Poly(dimethylsiloxane): A sustainable human skin alternative for transdermal drug delivery prediction.

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Abstract.

Despite the advantages of transdermal drug delivery (TDD), which makes it a fast-growing area of research in pharmaceuticals, numerous challenges affect their development, which limits exploring the full potential of this alternate drug delivery route. In trying to address one of these problems, it is strongly believed that the need for a sustainable skin alternative is paramount. Efforts made in an attempt to provide a sustainable alternative to employing skin in pharmaceutical analysis, by better utilising a polymer membrane, namely poly(dimethylsiloxane), also known as PDMS are discussed. Several combined properties of this polymer, which includes its relative stability in comparison with human skin, make it a good candidate for the replacement of skin. Modifications undertaken to this polymer membrane (to create an enhanced skin mimic for permeation analysis) are discussed and reviewed in this paper, including the improved ability to predict permeability for both hydrophobic and hydrophilic drugs. Optimisations related to studying TDD including limitations encountered are also documented and reviewed. It is hoped that such developments in this field will ultimately lead to researchers replacing skin with optimised polymer-based alternatives to predict transdermal drug delivery.

Keywords: Drug delivery; Permeability; Polymers; Transdermal; Skin

Introduction

Within the pharmaceutical industry there is a keen desire to operate sustainably. Sustainability simply means the ability to maintain the needs of the present in a way and manner as not to compromise the future. Researchers over recent years have tried to describe the concept of sustainability and lay some guiding principles around economic, environmental and social indexes¹⁻⁴. As scientists, the logic behind this concept is simple, research activities should be planned having the “sustainability clause” as the subject of all activities rather than an object within the research⁵. It is generally agreed that scientific activities should not be practiced at the expense of endangering ecological balance, it is argued that the whole idea after all, is making today's world a better place than it was before. Despite the progressive nature of this idea, and the technological advancements we presently have, a compromise is sometimes drawn to allow the practice of some scientific activities. For instance, a recent petition was offered to the European Commission calling for an immediate and total ban of animal testing.

Alternate methods that are animal-free must consider complexities associated with replicating specific biological environments, such as skin membrane⁶. A number of organisations maintain the requirement for strict regulation with regards to analysis, including authorities and licensing bodies such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and BPF (British Plastic Federation)⁷ amongst others. Such organisations must accept responsibility to ensure human safety yet simultaneously move towards the most sustainable and ethical forms of analysis. The authors of this article consider that implementation of sustainability must be taken into consideration when applied to the choice of membrane used in transdermal research equally alongside other factors such as economics and ethics.

Transdermal drug delivery (TDD) systems

Utilising the skin for drug delivery purposes, otherwise referred to as topical or transdermal drug delivery (TDD) systems⁸, has been practised for many years⁹. Within America alone, more than 35 transdermal products have been approved to treat conditions ranging from hypertension, female menopause, severe pain from local muscle injuries, contraception, eczema, psoriasis and nicotine dependence, amongst others¹⁰. Approximately 74 % of drugs taken orally have been reported not to be as effective as desired¹¹, this coupled with the many advantages of TDD¹² has led to increased appreciation in terms of the therapeutic usefulness of this route. Advantages include: increased patient compliance¹³, minimised systematic delivery to other sites, reduced likelihood of uncertain absorption from degradation by stomach acid and enzymes¹⁴, elimination of needle re-use particularly in developing countries^{15,16}, reduced hospitalisation and the release of drug that can be more carefully sustained and controlled¹⁷, thus encouraging development of alternate routes of drug delivery, such as TDD¹⁸. For any drug to make a good candidate for transdermal delivery, the major issue (in terms of research and development) lies in the ability of the drug to permeate the skin^{19,20}, i.e. at what rate it permeates, the mechanism of this permeation as well as the extent of the permeation. Considering these factors, scientist have been able to cascade the process of permeation into three main stages^{21,22}, namely liquid take-up, migration and desorption.

Transdermal Permeation Analysis

Permeation rate and extent are of paramount importance to scientists concerned with TDD and are established using permeation analysis. Permeation of drugs when applied to the skin can best be described by Fick's first law of diffusion. The law states that 'flux in a certain direction is directly proportional to the concentration gradient' (Equation 1), this is however, under the assumption of a steady state²³.

$$J = D \frac{dC}{dx} \quad (1)$$

Where J is diffusion flux (or sometimes referred to as the permeant flux), reported in units of $\text{mol m}^{-2} \text{s}^{-1}$ or $\mu\text{g cm}^{-2} \text{s}^{-1}$. D is the diffusion coefficient in units of $\text{m}^2 \text{s}^{-1}$ or $\text{cm}^2 \text{s}^{-1}$, C is the concentration of the permeant in mol cm^{-3} or $\mu\text{g cm}^{-3}$ and x is the spatial dimension of the membrane surface thus represented in units of m or cm.

The *in vitro* analytical permeation process is such that it comprises of a donor phase and receptor phase, where a pre-heated membrane is carefully mounted between these two phases and a solution placed on the membrane surface within the donor compartment. All openings are occluded to prevent the loss of solvent through evaporation²⁴. During the permeation process it is important to maintain sink conditions, whereby the receptor compartment would have zero-drug concentration. This condition is challenging to achieve when using static cells²⁵, and this led to the development of a system shown in Figure 1. The flow-through set-up still takes into consideration variables such as temperature, stirring, and flow rate²⁵. Temperature is an important property in permeation studies and is usually maintained at $32 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$. It has been reported that an increase in temperature affects permeation profiles, such as an approximately $8 \text{ }^{\circ}\text{C}$ increase in temperature alters the permeation of caffeine, methyl paraben and butyl paraben two-fold²⁶. Generally, two distinct approaches are employed in drug permeation analysis namely, a finite and infinite dose approach whereby the former requires a certain drug concentration that can be changed and varied according to suitability of the intended experiments, while the latter employs a constant concentration usually from the saturated form of the drug. Another important factor to consider is a constant agitation of the fluid in the receptor compartment using a magnetic stirrer at an optimum speed. Stirring should be such that the speed is not too fast to create a vortex, which may disturb the flow rate, leading to the formation of bubbles that can affect the permeation profile.

Figure 1. Schematic set-up of an analytical permeation cell

If a permeable compound is added to the donor chamber then a steady-state diffusion from the donor phase, across the membrane to the receptor phase, is expected. Hence, the concentration gradient across the membrane would be linear. Thus, Equation 1 can be re-written as:

$$J_{ss} = D \frac{[C^0 - C^h]}{h} \quad (2)$$

Where J_{ss} is the steady state flux, C^o is the initial concentration of permeant on the surface of the membrane (the donor compartment concentration) and C^h is the concentration of permeant within the membrane at a certain position, h , i.e. the receiver compartment. These permeant concentrations are difficult to ascertain in practical terms, hence a partition coefficient that describes the partitioning between the permeant and the aqueous solution is introduced for convenience, therefore, concentrations of the aqueous solution in the donor and receiver compartments are obtained practically²⁷. Equation 2 can therefore be written as:

$$J_{ss} = DK \frac{[C^D - C^R]}{h} \quad (3)$$

Where K is the introduced membrane partition co-efficient, C^D the donor compartment aqueous solution concentration and C^R the receiver compartment aqueous solution concentration. Equation 3 can be simplified further considering the fact that the diffusion coefficient (D) is still practically difficult to ascertain, hence, the kinetic parameter D , the thermodynamic and the membrane thickness parameters K and h respectively, can be combined to give the permeability coefficient parameter, K_p . The equation can then be written as:

$$J_{ss} = K_p (C^D - C^R) \quad (4)$$

Furthermore, the concentration of the receptor compartment is effectively zero, in comparison with the donor compartment. This is practically attainable if the permeant concentration in the receptor compartment does not exceed 10 % of the donor compartment²⁸. Hence, Equation 4 is simplified to;

$$J_{ss} = K_p C^D \quad (5)$$

Having met the conditions previously highlighted, the permeability coefficient (K_p) becomes experimentally obtainable and provides useful information regarding permeation. The convenience in comparing these experimentally determined datasets and existing skin data can be seen further in Flynn's work²⁹ and similar, more recent studies³⁰.

Specifications for *in vitro* permeation studies according to the *European Medicines Agency Guideline (EMA)* stipulate *in vitro* permeation analysis is meant to evaluate *the permeation of drugs across the skin*, and is not necessarily expected to establish a correlation to that of *in vivo* permeation. Permeation studies allow the use of artificial or synthetic membranes, as well as human skin or skin from animals such as rodents, snakes, pigs and other animals. It is expected that when using synthetic membrane, information such as membrane thickness, integrity of the membrane and at least six repeatable data sets should be provided.

Human, animal and skin equivalent alternatives

Human skin, and skin equivalent models, have been used in predicting percutaneous permeation³¹, and are popularly referred to as the gold standard for TDD³². However, availability, ethical related issues, storage limitations and variability of skin types are some of the reasons why animal membranes have been considered as an alternative to replace human

skin. The use of animal skin is widespread, for example, it has been estimated that about 115-127 million animals are used for scientific experiments annually³³⁻³⁶. However, there have been increased concerns pertaining to the use of animals in pharmaceutical analysis. Aside from its moral issues, the use of animal testing for research purposes suffers from a variety of issues yet a lot of scientists view this form of research as imperative³⁷ and unavoidable. Financial issues are also a disadvantage when using animals and more recently, there have been increased legal constraints on the allowance of animal testing. For example, the global debate in using animals for cosmetic testing³⁸, including a complete ban on animal testing for cosmetics in places such as the EU (cosmetic directive 76/768/EEC), poses a serious constraint³⁹. Furthermore, animal rights activists with strong and emotional messages encourages moral conversation around this practice. From a strictly scientific perspective, several papers have reported incoherence between animal and human data, demonstrating that animal data has been poorly predictive and not reliably replicated in human clinical research^{40,41}. Most frequently used in permeation analysis is the excised skin from mice, albino rats, pigs, guinea pigs and rabbits⁴²⁻⁴⁶. It is reported that tissues from snakes and fish are also used, but these suffer from a relative shortage in availability⁴⁷. Generally, the use of animal tissue results in the over-estimation of permeability values obtained and has found to be 10 to 100 times higher in nude mouse compared with cadaver skin⁴⁸. Other publications have also claimed that *in vitro* studies with animal skin may not provide information in agreement with *in vitro* human skin⁴⁹, and that human skin is less permeable than frequently used alternatives such as rat skin⁵⁰.

Success stories have been recorded to an extent, regarding the acceptance of cell culture models in place of laboratory animals⁵¹, especially with multinational co-operations such as the Organisation for Economic Co-operation and Development (OECD) accepting their usage for *ex-vivo* purposes in place of animal tissues. Notable amongst these models are reconstructed human epidermis (RHE) and living skin equivalent (LSE). These models try to mimic the human skin structure, for example, RHE consists of a three-dimensional framework, which is grown from keratinocytes and possesses stratum corneum equivalent on its surface. The LSE is more robust having an additional underlying structure which represents dermis⁵². These models have proven promising especially in histological studies and related research areas⁵³. Despite promising features of these models, barrier functions and by extension prediction of percutaneous absorption, has been found to have its limitations. For instance, skin equivalent models have overestimated permeation when compared with isolated human tissue⁵⁴. Similar findings have been reported elsewhere⁵⁵, where these limitations have been attributed to immaturity of the stratum corneum. Poor reproducibility, the delicate nature of the material, complexity of handling and high cost has contributed in limiting the widespread use and acceptance of such models. Most encouragingly, recent surveys have shown high support for the replacement of animals in research⁵⁶, such as those previously discussed. In summary, considering the advantages TDD can offer, and moving away from issues surrounding animal testing, the development of pseudo skin mimics for permeation analysis is

needed now more than ever. In particular, those that do not involve animal skin or cell culture models are ideal in helping address issues of financial, scientific and economic concern.

Poly(dimethylsiloxane) (PDMS)

Poly(dimethylsiloxane) (PDMS) addresses most of the major challenges encountered with the use of human or animal skin in permeation analysis, including availability, uniformity, stability, cost, handling, sufficiency for large scale studies, ethical and legal constraints. In addition, certain properties PDMS membrane offers makes it even more attractive for utilisation, particularly as a substitute for biological tissues in permeation analysis. While the advantages are clear, it is imperative to highlight some of the challenges (and numerous modifications recently developed to overcome such challenges) encountered in tailor-fitting the membrane to the desired outcome.

Since the 1940's, polyorganosiloxanes, a family of compounds to which PDMS belongs, have been produced on an industrial scale (via Muller–Rochow direct synthesis)⁵⁷. As far back as 2002 silicon based material synthesis was estimated to be over 2 million tonnes, a consequence of their un-paralleled properties, compared with other polymer membranes^{57,58}. Most of these properties are largely attributed to the Si-O-Si backbone of these polymers. PDMS has a Si-O-Si bond angle of 140° ⁵⁹ exhibiting a certain orientation in space and has a glass transition temperature of -123°C ⁶⁰ due to its molecular backbone orientation which allows a level of torsional motion. It also has low intermolecular forces, and therefore a large molar volume of $75.5\text{ cm}^3\text{mol}^{-1}$ ⁵⁹. The low cohesion energy, poor solubility and surface energy can all be attributed to this low intermolecular force. However, the value of the Si-O-Si bond is high (110 kCal mol^{-1}), compared with Si-C (76 kCal mol^{-1}), C-C ($82.6\text{ kCal mol}^{-1}$) and C-O ($85.5\text{ kCal mol}^{-1}$) bonds, which explains the excellent thermal stability of the polymer⁵⁹. In addition, methyl groups present on the membrane surface are largely responsible for the hydrophobic nature of the membrane. The basic structure of PDMS can be seen in Figure 2.

Figure 2. General structure of Poly(dimethylsiloxane), also known as PDMS.

Other remarkable properties of the membrane include: gas permeability making it transparent to visible and UV light⁵⁷, relative environmental friendliness (it does not bio-accumulate), low-cost and non-toxicity⁶⁰⁻⁶². These properties, amongst others, gives PDMS advantages over other polymeric membranes, making it the most commonly used polymer among the polyorganosiloxanes^{57,63}. These desirable features facilitate using it for biological mimic functions, such as human skin. For more than sixty years, there has been considerable research

regarding the use of polymeric membranes for drug delivery purposes⁶⁴⁻⁶⁷. However, in the last decade there has been an increase in PDMS-biological research, for example exploiting its optical transparency and gas permeability for eye lenses and glasses⁶⁸ as well as discreet oxygen sensors⁶⁹. More recently, powdered PDMS has been utilised for the delivery of genes into cultured animals⁷⁰ and as mimics in fluids analogous to blood particulates⁷¹. Alongside these applications, the selectivity and stability of PDMS prompted scientists to consider it as a potential candidate to mimic the barrier function of human stratum corneum. Skin owes its selective reabsorption and barrier functions mainly to the stratum corneum (the primary barrier). This function is possible as a consequence of the unique composition of the stratum corneum, and can be envisaged as a 'bricks and mortar' structure, comprising of the keratinocytes (corneocytes) 'bricks' and the intracellular 'lipids', which are both key determinants of skin permeability and, in turn, relate to drug permeation through the skin. It is this structure-activity relationship that permits prediction of drug permeation⁷². The corneocytes are comprised mainly of keratin macrofibrils, and are protected externally by a cornified cell envelop, held together by protein⁷³. The intercellular lamellar lipid membrane comprises of three different lipid components, found in approximately a 3:1:1 relative ratio, based on the stratum corneum - lipid composition (% by weight) which comprises of ceramide (50 %), free fatty acid (10-15 %) and cholesterol (25 %)⁷⁴. It is important to note that these lipids are produced from lamellar bodies within the stratum granulosum, some of which (upon entry into the stratum corneum, after extrusion) are converted enzymatically to ceramides⁷³. A summary of recent studies considering PDMS as a potential alternative to skin are listed in Table 1 whereby their findings related to percutaneous absorption are considered along with their limitations and advantages over the alternatives available.

Table 1. A summary of several studies proposing PDMS as a potential alternative to predict transdermal permeation.

Polymer model	Compared model	Model compounds used	Findings related to percutaneous absorption	Physicochemical Parameters used in the experiments				Limitations	Advantages	Ref
				Temperature	Medium pH	Flow rate	Experiment Duration			
PDMS – PC BCP	Human skin epidermis (Inner thigh)	14	Share similar mechanism of drug transport (solubility-diffusion process)	35°C ± 0.5 °C	-	100 rpm ± 1	48 hours	Over prediction of percutaneous absorption	Easily achieved	75
PDMS – PA Carbo wax	Porcine skin	32	Shows a good correlation with model compounds (R ² = 0.93)	37 °C	7.3 – 7.5	400 rpm	12 hours	Performs best under precise and fine conditions, lot-to-lot variabilities could lead to variable results	Provides a good relationship with porcine skin	76
PDMS - PEG	Human skin	12	Can be used to predict permeability of the human skin for both hydrophilic and lipophilic drugs	37° C	-	-	-	Less reliable when predicting hydrophilic compounds	Provides a suitable alternative to human skin	77
PDMS	Pig skin	1	Absorption was slightly higher compared to that of pig skin	37 ± 0.5 °C	7.4	-	24 hours	Limited sample size	Cost effective	78
PDMS	Human skin and Hairless rat skin	15	Results obtained show correlations with compounds having low Log P values yet more pronounced with drugs having high Log P values	32 °C	7.4 (except for 1 compound)			Overestimation of percutaneous absorption	Could serve as a screening test for permeation analysis	79
PDMS - MA	Human skin	11	Drug permeation was similar to human skin	37° C	7.4	-	-	The membrane was delicate, and more studies need to be carried out between polymer materials and the drug	Provides a linear relationship between membrane permeation and that of human skin, which could provide an avenue for a mathematical model	80
PDMS	Porcine skin	2	Percutaneous absorption of the synthetic membrane, fits a normal distribution curve	37 ± 0.5 °C	7.4		24 hours	A correlation wasn't achieved between synthetic membrane and porcine skin	The permeation profile was good, and results were reproducible	81

Based on papers presented in Table 1, along with many other examples that can be found in literature, it is fair to say that PDMS membrane has been receiving consideration over the past few years as a potential alternative to skin for such analysis. Factors affecting permeation whilst using PDMS must be carefully contemplated as the extent of permeation can be influenced by properties such as ionisation. For example, if PDMS is to be used to predict permeation then the degree of ionisation of the compound under analysis must be controlled as it has been found that the greater extent of permeation is experienced for the more 'neutral' form of the compound, i.e. the unionised, rather than the anionic or cationic species⁸². The potential of PDMS to determine permeation has recently expanded into considering modifying the surface of the polymer to create a new type of surface for the compound to permeate through. One recent study exemplifies this potential whereby PDMS was plasma treated to create a new form of polymer, referred to as plasma-modified silicone membrane. It was found that the plasma-modified silicone membrane was proven to be a suitable replacement for clinical studies when determining permeation of compounds through the skin⁸³. However, some scientists have shown reservations in accepting artificial membranes, proposing that it will not successfully model the lipid perturbation effects undergone by biological tissues⁵⁵.

Another interesting aspect of this particular polymer membrane is the well-developed silicon chemistry it exhibits, enabling one to replace the methyl group by a wide range of functional groups^{31, 57, 82-84}. It is also reported that tailored properties can be achieved by the judicious choice of the chemical substitute, including skin-like properties. A plethora of data have shown how PDMS possesses some intrinsic flexibilities, where modifications on the membrane can lead to an overall effect on how it works⁸⁵⁻⁹⁰. Interestingly, over the last decade research has been published that involves modifications carried out on the membrane which subsequently alters the permeation profile compared with the un-modified form of the membrane. Such studies highlight the possibility of tailoring the membrane to most successfully replicate human skin data, alongside factors such as donor compartment composition⁹¹. Other possibilities include chemical modification of the PDMS surface, such as a recent publication confirmed the extent and profile of drug permeation can be immensely altered by silanising the surface of PDMS⁸⁴. In this case it was found that there was an appreciable increase observed in the extent of permeation compared with standard PDMS, i.e. permeation could be controlled by modifying the membrane surface. These findings exemplify the importance of selecting specific experimental conditions and the potential of modifying PDMS membranes to optimise permeation data acquired. A comparison of PDMS with alternative options is summarised in Table 2 whereby the advantages of PDMS over such alternatives can be clearly seen. These include the reproducibility of the polymer, thus leading to more reliable data than can be acquired using human or animal skin as there is no variability in thickness or composition. When compared with skin equivalent based systems, the economic benefits are considerable as the polymer does not need to be 'grown' or maintained under any specific laboratory conditions to retain its efficacy. The careful storage conditions for such systems are one of the reasons they are renowned for being so costly which can limit their suitability for many research establishments. Developing a membrane that can meet all of these criteria is clearly a challenge for analysts as all available options have their own advantages and limitations and there is also the issue of experimental variation. This final point has been the subject of a considerable inter- and intralaboratory study where it was found that the coefficient of variation between laboratories was high at 35 % although far lower within a single laboratory

297 at 10 %⁹². This highlights the importance of developing and maintaining detailed
 298 methodologies before undertaking permeation analysis to attain reliable data.

299

300 **Table 2.** Comparative description between PDMS membrane and skin equivalent models.

Skin model	Advantages	Limitations
PDMS silicon membrane	-Cost effective -Reproducible -Modifiable	-Non-biological -Over prediction of percutaneous absorption
Reconstructed Human Skin equivalent (RHE)	Good correlation compared with human skin data	-Fragile -Expensive -Variable -Instability
Animal skin	Traditionally used	-Ethical constraints -Legal constraints -Questionable barrier property -Age, anatomical site and animal type influence percutaneous absorption
Human skin	Gold standard	-Ethical permission required - Availability and storage constraints -Variance resulting from anatomical site used, gender, age and race

301 Conclusion

302 Human skin and other skin equivalent models popularly used for analysing TDD suffer from
 303 several constraints, both scientific and humane. PDMS however, offers advantages of
 304 sustainability, suitability, stability, activity and most importantly, provides an avenue to be
 305 modified and tailor fitted to a desired outcome, with a careful interplay between cost
 306 effectiveness, ease of usage and availability. A plethora of established datasets are already
 307 available, and it is our belief that PDMS is a potential alternative for rapid screening and TDD
 308 optimisation, which in turn not only solves the ethical issues surrounding the use of animals
 309 but also helps provide a more suitable, economic, precise and accurate method to analyse new
 310 medicines intended for use as TDD systems. The authors believe that the future direction of
 311 the application of polymer membranes to determine permeation lies in their ability to be
 312 chemically modified, there are many more modifications yet to be considered which could help
 313 researchers identify the most optimised membrane structure possible to allow accurate and
 314 precise *in vivo* prediction using such an *in vitro* system.

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