ADMET

Review

Skin PAMPA: Application in practice

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Abstract

Transdermal drug delivery has been growing extensively in the past decades, therefore new, reliable and cost-effective in vitro models were demanded to support the research and development on this field. Model membrane of PAMPA, which can mimic skin penetration, was first described in 2006, but the need for a more bio-mimetic system has been arisen by new industrial tendencies and a bio-relevant system was published in 2012. Since its first publication, Skin PAMPA has already been applied by several universities and industrial groups successfully, and the first articles, podium and poster presentations have been appeared. The original Skin PAMPA model has been further developed in order to extend its application for formulations. Examples of liquid or semi-solid formulation projects and transdermal patch studies are available beside standard solution applications. The present review demonstrates the different approaches needed for various type of samples, provides examples of applications and practical conclusions for further improvement.

Keywords

skin penetration; in vitro; artificial skin model, formulation study

Introduction

Estimation of skin penetration of compounds has become crucial in the past decades, as the advantages of dermal usage and transdermal absorption route have been recognized by many pharmaceutical and cosmetic companies [1]. It is common that the best estimation is provided by *in vivo* human skin studies, however its availability and the ethical regulation make it impossible to apply routinely in product development. *In vitro* human skin experiments could be the most useful replacement to predict human *in vivo* environment, but accessibility is difficult, especially for cosmetic industry. Therefore researchers started to work on development of artificial membrane models that can mimic the features of skin, while having much better availability and being more cost effective. Depending on the need, silicon based membranes, cellulose acetate or cellulose nitrate filters are available for Franz diffusion cell method; however these models have limited quality of prediction, suffer from poor standardization and do not even meet the high throughput criteria, that would be useful in early stages of discovery and development [2]. The current review introduces the PAMPA method and the Skin PAMPA model, that provides a good estimation of skin penetration, does not suffer from pure standardization and reproducibility, while having a high throughput and low cost option [3].

With the introduction of the Biopharmaceutics Classification System [4], fast and reliable permeability measurement strategies were needed to classify the molecules at the earliest stages of discovery. In 1998, Kansy and co-workers have introduced the Parallel Artificial Membrane Permeability Assay (PAMPA) as a tool for rapid determination of passive membrane permeability of drugs [5]. The interest of the industry was growing extensively in the first decade of the method, mostly because of the method's low cost and high throughput performance. Since the first publication by Kansy and co-workers, several companies have developed their own variants and many articles were published, indicating that PAMPA is a good research tool for physico-chemical property screening in early discovery stage [6]. PAMPA models have been published for the prediction of gastrointestinal absorption (GIT) [7,8] and for modelling the blood brain barrier (BBB) [9]. There are two models available for the estimation of skin penetration as well.

The first PAMPA model for skin penetration estimation was published by Ottaviani et al. in 2006 [10]. This approach incorporates silicone oil and isopropyl myristate as membrane components, which are not the natural elements of the *in vivo* barrier. Also, that model was developed based on the Flynn database that contains human skin permeability results collected from many research groups using diverse experimental conditions. As it was shown in several studies [11-13], the difference between permeability data measured in various laboratories can exceed by an order of magnitude, therefore the prediction potential of this model is not proved yet.

Following the tendencies of model development in pharmaceutical industry, Skin PAMPA was designed to be bio-mimetic, i.e. the same or similar components have been applied as present in the most important barrier of human skin [3]. As the main barrier of human skin, it is known to be the outermost layer of epidermis, the Stratum Corneum (SC), Skin PAMPA has been designed to mimic the features of this layer. SC composes of corneocytes embedded into a multilamellar lipid layer. Lipid layer consists of a mixture of ceramides, cholesterol and free fatty acids as major components, and provides the main route of penetration, the paracellular pathway [1]. Skin PAMPA membrane was created by using cholesterol, free fatty acid and a ceramide-analog compound that mimics the features of ceramides in the lipid matrix [14]. The ceramide-analogue compound (certramide) has been studied extensively and its properties have been found to be suitable for PAMPA membrane [15].

During the 15 years history of PAMPA, researchers have used the system to predict the permeability of compounds through the GIT, BBB or later through Skin. In most cases, the main goal was to determine permeability as kinetic parameter of pure compounds in early stage of drug discovery without any formulation effect. Most of the protocols was developed to satisfy this need, therefore buffer solutions at various pH with 0.5-1(-5) % DMSO was studied as donor solution and pure pH = 7.4 buffer as acceptor solution [16]. Donor concentration was selected according to the physico-chemical (solubility, pK_a, log$P$) parameters of the compounds and permeability was calculated as the main parameter for comparison. Upon the expanding application of Skin PAMPA, an urgent need has been arisen for the investigation of drug formulations, including semi-solid dosage forms and transdermal patches. The following paragraphs describe the application possibilities of Skin PAMPA, provide some general considerations of assay design and data processing, and demonstrate some recent examples.

Applications

Permeability studies of compounds in solution

Samples of compounds dissolved in pure solvent or solvent mixture are typically applied in Skin PAMPA, when the permeation properties of active pharmaceutical ingredients (API) are investigated. In these studies, the performance of the API itself is important, without any penetration enhancing or controlling effect. Simple solvents, like water based buffers, PEG 400, ethanol or propylene-glycol are applied in these projects that have a well-defined and usually predictable effect on permeability. The protocol of these studies is the same or similar to general PAMPA protocols, i.e. the concentration and the applied dose are selected based on the physico-chemical parameters of the API and not based on the relevant *in vivo* dose. The donor volume/membrane area ratio is also significantly higher compared to *in vivo* environment. Permeability is determined and used for assay evaluation, that is normalized for donor concentration, for surface area and for time, therefore it is capable to rank the compounds own permeation property.

The performance of Skin PAMPA for API characterisation was first investigated by the inventors of the model. Two correlations were described and both were established as a result of careful human permeability data selection. Selection of appropriate, reliable reference dataset has fundamental importance in model validation, since several published databases contain results of different laboratories that can reduce the significance of the correlation.

The first selected dataset was published by Lee and co-workers in 2010 [17]. This dataset provided homogenous and normalized permeability data for more than 40 compounds. The results were measured on dermatomed human female back skin using Franz diffusion cell method. 45 % PEG 400 was added to donor solution to mimic the effect of a basic formulation and pH was selected to be 6.4. The Skin PAMPA experiment for 15 compounds was performed with similar parameters to Franz cell experiment. Permeability was calculated and used for the comparison as the performance of the APIs. High correlation (R^2 = 0.84) was found between human skin data and Skin PAMPA data as shown on Figure 1. The good correlation also indicates good ranking order of compounds, so the model was able to characterize the permeability and rank the APIs properly. The authors have also compared Skin PAMPA results measured with pure buffer solutions in the donor phase to the same dataset, and found a significantly weaker correlation that indicates the responsiveness of the system for the simple formulation effects of PEG 400.

The second database was the Full Validated dataset published by Hadgraft and Guy [18]. This database contains results measured in different laboratories and despite the careful validation criteria of selection the experimental conditions are very different. Therefore data were divided into small homogenous groups. Data were sorted based on the incubation temperature and the skin derivative used for the test. Skin PAMPA experiment was performed with the standard solution protocol, pure buffers solutions with 1 % DMSO were studied as donor phase, pure buffer at pH = 7.4 was used as acceptor, and permeability was calculated for the correlation study. Four comparisons were published and good correlation was found, however statistical evaluation was not possible for the reason of low number of compounds. A united correlation study performed on the published 4 small groups is shown on Figure 2. The obtained R^2 = 0.70 value is also an acceptable correlation regardless of the uncertainty caused by the human data.
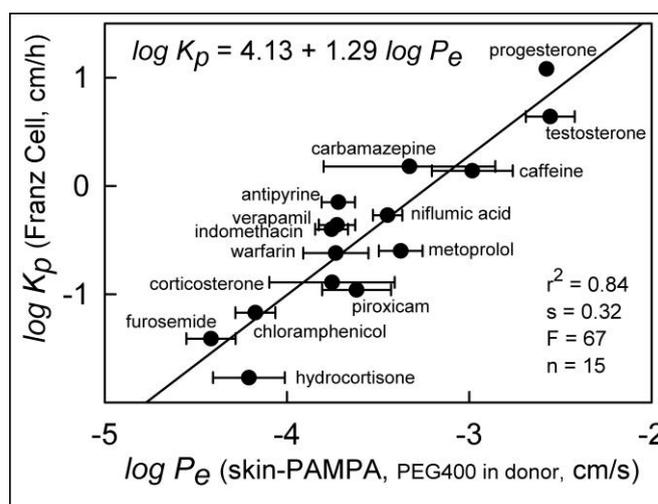
Figure 1. Comparison of human skin permeability data measured by Franz cell vs Skin PAMPA data.
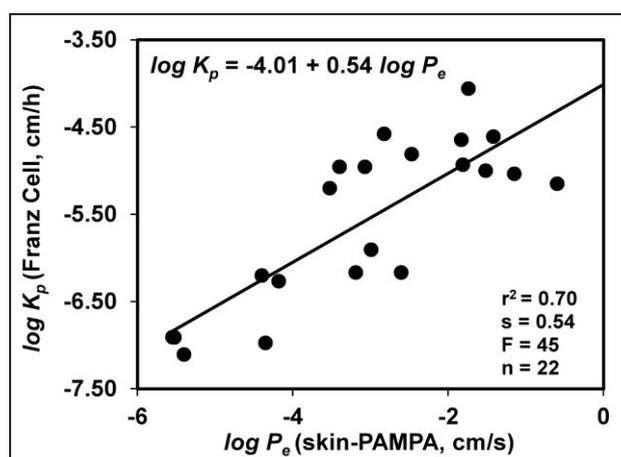
Figure 2. Correlation between human skin permeability data of Full Validated dataset and Skin PAMPA data.

Karadzovska and Riviere published a study of 96 well based skin penetration models in 2013 that included Skin PAMPA as well, beside the other, previously mentioned PAMPA skin model by Ottaviani and Strat-M membrane of Merck [19]. Six compounds dissolved in three different solvents (water, ethanol and propylene-glycol) were studied. Compounds were applied in both saturated and unsaturated solutions, but both applications can be considered as infinite for the reason of the total applied dose. Permeability was calculated and used for the correlation study. The assays with saturated solutions were not successful, but the results of unsaturated solutions were in good agreement with data measured on human skin. They investigated the membrane retention of 96 well plate based models, and found good correlation to skin retention as well, therefore, all three models were found to be useful for transdermal penetration prediction. Skin PAMPA has been acknowledged as the most bio-mimetic membrane model, where specific skin mimetic interactions are more probable.

In a paper published in 2013, Vizserálek and co-workers investigated the effect of incubation temperature on permeability using Skin PAMPA model among others [20]. The study concluded that Skin PAMPA results are more sensitive for incubation temperature compared to other, mostly phospholipid-based PAMPA models. Figure 3 demonstrates the temperature dependence of 7 compounds. As the slope of the lines indicates, as high as 0.35 log unit difference can occur by performing the assay either at 25 or 32 °C. Therefore, the authors concluded that proper incubation temperature is important for Skin PAMPA

assay, especially when formulations are investigated where the sample itself can have temperature dependent behavior.
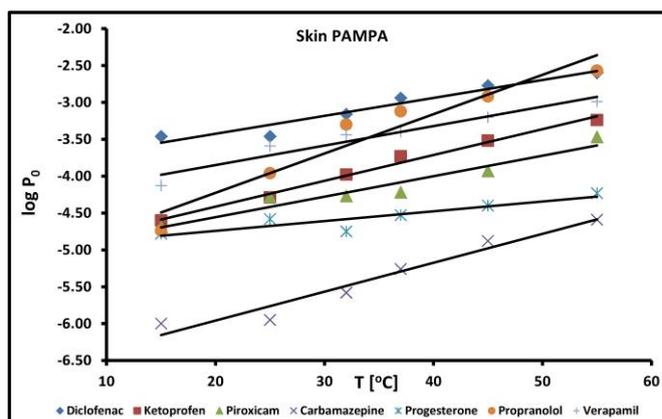
Figure 3. Temperature dependence of permeability in Skin PAMPA method.

Permeability studies with liquid or semi-solid formulations

Formulations require different approach compared to standard PAMPA application, as the aim of the study is to investigate the performance of the formulation, and not just the pure API. The modification affects the applied dose, the way of application and the calculated parameters as well.

As the goal is to investigate the performance of the formulation, keeping the applied dose close to *in vivo* environment would provide more reliable information. The previously described general PAMPA protocol or Skin PAMPA protocol for solutions apply "infinite dosing", that means significantly higher dose compared to *in vivo* case. Therefore, the dose should be reduced as much as possible to approach the "finite dosing", i.e. the *in vivo* relevant amount [21]. The currently available protocols and plates make it possible to apply 10 times finite dose, that is much closer to finite situation than standard PAMPA assay, and the development of lower dose applications are still in process. New bottom plate (Formulation plate) has also been developed by the manufacturer to make the '10 times finite dose' application possible.

As the performance of the formulation as a complex matrix is investigated, parameters like flux, concentration in the receiver solution or permeated amount of drug are calculated, that are not normalized for the applied dose. These parameters are suitable for direct comparison of the formulations. It is advantageous if the permeation is studied in wider time period, and the permeated amount *vs* time profile is used for evaluation. This graph includes all the main information like permeated amount at certain time point, flux and lag time.

As these considerations are the result of the latest developments, most of the cited studies below have not been designed according to all of these recommendations.

On a poster of Clough and co-workers published at AAPS in 2013, the permeability enhancing effect of a series of solvents, lipidic fluids and emulsifiers were investigated [22]. Infinite dosing approach was followed by the authors and standard PAMPA sandwiches were used for the study. All samples contained 5 % Ibuprofen-Na as API and 8 % of one of the investigated component beside water. The samples of lipidic fluids contained 45 % PEG 400 as well. Figure 4 demonstrates the receiver concentration after one hour of incubation where the authors have found significant differences in the permeability of Ibuprofen depending on the penetration enhancer. Unfortunately, correlation with human skin data is not available, but the

results itself suggest that the model is able to differentiate the samples, and therefore can be used for ranking the formulations.
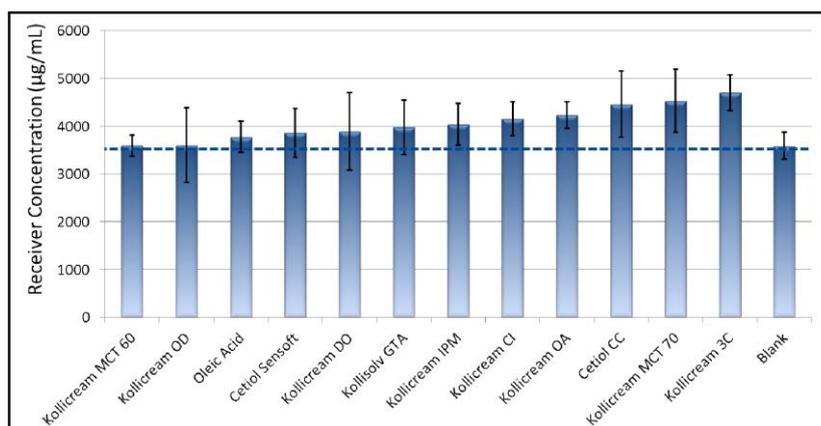
Figure 4. Permeability enhancing effect of different solvents, lipidic fluids and emulsifiers in case of ibuprofen measured by Skin PAMPA method [22].

Tsinman and co-workers presented their work with semi-solid formulation of ibuprofen at AAPS in 2012 [23]. Three formulations and one slurry of ibuprofen were investigated using both Skin PAMPA model and real human skin mounted in Franz cell system. They have applied 30 μg formulation/well that is equal to '10 times finite dose application' as mentioned before, and they used the receiver concentration for the comparison. Good agreement was found between Franz cell and Skin PAMPA results in terms of ranking order the samples, however Skin PAMPA model provided higher permeation results compared to human skin data.

In 2014 at AAPS conference, Luo and co-workers presented a comparison of Skin PAMPA results to porcine skin results measured with Franz cell method [24]. The study investigated the permeation of 3 APIs (ibuprofen, caffeine, uvinul A plus) using saturated solutions. They presented both infinite and '10 time finite' dosing studies and used the cumulative permeated amount *vs* time profiles for data interpretation and analysis. The comparison shows, that Skin PAMPA can provide an excellent ranking order of samples, while having the advantage of much lower cost and ease of standardization.

Permeability studies with transdermal patches

Similarly to formulation studies, the aim of transdermal patch testing is to evaluate the performance of the patch as transdermal therapeutic system and not just the API, therefore similar approach is needed. The dosing is easier in this case as the patch is applied directly on the membrane without any need for normal or modified bottom plate, so the applied dose is predefined by the patch. As a significant amount of available patches must not be cut into pieces, it is recommended to apply them as they are. This application involves the occurrence of "edge effect" [25], which means a possible lateral diffusion of API within the adhesive layer that can increase the flux.

It is recommended to determine the cumulative permeated amount *vs* time profile that can provide all the necessary information for comparison as detailed before. As most of the transdermal patches are designed to provide a zero order kinetic release of API, it is suggested to continue the Skin PAMPA study till the end of the proposed application period of the patch.

The first and only study of transdermal therapeutic systems has been presented by Vizserálek and co-workers at AAPS 2014 [26]. Transdermal patches of four APIs (nicotine, fentanyl, ketoprofen, rivastigmine) were applied on Skin PAMPA model. Figure 5 demonstrates the permeated amount *vs* time profile for

fentanyl as an example. The blue dashed line shows the *in vivo* delivery rate calculated from human blood level, while the red dots and black solid line demonstrate the Skin PAMPA results. Excellent agreement can be seen between Skin PAMPA results and *in vivo* human flux data, therefore the study concluded that Skin PAMPA is a good tool for ranking order of transdermal patches, and it can even estimate the human absorption rate with an acceptable precision. The detailed results of this study on transdermal patches will be published soon elsewhere.
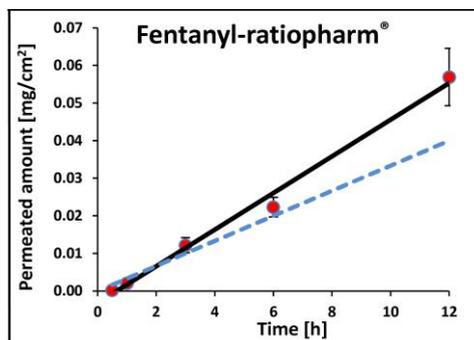
Figure 5. The permeated amount vs time profile of a marketed transdermal patch containing fentanyl.

Conclusions

In the past 2.5 years, Skin PAMPA as a bio-relevant artificial membrane based HT permeability model has been widely applied for the estimation of skin penetration. This paper has collected the most important publications, and grouped them based on the type of sample applied on the membrane. The driving forces of the studies, the ways of application and the criteria of calculated parameter selection have been considered and suggestions for assay planning have been provided. It can be concluded, that the careful definition of study expectation, the proper design of the assay and the calculation of suitable parameters are crucial for satisfying results and for good prediction potential.

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