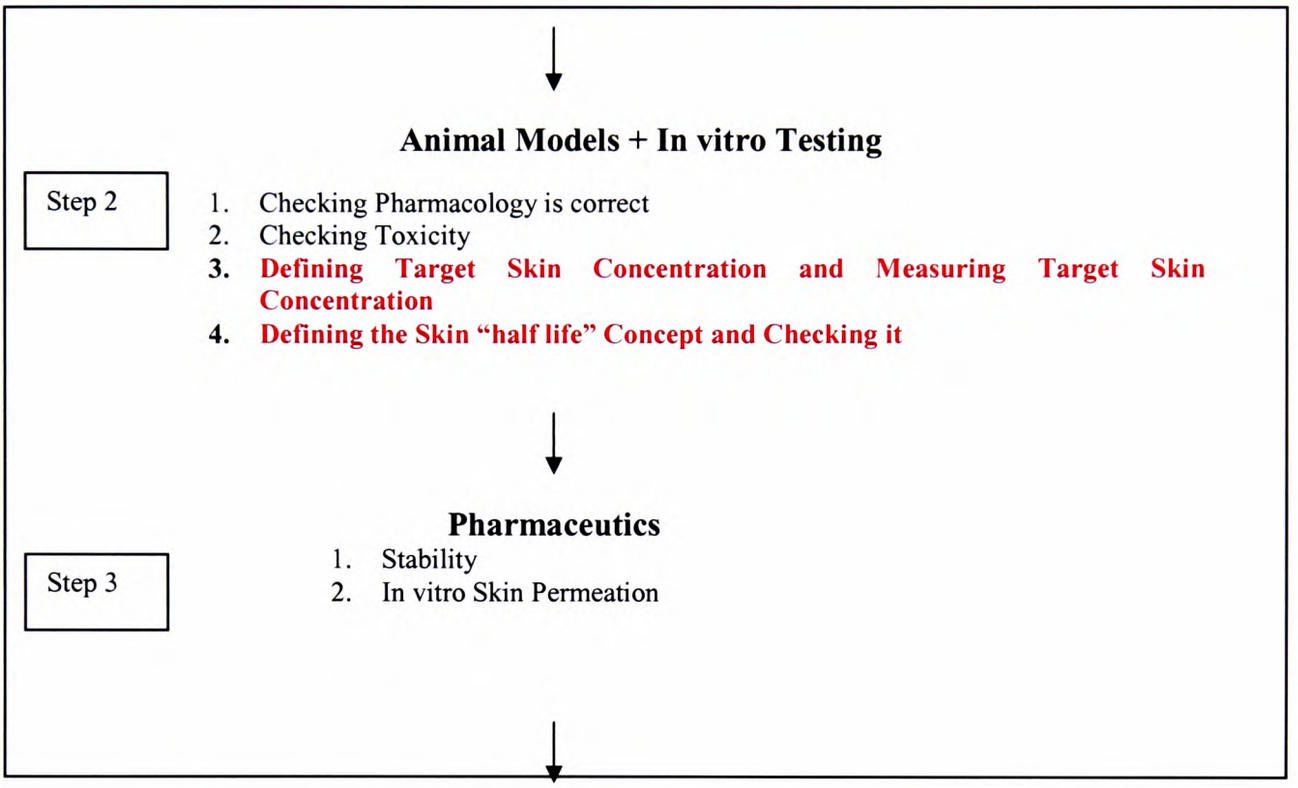

Figure 6. Improved Topical Drug Development Process

1.5. Key Factors Influencing the Efficacy of a Topical Drug Candidate: Learning from Past Topical Drug Development

In the previous section, the learning from the oral drug development process has been used to foresee how the topical drug development process could be improved. In this section, the focus is on what can be learned from the successes and failures of topical drug development over the last 50 years and draw some conclusions from there.

1.5.1. Skin Barrier Condition vs. "Easiness" of Topical Drug Development

In section 1.3.2.4 it has been shown that topical drugs are proven more effective in rodent animal models than in the human skin, and that this difference in effectiveness seems to be linked with a difference in topical drug delivery.

To investigate further the effect of drug delivery on the effectiveness of a topical drug, it is interesting to study the effect of the skin condition –its barrier properties- on the efficacy of a topical drug. This section aims to review each drug class with the specific skin diseases they treat in order to see whether some skin diseases are more easily treatable than others. In order to do that, the skin itself, as well as the different key skin diseases, needs to be better understood which is the object of 1.5.1.1 and 1.5.1.2.

1.5.1.1. The Skin

The aim of this section is to provide the required information about skin so that its barrier properties as well as its different parts and their functions are well understood.
This information known should allow us to understand better, how skin diseases can influence some of the key skin properties such as its barrier function.

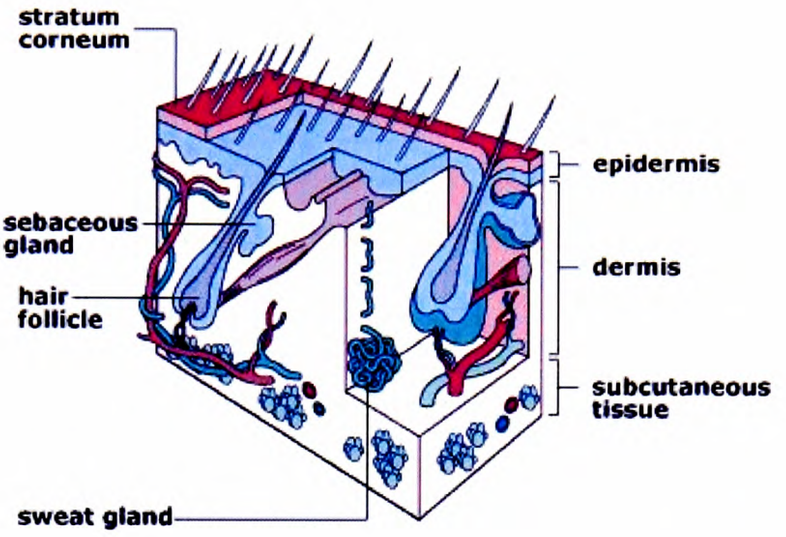

Figure 7. The Skin in 3D

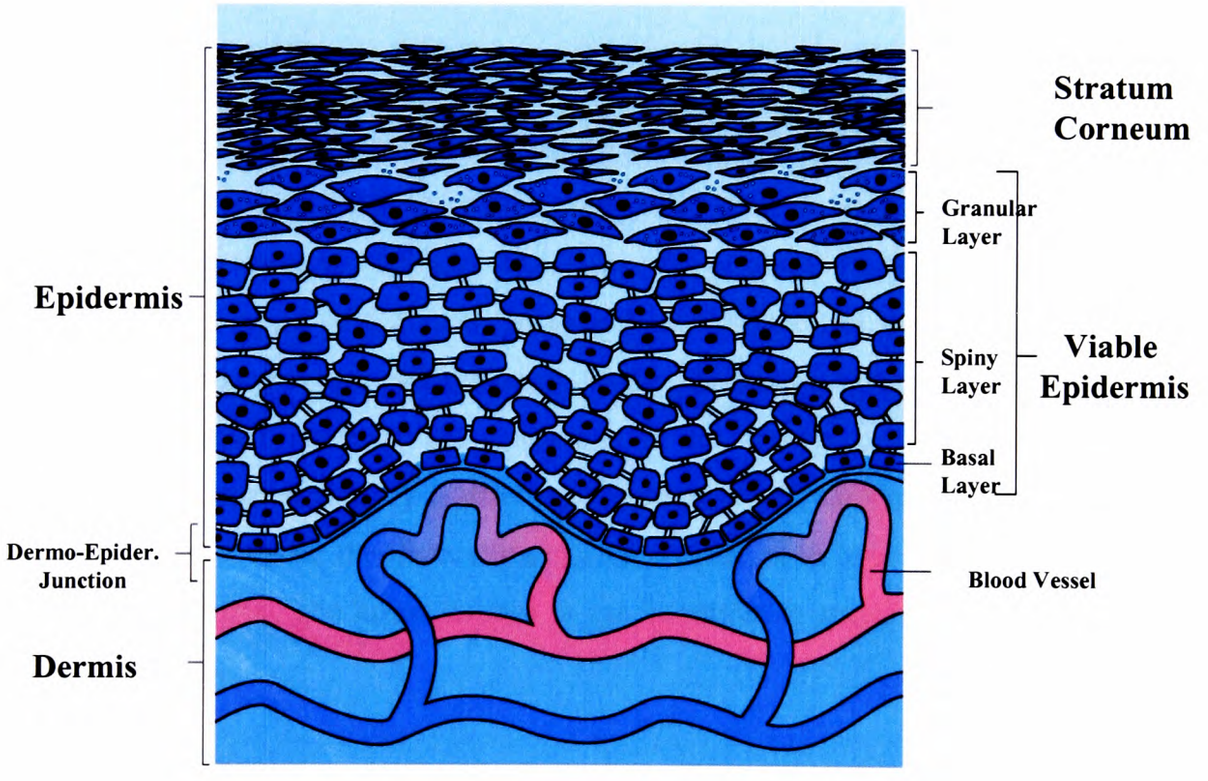

Figure 8. The Skin in Cross Section

1.5.1.1.1. The Skin Functions

The skin, one of the largest organ of the body with an average surface area of 1.8 m^2 for an adult, combines with the mucosal membrane of the respiratory, the gastrointestinal and the urogenital tracts to form a capsule which separates the internal part of the body from the external world.

The main functions of the skin are to provide a watertight barrier, which provides body heat control and protection from environmental hazards. The skin forms a great barrier against chemicals, micro-organisms, as well as a protection against radiation, heat, electrical and mechanical trauma. The skin vascular system helps to regulate body temperature, and the nerve network present in the skin mediates the sensations of touch, pain, heat and cold. The skin expresses as well social signals like anger, fear and anxiety.

1.5.1.1.2. The Stratum Corneum

The stratum corneum (SC) or horny layer, is the outermost layer of the skin and the main barrier to percutaneous absorption of chemical compounds despite being a very thin layer of an average thickness of 10-20μm. The barrier properties of the SC are attributed to the highly organised layers of flattened, polygonal corneocytes and specialised intercellular lipids. The corneocytes are cell remnants of the terminally differentiated keratinocytes found in the basal layer of the epidermis at the epidermal-dermal junction. The corneocytes are surrounded by a practically insoluble and very resistant cell envelope. Around the corneocytes, the intercellular space is filled with lipids organised in stacked bilamellar structures. The lipids located in the intercellular space play a key role in the barrier formation.

1.5.1.1.3. The Viable Epidermis

Below the SC is the viable part of the epidermis. Its thickness varies from 50-200μm. Its main function is the production and maintenance of the SC. As well, it does have a role as a metabolic barrier against exogenous substances (see 1.5.1.1.7). The viable epidermis constitutes a dynamic system in which the keratinocytes proliferate from the basal layer,

differentiate as they progress towards the SC and get transformed into corneocytes. The turnover time for a keratinocyte from the basal layer to the skin surface is about 28 days for normal skin. It also contains specialised cells like melanocytes, which protect the body against UV radiation. The viable epidermis does not contain blood vessels as it receives nourishment from the dermis by passive diffusion. The viable epidermis is not considered as having strong barrier properties.

1.5.1.1.4. The Dermis

The dermis is situated below the viable epidermis. It is approximately 1 to 3 mm thick and constitutes the bulk of the skin. It consists of a matrix of connective tissues made from fibrous proteins like elastin and collagen. The main functions of the dermis are to give mechanical strength and elasticity to the skin barrier, to supply oxygen and nutrients, and to remove waste products.

The dermis has an extensive vascular supply, which regulates temperature and pressure, delivers nutrients, removes waste products and mobilises defence forces. There are two networks of blood vessels in the dermis: the superficial vascular plexus in the upper dermis and the deep vascular plexus in the lower dermis. These plexuses are extensively branched and a particularly dense network of capillaries is formed around the appendages. The lymphatic system forms another important vascular network. It regulates the interstitial pressure, mobilises defence mechanisms, and removes waste, e.g. the removal of extracellular proteins. The importance of this second vasculature network for the clearance of foreign compounds does not appear to be well studied in the literature. Due to the presence of these networks, exogenous substances as well as skin waste products are well cleared from the skin. Therefore the local concentrations in the dermis, of a compound applied topically, are particularly low and a steep concentration gradient from the skin surface to the dermis region is formed. The dermis therefore acts very much as a "sink".

1.5.1.1.5. The Subcutaneous Tissue

The subcutaneous tissue is a fatty layer of connective tissue that anchors the dermis loosely to the underlying muscle or bone. The large fat content of this tissue provides the skin its heat insulator property, its shock absorbing property and provides, as well, a good source of energy. The presence of collagen and elastic fibres allows for a good skin flexibility and free movement over the underlying structures.

1.5.1.1.6. The Skin Appendages

In humans, it is estimated that skin appendages account for less than 0.1% of the skin surface area but have to be considered in the discussion of percutaneous routes as they produce an apparent discontinuity in the barrier integrity. There are two types of skin appendages: the follicles and the sweat glands. Their structures have some similarities. They all cross the SC barrier and run deep in the dermis. They all have a central part believed to be relatively permeable. The outer part of these appendages is a membrane of unknown permeability. Unless this membrane is relatively permeable or can be opened under certain conditions, the skin appendages will or will not constitute a route of entry of exogenous compounds.

• **The follicles**

There are three types of follicles in human skin:

- Terminal hair follicles (e.g. hair on the head) in which a large hair is associated with a large sebaceous gland. The root of this hair may extend more than 3mm below the skin surface into the subcutaneous fatty tissue.

- Vellus hair follicles (e.g. fine hairs on the face in women), in which only a small sebaceous gland is associated with a fine hair. Its roots extend less than 1mm in the dermis.

- Sebaceous follicles (or sebaceous glands) are characteristic of human beings and not present in animals. They are found mainly on the face and the central parts of

chest and back. It consists of 4 parts: the sebaceous gland lobes that secrete sebum (a mixture of lipids), the ducts that connect the lobes and the secretory duct, a small vellus hair and the secretory duct which is a long duct lined by keratinocytes that is the large duct that conducts the sebum from the sebaceous gland to the skin surface. The keratinocytes produce corneocytes that normally are ejected outwards. The sebaceous duct is situated approximately 0.5 mm below the skin surface.

• **The sweat glands**

Sweat glands are tubular glands distributed almost over the entire human body. Each gland has a secretory part located below the dermis in the subcutaneous tissue and an excretory duct that ultimately opens directly on the skin surface. These glands produce perspiration.

1.5.1.1.7. Skin Metabolism

The skin is not just a passive barrier to foreign compounds, it is as well an active metabolic barrier that degrades a wide variety of compounds [95]. This local phenomenon can therefore have an effect on the pharmacological activity of a compound: reducing its activity or increasing it if the metabolite is more active than the parent molecule.

The skin contains a wide range of enzymatic activities, including phase I functionalisation reactions (oxidative, reductive, hydrolytic) and phase II conjugative reactions [96].

Various attempts have been made to compare skin and liver metabolism. They suggest that skin activities ranges from 0.1% to 50% of liver activities [95]. However, significant skin enzymatic activity has only been well established for esterase activity. Cutaneous esterase activity has been reported for corticosteroid esters [97], metronidazole esters [98], parabens [99], and salicylates [100].

1.5.1.2. Some of the Key Skin Diseases

The aim of this section is not to give an exhaustive list of the existing skin diseases but to concentrate on the important ones that will be discussed in sections 1.5.1.3, 1.5.1.4 and Chapter 6.

1.5.1.2.1. Atopic Dermatitis (A.D.) (target site = top dermis)

Atopic dermatitis occurs in about 20% of the children population [101]. The disease starts early in life, most often in the first year of life, and will often disappear before adulthood. It is a chronic inflammatory skin condition. The classic feature is itchy skin. The skin is dry, flaky, rough, and can be secondarily infected and show oozing and crusts.
The skin barrier property of atopic dermatitis lesions is viewed as being diminished compared to normal skin.

The body surface area affected by Atopic Dermatitis depends on disease severity. 10% body surface area affected represents a moderate to severe case (~ 2000 cm^2 in adults).

1.5.1.2.2. Psoriasis (target site = top dermis / bottom epidermis)

Psoriasis affects about 13 million of people world-wide [102]. It is more prevalent in the population of the western world and is less common in tropical and sub-tropical climates. The involved sites for psoriasis are the "extensor" surfaces of the extremities, particularly the elbow and the knee as well as the lumbar area and the scalp, but psoriasis can occur in any part of the body. Psoriatic skin lesions are inflammatory, red, sharply delimited plaques of various shapes with characteristics silvery lustrous scaling. It has not yet been established whether psoriasis is primarily an epidermal or dermal disease. There are indeed two primary activities. The first one occurs in the epidermis. There is a large increase in the volume of the epidermis, which is 4 to 6 fold greater than normal, characterised by high keratinocyte proliferation and a very short keratinocyte life cycle. From basal layer to stratum corneum the keratinocyte life cycle is reduced to about 2 days as opposed to 28 days for normal skin. The second one occurs in the dermis where there is a strong inflammatory reaction with a network of capillaries expanding from the

one present in normal skin [103]. The barrier property of psoriatic plaque compared to normal skin has been studied with hydrocortisone *in vivo* [104]. The conclusion was that the barrier property of psoriasis plaque skin was comparable to normal skin.

The body surface area affected by psoriasis depends on disease severity. 10% body surface area affected represents a moderate to severe case (~ 2000 cm^2).

1.5.1.2.3. Acne (target site = sebaceous gland and duct)

Acne is one of the most common diseases in dermatology. It occurs at puberty in almost every one, although to different extents and it usually regresses in early adulthood. The preferred sites are naturally on the face as this is a disease affecting the sebaceous follicles. It is characterised by seborrhoea, disturbed keratinisation in the follicles with comedones (blockage of the sebaceous follicle), and subsequent inflammatory papules, pustules, and nodular abscesses and scars. One of the most important factors in acne is the superior production of sebum in acne sites as opposed to healthy skin sites. Sebaceous follicles are densely populated by bacteria and fungi. In the deeper anaerobic area of the sebaceous glands, one finds propionibacteria. These produce lipases and are regarded as substantial factors in the pathogenesis of acne. The first detectable sign of acne is increased production of corneocytes in the secretory duct, but these are no longer extruded outwards. Comedones occur therefore through hyperkeratosis associated with proliferation and retention. Bacteria proliferation and inflammation can then occur [105].

The body surface area affected by acne being usually the face the classic surface area treated will represent about 2% of the total body surface area (~ 400 cm^2 in adults).

1.5.1.2.4. Fungal Infection (target site = stratum corneum)

Skin fungal infections or dermatomycoses are diseases caused by fungi. There are 3 types of fungi: dermatophytes (of which Trichophyton, Microsporum and Epidermophyton are the most important causes of dermatomycoses), yeasts (of which Candida Albicans is by far the most important) and finally moulds (of which only few cause superficial

mycoses). Tinea pedis or mycosis of the foot is the most common fungal disease as its frequency in Europe and North America is estimated at 15%-30% [106]. In dermatomycoses, the fungi remain in the stratum corneum and do not invade the deeper layers of the skin [107].

The body surface area affected by fungal infection is generally limited to the plantar region and will represent about 2% of the total body surface area (~ 400 cm^2).

1.5.1.2.5. Viral Infection – Cold sore (target site = basal layer – broken skin during cold sore development)

A cold sore is a lesion caused by herpes simplex virus. The lesions usually occur around the lips. After the primary infection the virus hides within the nerve ganglion supplying the primary infected area. Following a trigger factor, the virus is activated and travels back to the skin and starts replicating at the base of the epidermis. That replication causes tissue damage and a strong host immune response that will further damage the local tissues. Within 24 hours, papules are formed. After about 48 hours, these papules further coalesce to form one large sore that will burst. At this stage the skin barrier is therefore absent. From day 3 onwards, the skin recovers slowly by forming a crust that will eventually disappear by day 7-10 [108].

The body surface area affected by a cold sore is very small and will generally not exceed 10 cm^2.

1.5.1.2.6. Dermal Anaesthesia (target site = dermis)

Dermal anaesthesia will generally be practised before local surgery onto skin with normal characteristics (not inflamed and with normal permeability).

The body surface area affected by such surgery is generally very small and will generally not exceed 50 cm^2.

1.5.1.2.7. Muscle Pain (target site = sub-cutaneous tissue)

Treatment of muscle pain will generally be practised onto a sore part of the body. Skin at this body site will be in normal condition (normal permeability) but at the target site some inflammation will be present.

The body surface area affected by muscle pain will vary and generally represent about 2 to 3% of the body surface area (~ 500 cm^2 in adults).

1.5.1.3. Success Rate of Oral Drugs Subsequently Developed as Topical Drug as well as First in Their Class: Differences from One Drug Class to Another

The review of the success rate of oral drugs developed as topical drugs is of interest as it may give some ideas of what kind of parameters may influence, positively or negatively, the successful development of a topical drug:

Table 4. Success Rate of Oral Drug Developed Topically and First in their Class Topical Drugs

Drug Class	Success	Mixed results (positive but limited results)	Failure	References
Antifungals	Etonam Ketoconazole Terbinafine			see 6.7
Antivirals		Acyclovir Penciclovir		[37-39;109-111]
Retinoids			Vitamin A (psoriasis)	see 6.4
Vitamin D3 derivatives		Calcitriol (effective but systemic side effect)		see 6.6
Immuno-Modulators	Tacrolimus (eczema, facial psoriasis)		Cyclosporin A – Tacrolimus (psoriasis)	see 6.9
Corticosteroids		Hydrocortisone (eczema)	Cortisone Hydrocortisone (psoriasis)	see 6.5
NSAIDs	Equivocal	Equivocal	Equivocal	see 6.2

The drug classes discussed above can be summarised into two categories:
1- Easy Development Drug Class, defined as a class in which all oral drugs tried topically were successfully developed
2- Difficult Development Drug Class, defined as a class in which some oral drugs tried topically failed or were only partially successful.

Easy Development Drug Class: The Antivirals (all oral compounds successful topically) and the Antifungals (Etonam (1^{st} azole): effective – Ketoconazole (oral => topical): effective – Terbinafine (1^{st} allyl): effective].

Difficult Development Drug Class: The local Anaesthetics (Benzocaine: not indicated on intact skin – Cocaine cannot be commented as not indicated for drug-abuse reason), the Retinoids (Retinol palmitate: ineffective in Psoriasis – Retinol: effective in ageing), the Vitamin D3 derivatives (Calcitriol: systemic exposure issue), the Immunosuppressors (Cyclosporin: ineffective – Tacrolimus: effective (atopic dermatitis)), the Corticosteroids (Cortisone: ineffective – Hydrocortisone: effective in atopic dermatitis but ineffective in Psoriasis).

The NSAIDs are a difficult family to discuss properly, as their clinical efficacy is variable and equivocal.

1.5.1.4. Merging Drug Class, Barrier Condition of the Skin Diseases, and Success Rate of Topical Drug Development

The learning of the two previous sections (1.5.1.2, 1.5.1.3) can be represented in a single table (Table 5).

Table 5. Barrier Condition of Skin Diseases and Success Rate of Topical Drug Development

Drug Class	Disease	Target Site	Skin Barrier	Easiness of Topical Drug Development *
Corticosteroids	1. A.D. 2. Psoriasis	Dermis Epidermis/Dermis	Partly damaged Not damaged	- - -
Antifungals	Fungal Infection	Stratum Corneum	Not damaged	+
Anaesthetics	Dermal Pain	Dermis	Not damaged Damaged (insect bite)	- 0
NSAIDs	Muscle Pain	Sub Dermis	Not damaged	0
Antivirals	Coldsore	Bottom Epidermis	Severely damaged (by day 2 of coldsore)	+
Retinoids	1.Psoriasis 2.Acne	Epidermis/Dermis Sebocyte	Not damaged Not damaged	- -
Vitamin D3 derivatives	Psoriasis	Epidermis/Dermis	Not damaged	-
ImmunoModulators	1.A.D. 2.Psoriasis	Dermis Dermis	Partly damaged Not damaged	 - -

*: + for easy, 0 for equivocal, - for difficult, -- for very difficult topical drug development

This table suggests that the ability to develop easily (or not) a topical depends on two things:

1. if target site is in the stratum corneum (= top of the skin) => easy development, but if target site is deeper, the development is more difficult
2. if skin is severely damaged => easy development, but if skin is however not damaged the development is more difficult.

It is interesting to note that in atopic dermatitis (A.D.) where the skin barrier is partly compromised the skin condition seems to be treated more easily than psoriasis. One could

reasonably argue that it is the pharmacology of the disease responsible for the difference but it could well be argued that drug delivery could play a major role.

1.5.2. Drug Potency

Drug potency is another key element that can be learned from past topical drug development experience as being critical in the efficacy of a topical drug. The examples below of three drug classes prove the importance of this parameter.

1.5.2.1.1. The Case of Corticosteroids

Potency ranking:

Table 6. Potency of 3 Corticosteroids

	Relative Receptor Affinity (RRA) to Dexamethasone						Mean Relative Potency
References	[112]	[113]	[114]	[115]	[116]	[117]	
Steroids							
Dexamethasone	100	100	100	100	100	100	100
Hydrocortisone	6.7	7.8	17	11	31	32	11.1
Betamethasone Valerate	440	1663	2550	220	400	353	622.1

Betamethasone Valerate > Hydrocortisone

Note: Dexamethasone = control corticosteroid in RRA assay.

Clinical Efficacy:

Hydrocortisone is classified as "mild" in the topical corticosteroids while betamethasone valerate is classified as "potent" [118].

betamethasone valerate > hydrocortisone

=> The comparison of the potency ranking of hydrocortisone and betamethasone valerate does match the clinical efficacy ranking.

1.5.2.1.2. The Case of the Immunomodulators

Potency ranking:

Table 7. Potency of 2 Immunomodulators

Potency	Mean unbound Drug Potency (nM)	unb. Drug Potency 1 (nM)	unb. Drug Potency 2 (nM)	unb. Drug Potency 3 (nM)	unb. Drug Potency 4 (nM)
Cyclosporin A	11	11.3 oral dose = 2.5mg/kg + PK [119]	10.9 [120] [IC 50 in vitro]	7 [121] [IC 50 in vitro]	20 [122]
Tacrolimus	0.17	0.1 oral dose = 0.1mg/kg +PK [123]	0.7 [120] [IC 50 in vitro]	0.07 [124] [IC 50 in vitro]	

Tacrolimus > Cyclosporin A

Clinical Efficacy:

Topical tacrolimus is effective in the treatment of atopic dermatitis [55] while Cyclosporin A has showed limited or no activity in atopic dermatitis [51;52].

Tacrolimus > Cyclosporin A

=> The comparison of the potency ranking of tacrolimus and cyclosporin A does match the clinical efficacy ranking.

1.5.2.1.3. The Case of the Local Anaesthetics

Potency ranking:

Table 8. Comparison of 4 Anaesthetics

Potency	Mean unbound drug Potency (μM)	Unbound Drug Potency (μM) [125]	Unbound Drug Potency (μM) [126]	Unbound Drug Potency (μM) [126]
Tetracaine	3.5	3.4	2.7	4.5
Lidocaine	155	240	141	110
Prilocaine	125	54	85	427
Benzocaine	910	910		

Tetracaine > Prilocaine > Lidocaine > Benzocaine

Clinical Efficacy:

Head to head comparison of these 4 anaesthetics is found in the literature. In a pinprick model[127], Ametop (tetracaine) is compared with EMLA (lidocaine+prilocaine) showing superiority of Ametop. In another study with the same model [128], saturated solution of tetracaine, lidocaine and benzocaine are compared showing that tetracaine is superior to lidocaine which is superior to benzocaine.

Tetracaine > Lidocaine + Prilocaine > Benzocaine

=> The comparison of the potency ranking of tetracaine, lidocaine, prilocaine and benzocaine does match the clinical efficacy ranking

Learning from these 3 examples suggests that the drug potency will have a major impact on the topical efficacy of a topical drug candidate.

1.5.3. Linking Drug Delivery, Drug Potency and Efficacy

The location of the target site for a skin disease as well as the influence of this disease on the skin barrier properties appear to be critically important for the successful development of a topical drug. Likely due to these factors, some skin diseases appear to

be indeed more easily treatable (fungal infections and possibly cold sores) than others (atopic dermatitis, psoriasis or acne).

It is possible to explain these findings by schematically describing qualitatively the concentration reached in the different tissues in intact vs. damaged skin. After topical application and because of the passive diffusion transport of drugs through skin, a concentration gradient exists through the three skin compartments. Using the mean concentration in each skin compartment, skin concentration with skin depth can be represented as followed in Figure 9.

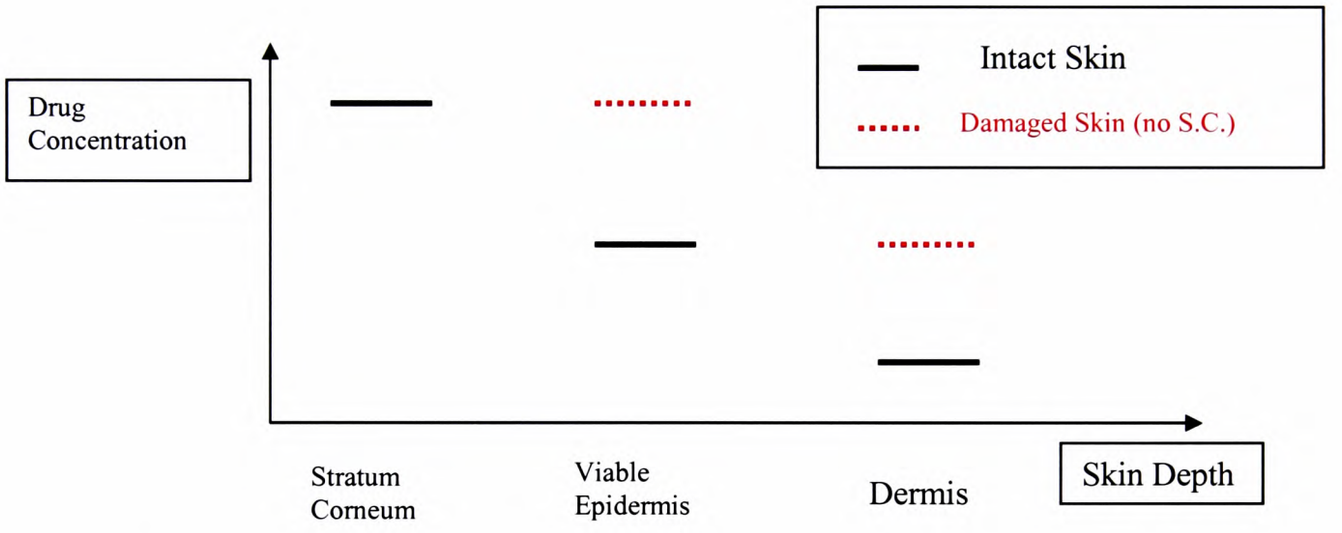

Figure 9. Schematic Skin Concentration in Intact and Damaged Skin

The stratum corneum being at the surface of the skin (and because of favourable drug partitioning into stratum corneum lipids for most drugs), drug concentration is higher than in the deeper tissues, hence a higher rate of success for drugs targeting this tissue.

As well, as represented on Figure 9, with damaged skin, local concentrations are higher for the same target tissue hence a higher success rate for drugs targeting a skin disease where the barrier is impaired.

Drug potency has been shown as well to be critically important for the successful development of a topical drug.

Using the same approach as above, the effect of the drug potency can be schematically described as in Figure 10.

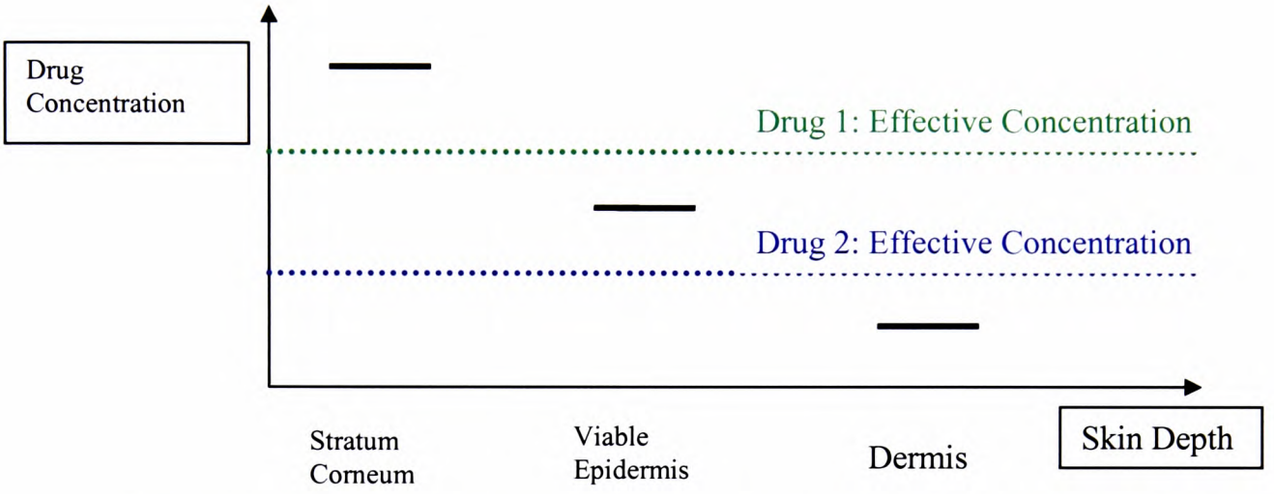

Figure 10. Schematic Representation of Drug Potency Effect with regards to Efficacy

In this second case, Drug 1 and 2 are hypothesised to have the same concentration profile in skin but both have a different potency (Drug 2 is more potent). For Drug 2, its viable epidermis concentration after topical application exceeds its effective concentration and therefore, a pharmacological effect of Drug 2 is expected in the epidermis. However, for Drug 1 as the epidermis concentration is inferior to its effective concentration, no pharmacological effect is expected in the epidermis.

In summary, the knowledge and use of both, drug concentration in skin (quantitatively), as well as, drug potency, appears to be key if one wants to rationalise the topical drug development process.

1.6. Drug Concentration in Skin

1.6.1. Existing Topical Pharmacokinetic Sampling Methods

The aim of this section is to review the most commonly used topical pharmacokinetic techniques. Numerous other techniques (e.g. the non-invasive spectroscopic techniques like confocal, fluorescence techniques as well as the NMR technique) are not described here as they will have one or more of the following characteristics: very specific for a drug or disease, possibly impractical, possibly not quantitative, possibly lacking sensitivity or possibly at a too early stage of development [71;129;130].They cannot therefore be applicable as versatile techniques for studying topical pharmacokinetics.

1.6.1.1. Tape Stripping

Description + Use of the method

This method is a very widely used technique. It can be used either *in vitro* or *in vivo* and has an aim of determining the concentration in the stratum corneum of a drug applied topically. It has been used as well as a method for defining bioequivalence between two formulations. It is performed as follows: 1- a drug is applied to the skin surface for a fixed time period; 2- the drug remaining on the skin surface is removed (where possible) by wiping or washing; 3- a succession of stratum corneum layers are removed by sequential tape strips using adhesive tape; 4- the drug content of the tape strips is determined [131].

Pros

The method has been improved significantly over the years, the quantification of the amount of stratum corneum removed improves the quality of the data generated [132]

The location of the stratum corneum makes it easy to sample and the concentration within this first skin layer are high which does not generally cause issues regarding analytical detection limits.
The method can be use *in vivo* and is relatively non-invasive.

Cons

The stratum corneum is not a skin tissue of much interest for the development of topical treatment, as it is only the target site of antifungals or sunscreen agents.

In this technique the controversial issue of the washing procedure and the accounting or not of the first tape strip remains. This issue could be summarised as the "contamination issue". As it will be seen for most of the other skin pharmacokinetic sampling methods, the "contamination issue" is an important factor.

The "contamination issue" is caused by the following situation: while applying on the skin surface a topical formulation, the amount of the drug applied will be several orders of magnitude more than the amount of drug present in the skin tissue. As a consequence, the washing procedure used to remove the drug applied has to be extremely effective and should be in theory equal to 100%. At least, if the amount of drug sampled in the skin tissue is 100 times inferior to the amount of drug applied, then the washing procedure recovery should be superior to 99%. That task is in theory achievable on a surface that is smooth, tough and impermeable but less so on the skin surface that is rough, soft and permeable. The task is more complicated by the fact that classically a small amount of the topical formulation is applied that will dry over the time course of the experiment. This makes any validation, strictly speaking, impossible to perform. Indeed if one performs the validation at t=0, the topical formulation has not dried yet which makes the washing procedure easier to perform. If however the validation is done at t=t1, an unknown amount of drug will have already permeated into the skin, which makes the calculation of the washing procedure recovery impossible to perform.
In the case of the stratum corneum tape stripping technique, the first tape is, depending on the protocol, counted or not, which is very much a recognition of the presence of the "contamination issue" described above.

1.6.1.2. Blister Suction

Description + Use of the method

The method consists in separating the epidermis from the dermis by the use of a special dome shaped Dermovac cap as describe by Kiistala et al. [133;134]. A suction of about 200 mm Hg (2.66 Pa) below the atmospheric pressure is employed for a 2-3 hour period after which 50-150 μl blister fluid and small corneum-epidermal sheet can be harvested. The blister fluid corresponds roughly to the interstitial fluid. The protein content of suction blisters is about 60-70% of the corresponding serum value [135]. The aim of the method is to measure interstitial fluid concentration at the epidermal/dermal junction. The method has been used after topical application of different molecules [136-140].

Pros

The sample collected is sampled from the viable epidermis/dermis junction and a large number of the skin pharmacological targets are situated either side of this junction.
The sample collected is a liquid that will make the analysis easier than for a solid sample.
The method is used *in vivo* and is relatively non-invasive.

Cons

The proportionality factor in between the drug concentration in the blister liquid and the drug concentration in the epidermis or dermis is not known especially as the large volume of fluid created in the blister is likely to dilute the concentration truly present in the interstitial fluid at the epidermal-dermal junction.
The "contamination issue" is present here as well. While inserting the needle to collect the blister liquid the needle will transport from the surface some drug. However the small surface area of the needle makes this issue less severe than in other skin PK sampling methods.

1.6.1.3. Skin Biopsy

Description + Use of the method

This is the most invasive technique of the methods described in this section. It consists, after removal (optional) of the stratum corneum by an appropriate tape stripping method, of cutting deep into the skin. The punch biopsy will contain parts of the subcutaneous tissues, dermis and epidermis while the shave biopsy will contain epidermis and some dermis. Parts of the stratum corneum may remain depending on the method used for stratum corneum removal. The biopsy can be frozen which allows cryo-sectioning of the biopsy [141].

Pros

This technique provides *in vivo* drug concentration in all skin tissues.

Cons

This is a very invasive technique. Volunteers will have a small scar usually for the rest of their life on every sampling point of the punch biopsy. The risk of scaring for shave biopsy is decreased, as some of the dermis is not sampled.

The "contamination issue" is present here, as the tool used to cut into the skin may carry some drug from the surface into the deep layers of the skin. The contamination issue is discussed by Surber et al [129].

The skin concentration obtained represents the bound + unbound drug fractions, while only the unbound concentration is of interest (only unbound drugs will be able to cross cell membranes or/and be presented in the right conformation to a receptor) [142].

1.6.1.4. Plasma / Excreta Collection

Description + Use of the method

This technique consists in collecting plasma samples or (and) excreta samples. If the samples collected are only plasma samples, the flux through human skin can be calculated providing one knows the total systemic clearance of the drug studied [143]. If only excreta samples are used, the applied drug needs to be radiolabelled (or a large proportion of the drug needs to be excreted unchanged and this proportion needs to be well defined), the total bioavailability of the drug topically applied can be estimated.

Pros

These techniques are the standard pharmacokinetic techniques used for the development of oral drugs.

There is no "contamination issue".

The method is used *in vivo* and is relatively non-invasive.

Cons

For a drug applied topically, the proportionality factor in between the drug concentration in the plasma or urine and the drug concentration in the different skin tissue is not known. The concentration of the samples is generally extremely low especially if the topical formulation was applied on a small body surface area. Measurement of such low concentrations will require normally the use of very sensitive analytical techniques.

1.6.1.5. Microdialysis

Description + Use of the method

Cutaneous microdialysis allows the measurement of drug concentration in the extra cellular space of the dermis. The technique consists in inserting a microdialysis fibre below the skin surface into the dermis and back. The dialysis fibre is then perfused with a physiological fluid that can collect the small molecules present in the area around the fibre. Due to the small pores of the microdialysis fibre, only small molecules can diffuse across the fibres, the sample therefore recovered is protein-free. After a defined period of time, which is used to let the inflammation caused by the insertion of the fibre to decrease, the topical drug is then applied above the area where the fibre has been inserted. A small sample size can then be collected over time [144].

Two approaches can be used:

Case 1: Simple measurements of the dialysate are made. The concentration is proportional to the dermis free concentration. The "microdialysis fibre's recovery factor" (= the proportionality constant) needs to be defined separately so that true extra cellular dermal concentration can be estimated.

Case 2: The point of No Net Flux method (NNF) is used. It consists in infusing in 4-6 fibres a range of concentrations that are believed to cover the actual dermis drug concentration. The dermis concentration corresponds to the concentration infused in the fibre where no change was observed between the concentration infused in and the recovered sample.

Pros

The method is used *in vivo*. There is no "contamination issue" providing the point of entry/exit of the fibre in/out of the skin are well isolated from area where the topical drug is applied

Case 1: A concentration, which is proportional to the dermis free concentration *in vivo*, is generated which is the right information in an important pharmacological target skin tissue.

Case 2: The free concentration in the dermis is determined which is the right type of information wanted in a relevant skin tissue. There is no "microdialysis fibre's recovery factor" issue.

Cons

This is a relatively invasive method and difficult method to put in place.

The drug concentration in the samples is generally low especially with lipophilic permeants. Measurement of such low concentrations will require normally the use of very sensitive analytical techniques.

Case 1: The validity of the concentration defined is dependent on the "microdialysis fibre's recovery factor" used and this factor determined outside of the *in vivo* condition is often poorly estimated [145]. It should be added however that the retrodialysis method when used *in vivo* as described by Stahle et al [145], is recognised as a good method for estimating *in vivo* recovery and is used by a growing number of microdialysis groups as an abbreviated method of the NNF method as it does not require equilibration [146].

Case 2: It requires long equilibration time and reliable microdialysis fibres (that do not block over time). It assumes as well that the concentration to be measured is approximately known to "bracket" the different concentrations to be infused.

1.6.1.6. *In vitro* Percutaneous Studies: Flux Determination

Description + Use of the method

The method consists of studying the percutaneous flux of a topical drug. Skin samples are mounted on a diffusion cell that consists of two different compartments. The donor compartment is the part where the topical drug is applied. The receptor compartment is filled with a fluid that will collect the drug diffusing from the donor chamber through the skin membrane. Receptor medium samples are collected over time and a flux profile is generated.

Pros

This is an *in vitro* technique and therefore easy to perform.
There is no "contamination issue", as the collected receptor sample has no direct contact with the skin surface.

Cons

This is an *in vitro* technique (the *in vivo* equivalent would be the plasma collection seen in 1.6.1.4).
The proportionality factor between the percutaneous flux and the drug concentrations in the different skin tissue is not known.
The drug concentration in the samples is generally low. Measurement of such low concentrations will normally require the use of very sensitive analytical techniques.

1.6.1.7. *In vitro* Percutaneous Studies: Skin Tissue Concentration Determination

Description + Use of the method

The method consists of determining the drug concentration in the different skin tissues after topical drug application *in vitro*. The methodology is the same as for 1.6.1.6, this time all the different skin tissues are sampled. Classically, after removal of the studied topical formulation from the skin surface, the stratum corneum is tape-stripped, the

epidermis and dermis tissue are then separated. Concentration in the three different tissues is then determined.

Pros

This is an *in vitro* technique so easy to perform.

Cons

This is an *in vitro* technique.

The "contamination issue" is present here, as the cutting tool used to recover the skin tissue will carry some drug from the surface in the deep layer of the skin.

The skin concentration obtained represents the bound + unbound drug fractions, while only the unbound concentration is of interest (only unbound drugs will be able to cross cell membranes or/and be presented in the right conformation to a receptor) [142].

1.6.2. Use of the Skin Pharmacokinetic Sampling Methods

The next table summarises for which purposes the different skin pharmacokinetics sampling methods described in 1.6.1 are used. It is interesting to note that all of them seem to be suitable for bioequivalence type of studies but only half of them seem to be suitable for tissue concentration type studies.

Table 9: Classical use of PK skin sampling techniques

Techniques	**Use for Bioequivalence**	**Use for Tissue Concentration**
Tape stripping	Yes	No
Blister suction	Yes	Yes (pseudo tissue)
Skin Biopsy	Yes	Yes
Plasma or Excreta collection	Yes	No
Microdialysis	Yes	Yes
In vitro percutaneous permeation	Yes	No
In vitro percutaneous skin tissue concentration	Yes	Yes

1.6.3. Pros and Cons of the different Skin Pharmacokinetics Sampling Methods Regarding the Determination of Tissue Concentration

The next table summarises the Pros and Cons of the different skin pharmacokinetics techniques reviewed in 1.6.1.

Table 10: Pros and Cons of the different PK skin sampling techniques regarding the determination of tissue concentration

Techniques	*In vitro* / *In vivo*	Information given	Issues	Feasibility (1 => 4) (easy to hard)
Tape stripping	*In vitro* [or *in vivo*]	Concentration in stratum corneum	Surface contamination + Only Stratum Corneum + Bound drug	2 [3]
Blister suction	*In vivo*	Pseudo tissue concentration (epidermal / dermal junction)	Surface contamination + Not true tissue concentration	3
Skin Biopsy	*In vivo*	Concentration in all tissues	Surface contamination + Bound drug	4
Plasma or Excreta collection	*In vivo*	Input (flux)	Detection limit + No tissue concentration information	3
Microdialysis: Case 1:	*In vivo*	-1- Dialysate concentration	-1- *In vivo* fibre's recovery factor difficult to define + Detection limit	3 – 4
Case 2:		-2- Dermis free concentration	-2- Long time for equilibration + Detection limit	4
In vitro percutaneous permeation	*In vitro*	Percutaneous flux	Not tissue concentration	1
In vitro percutaneous skin tissue concentration	*In vitro*	Concentration in all tissues	Surface contamination + Bound drug + *In vitro* only	2

From the quality and relevance of the data given, the microdialysis technique sounds the most attractive technique as it does give the information wanted. However, the practicality issues added to the tough analytical issues does not make microdialysis a suitable technique to form part of the topical drug development process as pointed out by Simonsen from Leo Pharmaceuticals [147].

All the other techniques that do give skin tissue concentration information have the surface contamination issues coupled with the fact that the concentration measured is the total drug concentration (bound+unbound fraction). This last comment is not true for blister suction as the level of protein can be measured [135].

The only techniques that do not have the issue of surface contamination are the techniques that measure flux either *in vivo* (plasma/excreta collection) or *in vitro* (*in vitro* percutaneous studies: flux determination). The problem is that the link between flux and skin tissue concentration is not known.

Overall, no currently available techniques appear to be satisfactory to estimate drug concentration in skin tissue after topical application. They are either flawed because of surface contamination coupled with a concentration determination which is unsatisfactory as the total (bound+unbound) concentration is generated (and not the unbound concentration as desired); or they are too complex to be used effectively as a key tool of the drug development process.

1.7. Conclusion

Developing drug treatment is a long and complex process as well as an expensive one. If possible, it would be appealing for the industry to develop new treatments at reduced cost or increased speed.

Moving towards topical drug development can therefore be appealing for the pharmaceutical industry, for two reasons that are additive to the potentially large dermatological market size. The first reason is that trying to use existing oral drugs and using them topically does not require a large amount of toxicological studies and the development time appears shorter as opposed to a fully new pharmacological target. Secondly, most topical drug classes originated from an existing oral/systemic drug. This second point could suggest that developing a topical drug from an existing oral drug is easy (1.3.1).

However, looking more carefully at the history of topical drug development, it appears that there are a lot of pitfalls (1.5.1.3). Indeed a lot of oral drugs were not successful topically if the skin disease targeted had a relatively intact skin barrier and that the target site was deeper than the stratum corneum.

These failures seem to be influenced by the poor topical drug development process used that largely rely on pharmacokinetically flawed rodent animal models (1.3.2.2, 1.3.2.4). In other words, these failures could potentially be explained by the lack of early involvement of pharmacokinetics in the topical drug development process. If for oral drugs, pharmacokinetics forms part of the core disciplines influencing early drug candidate selection (1.2.2), for the development of topical drugs, pharmacokinetics is used only at the pharmaceutics stage (1.4.2.2). By that stage, the scope to improve a bad topical candidate is limited and **unsatisfactory** (1.4.2.2).

This poor use of pharmacokinetics for topicals is likely due to the difficulty of reliably and effectively quantifying free drug concentration in the skin tissues after topical application (1.6.3).

The knowledge of target tissue concentration appears indeed to be key for the development of topical drugs if one considers the way oral drugs are developed (1.4.3).

The review in section 1.5 of what key factors influence a successful drug development concludes that the knowledge of both skin tissue concentration as well as drug potency is critical to the topical drug development success.

Overall, the introduction suggests that effectively quantifying free drug concentration in skin is a key requirement if one wants to develop effectively new topical drugs.

1.8. Objectives of the PhD Thesis

In order to fill the gap in between oral and topical drug development process, the primary objective of the thesis was:

- **To define a way of measuring drug concentration in skin tissue after topical application that is reliable, effective and practical**

The secondary objective was then:

- **From the knowledge of the skin tissue concentration, to develop a topical PK/PD model to predict likely efficacy for a topical drug candidate**

Chapter 2.

Materials and Methods

2.1. Drug Permeants Used

2.1.1. Amitriptyline

• **Materials:**

Compound	Batch N°	Supplier
Amitriptyline	963	Di Pharma

• **Analysis: LC-MS-MS**

No Sample Preparation.

Column: Phenomenex – Luna 5μ C18(2) – 30 x 2.0 mm
Eluent A: 0.1% Formic acid
Eluent B: Acetonitrile
Injection: 20μl

Gradient:

Time	%A	%B	Flow Rate (ml/min)
0	96	4	0.35
1.0	96	4	0.35
2.5	30	70	0.35
3.5	30	70	0.35
3.51	96	4	0.35
9.0	96	4	0.35

Compound	Parent	Daughter	ESI	Collision energy (eV)	Cone (volts)
Amitriptyline	278	233	+	18	30

2.1.2. Corticosteroids

• **Materials:**

Compounds	Batch N°	Supplier	Formulation Names	Batch N°	Supplier
Betamethasone dipropionate	20K1602	SIGMA	Diprolene AF cream Diprosone cream	1EEW303 9KG01	Schering Schering
Betamethasone	87H0179	SIGMA	NA	NA	NA
Fluticasone Propionate	NA	GSK	Cutivate ointment 0.005% Cutivate cream 0.05%	1B389 1G281	GSK GSK
Fluocinolone Acetonide	69H0524	Sigma	Synalar Cream 0.025%	RAC019	Medicis
Hydrocortisone	20K1230	Sigma	Hydrocortisone cream USP 1% with Aloe	L01064	Alpharma
Clobetasone Butyrate	NA	GSK	Eumovate Cream 0.05%	C042779	GSK
Clobetasol propionate	29H1327	Sigma	Temovate Ointment 0.05% Temovate Cream 0.05% Temovate Cream E 0.05%	OL210 OG295 OL209	GSK GSK GSK

• **Analysis: LC-MS-MS**

Sample Preparation: Solid Phase Extraction
- Cartridges: Isolute C-8 100mg/3ml [Jones Chromatography - UK]
- Equipment: Rapid Trace + Turbovap [Zymark UK]
Condition with methanol, then water / Load 3ml of sample / Rinse with water / Elute with 1.5 ml Methanol => Evaporate + Reconstituted with 70/30 0.01% Acetic Acid/Methanol

Column: Phenomenex – Luna 5μ C18(2) – 30 x 2.0 mm
Eluent A: 0.01% Acetic acid
Eluent B: Methanol
Injection: 100-300μl

Gradient:

Time	%A	%B	Flow Rate (ml/min)
0	70	30	0.35
0.1	70	30	0.35
2.5	10	90	0.35
6.0	10	90	0.35
6.01	70	30	0.35
9.0	70	30	0.35

Compounds	Parent	Daughter	ESI	Collision energy (eV)	Cone (volts)
Betamethasone DiPropionate	563	59	-	17	24
Betamethasone	451	361	-	18	20
Fluticasone Propionate	559	413	-	22	26
Fluocinolone Acetonide	511	431	-	18	24
Hydrocortisone	421	331	-	17	18
Clobetasone Butyrate	477	441	-	14	19
Clobetasol Propionate	525	465	-	13	22

Note: Formation of drug-adduct with acetic acid. The drug adduct is monitored as sensitivity is largely improved.

2.1.3. Loperamide

- **Materials:**

Compound	Batch N°	Specific Activity	Radiochemical Purity	Supplier
[^{14}C] Loperamide	3362-021	54 mCi/mMol	99.6%	NEN
Loperamide	78H1043			Sigma

- **Analysis: Scintillation counting and LC-MS-MS**

No Sample Preparation.

• **Study: "From Radiochemical Counting to MS-MS"**

Column: Phenomenex – Luna 5μ C18(2) – 30 x 2.0 mm
Eluent A: 0.1% Formic acid
Eluent B: Acetonitrile
Injection: 20μl

Gradient:

Time	%A	%B	Flow Rate (ml/min)
0	96	4	0.35
1.0	96	4	0.35
2.5	30	70	0.35
3.5	30	70	0.35
3.51	96	4	0.35
9.0	96	4	0.35

Compound	Parent	Daughter	ESI	Collision energy (eV)	Cone (volts)
Loperamide	477	266	+	28	39

• **Study: "N in 1"**

Column: Phenomenex – Luna 5μ C18(2) – 30 x 2.0 mm
Eluent A: 0.1% Formic acid
Eluent B: Acetonitrile
Injection: 4x100μl – Use of Trap Column

Gradient:

Time	%A	%B	Flow Rate (ml/min)
0	96	4	0.35
0.05	96	4	0.35
1.5	30	70	0.35
2.5	30	70	0.35
2.51	96	4	0.35
6.0	96	4	0.35

Compound	Parent	Daughter	ESI	Collision energy (eV)	Cone (volts)
Loperamide	477	266	+	28	39

Note: The other LC gradient as described for the analysis of rosiglitazone/amitriptyline could be used to analyse in one run all 3 drugs (loperamide/ rosiglitazone/ amitriptyline). The method described above had however to be used as it was more sensitive.

2.1.4. Nicotine

- **Materials:**

Compound	Batch N°	Supplier
Nicotine	127H1067	Sigma

- **Analysis: HPLC UV**

No Sample Preparation.

Column: 250x4.6 mm-C18-Ecosinil [Waters, UK]
Eluent: 65/2/29/4 Water/Acetonitrile/Methanol/Acetate Buffer 0.5M
Injection: 20μl
Flow rate: 1ml/min
Wavelength: 260 nm

2.1.5. Penciclovir

- **Materials:**

Compound	Batch N°	Specific Activity	Radiochemical Purity	Supplier
[^{3}H] Penciclovir	KW97059-092A1	94.6 μCi/mg	97.6%	ICN Biomedicals

- **Analysis: Scintillation counting**

2.1.6. Propylene Glycol

- **Materials:**

Compound	Batch N°	Specific Activity	Radiochemical Purity	Supplier
[^{14}C] Propylene Glycol	5776109	55 mCi/mMol	97%	ICN Biomedicals

- **Analysis: Scintillation counting**

2.1.7. Rosiglitazone

• **Materials:**

Compound	Batch N°	Supplier
Rosiglitazone	00-AS-511	GSK

• **Analysis:**

No Sample Preparation.

Column: Phenomenex – Luna 5μ C18(2) – 30 x 2.0 mm

Eluent A: 0.1% Formic acid
Eluent B: Acetonitrile
Injection: 20μl

Gradient:

Time	%A	%B	Flow Rate (ml/min)
0	96	4	0.35
1.0	96	4	0.35
2.5	30	70	0.35
3.5	30	70	0.35
3.51	96	4	0.35
9.0	96	4	0.35

Compound	Parent	Daughter	ESI	Collision energy (eV)	Cone (volts)
Rosiglitazone	358	135	+	25	39

2.2. In vitro Percutaneous Studies

2.2.1. *In vitro* Percutaneous Studies

The aim of an *in vitro* percutaneous study is to quantify the amount of drug that will have permeated into the skin and through the skin.

Permeation into the skin: Skin tissue concentration studies:

The method consists of determining the drug concentration in the different skin tissues after topical drug application *in vitro*. The methodology is the same as for 1.6.1.6 and all the different skin tissues are sampled. Classically, after removal of the studied topical formulation from the skin surface, the stratum corneum is tape-stripped, the epidermis and dermis tissue are then separated. Concentration in the three different tissues is then determined.

Permeation through the skin: Percutaneous flux studies:

The method consists of studying the percutaneous flux of a topical drug. Skin samples are mounted on a diffusion cell that consists of two different compartments. The donor compartment is the part where the topical drug is applied. The receptor compartment is filled with a fluid that will collect the drug diffusing from the donor chamber through the skin membrane. Receptor medium samples are collected over time and a flux profile is generated.

2.2.2. Static Diffusion Cells

The name of such cells indicates that the receptor fluid is static and will remain in the diffusion cell.

The cells used were custom-made within GSK and are represented in the figure below:

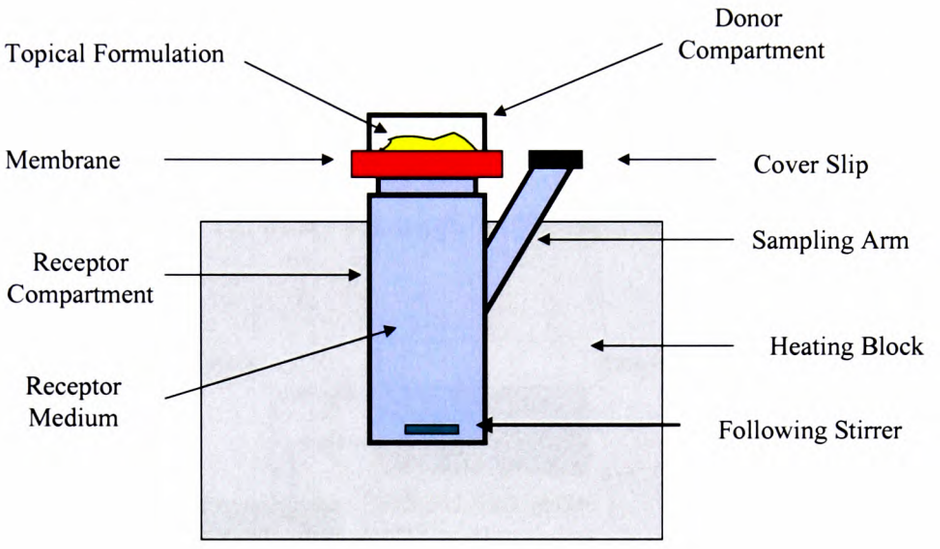

Figure 11. Static Diffusion Cells

Such cells are used for measuring skin tissue concentration.

Pros: Their design allows to isolate the skin surface from the lower skin tissue therefore minimising contamination of the lower skin tissue.

Cons: The need of manual sampling of the receptor fluid limits their use for flux studies.

2.2.3. Flow Through Diffusion Cells

The name of such cells indicates that the receptor fluid flows through the receptor compartment.

The diagram of the cells used as well as the full diffusion cell system are represented in Figure 12 and Figure 13:

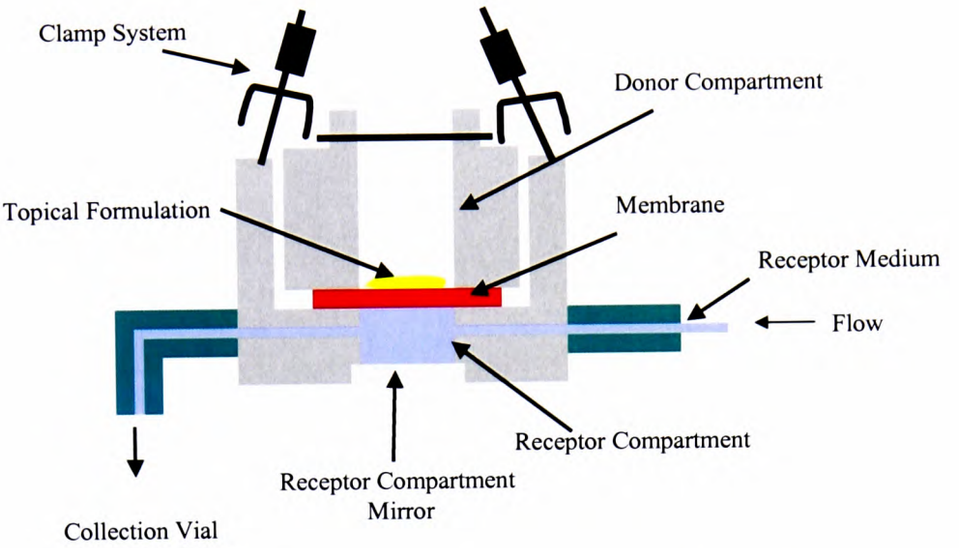

Figure 12. Flow Through Diffusion Cells Diagram

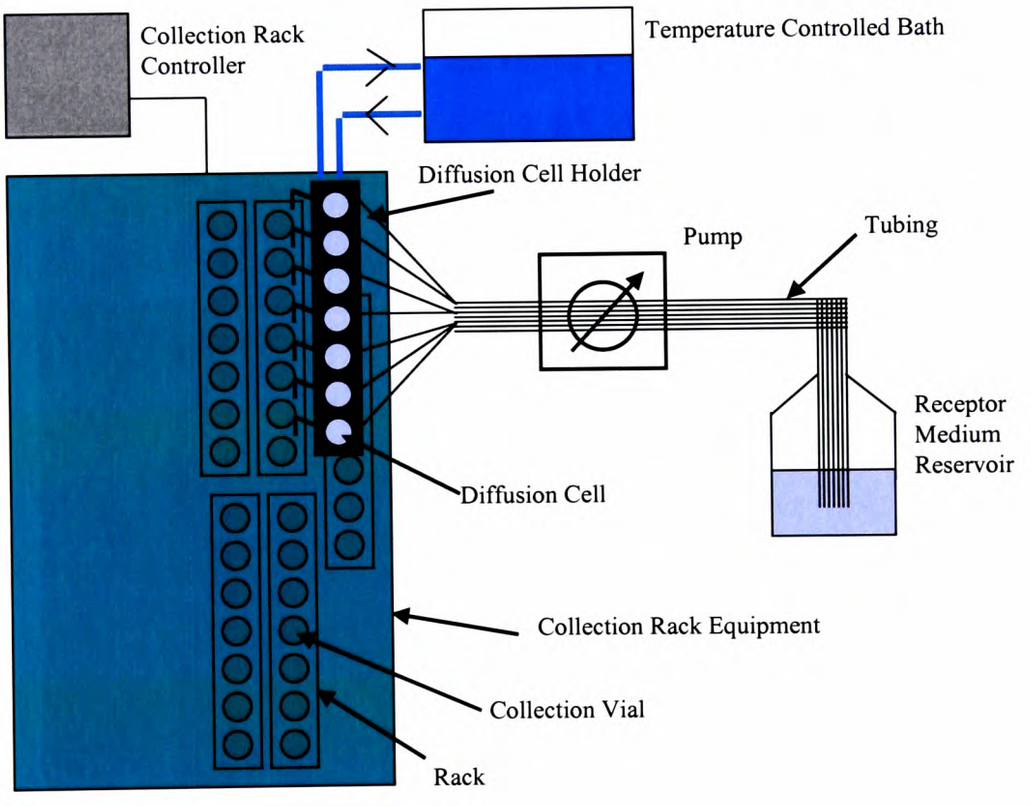

Figure 13. Flow Through Diffusion Cells System

Two types of flow through diffusion cells have been used both from Permegear (In-Line Cells). The smaller ones had a diffusion area of 0.64cm^2. The larger ones were custom-

made with a diffusion area of 3.14cm^2 to allow the application of clinically relevant doses [see 7.1].

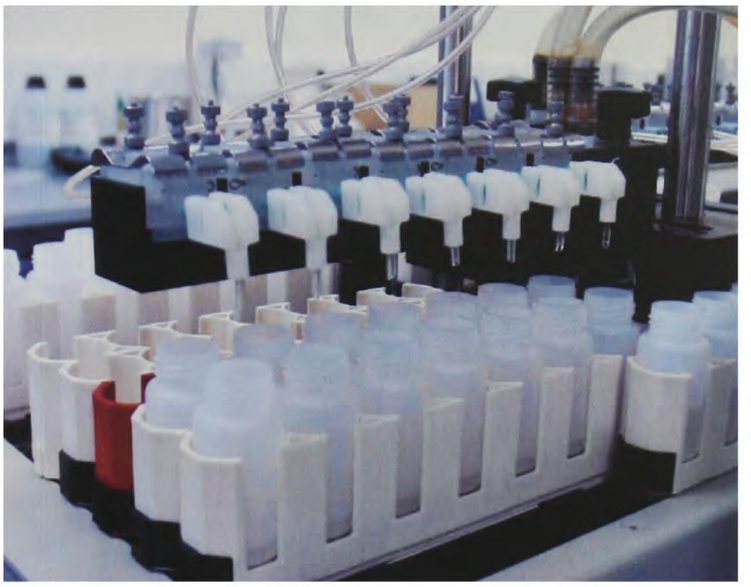

Figure 14. Small Diffusion Cells

Figure 15. Large Diffusion Cells [Full System]

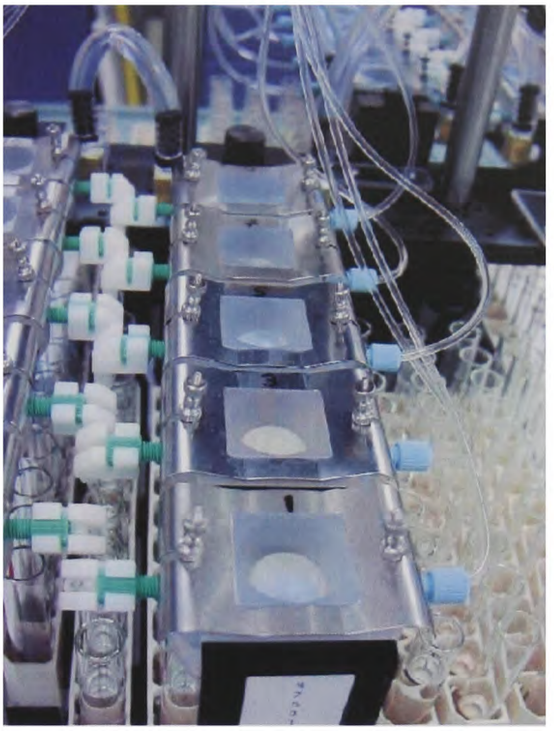

Figure 16. Large Diffusion Cells

Such cells are used for percutaneous flux studies.

Pros: The automatic sampling of the receptor fluid. Large number of time points can be collected.

Cons: Their design induces drug contamination from the surface towards the lower skin tissue when the cells are unclamped. This prevents the use of such cells for skin tissue concentration determination.

2.2.4. Protocol for Skin Tissue Concentration Studies

See 3.1.1 for the protocol and see 3.1 for the rationale behind this protocol.

2.2.5. Protocol for Percutaneous Flux Studies

Bronaugh flow through type diffusion cells [Permegear – USA – In line Cells II], having an available diffusion area of 3.14 cm^2 or 0.64 cm^2 and a receptor volume of 1.2ml or 0.3ml, are employed [see 2.2.3]. These cells seat on a cell holder temperature controlled by circulating water heated at 37±1°C leading to a measured temperature [measured with a spring loaded surface probe from Hannah Instrument, UK] of 32±1°C on the skin surface.

The skin membrane used in percutaneous studies is dermatomed human skin (approximately 300-500μm thick) from the back (unless mentioned)(stored at –20°C after collection until used) [see 2.2.6].

Cut skin samples are placed in the diffusion cells. If the large diffusion cells are used, the cut skin sample is placed over a high flow filter paper (Whatman - 541).

The receptor phase is generally phosphate buffer saline [pH=7.4] [Sigma] (unless mentioned) and is pumped at a rate of 1.5 ml per hour.

Prior to application of the formulation, the diffusion cells are left to equilibrate for approximately 2 hours.

The number of replicates per formulation varies from one study to another but is generally around 5 with the large cells and 6 or 7 for the small diffusion cells.

When semi-solid formulations are used (creams or ointments), accurately weighed quantities of creams are applied to the surface of the skin and spread by means of a small bent metal spatula. When liquid formulations are used, a pipette is used to define the volume to add and the solution is left as is on the skin surface.

The dose of the topical formulation varies for semi-solid from 2 mg/cm^2 to 40 mg/cm^2 and is mentioned in the summary of the study. For semi-solid preparation the cells are left unoccluded while for solution preparation the cells are occluded to avoid evaporation.

Receptor samples are taken at several time points over a maximum of 24 hours.

2.2.6. Skin Membranes Used

• **Human Dermis membrane**

This membrane comes from human full thickness skin. . The skin was kept frozen at -20°C (without the addition of any solvent) till used. After removal of the subcutaneous fat the membrane is immersed for 45 seconds in a bath at 60°C. After that, the membrane is laid flat on a flat surface epidermis side up. The epidermis is rolled by the operator finger and separated from the dermis.

• **Human Dermatomed skin**

These cadaver skin specimens came from the back region. Within two days of death, the skin from the back was collected with a dermatome and then stored without any addition of solvent at –20°C till used. On day of use, the membrane is left to defrost for about 10-15 minutes and can be used as is.

2.3. Analytical Equipment

2.3.1. Radiochemical Counting

• **Equipment used:**

Rackbeta 1209 [EG&G Wallac, UK]

• **Procedure:**

8 ml of scintillation cocktail (Hi Phase Supermix - EG&G Wallac, UK) is added to the samples, mixed with the sample and placed in the scintillation counter for analysis.

2.3.2. LC-UV

• **Equipment used:**

JASCO 900 series HPLC system [Jasco, UK]

2.3.3. LC-MS-MS

• **Equipment used:**

-Autosampler: HTS Pal [CTC, Switzerland]
-Pump: Agilent 1100 [Agilent, UK] (+ Jasco 900 pump as loading pump if trap column)
-MS-MS: LC-Quattro [Micromass, UK]

LC-MS-MS is a very sensitive and very versatile technique that is nowadays the key technique used in the pharmaceutical industry for pharmacokinetic studies.
Samples are first introduced into an ion source [Figure 17] where ions [Figure 18] are generated before being passed into a mass analyser prior to detection and data processing [148;149].

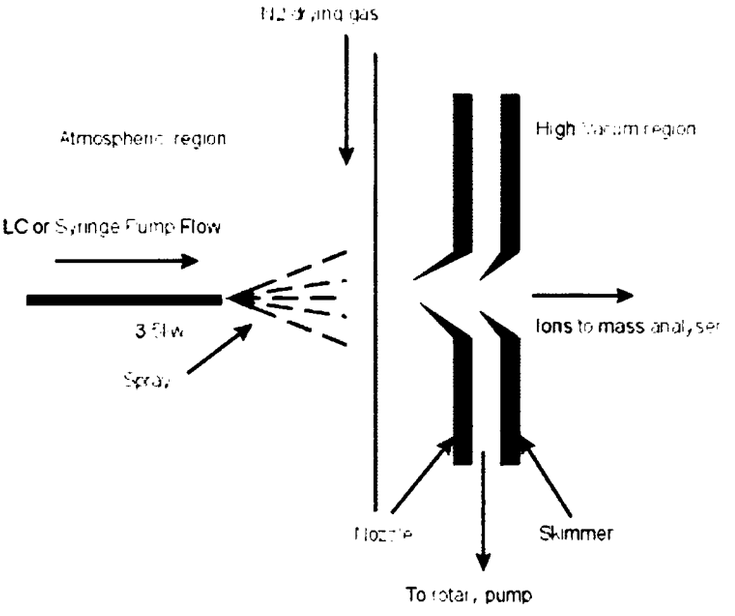

Figure 17. Schematic figure of the source of an LC-MS

The use of high performance liquid chromatography coupled to mass spectrometry requires atmospheric pressure ionisation techniques such as electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APcI) [in all the analysis conducted the ESI mode was used]. These ionisation techniques allow the generation of ions from a liquid

phase such as a HPLC eluent. The solvent (volatile HPLC eluent) is pumped into a capillary of the MS source that carries a high potential, typically 3 –5 kV. The strong electric field, in conjunction with a nebulising gas, induces highly charged droplets to be sprayed from the end of the capillary [148].

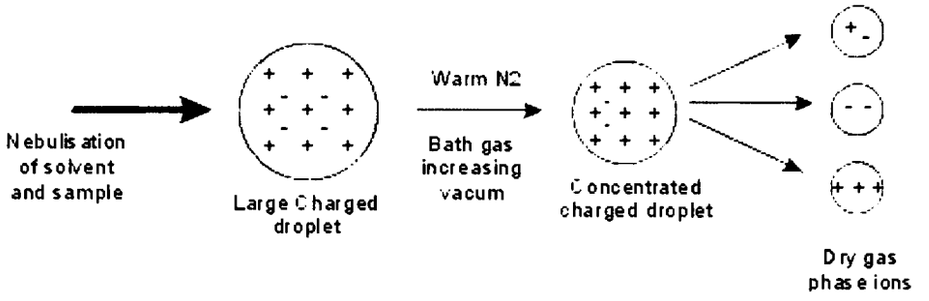

Figure 18. Transformation of the liquid sample into gaseous ions

Desolvation of these charged droplets occurs with the aid of a drying gas, a stream of warm nitrogen, until charge repulsions induce a 'Coulombic explosion'. The resulting desolvated analyte ions are accelerated into a mass analyser.

ESI as well as APcI ionisation techniques are known as soft ionisation techniques producing usually a molecular ion plus or minus a hydrogen, i.e.: $[M+H]^+$ for a basic drug and $[M-H]^-$ for an acidic one.

The mass analyser is used to determine the resulting mass to charge ratio of the ions produced in the source. The mass analyser, for the LC-Quattro used [Micromass, UK] during these studies, is a quadrupole that acts as an ion filter only allowing ions of specific mass to charge ratio to reach a detector.

The soft ionisation techniques used with LC-MS usually produce molecular ions that will not reveal much structural information. To overcome this, structural information can be achieved by collision inducted dissociation (CID) in the mass spectrometer source or preferably by CID between two coupled mass analysers (via a collision cell) [Figure 19]. This allows the molecular ion of the target analyte to be fragmented to reveal structural fragments, which can be used qualitatively or quantitatively [149].

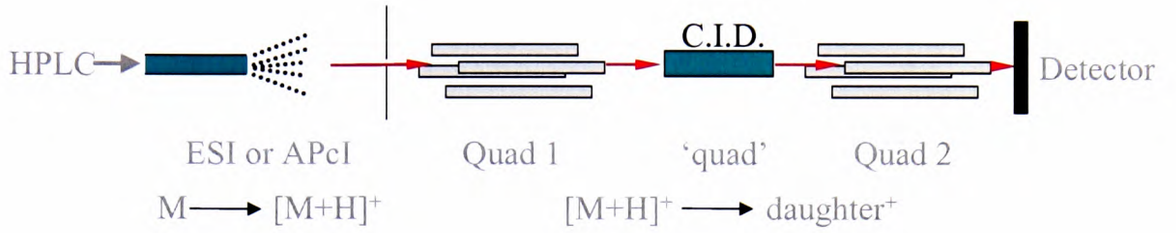

Figure 19. Diagram of a triple quadrupole [MS-MS]

The MS-MS fragment coupled with HPLC retention time can make highly specific sensitive detection systems for biological samples. LC-MS-MS can thus be used quantitatively and qualitatively.

2.3.3.1. Use of Trap Column

The aim of a trap column is to allow direct injection of a complex matrix (large content of protein in sample e.g. plasma) into a LC-MS-MS system.

The concept is to trap the analyte onto a "pre-column" (positioned before the analytical column) that has a function similar to a loop. However, while the analyte is retained into this "pre-column", the proteins are not. In effect, this cleans the sample from proteins. This therefore allows direct injection onto an analytical column of larger volume of sample containing proteins without the need to do any sample preparation.

The principle of a trap column is as followed: Turbulence created within the column by the high flow rate causes the large polar molecules (proteins) not to bind to the column while the smaller molecules (the analyte) will bind with the column packing.

The 3 following graphs explain the practical settings to use a trap column.
The flow from the trap column is initially diverted to waste to avoid sending the protein and buffer to the MS-MS.
The valve then switches the flow to the Analytical Column where the analyte is retained.
As, the eluent (with higher organic content than the loading eluent) flows into the pre-column, it removes the drug from the pre-column and loads it onto the analytical column.

As the organic content of the eluent keeps increasing the analyte is eventually eluted from the analytical column.

Trap Column used: Turboflow HTLC Cyclone [Cohesive Technologies, UK].
LC requirements: Two pumps (one to load at a high flow rate the analyte onto the trap column while the other acts as the classic analytical pump) and two 6-port valves.

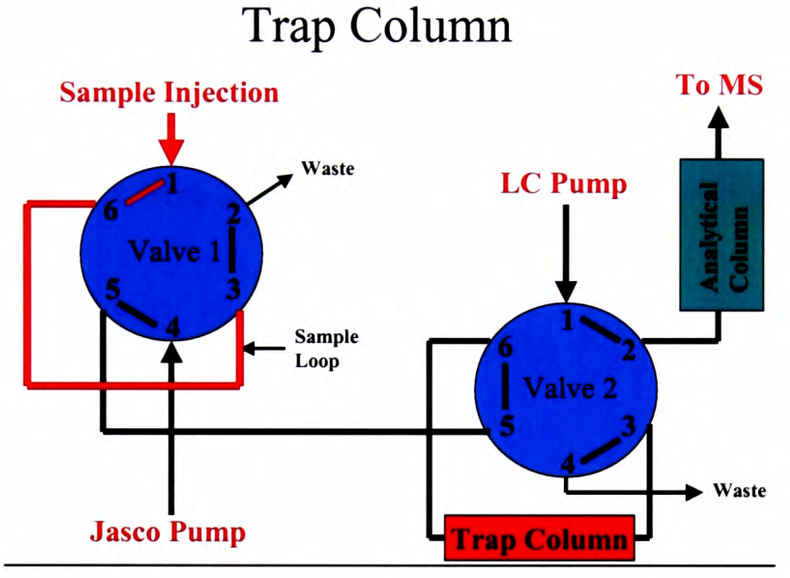

Figure 20. Step 1: Injection => Loading of sample loop in valve 1

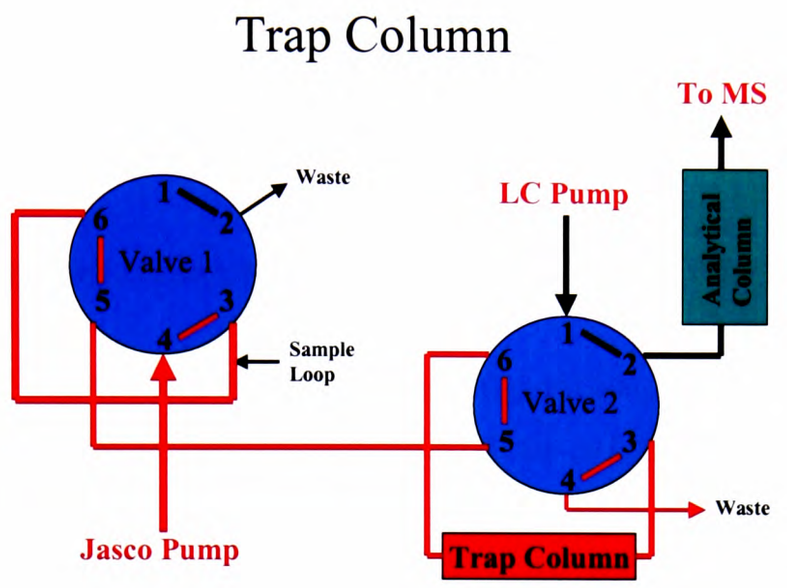

Figure 21. Step 2: Loading of the trap column in valve 2

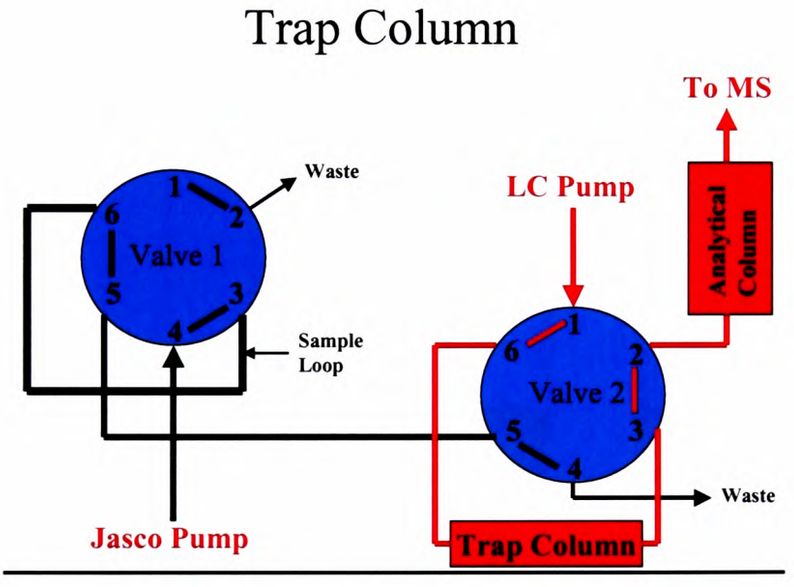

Figure 22. Step 3: The analyte presents in the trap column is eluted out of this "pseudo-loop" and loaded onto the analytical column

2.3.3.2. Solid Phase Extraction

The aim of conducting Solid Phase Extraction (SPE) is to clean the sample prior to injecting it onto the LC-MS-MS.

SPE can be divided into 4 different steps [Figure 23].

Step 1: Column Conditioning & Equilibration - Conditioning is necessary to wet the sorbent bed & equilibration is required to maximise analyte retention

Step 2: Sample Application

Step 3: Interference Elution - Selectively removes undesired compounds from sorbent without eluting analytes

Step 4: Analyte Elution - A solvent in which the analyte is readily soluble is used to overcome the sorbent/analyte interactions

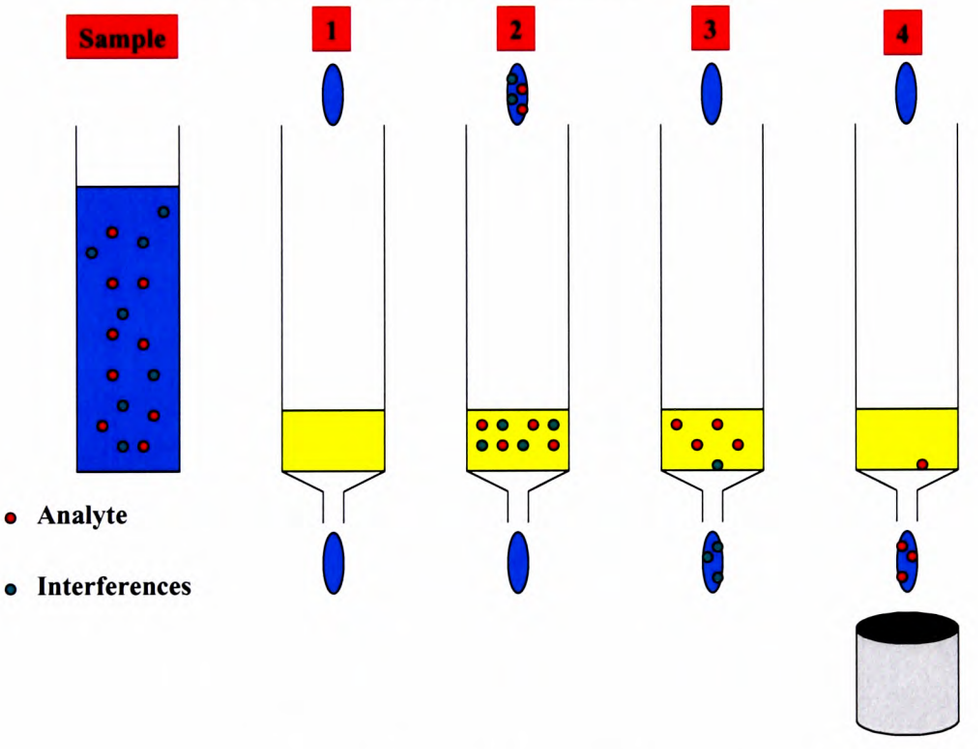

Figure 23. The 4 Steps of Solid Phase Extraction

These samples are then blown dry using a Turbovap Unit [Zymark, UK] and reconstituted in the appropriate solvent mix ready for analysis by LC-MS-MS.

Chapter 3.
Direct Measurement of Skin Concentration

3.1. Direct Measurement Proposed Approach

As seen in 1.6.3, none of the currently used skin PK sampling methods can satisfactory estimate skin concentration as there are two elements that need to be solved:

1- suppression of surface contamination

2- increase of experimental practicality

This chapter describes a direct measurement approach that addresses these two issues.

3.1.1. Minimising the Surface Contamination Issue

The problem related to skin surface contamination, as detailed in 1.6.1.1, comes from the large difference in between the amount of drug applied and the amount of drug recovered in the skin tissue. For compounds which are poorly absorbed, this difference can be of the order of a thousand-fold.

In order to avoid or limit surface contamination, it is theoretically possible to use a very efficient washing procedure that would not extract the drug present in the top layers of the skin surface, nor push the drug still on the skin surface into the skin.

Figure 24 describes the three factors preventing the washing procedure to be reliable and as well the combined solutions to fix these problems.

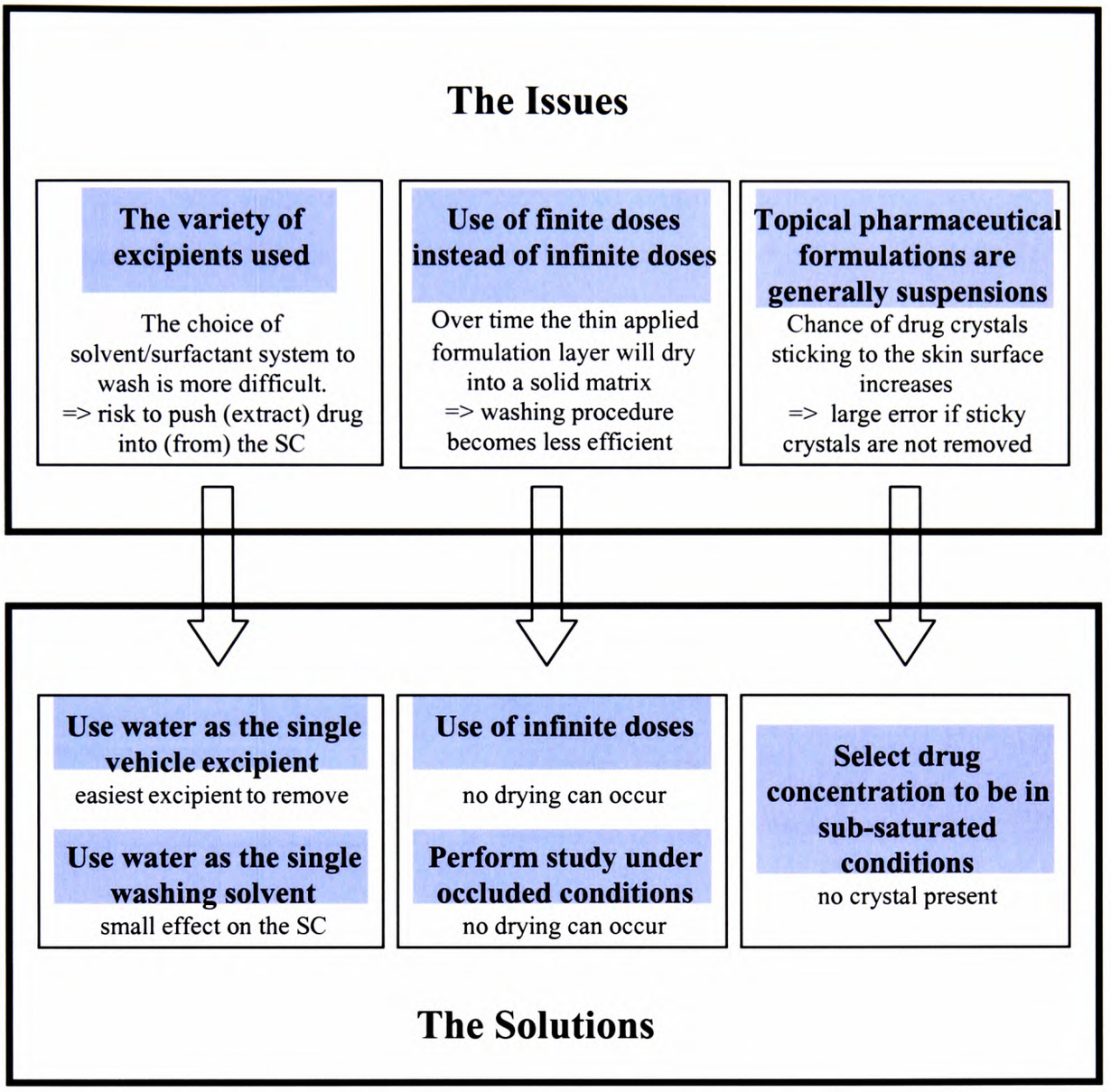

Figure 24. Solving the Surface Contamination Issue

3.1.2. Improving Experimental Practicality

This section demonstrates that skin tissue concentration study can be replaced by flux studies providing one skin tissue concentration study is performed once.

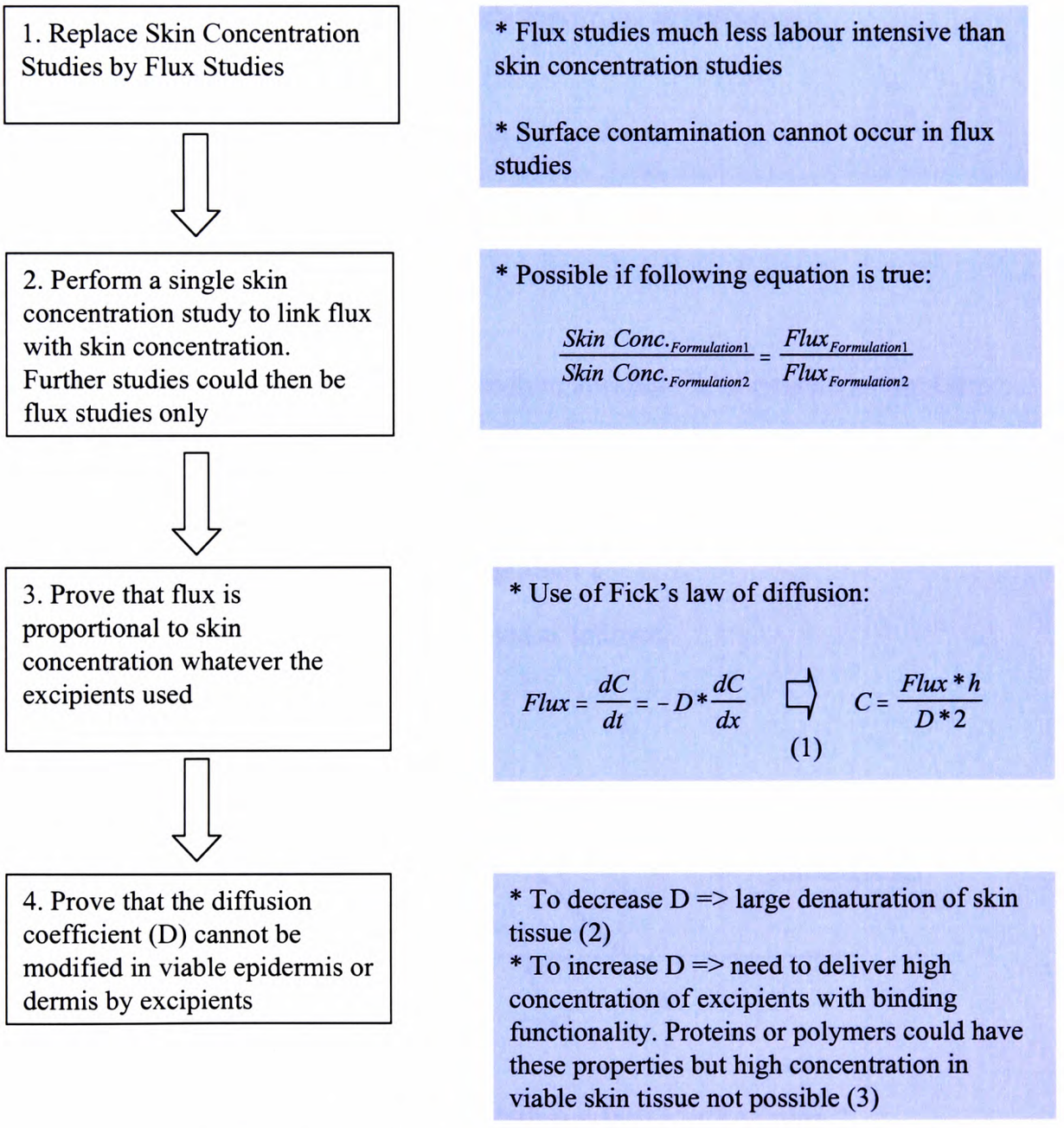

Figure 25. Rationale for Improving Experimental Practicality

- (1)

The aim is to find out the correlation in between flux and tissue concentration. For simplification of the analysis, only the steady state case is considered. At steady state, Fick's first law of diffusion applies.

$$Flux = \frac{dC}{dt} = -D * \frac{dC}{dx}$$

Equation 1 - Fick's First Law of Diffusion

Where

D is the diffusion coefficient of the drug in the medium.

C is the drug concentration

x is a distance (thickness of the diffusion medium)

t is time

At steady state, by integrating over the medium thickness 'h' the equation becomes:

$$C_{free}[mean] = \frac{Flux * h}{D * 2}$$

Equation 2

This equation can be graphically represented as follows:

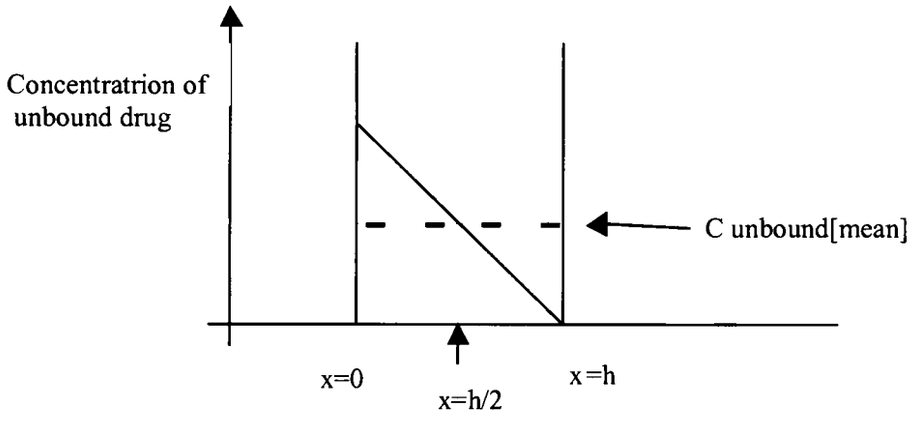

Figure 26. Graphical Representation of Equation 2

Equation 2 shows that, in order to lose the proportionality, in between flux and the concentration:

⇨ The diffusion coefficient of the diffusing molecule in the medium needs to be modified (as h cannot be modified)

- **(2)**

Diffusion coefficients of molecules in gas, liquids and solids are very different. In solids the diffusion coefficients will be very low and inferior to 10^{-10} cm^2/s while in gas they will be superior to 10^{-5} cm^2/s and in between these values for liquids [150]. In particular, in water, the diffusion coefficient values of small molecules is close 1 to 2 10^{-5} cm^2/s [150]. The nature of biological membranes brings the diffusion coefficient values in between the water diffusion coefficients and solids diffusion coefficients. This means, as well, that if the biological tissue was to resemble water, diffusion coefficients through that tissue could not get higher than 1 to 2 10^{-5} cm^2/s. Small molecules diffusion coefficients in dermis are close to 10^{-6} cm^2/s [see section 10.2]. Only a small increase in diffusion coefficient can therefore be expected in the dermis.
As well, in order to resemble water, the dermis would very likely lose its physical characteristics and would be unlikely to hold together after such a destructuration, as bonds/links holding these tissues together would have disappeared.

=> Increase of the diffusion coefficient in viable tissues seems unlikely.

The same conclusion is not made for the stratum corneum, as it is known that some excipients do affect the diffusion coefficient of molecules into that membrane.

- **(3)**

To decrease the diffusion coefficient (which would then increase local concentration for the same flux) the foreseeable way is to get the tissue looking more like a solid. Bringing either new proteins or polymers in these viable tissues are the most probable options to achieve a more solid state membrane (because of the biological nature of viable epidermis and dermis, and because of the nature of the excipients used in topical formulations).

The very large size of proteins or polymers suggests, however, that a flux larger than 1ng/cm^2/hr cannot be reached (use of the Potts and Guy equation [151] = Equation 3).

$$Log\,kp(cm/hr) = -2.72 + 0.71\,Log\,Koct - 0.0061\,MW$$

Equation 3. Potts and Guy Equation to Predict Drug Permeability Through Skin

To calculate the flux of the permeant, the permeability value (kp) has to be multiplied by the saturated solubility of the permeant (S) in water.

$$Flux\left(ng/cm^2/hr\right) = kp(cm/hr) * S\left(ng/cm^3\right)$$

Equation 4. Flux vs. Permeability Coefficient vs. Water Saturated Solubility

Concentration of polymer or protein in the dermis are then expected to be less than 0.0000005% (use of Equation 2 with: Thickness ~ 400 μm (top part only of dermis being considered) and using a Diffusion Coefficient in the Dermis of 10^{-6} cm^2/s) and more likely inferior to 0.00005% (if using a Diffusion Coefficient in the dermis of 10^{-8} cm^2/s as these are extremely large molecules - Diffusion coefficient of proteins in water are 10 to 50 fold lower than for small solutes [150]).

The same approach can be used for the viable epidermis. If one assumes that the viable epidermis diffusion coefficient is about 10 fold inferior to the dermis one: => Concentration of polymer or protein in the viable epidermis are then expected to be less

than 0.002% (thickness ~ 150 μm - using a Diffusion Coefficient in the viable epidermis of 10^{-9} cm2/s).

At such low concentrations (0.002%) it seems very unlikely that a polymer/protein would have a substantial effect on the diffusion coefficient of a drug in the viable epidermis or dermis.

The lack of strong knowledge on diffusion modification by polymers or proteins prevents a stronger conclusion.

=> Decrease of the diffusion coefficient in viable tissues seems unlikely.

The same conclusion is not made for the stratum corneum, as it is known that some excipients do affect the diffusion coefficient of molecules into that membrane.

3.1.3. Summary for the direct measurement approach

Using the learnings from section 3.1.1 and 3.1.2, it is possible to generate drug concentrations in skin tissue of different formulation by performing the following studies:

Step 1: To perform a skin tissue concentration study using principles described in section 3.1.1

Step 2: To perform flux studies of the different formulations of interest at finite doses

Step 3: By using Equation 5, the percutaneous flux measured in step 2 can be transformed into skin tissue concentration.

$$\frac{Skin\ Conc._{Formulation1}}{Skin\ Conc._{Formulation2}} = \frac{Flux_{Formulation1}}{Flux_{Formulation2}}$$

Equation 5

This approach allows generation of skin tissue concentration data with a limited risk of having skin surface contamination and with limited resources as most studies performed are percutaneous flux studies which are less demanding in resources.

3.2. Direct Measurement Protocol

Due to the static diffusion cell design [2.2.2](that limits contamination of lower skin tissue during skin removal at the end of the experiment – as opposed to the flow through diffusion cells [2.2.3]), static diffusion cells are used for these skin tissue studies. Full thickness skin tissue is clamped in the diffusion cells. A sub-saturated drug solution (buffered water) is applied at infinite dose in the donor chamber of the cell. The donor compartment is then occluded (glass cover sealed over the donor chamber using silicone sealant).

At the desired time points, the different samples are taken as follows:

1- After removal of the glass cover, the donor solution is removed using a disposable plastic Pasteur pipette.

2- With the same disposable plastic Pasteur pipette, about 1 ml of water is added to the empty donor compartment in order to rinse the donor compartment. The 1-ml of water introduced is then removed. This procedure is repeated 5 times. A cotton wool is placed on the surface of the skin to absorb any remaining water present.

3- All the elements (glass cover + donor solution + 5 ml rinsing solution + pipette + cotton) are grouped together as the "donor sample" in a scintillation vial.

4- The receptor solution is completely removed (via the side arm of the diffusion cell) and 8 ml of scintillation cocktail, Super Mix [EG&G Wallac, UK] is added.

5- The diffusion cell is unclamped and the donor part of the cell is removed. A second cotton wool pad is used to absorb any remaining water still present and is

added to the pooled "donor sample" and 8 ml of scintillation cocktail, Super Mix [EG&G Wallac, UK] is added.

6- The skin sample still placed on the receptor part of the cell has its stratum corneum stripped 15 times with a 3M Blenderm sellotape. The pooled sellotapes constitute the "stratum corneum sample" and 8 ml of scintillation cocktail, Super Mix [EG&G Wallac, UK] is added. [Note: as the surface rinsing process is considered very efficient all the strips are used, the first one is not discarded].

7- The skin is then removed from the diffusion cell and the part through which diffusion occurs is cut with a knife. The cut skin sample is then placed for few seconds on a hot plate. With the use of forceps the viable epidermis and dermis are separated, weighed and put in separate vials. They constitute the "viable epidermis sample" and the "dermis sample". In each vial 2.5 ml of tissue solubiliser, Optisolv [EG&G Wallac, UK] is added, and the vials are placed overnight into an incubator at 40°C. Then 200µl of acetic acid (10%) is added to the vials [neutralisation to avoid chemino-luminescence during the scintillation counting]. 8 ml of scintillation cocktail, Super Mix [EG&G Wallac, UK] is then added.

3.3. Experimental Results: Study of Loperamide Skin Concentration

The experimental details are described in 10.1. Only the main data of the study are reported here.

Table 11. Direct Skin Tissue Concentration Measurement: The case of Loperamide

	Donor (μg/ml)	Concentration in Tissue (μM)	**Ratio in between doses used**
Donor	12.3 (n=6)	NA	**5.5**
	68 (n=5)	NA	
Stratum Corneum	12.3 (n=6)	131 (+48/-35)	**6.3**
	68 (n=5)	820 (+369/-254)	
Viable Epidermis	12.3 (n=6)	23.7 (+7.4/-5.6)	**7.9**
	68 (n=5)	187 (+39/-32)	
Dermis	12.3 (n=6)	0.41 (+0.19/-0.13)	**5.4**
	68 (n=5)	2.2 (+1.0/-0.7)	
		Flux (ug/cm^2/hr)	
Receptor	12.3 (n=6)	1.02 (+0.42/-0.30)	**4.2**
	68 (n=5)	4.3 (+2.4/-1.5)	

⇨ This experiment shows a relatively good proportionality between, flux and the different skin tissue concentrations.

However, in this experiment, the ratio drug applied and drug absorbed is the same. This therefore does not prove that the approach does not suffer from surface contamination.

This would have been proven by conducting a second experiment, where, the concentration in the donor phase would have been chosen different while their saturation level would have been chosen equal. The amount of drug absorbed should have been similar in both cases but the amount applied would have been different. Such a change in the drug concentration in the donor solution would have been possible by changing the pH of the solution [i.e. keeping the concentration constant but changing the saturation level by varying the pH].

This experiment has however not been conducted because the direct tissue concentration determination approach was abandoned as discussed in the conclusion below.

3.4. Conclusion

This approach to measure skin concentration appears to be satisfactory, as it seems to solve or largely reduce the surface contamination issue. It appears as well to be a more effective use of resources. However, it does not provide the ideal solution. The reason is that the measured concentration is the total concentration in the tissue (free + bound drug) and for a PharmacoKinetic/PharmacoDynamic (PK/PD) assessment only the free drug concentration is of interest. The outcome of such an approach is therefore unsatisfactory and this approach was abandoned in favour of the indirect approach described in the following chapter.

Note: Despite the limited experiment carried out with that protocol it was felt that the rationale behind the approach deserved to be described in a chapter. As well the attempt to demonstrate the lack of possible effect of excipients in the viable skin tissues on the ratio tissue concentration / flux - is new to the literature. This latter point should help make the case for the use of percutaneous flux for topical bioequivalence as a simple and reliable measurement.

Chapter 4.
Indirect Measurement of Skin Concentration

4.1. Methodological approach

In the previous chapter, the direct measurement of drug concentration in skin has been attempted to improve the quality of the measurement. However, the proposed methodology does not lead to the pharmacologically relevant concentration: the free drug concentration in skin. As well, the measured concentration remains an *in vitro* concentration while ideally, drug concentration should be measured *in vivo* possibly in diseased skin.

This chapter describes another approach to measure free drug concentration in skin *in vivo* (in diseased skin).

In order to calculate the free drug concentration at the target site of diseased skin the following three-step approach is conducted.

1- **in vitro skin concentration** is defined from a percutaneous *in vitro* flux measurement using Fick's first law of diffusion

2- **in vivo skin concentration (normal skin)** is derived from the *in vitro* skin concentration by correcting for the local capillary clearance

3- ***in vivo* skin concentration (disease state)** is derived from the *in vivo* skin concentration (normal skin condition) by correcting for the difference in –1- the permeability (impaired barrier and body site) and –2- the local capillary clearance (inflammation).

4.2. *Step 1: From percutaneous flux to* in vitro *drug concentration in the dermis*

At steady state:

Flux through stratum corneum = Flux through viable epidermis = Flux through dermis = Flux through skin.

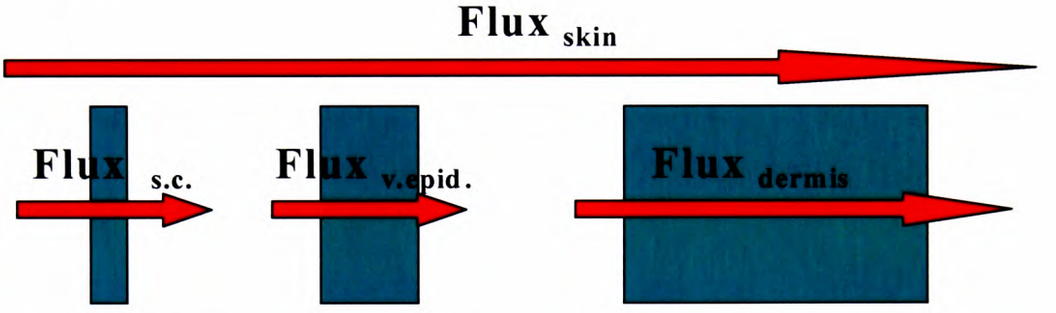

Figure 27. Fluxes in the Different Skin Tissue at Steady State

Fick's first law of diffusion through membranes says:

$$Flux = J = -D * \frac{dC}{dx}$$

Equation 1

Where J= Percutaneous Flux (ng/cm^2/hr)

D= diffusion coefficient in the tissue (cm^2/hr)

C= free drug concentration (ng/cm^3)

x= membrane depth (cm)

At steady state because dC/dx =constant, by integrating Equation 1, at half thickness (x=h/2).

$$C_{free}[mean] = \frac{J}{D} * \frac{h}{2}$$

Equation 2

The membrane studied in the present case is skin and the specific tissue of initial interest is the dermis as direct diffusion coefficient measurement of the viable epidermis is not easily performed (too thin membrane). However the diffusion coefficient in the dermis can be measured.

The diffusion coefficient in the dermis has been measured for several compounds covering a large range of molecular weight and was found to be approximately constant [see Table 12 for the experimental results and see 10.2 for the details of this experimental measurement].

In the present experiment only the lag time to reach steady state through dermis tissue is measured. The diffusion coefficient in the dermis is then calculated using Equation 6 (where h is the membrane thickness and L the lag time) [152].

$$D = \frac{h^2}{6 * L}$$

Equation 6

Table 12. Diffusion Coefficient in the Dermis

	Molecular Weight	Diffusion Coefficient in the Dermis (cm^2/s)
Nicotine	162	$1.6.10^{-6}$
Penciclovir	253	$1.3.10^{-6}$
Loperamide	477	$9.5.10^{-7}$

The diffusion coefficient in the dermis for a drug-like molecule (MW = 200 - 500) can be therefore considered as reasonably constant:

$$D_{Dermis} \approx \text{Constant} = 10^{-6}\ cm^2/s$$

Equation 7

The thickness has to correspond with the part of the tissue considered and with the portion of the tissue from which the flux is measured. As a large proportion of the drug is cleared in the top section of the dermis due to the high capillary network, only the top thickness of the dermis is considered (the top thickness is considered to be 20% of the dermis thickness).

$$Thickness = h = 400\,\mu m$$

Equation 8

Note: The flux value used needs to be derived from *in vivo* pharmacokinetic measurement or from flux value derived from *in vitro* skin permeation measurement where only the top portion of the dermis is present (= dermatomed skin but not full thickness skin).

Using Equation 2, Equation 7 and Equation 8:

$$C_{free\ top\ dermis\ in\ vitro}\,[ng/ml] = J\,[ng/cm^2/hr] * 5.5\,[hr/cm]$$

Equation 9

4.3. *Step 2: From percutaneous flux to* in vivo *drug concentration in normal skin*

4.3.1. *In vitro* to *in vivo* for the Top Dermis Tissue

In step 1, the evaluation of the free drug concentration in the dermis *in vitro* is done with the assumption that only passive diffusion occurs. However, *in vivo,* the drug is cleared by one and possibly two active processes. The first one will occur for every drug: active drug removal by local capillary clearance. The second one will occur as well for every drug but at a rate that may not be significant: drug metabolism (see 1.5.1.1.7).

Therefore, in order to evaluate the drug concentration in the dermis *in vivo*, two correcting factors are added to Equation 9:

- capillary clearance factor ($Cl_{capillary}$Factor)*
- skin metabolism clearance factor ($Cl_{metabolism}$Factor)

* Some clearance from lymph drainage may occur in skin but its relative proportion is unknown. Studies in alive and sacrificed rats by Singh and Roberts (see next page in the section $Cl_{capillary}$Factor determination) do in fact take into account of both capillary and lymph drainage. For simplicity, it will be assumed that the lymph clearance is negligible compared to the capillary clearance.

Both $Cl_{capillary}$Factor and $Cl_{metabolism}$Factor will have a value between 0 and 1.

$$C_{free\ top\ dermis\ in\ vivo} = C_{free\ top\ dermis\ in\ vitro} * Cl_{capillary}Factor * Cl_{metabolism}Factor$$

Equation 10

To simplify the need for evaluating these correcting factors for each drug some assumptions are made:

1- $Cl_{metabolism}$Factor is considered as negligible for all non-ester drug molecules (see 1.5.1.1.7) and will be considered as equal to 1.

For ester drug molecules, some assessment of its value will be required.

2- $Cl_{capillary}$Factor is considered as constant for non-vasoactive drugs based on the literature review described below.

3- For vasoactive drugs, a supplementary factor not described in Equation 10 is added to take into account the effect of the drug itself on the capillary clearance. For a drug with vasoconstrictor properties, this factor will be greater than 1. For a drug with vasodilator properties, this factor will be less than 1.

$Cl_{capillary}$Factor determination:

The determination of the $Cl_{capillary}$Factor can be done by envisaging an experimental *in vivo* situation where the permeation of a drug through the dermis is conducted in two ways: 1-with a normally functioning capillary network and 2- with a "shut down" capillary network. This could be obtained by the use of local vasoconstrictor and has been shown with the microdialysis technique. With this technique, two different studies in the same subject are conducted: in both cases the drug is applied on skin and collected in the dermis by a microdialysis fibre, but in one case the dialysate contains a vasoconstrictor (noradrenaline) and in the other one it does not. For malathion and penciclovir the difference of drug recovery within the dialysate was 8-fold [153] and 16-fold [154] respectively.

In rats, a large number of similar studies have been conducted by Singh and Roberts [155-159]. In their model, the rate of disappearance of drug from a donor solution applied on the dermis surface (epidermis previously removed) of the animal is monitored. Again, two different studies are performed: one when the animal is anaesthetised and alive (with capillary clearance) and the other when the animal has been previously sacrificed (without capillary clearance). In these experiments the difference found in between the

rate of disappearance of the drug was about 5 to 10-fold in between the sacrificed and anaesthetised animal groups. It is however important to mention that large variations were observed from one drug to another: 1 to 2-fold for haloperidol to 30 to 35-fold for water.

The procedures used in these experimental models, do induce locally an increase in blood flow (insertion of microdialysis fibre or removal of epidermis) and therefore overestimate the effect of the active capillary clearance. Based on the data available and taking into account the overestimation of these models, the $Cl_{capillary}$Factor is assumed to be similar for all drugs (for simplification) and equal to 1/3.

Therefore:

$$C_{free\ top\ dermis\ in\ vivo\ (normal\ skin)} = \frac{C_{free\ top\ dermis\ in\ vitro} * Cl_{metabolism}Factor}{3}$$

Equation 11

4.3.2. From Top Dermis Tissue to Surrounding Tissues

The *in vivo* concentration in the top layers of the dermis is established. The concentration in neighbourhood tissues (bottom epidermis, bottom dermis and subcutaneous tissue) (for the key target skin tissue, see 1.5.1.2) is calculated from this concentration by applying a tissue correction factor.

$$C_{free\ skin\ tissue\ X\ (normal\ skin)} = C_{free\ top\ dermis\ (normal\ skin)} * Tissue\ Correction\ Factor$$

Equation 12

Bottom Epidermis

A correction factor of 4 is chosen for the bottom of the epidermis tissue using the following physiologically based qualitative observations:

- The epidermis is closer to the surface compared to dermis so, from Fick's law, the concentration due to passive diffusion is greater.
- The epidermis texture is different compared to the dermis texture. The dermis will have the diffusion properties of an aqueous gel full of proteins while the viable epidermis is likely to have diffusion properties in between the dermis and the stratum corneum but much more like the dermis especially in the basal layer (bottom part of the epidermis). The diffusion coefficient is therefore likely to be slightly higher in this tissue than in the dermis, impacting favourably the concentration.
- The effect of the clearance of the drug in the epidermis due to local capillary network is inferior to its effect in the dermis as the capillary network is situated below the epidermis tissue. However it is still likely to have an important effect especially very close to the interface with the dermis, but less so, higher in the epidermis. In any case the local free concentration will be impacted favourably compared to the dermis.

The corrected free concentration at the bottom of the epidermis will therefore follow the equation:

$$C_{free\ bottom\ epidermis\ in\ vivo\ (normal\ skin)} = C_{free\ top\ dermis\ in\ vivo\ (normal\ skin)} * 4$$

Equation 13

Bottom of Dermis

A corrective factor of 1/5 is chosen for the deep dermis tissue using the following physiologically based qualitative/quantitative observations:

- The deep dermis is further away from the surface compared to top part of the dermis so from Fick's law the concentration due to passive diffusion is inferior.

- The depth considered in the top dermis calculation is 400μm. The average thickness of the dermis in man is about 2mm, a 1/5th of the thickness considered to calculate the top dermis concentration

The corrected free concentration in the deep dermis will therefore follow the equation:

$$C_{free\ bottom\ dermis\ in\ vivo\ (normal\ skin)} = \frac{C_{free\ top\ dermis\ in\ vivo\ (normal\ skin)}}{5}$$

Equation 14

Subcutaneous tissue (superficial)

A corrective factor of 1/20 is chosen for the superficial subcutaneous tissue based the following observation:

The subcutaneous tissue is located deeper in the skin than the bottom of the dermis; therefore the factor to apply will have to be inferior to the one applied to the bottom of the dermis.

- Singh and Roberts suggest such a corrective factor of that order in rats [156-158].

Note 1: Drug molecules present in this deep tissue may have been transported not only by passive diffusion but as well by a redistribution of drug cleared by the capillary in the dermis.

Note 2: This factor should be looked at as a guide as no substantial observations are used for its assessment.

The corrected free concentration in the subcutaneous tissue will therefore follow the equation:

$$C_{free\ subcutaneous\ tissue\ in\ vivo\ (normal\ skin)} = \frac{C_{free\ top\ dermis\ in\ vivo\ (normal\ skin)}}{20}$$

Equation 15

Drug concentration in the stratum corneum

Drug concentration in this surface membrane is very high compared to the viable layers and the fundamental approach followed for the dermis should be reapplied without using correction factor that could be too approximate. Equation 2 is therefore used again with the addition of drug diffusion coefficient in the stratum corneum and stratum corneum thickness (~15μm):

$$C_{free}[mean] = \frac{J}{D} * \frac{h}{2}$$

Equation 2

The diffusion coefficient of drug in the stratum corneum is more difficult to estimate because of drug binding or drug partitioning. Measurement of stratum corneum diffusion coefficient has been established for different drugs by estimating lag time and calculating the diffusion coefficient (using Equation 6). However the binding or partitioning effects were not considered and this would artificially increase lag time (hence decrease diffusion coefficient). For estimate purposes using a more conservative figure of only two hours for the lag time (assuming limited binding or partitioning) a drug diffusion coefficient in the stratum corneum can be defined using Equation 6.

$$D_{stratum\ corneum} \approx 5.2 \,.\, 10^{-11}\ cm / s$$

Equation 16

The active drug clearance is considered here negligible as this tissue is too remote compared to the dermis tissue and metabolism is considered non-existent (=> purely passive diffusion mechanism to clear the drugs is considered relevant).

Using Equation 2, Equation 16, a thickness of 15μm and a purely passive diffusion mechanism:

$$C_{free\ stratum\ corneum\ in\ vivo}[ng/ml] = J[ng/cm^2/hr] * 4000[hr/cm]$$

Equation 17

Drug concentration in skin tissues at different body site

Skin permeability will vary from skin site to skin site and this therefore needs to be taken into account as reported by Feldmann and Maibach [160].

Table 13. Hydrocortisone Absorption - Effect of Anatomic Region (from [160])

Anatomic Region	Ratio
Forearm (ventral)	1.0
Forearm (dorsal)	1.1
Foot arch (plantar)	0.14
Ankle (lateral)	0.42
Palm	0.83
Back	1.7
Scalp	3.5
Axilla	3.6
Forehead	6.0
Jaw Angle	13
Scrotum	42

To simplify the equations considering that most skin disease affects the leg, arms, back or trunk (for which the permeability is similar) as well as the face (for which the permeability is substantially increased by 3-6 fold compared to these other sites) and the

foot (for which the permeability is substantially decreased by 6 fold compared to these other sites), the effect of the skin site is simplified to that following equation:

$$C_{free\ skin\ tissue\ (face)} = C_{free\ skin\ tissue\ (leg,arm,back,trunc,normal\ skin)} * 5$$

Equation 18

$$C_{free\ skin\ tissue\ (foot)} = C_{free\ skin\ tissue\ (leg,arm,back,trunc,normal\ skin)} / 6$$

Equation 19

4.4. *Step 3: From percutaneous flux to* in vivo *drug concentration in disease skin*

The presence of an abnormality in the skin tissue (disease skin) will affect the local drug concentration as two parameters may be modified:

1. skin permeability may differ compared to normal skin
2. local blood flow may differ compared to normal skin (largely influence by the inflammatory response)

$$C_{free\,skin\,tissue\,X[disease\,skin]} = C_{free\,skin\,tissue\,X[normal\,skin]} * Correct.Factor_{skin\,permeability} * Correct.Factor_{blood\,flow}$$

Equation 20

For each skin disease of interest the Skin Permeability and Blood Flow correction factor will need to be defined.

4.4.1. Atopic dermatitis

The atopic dermatitis skin condition is an inflamed skin condition and a subsequent factor attributed to the disease needs to be added. This local inflammation in atopic dermatitis skin has been studied [161]. It was concluded that there is a 3-fold increase in cutaneous blood flow compared to normal skin. Local concentration needs therefore to be divided by an extra factor equal to 3.

Together with the effect of the disease on cutaneous blood flow, it is required to take into account whether or not the skin disease has any effect on the barrier function of the skin as the measurement of flux, whether *in vitro* or *in vivo*, are conducted on normal skin.

Patients with atopic dermatitis have a reduced skin barrier function as compared to people with normal skin and this seems to be dependent on the disease severity. In a number of papers [162-170], the effect of the atopic dermatitis disease on the skin barrier property of the skin has been studied. The review of these papers suggests that unless in the case of patients with erythroderma -where the skin barrier has virtually disappeared- the skin barrier is decreased by about 10 fold in patients with severe atopic dermatitis and by about 2 fold in patients with mild atopic dermatitis. Such a factor must therefore be used to consider the flux through these types of skin. To simplify, a factor of 3 will be considered as the factor to represent the increase in skin permeability due to this skin disease condition [the large majority of patients have mild or moderate atopic dermatitis].

The overall effect of the disease state (change in blood flow [factor = 1/3] and in skin barrier property [factor = 3]), on the local concentration in the dermis, leads to a correcting factor equal to 1:

Equation 11 (assuming $Cl_{metabolisation}$ factor = 1) and this factor of 1 lead to:

$$C_{free\ top\ dermis\ in\ vivo\ in\ atopic\ dermatitis\ skin} = \frac{C_{free\ top\ dermis\ in\ vitro}}{3}$$

Equation 21

Using Equation 9 and Equation 21:

$$C_{free\ top\ dermis\ in\ vivo\ in\ atopic\ dermatitis\ skin}\,[ng/ml] = J\,[ng/cm^2/hr] * 1.8\,[hr/cm]$$

Equation 22

4.4.2. Psoriasis

Psoriasis is an inflamed skin condition and a blood flow corrective factor attributed to the disease needs to be added. This local inflammation in psoriatic skin has been studied [171]. It was concluded that there is a 10 fold increased in cutaneous blood flow compared to normal skin. Local concentration needs therefore to be divided by an extra factor equal to 10.

While taking into account the effect of the disease on cutaneous blood flow, it is necessary to take into account whether or not the skin disease has any effect on the barrier function of the skin. This is a requirement as the measurement of flux, whether *in vitro* or *in vivo*, is conducted on normal skin. Psoriatic skin seems to retain the same barrier function as normal skin, this is suggested by a study with hydrocortisone [104].

The overall effect of the disease state, on the local concentration in the dermis, is a decrease of 10 fold compared to normal skin.

Equation 11 (assuming $Cl_{metabolisation}$ factor = 1) and this factor of 10 lead to:

$$C_{free\ top\ dermis\ in\ vivo\ in\ psoriasis\ skin} = \frac{C_{free\ top\ dermis\ in\ vitro}}{30}$$

Equation 23

Using Equation 9 and Equation 23:

$$C_{free\ top\ dermis\ in\ vivo\ in\ psoriatic\ skin}\,[ng/ml] = J\,[ng/cm^2/hr] * 0.18\,[hr/cm]$$

Equation 24

However, as seen in 1.5.1.2.2, the bottom of the epidermis as well as the top of the dermis is a target site in psoriasis.

Using Equation 13 and Equation 24:

$$C_{free\ bottom\ epidermis\ in\ vivo\ psoriatic\ skin}[ng/ml] = J[ng/cm2/hr] * 0.73$$

Equation 25

4.4.3. Acne

Acne is an inflamed skin condition and a blood flow correction factor attributed to the disease needs to be added. This local inflammation in acne has not been studied so only a comparison to psoriatic skin or atopic dermatitis skin conditions is possible. A qualitative observation suggests that the inflammation would be similar to the one observed in atopic dermatitis. Local concentration needs therefore to be divided by an extra factor equal to 3.

While taking into account the effect of the disease on cutaneous blood flow, it is necessary to take into account whether or not the skin disease has any effect on the barrier function of the skin. This is a requirement as the measurement of flux, whether *in vitro* or *in vivo*, is conducted on normal skin. There is no clear evidence in the literature that suggests that the permeability of the acne skin condition is impaired. However, as the most important skin location for a person suffering from acne is the face, the particular permeability of this skin site needs to be taken into account and Equation 18 is used (correction factor of 5).

The overall effect of the disease state (change in blood flow and in skin barrier property and skin site [face]), on the local concentration in the dermis, is an increase by a factor of 5/3:

Equation 11 (assuming $Cl_{metabolisation}$ factor = 1) and this factor of 5/3 lead to:

$$C_{free\ top\ dermis\ in\ vivo\ on\ face\ in\ acne\ skin} = \frac{C_{free\ top\ dermis\ in\ vitro}}{9} * 5$$

Equation 26

Using Equation 9 and Equation 26:

$$C_{free\ top\ dermis\ in\ vivo\ on\ face\ in\ acne\ skin}[ng/ml] = J\,[ng/cm^2/hr] * 3\,[hr/cm]$$

Equation 27

However, as seen in 1.5.1.1.6 and 1.5.1.2.3, a drug can have two targets in acne: 1- a drug may be able to affect the hyperkeratinisation of the sebaceous duct (top dermis) and/or 2- the sebaceous gland itself (situated quite deep into the dermis). This requires as well to consider the concentration in the deep dermis.

Using Equation 14 and Equation 27:

$$C_{free\ bottom\ dermis\ in\ vivo\ on\ face\ in\ acne\ skin}[ng/ml] = J[ng/cm2/hr] * 0.6[hr/cm]$$

Equation 28

4.4.4. Fungal Infection

For this skin condition, the barrier function will be considered as the skin barrier of the foot as the most common skin fungal infection is the athlete's foot infection. As active clearance is not considered relevant Equation 17 and Equation 19 are used.

$$C_{free\ stratum\ corneum\ in\ vivo\ fungal\ athlete's\ foot\ skin}[ng/ml] = J[ng/cm^2/hr] * 666[hr/cm]$$

Equation 29

4.4.5. Dermal Anaesthesia

In dermal anaesthesia, the barrier function is considered as the normal skin barrier. As the site is not inflamed no clearance factors are considered (topical anaesthesia is generally requested prior to a minor local surgery affecting the skin tissues). Equations related to both top and deep dermis are considered (Equation 11 and Equation 14).

$$C_{free\ top\ dermis\ in\ vivo\ normal\ skin\ before\ surgery}[ng/ml] = J[ng/cm^2/hr] * 1.8[hr/cm]$$

Equation 30

$$C_{free\ bottom\ dermis\ in\ vivo\ normal\ skin\ before\ surgery}[ng/ml] = J[ng/cm^2/hr] * 0.37[hr/cm]$$

Equation 31

4.4.6. Muscle Pain

For muscle pain, the barrier function is considered as the normal skin barrier. As the site is inflamed a factor 1/3 (qualitative) is considered and Equation 17 coupled with Equation 15 (subcutaneous target) are considered.

$$C_{free\ sub-cutaneous\ in\ vivo}[ng/ml] = J[ng/cm^2/hr] * 0.028[hr/cm]$$

Equation 32

4.5. Summary of the Indirect Approach

The indirect approach is conducted in 3 steps using a single flux measurement:

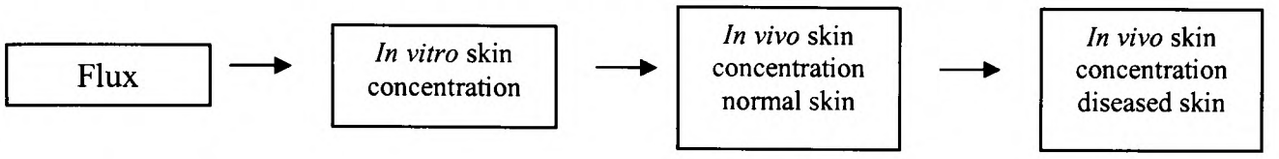

Figure 28. Indirect Approach Summary

Equations linking flux and tissue concentration for atopic dermatitis, psoriasis, acne, fungal infection, dermal anaesthesia and muscle pain are summarised below:

Note: Flux means the percutaneous flux measured through intact skin and from abdominal, back, leg or arm skin site, not from diseased skin or from a skin site where the barrier is largely down (e.g. the face) or up (e.g. the plantar).

Table 14. Summary of the Equations Linking Flux and Skin Tissue Concentrations for Atopic Dermatitis, Psoriasis, Acne, Fungal Infections, Dermal Anaesthesia and Muscle Pain

	Concentration (ng/ml) = Flux (ng/cm^2/hr) * X (hr/cm) Where X = …				
	Stratum Corneum	Bottom of Epidermis	Top of Dermis	Bottom of Dermis	Sub-Cutaneous
Atopic Dermatitis			1.8		
Psoriasis		0.73	0.18		
Acne (face)			3	0.6	
Fungal Infection (athlete's foot)	666				
Dermal Anaesthesia			1.8	0.37	
Muscle Pain					0.028

Chapter 5.

PK/PD Model Development

5.1. The Efficacy Index

The aim of this index is to predict likely efficacy of a drug applied topically. As the key free drug concentration in the skin tissue has been defined, a classic PK/PD approach can now be followed.

The principle consists therefore in comparing the predicted free concentration in the skin tissue with the required free effective concentration to get the pharmacodynamic response.

The efficacy index is calculated as followed:

$$Efficacy\ Index = \frac{C_{free\ \text{target}\ tissue\ achievable}}{C_{free\ effective\ required}}$$

Equation 33

This leads to:

$$Efficacy\ Index = \frac{Flux[ng/cm^2/hr]*X[hr/cm]}{Free\ Effective\ Concentration[ng/ml]}$$

Equation 34

Where: X value is as described in Table 14.

Taking into account all assumptions/approximations made, an efficacy index superior or close to 1 for a drug A in a specific formulation would then mean that drug A in that formulation should induce some of its pharmacological action.
On the other end, an efficacy index largely less than 1 for a drug B in a specific formulation would then mean that no pharmacological action should be expected as the local concentration reached after topical application would be far from its required effective level.

5.2. *The Systemic Safety Index*

This index evaluates the systemic exposure of a compound when applied topically. It is represented by the ratio of the free concentration in the target skin tissue with the free concentration in plasma. The higher the ratio, the safer is the drug, or the less likely systemic side effects are to be expected.

$$Systemic\ \ Safety\ Index = \frac{C_{free\ target\ tissue}}{C_{free\ plasma}}$$

Equation 35

The free target skin tissue concentration has been established in Chapter 4. The free plasma concentration achieved after topical application is obtained using the classic PK equation:

$$C_{plasma}[ng/ml] = \frac{Input\ Dose\,[ng/hr]}{Total\ Systemic\ Clearance\,[ml/hr]}$$

Equation 36

The input dose for a topical relates to:

$$Input\ Dose\,[ng/hr] = Flux\,[ng/cm^2/hr] * Surface\ Area\,[cm^2] * Corr.Factor_{skin\ permeability}$$

Equation 37

As well:

$$C_{free\ plasma} = C_{total\ plasma} * (1 - \frac{protein\ binding}{100})$$

Equation 38

This leads to:

$$Systemic\ Safety\ Index = \frac{Flux[ng/cm^2/hr]*Clearance[ml/hr]*X[hr/cm]}{Flux[ng/cm^2/hr]*Surface\ area[cm^2]*(1-\frac{prot.binding}{100})*Corr.Factor_{skin\ permeability}}$$

And finally to:

$$Systemic\ Safety\ Index = \frac{Clearance[ml/hr]*X[hr/cm]}{Surface\ area[cm^2]*(1-\frac{protein\ binding}{100})*Corr.Factor_{skin\ permeability}}$$

Equation 39

Where: X value is as described in Table 14

Corr. Factor $_{\text{skin permeability}}$ is as described in:

$$C_{free\ skin\ tissue\ X[disease\ skin]} = C_{free\ skin\ tissue\ X[normal\ skin]} * Correct.Factor_{skin\ permeability} * Correct.Factor_{blood\ flow}$$

Equation 20

The surface area value is as described for each disease (see 1.5.1.2) and is summarised in Table 15.

Table 15. Summary of Body Surface Area per Skin Diseases

	Body Surface Area generally Affected and Treated (cm^2)
Atopic Dermatitis	2000
Psoriasis	2000
Acne (face)	400
Fungal Infection (athlete's foot)	400
Dermal Anaesthesia	50
Muscle Pain	500

This index (assuming efficacy index =1) is independent of the flux of the drug through skin but only dependent on surface area, target site, systemic clearance and plasma protein binding.

Note 1: Interpretation of the Systemic Safety Index: The higher the index figure the safer... However one needs to remain careful, as by its definition (Equation 35), this Systemic Safety Index figure is true when the Efficacy Index figure is equal to 1. Therefore, it represents the potential safety window of a drug if applied topically. If however the drug is overdosed (Efficacy Index >>1), the safety window will be reduced. Example: Drug X has an Efficacy Index=100 and Systemic Safety Index=100 (apparently quite safe). If it is used in a topical formulation in a way such that the flux of the drug is not decreased compared to its maximum potential, the real systemic safety index in this topical formulation is only 1 (=> systemic exposure will occur).

Note 2: In some cases a drug maybe used for a specific pharmacological activity and may have another more potent pharmacological activity. In this case the systemic index will have to be corrected (decreased) to take into account this fact as described by the following equation:

$$Systemic\ Safety\ Index = Systemic\ Safety\ Index * \frac{Effective\ \ Conc._{free\ pharmacol.2}}{Effective\ \ Conc._{free\ pharamcol.1}}$$

Equation 40

Chapter 6.

PK/PD Model Validation

6.1. Selection Criteria of the Data Used

- ***Flux:***

Flux obtained from *in vitro* studies:

* Membrane: human skin. No rejection criteria are used on the type of human skin used, providing that the skin membrane contained unstripped/undamaged stratum corneum.

* Donor phase:

  - drug at saturation or in suspension or with known saturation level (corrected to saturated level equal to 1 thereafter)
  - formulation not involving strong penetration enhancers or a high proportion of them (ethanol, propylene glycol, DMSO, azone)

Usually marketed formulations or pure aqueous solutions are used. If a radiolabelled drug is used then correct incorporation of the radiolabelled drug into the formulation is assessed (spiking methods are assessed critically).

* Receptor phase: receptor consisting of pure water or water with salts or with protein or with small percentage of surfactant are acceptable but media containing ethanol or equivalent are rejected.

Flux obtained from *in vivo* studies:

Flux values are calculated using either plasma sample measurements or excretion measurements.

In either case, one assumption made is that depletion of the drug applied does not occur. Studies using occlusion are not taken into account (unless mentioned).

* Plasma concentration measurements:

Systemic clearance (ml/hr), surface area (cm^2) over which the drug is applied, time for which the drug is applied, are needed as well to calculate the flux.
At steady state, the following equation is used for flux determination:

$$Flux\ (\mu g / cm^2 / hr) = \frac{Plasma\ Conc.\ (\mu g / ml) * Clearance\ (ml / hr)}{Surface\ Area\ (cm^2)}$$

Equation 41

* Excretion measurements:
Dose applied (mg/cm^2), concentration of the drug in the formulation (%), surface area (cm^2) are used to calculate first the total amount of drug applied (mg/cm^2).

$$Total\ Amount\ Applied\ (mg / cm^2) = Dose\ Applied\ (mg / cm^2) * Conc.\ in\ Formulation\ (\%)$$

Equation 42

Note 1: For studies with radiolabelled drugs, one assumption is made (unless mentioned): amount excreted = amount absorbed (it is assumed that no other route than urine or faeces are route of elimination)

Note 2: For studies with non-labelled drugs, a correction is performed to take into account the ratio of unchanged drug recovered compared to the amount of drug administered.

At steady state, the following equation is used for flux determination:

$$Flux\ (\mu g / cm^2 / hr) = \frac{\%\ Absorbed\ *\ Total\ Amount\ of\ Drug\ Applied\ (\mu g)}{Surface\ Area\ (cm^2) * Time\ of\ Topical\ Application\ (hr)}$$

Equation 43

- ***Effective concentration:***

In all cases the effective concentration used is free (non-protein bound) drug concentration in order to be able to correlate it with the concentration evaluated with the kinetic model which is a non-bound drug concentration.

Drug potency is calculated using preferentially, when applicable and when available, mean plasma level of the drug used systemically in human and then protein bound corrected:

$$EffectiveConc._{total\ plasma}[\mu g/ml] = \frac{Dose[mg]*1000*N°of\ dose\ per\ day*Bioavailability[\%]}{100*24[hr]*Clearance[ml/hr]}$$

Equation 44

Unbound drug correction:

$$EffectiveConc._{free} = EffectiveConc._{total\ plasma} * fraction\ \ unbound$$

Equation 45

$$Effective\ Conc._{free} = Effective\ Conc._{total\ plasma} * (1 - (\frac{protein\ binding}{100}))$$

Equation 46

When no *in vivo* data are available, *in vitro* effective concentration data are used. For each *in vitro* assay, the presence or not of albumin is investigated to correct accordingly for unbound drug. For example, in case the medium used is 10% serum (~ 0.4% albumin

in medium), the fraction unbound is corrected by multiplying its value by 10 (providing it remained below 1, otherwise the fraction unbound value is taken equal to 1).

- ***Systemic clearance:***

Human systemic clearance (ml/hr) is used from literature data. When given in ml/min/kg it is translated in ml/min by multiplying by 70, 70kg being chosen as the average weight of a human adult. In some rare cases where data are not available the clearance is calculated from the half life and volume of distribution using the equation (assumption: first order kinetics):

$$Clearance(ml/hr) = \frac{Vd(l) * 1000}{t_{1/2}(hr)} * 0.693$$

Equation 47

- ***Calculation of Average for Sets of Data (example: flux or potency data)***

Due to possible high variation in the same set of data, geometric mean is used rather than arithmetic mean (to avoid having large values dominating the smaller ones).

6.2. NSAIDs

Choice of drugs: diclofenac, ketoprofen, ibuprofen, piroxicam and indomethacin are among the most common NSAIDs used topically and clinical comparison is available.

Flux (see Table 16*):* Relatively good literature covering these drugs is available and there is a relatively good agreement among the data apart for piroxicam and ibuprofen.

Table 16. NSAIDs Flux from literature

Flux	Mean Flux ($\mu g/cm^2$ /hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)	Flux ($\mu g/cm^2$/hr)
Diclofenac	0.57	8 [172]	1.4 [173]	0.38 [174]	0.39 [175]	0.42 [176]	0.17 [177]	0.044 [178]	2.25 [179]
Ketoprofen	3.42	10.5 [172]	2.1 [174]	5 [180]	16 [173]	1 [181]	7.5 [182]	0.41 [178]	
Ibuprofen	6.97	22 [173]	117 [174]	1 [183]	1.6 [184]	4 [185]			
Piroxicam	0.16	0.08 [183]	1.3 [184]	0.062 [178]	0.1 [186]				
Indomethacin	0.38	0.5 [172]	0.7 [173]	0.37 [76]	0.13 [187]	0.29 [188]	0.66 [189]		

Effective Concentration (see Table 17*):* Oral dosage as well as *in vitro* IC 50 against COX-2 receptor are used. There is good agreement in between *in vitro* and *in vivo* determination of drug potency.

Table 17. NSAIDs. Potency /Effective concentration

Potency	Mean Unb. Drug Potency (μM)	Unb. Drug Pot. (μM)	Oral Dose + PK	Unb. Drug Pot. (μM) [190]	Unb. Drug Pot. (μM) [191]	Unb. Drug Pot. (μM) [192]	Unb. Drug Pot. (μM) [193]	Unb. Drug Pot. (μM) [194]	Unb. Drug Pot. (μM) [195]	Unb. Drug Pot. (μM) [196]	Unb. Drug Pot. (μM) [197]
Diclofenac	0.0011	0.0022	Oral dose+ PK	0.001	0.0013	0.001	0.0006	0.0003	0.006		0.0010
Ketoprofen	0.029	0.052	Oral dose+ PK		0.004				0.12		
Ibuprofen	1.0	0.62	Oral dose+ PK	15.72	0.67	0.465	0.56			0.183	3.5
Piroxicam	0.15	0.13	Oral dose+ PK	0.98	0.35		0.073	0.0899	0.53	0.0093	
Indomethacin	0.031	0.058	Oral dose+ PK	0.03	0.026	0.016	0.068	0.0184	0.052	0.0112	0.044

Protein Binding Correction (below = IC 50 in full blood = Bound + Unbound drug):

	% Prot. Binding	Bound Drug Pot. (μM)	[118] + [198]	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)	Bound Drug Pot. (μM)
Diclofenac	99.5	0.44	Oral dose+PK [dose=25mgx4-Cl=17.6l/hr-99.5% bound-Bioav.=54%]				0.12	0.05			
Ketoprofen	99.2	6.5	Oral dose+PK [dose=50mgx4-Cl=5.0l/hr-99.2% bound-Bioav.=100%]								
Ibuprofen	99	62	Oral dose+PK [dose=400mgx3-Cl=3.15l/hr-99% bound-Bioav.=80%]				56			18.3	
Piroxicam	99	13	Oral dose+PK [dose=20mgx1-Cl=0.15l/hr-99% bound-Bioav.=80%]				7.3	8.99		0.93	
Indomethacin	96	1.45	Oral dose+PK [dose=25mgx3-Cl=5.9l/hr-96% bound-Bioav.=98%]				1.7	0.46		0.28	

Target site: Muscle/Subcutaneous tissues

PK/PD Equation: Muscle Pain [4.4.6]

Efficacy Index = Flux (ng/cm^2/hr)*0.028 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.028 / [500*(1-(Prot. binding/100)]

Pharmacokinetic data: Clearance and plasma protein binding data are all available.

The summary data on NSAIDs is presented in Table 18.

Table 18. NSAIDs summary

Compounds	MW	Potency (μM)	Flux (μg/cm^2/hr)	Efficacy Index	Systemic Safety Index
Diclofenac	296	0.001	0.57	49	197
Ketoprofen	254	0.029	3.42	13	35
Ibuprofen	206	1.012	6.97	0.9	18
Piroxicam	331	0.146	0.16	0.1	1
Indomethacin	358	0.031	0.38	1.0	17

Discussion: Diclofenac and ketoprofen seem to be efficacious. On the other hand, ibuprofen and indomethacin seem to be borderline for efficacy, while piroxicam should be ineffective. On the safety side, diclofenac appears to be the safest of all these NSAIDs.

Correlation with clinical trial safety / efficacy / comparison: Large number of trials exist on topical NSAIDs and for that reason the best way to examine them is to use available meta-analysis or direct head to head comparison. The most recent meta-analysis [199] shows the following rating among the chosen topical NSAIDs: ketoprofen >ibuprofen >piroxicam >indomethacin where indomethacin was considered not effective. Diclofenac was not included, as there were not a sufficient number of clinical trials available. In a head to head comparison of diclofenac, ketoprofen and piroxicam [200], the outcome was: diclofenac ~ ketoprofen > piroxicam.

The PK model predicts well for diclofenac, ketoprofen and ibuprofen. However, it seems to overestimate the efficacy for piroxicam and underestimate it for indomethacin

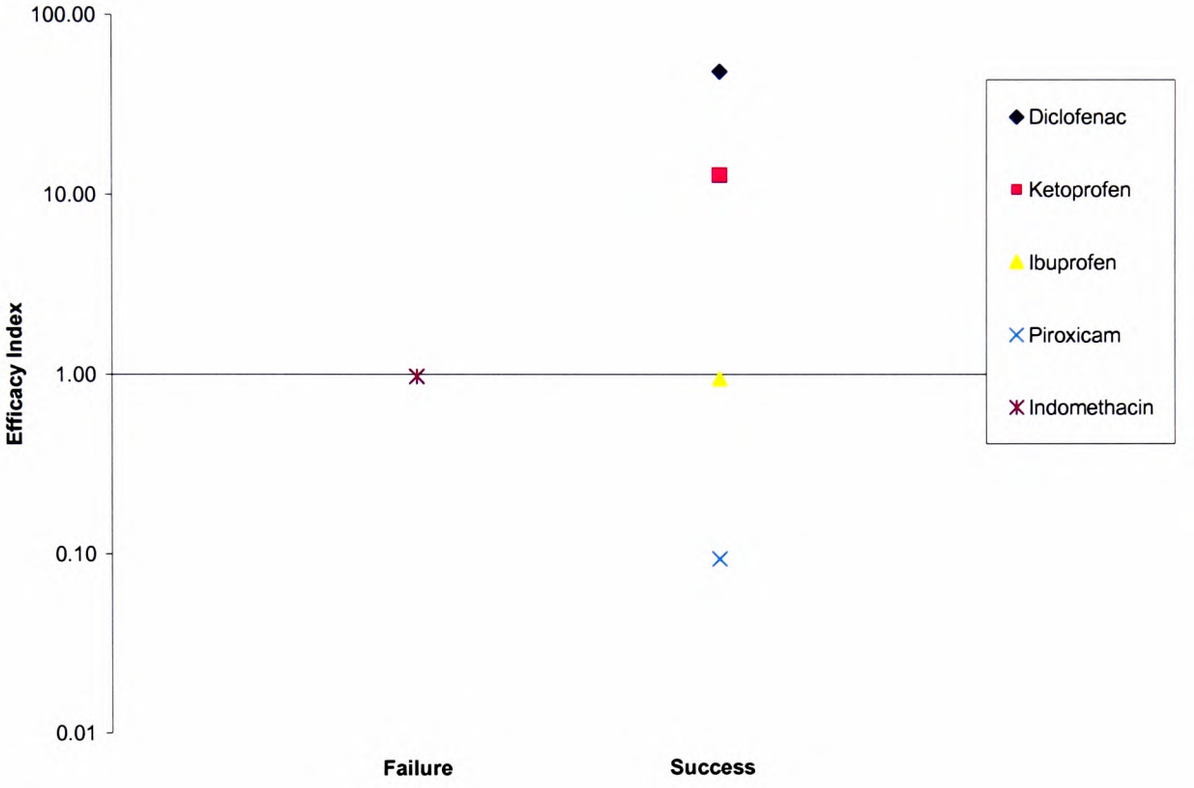

Figure 29. NSAIDs Chart Summary

For NSAIDs, the PK model developed fits partly with what has been observed in the clinic (60% good prediction).

6.3. *Anaesthetics*

Choice of drugs: Tetracaine, lidocaine, prilocaine are commonly used topically on skin, benzocaine is used topically (not intact skin), etidocaine has been studied in one clinical comparison.

F*lux (see* Table 19*):* Flux data are relatively reproducible for three out of the five compounds. Only one flux data for prilocaine and etidocaine is available.

Table 19. Anaesthetics Flux from literature

Compounds	Mean Flux ($\mu g/cm^2/hr$)	Flux ($\mu g/cm^2/hr$)	Flux ($\mu g/cm^2/hr$)	Flux ($\mu g/cm^2/hr$)
Tetracaine	30	20 [201]	10 [202]	130 [203]
Lidocaine	44	55 [172]	63 [203]	25 [204]
Prilocaine	50	50 [204]		
Benzocaine	8	5 [205]	14 [203]	
Etidocaine	8	8 [203]		

Effective Concentration (see Table 20*):* As these drugs are not given orally, IC_{50} values are used. (BTX binding, Sodium flux, Phosphoinositide breakdown) [The assumption is that the medium does not contain a high level of protein/albumin]

Table 20. Anaesthetics Potency /Effective concentration

Potency	Mean unbound drug Potency (μM)	Unbound Drug Potency (μM) [125]	Unbound Drug Potency (μM) [126]	Unbound Drug Potency (μM) [126]
Tetracaine	3.5	3.4	2.7	4.5
Lidocaine	155	240	141	110
Prilocaine	125	54	85	427
Benzocaine	910	910		
Etidocaine	7.0	3.5	8	12

Target site: Dermis

PK/PD Equation: Dermal Anaesthesia [4.4.5]

Efficacy Index = Flux (ng/cm^2/hr)*0.37 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.37 / [50*(1-(Prot. binding/100)]

Pharmacokinetic data: Clearance and plasma protein binding data are available for the amino esters [Lidocaine: Cl= 38.6l/hr / Prot. Bind.=70% -- Prilocaine: Cl=269 l/hr / Prot. Bind.=55% -- Etidocaine: Cl=66.8 l/hr / Prot.Bind.=95%] but not for the amino amides (tetracaine and benzocaine).

The summary data on Anaesthetics is presented in Table 21.

Table 21. Anaesthetic summary

Compounds	MW	Potency (μM)	Flux ($\mu g/cm^2/hr$)	Efficacy Index	Systemic Safety Index
Tetracaine	264	3.5	30	12	Very High*
Lidocaine	234	155	44	0.4	952
Prilocaine	220	125	50	1	4424
Benzocaine	165	910	8	0.02	Very High*
Etidocaine	276	8	8	2	9886

*: Unknown but very high as these anaesthetics (amino esters) are metabolised much more quickly (minutes) than lidocaine/prilocaine (amino amides) (hours) [206].

Discussion: The model results suggest that if these local anaesthetics are used for dermal pain, tetracaine should give a good response. Etidocaine, prilocaine and lidocaine should have some pharmacological activity. However nothing should be expected from benzocaine.
The Systemic Safety Index is extremely high.

Correlation with clinical trial safety /efficacy / comparison: Head to head comparison of these 4 anaesthetics is found in the literature. In a pinprick model [127], Ametop (tetracaine) is compared with EMLA (lidocaine+prilocaine) showing superiority of Ametop. In another study with the same model [128], saturated solution of tetracaine, lidocaine and benzocaine are compared showing that tetracaine is superior to lidocaine which is superior to benzocaine. In a study observing the effect of local anaesthetics on pain reduction during pulse dye laser treatment of portwine strains, tetracaine performed better than EMLA [207]. In a study on nociceptors and thermoreceptors [203], solutions of tetracaine, lidocaine, benzocaine and etidocaine at similar saturation level (50%) are compared and show tetracaine >lidocaine >>benzocaine >etidocaine. In the U.S., only

topical lidocaine+prilocaine and tetracaine are indicated to be used on the skin while topical benzocaine is indicated to be used on mucosal membranes but not skin [208].

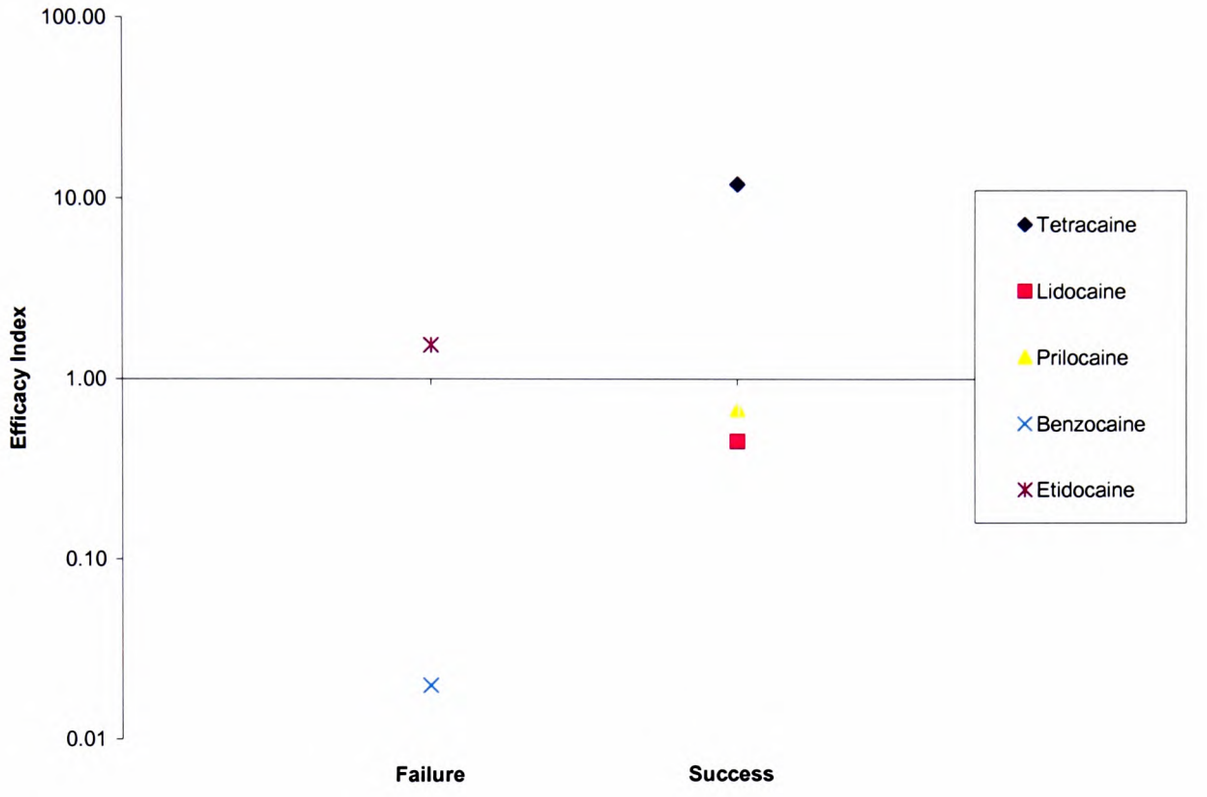

Figure 30. Anaesthetics Chart Summary

Among the group of these 5 anaesthetics, for 4 of them, tetracaine, lidocaine, prilocaine and benzocaine, the PK model fits well with what has been observed in the clinic. Etidocaine, using the PK model with the data used, was believed to have an action similar to lidocaine or prilocaine but it did not in the clinical model used.

As prilocaine and lidocaine in EMLA consists of a mixture of both, one could consider them as 1 compound. So the model predicts for ~3/4 of compounds (75% correct prediction).

6.4. Retinoids

Choice of drugs: Tretinoin (trans-retinoic acid) and isotretinoin (cis-retinoic acid) are the oldest retinoids known and used as they occur naturally endogenously. They have been used both systemically and topically which makes them good candidate to be assessed by this PK/PD model (=>PK data available, => oral dose available => effective plasma level available). A synthetic retinoid like acitretin could potentially be used in this model as this synthetic retinoid is given orally (effective plasma level known). However it has not been used topically so cannot be assessed in the model. Newer retinoids, like tazarotene or adapalene, developed for topical use, lack systemic data for potency, which means that large errors could be made during their potency determination. As for retinoids, the mechanism of action remains unclear [209], the use of *in vitro* binding data or other *in vitro* data could be poorly predictable of the potency of the compound. An extra difficulty in using *in vitro* data with retinoids comes from their high protein binding properties (>99%)(see pharmacokinetic section). To use *in vitro* data the protein binding for these drugs would need to be known (not found in literature) and the level of protein (albumin) in the *in vitro* assay would need to be carefully identified. For these reasons tazarotene and adapalene are excluded from this analysis.

F*lux (see* Table 22*):* There is a good consistency in the flux data of tretinoin. No flux data for isotretinoin appears to be available. However because chemically isotretinoin and tretinoin are very similar (same molecule but one double bond which is -cis- for isotretinoin and which is -trans- for tretinoin), the same flux value is used for both drugs.

Table 22. Retinoids Flux from literature

Compounds	Mean Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)
Tretinoin	0.20	0.1 [210] (vitro) $5mg/cm^2$ 0.025%	0.26* [211] (vivo) $2mg/cm^2$ 0.05%	0.75 [212] (vitro) $10mg/cm^2$ 0.1%	0.2** [213] (vivo) $2mg/cm^2$ 0.01%	0.22 [214;215] (vitro) $10mg/cm^2$ 0.025%	0.08*** [216] (vivo) $2mg/cm^2$ 0.05%

* ^{14}C tretinoin based on urine excretion, corrected from IV study in monkey

** ^{3}H tretinoin based on urine+faeces excretion (assumption this is total excretion)

*** Corrected flux for non facial application site + ^{3}H tretinoin based on urine+faeces excretion (assumption this is total excretion)

Effective Concentration (see Table 23*):* The oral dose is used to define effective concentration. It is noteworthy that accurate protein binding data are missing for tretinoin, which inevitably impairs the true value of the effective concentration.

Table 23. Retinoids Potency /Effective concentration

Potency	Mean unbound drug Potency (nM)	Oral Dose + PK
Tretinoin	~ 0.56	Oral dose in psoriasis 1mg/kg/day [217] Pharmacokinetics [218]: 45mg/m2/day => 249 < AUC < 537 ng.hr/ml Prot. Bind.~99%? Cl ~ 132 l/hr -- F ~ 66% -- 0.5<t1/2<2hr
Isotretinoin	~ 0.69	Oral dose in acne 1mg/kg/day [219] Pharmacokinetics [219]: 40mgx2/day => AUC steady state~5,000 ng.hr/ml Prot. Bind.~99.9% Cl ~ 12 l/hr -- F ~ 75% -- 10<t1/2<20hr

Target site: Sebocyte gland (likely external part of the sebocyte gland contrary to the antimicrobials which would be the internal part) =>Dermis (top and deep).

PK/PD Equation: Acne [4.4.3]

Efficacy Index = Flux (ng/cm^2/hr)*0.6 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.6 / [400*(1-(Prot. binding/100)]

Note: The deep dermis acne PK/PD equation is used as the target site of retinoids is likely to be at the bottom of the sebocyte for sebum suppression (some action will as well occur at the top of the dermis to prevent keratinocyte proliferation in the sebocyte duct).

Pharmacokinetic data: AUCs after oral administration are available for both drugs at their clinical dose. It should be noted that the half-life for isotretinoin (10-20 hours) is much higher compared to tretinoin (0.5-2 hours). The bioavailability appears to be about 66% for tretinoin based on IV radiolabelled study and 75% based on oral radiolabelled study investigating metabolites (both studies remain not accurate). Plasma protein binding data exists for isotretinoin (~99.9%) but a very imprecise value is available for tretinoin. Protein binding of tretinoin is superior to 95% but no more information is found, so 99% is chosen as 99.9% is the binding for isotretinoin and Sani et al. [220] suggest higher binding efficiency for albumin for isotretinoin Vs. tretinoin. This unknown data can potentially induce very different value for the Efficacy Index and the Systemic Safety Index depending on the true protein binding value. As well, a very high protein binding of the order of isotretinoin is difficult to measure experimentally, such data need therefore to be taken with care. Overall it should be noted that the PK data extrapolated from what is published could lead to substantial error on potency as well as systemic safety index.

The summary data on Retinoids is presented in Table 24.

Table 24. Retinoids summary

Compounds	MW	Potency (nM)	Flux (ng/cm^2/hr)	Efficacy Index	Systemic Safety Index
Tretinoin	300	0.56	0.20	0.7	19800
Isotretinoin	300	0.69	0.20	0.6	18000

Discussion: Taking into accounts the uncertainty of the protein binding data and therefore on the potency of the drugs, the results suggest that these two drugs would be in the right range to give efficacy in acne. The systemic safety index is very high and therefore it should be confidently postulated that these drugs applied topically should not induce the same systemic exposure as observed when the drugs are given orally. However one should consider the fact that some of the metabolites, potentially toxic, have longer half-lives such as the 4-oxo metabolite.

Correlation with clinical trial safety / efficacy / comparison: Topically both drugs are effective and this would be predicted by the PK/PD model assuming that the pharmacokinetic (flux) is the same for both drugs. Only oral isotretinoin is used, probably because of the much longer half-life. As well, oral isotretinoin outperforms the topical treatments of isotretinoin or tretinoin. The relatively low efficacy index may explain the discrepancy between topical and oral efficacy, however the error introduced by the high protein binding does not allow a firm conclusion.

On the safety aspect, several publications have shown that topical administration of tretinoin does not modify the endogenous level of retinoic acids [216;221] as predicted by the model. As well, topical tretinoin is now approved for photo-aging (wrinkles) by the FDA, suggesting further that the risk/benefit ratio for a life style condition such as photo-aging is good for retinoids despite their toxic profile.

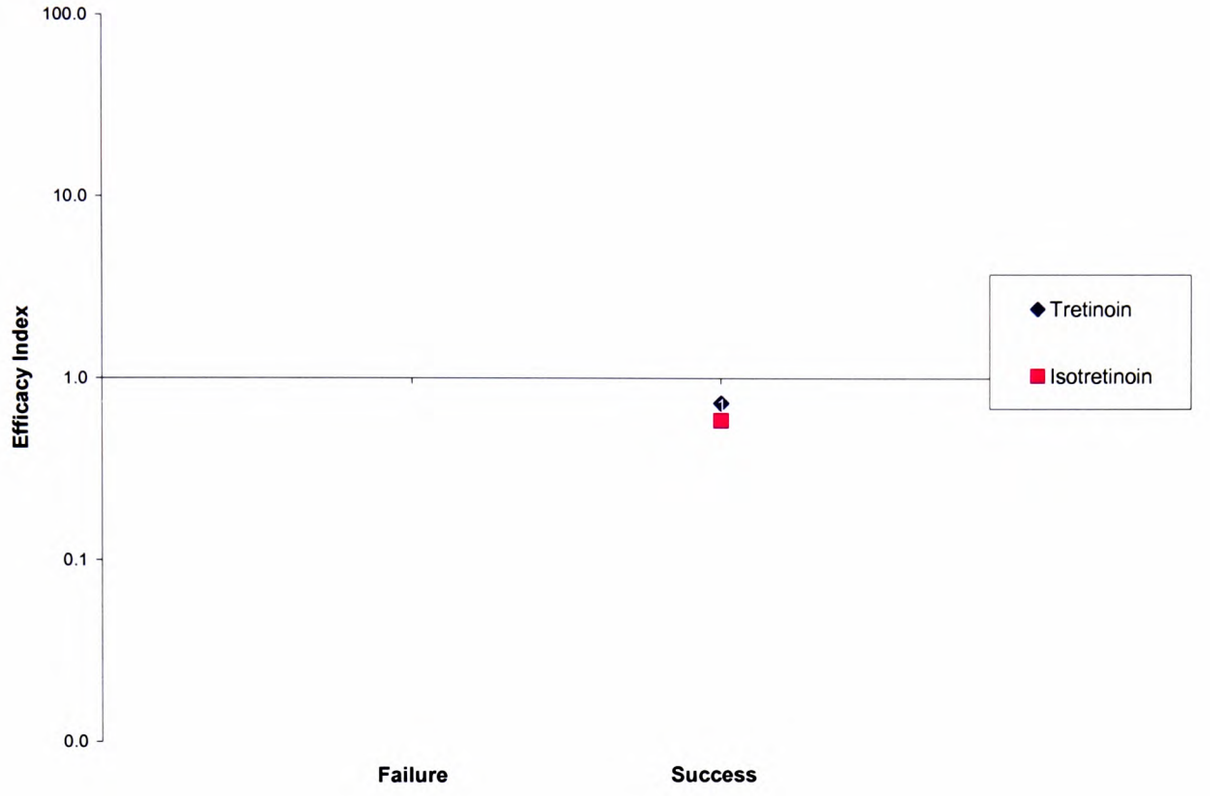

Figure 31. Retinoids Chart Summary

Overall, the PK/PD model seems to predict well the efficacy and safety of tretinoin and isotretinoin.

6.5. *Corticosteroids*

Choice of drugs: The corticosteroids chosen are among the most common.

Flux (see Table 25*):* Because of a lack of available data in the literature allowing the determination of flux from different drugs and formulations, the flux of 10 corticosteroid formulations has been measured experimentally (see 10.6 for details).

Table 25. Corticosteroids Flux GSK *in vitro* Measurements

Formulations	Flux ($ng/cm^2/hr$)
Temovate Ointment (0.05% Clobetasol Propionate)	0.95 (+0.20/-0.16)
Temovate Cream (0.05% Clobetasol Propionate)	0.36 (+0.17/-0.11)
Temovate Cream E (0.05% Clobetasol Propionate)	0.15 (+0.03/-0.03)
Diprolene Cream AF (0.05% Betamethasone Di- Propionate)	0.37 (+0.36/-0.18)
Diprosone (0.05% Betamethasone Di- Propionate)	0.18 (+0.10/-0.06)
Synalar Cream (0.025% Fluocinolone Acetonide)	0.043 (+0.072/-0.026)
Eumovate Cream (0.05% Clobetasone Butyrate)	0.059 (+0.067/-0.031)
Hydrocortisone (1% Hydrocortisone)	0.29 (+0.86/-0.20)
Cutivate Ointment (0.005% Fluticasone Propionate)	1.1 (+1.07/-0.44)
Cutivate Cream (0.05% Fluticasone Propionate)	0.024 (+0.015/-0.009)

EC 50 (see Table 29*):* As corticosteroids are usually compared with dexamethasone for competitive binding, results are given as Relative Receptor Affinity (RRA) (Table 26).

Table 26. Corticosteroids Relative Potency

Corticosteroids	Relative Receptor Affinity (RRA) to Dexamethasone													Mean Relative Potency
	[222]	[223]	[224]	[113]	[225]	[226]	[227]	[115]	[116]	[117]	[228]	[229]	[230]	
Hydrocortisone		6.7	17	7.8	4	11	17	11	31	32	9		5	11.1
Hydrocortisone Butyrate							99	68	111					90.7
Methyl Prednisolone	42													42.0
Prednisolone	16	11.4	33	10.4		14	17		69		16			18.9
Prednicarbate				7.3		0.04	16							1.7
Prednisolone 17 Ethyl Carbonate				103		74	181							111.3
Dexamethasone	100	100	100	100	100	100	100	100	100	100	100	100	100	100.0
Beclomethasone				76										76.0
Beclomethasone Monopropriate				1345							1010			1165.5
Beclomethasone Dipropionate				53									188	99.8
Triamcinolone				1.1		3					9			3.1
Triamcinolone Acetonide	233	278	462	361	380	255				195	233	164		270.7
Budesonide				935	780					147	390	258		404.2
Betamethasone				55			96	119	66		58			75.2
Betamethasone Valerate		440		1663			2550	220	400	353				622.1
Betamethasone Dipropionate										313			75	153.2
Fluocortolone				82							64			72.4
Fluocinolone Acetonide		674		478						243				427.8
Fluocinonide										147				147.0
Fluticasone Propionate				1800								813		1209.7
Clobetasol Propionate		844	1500					170		151				424.6
Clobetasone Butyrate		509							266	439				390.3
Halcinonide										230				230.0
Mometasone Furoate												1235		1235.0

The RRA represents a relative potency but not a concentration. In order to define the corresponding concentration the mean free plasma level of the most classic systemic (oral) corticosteroid is calculated. As there is a well-established equivalent oral dosage for these oral steroids and because their pharmacokinetic parameters are known, it is expected that their plasma free effective concentration would be more accurate rather than using cell assay potency data.

The six most classic oral corticosteroids are: hydrocortisone, prednisone, methyl-prednisolone, triamcinolone acetonide, dexamethasone and betamethasone. Of the six, the pharmacokinetics of prednisone and its active metabolite prednisolone is complex (reversible metabolism) [231;232], prednisone is therefore not considered.

Table 27. Effective Free Concentration of 5 Oral Corticosteroids

Compounds	Equivalent Oral Dose (mg) [118]	Bio Availability (%) [198]	Clearance (l/hr) [198]	Plasma Protein Binding (%) [198]	Mean Plasma Conc. (ng/ml)	Mean Free Plasma Conc. (ng/ml)
Hydrocortisone	20	90	25	92.5	30.0	2.25
Methylprednisolone	4	82	26	78	5.3	1.16
Triamcinolone acetonide	4	23	32	40	1.2	0.72
Dexamethasone	0.75	78	15	68	1.6	0.52
Betamethasone	0.75	72	12	64	1.9	0.68

The next step consists in assessing what factor to apply to link the relative corticosteroid potency and the free drug concentration. This is achieved by fixing a concentration to dexamethasone and then using the relative potency value to calculate the free effective concentration. The concentration for dexamethasone is defined by trial and error such that the overall "difference ratio" of the five drugs equals to 1.0 as shown in Table 28. This leads to the free effective concentration for dexamethasone: 0.58ng/ml.

Table 28. Linking Relative Potency with Effective Free Concentration

Compounds	Free Plasma Concentration (ng/ml)	Mean Relative Potency	Calculated Free Effective Concentration from RRA (ng/ml)	*Difference Ratio (Calculated from RRA vs. Mean Free Plasma)*
Hydrocortisone	2.25	11	5.2	*2.30*
Methylprednisolone	1.16	42	1.4	*1.19*
Triamcinolone acetonide	0.72	271	0.21	*0.30*
Dexamethasone	0.52	100	0.58	*1.11*
Betamethasone	0.68	75	0.77	*1.13*
		Overall Difference Ratio		***1.0***

This leads to a modified Table 26 where the RRA can be transformed into a free effective concentration.

Table 29. Corticosteroids Potency

Corticosteroids	M.W.	Mean Relative Potency	Free Effect. Conc. [ng/ml]	Free Effect. Conc. [nM]
Hydrocortisone	362	11.1	5.21	14.39
Hydrocortisone Butyrate	433	90.7	0.64	1.48
Methyl Prednisolone	374	42.0	1.38	3.69
Prednisolone	360	18.9	3.06	8.51
Prednicarbate*	489	1.7	34.69	70.95
Prednisolone 17 Ethyl Carbonate	433	111.3	0.52	1.20
Dexamethasone	392	100.0	0.58	1.48
Beclomethasone	409	76.0	0.76	1.87
Beclomethasone Monopropriate	465	1165.5	0.05	0.11
Beclomethasone Dipropionate	521	99.8	0.58	1.12
Triamcinolone	394	3.1	18.73	47.53
Triamcinolone Acetonide	434	270.7	0.21	0.49
Budesonide	431	404.2	0.14	0.33
Betamethasone	392	75.2	0.77	1.97
Betamethasone Valerate	477	622.1	0.09	0.20
Betamethasone Dipropionate	478	153.2	0.38	0.79
Fluocortolone	376	72.4	0.80	2.13
Fluocinolone Acetonide	452	427.8	0.14	0.30
Fluocinonide	495	147.0	0.39	0.80
Fluticasone Propionate	501	1209.7	0.05	0.10
Clobetasol Propionate	467	424.6	0.14	0.29
Clobetasone Butyrate	460	390.3	0.15	0.32
Halcinonide	455	230.0	0.25	0.55
Mometasone Furoate	521	1235.0	0.05	0.09

Target site: Top Dermis.

PK/PD Equation: Atopic Dermatitis (A.D.) and psoriasis equations are used with a correction factor. As corticosteroids have vasoconstricting properties, the capillary clearance is decreased and therefore local concentrations are increased. PK/PD equations used need therefore to be modified to take into account the vasoactive properties of this class of drugs. A factor is used to correct these equations. This factor is chosen constant for all corticosteroids for simplicity, and chosen equal to 3 based on the two points below that appear reasonable:

1- By choosing the corticosteroid correction factor equal to 3, the local clearance in A.D. with a corticosteroid becomes equivalent to the clearance for normal skin:
Factor [A.D. with corticosteroid] = 1/3 [A.D. factor] * 3 [corticosteroid factor] = 1
[see 4.4.1 for A.D. correcting factor].

2- The factor associated with active capillary clearance is equal to 3 (see section 4.3.1). The correction factor used with corticosteroids cannot be superior to that value.

This change in concentration due to the vasoactive properties of corticosteroids has two effects:
1- efficacy index is increased by 3 fold
2- systemic safety index is increased by 3 fold

This leads to:
Efficacy Index [A.D.]= Flux (ng/cm^2/hr)*5.5/ free Effective Conc.(ng/ml)
Efficacy Index [Psoriasis]= Flux (ng/cm^2/hr)*0.55/ free Effective Conc.(ng/ml)
Systemic Safety Index [A.D.]= Clearance (ml/hr)*1.65/ [2000*(1-(Prot. binding/100)]
Systemic Safety Index [Psoriasis]=Clearance (ml/hr)*0.55/ [2000*(1-(Prot. binding/100)]

Pharmacokinetic data (see Table 30): As most of corticosteroids have been developed for topical use on skin in the 60's and 70's, systemic PK data (clearance or protein binding) are usually not available. However when the steroids were developed for oral delivery or lung delivery these data are available.

Table 30. Corticosteroids: Systemic Safety Index

	MW	CL (ml/hr)	Prot. Binding (%)	Systemic Safety Index (A.D.)	Systemic Safety Index (Psoriasis)
Hydrocortisone*	362	25000	92.5	275	92
Triamcinolone*	394	69000	40	95	32
Dexamethasone*	392	15000	68	39	13
Betamethasone*	392	10000	64	23	8
Triamcinolone Acetonide**	434	37000	71	105	35
Budesonide**	431	84000	88	578	193
Fluticasone Propionate**	501	69000	90	569	190

*: Clearance and Protein binding data from [198]

**: Clearance and Protein Binding data from [233]

The summary data on Corticosteroids Efficacy is presented in Table 31, Table 32, Figure 32 and Figure 33:

The last column represents the US classification of topical corticosteroids [234]: Class I represents the most potent corticosteroids while Class VII represents the least potent corticosteroids. The classification of clobetasol butyrate not marketed in the US is based on the positioning of this cream by GlaxoSmithKline as well as a paper from August [235].

Table 31. Corticosteroids Summary Atopic Dermatitis

Corticosteroid Formulation	Mean Relative Potency	Free Effect. Conc. [ng/ml]	Flux [ng/cm^2 /hr] *	Dermis Free Conc. A.D. [ng/ml]	Efficacy Index in A.D.	Stoughton U.S. classification
Dexamethasone	100	0.58	NA	NA	NA	NA
Hydrocortisone cream	11	5.21	0.29	1.60	0.31	7
Clobetasone Butyrate: Eumovate cream	390	0.15	0.059	0.32	2.2	6
Fluocinolone Acetonide: Synalar cream	428	0.14	0.043	0.24	1.7	5
Fluticasone Propionate: Cutivate cream	1210	0.05	0.024	0.13	2.7	5
Fluticasone Propionate: Cutivate ointment	1210	0.05	1.1	6.05	126	3
Betamethasone Di Propionate: Diprosone cream	*1165*	0.05	0.18	0.99	19	3
Betamethasone Di Propionate: Diprolene AF cream	*1165*	0.05	0.37	2.04	41	2
Clobetasol Propionate: Temovate cream E	425	0.14	0.15	0.83	6.0	1
Clobetasol Propionate: Temovate cream	425	0.14	0.36	1.98	15	1
Clobetasol Propionate: Temovate ointment	425	0.14	0.96	5.28	39	1

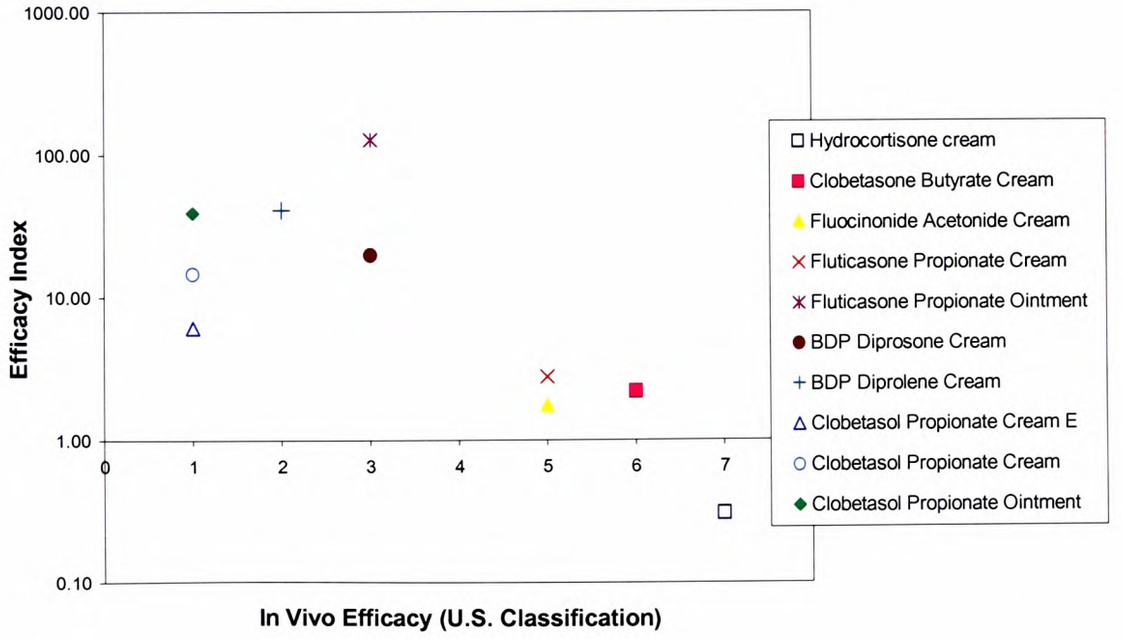

Figure 32. Predicted Topical PK/PD vs. Clinical Efficacy in Atopic Dermatitis

Table 32. Corticosteroids Summary Psoriasis

Corticosteroid Formulation	Mean Relative Potency	Free Effect. Conc. [ng/ml]	Flux [ng/cm^2 /hr]*	Dermis Free Conc. Psoriasis [ng/ml]	Efficacy Index in Psoriasis	Stoughton U.S. classification
Dexamethasone	100	0.58	NA	NA	NA	NA
Hydrocortisone cream	11	5.21	0.29	0.16	0.03	7
Clobetasone Butyrate: Eumovate cream	390	0.15	0.059	0.03	0.22	6
Fluocinolone Acetonide: Synalar cream	428	0.14	0.043	0.02	0.17	5
Fluticasone Propionate: Cutivate cream	1210	0.05	0.024	0.01	0.28	5
Fluticasone Propionate: Cutivate ointment	1210	0.05	1.1	0.61	13	3
Betamethasone Di Propionate: Diprosone cream	*1165*	0.05	0.18	0.10	2.0	3
Betamethasone Di Propionate: Diprolene AF cream	*1165*	0.05	0.37	0.20	4.1	2
Clobetasol Propionate: Temovate cream E	425	0.14	0.15	0.08	0.60	1
Clobetasol Propionate: Temovate cream	425	0.14	0.36	0.20	1.4	1
Clobetasol Propionate: Temovate ointment	425	0.14	0.96	0.53	3.9	1

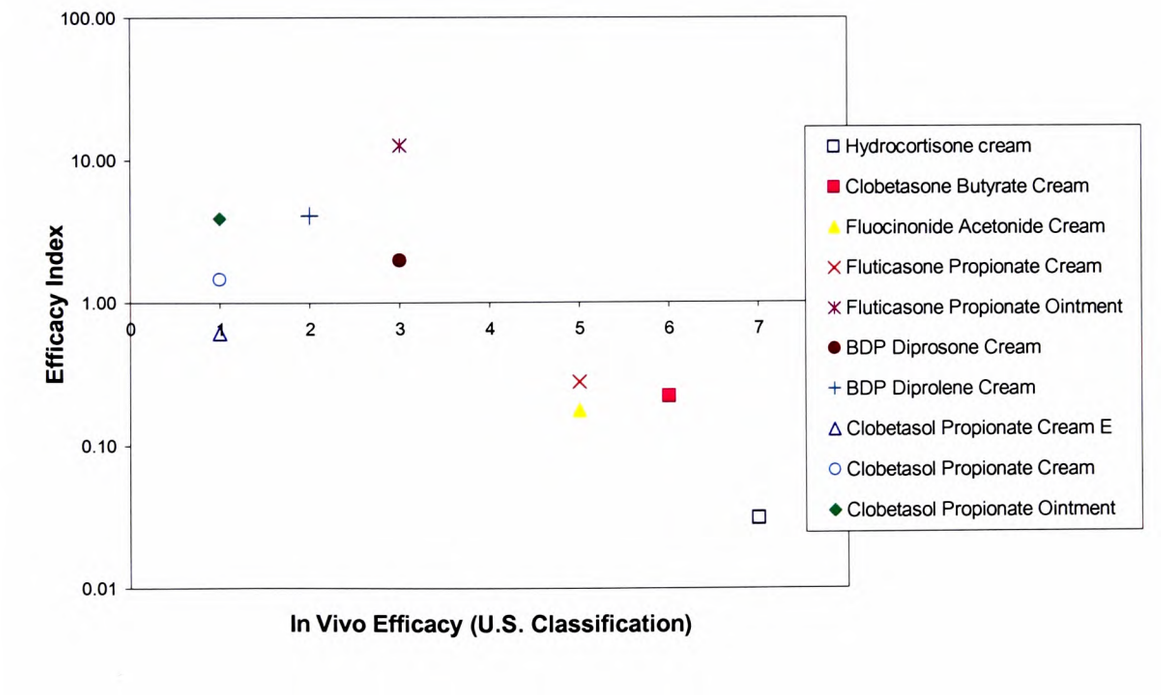

Figure 33. Predicted Topical PK/PD vs. Clinical Efficacy in Psoriasis

Notes:

1- The value of the RRA used for Betamethasone-DiPropionate is the one for Betamethasone MonoPropionate. The activity of the monopropionate and not of the dipropionate is demonstrated for beclomethasone dipropionate (enantiomer of betamethasone dipropionate). It may appear reasonable to make the same assumption for betamethasone. However, it is here assumed that there is a 100% conversion to the monopropionate. This therefore may lead to an overestimation of the efficacy index of the BDP creams.
2- The percutaneous bioavailability of Cutivate ointment is close to 30% through normal skin [see 10.6]. This could lead to an overestimation of flux in a clinical condition as drug depletion is likely to occur at least for AD (more permeable skin membrane).
3- The substantial long lag time for the flux of the clobetasol propionate formulations observed [see 10.6] is a factor not taken into account in the efficacy index PK/PD approach. It is likely however to play a role. The longer a drug stays in the stratum corneum, the longer the drug will be released to the lower target skin tissues. Therefore the longer the pharmacological effect of the drug will last. If the longer lag time for the flux of clobetasol propionate against other corticosteroids was demonstrated in a same flux study, this would suggest that the real efficacy of clobetasol is underevaluated by using the efficacy index alone. For a better assessment the retention time in the stratum corneum should be taken into account.

Discussion:

The strict use of the Efficacy Index described in Table 31 and Table 32 suggests that the most effective corticosteroid preparations would be the three clobetasol propionate formulations, the two betamethasone creams and the fluticasone propionate ointment. On the other end, the hydrocortisone cream would be the least effective topical corticosteroid. On that last drug, the low efficacy index would suggest a lack of efficacy in psoriasis.

On the systemic safety side, newer steroids (inhaled steroids) like budesonide, fluticasone propionate would appear to be potentially safer than older corticosteroid if dosed

accordingly (not too much overdosage to avoid a minimisation of the Systemic Safety Index (S.S.I.) [see 5.2]). Hydrocortisone has an inherent good S.S.I. and because its efficacy index is less than 1 it appears that overdosing is not possible and that therefore systemic exposure looks very unlikely under normal dosage conditions.

Correlation with clinical trial safety / efficacy / comparison:

The Efficacy Index of the drugs quoted in Table 31 and Table 32 show good agreement with the US Classification of Topical Corticosteroids (see Figure 32 and Figure 33).
The notes made in the previous paragraph regarding the potential misrepresentation of the efficacy index for clobetasol propionate formulations, betamethasone dipropionate formulation and fluticasone propionate may explain the difference with the ranking from the US classification.
In the clinic it is easier to differentiate corticosteroids in psoriasis than in A.D. The large Efficacy Index seen in A.D. with most corticosteroids may explain this lack of response differentiation due to saturation of the response. On the other end, for psoriasis differentiating one drug from another is easier and this may be explained by the lower efficacy index closer to 1. It should be noted that there is a good fit for hydrocortisone: efficacy in A.D. (E.I.=0.3) and lack of efficacy in Psoriasis (E.I.=0.03).

On the systemic safety predictive aspect, it is more difficult to draw conclusions as for most of these compounds the PK data (clearance and protein binding) are not available or the flux of the formulation is not available. Comments can however be made as clearance or protein binding data are available for both hydrocortisone and fluticasone propionate:

1. Hydrocortisone:

Hydrocortisone was the first topical corticosteroid switched from Rx to OTC, and this fits again with the fact that it has a low Efficacy Index (=0.3 in A.D.) and a high Systemic Safety Index (=92).

2. Fluticasone Propionate:

Despite the fact that fluticasone propionate is a very potent drug [see Table 29], adrenal suppression following topical application has not been reported/demonstrated under normal clinical conditions. Interestingly this could be explained with the high Systemic Safety Index [see Table 30] coupled with either a low flux (cream) or a very low dose (ointment).

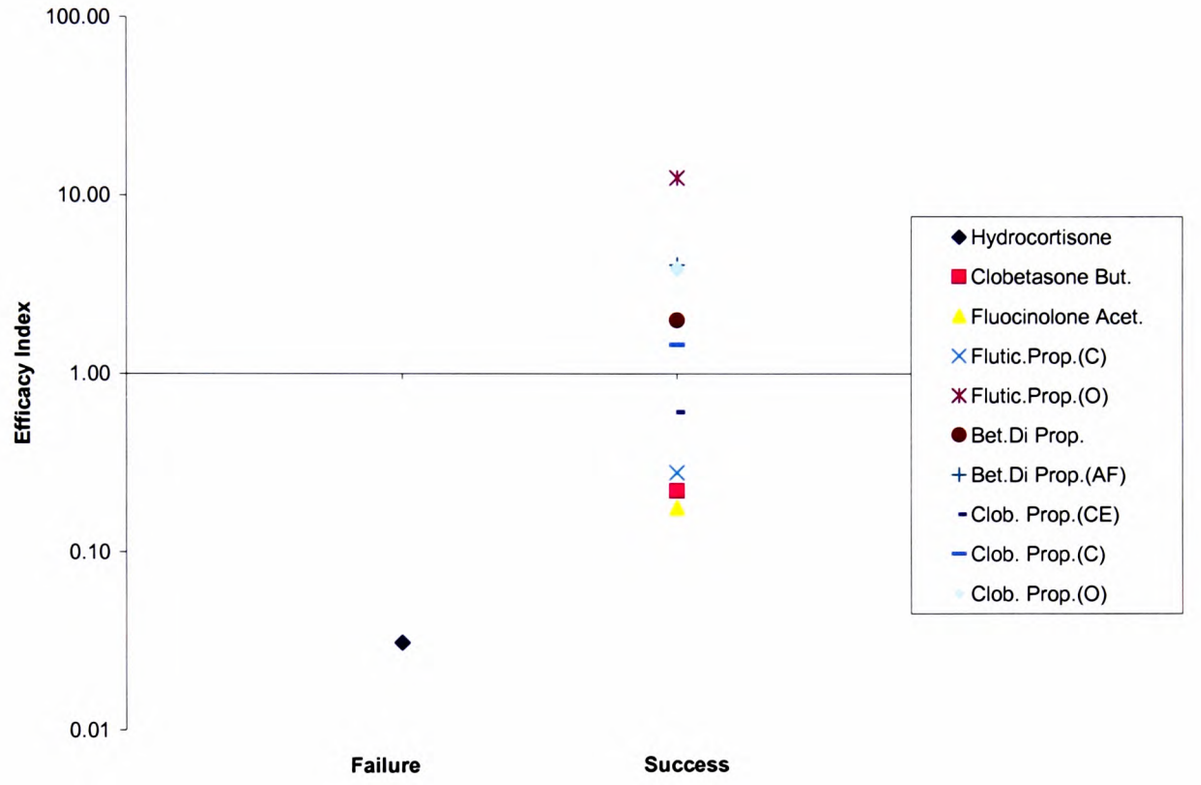

Figure 34. Corticosteroids Chart Summary (Psoriasis)

Overall from an efficacy or systemic safety perspective, the important corticosteroid family seems to fit well the PK/PD model.

6.6. *Vitamin D3 Derivatives*

Choice of drugs: Calcitriol, calcipotriol and tacalcitol are the 3 Vitamin D3 derivatives marketed for the treatment of psoriasis. Calcitriol is the natural/endogenous active form of Vitamin D3.

F*lux (see* Table 33*):* Limited data are available for the flux for these drugs, probably because of the extremely low delivery: ~10-50 pg/cm^2/hr. Only Tacalcitol *in vitro* data are published (marketed ointment). *In vitro* data for Calcipotriol can be found in the NDA application [236][Note: *in vivo* exposure to calcipotriol ointment is available as well but the application was made under occlusion so the data are not considered. Under these conditions, the flux calculated from the excreted amount reaches 450pg/cm^2/hr]. No data on calcitriol were found. The close chemistry of the three compounds considered suggests that the flux of calcipotriol and tacalcitol can be used to estimate the flux of calcitriol.

Table 33. Vitamin D3 Derivatives. Flux from literature

Compounds	Mean Flux (ng/cm^2/hr)	Flux (ng/cm^2/hr)
Calcitriol	0.02	0.02*
Calcipotriol	0.04	0.04** [236]
Tacalcitol	0.01	0.01*** [237]

* No data: geometric mean of the other two compounds is used

** *In vitro* flux for ointment (lower flux for lotion or cream)

** *In vitro* flux for ointment

Effective Concentration (see Table 34*):* Only *in vitro* data are available: keratinocyte differentiation and keratinocyte proliferation. There are issues as: 1- true level of protein

is not known: assumption is that the media contained 10% serum equivalent (=1/10 of normal amount of protein) as this is often the medium used in keratinocyte cell cultures); and 2- true protein binding for calcipotriol and tacalcitol is not known. Protein binding for calcitriol has been investigated in two publications [238] (99.5%) and [239] (99.92%), the last study appearing as the most rigorous one (protein binding data chosen: 99.8%). For tacalcitol, the reported [240] in vitro human plasma data is 100% which can only be interpreted as very high binding. With this single information, tacalcitol protein binding is chosen to be equal to calcitriol: 99.8%. For calcipotriol, the reported data are >95% which again is not a precise figure. However, one publication [241] suggests that calcitriol is about 3 fold more bound than calcipotriol. So if calcitriol protein binding ~ 99.8% => calcipotriol protein binding ~ 99.4%.

Second pharmacological action: calcium homeostasis (=increase in calcium level) at vitamin D3 (calcitriol) plasma level slightly above the calcitriol endogenous level. This second pharmacological action potency figure is taken as equivalent to an increase of Vitamin D3 derivative in blood of 0.06nM (blood). [Note: endogenous calcitriol level ~ 40pg/ml. By adding 25pg/ml (0.06nM) => x 1.5 endogenous level]. This second much more potent pharmacological action induces a large reduction of the safety window of these drugs: Potency of differentiation or proliferation pharmacology is about 15-35nM (100% serum corrected)...compared to 0.06nM. The Systemic Safety Index needs to be divided by a factor 250 to 580 (= 400 to simplify) (see Equation 40).

Table 34. Vitamin D3 Derivatives. Potency /Effective concentration

Potency	Mean Unbound* drug Potency (nM)	Mean Bound** drug Potency (nM)***	Drug Potency (nM) [242]	Drug Potency (nM) [243]	Drug Potency (nM) [cell diff.] [242]	Drug Potency (nM) [cell prolif.] [242]	Drug Potency (nM) [cell diff.] [243]	Drug Potency (nM) [cell prolif.] [243]
Calcitriol	0.070	3.5	1.5	8.0	3.4	0.7	28	2.3
Calcipotriol	0.10	1.7	0.46	6.5	2.1	0.1	14	3
Tacalcitol	0.030	1.5	0.57	NA	0.2	1.6		

*: Protein binding used: calcitriol ~ 99.8%, calcipotriol ~ 99.4%, tacalcitol ~ 99.8%

**: Partially bound, as medium believed to contain 10% serum (1/10 of normal amount of protein)

***: Corrected for tacalcitol as only in one reference

Target site: Bottom epidermis

PK/PD Equation: Psoriasis [4.4.2]

Efficacy Index = Flux (ng/cm^2/hr)*0.73 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.73 / [2000*(1-(Prot. binding/100)] / 400

Pharmacokinetic data: Only clearance data for calcitriol [Cl=2.1 l/hr] are available. The other clearance data found correspond to studies in rats: 127 fold superior clearance for calcipotriol vs. calcitriol [244]. For tacalcitol the half-life in rats was of the order of hours [240] while it was minutes for calcipotriol and minutes to hours for calcitriol.

The summary data on Vitamin D3 derivatives is presented in Table 35.

Table 35. Vitamin D3 derivatives summary

Compounds	MW	Potency (nM)	Flux (ng/cm^2/hr)	Efficacy Index	Systemic Safety Index
Calcitriol	417	0.070	0.02	0.5	0.6
Calcipotriol	412	0.103	0.04	0.7	41*
Tacalcitol	417	0.030	0.01	0.6	Low**

*: Assuming clearance in rats difference in between calcitriol and calcipotriol (~127 fold) is equivalent in human and assumed similar potency against hypercalcemia.

**: Based on half-life in rats (see pharmacokinetic data section above)

Discussion: Taking into account the uncertainty in the protein binding data and on the flux, the results suggest that these three drugs would deliver some limited efficacy and be about equally potent. Only calcipotriol would appear to be relatively safe, as the other 2 drugs would likely induce some systemic level leading to hypercalcemia.

Correlation with clinical trial safety / efficacy / comparison: The three drugs applied topically do improve psoriasis[245-247]. However calcipotriol seems superior to calcitriol [248]. As well calcipotriol ointment twice a day has been shown to be superior to tacalcitol ointment once a day [249].

The efficacy of drugs in psoriasis seems to fit with the prediction. However, calcipotriol appears to be superior to the other 2 drugs (at least to calcitriol). This was not predicted by the model, which was predicting an equal efficacy. Better data, especially on flux and protein binding, would be helpful to decide whether this variance is a model problem or not.

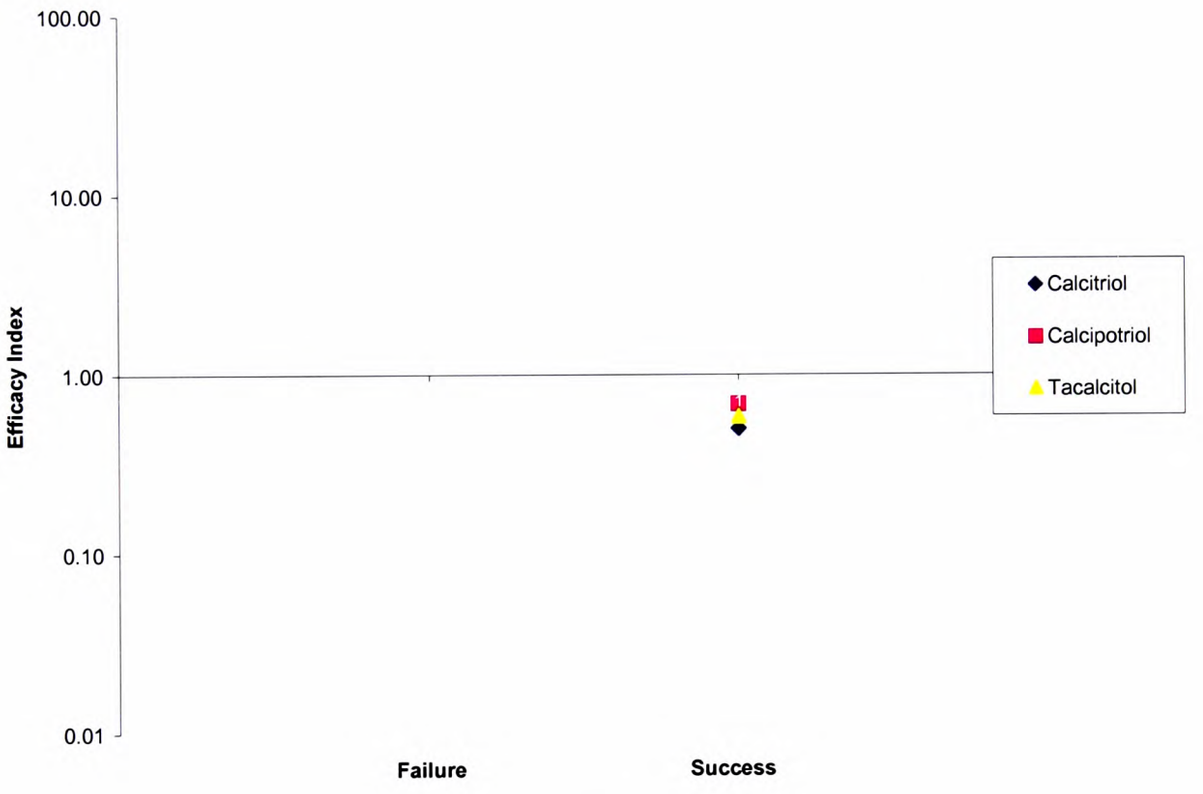

Figure 35. Vitamin D3 Derivatives Chart Summary

On the safety aspect, calcipotriol does not lead to systemic calcium homeostasis at dose less than to 100g per week (< 15% Body Surface Area (BSA): if twice a day and application of 2.5mg/cm^2) [250]. At higher dose, hypercalcemia appears consistently [250]. For calcitriol, formulation containing 3μg drug per g of ointment applied at a dose

of 50g per week (~ 7-8% BSA) did not induce hypercalcemia. However formulation containing 15μg calcitriol per g applied on more than 600cm^2 (3% BSA) induced hypercalcemia [250]. Little is published on tacalcitol safety.

The model was predicting systemic toxicity issue with calcitriol, which is proven correct. The larger safety window predicted for calcipotriol seems to fit as well with the observed clinical side effects for that drug (safe if BSA<15%). It must be stressed however that true human clearance data coupled with good protein binding data would be required to really assess this safety window for calcipotriol.

6.7. Antifungals

Note: Superficial fungal infections are caused generally by one of the following three fungi family: Dermatophytes (the majority of superficial fungal infection [251]), Candida species and Malassezia furfur. Antifungals will act differently against these families: the imidazoles are active against the 3 species while the "allylamines/benzylamines" are active against the first 2 only.

Choice of drugs: ketoconazole, clotrimazole, miconazole (imidazole family), terbinafine and butenafine ("allylamine/benzylamine" family) are chosen as they represent the two main families of antifungals used to treat superficial fungal infections.

F*lux (see* Table 36*):* Only data on ketoconazole and terbinafine meeting the flux criteria appear to be available. Similar fluxes are found for both drugs (~0.1$\mu g/cm^2/hr$). Because of that, equal fluxes were used for the other antifungals (for azoles equal to ketoconazole and for butenafine equal to terbinafine). [Note: due to the large efficacy index found for antifungals, the quality of the flux data does not appear to be crucial for the overall analysis].

Table 36. Antifungals. Flux in literature

Flux	Mean Flux ($\mu g/cm^2/hr$)*	Flux ($\mu g/cm^2/hr$)	Flux ($\mu g/cm^2/hr$)
Ketoconazole	0.15	0.15 [252] vivo	0.15 [253] vivo
Terbinafine	0.071	0.1 [254] vivo	0.05 [255] vivo under occlusion*

* Antifungal clinical usage is often partly occluded (because of the fungal infection location: plantar), this data can therefore be included (the large efficacy index found for the antifungals does not make the quality of such data critical).

Effective Concentration (see Table 37 *and* Table 38*):* Plasma level (oral dose + oral PK) as well as *in vitro* data (MIC) are used to define the effective concentration. As in all antimicrobial agents, the term MIC can be misleading as one can study MIC (as total inhibition) or partial inhibition for example 50% inhibition (MIC_{50}). This added to the fact that *in vitro* methods will vary in time of exposure to the antifungal agents, it induces large variation in the effective concentration. More recent publications are often more relevant. Similarly, using plasma concentration (after oral administration) for these drugs is not ideal either, as for quite a few fungal infections, the stratum corneum is the target site. As these drugs are lipophilic, the partition coefficient between plasma and the specific tissue can be important [256]. Therefore using the plasma level can underestimate the true effective concentration. Taking all this into account, the potency data presented here need to be interpreted with caution.

Table 37. Antifungals. Potency /Effective concentration against Dermatophytes

Potency	Mean Unbound Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)
Ketoconazole	0.040	0.008 oral dose (300mg) [Bioavail.= 75% / Cl=35.3 l/hr / Prot. Bind. = 97%]	0.2 [257] MIC	
Miconazole	0.048	0.048 I.V. dose (800mg) [Cl=58.4 l/hr / Prot. Bind. = 91.5%]		
Clotrimazole	0.25	0.25 [258] MIC		
Terbinafine	0.0021	0.0011 oral dose (250mg) [Bioavail. = 80% / Cl=74.8 l/hr / Prot. Bind. =99%]	0.003 [259] MIC	0.003 [260] MIC
Butenafine	0.012	0.012 [258] MIC		

Table 38. Antifungals. Potency /Effective concentration against Candida Albicans

Potency	Mean Unbound Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)	Unb. Drug Potency (μg/ml)
Ketoconazole	0.79	0.51 [257] MIC	16 [260] MIC	0.06 [261] MIC_{50}	0.03 [262] MIC_{50}
Clotrimazole	0.72	6.4 [258] MIC	0.08 [262] MIC_{50}		
Terbinafine	2.8	8 [258] MIC	1 [261] MIC_{50}		
Butenafine	25	25 [263] MIC			

Target site: Stratum Corneum.

PK/PD Equation: Fungal Infection [4.4.4]

Efficacy Index = Flux ($ng/cm^2/hr$)*666 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*666 / [400*(1-(Prot. binding/100)]

Pharmacokinetic data: Systemic clearance and protein binding data exists (see Table 37), for all drugs but butenafine and clotrimazole (developed for topical use only).

The summary data on antifungals is presented in Table 39 and Table 40.

Table 39. Antifungals summary against Dermatophytes

Compounds	MW	Potency (μg/ml)	Flux (μg/cm^2/hr)	Efficacy Index	Systemic Safety Index
Ketoconazole	531	0.04	0.15	2498	1959150
Miconazole	416	0.048	0.15	2081	1143953
Clotrimazole	345	0.25	0.15	400	NA
Terbinafine	291	0.0021	0.071	22517	12454200
Butenafine	317	0.012	0.071	3941	NA

Table 40. Antifungals summary against Candida Albicans.

Compounds	MW	Potency (μg/ml)	Flux (μg/cm^2/hr)	Efficacy Index	Systemic Safety Index
Ketoconazole	531	0.79	0.15	799	9800000
Miconazole	416	?	0.15	?	5731765
Clotrimazole	345	0.72	0.15	880	NA
Terbinafine	291	2.8	0.071	105	62743278
Butenafine	317	25	0.071	12	NA

Discussion: Due to the location of the target site, the concentrations achieved in this target site are very large. This induces extremely high efficacy index for all drugs even for terbinafine against C. Albicans despite being relatively poorly potent against this fungus.

Due to the target site, again, the uncorrected safety index is very large. Once more, these data should be interpreted with care, as this value will decrease dramatically (division by the efficacy index if these drugs are applied without controlling their flux). The systemic exposure will remain high even if the dose is uncontrolled.

Overall, the particular case of this PK/PD approach with topical antifungals suggests that against dermatophytes, antifungals are very largely overdosed (efficacy index > 400 with all drugs investigated).

Correlation with clinical trial safety / efficacy / comparison: In the clinic, when applied topically, all the antifungals presented here [251;264-277] perform well after 4 weeks of treatment or less. However, numbers of studies have compared the efficacy of allylamine/benzylamine-type drugs with imidazoles. It appears that terbinafine and butenafine are superior to the imidazoles. It requires a shorter treatment period to get similar efficacy (treatment for 1 week with terbinafine resulted in mycological cure rate of 76% at week 12 but 4 weeks of treatment with clotrimazole were required to achieve 70% at the same time point [269]) and relapses are less frequent for allylamine/benzylamine-type drugs than for imidazoles. Long term studies with patients treated topically with either terbinafine or clotrimazole for 1 or 4 weeks showed that relapses in patients treated with terbinafine was less than in the clotrimazole group [275]. The superior clinical efficacy of terbinafine or butenafine has been explained by the different mode of action of the two types of drugs. The imidazoles are fungistatic while the allylamine/benzylamine-type drugs are fungicidal. As arthrospores, the hyphal fragment of dermatophytes associated with contagion, can remain in skin scales for years [278], fungicidal drugs would theoretically have an advantage over fungistatic therapies by more quickly eradicating the fungi. This would then explain the shorter time for treatment required.

The topical overdosing concept suggested by the PK/PD approach deserves to be investigated more thoroughly as it does not appear to be described or explained in the literature. In order to assess this overdosing concept, two different questions can be asked:

Question N°1: Does the difference in potency of an antifungal against two different fungi lead to a difference in clinical efficacy against these two fungi?

If NO, the antifungal is overdosed against the fungus for which the antifungal is the more potent.

The case of fungicidal agents (allylamine) is of interest as there is a 100 fold difference in potency against dermatophytes fungi compared to candidasis fungi (see Table 37 and Table 38). Despite this very large difference, a review of clinical experience with topical terbinafine [266] shows that cure rate is similar against both fungi [75-85%]. This suggests that terbinafine is overdosed against dermatophytes.

Question N°2: Is the dose regimen critical for efficacy with a topical antifungal?
If YES, it suggests that an effective concentration cannot be maintained for a long period in the stratum corneum. If the concentration was very substantially above (> 400 fold above as suggested by the efficacy index), an effective concentration should be maintained for a few days. Therefore, if the answer is NO, the topical antifungal would be overdosed.

The case of terbinafine: one day vs. seven-day treatment
As seen previously the fungicidal action of allylamine drugs does not require the long-term treatment of the imidazoles, which are fungistatic agents, and the prescribed treatment regimen for terbinafine is equal to 7 days.
In a short duration therapy, with terbinafine in dermatophyte infections of tinea pedis [272], 78 patients were divided into 4 groups to treat once a day for either 1,3,5 or 7 days. After 4 weeks, the mycological cure rates were high in all four treatment-groups ranging from 78% in the 1-day active drug group to 83% in the 7-day active drug group (not statistically different).
The similar cure rates for a single application or for a 7-day therapy suggest three hypothesis:

- hypothesis 1:a short duration of an effective concentration is enough to lead to high cure rate
- hypothesis 2: an effective drug concentration is maintained for few days

- hypothesis 3: both hypothesis 1 and 2 are critical to lead to substantial cure rate.

It should be noted that single oral administration of terbinafine studies has not been reported so hypothesis 1- cannot be proven or ruled out. If hypothesis 2 or 3 were correct, this would suggest that overdosage occurs for terbinafine against dermatophytes.

Once daily Vs twice-daily treatment

5 studies comparing the dose regimen of antifungals were retrieved from the literature.

Table 41. Twice a day vs. once a day for antifungals

Antifungal	Number of Patients	Results (cured/improved or mycologically cured)	References
Naftifin (allylamine)	116	Clinically: Once (83.1%) Vs. Twice (82.1%)	[264]
Tioconazole (imidazole)	97	Clinically: Once (96%) Vs. Twice (98%)	[279]
Ketoconazole (imidazole)	62	Clinically: Once (90%) Vs. Twice (83%)	[265]
Oxiconazole (imidazole)	161	Mycologically: Once (82%) Vs Twice (82%) Clinically: Once (87%) Vs Twice (87%)	[280]
Oxiconazole (imidazole)	101	Mycologically: Once (74%) Vs Twice (71%) Clinically: Once (78%) Vs Twice (82%)	[280]

In these 5 relatively large studies for antifungals, once daily dosing is equivalent to twice daily dosing. These results suggest that a substantial dose is being delivered per application that is large enough to last for at least 24 hours.

Answers to the two questions asked above suggest likely overdosing of topical antifungals based on clinical experience with topical antifungals.

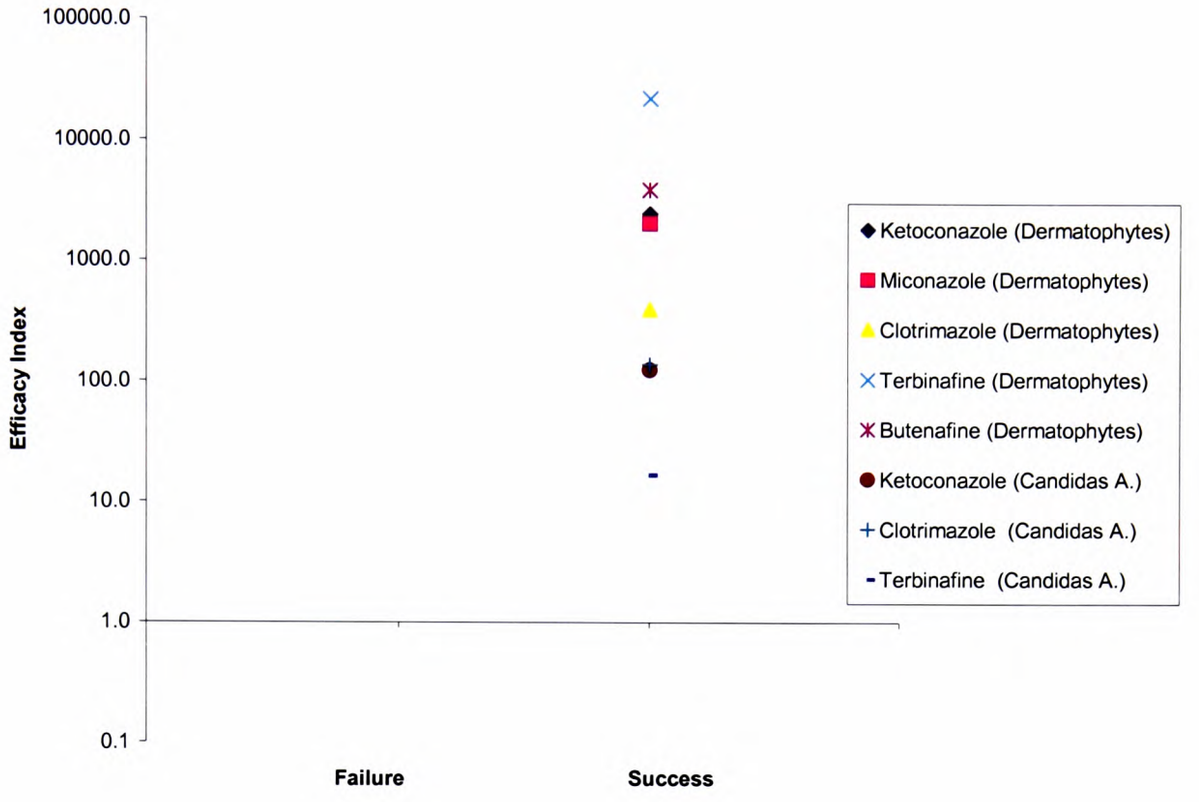

Figure 36. Antifungals Chart Summary

Overall, the model predicts the efficacy of topical antifungals. The overdosing concept predicted by the model seems to be suggested by clinical trials as well. It is however not understood why the clinical cure rates are not closer to 100% in the clinical studies as the efficacy index is very high. One potential explanation could be linked with the aetiology of the disease and the real location of the fungi. If in some cases/patients, the fungi were located in a deeper tissue than the stratum corneum, the concentration of the antifungal in that deeper tissue could prove insufficient to totally eliminate the fungi.

6.8. *Antibacterials (Acne)*

Choice of drugs: Erythromycin and clindamycin are specific antibacterial agents used topically to treat acne. Benzoyl peroxide is a non-specific antibacterial agents used topically to treat acne.

F*lux (see* Table 42*):* There are only data available for benzoyl peroxide and for clindamycin. Erythromycin is nearly twice as large as clindamycin, so one would expect to see its flux inferior to the flux of clindamycin: <4ng/cm^2/hr.

Table 42. Antibacterials. Flux from literature

Compounds	Mean Flux (μg/cm^2/hr)	Flux (μg/cm^2/hr)
Erythromycin	<0.004	?
Benzoyl peroxide	1.77	1.77 [281]
Clindamycin	0.004	0.004 [282]

Effective Concentration (see Table 43*):* MIC data against Propionibacterium Acnes are available. These data are preferred to plasma level, as these agents will have different susceptibility against different bacteria.

Table 43. Antibacterials. Potency /Effective concentration

Compounds	Mean unb. drug Potency (μM)	unb. Drug Potency (μM)	unb. Drug Potency (μM)	unb. Drug Potency (μM)	unb. Drug Potency (μM)
Erythromycin	0.58	0.126 [283] MIC_{100}?	2.7 [284] MIC_{90}		
Benzoyl peroxide	397.00	397 [283] MIC_{100}?	397 [285] MIC_{100}?		
Clindamycin	0.49	0.07 [286] MIC_{90}	1.18 [287] MIC_{70}	0.59 [288] MIC_{100}	1.18 [284] MIC_{90}

Target site: Sebaceous Gland* (in the dermis)/ On the face

*Assumption is that the drug enters the sebaceous gland via the dermis and not via the sebaceous canal, dermis could then be considered as the target site [this important point is discussed later in the conclusion of that section].

PK/PD Equation: Acne [4.4.3]

Efficacy Index = Flux (ng/cm^2/hr)*0.6 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.6 / [400*(1-(Prot. binding/100)]

Pharmacokinetic data: Erythromycin and clindamycin are given orally [Erythromycin: Cl=32 l/hr - Prot. Bind.=84% / Clindamycin: Cl=22.7 l/hr - Prot. Bind.=88%]. No data for Benzoyl Peroxide are available.

The summary data on Antibacterials is presented in Table 44.

Table 44. Antibacterials summary

Compounds	MW	Potency (μM)	Flux (μg/cm^2/hr)	Efficacy Index	Systemic Safety Index
Erythromycin	734	0.58	<0.004	< 0.01	1500
Benzoyl Peroxide	242	397	1.77	0.01	?
Clindamycin	425	0.49	0.004	0.01	1419

Discussion: The model predicts very unlikely efficacy for the 3 drugs. This is especially true for erythromycin, as the flux could be less than clindamycin by possibly an order of magnitude (a lot of functional groups and a nearly twice as big molecule). In such a case, the efficacy index would become very small for erythromycin.

Correlation with clinical trial safety / efficacy / comparison: Despite the very poor efficacy prediction for the 3 compounds, these agents are effective topically. For these 3 drugs, the efficacy index as calculated is very low. For erythromycin, the efficacy index is likely to be even lower than the value calculated as the true flux could be 10 fold less than the flux of clindamycin. For benzoyl peroxide a new formulation has been developed. In this new formulation, the active is entrapped which induces a flux reduction by about 5 fold. This new formulation seems to exhibit similar activity in vivo [281]. For erythromycin and benzoyl peroxide the efficacy index could therefore be as low as 0.002.

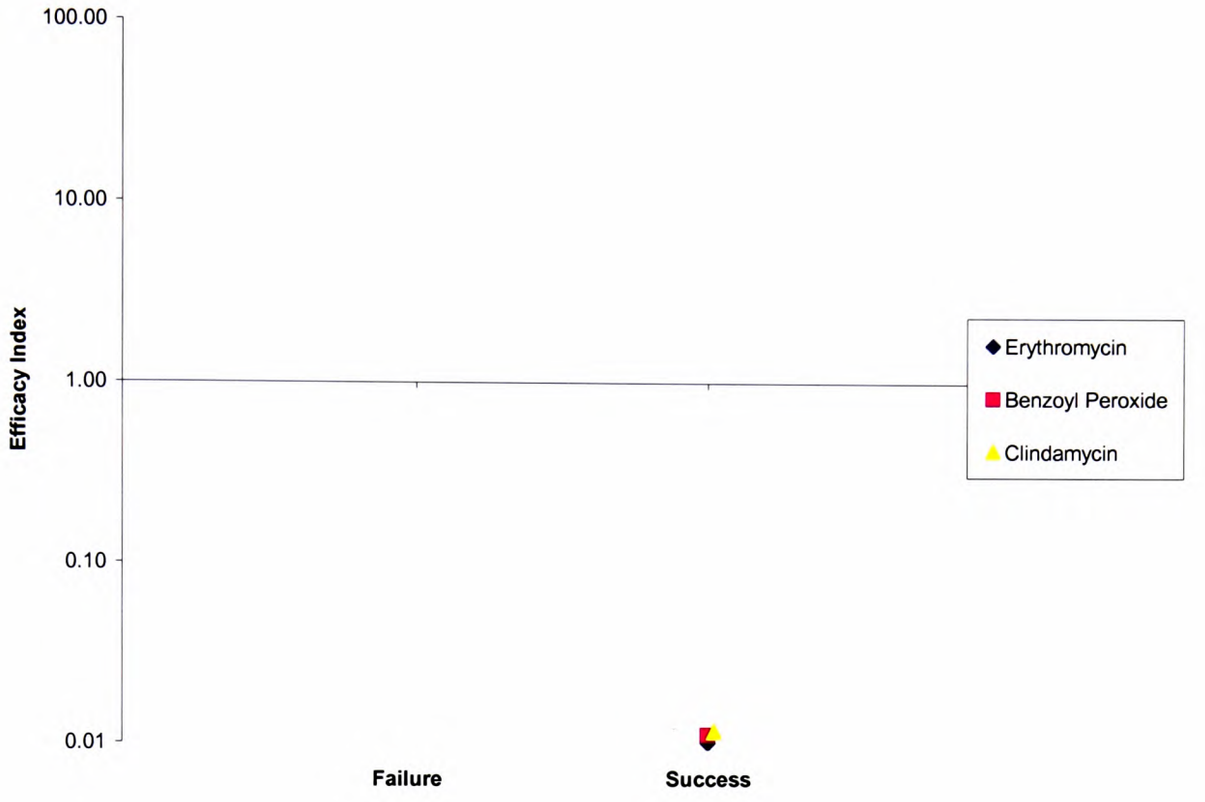

Figure 37. Antibacterials (Acne) Chart Summary

The antibacterial nature of these agents needs to be considered. The effective concentrations used are usually MIC_{100} or MIC_{90}, which assume very large eradication of the bacteria. MIC_{50} (lower concentration required) could be still relevant clinically. Unfortunately, this clinically relevant concentration is not known. An MIC_{50} 100 fold lower than the MIC_{100} is however quite unlikely.

The model therefore does not seem to predict the efficacy of these antibacterial agents for acne: Efficacy is largely under-evaluated.

In the validation exercise of Chapter 6, antibacterials for acne appear to be the first class failing in the model. Understanding of the poor prediction would be of value.
It should be noted that the target site for of antimicrobials in acne - the internal part of the sebaceous gland - is relatively isolated. This could lead to two potential explanations for this poor prediction of the PK/PD model:

1- These drugs would partition very favourably into the sebaceous gland compared to the dermis.

2- The route of entry in the sebaceous gland could be via the sebaceous duct (Figure 38) and not via the stratum corneum (Figure 39). This route of entry is likely to be a more permeable route than the dermis route, as the stratum corneum barrier does not have to be crossed.

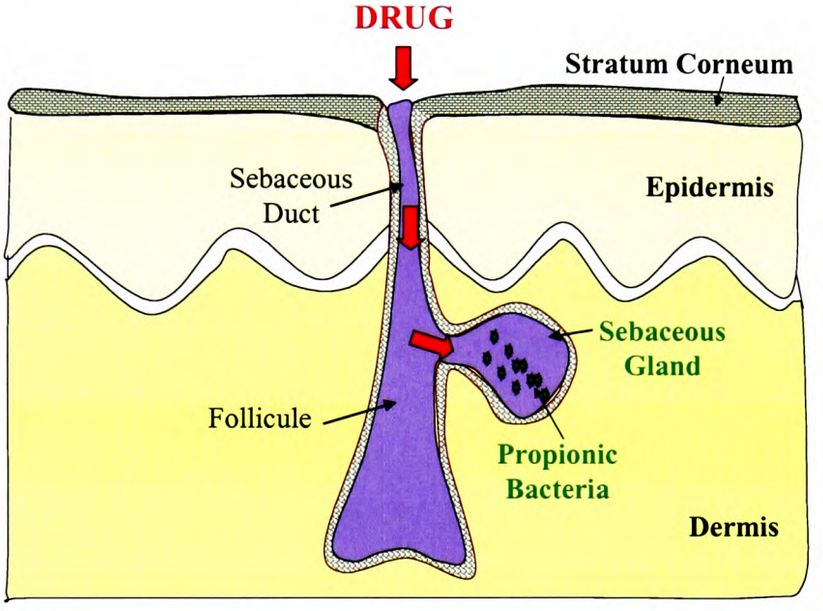

Figure 38. Antibacterial Route of Entry = Via Sebaceous Duct

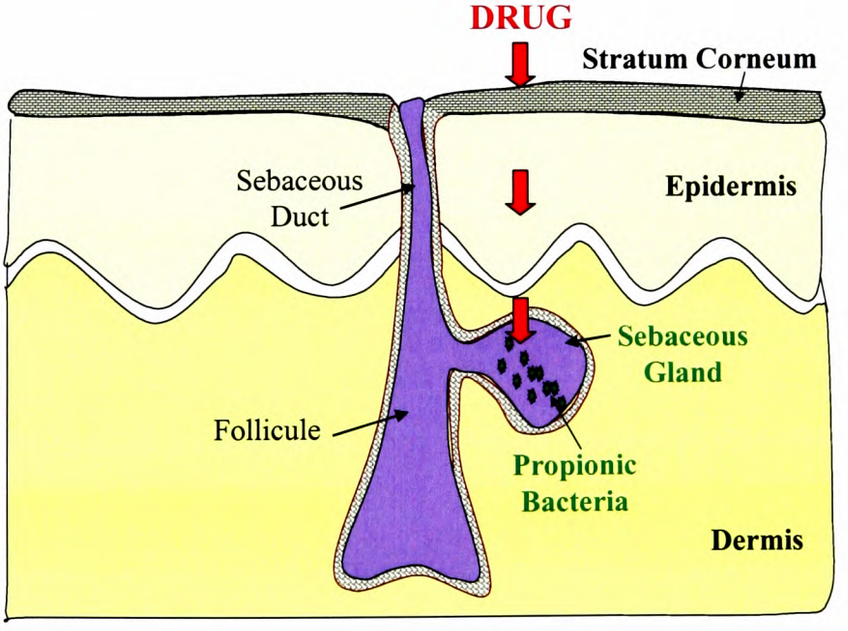

Figure 39. Antibacterial Route of Entry = Dermis Via Stratum Corneum

Of the two hypothesis, the first one appear less likely as the crossing process from the dermis to the internal side of the sebaceous gland is likely to be slow. The barrier to cross is to some extent of the same nature as the stratum corneum (a relatively impermeable membrane).

This topical PK/PD model assumes a route of entry via the stratum corneum. The under evaluation seen with this model could suggest that the route of entry of molecules acting inside the sebaceous gland is via the sebaceous duct as presented in Figure 38.

6.9. *ImmunoModulators*

Choice of drugs: Cyclosporin A, tacrolimus and pimecrolimus are chosen as immunomodulators drugs, as they have been used/tried to treat eczema or psoriasis topically. Tacrolimus and pimecrolimus are now marketed for the treatment of atopic dermatitis topically.

F*lux (see* Table 45*):* Some good data could be found for tacrolimus (3 *in vivo* studies). For cyclosporin A, all but two of the *in vitro* flux experiments reviewed did not fit the protocol requirement set in section 6.1. One of the two found which appeared to have been well conducted [289] had very high flux (~200 ng/cm^2/hr). Such a high flux would not be anticipated for a molecule of the size of cyclosporin A. As well, in the other study [290] comparing cyclosporin A and tacrolimus (well performed but with an unknown sub-saturated level) with cyclosporin and tacrolimus, it was found that tacrolimus was permeating at a higher rate than cyclosporin A. This would indeed fit with the size of the two molecules. $MW_{Cyclosporin\ A}$ = 1203 Vs. $MW_{Tacrolimus}$ = 804. For that reason, no flux can be chosen for cyclosporin A, it is only assumed that its flux would be equal or less than the flux of tacrolimus ~ 1.25 ng/cm^2/hr.

With pimecrolimus, despite the recent development of the molecule, the quality of the topical pharmacokinetic studies prevents a good assessment of the flux of the 1% pimecrolimus cream. The *in vivo* PK data available were collected through population PK studies where the body surface area was not or poorly controlled. Using the most controlled study [291], it is suggested that the flux of this cream is of the same order of the flux for tacrolimus possibly lower.

Table 45. Immunomodulators. Flux from literature

Flux	Mean Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)	Flux ($ng/cm^2/hr$)
Cyclosporin A	< 1.25 * (A.D. vivo)					
Tacrolimus	~ 1.25 (A.D. vivo) 0.1%	2.05 [292] (A.D. vivo) 0.3%	1.55 [292] (A.D. vivo) 0.1%	0.79 [292] (A.D. vivo) 0.03%	~ 0.5-1 [293] (A.D. vivo) pulled 0.03 and 0.1%	1.2 [294] (A.D. vivo) 0.3%
Pimecrolimus	~ 0.65** (A.D. vivo) 1%	~ 0.65 [291;295] (A.D. vivo) 1%	< tacrolimus [296] (vitro)			

*: Cyclosporin is a bigger molecule than tacrolimus, as well one paper [290] suggests that about 10 times less cyclosporin is delivered compared to tacrolimus.

**: Very difficult to estimate flux as PK study poorly conducted based on body surface area... in the most controlled published study [291] 15-59% BSA, 77% blood sample below 0.5ng/ml and 443/444 samples below 1.4ng/ml. Using the distribution of plasma level seen in the Tacrolimus studies, the median blood sample could be around 0.15 ng/ml, using a median BSA of about 40% and a Cl of 32l/hr => Flux ~ 0.65 $ng/cm^2/hr$. As well, *in vitro* studies comparing non final formulations suggests that pimecrolimus permeates less than tacrolimus [296].

Effective Concentration (see Table 46*):*

Table 46. Immunomodulators. Potency /Effective concentration

Potency	Mean unb. Drug Potency (nM)	unb. Drug Potency (nM)	unb. Drug Potency (nM)	unb. Drug Potency (nM)	unb. Drug Potency (nM)
Cyclosporin A	11	11.3 oral dose = 2.5mg/kg + PK [119]	10.9 [120] IC 50 in vitro	7 [121] IC 50 in vitro	20 [122]
Tacrolimus	0.17	0.1 oral. dose = 0.1mg/kg +PK [123]	0.7 [120] IC 50 in vitro	0.07 [124] IC 50 in vitro	
Pimecrolimus	0.23	0.35 oral. dose = 0.55mg/kg +PK [295]	0.3 [121] IC 50 in vitro	0.11 [124] IC 50 in vitro	

Target site: Top Dermis

PK/PD Equation: Atopic Dermatitis [4.4.1]

Efficacy Index = Flux (ng/cm^2/hr)*1.8 / free Effective Conc.(ng/ml)

Systemic Safety Index = Clearance (ml/hr)*0.6 / [2000*(1-(Prot. binding/100)]

As the flux data used are based on the *in vivo* flux through atopic dermatitis skin, in the efficacy index equation, the correction due to skin permeability has to be removed: the corrective factor in AD is equal to 3.

=> Efficacy Index = Flux [AD skin] (ng/cm^2/hr)*0.6 / free Effective Conc.(ng/ml)

Pharmacokinetic data:

Cyclosporin A:

Oral Bioavailability (%) : *~40% (10-92%) [119]*

Clearance (l/hr) : *~10.2 (l/hr) from Vd and t1/2 assuming first order [119]*

Protein Binding (%) : *90% [119]*

Tacrolimus:

Oral Bioavailability (%) : *18% [297]*

Clearance (l/hr) :*8.4 l/hr [2 ml/min/kg] (paediatrics) [298]*

Protein Binding (%) : *99% [297]*

Pimecrolimus:

Oral Bioavailability (%) : *assumed to be about 40%*

Oral bioavailability is unknown, but some animal data suggests that the oral bioavailability of pimecrolimus is superior to the one of tacrolimus. Potency of oral Pimecrolimus (25mg/kg) > Tacrolimus (no effect at 25mg/kg); while subcutaneous Tacrolimus (0.3mg/kg) > Pimecrolimus (20mg/kg) [299;300]. If pimecrolimus oral bioavailability ~ 2 x tacrolimus oral bioavailability => pimecrolimus oral bioavailability ~ 40%.

Clearance (l/hr) : *assumed to be about 32 l/hr [7.6 ml/min/kg] [301]*

Apparent clearance [Cl/F] in 30mg and 60mg oral dosage group: 71 and 91 l/hr

Clearance will depend on oral bioavailability. (if bioavailability = 40%, then Cl = 28 and 36 l/hr).

Protein Binding (%) : *assumed to be equal to tacrolimus ~ 99%.*

The summary data on Immunomodulators is presented in Table 47.

Table 47. Immunomodulators summary

Compounds	MW	Potency (nM)	Flux (in AD) ($ng/cm^2/hr$)	Efficacy Index	Systemic Safety Index
Cyclosporin A	1203	14	< 1.25	< 0.04	96
Tacrolimus	804	0.17	1.25	5.5	252
Pimecrolimus	810	0.23	~ 0.65	~ 2.1	~ 960

Discussion:

The potency difference between the cyclosporin A and the other two drugs (50-100 fold) is the major reason why tacrolimus and pimecrolimus would be superior to cyclosporin A. One could suggest as well that tacrolimus and pimecrolimus would get some better

efficacy due to their better permeation. However the lack of relevant flux data for cyclosporin A prevents making this statement.
The model predicts for these three compounds that tacrolimus and pimecrolimus should give some efficacy and that cyclosporin A should not. Because the efficacy index is close to 1 for both tacrolimus and pimecrolimus, and because the systemic safety index is relatively high, these drugs are unlikely to be overdosed and unlikely to induce systemic exposure.

Correlation with clinical trial safety / efficacy / comparison:
Tacrolimus and pimecrolimus are registered for their topical use for the treatment of atopic dermatitis, while attempts with cyclosporin A in contact/atopic dermatitis or psoriasis have failed [51;52]. These results are well predicted by the model. It should be noted as well that topical tacrolimus does not seem to work in non-facial psoriasis [302] but would work in facial psoriasis [303]. As the efficacy index in A.D. for tacrolimus is about 5, using the psoriasis PK/PD equation (see Equation 24) would predict an efficacy index close to 0.5 in psoriasis. This value would suggest the treatment to be borderline for efficacy. For facial psoriasis however, the skin barrier on the face is down by a factor approximated to 5 during PK/PD model development (see Equation 18). Such difference in barrier property has been measured for tacrolimus [294]. It was found that there was a 5-fold difference in permeability in between patients with AD on the face vs. patients with AD on the trunk and limbs. With this corrected factor the efficacy index for tacrolimus on facial psoriasis would be 2.5, therefore suggesting efficacy.

The systemic exposure fits as well. In clinical trials with tacrolimus [304-306] as well as with pimecrolimus [307], plasma level exposure in AD patients has been showed to be consistently below 2ng/ml for 99% of collected samples, while relevant plasma level for having systemic immunosuppressing effect ranges from 10-40ng/ml [123;295].

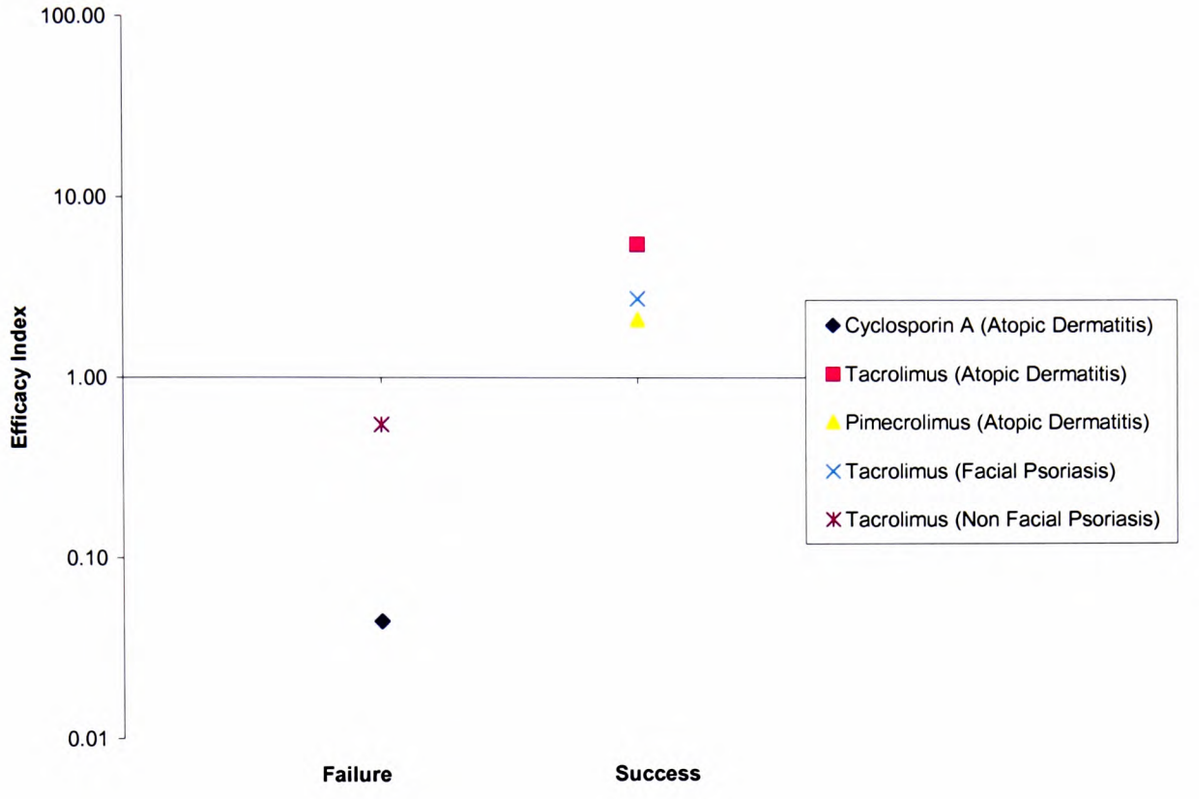

Figure 40. Immunomodulators Chart Summary

Overall the efficacy and safety prediction looks good for these three drugs.

6.10. Model Validation Summary

6.10.1. Prediction of Topical Efficacy

With all the limitations of the quality of the data used for this validation, the topical PK/PD model developed in sections Chapter 4 and Chapter 5, appears to predict relatively well the efficacy for 7 topical drug classes (NSAIDs, anaesthetics, retinoids, corticosteroids, vitamin D3 derivatives, antifungals and immunomodulators) out of the 8 large drug classes considered in this validation. For the last class (antibacterials for acne), the model does not predict the efficacy. For the latter it is suggested that the model should not be applied as the route of entry to the target site (internal side of the sebocyte) may not be the same as the classic stratum corneum path.

Figure 41 summarises the overall findings of the validation for the drugs acting below the stratum corneum (the antifungals class is not showed in this graph).

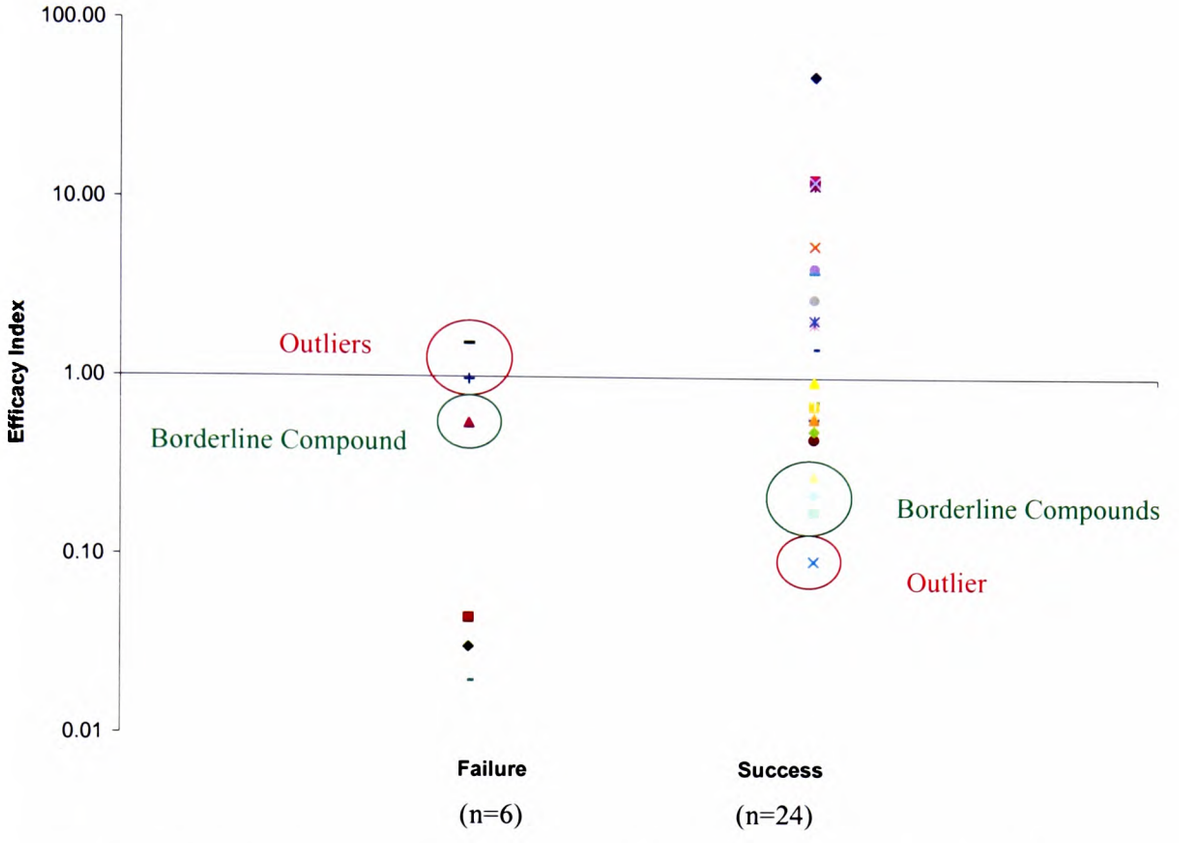

Figure 41. NSAIDs, Anaesthetics, Retinoids, Corticosteroids, Vitamin D3 Derivatives and Immunomodulators Efficacy Chart Summary

6.10.2. Prediction of Systemic Exposure

The prediction of the systemic exposure is more difficult to demonstrate as for most of the drugs considered, the relevant pharmacokinetic parameters (clearance and plasma protein binding) are not known. However when these data are available the model seems to predict the risk of systemic exposure:

Table 48. Systemic Exposure Risk Summary

	Predicted Risk (Systemic Safety Index)	Clinically Observed Risk
Corticosteroids:		
- Hydrocortisone	Low (275) (in A.D.)	Low
- Fluticasone Propionate	Low (569) (in A.D.)	Low
Vitamin D3 derivatives:		
- Calcitriol	High (0.6)	High
- Calcipotriol	Medium (41)	Medium
Retinoids:		
- Tretinoin	Low (19800)	Low
Immunomodulators:		
- Tacrolimus	Low (252)	Low
- Pimecrolimus	Low (960)	Low

6.10.3. Consequences For Future Topical Drug Development

Based on the validation exercise conducted on these topical drug classes it appears that the PK/PD model could be used to select drug candidates in order to increase the chance of success of future topical drug development programs.

Four key rules could be applied to increase chances of success:

1. Select the drug candidate with ideally the highest efficacy index of the series of drugs of the program
2. Discontinue drug candidates that have an efficacy index less than 0.1
3. Select the drug candidate with one of the highest systemic safety index of the series of drugs of the program
4. Discontinue drug candidates that have a systemic safety index less than 50

In order to be able to apply this topical PK/PD model, the pharmaceutical industry would need to change the way it selects topical drug candidates:

1. *In vitro* percutaneous flux measurement would have to be conducted (it is believed that this is not the case every time). These studies would have to be performed early enough (before candidate selection) in order to select the likely most effective drug candidate among a set of drugs. The drug with the highest potency is not necessarily the best candidate even if the potency parameter remains key. Potency has to be balanced with the percutaneous flux of the drug.
2. Determination of total systemic clearance and especially plasma protein binding will have to be done. These data would have to be known early enough (before candidate selection) in order to assess the risk of systemic exposure. As nowadays drug candidates are more and more bound to plasma proteins and as the techniques to measure plasma protein binding show some limits, it will be required to improve and select the best techniques to measure plasma protein binding.

Chapter 7.
Effective Use of the Model: Improving Percutaneous Flux Measurements

7.1. Introduction

In the previous chapters it has been demonstrated that the percutaneous flux has to be measured to be able to use the topical PK/PD model.

For the pharmaceutical industry, in order to use and trust such *in vitro* measurements two aspects of this technique have to be further considered:

1- Quality and relevance of the data generated

2- Access to the technique and throughput of the technique

In order to answer some of these aspects, three pilot studies have been conducted:

Study 1: Influence of the amount of formulation applied => to improve quality of measurements

Study 2: Use of LC-MS-MS assay as opposed to radiochemical assay => to improve access to the technique

Study 3: Use of cassette dosing => to improve throughput

7.2. Influence of the amount of formulation applied on the flux measurement

7.2.1. Introduction

Drugs delivered orally have a well-defined dose because the solid dosage form limits what a patient can do with the dose. With topicals the dose is subject to the way the patient will use their treatment. Depending on the disease and on the patient, the amount applied per cm^2 (or thickness) will vary.

Surber and Davis [308] have reviewed this field. They show that the amount applied varies from 0.7 to 4 mg/cm^2 for diseases affecting relatively large body surface area (e.g. atopic dermatitis or psoriasis). This range of dose applied seems to vary even more if one considers the surface area treated. Some data suggest that the larger the surface, the lower the dose. For sunscreens, for example where most of the body is treated, the amount applied falls to 0.5 mg/cm^2 [309;310]. With cold sores, a very small surface area is treated (~ 2 cm^2), and a 2g tube will last on average for 2 cold sore episodes (5 applications per day for 5 days). This leads to an average amount applied of 20 mg of product per cm^2. For these two extreme cases, there is a 40-fold range in the amount of formulation applied.

This variation observed with different skin diseases is not helpful, as it does not fix clearly which dose should be applied while performing an *in vitro* percutaneous flux study. This generally leads laboratories performing flux studies to define a dose that will often remain constant whatever the skin condition to treat is. The likely reason behind this constant dosing is probably related to the three following factors:

1- No need to search for the likely clinically relevant amount of formulation to apply.

2- No need to change the diffusion cells used in order to apply a smaller dose [diffusion cells have often a relatively small diffusion area (<1 cm^2) preventing the application of doses less than 5mg/cm^2].
3- No clear evidence in the literature suggesting that dose applied has an effect on the percutaneous flux.

As the topical PK/PD model requires the use of flux data, it appears critical to clarify the effect of the loading dose on flux. This should improve the quality of the flux data generated to input into the PK/PD model.

A pilot study was therefore designed to assess whether the dose applied had an effect on the percutaneous flux.

7.2.2. Aim of the Study and Summary Results

Note: Details of the study protocol and results can be found in section 10.3.

The aim of this pilot study was to investigate whether a difference in the amount of topical applied leaded to a difference on the percutaneous flux. A non-aqueous gel containing 12% propylene glycol and 3% loperamide hydrochloride was used. As the diffusion cells available in house at that time were relatively small (0.64 cm^2), it was not possible to apply less than 10mg/cm^2. The two doses applied were as followed: 10 and 40 mg/cm^2.

The results (see Figure 42) suggest proportionality in between the amount of formulation applied and the percutaneous flux.

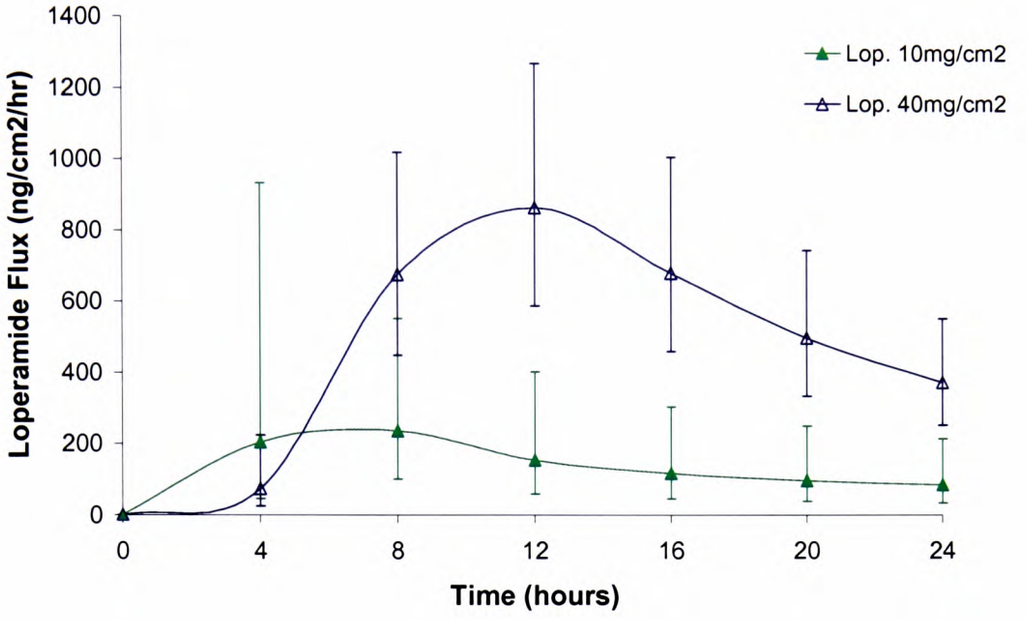

Figure 42. Effect of the Amount of Formulation Applied on the Flux of Loperamide

7.2.3. Conclusion

The observed proportionality in between the amount of formulation applied and the percutaneous flux in this pilot study suggests that in order to best use the PK/PD model, flux data needs to be generated with a clinical relevant amount of formulation applied.

Note: With the findings from this study, new larger diffusion cells (custom made) (3.14cm^2 - [2.2.3]) were manufactured in order to be able to apply smaller amount of formulation in the diffusion cells (e.g. 2mg/cm^2) [see 10.6].

7.3. From radiochemical to MS-MS analysis

7.3.1. Introduction

Skin is a very impermeable membrane. This leads to poor percutaneous fluxes as seen in Chapter 6 (and summarised in Table 49).

Table 49. Percutaneous Flux Range of 8 Topical Drug Class

Topical Drug Class	Flux ($ng/cm^2/hr$)
Anaesthetics	8000 - 50000
NSAIDs	150 - 7000
Antifungals	70 150
Antibacterials	4 - 1800
Immunomodulators	0.6 - 1.3
Retinoids	0.2
Corticosteroids	0.02 - 1.1
Vitamin D3 Derivatives	0.01 - 0.04

Flux below 100 $ng/cm^2/hr$, are difficult to measure with a classic HPLC-UV assay as they would lead to concentrations in the receptor fluid ranging from 50-500 ng/ml depending on the protocol and diffusion cells used. Table 50 details the link between the percutaneous flux and the concentration obtained in the receptor.

Table 50. Percutaneous Flux and Analytical Assay Requirements

Flux ($ng/cm^2/hr$)	Diffusion Cell Characteristics and Protocol (cm^2*hr/ml)		Receptor Sample Concentration (ng/ml)	
	Favourable *	Non Favourable **	Favourable	Non Favourable
0.01	5	0.5	0.05	0.005
0.1	5	0.5	0.5	0.05
1	5	0.5	5	0.5
10	5	0.5	50	5
100	5	0.5	500	50
1000	5	0.5	5000	500
10000	5	0.5	50000	5000

**: -1- For static diffusion cells (e.g.): diffusion area=2cm^2, time point interval=12hr, receptor volume=5ml*

-2- For flow through diffusion cells (e.g.): diffusion area=3cm^2, receptor flow rate=0.6 ml/hr

***: -1- For static diffusion cells (e.g.): diffusion area=0.64cm^2, time point interval=6hr, receptor volume=7ml*

-2- For flow through diffusion cells (e.g.): diffusion area=0.64cm^2, receptor flow rate=1.5ml/hr

As the percutaneous flux of most drug classes falls below 100ng/cm^2/hr, a classic HPLC-UV assay is not the appropriate analytical assay.
Historically therefore, the use of radiolabelled drugs has been favoured in percutaneous studies.

The use of radiolabelled materials has however several issues:

1. Cost: 1g of custom made ^{14}C radiolabelled material at 50mCi/mMol will likely cost more than $20,000.
2. Time: Synthesising radiolabelled material will add 4-8 weeks to study timelines if the radiolabelled drug is not already available.
3. Practicality: As small quantity of radiolabelled drug is available, the batch size is very small (down to 1g batch size). It is therefore difficult to match the way a larger batch would be manufactured (especially difficult to solubilise the drug and to make emulsion). Spiking should be avoided, as the ratio of radiolabelled drug in solution vs. in suspension may not match the ratio of non-labelled drug in solution vs. in suspension.
4. Safety: Further care is required when using radiolabelled material.
5. Validity of the results: Drug impurities are always present in any dosage form at small levels. This applies as well to topical dosage form with radiolabelled drug. The further issue with topicals is that drug permeation is very dependent on the size of the molecule. If a drug impurity present was very small compared to the studied molecule, the permeated amount of this impurity could be substantial. As most often, a scintillation counting assay is used as opposed to a LC-radiolabel assay, the percutaneous flux is based on the total amount of radiolabelled material crossing the skin. The percutaneous flux can therefore be substantially overestimated.

7.3.2. Aim of the Study and Summary Results

Note: Details of the study protocol and results can be found in section 10.4.

The aim of this pilot study is to investigate whether LC-MS-MS analysis can replace scintillation counting analysis and solving the five issues listed above.

A gel formulation was tested once using a scintillation counting assay and then twice with a LC-MS-MS assay.

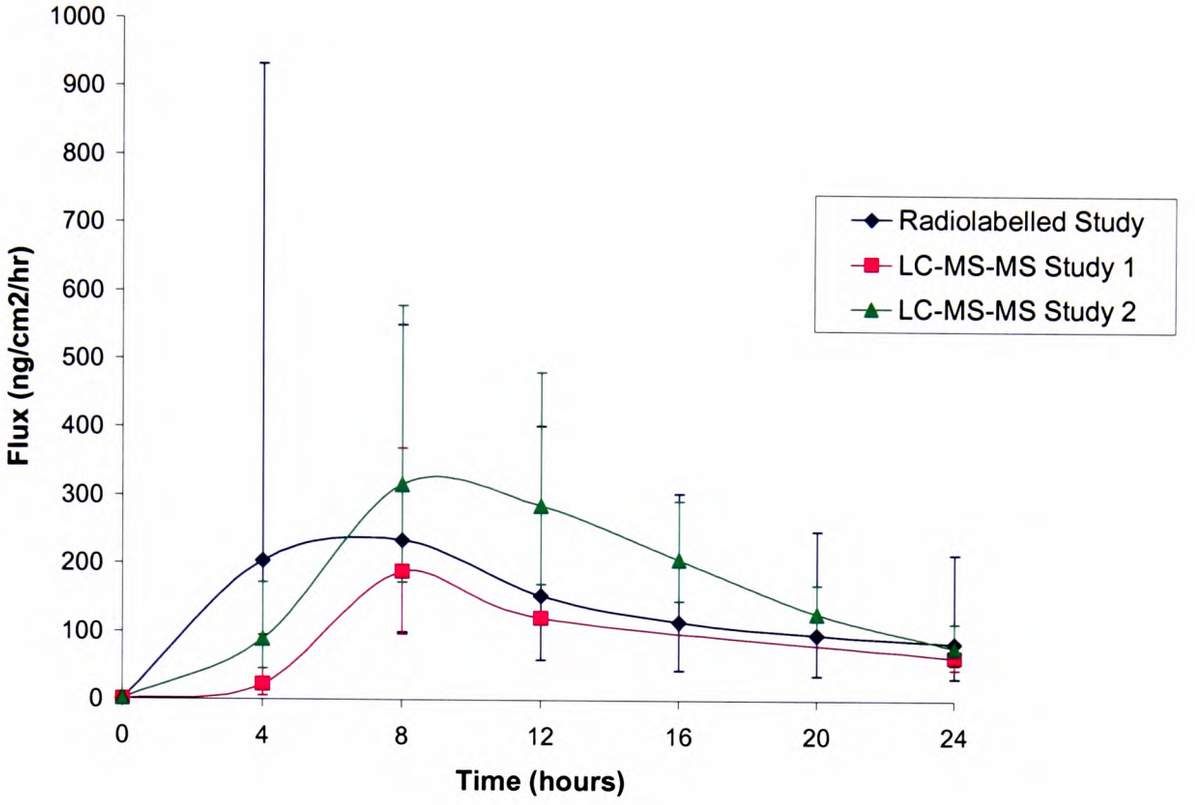

Figure 43. Radiolabelled vs. LC-MS-MS Studies

7.3.3. Conclusion

The similar fluxes obtained through the three different studies show the reliability of the LC-MS-MS technique and suggest that in this particular study, radiolabelled impurity was not an issue (possibly at the first time point). The fluxes generated in these studies are relatively large (100-300 ng/cm^2/hr). The LC-MS-MS assay appears reliable and sufficient for these studies. As can be seen in other experiments (see sections 10.5 and 10.6) much lower fluxes than the one observed in these studies can be observed (down to 0.024 ng/cm^2/hr), which proves further the large applicability of LC-MS-MS analysis for skin permeation studies.

Current sensitivity of the latest generation of MS-MS equipment (API-4000 [Sciex], Platinum [Micromass], Quantum [Finnigan]) are down to 0.1-0.5 ng/ml for most drugs.

This high sensitivity coupled with the possibility to concentrate receptor samples 10 fold (several ml collected can be concentrated into 100µl) push the realistic limit down to 0.01-0.05 ng/ml. If the right protocol and diffusion cells are used (see Table 50– "favourable characteristics") flux down to 0.01 ng/cm^2/hr can be measured. This suggests that the percutaneous flux of virtually all drugs can be studied (see Table 50).

As the sensitivity of MS-MS technology will improve, the determination of percutaneous flux will become easier without even the need to concentrate receptor samples prior to analysis which remains needed for the least permeable drugs.

7.4. N in 1 approach: Several drugs studied in a single formulation

7.4.1. Introduction

N-in-1 approach (or "cassette approach") is widely used in the pharmaceutical industry to study the pharmacokinetic characteristics of drug candidate after oral administration in the animals. It consists in administering to the animals a cocktail of drugs instead of one at a time. This allows higher throughput as more compounds can be screened.

The large use of scintillation counting analysis in the topical PK field has largely prevented the translation of this approach to skin percutaneous studies.

The use of LC-MS-MS in topical PK studies may now allow the N-in-1 approach to be used in the selection of drug candidate as part of a more rationale topical drug development process.

A pilot study was therefore designed to assess whether the N-in-1 approach could be used for percutaneous flux studies.

7.4.2. Aim of the Study and Summary Results

Note: Details of the study protocol and results can be found in section 10.5.

The aim of this experiment was to investigate the practicality of this approach. For that purpose, three model drugs were used and four formulations with the same base were

manufactured (three containing each, one of the three drugs, and one containing the three drugs together).

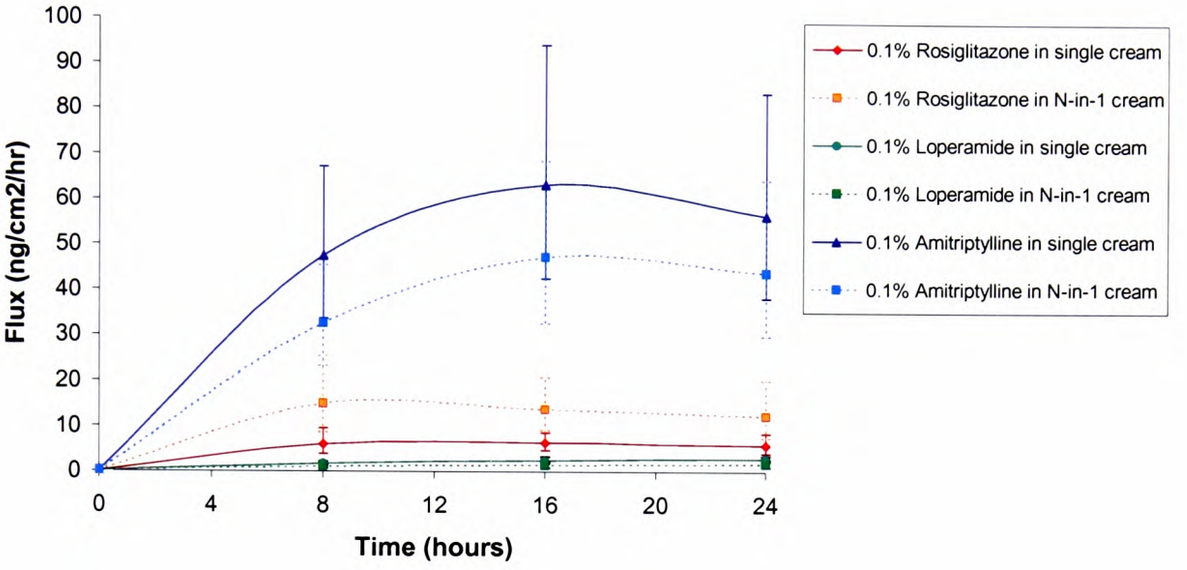

Figure 44. Summary of the N in 1 Percutaneous Absorption Study

Of the three drugs tested, loperamide and amitriptyline had relatively similar flux profile whether tested alone or in the N-in-1 formulation. Rosiglitazone however showed some substantial difference in between the single drug formulation and the N in 1 formulation.

7.4.3. Conclusion

This pilot study demonstrates the potential for this approach despite some variation.

Depending on the expectation from such an approach, the N-in-1 will or will not have some value:

Case 1: If the expectation is to find out whether the drug candidates have a percutaneous flux of 0.1 or 1 or 10 ng/cm^2/hr. The approach should offer value.

Case 2: If the expectation is to find out within 2-3-fold error the flux of drug candidates, the approach does not appear suitable.

To improve the reliability/predictability of this approach, better knowledge in two areas should be gathered:

- Drug-drug interaction effect on permeation (analogy with the P-450 inhibition issue when testing cocktails of drug candidates in animals)
- Flux extrapolation from sub-saturated systems to saturated systems (in this study the three drugs were sub-saturated)

7.5. Conclusion

The three pilot studies and their rationale suggest that access, throughput and quality of the technique can be improved.

Other studies could be conducted to further assess/use the *in vitro* flux measurement technique. Chapter 9 describes some of these studies.

The likely key aspect not covered here and poorly covered in the literature is the need to generate *in vitro* to *in vivo* percutaneous flux measurement correlation in order to improve the *in vitro* flux measurement technique. Good correlation exists with transdermal patches in human or topical formulation in rodents. In both cases the bioavailability observed is relatively large (due to either occlusion with patch, or poor skin barrier in rodents) which limits possible discrepancy between *in vitro* and *in vivo* data. With a classic pharmaceutical drug (MW>350), in a classic pharmaceutical preparation, applied at finite dose, in human, the bioavailability is expected to be close to 1%. Such low bioavailability is likely to induce higher discrepancy in between an *in vitro* and an *in vivo* measurement. Generating such *in vitro* and *in vivo* data would therefore be invaluable if one wants to improve the *in vitro* flux measurement protocols.

Chapter 8.
Conclusion

The aim of the thesis was to develop a method to define drug concentration in the skin after topical application. In other words, the objective was to give a real meaning to the pharmacokinetic data that can be generated by a topical pharmacokinetic sampling method. Indeed most topical pharmacokinetic sampling data are difficult to understand (e.g. flux data, tape-stripping measurement). This is the likely reason why topical pharmacokinetics is not considered in the selection of topical drug candidates.

For the drug concentration determination, to be meaningful, the choice of the pharmacologically relevant pharmacokinetic data – the unbound drug concentration - is critical. Indeed this allows bringing together two separate disciplines: pharmacokinetics and pharmacodynamics. PK/PD modelling can therefore begin from then.

The approach taken to predict the unbound drug concentration is an indirect approach in three steps that links first the *in vitro* percutaneous flux with the unbound drug concentration in the dermis. The *in vivo* drug concentration in all the skin tissues is then defined using different physiological parameters. Finally, taking into account some skin disease parameters, the *in vivo* unbound drug concentration in skin tissues in diseased skin is calculated.
A big advantage of this method is that the predicted concentration is the unbound drug concentration and it is directly proportional to the percutaneous flux. The only experimental data used are therefore accessible and reliable.
However, throughout this indirect approach some assumptions are made and cannot be checked directly.

The topical PharmacoKinetic/PharmacoDynamic model built from there is attractive as well, as it is simple to use. -1- To calculate the "efficacy index" (a prediction of efficacy) only percutaneous flux and drug potency data are required. –2- To calculate the "systemic safety index" (an assessment of systemic exposure) only total systemic clearance and plasma protein binding data are required.

More importantly, a predictive tool for topical drug efficacy does not exist. As the animal model (when they exist) for skin diseases are generally rodent models, they poorly predict topical efficacy in human (their skin is far more permeable than human skin). Such a tool can therefore become the only way to select or terminate a drug candidate and discharge risk before the expensive clinical trial phase starts.

The validation of this new model with eight topical drug classes (NSAIDS, anaesthetics, retinoids, corticosteroids, vitamin D3 derivatives, antifungals, antibacterials for acne and immunomodulators) brings value to the newly built model. Indeed for seven out of the eight classes, the validation of the model is good. For the last class, the antibacterials for acne, the model underpredicts efficacy but it is suggested that the route of entry of antimicrobials in acne could be via the sebaceous duct as opposed to a more classic stratum corneum pathway and therefore the model should not be used.

Finally, the three pilot studies presented in the last chapter suggest that quality and relevance of the data generated with *in vitro* percutaneous flux studies as well as the access to this technique and throughput of this technique can be improved. All these factors are important to allow the industry to adopt topical pharmacokinetics in their drug development process.

To conclude, the work described in this thesis is not complete and more research is likely to be required to convince the industry to adopt fully pharmacokinetics into their early topical drug selection process. It is hoped, however, that enough material, evidence and enthusiasm has been put together to motivate other researchers to investigate further this topical PK/PD bridgehead and bring fully pharmacokinetics to play the key role it should play in the development of better topical treatments.

Chapter 9.
Future Work

The present thesis suggests that pharmacokinetics as well as pharmacokinetics/pharmacodynamics could bring value in the development of a topical drug. However, a number of further studies could convince the industry to consider this approach further. These different possible areas of research can be divided into the different development phases of a drug.

- **Lead Optimisation (or Lead Candidate Selection)**
  - What: **To generate better *in silico* tools for the prediction of percutaneous flux** (larger drug set than the current one used in the Potts and Guy equation [151] + in a pharmaceutical vehicle as opposed to water).
  - ⇨ Aim: To limit the number of drugs to screen *in vitro* as the *in silico* tool would be more predictive.

  * How: By generating for a library of drugs, percutaneous flux studies using the same protocol and a pharmaceutical vehicle.

  - What: **To perform percutaneous flux measurement with very small amount of drug (less than 50mg and ideally less than 10mg).**
  - ⇨ Aim: To limit the chemistry effort due to resynthesis requirements when a drug appears interesting after *in silico* assessment. Nowadays when a new molecule is first synthesised less than 100mg is generally available. If a basic oral pharmacokinetic study in rat can be performed with few mg, performing an *in vitro* percutaneous study requires substantial amount of drug material preventing a screen to occur without a further resynthesis.

  * How: -1- By investigating the use of small manufacturing tools and new manufacturing processes that would allow to manufacture down to 0.5g batch size reliably. –2- By investigating whether or not it is possible to use a fix drug concentration in a vehicle and then predict achievable flux with higher drug concentration in this vehicle.

- What: **To predict residence time in the stratum corneum in order to select a drug that crosses slowly the stratum corneum.**

⇨ Aim: To select drugs that would be likely superior to others because their effect would be more prolonged. This would be the concept of half-life applied to a topical drug (as seen in section 1.4.2.1.2, this concept does not exist for topical drugs and is ignored during drug selection).

* How: By generating a library of drugs, percutaneous flux studies using the same protocol and water as the vehicle (to limit modification of the diffusion coefficient in the stratum corneum) and defining the lag time for each of them. The next objective would be to investigate whether simpler parameters (e.g. plasma protein binding, logD, Abraham's parameters, retention time through a keratinocyte packed column...) could predict this retention time.

- **Candidate Selection => First Time In Human => Proof of Concept**
  - What: **To make *in vitro* to *in vivo* percutaneous flux correlation in human.**

⇨ Aim: To be able to predict better percutaneous flux *in vivo* based on *in vitro* measurements. Good *in vitro* to *in vivo* correlation exists with transdermal patches and some reasonably good studies with topicals in animals have been published. However, *in vitro* to *in vivo* correlation studies in man with a topical at finite dose are absent in the literature.

* How: To generate *in vitro* and *in vivo* percutaneous flux studies in human for several drugs. To study the different parameters (e.g. diffusion cell design, effect of clothes, amount applied...) that can explain the discrepancy in between the *in vitro* and the *in vivo* percutaneous fluxes observed.

- What: **To investigate whether or not a universal topical formulation (mildly [ideally not[irritant and with good drug delivery property) exists?**

⇨ Aim: A large amount of time can be spent defining the best formulation for a drug. This can exhaust pharmaceutical development resources that could be

possibly better used at the selection stage of the drug candidate. If such a formulation existed the pharmaceutical development phase would be shorter and less costly in resources. As well, the risk to end up with an irritant base formulation would disappear.

* How: To investigate for a series of drugs, a set of formulation vehicles with known penetration enhancers and with limited irritation. Drug delivery ranking for the different formulations would then be studied.

- What: **To investigate whether or not the technologies and concepts used in the topical formulations of the cosmetic industry deliver reasonable fluxes?**

⇨ Aim: To improve patient compliance with topical treatment. The aesthetics and customer satisfaction of the cosmetic industry formulations outperforms the pharmaceutical industry formulations. Integrating the aesthetics of the cosmetic industry in pharmaceutical preparations could increase patient compliance and therefore treatment performance.

* How: To study the key technologies and concepts of the cosmetic industry topical formulations. To formulate drugs into key concept formulations and perform percutaneous flux studies. To learn from these studies to retain the key technologies or concepts which do not impair too much topical drug delivery.

- What: **Is residence time in skin observed *in vitro* predictable of *in vivo* residence time?**

⇨ Aim: To predict the posology of a topical drug. Most often during the design of the Proof of Concept clinical study, the posology for a topical is set to twice a day. This is historical and partially empirical. The nature of the skin disease or the body site should likely influence the posology as well as the drug retention time in the stratum corneum. For example, skin is more permeable on the face or in atopic dermatitis patients. Drug residence time in skin is therefore likely to be shorter in acne or atopic dermatitis than in psoriasis. As applying a topical is less patient-friendly than taking a tablet, careful

definition of the topical posology could be a key marketing advantage and key to a more successful treatment therapy.

* How: To investigate, for a series of drug, the relationship between time to reach maximum flux *in vitro* and *in vivo* based on plasma drug concentration profile and other systemic pharmacokinetic parameters of these drugs.

- **After drug Registration or/and Patent Expiry**
  - What: **To make the case that a percutaneous flux study is the most appropriate way to assess bioequivalence for topical formulations.**
  - Aim: To get a reliable and accessible assessment of topical bioequivalence for the registration of generic formulations. Current practices appear to be inappropriate. In Europe, the standards to get a topical generic drug on the market are low, as no pharmacokinetic or pharmacodynamic studies are required prior to registration. In the U.S., the only way to register a topical generic formulation (unless it is a corticosteroid formulation) is to perform a clinical trial. In the latter case the hurdle is very high for the generics. The most likely future approach to be approved to perform topical bioequivalence study is the tape stripping technique. As seen in section 1.6.1.1, surface contamination is possible and proving the absence of surface contamination is not possible. The relevance of this approach remains therefore questionable.

* How: To generate further percutaneous flux studies (*in vi*tro as well as *in vivo)* of formulations (with the same active) proven to be not bioequivalent clinically. By increasing the library of correlations as showed in section 10.6, the amount of evidence should convince the regulatory bodies of the relevance of the percutaneous flux approach.

Chapter 10.
Appendix: Experimental Section

10.1. Direct Tissue Concentration Measurement

Objective:

To demonstrate proportional increase in skin tissue concentration as flux is increased.

Protocol:

Skin tissue concentration protocol as described in 3.1.1.

Membrane	Dermatomed – Cadaver - Male – Back – 55 years old
Number of Replicates	n=6 for solution 1 + PBS n=5 for solution 2 + PBS n=5 for solution 1 + PBS/Albumin
Drug Studied	Loperamide Hydrochloride
Donor	solution 1:Phthalate buffer (0.1M) - 12.3 μg/ml (~15% sat.) - 600μl/cm^2 (PBS) and 420μl/cm^2 (PBS+Albumin) solution 2: Phthalate buffer (0.1M) - 68 μg/ml (~85% sat.) - 600μl/cm^2 (PBS)
Formulation loading	Infinite dose (see above for details) with occlusion
Time of Application	For the whole duration of the study
Receptor	Phosphate Buffer Saline [Sigma,UK] Phosphate Buffer Saline [Sigma,UK] + 4% Bovine Serum Albumin (fraction V) [Sigma,UK]
Diffusion Cells	Static cells (0.64cm^2)
Sampling Time	One time point at 24 hours
T°	32°C
Analysis	Scintillation Counting (see 2.3.1)

Comments	None

Note: Because of the uncertainty of having removed well the stratum corneum, the stratum corneum is as well counted with the viable epidermis

Results:

Table 51. Direct Tissue Concentration Measurements – Difference in Donor Saturation Level.

	Donor (ug/ml)	Drug Recovered (ug)	% Dose Recovered	Concentration in Tissue (uM)	**Factor**
Donor	12.3	NA	NA	NA	
	68	NA	NA	NA	**5.5**
Wash	12.3	4.4 (+0.3/-0.3)	89.6*	NA	
	68	21.5 (+1.8/-1.6)	78.7*	NA	**NA**
Stratum Corneum	12.3	0.074 (+0.03/-0.02)	1.5	131 (+48/-35)	
	68	0.46 (+0.21/-0.14)	1.7	820 (+369/-254)	**6.3**
Viable Epidermis	12.3	0.10 (+0.06/-0.04)	2.1	23.7 (+7.4/-5.6)	
	68	0.77 (+0.22/-0.17)	2.8	187 (+39/-32)	**7.9**
SC + Viable Epidermis	12.3	0.18 (+0.07/-0.05)	3.6	41 (+9.8/-7.9)	
	68	1.16 (+0.27/-0.22)	4.5	280 (+59/-49)	**6.7**
Dermis	12.3	0.0081 (+0.0056/-0.0033)	0.2	0.41 (+0.19/-0.13)	
	68	0.041 (+0.009/-0.007)	0.2	2.2 (+1.0/-0.7)	**5.4**
		Drug Recovered (ug)	% Dose Recovered	Flux (ug/cm^2/hr)	
Receptor	12.3	0.016 (+0.006/-0.005)	0.3	1.02 (+0.42/-0.30)	
	68	0.066 (+0.036/-0.023)	0.2	4.3 (+2.4/-1.5)	**4.2**

Table 52. Direct Tissue Concentration Measurements – Difference in Receptor Composition.

	Donor (ug/ml)	Receptor	Drug Recovered (ug)	% Dose Recovered	Concentration in Tissue (uM)
Donor	12.3	PBS	NA	NA	NA
	12.3	PBS + 4% BSA	NA	NA	NA
Wash	12.3	PBS	4.4 (+0.3/-0.3)	89.6	NA
	12.3	PBS + 4% BSA	21.5 (+1.8/-1.6)	78.7	NA
Stratum Corneum	12.3	PBS	0.074 (+0.03/-0.02)	1.5	131 (+48/-35)
	12.3	PBS + 4% BSA	0.080 (+0.37/-0.25)	2.3	142 (+66/-45)
Viable Epidermis	12.3	PBS	0.10 (+0.06/-0.04)	2.1	24 (+7/-6)
	12.3	PBS + 4% BSA	0.041 (+0.032/-0.018)	1.2	19 (+5/-4)
SC + Viable Epidermis.	12.3	PBS	0.18 (+0.07/-0.05)	3.6	41 (+9.8/-7.9)
	12.3	PBS + 4% BSA	0.13 (+0.01/-0.01)	3.5	60 (+55/-29)
Dermis	12.3	PBS	0.0081 (+0.0056/-0.0033)	0.2	0.41 (+0.19/-0.13)
	12.3	PBS + 4% BSA	0.0028 (+0.0021/-0.0012)	0.2	0.22 (+0.09/-0.07)
			Drug Recovered (ug)	% Dose Recovered	Flux ($ug/cm^2/hr$)
Receptor	12.3	PBS	0.016 (+0.006/-0.005)	0.3	1.02 (+0.42/-0.30)
	12.3	PBS + 4% BSA	0.0097 (+0.0021/-0.0018)	0.3	0.63 (+0.14/-0.11)

Conclusion:

- Proportionality is observed in between flux and epidermis or dermis tissue concentration.
- The addition of 4% BSA in the receptor phase does not appear to affect significantly the outcome of the study.

10.2. Diffusion Coefficient in the Dermis Measurement

Objective:

To measure the diffusion coefficient in the dermis of 3 drugs with varied physico-chemical properties (3 drugs selected: nicotine, penciclovir, and loperamide).

Drugs Studied:

Table 53. Drug Characteristics for the N-in-1 Study

Drugs	MW	ACD-Log P [ACD Corporation -Version 1.0]	Plasma Protein Binding (%)	Radiolabeling
Nicotine	162	0.72	< 20% [198;311]	N.A.
Penciclovir	253	-2.12	< 20% [312]	^{3}H
Loperamide	477	5.08	96.5% [313]	^{14}C

Dermis Density Measurement:

The principle of the dermis density measurement is described in the diagram below.

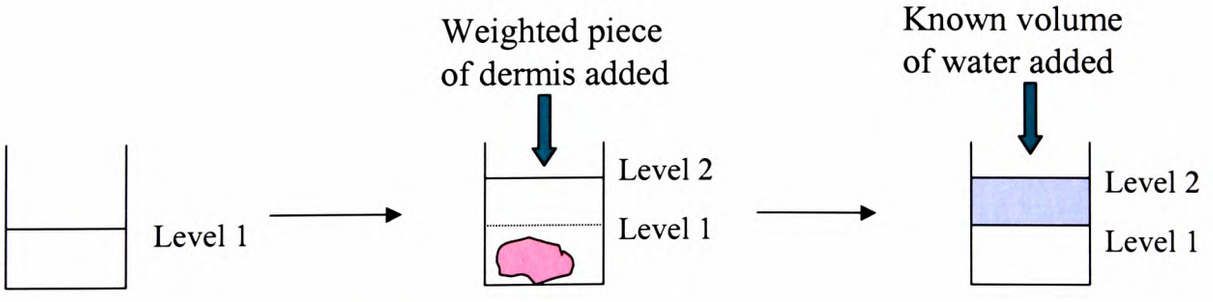

Figure 45. Diagram of the Experimental Measurement of Dermis Density

Number of replicates: 6

Dermis Swelling Measurement:

Piece of dermis added to receptor solution (PBS)

Measurement of weight change over time

Time point: 0, 24 and 48 hours

Number of replicates: 2

Note: Aim is to correct error in post weighing measurement

Percutaneous Protocol:

Membrane	Full Thickness Dermis – Cosmetic Surgery – Abdominal - 55 years old
Number of Replicates	n=6
Donor	Phosphate Buffer Saline [*] (pH=7.4) sub-saturated solution of: Nicotine: 45 mg/ml [acid added to correct for pH changed] Penciclovir : 1 mg/ml Loperamide HCl: 3.2 μg/ml
Formulation loading	Infinite dose (600µl/cm^2) with occlusion
Time of Application	For the whole duration of the study
Receptor	Phosphate Buffer Saline [*]
Diffusion Cells	Small flow through cells (0.64cm^2)
T°	32°C
Sampling time	0.5,1,2,3,4,6,8,10,12 hours [**]
Analysis	Nicotine (HPLC), Loperamide/Penciclovir (Scintillation counting) (for analytical methods details see 2.1)

Comments	[*] Need to have the donor solution equivalent to the receptor as the dermis is very permeable and the receptor could buffer the donor and change the saturation level of the drug in the donor solution. [**] For Nicotine, some time points are not available [***] All dermis samples weighed post experiment

Results:

*** Density Measurement:**

Dermis Density = 1.08 (+/- 0.06) g/ml

*** Dermis Swelling:**

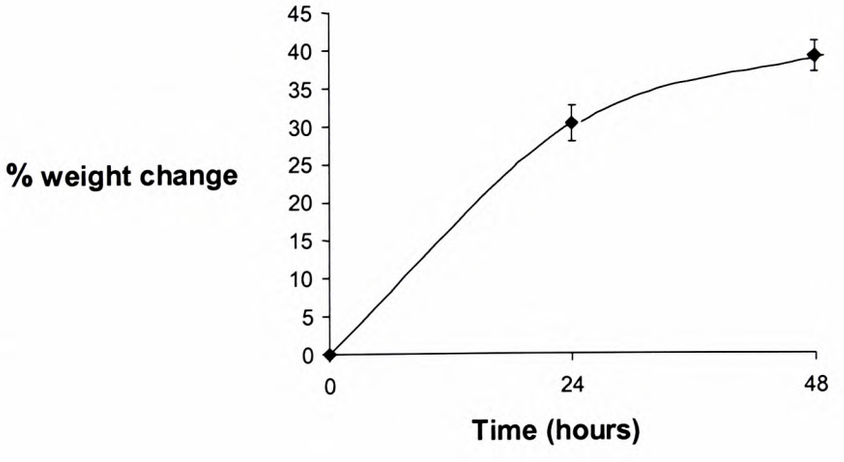

Figure 46. Dermis Swelling in PBS Buffer - % Weight Change Over Time

*** Dermis Diffusion**

Treatment of percutaneous data for each of the 6 replicate cells:

1. Lag Time to reach steady state is defined with fit equation from Microcal Origin Software (lag time corresponds to the intersection of red line with the x-axis on the permeation graphs in Figure 47,Figure 48 and Figure 49)

2. Membrane thickness calculated with Equation 48

$$Thickness = \frac{Weight_{\ of\ cut\ sample\ \ predicted\ at\ t=0}}{Dermis\ Density * Surface\ Area_{of\ cut\ sample}}$$

Equation 48

Note: Weight $_{\text{of cut sample post experiment}}$ = Weight $_{\text{of cut sample predicted at t=0}}$ * 1.30
This is due to 30% swelling after 24 hours (see the previous "dermis swelling" section).

3. D, the diffusion coefficient is defined from Equation 6

$$D = \frac{h^2}{6 * L}$$

Where;
h= membrane thickness
L= lag time to reach steady state

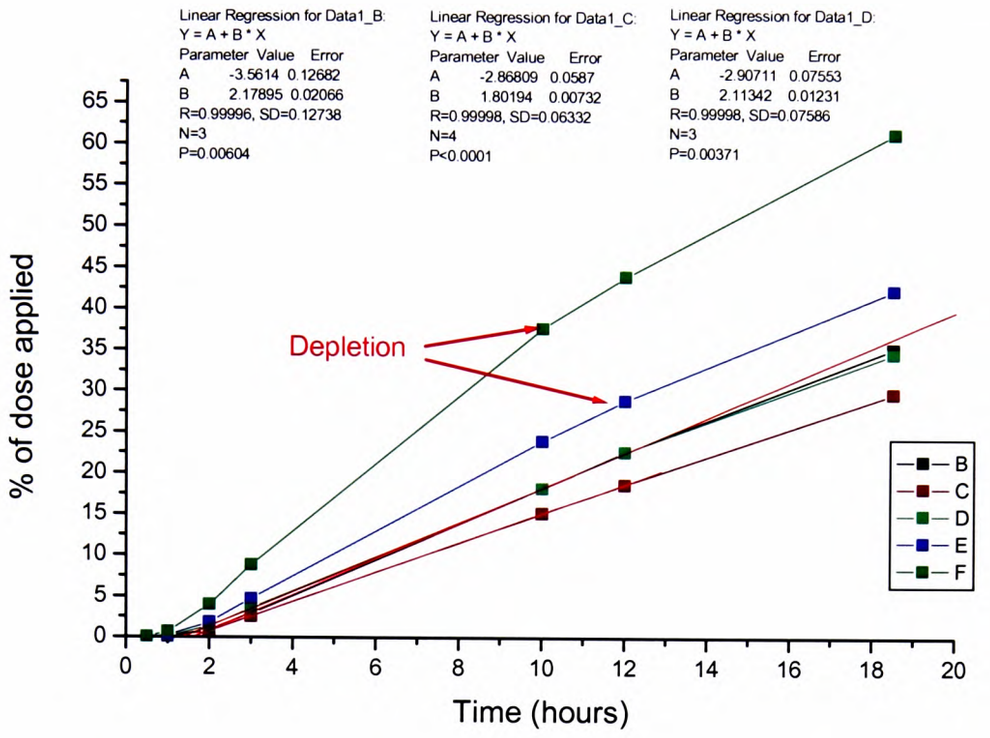

Figure 47. Nicotine Permeation Through Full Thickness Dermis

Only time points where the dose permeated was inferior to 20% of the dose applied were considered. Two diffusion cells (E and F) were excluded from the calculations as depletion occurred early (<10 hours).

Table 54. Nicotine Dermis Diffusion Coefficient Determination

Cell	**1**	**2**	**3**
Thickness determination (cm)			
dermis sample weight (mg) (at 24 hours)	196	165	204
dermis samples weight (mg) (predicted at t=0)	151	127	157
Density	1.08	1.08	1.08
surface area (cm^2)	0.594	0.594	0.594
(thickness= weight/(area*density)			
Thickness (cm)	0.24	0.20	0.24
Lag Time Determination (hr)			
A	3.56	2.86	2.91
B	2.17	1.8	2.11
Lag Time (hr)	1.64	1.59	1.38
Diffusion (cm^2/hr)	0.0056	0.0041	0.0072
Mean Dermis Diffusion Coefficient (cm^2/s)	**1.6E-06**		
Std Dev Dermis Diffusion Coefficient (cm^2/s)	**4.3E-07**		

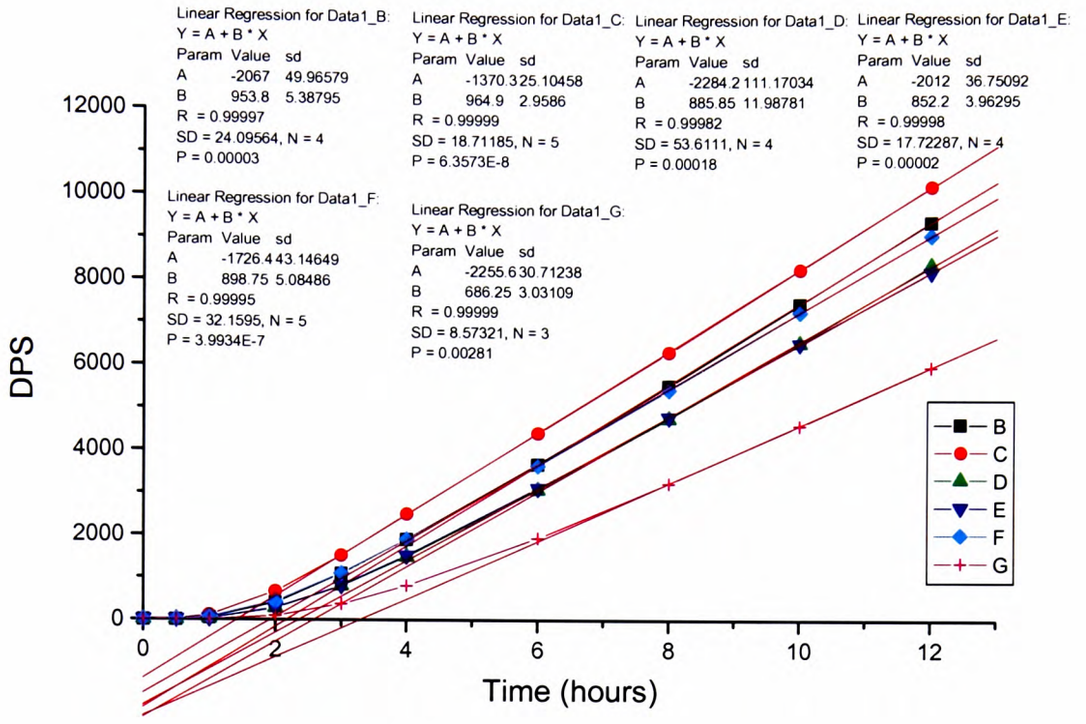

Figure 48. Penciclovir Permeation Through Full Thickness Dermis

Table 55. Penciclovir Dermis Diffusion Coefficient Determination

Cell	**1**	**2**	**3**	**4**	**5**	**6**
Thickness determination (cm)						
dermis sample weight (mg) (at 24 hours)	192	156	214	266	187	222
dermis samples weight (mg) (predicted at t=0)	148	120	165	205	144	171
Density	1.08	1.08	1.08	1.08	1.08	1.08
surface area (cm^2)	0.594	0.594	0.594	0.594	0.594	0.594
(thickness= weight/(area*density)						
Thickness (cm)	0.23	0.19	0.26	0.32	0.22	0.27
Lag Time Determination (hr)						
A	2067	1370	2284	2012	1726	2256
B	954	965	886	852	899	686
Lag Time (hr)	2.17	1.42	2.58	2.36	1.92	3.29
Diffusion (cm^2/hr)	0.0041	0.004	0.0043	0.0072	0.0044	0.0036
Mean Dermis Diffusion Coefficient (cm^2/s)	**1.3E-06**					
Std Dev Dermis Diffusion Coefficient (cm^2/s)	**3.6E-07**					

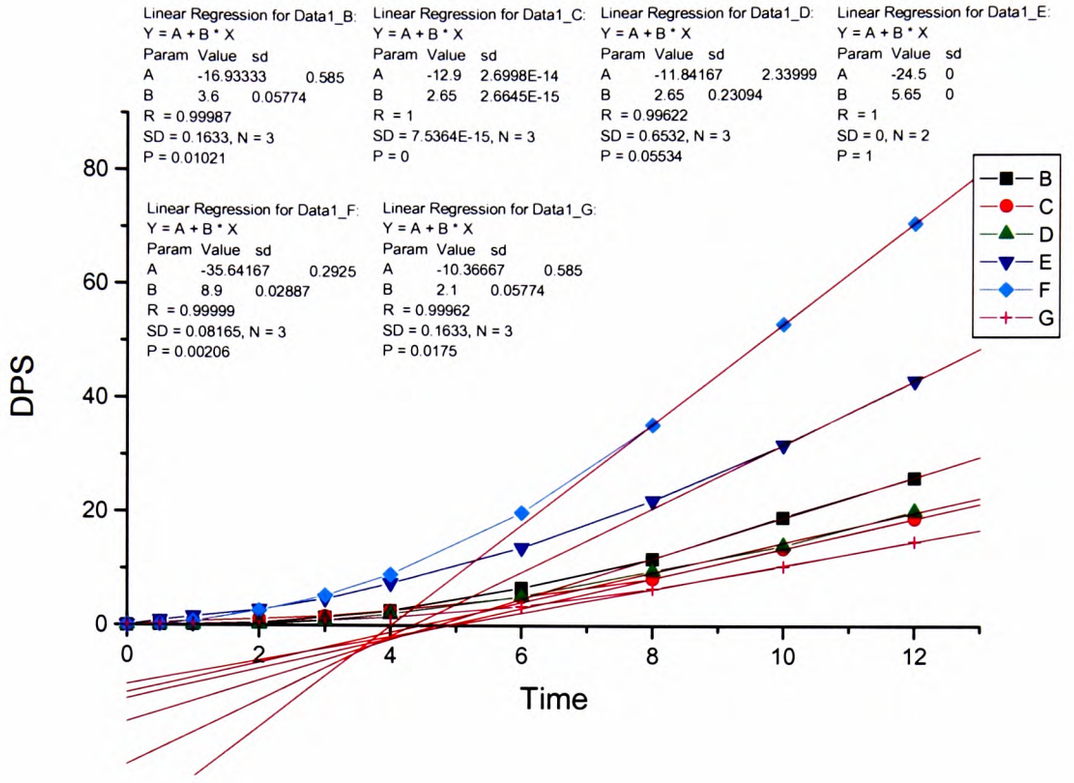

Figure 49. Loperamide Permeation Through Full Thickness Dermis

Table 56. Loperamide Dermis Diffusion Coefficient Determination

Cell	**1**	**2**	**3**	**4**	**5**	**6**
Thickness determination (cm)						
dermis sample weight (mg) (at 24 hours)	270	317	245	245	138	293
dermis samples weight (mg) (predicted at t=0)	208	244	188	188	106	225
density	1.08	1.08	1.08	1.08	1.08	1.08
surface area (cm^2)	0.594	0.594	0.594	0.594	0.594	0.594
(thickness= weight/(area*density)						
Thickness (cm)	0.32	0.38	0.29	0.29	0.17	0.35
Lag Time Determination (hr)						
A	16.9	12.9	11.84	24.5	35.6	10.36
B	3.6	2.65	2.65	5.65	8.9	2.1
Lag Time (hr)	4.69	4.87	4.47	4.34	4.00	4.93
Diffusion (cm^2/hr)	0.0037	0.004	0.0032	0.0033	0.0011	0.0042
Mean Dermis Diffusion Coefficient (cm^2/s)	**9.5E-07**					
Std Dev Dermis Diffusion Coefficient (cm^2/s)	**3.6E-07**					

Diffusion coefficient in the dermis summary

Table 57. Diffusion Coefficient of 3 Drugs in the Dermis

	Molecular Weight	Dermis Diffusion Coefficient (cm^2/s)
Nicotine	162	$1.6.\ 10^{-6}$
Penciclovir	253	$1.3.\ 10^{-6}$
Loperamide	477	$9.5.\ 10^{-7}$

Conclusion:

Despite the large difference in the physicochemical characteristics of the three drugs tested, the diffusion coefficient in the dermis was similar for the three molecules and closed to the value: 10^{-6} $cm^2.s^{-1}$. This study suggests that contrary to the large differences observed for the stratum corneum, the diffusion coefficient in the dermis of drug molecules maybe very similar.

10.3. Influence of Formulation Loading on Percutaneous Flux Measurement

Objective:

To investigate whether or not the amount of formulation applied had an effect on the amount of drug delivered.

Protocol:

Membrane	Dermatomed – Cadaver - Back
Number of Replicates	n=5 or 6
Drug/Molecule Studied	Loperamide Hydrochloride and Propylene Glycol
Donor	- 1 Non Aqueous Gel [see Table 58] – dosed at 10mg/cm^2 and 40mg/cm^2 – without occlusion - 1 Control Solution (PG/Water 50/50) with no drug added – infinite dose (600µl/cm^2) – with occlusion
Formulation loading	See details above (10 and 40mg/cm^2 as well as infinite dose)
Time of Application	For the whole duration of the study
Receptor	Phosphate Buffer Saline
Diffusion Cells	Small Flow Through Cells (0.64cm^2)
T°	32°C
Sampling Time	2 (PG only),4,8,12,16,20,24 hours
Analysis	Scintillation Counting (see 2.3.1)

Comments	• The experiment was repeated twice with the same skin. For the first experiment, the gel was made with [^{14}C] drug, while in the second experiment the gel was made with [^{14}C] propylene glycol. • For the study with [^{14}C] propylene glycol, recovery of propylene glycol in donor and in skin was carried out at the end of the study. At 24 hours, the donor compartments of the diffusion cells

	were washed with 400μl of Transcutol (Gattefossé) followed by 2 washes of 400μl of water and then wiped with tissue. The diffusion cells were then dismounted and wiped again. The 3 washes and the 2 wiping tissue samples were pulled together and 8ml of scintillant was added and the sample counted. The skin was then placed in a scintillation vial filled with 8ml of water to extract the propylene glycol present in the skin. After overnight extraction, 8ml of scintillant were added and the sample counted.

Table 58. Loperamide HCl Gel Composition

Formulation Components	% (w/w)
Loperamide HCl	3
Propylene Glycol	12
Co-solvent 1*	10
Co-solvent 2*	19
Co-solvent 3*	48
Silica (Thickener)	8
Total	100

* Co-solvent mixture is proprietary to GlaxoSmithKline.

Flux data calculations:

Because of the log normal distribution of skin permeability as described by Williams et al [314], flux data were analysed by the method proposed by the same authors. Error bars plotted represent standard deviation.

Results:

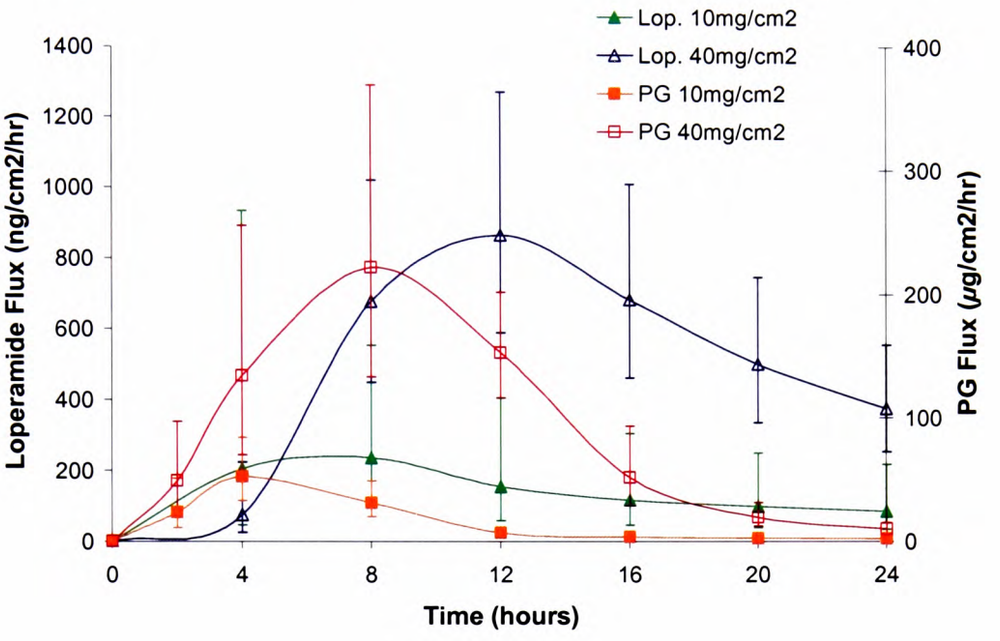

Figure 50. Effect of Dose applied on Propylene Glycol and Drug Permeation

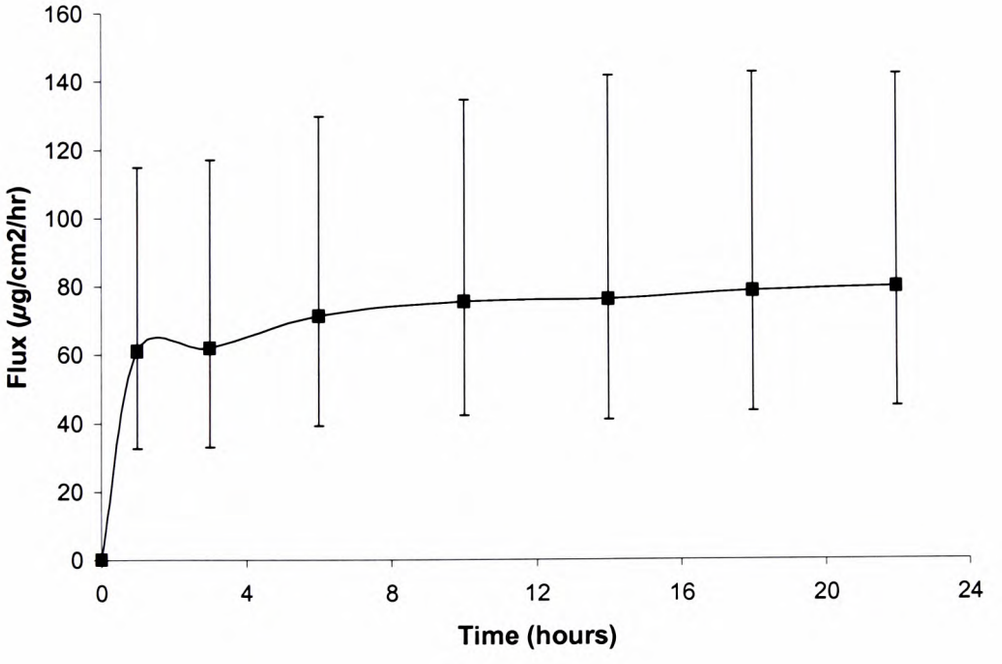

Figure 51. Permeation of Propylene Glycol from a Control Solution (PG/Water 50/50) at Infinite Dose under Occlusion

Table 59. Propylene Glycol Mass Balance Table

	PG/Water 50/50 Infinite Dose	12% PG Gel 10 mg/cm^2	12% PG Gel 40 mg/cm^2
% dose in receptor (std error)	0.65 (0.35)	29.9 (8.5)	45.4 (5.4)
% dose in donor (std error)	95.9 (8.1)	27.4 (25.6)	30.6 (26.1)
% dose in skin (std error)	0.16 (0.03)	4.2 (1.1)	2.9 (0.4)
% dose in receptor+donor+skin (std error)	96.7 (8.5)	61.4 (35.1)	78.9 (31.9)
% missing	3.3	38.6	21.1

For both permeants, loperamide and propylene glycol, the fluxes are roughly proportional to the dose applied (about 4-fold difference) [Figure 50]. For both permeants, the permeation does not reach a steady state with a peak flux occurring earlier for the propylene glycol than for the loperamide. The shape of the flux curves seems to indicate that there is depletion of propylene glycol and possibly of the drug with time. The mass balance of propylene glycol [Table 59] confirms the depletion of propylene glycol while less than 5% of the drug applied has permeated after 24 hours. The permeation of propylene glycol is about 250-fold higher than the permeation of loperamide. This difference can be explained by the large difference in size of these two molecules (MW=88 and 477 respectively, for propylene glycol and loperamide).

In the controlled environment (infinite + occluded condition) of the 50/50 propylene glycol/water solution (= at half its maximum saturation level), the flux of propylene glycol reaches steady state [Figure 51]. The mass balance of propylene glycol permeation is presented in Table 59. In the finite condition, the recovery of propylene glycol is not total and high variation in the donor compartment recovery is observed. On the other hand, in the infinite + occluded condition, the recovery is virtually 100% and only small variations are observed.

Conclusion:

The formulation loading effect on flux is demonstrated for both loperamide and propylene glycol.

Furthermore, the penetration enhancement effect of propylene glycol on the drug is demonstrated and profiles of both permeants suggest strongly that it is the permeation of propylene glycol that drives the permeation of the drug. There are several indications for that: earlier peak flux of propylene glycol compared with the drug, depletion of propylene glycol in donor but not of the drug, penetration enhancement of the drug linked with propylene glycol dose loading.

As well, the results of the mass balance of propylene glycol fits with the findings of Tsai et al [315] who studied the evaporation of propylene glycol from propylene glycol/ethanol/water mixtures. They showed that with a semi-finite loading dose, propylene glycol did evaporate. As well, they observed that the smaller the loading dose, the higher was the degree of evaporation. The results in this study suggest the same outcomes as not all the propylene glycol is recovered from donor+skin+receptor. Additionally, with the high loading dose of $40mg/cm^2$ only 21% of propylene glycol evaporated while for the lower dose of $10mg/cm^2$, more propylene glycol has: 39%.

Finally, the high variability in the recovery of the donor is likely to be due to a practical issue as some gel will not be applied to the surface of the skin but on the internal side of the donor chamber (difficulty in applying small doses on small surface area). This issue is likely to be minimised in permeation studies using cells of larger surface area.

The effect of propylene glycol loading dose in these *in vitro* studies confirms the overestimation error suggested by Smith and Maibach [316] if the *in vitro* study is not conducted with a clinically relevant dose. This emphasises the significance of formulation excipients in the optimisation of topical formulations. Perhaps more importantly, it is of significance in the experimental design of *in vitro* permeation studies.

Those studies in which the dose applied is significantly superior to the clinical one can give erroneous and therefore misleading results.

10.4. From Radiochemical Detection to MS-MS Detection

Objective:

To investigate whether LC-MS-MS analysis can replace scintillation counting analysis. If successful, the aim is to switch from radiolabel studies to non-radiolabel studies, as this would be quicker, cheaper, safer and more reliable.

A gel formulation is tested once using a radio-scintillation counting assay and twice with a LC-MS-MS assay.

Protocol:

Membrane	Dermatomed – Cadaver – Back
Number of Replicates	n=6
Drug Studied	Loperamide Hydrochloride
Donor	Gel Formulation [see Table 58] - Study 1: [14C] drug batch - Study 2: Cold drug batch 1 - Study 3: Cold drug batch 2
Formulation loading	10 mg/cm^2 without occlusion
Time of Application	For the whole duration of the study
Receptor	Phosphate Buffer Saline
Diffusion Cells	Small Flow Through Cells (0.64cm^2)
T°	32°C
Sampling Time	4,8,12,16,20,24 hours
Analysis	- Study 1: Scintillation counting (see 2.3.1) - Study 2 and 3: LC-MS-MS (see 2.1.3)
Comments	None

Flux data calculations:

Because of the log normal distribution of skin permeability as described by Williams et al [314], flux data were analysed by the method proposed by the same authors. Error bars plotted represent standard deviation.

Results:

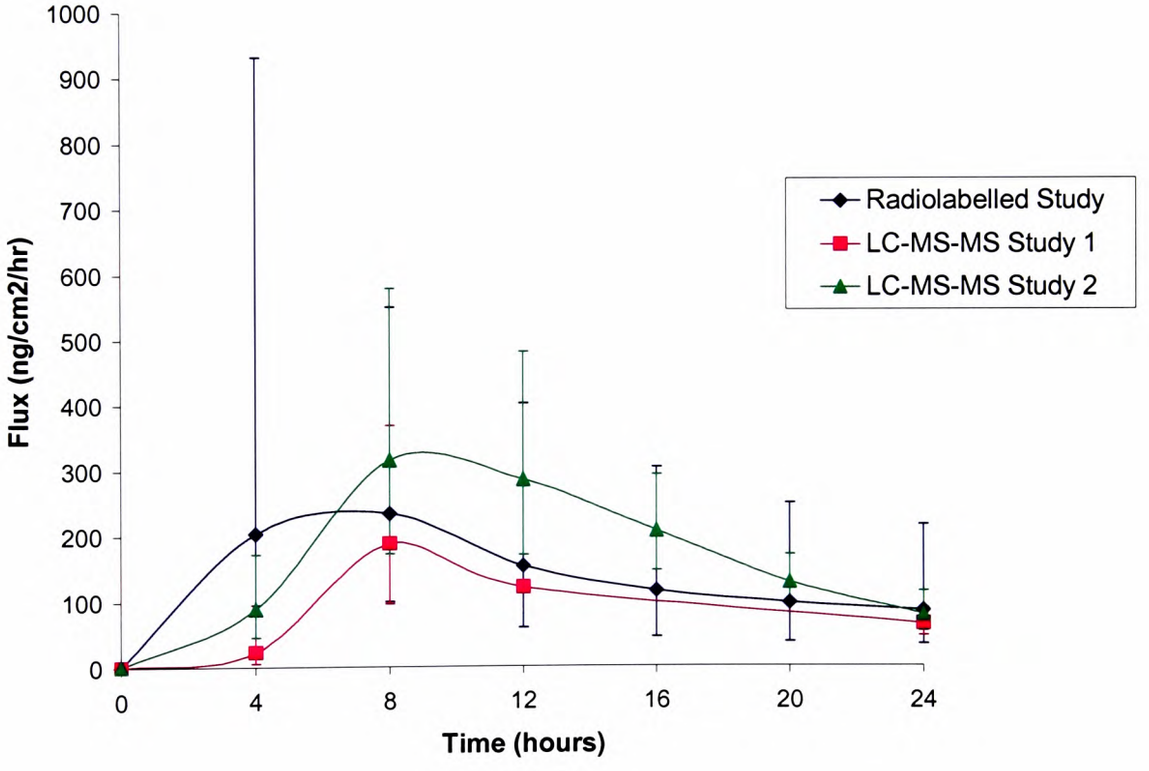

Figure 52. Percutaneous Flux of Loperamide HCl in a Gel using a LC-MS-MS and a RadioCounting Analytical Assays

The two LC-MS-MS studies gave similar results as the radiolabelled one.
The large variation at 4 hours for the radiolabelled study is not explained.

Conclusion:

The similar fluxes obtained through the three different studies show the reliability of the LC-MS-MS technique. The fluxes generated in these studies are relatively large (100-300 $ng/cm^2/hr$); the LC-MS-MS assay is therefore sufficient for these studies. As can be seen in other experiments (10.5 and 10.6) much lower fluxes than the one observed in these studies can be observed (down to 0.04 $ng/cm^2/hr$), which proves further the large applicability of LC-MS-MS analysis for skin permeation studies.

Further discussions and conclusions are given in section 7.3.

10.5. N-in-1 Formulation

Objective:

The aim of this experiment is to conduct a pilot study to investigate, whether or not, the "cassette" (or N-in-1) approach used to study the pharmacokinetics of systemic drugs can be used for skin pharmacokinetic studies.

Protocol:

- Three model drugs were investigated in that study.

Table 60. N in 1 Drug Characteristics

	MW	ACD-Log P [ACD Corporation - Version 1.0]	Plasma Protein Binding (%)
Loperamide	477	5.08	96.5% [313]
Amitriptyline	277	6.14	94.8% [198]
Rosiglitazone	357	2.56	99.8% [317]

- Formulation details (all sub-saturated).

Table 61. N in 1 Formulation Details

	Base	Loperamide	Amitriptyline	Rosiglitazone
Formulation 1	Aqueous cream BP – pH=6.5	0.1%		
Formulation 2	Aqueous cream BP – pH=6.5		0.1%	
Formulation 3	Aqueous cream BP – pH=6.5			0.1%
N in 1 Formulation	Aqueous cream BP – pH=6.5	0.1%	0.1%	0.1%

Membrane	Dermatomed – Cadaver – Male – Back – 97 years old
Number of Replicates	n=6
Donor	See Formulation Details in Table 61
Formulation loading	10 mg/ cm^2 without occlusion
Time of Application	For the whole duration of the study
Receptor	Phosphate Buffer Saline
Diffusion Cells	Small diffusion cells (0.64cm^2)
Equilibration Time	1 hour
T°	32°C
Sampling Time	8,16,24 hours
Analysis	LC-MS-MS (for analytical methods details see 2.1)
Skin Integrity Check	N.A.

Flux data calculations:

Because of the log normal distribution of skin permeability as described by Williams et al [314], flux data were analysed by the method proposed by the same authors. Error bars plotted represent standard deviation.

Results:

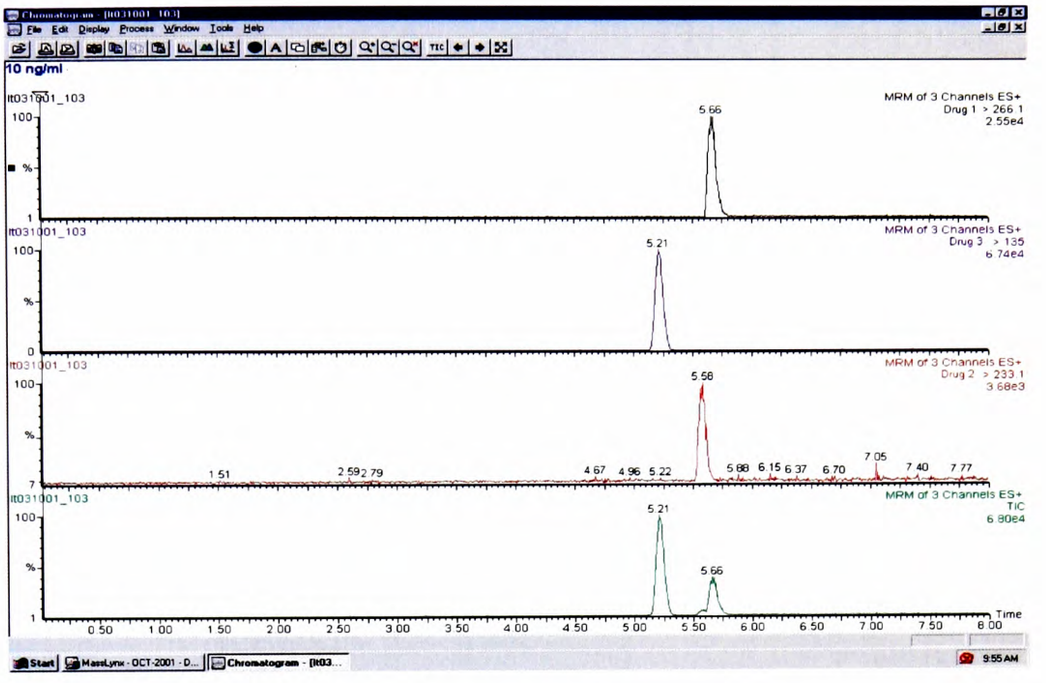

Figure 53. LC-MS-MS Chromatogram of the Three Drugs Analysed in One Assay

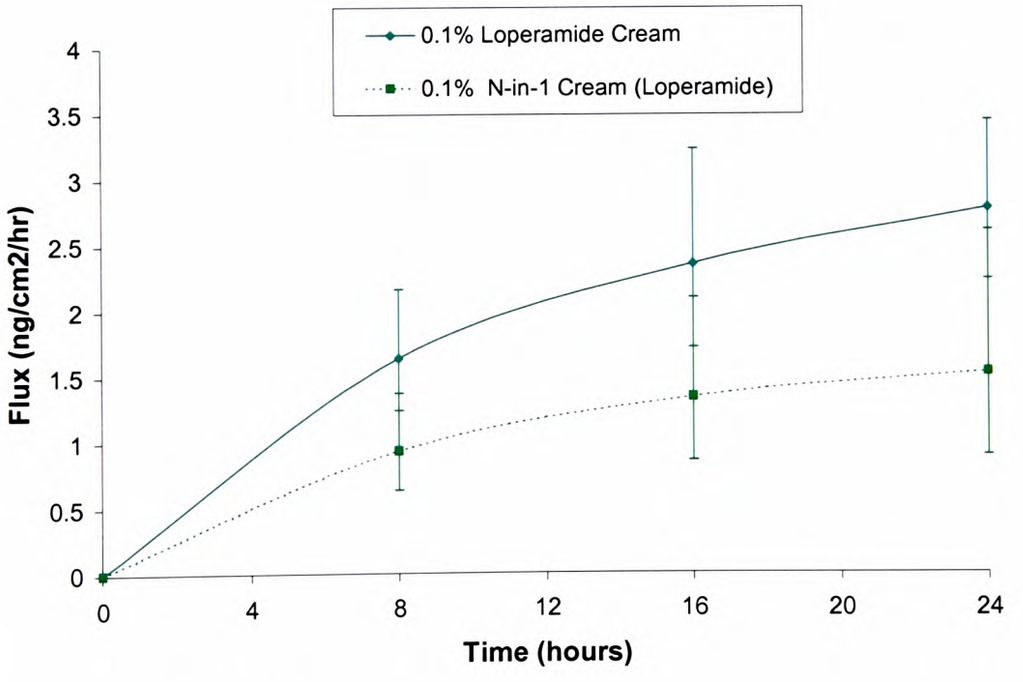

Figure 54. Percutaneous Absorption of Loperamide from the single drug and N in 1 Formulation

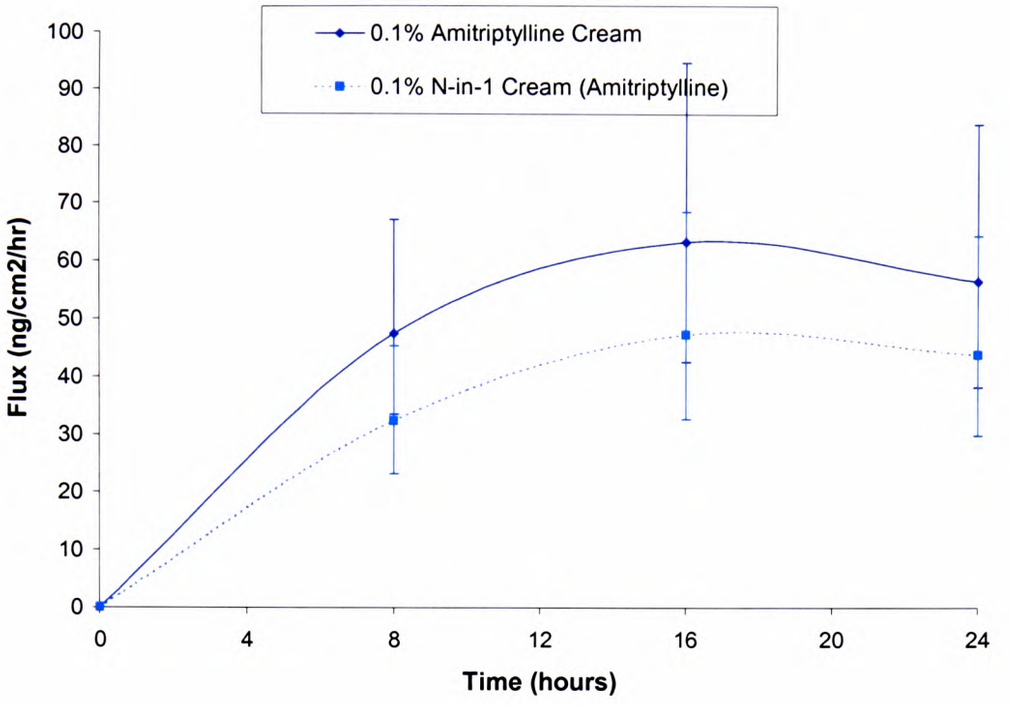

Figure 55. Percutaneous Absorption of Amitriptyline from the single drug and N in 1 Formulation

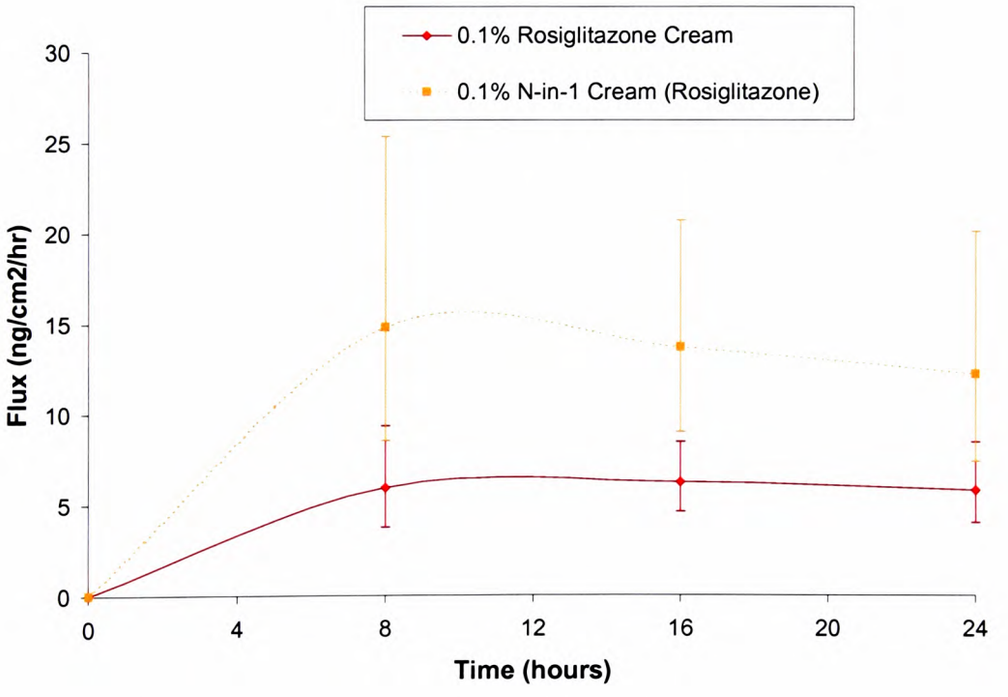

Figure 56. Percutaneous Absorption of Rosiglitazone from the single drug and N in 1 Formulation

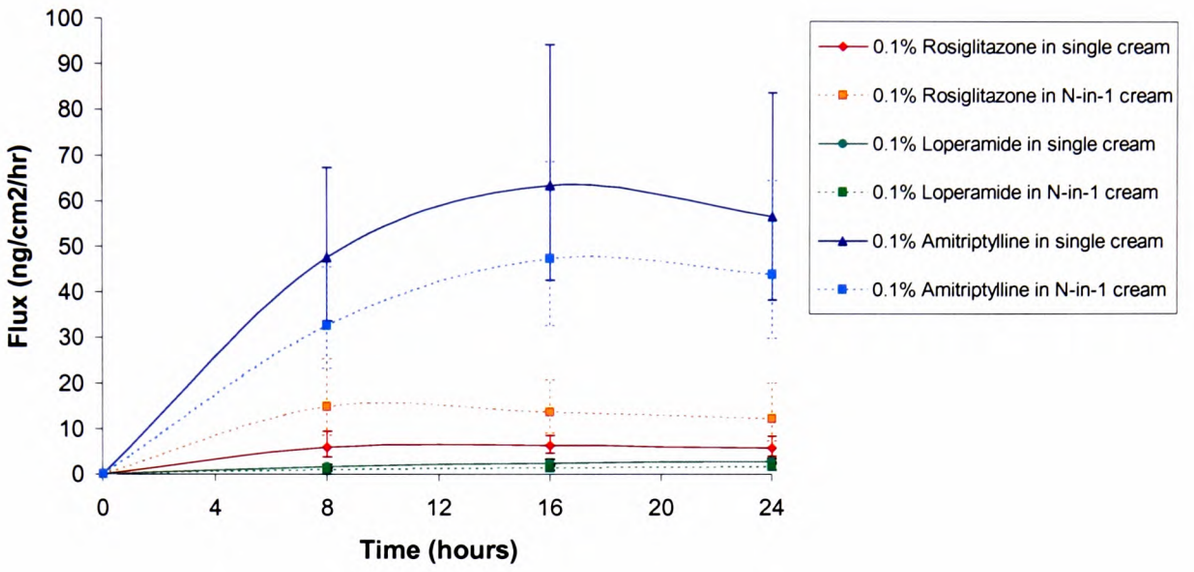

Figure 57. Summary of the N in 1 Percutaneous Absorption Study

Conclusion:

Of the 3 drugs tested, loperamide and amitriptyline had relatively similar flux profile whether tested alone or in the N-in-1 formulation. Rosiglitazone however showed some substantial difference in between the single drug formulation and the N in 1 formulation.

Further discussions and conclusions are given in section 7.4.

10.6. Percutaneous Flux of 10 Corticosteroid Formulations

Objective:

The aim of this series of experiments is to perform percutaneous flux studies of 10 formulations (6 corticosteroid drugs in total). Existing data in the literature are difficult to interpret for an optimum input into the PK/PD model. Clinical comparison of the 10 formulations exists (via the blanching assay), efficacy prediction versus clinical observation should therefore be possible if flux data were available.

Protocol:

Membrane	Dermatomed – Cadaver – Male – Back - 56 years old (study 1) - 61 years old (study 2 and 3) (same skin)
Number of Replicates	n=5
Donors (see details in 2.1.2)	0.05% Cutivate Cream (Fluticasone Propionate) (1) 0.005% Cutivate Ointment (Fluticasone Propionate) (1) 0.05% Diprolene AF Cream (Betamethasone DiPropionate) (1) 0.05% Diprosone AF Cream (Betamethasone DiPropionate) (1) 1% Hydrocortisone Cream (Hydrocortisone) (2) 0.05% Eumovate Cream (Clobetasone Butyrate) (2) 0.025% Synalar Cream (Fluocinolone Acetonide) (2) 0.05% Temovate Ointment (Clobetasol Propionate) (3) 0.05% Temovate Cream (Clobetasol Propionate) (3) 0.05% Temovate Cream E (Clobetasol Propionate) (3)
Formulation loading	2 mg/ cm^2 without occlusion
Time of Application	For the whole duration of the study

Receptor	Phosphate Buffer Saline in Study 1 and Study 2 Phosphate Buffer Saline with 0.05% sodium azide in Study 3 (used as an esterase inhibitor)
Diffusion Cells	Large Flow Through
T°	32°C
Sampling Time	Fluticasone Propionate: 4,8,12,16,20,24 hours Betamethasone DiPropionate: 8,16,24 hours Hydrocortisone, Clobetasone Butyrate, Fluocinolone Acetonide

	and Clobetasol Propionate: 4,10,16,22 hours
Analysis	LC-MS-MS (see 2.1.2)

Comments	For betamethasone dipropionate, both betamethasone dipropionate and betamethasone concentrations are monitored and pulled together.

Flux data calculations:

Because of the log normal distribution of skin permeability as described by Williams et al [314], flux data were analysed by the method proposed by the same authors. Error bars plotted represent standard deviation.

Results:

•**Study 1**

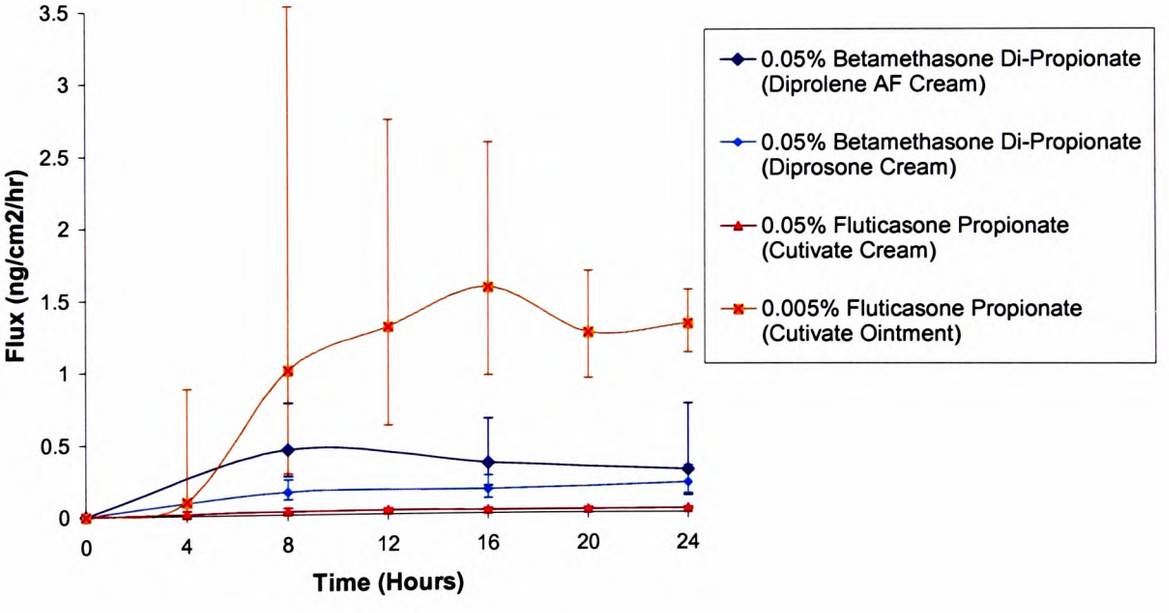

Figure 58. Percutaneous Flux of Fluticasone Propionate (Cutivate Cream and Ointment) and Betamethasone DiPropionate Creams (Diprolene AF and Diprosone)

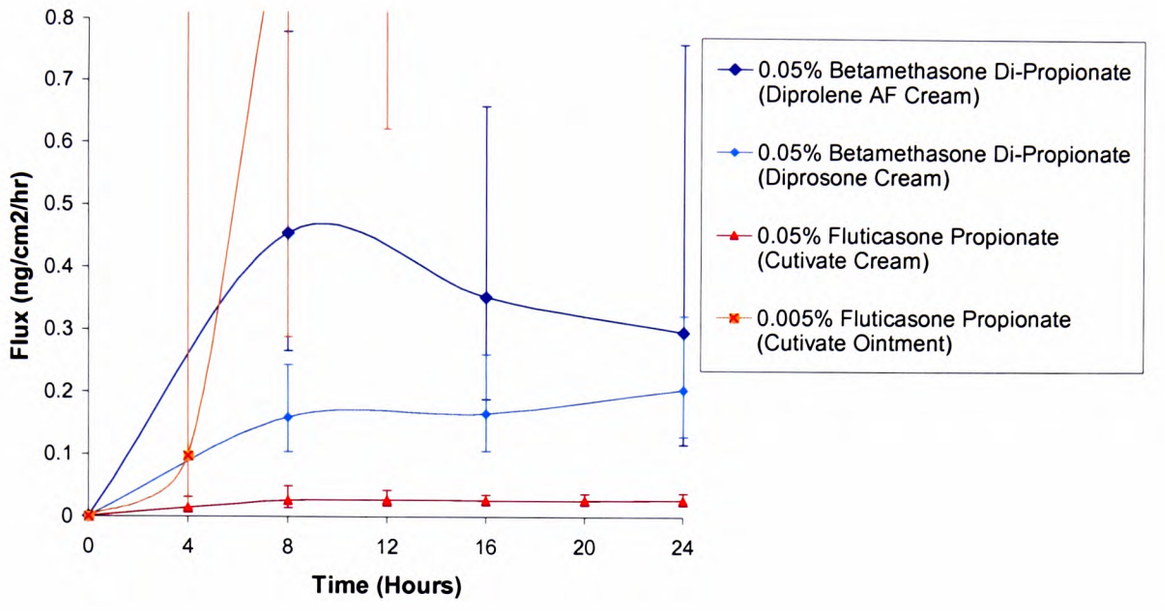

Figure 59. Percutaneous Flux of Fluticasone Propionate (Cutivate Cream and Ointment) and Betamethasone DiPropionate Creams (Diprolene AF and Diprosone) [small scale]

Table 62. Corticosteroid Percutaneous Flux Study 1 Summary

Formulations	Average Flux over 24 hours ($ng/cm^2/hr$)	% Dose Applied Permeated over 24 hours
0.05% Fluticasone Propionate (Cutivate Cream)	0.024 (+0.015/-0.009)	0.058%
0.005% Fluticasone Propionate (Cutivate Ointment)	1.1 (+1.07/-0.44)	27%
0.05% Betamethasone Di-Propionate (Diprolene AF Cream)	0.37 (+0.36/-0.18)	1.1%
0.05% Betamethasone Di-Propionate (Diprosone Cream)	0.18 (+0.10/-0.06)	0.53%

• **Study 2**

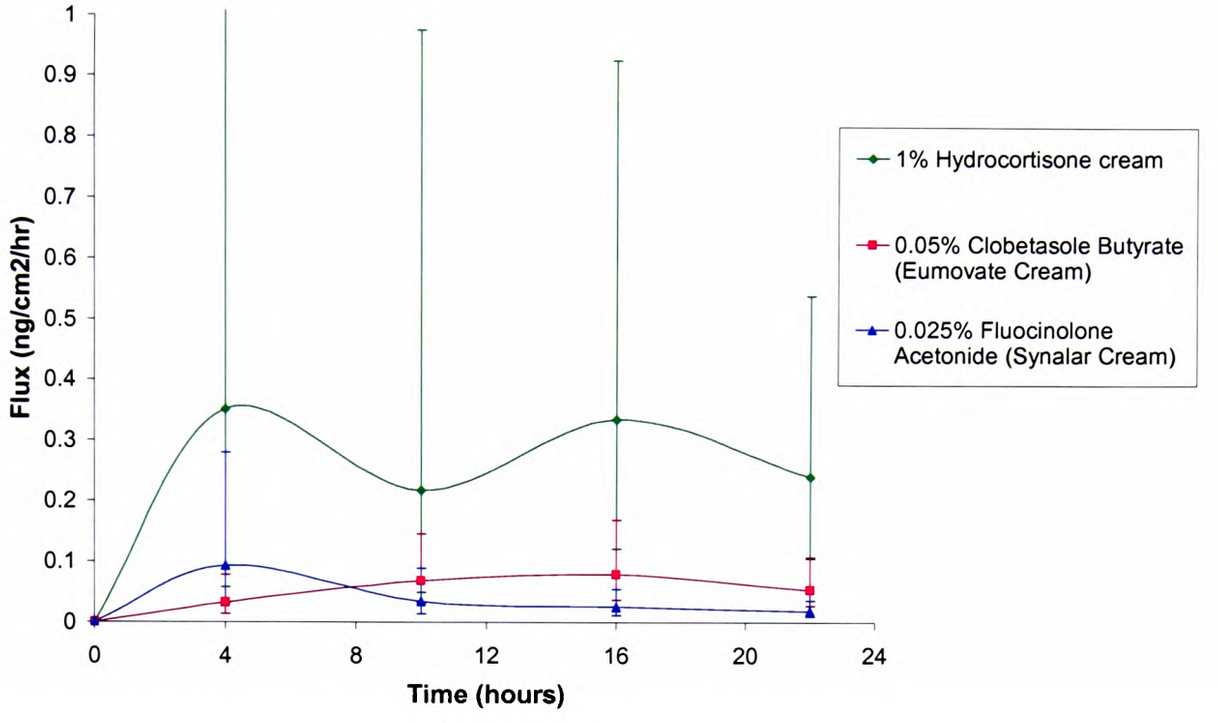

Figure 60. Percutaneous Flux of Hydrocortisone Cream, Clobetasone Butyrate Cream (Eumovate) and Fluocinolone Acetonide Cream (Synalar)

Note: For 1 diffusion cell (clobetasone butyrate), the concentration in the receptor samples assay were below the limit of quantification.

Table 63. Corticosteroid Percutaneous Flux Study 2 Summary

Formulations	Average Flux over 22 hours ($ng/cm^2/hr$)	% Dose Applied Permeated over 22 hours
1% Hydrocortisone Cream	0.29 (+0.86/-0.20)	0.031%
0.05% Clobetasone Butyrate (Eumovate Cream)	0.059 (+0.067/-0.031)	0.13%
0.025% Fluocinolone Acetonide (Synalar Cream)	0.043 (+0.072/-0.026)	0.17%

• **Study 3**

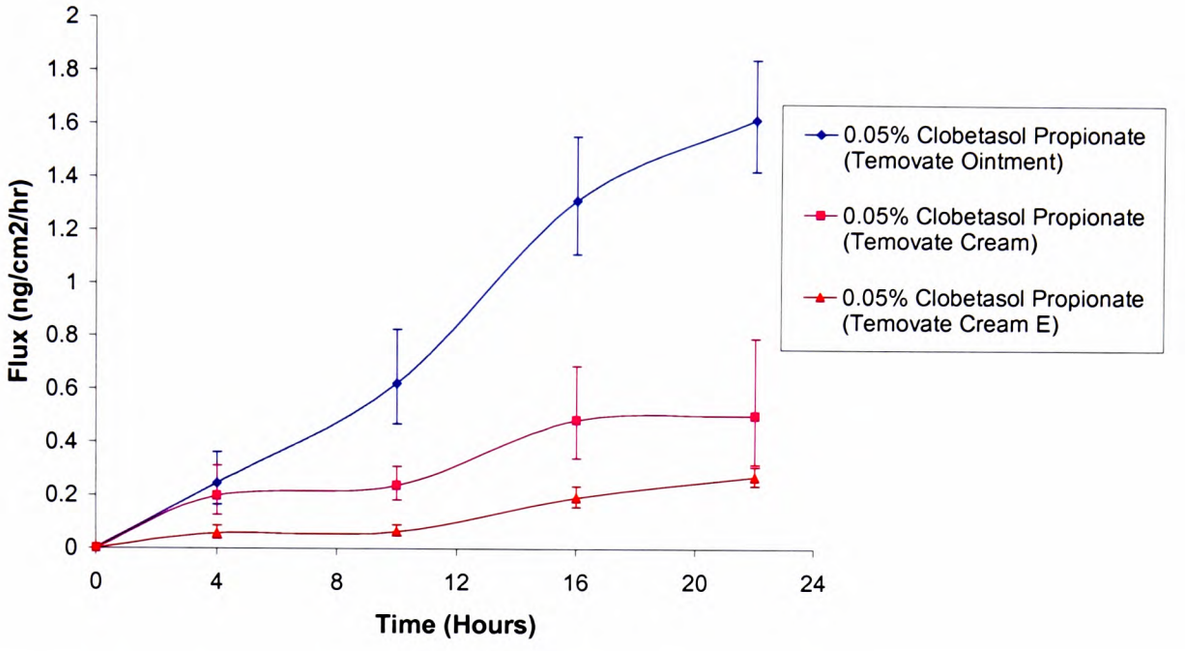

Figure 61. Percutaneous Flux of Clobetasol Propionate (Temovate Ointment, Temovate Cream and Temovate Cream E)

Table 64. Corticosteroid Percutaneous Flux Study 3 Summary

Formulation	Average Flux over 22 hours ($ng/cm^2/hr$)	% Dose Applied Permeated over 22 hours
0.05% Clobetasol Propionate (Temovate Ointment)	0.95 (+0.20/-0.16)	2.2%
0.05% Clobetasol Propionate (Temovate Cream)	0.36 (+0.17/-0.11)	0.8%
0.05% Clobetasol Propionate (Temovate Cream E)	0.15 (+0.03/-0.03)	0.3%

Note. A relatively large variability for the 3 creams studied in study 2 was observed but is not explained.

Conclusion:

- The percutaneous flux of all corticosteroid formulations were monitored but were very low (0.04 – 1.1 ng/cm^2/hr)

- Different drugs lead to different fluxes

- Different formulations lead to different fluxes (betamethasone dipropionate, clobetasol propionate and fluticasone propionate)

- Bioavailability can vary greatly from formulation and drug to another (0.03% for the hydrocortisone cream to 27% for the fluticasone propionate ointment)

- PharmacoKinetic/PharmacoDynamic : Blanching Assay Vs. Percutaneous Flux

    - Betamethasone Di-Propionate: Diprolene AF belongs to Group II topical corticosteroids [in US Classification] while Diprosone Cream belongs to Group III (= less potent) [234;318]
        - ⇨ PharmacoDynamic ranking = Percutaneous Flux ranking

    - Fluticasone Propionate: Cutivate Ointment belongs to group III topical corticosteroids [in US Classification] while Cutivate Cream belongs to Group V [234]
        - ⇨ PharmacoDynamic ranking = Percutaneous Flux ranking (however the very large difference in flux (50 fold) would suggest a bigger efficacy difference that is not seen in the clinic)

- Clobetasol Propionate: That drug falls into the Group I of topical corticosteroids (=most potent group) so group differentiation cannot be made from the group number, however the Temovate formulations have been reported to rank in the blanching assay as followed:
  Temovate Ointment > Temovate Cream > Temovate Cream E [319;320]
  - ⇨ PharmacoDynamic ranking = Percutaneous Flux ranking.

- Clobetasol propionate appears to be delivered at a relatively high flux but more slowly than the drugs studied in Study 1 and Study 2 as the highest flux for the three formulations is reached after 22 hours at least. This finding is possibly artefactual but could possibly explain in part the good performance of clobetasol propionate in the clinic. This suggests that the time to maximum flux, that is not taken into account in the PK/PD model, would deserve to be considered further to fully evaluate a topical drug.

- The use these percutaneous flux data into the topical PK/PD model is described in section 6.5.

Chapter 11.

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