Modelling the diffusion of xenobiotic compounds in the skin: A review

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Abstract

Interest for in silico skin xenobiotic absorption models has been growing in the last years owing to the economic advantage of computer simulations over costly experimental procedures. This review presents a classification of the modelling categories reported in the scientific literature so far, discussing their capabilities and limitations. First, Quantitative Structure Permeability Relationships (QSPR) are presented and discussed, followed by Physiologically-Based Pharmacokinetic Models (PBPK). PBPK models include the compartmental approach, and modelling approaches based on the solution of the discretized diffusion equations in geometric representations of the skin in a Computational-Fluid-Dynamics-like manner. The latter modelling category allows the user to represent relevant morphological elements of the skin (such as the stratum corneum, the hair follicles, and the pilosebaceous glands), and are expected to help with the development of efficient delivery of cosmetic products and medical drugs. The review highlights also several interesting directions for future research in the area of skin xenobiotic penetration made possible by the Computational-Fluid-Dynamics-like modelling category.

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Keywords: xenobiotic diffusion; in silico; appendageal route; skin absorption; hair follicle; stratum corneum
1. Introduction.

The skin is the largest organ in the human body and also provides the primary interface to the external environment, an adult typically having a skin surface area of between 1.5 and 2 m$^2$ [1]. Maintaining healthy skin is therefore a very important aspect of maintaining health of the organism as a whole.

Although the skin outer, epidermal layer, is mostly comprised of keratinised material which acts as a barrier against potentially disadvantageous substances, there are applications where it is important to ensure that the sub-layers of the skin structure (dermis and hypodermis) receive a sufficient amount of the xenobiotic at hand, which can either be provided internally, via the blood circulatory system, or externally via adsorption through the epidermis. A significant potential advantage of topical application is that the active material can be provided in a local targeted fashion (c.f. with delivery via the circulatory system, which is non-specifically targeted and maintains a nominal concentration throughout the body).

Nevertheless, there are significant challenges to optimising molecular transport across the skin, and to be more specific, across its outermost layer (the stratum corneum), which has the lowest permeability among the different sublayers of the skin owing to its specific morphology. Understanding the kinetics of such transport processes in either an \textit{in vitro} or an \textit{in vivo} experiment is far from simple and as such, mathematical modelling and simulation becomes an attractive approach for nutrient, cosmetic and pharmaceutical design. This is the context of the current review.

We begin with a description of the modelling categories found in the literature in order of increasing detail, and follows with the specifics for the modelling of the sublayers that compose the skin. The document closes with a general summary of the current state-of-the-art and future perspectives in the field.

2. Evolution of xenobiotic absorption models (from QSPR to \textit{in silico} physiologically-based pharmacokinetic (PBPK) modelling)

Human skin exposure to chemicals can be intentional (this is case of cosmetics or medical drugs of topical application), or unintentional (industrial hazards derived from chemicals, pollutants, and cleaning products). Upon application on the skin, permeation occurs by passive diffusion through the successive skin sublayers and its appendages, e.g. hair follicles and pilosebaceous glands [2]. The hair follicle in particular constitutes a shortcut to
reach the deepest parts of the skin, having subsequently received considerable attention as of late [3–5]. The skin is formed of three main sublayers, i.e. the epidermis, the dermis, and the hypodermis, which lie on an adipose layer before muscular tissue. The epidermis lies in contact with the open air, and provides waterproof barrier as well as pigmentation. Two sublayers can be clearly differentiated in the epidermis: the stratum corneum, which constitutes the actual barrier given its low value of permeability, and the viable epidermis. The dermis is a complex combination of blood vessels, hair follicles, sebaceous glands, and fibroblasts. Collagen and elastin, substances which are crucial to a healthy skin, are synthesised in the dermis. Nerves are also found in the dermis. Moreover, its rich vascularisation makes the dermis the target for effective distribution of topically applied products to the rest of the organism via the circulatory system. The hypodermis consists of adipose and connective tissue, and is also highly vascularised. Clear differentiation of these layers, and their morphology and physical properties are crucial to the development of detailed PBPK modelling of the absorption of xenobiotics into the skin.

The viable epidermis and dermis are commonly simplified in terms of modelling as homogeneous layers composed of approximately 40% water, 40% proteins, and 20% lipids [6,7]. Most research efforts however, focus on the stratum corneum, which is the outermost layer of the epidermis, and constitutes an effective barrier against penetration of alien substances, given its morphology. The stratum corneum is formed of two phases with different effects on the mass absorption rate: one formed mainly by keratinocytes, and the other by lipids. Most of the mass transfer occurs through the lipidic route given its greater permeability relative to the keratinocyte route. The complexity and relevance of the appropriate modelling of the stratum corneum is treated in Section 2.3.2 of this review.

Skin appendages are weak points through which substances can diffuse rapidly, skipping the keratin barrier in the epidermis and reaching the blood stream (in the case of small molecules as is the case of most pollutants and cosmetics) and/or the lymphatic system (larger molecules as snake venom). Skin appendages have thus been intensively targeted in recent investigations as the primary route to accelerate drug and cosmetics administration, and as the sole route for big molecules to access the circulatory system [8].

The pharmacokinetics of a xenobiotic substance after skin absorption is influenced by physiologic characteristics of the skin, the physico-chemical nature of the compound, and environmental effects, which must be considered from the modelling point of view so as to get accurate results. Thickness, hydration, and temperature are among the skin-specific factors, and have an effect on both the geometry of the different layers—so is the case of
the stratum corneum, which presents greater thickness and permeability as the moisture content grows, affecting the mass transfer rate. The permeability of the skin depends on the location in the body as well. Evidence provided in the literature establishes that the absorption rate and final concentration values of a particular compound in humans depends on the anatomical site considered [9], although the assumption of a constant overall permeability coefficient is often considered [10]. As for the effect of the environmental factors, Reifenrath and Spencer [11] concluded that wind, humidity, temperature, and vapour pressure have an effect on the quantity of product absorbed by the skin owing to loss of product to the environment through evaporation. A frequent source of error, for instance, when determining xenobiotic absorption rates in \textit{in vitro} cell diffusion studies comes from covering the application site with an occlusive wrap to prevent loss of the compound towards the environment. Extra hydration of the stratum corneum may occur as a result as well, hence affecting the overall absorption rate [12].

Regarding the nature of the xenobiotic compound, the concentration of that particular chemical in the environment is usually a factor to be considered, whereas in the case of topical application the vehicle, i.e. the formulation used for topical application, is often considered as an infinite source. Diffusive properties, lipophilicity, polarity, etc. are also key to determine the final reach of the particular compound within the skin [13].

The different approaches used to determine and use the above mentioned properties result in different types of models available in the scientific literature. The properties of a given compound and their effect in the permeability can simply be measured experimentally, subsequently fitted to a model, and used to predict the effect of new xenobiotics, i.e. this approach is known as a QSAR or QSPR models. Another option is to include basic data such as diffusion and partition coefficients into mechanistic models to obtain a more accurate description of the concentration depth-profiles of a given compound by solving the diffusion equations, the latter approach being called \textit{in silico} PBPK models, which comprise the compartmental approach and models based on the application of numerical discretisation models [14] and those based on the solving of the diffusion equations. QSAR models have been used for identification of potential hazards and risk assessment [15]. Lack of detail on the actual concentration depth-profile generated by the application of a xenobiotic component to the surface of the skin is a drawback, since QSPR models do not use a mechanistic approach to describe the xenobiotic penetration and also lack skin morphology details. On the other hand, holistic and fully transferrable models might be obtained when physics principles are applied to
calculate the values of the diffusion and partition coefficients which govern the mass transfer through the different layers of the skin, but in this case the validation process must be more comprehensive, with rigorous error assessments and providing space-time discretization confidence intervals. Moreover, in most cases the relation between the parameters needed to solve the diffusion equation and basic principles such as the radius of the molecules being implied are simply not available, and experimental correlations must be used instead. As a result, there is always a certain degree of dependency on experimental data in skin absorption modelling, although the latter reduces the experimental expenditure. The pursuit of ever increasing accuracy of numerical models must be the result therefore, of considering more complicated features of the skin morphology, i.e. accurate modelling of skin appendages, skin sublayers and vascularisation, and the pharmacokinetics of the product at hand, especially metabolism.

The combination of all the above mentioned factors results in a complex mixture of physical phenomena, with simultaneous evaporation from the surface of the skin, sorption into the stratum corneum, with and without reversible or irreversible binding; and finally penetration into the viable epidermis, followed by either metabolism or transfer into the blood/lymph stream with subsequent distribution throughout the organism. Modelling is therefore, a valuable tool capable of simplifying the design process, and provide added value from conclusions that otherwise might be difficult to reach only via experimental work.

Pharmacokinetics can therefore be investigated in vitro, i.e. using an experimental approach, and/or in silico, i.e. computer simulations. Both in vitro and in silico approaches are complementary, either because data from experiments have been used to develop the model, or to validate predictive models. Regarding in vitro, different approaches exist including empirical testing in bioengineered human tissue specifically grown for that purpose, in ex vivo skin samples obtained from living organisms by excision, or in animals (in vivo) [16,17]. Numerical modelling on the other hand, reduces the cost associated to experiments, and avoids the ethical conundrum of animal and human testing.

The pharmacokinetics of a xenobiotic compound in the organism is divided in four stages: absorption (A), deposition (D), metabolism (M) and excretion (E), which are known in the literature with the acronym ADME. Numerous models have been reported in the literature dealing with absorption, which to date appear to be well validated against experimental data for some specific purposes [18]. While predictive pharmacokinetic absorption models are well established, those dealing with the rest of the aspects involved, namely
disposition, metabolism, excretion and others as proposed further down in this document (section 4) are less frequent [19], being the work reported by Y. Dacik et al. [20] a notable exception. In the latter, the authors introduced a term in the differential equation governing diffusion, to account for elimination via systemic circulation, with the objective of interpreting previously published microdialysis data. Among the three least explored aspects of pharmacokinetics, some studies regarding metabolism have been reported as of late, via QSAR models of data on metabolic routes obtained from the liver, the main organ responsible for metabolism. The skin however, is also an important route of exposure, and therefore modelling the skin metabolism is key to predict and prevent the activity of harmful substances to the human body. Comprehensive, fully predictive in silico models of skin metabolism are yet to be developed, which opens an important alley in numerical modelling research. For deposition, only the model of Jones et al. [21] has been found.

*In silico* models have gained in complexity over the past years/decades owing to ever increasing computational capacity and research ambitions. The evolution in complexity of *in silico* models for skin permeation towards predictive mechanistic models, capable of being used in any given scenario is shown in the graphical abstract. Fully predictive *in silico* models have not been attained yet however, and even state-of-the-art models such as that reported by Kattou et al. [22] rely on experimental correlations for the calculation of basic diffusion parameters. The figure shows the three main groups of pharmacokinetic models, namely QSPR, compartmental (skin divided in homogeneous compartments which match skin sublayers), and Computational-Fluid-Dynamics-like (shortened to CFD-like in the remainder of this review) models. The latter two are commonly grouped as physiologically-based pharmacokinetic (PBPK) mechanistic models. Quantitative Structure Permeability Relationships (QSPR) first appeared to predict skin permeability of chemicals through the different layers which form the skin by using the molecular weight, the octanol-water partition coefficient, and the solubility in water of a given component as independent variables (input data). The compartmental approach followed in complexity and introduced mass exchange between the layers, i.e. treated as separate compartments, at a rate determined by experimental data fittings. The common feature of these two model categories consists in treating each skin sublayer as a homogeneous medium with no distinct internal features [23,24]. Finally, CFD-like PBPK models have been introduced in an attempt to gain more detail in the description of pharmacokinetics and ultimately full versatility. A CFD-like PBPK modelling approach consists in reproducing the actual
geometry of the skin through a Computer Aided Design (CAD) software, and discretize the domain analogously to the modelling process followed in Computational Fluid Dynamics. The diffusion equations, or a combination of the latter and the Navier-Stokes equation, can be solved in the discretized domain to provide a more realistic visualization of the xenobiotic absorption. CFD-like models still rely, however, on experimental data of the diffusion and partition coefficients. These models are therefore, useful to provide insight on some specifics of the problem, such as concentration depth-profiles, which would be otherwise difficult to get using experimental techniques. Full validation of specific purpose CFD-like models to describe any scenario other than that used to obtain basic mass transfer quantities has not been achieved so far however, limiting the application range, and making it necessary to complement the simulations with costly, or directly not available, experimental data. The following subsections provide further detail on the above mentioned *in silico* modelling approaches found in the literature, with the intention of reinforcing the need for the improvements proposed in section 4 as future work.

### 2.1. Quantitative Structure Permeability Relationships (QSPR models)

QSPR models are regression models frequently used in chemistry and biology to determine hazards posed by substances used in industry. Being based on regression of data obtained experimentally from *in vitro* assays at guaranteed steady-state conditions, QSPR models are restricted to the application being considered, and cannot be extrapolated to other conditions or simply to describe particular features of the skin described further down in this document, such as the appendageal shortcut. QSPR models aim on the other hand, to obtain accurate predictions of the permeability of the skin to a given substance, and thus constitute a useful tool for risk assessment. The model provided by Potts and Guy for instance has been recently used to evaluate the extension of the Threshold of Toxicological Concern (TTC) guidelines issued by the European Union [25–27]. They have also been used in the frame of the European Union COSMOS project, which aims to study the toxicity of cosmetic products. The input data (independent variables) to QSPR models are the measurements of the solubility in lipids, the partition coefficients and the molecular weight. Measuring solubility in lipids presents some experimental difficulties however, and surrogates are often sought, although providing a source of error (octanol is a common surrogate). The partition coefficients between the vehicle and the lipid are necessary to determine the concentration of the xenobiotic component at the outermost part of the stratum corneum, given the concentration in the vehicle. Once the permeability is established, the next step in the development of the
model is the application of the diffusion laws to obtain the mass transfer rate, total amount of substance being absorbed, etc., thus assessing the penetration potential of a given substance and the related hazards. The solute transport through the skin under steady-state conditions can be calculated by integrating Fick’s first law, which allows for the calculation of the maximum flux. The integrated expression for the first Fick’s law gives the total amount of substance absorbed by the skin as a function of time and depth into the skin. Immediately after the application of the topical solution, there is, however, a lag time which varies considerably with the solute size—from 0.5 h for the permeation of water through the stratum corneum to hundreds or thousands of hours for larger solutes.

The value of the maximum flux should however be corrected depending on the characteristics of a given xenobiotic. The European Commission has issued recommended values of the percentage of absorption rate in order to set guidelines for risk assessment policies. A value of 10% is used in case of solutes with a molecular weight greater than 500 Da and octanol-water partition coefficient smaller than -1 or higher than 4, otherwise 100% of the maximum flux applies [28]. Considering that the vehicle can be approximated to an infinite source, the maximum flux is the result of multiplying the permeability coefficient and the solubility of the xenobiotic.

QSAR models have been reported to obtain accurate calculations of the permeability of a number of substances via in vitro experimentation, being a widespread assumption that the stratum corneum behaves as a homogeneous layer. Details on the morphology of the stratum corneum are neglected, and thus, the quantification of the relative importance between the three different absorption routes within the stratum corneum (intracellular, lipidic, and appendageal) cannot be assessed, which might result in errors and lack of useful knowledge as to how skin appendages facilitate the penetration of large molecules. Another common assumption is that the resistance to skin absorption comes only from the stratum corneum, neglecting the resistance posed by the viable epidermis and upper avascular dermis, and possibly resulting in an over-prediction of the solute concentration reached in the highly vascularised layers of the skin.

A comprehensive amount of information related to experimental data on permeation coefficients of hazardous substances has been gathered in data bases, although they present significant variations owed to a number of factors related to the subject or to specific conditions of the experiment. Some of these data bases published in the literature in the last three decades are summarised in Table 1.
Consequently, being the result of experimental data fittings, QSPR models do not constitute mechanistic tools to be validated against other experimental data sets obtained a posteriori, and therefore use them as predictive tools. QSPR models do not describe any particularity of the skin morphology either, losing their applicability to relevant cases such as the use of the appendageal route as a shortcut or skin metabolism. Some QSPR models are, thus black box models with limited application. That is the case of those models based on statistical learning approaches such as the k-nearest neighbour model [41]. These need to be catalogued and curated, highlighting the exact conditions of the *in vitro* procedures, in order to constitute a source of information of the hazards related to a given substance. Overcome their limitations with the aid of mechanistic tools however, would result in a substantial gain of information useful for many purposes ranging from design to hazard prevention. Furthermore, QSPR models cannot be extrapolated to conditions other than those upon which the data have been obtained, and therefore they cannot be used as predictive tools with the potential of saving money and resources for experimentation. QSPR models are still needed though, to support mechanistic models by providing the values of the parameters needed to discretise and solve the diffusion equations.

As for the quality of these models to represent the penetration of xenobiotic substances within the skin, some QSPR models present a high degree of variability, reflected in the poor correlation coefficients resulting from fitting the data from *in vitro* assays. The simplest QSPR models obtain the permeation coefficient from the linear regression of basic structural descriptors, namely the molecular size (denoted by the molecular volume or the molecular weight) and the hydrophobicity (expressed by the octanol-water partition coefficient), although others utilise complex parameters such as quantum chemical indices.

Table 2 presents a summary of several QSPR models with their correlation coefficients in order to measure the goodness of the fitting. The models of Potts and Guy [26,27], Mass and Cronin [42], and Magnusson et al. [39] are obtained using only the hydrophobicity and the molecular size and present correlation coefficients which range from 0.670 to 0.847. The model of Basak et al. [43] considers more than two descriptors, thus taking into account more complex molecular morphology aspects, although the goodness of the fitting does not necessarily improve with respect to the other models. This variability in the results needs to be taken into account when considering mechanistic models which use
QSPR models as a source of diffusion-related data so as to consider potential prediction errors.

2.2. Compartmental PBPK models

In the compartmental approach, concentration values are obtained by solving linearized diffusion equations of each compartment, which results in good economy of computational resources. These models consider each layer of the skin as a separate compartment with homogeneous properties. Compartmental models give, as a result, a single value of the xenobiotic concentration for each compartment, i.e. one compartment per skin sublayer. Although these models do not give a concentration depth-profile as a function of the depth in each compartment, they can be considered as an approximate attempt to provide a description of the concentration depth-profiles caused by the absorption of a xenobiotic component within the skin if one considers the whole assemble of compartments. In the limit, an infinite number of compartments would provide a well described and smooth concentration depth-profile.

Each layer has therefore, an associated mass-balance ordinary differential equation in order to represent the movement of the chemical in and out of the compartment, resulting in an acceptable approximation of the concentration reached in the target layer or the systemic circulation. Generalisation of the compartmental model to any number of layers was introduced by Anissimov, who determined that the mass transfer rate constants between the different compartments can be expressed as a function of the diffusion time [44].

Compartmental models present thus an approximation to the concentration depth-profiles found and focus on the calculation of the mass transfer rate between layers, contrarily to previously discussed QSAR models, the objective of which is the calculation of permeability by fitting experimental data, with no solving of the diffusion equations. Extra compartments can be added to these models in order to account for storage of a given chemical into the skin and volatilisation of the component after topical application. Compartmental models are useful to represent the latter features, which would otherwise result in excessive computational load when represented in QSPR and PBPK models [45]. The complexity of compartmental models lies therefore, in determining the number of compartments needed to describe each application with an acceptable degree of accuracy. Simple compartmental models were limited to the study of the mass exchange between two compartments, namely the low permeability stratum corneum, and a high permeability second compartment which represents the viable epidermis and the upper
avascular dermis [46,47]. More complex models, featuring up to five compartments in a cascade disposition have been reported in the literature, too, in order to get a more accurate prediction of xenobiotic concentration at the target layer [48,49].

2.3. Computational-Fluid-Dynamics-like PBPK models

PBPK models follow in complexity and, contrarily to the previous categories, are based on the inclusion of the physiological characteristics of the skin such as the depth of each layer to solve the diffusion equations [50]. The spatial dependence of the concentration is therefore represented instead of considering separate black-box compartments characterised by a constant value of the concentration. The diffusion equation can be solved analytically upon some assumptions or numerically by means of commercial or open codes based on finite differences, finite element methods, or finite volume methods. CFD-like PBPK models aim therefore to provide a detailed description of the concentration depth-profile within each skin sublayer. CFD-like PBPK models, however, are often supported by experimental data sets to fix the values of the mass transfer parameters—through QSPR models previously developed for instance—and therefore, their field of use are often limited to the extent of the in vitro assays used to develop those QSPR models. Their capability to be extrapolated to describe any modelling scenario remains limited and depends on the input data to the model and ulterior validation against specific-purpose data. CFD-like models feature thus, the mathematics needed to reach an accurate description of the diffusion process, with more physiological detail than the compartmental models described in section 2.2. To gain accuracy, CFD-like PBPK models need to be complemented with a description of the skin geometry and specifics such as appendages or distribution of blood vessels. Today, research efforts are directed towards the development of polyvalent models capable of being applied to any potential scenario, as well of including both further pharmacokinetic features such as metabolic routes and excretion, and morphology specifics of the skin such as hair follicles and pilosebaceous glands. A schematic of the structure of a QSPR-supported CFD-like PBPK approach is described in Figure 1. The flow of information begins with the fitting of experimental data to calculate the parameters needed to solve the diffusion equations, and the morphology of the skin sublayers considered in the model. The output consists in the concentration depth-profiles, the accuracy of which increases with the degree of detail of the morphology data introduced in the model and the quality of the QSPR fitting. Data on the validation of PBPK models in the literature are nonetheless scarce. Kattou et al. [22] reported a comparison between their PBPK model and the experimental data of Otberg et al. [51],
who carried out in vivo assays in which a solution with ethanol and propylene glycol containing 2.5% caffeine was topically applied to the chest of six volunteers. The hair follicles were blocked using wax, and the concentration of caffeine in the blood was measured by taking blood samples from the volunteers. In the simulations, only the propylene glycol was considered for simplification. The comparison between in silico and in vitro series of data with and without hair follicle blocking shows good agreement, providing confidence in this modelling category. The experimental data show a high degree of variability attributed to the particular characteristics of the individuals from which data are sampled though, but generally speaking, the order of magnitude is the similar in both data series. An offset between the in vitro and the in silico concentration peak times is observed, which appears delayed in the case of the simulations. With these results in mind and in order to increase the accuracy of the model, the next step in its development might well be the inclusion of metabolic routes, i.e. by means of the introduction of mass source terms in the discretised equations, to represent the generation/consumption of substances derived from the interaction between substances already present in the skin and a given xenobiotic.

The model of Kattou et al. [22] focuses on both the epidermis (considering the stratum corneum and the viable epidermis) and the dermis, for which the mathematical terms describing convective transport are defined so as to accommodate the diffusion within the systemic circulation. The main transport mechanism in the stratum corneum and the viable epidermis is passive diffusion and therefore, the convective term is omitted in the mathematical description of these skin sublayers. The model includes three possible penetration pathways: the route through the lipids in the stratum corneum and the subsequent viable epidermis and dermis, the intracellular route through the low permeability corneocytes which form the stratum corneum (the structural description of the stratum corneum by means of the bricks and mortar model is included in the following subsection 2.3.2), and through the hair follicle, which constitutes a shortcut for xenobiotic penetration into the highly vascularised dermis. As boundary conditions, Kattou et al. [22] considered a 2D geometry with zero flux outer boundaries, which means no loss of active component through evaporation to the environment and overall unidirectional diffusion towards the dermis, the latter being a small source of error when a sufficient portion of skin is considered. A structured grid was obtained by discretisation of the 2D domain. The method of lines was applied to simplify the governing partial differential equations into linear differential equations [52]. The simplifications introduced in this model were the
suppression of the bending of the stratum corneum in the region nearby the hair follicle and the funnel-shaped infundibulum [53].

Considering that CFD-like PBPK models rely on the morphological description of the skin layers, a detailed description of the modelling specifics of each layer is included in the following subsections, e.g. vehicle, stratum corneum, viable epidermis, and appendages, in order to provide the reader with an idea of the reach of state-of-the-art modelling and the potential gaps which arise.

2.3.1. Modelling of diffusion through the vehicle

In some cases, the vehicle can be approximated as an infinite source of the xenobiotic studied, and so has been implemented in the boundary conditions in some PBPK models reported in the literature. In the in silico calculation presented by Chen et al. [13] for instance, the vehicle is simulated as an infinite source with a constant concentration of the active component, with a thickness of 0.7 mm. The approximation of an infinite source is appropriate provided its initial concentration is far above the saturation point and that concentration in the vehicle during the experimental period (~30 min) does not drop below the solubility value. This opens the question as to what can occur if a vehicle layer with a finite thickness and active component concentration is implemented.

New directions in the modelling of the vehicle (also discussed in section 4) would consist in including other specific components that have been as carefully selected as the drug/chemical itself. These added components might help with improving the delivery, stability or activity of the active ingredient, or simply by facilitating the delivery via an increase in moisture content in the skin. Commonly used components include surfactants and penetration enhancers, which increase the diffusion coefficient of the xenobiotic component by disrupting the barrier which constitutes the stratum corneum. Penetration enhancers may also improve the partitioning between the formulation and the stratum corneum, or decrease the skin thickness to facilitate permeation [54].

2.3.2. Modelling of the stratum corneum. The ‘bricks and mortar’ approach

The stratum corneum represents the main barrier to xenobiotic penetration, and is therefore rate-limiting. Detailed description of its geometry and the three possible pathways through it (intracellular route through low permeability corneocytes, high permeability lipidic route, and appendageal shortcut) is necessary in order to attain small comparative errors between in vitro and in silico data. Lately-developed diffusive-based models have incorporated a detailed description of the morphology of the stratum corneum
known as the ‘brick and mortar’ model [55,56]. The stratum corneum is mainly formed of several consecutive layers of low permeability keratin cells named corneocytes, which result from the replacement of intracellular organelles by a compact proteinaceous cytoskeleton, and lipids. In the ‘bricks and mortar’ description, each corneocyte is treated as a block with near-zero permeability linked to the adjacent block through a lipidic gap where diffusion occurs, i.e. the mortar. The composition of the stratum corneum depends on the area of the anatomical location considered though, but on average is formed of 15–25 layers of flattened, hexagonal corneocytes. Each corneocyte has an approximate diameter of 30–50 μm and a thickness which varies between 0.2–0.5 μm [57]. The mortar constitutes between 10% and 15% of the dry mass of the stratum corneum, and therefore, having a high proportion of water, it serves as the main channel for xenobiotic permeation. The implementation of a PBPK model featuring the geometry of the stratum corneum as a ‘bricks and mortar’ zone can shed light on the relative importance of those three routes based on a number of factors such as hydration degree. In this direction, Naegel et al. and D. Feuchter et al. [58,59] developed a 3D computational model to account for the effect of the corneocyte geometry on the overall diffusion of xenobiotic components through the stratum corneum. The authors implemented corneocytes with, in increasing order of realism, ribbon, cuboid, and tetrakaidekahedral shapes based on previously reported literature data [60,61], reaching the conclusion that the morphology of the corneocytes plays a key role on the permeability of the stratum corneum. A tetrakaidekahedral corneocyte resulted in skin permeability that doubles that found for 2D ribbons. Their work has application in the understanding of skin diseases such as psoriasis, which affect the morphology of the stratum corneum, and thus, results in a change of skin permeability. The treatment of the stratum corneum as a heterogeneous layer due to the existence of both corneocytes and the lipidic gap implies a considerable computational effort. Muha et al. [62] developed in this regard a mathematical method to implement a homogeneous equivalent to the brick-and-mortar model.

Common defining values of geometry and composition of the stratum corneum are provided by Johnson et al. [63] and Mitragoti et al [64]. Apart from the particular location in the body considered, the thickness of the stratum corneum presents a strong dependency on the hydration degree, varying from 52 μm to 16 μm upon low hydration, which affects the diffusion of any xenobiotic component strongly.

To model the stratum corneum, the properties of the ‘bricks’ and the ‘mortar’ need to be established separately. In particular, the diffusion coefficient of a given component through
the corneocytes (intracellular route), and through the mortar (lipidic route), as well as the partition coefficients between the lipids and the corneocytes, need to be determined. Anisotropic diffusion in the lipids of the stratum corneum, which is caused by the composition which is formed by crystalline and liquid parts, is usually neglected in the models reported in the literature [65], which consider the lipid phase in the ‘bricks and mortar’ model as homogeneous, resulting in an approximation which has proved to be accurate enough.

2.3.3. Modelling of appendageal route: The hair follicle

The hair follicle can act as a shortcut for xenobiotic penetration when active, that is to say, sebum is being produced and the hair follicle is growing [66]. Skin appendages, i.e. hair follicles, have not been traditionally considered as penetration routes owing to the small proportion of area they occupy—approximately 0.1% [67]. Hair follicles possess high vascularization however, which makes them even more attractive to be used as a shortcut for xenobiotic administration. They also give as a result, higher area than initially thought owing to the fact that they reach deep into the dermis, increasing the actual area available for xenobiotic permeation. Hair follicles present also diffusion coefficients significantly higher than the stratum corneum, making the follicular route more interesting from the point of view of xenobiotic absorption than the rest of the skin surface [68]. Some studies consequently suggest that the follicular route is particularly favourable for hydrophilic and high molecular weight molecules, as well as in the case of drugs delivered by using particles, owing to the difficulty of these molecules to cross the stratum corneum [69]. In spite of those advantages, models of the follicular absorption route are scarce in the literature, being the reported models presented by Liu et al. [70], Bookout et al. [71], and Frum et al. [72] summarised in Table 3. Liu et al. [70] compared the penetration of caffeine in human skin blocking and without blocking hair follicles using two different methods including a compartmental model with first order absorption and elimination. Bookout et al. [71] also reported a compartmental model, an interesting feature of their model being the inclusion of parallel subcompartments to represent the hair follicles. These models highlight the importance of the follicular route in xenobiotic penetration, with the absorption from the stratum corneum showing an approximately 10 min delay while there was no delay for absorption from hair follicles. An interesting modelling approach is that of Radtke et al. [73] who presented an approach based on Brownian dynamics instead of solving diffusion equations.

2.3.4 Modelling the transport through viable epidermis and dermis.
In terms of modelling both the viable epidermis and the dermis behave similarly, since they have almost identical multiphase compositions. The dermis consists of collagen and elastin fibres immersed in an aqueous matrix, with a thickness which depends on the anatomical location and reaches 4 mm at its maximum. The hair follicles and sweat glands are embedded within the dermal layer, and provide a fast route towards the rich vascularisation located in the transition between the epidermis and the dermis [74]. Relevant to mass transport and therefore to be accounted for, are also the presence of proteins where drugs can bind and small ramifications of the blood and lymphatic system which might be modelled as negative mass sources in the transport equation. Dermic blood vessels are concentrated in the upper 100–200 μm of the dermis, i.e. the papillary dermis, and decrease considerably in the lower part of the dermis, i.e. the reticular dermis. These aspects are also important in order to define areas of the computational domain where convection should be considered. The overall structure of the vessels consists of a dense aggregate of blood vessels running along the epidermal–dermal junction, a less dense plexus found much deeper in the lower (reticular) dermis, and connecting vessels that also connect to the hair follicles and sweat ducts [75].

2.4. Experimental methods for model validation

A brief description of experimental visualization techniques is given in this section so as to provide some information about the types of techniques that might be used for that purpose, with intention of not limiting the contents of this work to a purely numerical approach and also to provide some guidelines as to the type of data sources to be used for validation. Data obtained by in vivo/ex vivo studies and data sets from previous in silico methods can also be used for preliminary validation of skin permeation models. Ex vivo characterisation is usually combined with imaging methods as an effective method to use clinical waste for skin permeation studies, and thus avoiding ethical considerations. Sample preparation and preservation for ex vivo studies is critical, i.e. including removing the subcutaneous adipose tissue, and histological characterisation, and needs to be performed to determine the exact thickness and composition as a function of the location in the body. Pre- and post-examination integrity checks need to be performed as well to assess any damage and to ensure a reliable experimental basis to obtain good data [76]. Imaging techniques are commonly used to assess xenobiotic dermal penetration, allowing for the obtaining of concentration values in the receptor fluid. Among the available techniques, Dennerlein et al. used liquid chromatography linked with inductively coupled plasma-mass spectrometry (LC-ICP-MS), which allowed for the obtaining of the evolution
of concentrations over time [77]. Otberg et al [51], obtained similar data by applying a new surface ionization mass spectrometry (SI/MS) technique, and measured the intensity of transcutaneous permeation and the transient evolution of the concentration of caffeine in the blood stream. The authors focused on the effect of hair follicles on the permeation of aqueous caffeine solutions. Varnish-wax microdrops allowed the authors to modulate the access through the follicular route, establishing a comparison method to assess the effect of the hair follicle on the overall absorption rate. The predictive capability of the model reported by Chen et al. [13] was checked by comparison against data obtained in vivo after intentional exposure of human volunteers to 4-cyanophenol. Cleansing of remaining 4-cyanophenol and excision followed, so as to obtain samples of the stratum corneum. Subsequent measurement of the concentration depth-profiles followed by using Fourier transform infrared spectroscopy (ATR-FTIR) were performed [78].

3. Conclusions

This review summarises the most common approaches developed so far to model the pharmacokinetics of xenobiotic substances within human skin. Models are classified in three categories according to the level of detail with regard to the skin morphology: Quantitative Structure Permeability Relationships (QSPR) and Physiologically-Based Pharmacokinetics (PBPK), which comprises compartmental models and diffusions-based approaches. A considerable number of these models are available in the literature on the absorption characteristics of xenobiotic components (mostly QSPR and compartmental models), whereas models describing other aspects of the pharmacokinetic activity, namely deposition, metabolism and excretion (summarised in the literature with the acronym ADME), are still relatively scarce.

An approach based on the discretization of the diffusion equations was used to consider specific morphological elements of the skin.

QSPR models—also known in the literature as QSAR models—are the result of experimental fittings that relate the permeability of the skin to a given substance, i.e. usually considering only the stratum corneum, to parameters which define the size of the active principle and its solubility in lipids, among others. QSPR models offer an overall estimation of the penetration potential of the substance in study and thus, they constitute useful tools for risk assessment purposes. The absorption process is however treated as a black box, in which the physico-chemical characteristics of the compound are introduced and the model estimates a penetration potential and these models lack a detailed physical description of the skin morphology.
Compartmental models aim to close the gap of the loss of detail by introducing and solving linearized diffusion equations. This methodology consists in dividing the skin in different compartments, that is, one compartment per each skin sublayer with similar physico-chemical characteristics. Each compartment is characterised by one simplified diffusion equation, which gives a constant value of concentration as a result. Compartmental models provide thus an estimation of the concentration in each of the layers which form the skin, as well as the transfer rate between compartments. Compartmental models represent a first estimation of the actual distribution of solvent within the skin, but lack detail since they do not take into account crucial morphology aspects of the skin, such as hair follicles and the pilosebaceous glands.

To bridge this gap, Physiologically-Based PharmacoKinetic (PBPK) CFD-like models were developed. These models consider the morphology of the skin, e.g. the thickness of each sublayer, the distribution of blood vessels, etc., as they include a discretization of the computational domain in a similar manner to a Computational Fluid Dynamics. CFD-like PBPK models can therefore be used to include the morphology of the main barrier to xenobiotic penetration, i.e. the stratum corneum, through the inclusion of the ‘bricks and mortar’ approach, and other routes such as the follicular route, which is of high interest to the cosmetic industry and for drug delivery purposes since it has been experimentally proved as an effective shortcut to reach the highly vascularised dermis. CFD-like PBPK models also allow for insight on the different penetration routes (low permeability intracellular route, high permeability lipidic route, and appendageal route), establishing the relative importance between them as a function of the physico-chemical nature of the particular compound studied, mainly the size of the molecule, its lipophilicity, and its solubility in water. Although the increased degree of morphological detail, the capabilities of PBPK models have not been widely exploited as of yet.

As a final conclusion, the modelling of xenobiotic penetration into human skin appears to be in an early stage of development. A view on the current panorama of the modelling of in silico xenobiotic absorption into human skin reveals itself as rather incomplete, with many of the models consisting in the fitting of gathered experimental data, i.e. these are known as QSPR models. The next step in complexity are compartmental models, which consist on the resolution of a set of linearized diffusion equations, giving specific constant concentration values in each layer of the skin (compartment) as a result. To date, both models present dependence to some extent, on experimental data obtained previously, whether on data constituting the basis to the obtaining of an experimental correlation in the
case of QSPR models, or on the obtaining of the basic parameters needed to solve the diffusion equation in the case of compartmental and CFD-like PBPK models or simply for validation purposes. The pathway towards comprehensive and fully predictive \textit{in silico} models seems to include the use of software offering the user the flexibility to implement in those models characteristics not explored in the field as of yet, paving the way to a more accurate virtual representation of the skin and its behaviour.

4. Future research directions.

This section proposes different directions to expand the capabilities of CFD-like PBPK models by considering other key features that could provide a competitive advantage when designing products applied on the skin. These different potential research directions are summarised in Figure 2 and commented as follows:

a) Effect of vehicle thickness, microstructure and physical properties on xenobiotic transfer.

The capabilities of CFD-like PBPK models could be expanded by considering the microstructure of the vehicle and the slow release of active principles or the evolution of the vehicle properties while its solvent evaporates or diffuses into the skin. Furthermore the transfer of nano-particles through the skin to the blood, that is a potential major side effect of their use in cosmetics (e.g. TiO$_2$ and ZnO$_2$ in sunscreens [79, 80, 81]), could be included in the discretization of the diffusion equations. This could support the development of novel cosmetic formulations able to slow down or to stop the transfer of nanoparticle to the skin and prevent/limit their negative effects on the skin and on the body.

b) The effect of skin hydration on the transfer of xenobiotics and on the skin microstructure

Skin hydration causes the swelling of the corneocytes, significantly changing the geometry of the stratum corneum, and its permeability to the active principles found in a particular formulation, but also the diffusion of undesired xenobiotics (e.g. air pollution). Experimental evidence suggests that the swelling of the stratum corneum—the main barrier to xenobiotic penetration—is mainly due to the change in shape of the corneocytes, particularly in the direction perpendicular to the skin surface, resulting in a reduction of their aspect ratio. Bouwstra et al. [82] provide quantification of this phenomenon, and suggest that not all the corneocytes swell to the same extent, with those near the
outermost part of the stratum corneum showing less aspect ratio than those at deeper parts of the stratum corneum as a result.

The change in morphology and composition of the stratum corneum owed to hydration results in the modification of the diffusion coefficient [83]. Owing to the moisture increase causing a reduction of the proportion of low-permeability keratine in the stratum corneum, there is an exponential increase on the diffusion coefficient with the moisture content. There is thus a combined effect of moisturization, with an increase of thickness of the stratum corneum on the one hand and an increase in the diffusion coefficient on the other. The former should result in hindered xenobiotic penetration owing to the increase in the thickness of the main barrier to penetration, whereas the latter should enhance it. The solving of the diffusion equations in a discretized geometry representing the skin should constitute a powerful tool for the analysis of this double effect.

c) Modelling perspiration and its effects on xenobiotic transfer

Skin perspiration consists in the secretion of sweat by the sweat glands, its transport through the pores toward the skin surface and the formation of a liquid layer at the surface of the skin. The effect of sweat secretion on the skin moisture content, on the delivery of actives and on the undesired influx of nanoparticles should be evaluated. If the sweat duct can act as a preferential path for xenobiotic ingress, then the effect of the outflow of sweat should play an important role in modulating the xenobiotic transfer into the skin. The modelling of this effect can be easily accomplished by adding mass source terms in the diffusion equations, defined only in the relevant computational cells.

d) Introduction of metabolic pathways

Metabolic reactions of the compounds absorbed in the human skin can also be modelled by adding mass source terms to the mass governing the diffusion within the skin layers. Those mass source terms are posed as a function of the reaction rate and account for the generation/consumption of components owing to chemical reactions. The addition of metabolic routes, which will potentially reduce comparison errors with experimental data or the deposition of xenobiotic substances by chemical binding to components which form the different layers of the skin [84]. Cutaneous metabolism of a chemical applied to the skin is difficult to differentiate in vivo from the systemic metabolism occurring mainly in the liver by analysing blood and excreta samples. In vitro approaches can provide accurate insight on the specifics of skin metabolism by isolating the skin from the metabolic activity in the rest of the body, providing a source of data for validation purposes.
Acknowledgements

The authors would like to express their gratitude to Nestlé Research Center for the funding provided.
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Figure 1 This input/output schematic diagram of a sorption-only CFD-like PBPK model shows the data needed to run the model and the target output. The main difference with respect to the previously mentioned model categories is that, in this case, the physiology of the skin layers is also included, meaning that not only are the diffusion and partition coefficients used as an input, but also the geometric dimensions and morphology are fundamental to obtain a discretized representation of the skin.
Figure 2 Schematic view of future research directions and capabilities of the CFD-like PBPK modelling approach
Table 1 Summary of available databases with permeability coefficients available for QSPR models published in the scientific literature as of 2017:

| Author                  | No. of partition coeff. \((K_p)\) values | Molecular weight \((M_w)\) [g/mol] | \(\log(K_{ow})\) \((K_{ow} \text{ being the octanol-water partition coeff.})\) | Further comments |
|-------------------------|------------------------------------------|----------------------------------|--------------------------------------------------------------------------------|------------------|
| Brown et al. (2016) [29]| 392 from 245 compounds                   | 18–765                           | -6.8 to 7.6                                                                     | Only from human skin and using water as the vehicle. |
| Alves et al. (2015) [30]| -                                       | -                                | -4.85 to -0.94                                                                  | Data from human and rodent skin samples. 185 and 96 compounds considered, respectively. |
| Chen et al. (2013) [31] | 71 from 35 compounds                     | -                                | -                                                                               | Only from human skin and hydrophilic chemicals. |
| Flynn (1990) [32]       | 97 from 94 compounds                     | 18–765                           | -3 to 6                                                                         | High variability owed to use of samples of various parts of the body, which affects skin composition and morphology. This database was revised, double-checked and expanded by Johnson et al. (1995) [33] and Degim et al. (1998) [34] |
| Wilschut et al. (1995) [35] | 123 from 99 compounds                   | -                                | -                                                                               | Various chemical classes considered including monoaromatic hydrocarbons, volatile halogenated hydrocarbons, phenols and steroids. Useful for industrial hazard evaluation, but lacking components used in pharmaceutical and cosmeceutical industries. |
| Kirchner et al. (1997) [36] | 114 from 51 compounds                   | -                                | -                                                                               | A number of results compiled in this database are not the result of in vitro experiments but from the application of the Guy and Potts correlation [26,27]. |
| Patel et al. (2002) [37] | 186 from 158 compounds                   | -                                | -                                                                               | High diversity of compounds structure. |
| Vecchia et al. (2003) [38] | 170 from 127 compounds                   | 18–584                           | -3.1 to 4.6                                                                     | - |
| Magnusson et al. (2004) [39] | -                                       | 18–765                           | -5.7 to 8.7                                                                     | The database directly provides the values of the maximum flux. |
| EDETOX (2004) [40]      | 4800 from 320 compounds                  | -                                | -                                                                               | Considerable additional information (description of the skin samples, lag time, exposure time, etc. |
**Table 2 Summary of QSPR models based on linear regression and the quality of the fit**

| Author                     | Correlation coefficient ($R^2$) | Comment                                                                 |
|----------------------------|---------------------------------|-------------------------------------------------------------------------|
| Potts and Guy (1992) [26,27] | 0.670                           | Hydrophobicity and molecule size as structural descriptors              |
| Moss and Cronin (2002) [42] | 0.820                           | Id.                                                                    |
| Magnusson et al (2004) [39]  | 0.847                           | Id.                                                                    |
| Basak et al. (2007) [43]    | 0.67–0.87                       | Model including not only two structural descriptors but also shape descriptors and quantum chemical indices. |
Table 3 Summary of relevant literature on hair follicle modelling

| Reference       | Method                                                                 | Conclusions                                                                                                                                       |
|-----------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Liu et al. [70] | Wagner-Nelson method and compartmental with first order absorption and elimination. | • Tenfold increase of absorption rate through follicles with respect to absorption rate through stratum corneum.  
• No absorption delay in follicles; whilst 10 min delay in the stratum corneum.       |
| Bookout et al. [71] | Enhanced PBPK model obtained with measurable parameters and featuring parallel layers representing the skin appendages | • Improved predictions of chemical concentrations are obtained with the multi-layered model rather than with the previous homogeneous model in which the present model is based. |
| Frum et al. [72] | Skin sandwich technique.                                                | • Above a critical value of octanol-water partition coefficient ($K_{ow}$), the relation between the % follicular contribution and the drug absorption rate depends on the lipophilicity. |
| Kattou et al. [22] | Predictive model using open code.                                      | • Data with which the model is compared is not used to predict the diffusion parameters which define the model.                                    |