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1 Recent developments in skin mimic systems to predict transdermal permeation

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6 **Abstract**

7 In recent years there has been a drive to create experimental techniques that can facilitate the
8 accurate and precise prediction of transdermal permeation without the use of *in vivo* studies. This
9 review considers why permeation data is essential, provides a brief summary as to how skin acts as a
10 natural barrier to permeation and discusses why *in vivo* studies are undesirable. This is followed by an
11 in-depth discussion on the extensive range of alternative methods that have been developed in recent
12 years. All of the major ‘skin mimic systems’ are considered including: *in vitro models* using synthetic
13 membranes, mathematical models including quantitative structure-permeability relationships
14 (QSPRs), human skin equivalents and chromatographic based methods. All of these model based
15 systems are ideally trying to achieve the same end-point, namely a reliable *in vitro-in vivo* correlation,
16 i.e. matching non-*in vivo* obtained data with that from human clinical trials. It is only by achieving
17 this aim, that any new method of obtaining permeation data can be acknowledged as a potential
18 replacement for animal studies, for the determination of transdermal permeation. In this review the
19 relevance, and potential applicability, of the various model systems will also be discussed.

20 **Introduction**

21 Skin is a natural barrier yet, for many years has been a desirable route of administration for
22 therapeutic drugs. For any cosmetic skincare product, environmental, or pharmaceutical compound, it
23 is vital to know both the rate, and extent, of transdermal permeation to satisfy regulatory authorities.
24 There are several ways the required data can be acquired, broadly categorized into *in vivo*, *ex vivo* and
25 *in vitro* models (1). For all products that are intended for percutaneous permeation the main intention
26 is optimisation of the drug and formulation to achieve maximum *in vivo* performance. Ideally, human
27 studies would be undertaken to ascertain such information. However, this is not normally feasible
28 during development. Thus, researchers resort to the aforementioned models. Until recent years, the
29 majority of studies in this area utilised a wide variety of animals, mainly rodents (2), to obtain drug
30 permeation data which can then be used as a basis to predict clinical outcomes. Although the volume
31 of data that has arisen from such *in vivo* work has been beneficial in the development of some
32 pharmaceutical products, there is a clear trend to move away from animal studies (3) for three main
33 reasons. Firstly, ethical issues surrounding the use of live animals is a major incentive to consider
34 other testing methods. This is especially true in the EU and several other markets where it is already
35 no longer permissible for cosmetic product testing to involve the use of animals, with further
36 restrictions to follow (4). Although their use in the pharmaceutical industry is still permissible, there is

37 a clear move towards avoiding animal studies where possible. Secondly, researchers are adopting skin
38 mimic systems because the animal study data that does exist cannot easily be compared and analysed
39 across experimental studies as a result of the diverse range of animals used in research. Even within
40 datasets from similar species the results may vary so considerably that comparative analysis is
41 impossible, even within human studies inter-individual variation can be an issue (5, 6). The third, and
42 possibly the most compelling, reason that transdermal permeation studies may avoid the use of
43 animals is a lack of clear correlation between animal and human clinical trial data. For example, it has
44 been shown that rodent skin generally shows higher permeation rates than human skin, often leading
45 to incorrect conclusions from experimental data (7). Porcine skin, particularly that from the ear, is
46 used in permeation studies as it has been shown to have similar properties to human skin (8) yet full
47 animal studies with pigs are not ideal for the reasons previously outlined.

48 The outermost layer of human skin is the stratum corneum and is the main barrier for
49 transdermal permeation. In human stratum corneum, the major lipid classes are ceramides, cholesterol
50 and saturated long chain free fatty acids (9). Lipid organisation is fundamental to skin barrier
51 function, for example in diseased or dry skin the lipid composition is different from that in healthy
52 subjects. However, the specific details regarding lipid organisation within the stratum corneum are not
53 fully understood, although it is accepted they will have an influence on transdermal permeation (10).
54 It should be noted that in some cases it is not transdermal permeation that is desired but dermal
55 absorption, i.e. delivery within particular regions of the skin itself – see ref. (11-13) for examples. In
56 such circumstances transdermal permeation must still be measured, and considered, to satisfy
57 regulators regardless of whether permeation is the intended outcome or not. For dermal delivery
58 studies, drug permeation and distribution is not as specifically focused on the stratum corneum
59 permeation as transdermal studies, but will also consider the deeper layers individually, namely viable
60 epidermis and dermis. These are not so relevant to transdermal research as it is often assumed that
61 stratum corneum penetration is the rate limiting step in permeation and must therefore be the focus of
62 such studies.

63 Skin permeability is closely linked with the hydrophobicity/hydrophilicity of the molecule in
64 question. In general, researchers have shown that skin permeability decreases with increasing
65 hydrophilicity, as expected based on the lipidic structure of the skin (14). Furthermore, the rate and
66 extent of transdermal permeation is dependent upon several other factors including product
67 formulation, location of application, temperature (15, 16), volume applied and skin integrity. Recent
68 studies have begun to substantiate the importance of formulation and the considerable impact it may
69 have on permeation (17, 18) yet much work is still to be done to fully understand the relationships
70 involved.

71 This article reviews recent developments and current trends in the variety of methods reported
72 to determine transdermal permeation data, avoiding the use of *in vivo* studies. Collectively, all such
73 techniques can be referred to as ‘skin mimic systems’ yet individually they are comparatively diverse,
74 subdivided into:

- 75 • *In vitro* models using synthetic membranes
- 76 • Mathematical models including quantitative structure-permeability relationships (QSPRs)
- 77 • Human skin equivalent models
- 78 • Chromatographic models

79 **In vitro models using synthetic membranes**

80 For the past ten years, there has been general acceptance of the Organisation for Economic
81 Cooperation and Developments (OECDs) guidelines for *in vitro* methods in the examination of skin
82 permeation and distribution (No. 428). These guidelines set out a basic study design and requirements
83 to justify certain experimental parameters such as membrane choice, dose concentration and assay

84 validations. For all major *in vitro* systems, the basic experimental setup includes a phase to replicate
85 the skin surface, a separating membrane barrier and a solution phase to replicate beneath the skin –
86 see reference (19) for a full description. In all studies the sample is applied to the skin surface (donor)
87 phase, given time to pass through the membrane barrier and extracted from the second (receptor)
88 phase at given time intervals to then be analysed to determine concentration. From such data it is
89 possible to calculate a permeability coefficient, assuming ‘infinite’ doses are considered, most
90 commonly using a Franz-type diffusion cell or flow-through cell design (20). Modifications of this
91 set-up have been published, such as a novel diffusion cell which allows study of membrane diffusion
92 processes without the need for sampling of the receiver compartment (21). The proposed method
93 employs a spectrophotometer quartz cuvette containing the receiver solution with a small PTFE cap
94 containing the membrane and an injection port through which compounds can be applied. The
95 obvious benefit of this more advanced system is the non-invasive nature of obtaining permeation data
96 and its potential for continual and automated permeation measurements. Other researchers have
97 published methods to study membrane transport processes that also avoid the necessity to remove
98 samples such as surface enhanced Raman scattering (SERS) which again provides non-invasive
99 advantages compared with traditional methods (22). More recently, analysis has begun to move away
100 from the standard choice of UV-based techniques to include alternatives, such as attenuated total
101 reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and target factor analysis where it
102 was found data could be successfully deconvoluted and different components of formulations
103 identified (23).

104 Drug diffusion studies are often used to measure the movement of selected compounds
105 through a specific membrane, chosen by the researcher to mimic the *in vivo* scenario. Data acquired
106 from such studies can then be correlated with that obtained from human clinical trials. In some cases
107 the membrane selected for use may be of human origin (24, 25), although this is infrequently
108 undertaken for two main reasons, namely economic constraints and data variability. Skin for these
109 studies is often acquired following surgical procedures. Samples can vary significantly in thickness
110 and composition (often frozen for transport), which is not always a problem (26-28), although, it has
111 been reported to be an issue in many studies - as highlighted in the low reproducibility in data
112 obtained (29). Rather than using human skin, some experimental work has employed animal skin as
113 the membrane to separate the two phases. Again, this can lead to significant variability in data
114 depending upon the sample location and the species used. For example, one particular study compared
115 permeability coefficients for several commonly used drugs between hairless mouse skin and human
116 skin and found the human skin values to be far lower than the mouse skin, exemplifying the poor *in*
117 *vitro-in vivo* correlation (30).

118 Although the economic costs of using animal skin are far lower than those for human skin,
119 issues of data variability and ethical concerns have led scientists to develop many forms of synthetic
120 membranes to overcome these problems. The vast majority of work in the field of synthetic
121 membranes for transdermal and topical delivery studies has focused on the use of polymeric
122 membranes (31), such as silicone-based membranes (32). Such membranes are ideal for replacing *ex*
123 *vivo* skin as they can be synthesised to a desired thickness, are easy to handle and store, are
124 comparatively cheap, are inert, and provide reproducible data. For all of these advantages, many
125 studies have considered the suitability of replacing both human and animal skin with silicone
126 membrane for *in vitro* studies, attempting to develop a successful *in vitro* – *in vivo* relationship to
127 ensure such a membrane can be truly considered as an acceptable model system for the prediction of
128 transdermal permeation in humans. Silicone-based membranes, such as polydimethylsiloxane
129 (PDMS) are generally hydrophobic in nature, and provide a rate limiting step in drug permeation.
130 Many of the topical drug delivery diffusion studies that have employed synthetic membranes are
131 summarised in reference (31), illustrating the vast range available to researchers, from the standard
132 silicone membrane, to polyethylene to cellulose ester. The membranes exhibit variability in their pore
133 size, thickness and permeation resistance, thus affecting the rate and extent of drug penetration, which

134 in turn leads to variations in calculated permeability coefficients. Some studies involve porous
135 membranes in which case pore size in particular can be an incredibly influential factor, indicated by
136 its alternative name – ‘molecular weight cut-off’, identifying the relationship between the size of the
137 permeating molecule and the likelihood of permeation occurring. More specifically, porosity and
138 tortuosity are frequently used to define porous synthetic membrane structures, which is vital when
139 considering a porous membrane for *in vitro* studies.

140 For all synthetic membranes, the particular physicochemical properties displayed by the
141 membrane are mainly derived from the preparation method used, thus it is vital to have a reproducible
142 manufacturing technique to avoid inter-experimental variability. For a non-porous membrane,
143 permeation occurs in three stages – firstly the permeant dissolves in the membrane, secondly it
144 diffuses through the membrane and finally, it emerges from within the membrane. This process
145 follows Fick’s First Law of diffusion and permeation depends upon interactions between the
146 compound and the membrane, in a similar manner to that seen between the compound and
147 hydrophobic stratum corneum.

148 Synthetic membranes are used in transdermal studies for two specific purposes; *in vivo*
149 prediction and qualitative analysis. The latter is routinely measured using Franz-type diffusion cells as
150 part of the quality control process to ensure new products display comparable diffusion profiles to
151 those previously measured. The low cost and simplicity of the technique are ideal to ensure batch to
152 batch consistency in formulations with minimal complications from biological variations that would
153 be encountered with *in vivo* analysis. The former, i.e. the use of synthetic membranes to predict
154 transdermal permeation *in vivo*, is open to more debate as researchers attempt to correlate the
155 experimental data obtained with that expected from clinical studies. Through simplifying the
156 membrane structure to a synthetic material increases the reproducibility of the data yet also in turn,
157 removes the finer detail, such as more complex phenomena including metabolic epidermal activity.
158 For example, it has been found that introducing metabolic inhibitors can have a pronounced effect on
159 transdermal drug delivery (33), i.e. implying synthetic membranes may not be complex enough for
160 such studies. However, many feel the benefits from such a compromise outweigh the costs, for
161 example, even when replicating inflamed skin (34). It has been suggested that artificial membranes
162 can only provide a useful forecast of *in vivo* transdermal delivery when the following criteria are met
163 (35):

- 164 ○ Passive diffusion through the stratum corneum is the major resistance to transport
- 165 ○ The drug under investigation is known to be metabolically inert and not specifically
166 bound in viable skin
- 167 ○ The formulation does not contain a permeability enhancer which can interact with skin
168 but not membrane and
- 169 ○ *In vivo* experiments can be performed and correlated with *in vitro* results.

170 Investigations into the formulation aspects of the applied product have further confirmed the
171 variability that can occur as a result of modifications in the chemical nature of the excipients selected
172 (36-41). For example, research within our own group has found substantial effects based upon the
173 simple addition of an anionic surfactant to the formulation, yet no effect upon the addition of a
174 cationic surfactant (37). For example, a study into the influence of ethanol on the solubility, ionisation
175 and permeation characteristics of a model drug (ibuprofen) found the flux through silicone
176 membranes increased up to a maximum of 100 % ethanol yet, in human skin, flux was optimal at
177 lower ethanol percentages (42). Following on from this study, researchers investigated the influence
178 of propylene glycol (PG) using binary (PG:water) and ternary (ethanol:PG:water) solvent systems.
179 Fluxes were maximum for 70:30 PG:water systems in silicone membrane; however, for experiments
180 conducted with skin, the flux of ibuprofen systematically increased with increasing amounts of PG.
181 For silicone membrane, the flux values of ibuprofen from ternary systems were higher than the

182 highest values observed from the binary systems (43). Furthermore, permeation from mineral oil
183 (MO), Miglyol® 812 (MG) and binary mixtures of MO and MG found the solubility of ibuprofen to
184 be higher in MG than in MO. However, the permeation of ibuprofen from the pure vehicles and
185 combinations of both was comparable in silicone membrane. Additionally, when the permeation of
186 various hydrophilic and lipophilic vehicles was considered, a trend between flux values for the model
187 membrane and skin was evident suggesting that silicone membrane may provide information on
188 qualitative trends in skin permeation for vehicles of diverse solubility and partition characteristics
189 (44). Other studies have also demonstrated the effects excipients may have on drug solubility and
190 permeation. One such example highlights this phenomenon where drug-polymer dispersions were
191 clearly shown to improve flux for a poorly soluble drug, namely artemisinin (45). However, not all
192 excipients incorporated within transdermal formulations are capable of influencing drug transport or
193 permeating with the drug. For example, concerns had been raised about the possible implications that
194 dermal exposure to nanoparticles may have for human health. The maximum flux of such systems
195 was calculated, and the results confirmed that they are too large to permeate skin (46). Based on all of
196 these findings it can be said that the permeation of compounds through skin *may* be affected by the
197 additional compounds within a formulation, but determining the specific details of such an influence
198 is not a simple matter.

199 Some researchers have attempted to expand the applicability of using synthetic membranes to
200 maximise their suitability for permeation studies. Ng et al. (47) investigated diffusion rates of
201 ibuprofen across thirteen membranes finding variable drug fluxes, thus categorising the membranes
202 into high-flux and low-flux membranes. For example, they found that porous membranes derived
203 from various polymers demonstrated different degrees of diffusional resistance to ibuprofen,
204 indicating that there would be wide discrepancies in results obtained from different laboratories using
205 different porous membranes. They suggest that when selecting a membrane for formulation analysis it
206 is best to choose one with high porosity, a tortuosity of one and be relatively thin. Furthermore, the
207 compatibility of the membrane with the donor, and receptor, components must be considered, along
208 with the cost effectiveness of the membrane. In transdermal permeation studies, it is common to
209 measure drug release rates by monitoring the cumulative mass of drug appearing in the receptor
210 solution (M_R). However, if a synthetic membrane is placed between the donor and receptor phases
211 then there is a delay in drug transfer, and M_R is not immediately linear with respect to time. As a result
212 of this non-linearity it is vital that permeation studies are conducted for long enough that the
213 relationship achieves linearity to ensure calculated drug diffusivity values are correct (48).

214 More complex synthetic membranes have shown promise to provide a better representation of
215 the stratum corneum barrier, for example, using a polydimethylsiloxane-polycarbonate block
216 copolymer membrane (Carbosil) (49). In one such study, permeability was examined as a function of
217 permeant molecular weight, melting point, solubility, partition coefficient and diffusivity for 14 drugs
218 covering a wide range of chemical structures. It was found that Carbosil provided a higher drug
219 solubility, and consequently, higher permeability compared with human skin. However, by varying
220 the block copolymer membrane the diffusivity could be significantly modified, implying a more
221 appropriate *in vivo* model can be created. Further work in this field has led researchers to establish the
222 importance of considering membrane hydration in such studies (50). In another study two synthetic
223 lipid models (designed to replace human stratum corneum) were studied to investigate the impact of
224 volatile organic chemicals on the molecular organization of the skin barrier lipids (51). The models
225 built upon previously developed self-assembled lipid membranes, which have a composition and 3D
226 organisation similar to those of the *in vivo* lipid matrix. In one model the target chemicals were
227 incorporated in the lipids before their self-assembly, and in the other one they were applied on top of a
228 preformed lipid membrane. Encouragingly, the dose-dependent effects of the chemicals on the lateral
229 molecular organization in the models were qualitatively identical to those observed by infrared
230 spectroscopy in human skin. The study concluded that these model systems are suitable for *in vitro*
231 studies in the areas of skin biophysics, dermatology, transdermal drug delivery, and risk assessment.

232 Natural membranes have also been considered to measure permeation for model drugs,
233 including those of different molecular weights and lipophilicities using Franz-type diffusion cells. For
234 example, membranes can be taken from the outer layers of peach and tomato, the middle layers of
235 onions and with the inner layer of eggs (52). Encouragingly, results showed that the rate and amount
236 of diclofenac permeated through onion skin, metronidazole through tomato skin and erythromycin
237 through egg membrane was not significantly different from that with human skin. From these results
238 it was concluded that natural membranes have pores and channels with hydrophilic properties,
239 permitting permeation of small to middle size hydrophilic drugs to diffuse in a manner similar to
240 human skin. Other natural membranes have also been considered for transdermal studies, and
241 comparisons made with the more standard membrane options (53).

242 Within the last ten years a parallel artificial membrane permeability assay (PAMPA) has been
243 developed for the rapid determination of passive transport permeability *in vitro*, gaining acceptance in
244 pharmaceutical research. In PAMPA, a 96-well filter plate coated with a liquid artificial membrane is
245 used to separate two compartments: one containing a buffer solution of compounds to be tested
246 (defined as donor compartment) and the other containing an initial fresh buffer solution (defined as
247 acceptor compartment). In one of the earliest studies, isopropyl myristate, silicone oil, and mixtures of
248 the two components were immobilised on filters and tested as liquid supported membranes in
249 PAMPA to evaluate their potential to mimic the human skin barrier (54). Effective permeability
250 coefficients (P_e) were determined for a set of compounds using the PAMPA technique and compared
251 with the corresponding human skin permeability coefficient values (K_p). A good correlation between
252 P_e and K_p was found for compounds tested through a membrane consisting of 70 % silicone and 30 %
253 IPM. Moreover, a positive correlation between the membrane retention of compounds and stratum
254 corneum/water partition coefficients (P_{sc}) was established, implying PAMPA can be used for the
255 prediction of passive human skin permeability coefficients. Further studies in this area have further
256 confirmed the validity of the technique (55) and, along with the low cost, versatility and good
257 reproducibility (56) of the system ensure it is a feasible membrane mimic system. In 2012, Sinko et al.
258 (57) attempted to match the permeability of the rate-limiting barrier in human skin using synthetic
259 analogs of the ceramides present in the stratum corneum. The final skin-PAMPA membrane lipid
260 mixture (ceramide, free fatty acid, and cholesterol) was selected and optimized based on data from
261 three different human skin databases and the final model was found to correlate well to all of the
262 databases. The reproducibility of the skin-PAMPA model was investigated and compared to that of
263 other PAMPA models, confirming it to be a quick and cost-effective research tool that can serve as a
264 useful model of skin penetration in pharmaceutical and cosmetic research. More recently, several
265 variations of the artificial membrane employed in the PAMPA study system were analysed (isopropyl
266 myristate (IPM), ceramides and Strat-M™) (58). These were evaluated for their ability to predict the
267 skin permeability of caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone
268 applied in propylene glycol, water and ethanol as unsaturated and saturated concentrations. Resultant
269 absorption data was compared to skin diffusion cell data. The correlations between membrane and
270 diffusion cell data from saturated and unsaturated concentrations were rather low, although this
271 relationship improved when only saturated concentrations were evaluated. These results suggest the
272 potential of PAMPA as an initial screening approach to assist in narrowing the selection of
273 formulations to be evaluated, thereby assisting in the development of new topical formulations. Based
274 on these findings and others in the field (59), PAMPA has been accepted as a suitable skin mimic
275 system that can provide significant benefits for *in vitro* analysis compared with more simplistic
276 artificial membrane systems. Furthermore, results of PAMPA permeability and retention have been
277 used to create mathematical models that could be employed for the design of novel derivatives with a
278 favorable skin retention/permeability ratio.

279 In summary, *in vitro* models for predicting transdermal permeation have been thoroughly
280 studied over the years, including the effects of factors such as the formulation and choice of

281 membrane system. In recent years, researchers have successfully developed more complex artificial
282 membrane systems that are able to provide precise and accurate predictions of transdermal permeation
283 without the need to use human or animal skin. The benefits of such non-*in vivo* systems are
284 significant, for economic, reproducibility and ethical reasons amongst others.

285

286 **Mathematical models including quantitative structure-permeability relationships (QSPRs)**

287 Using models to predict the permeability of a compound through skin is particularly complex
288 as a result of the intricate nature of the structures and mechanisms that dictate the delivery pathway.
289 While the stratum corneum barrier, which serves as the major rate-limiting component to skin
290 penetration for most drugs, has been the focus of most penetration models, much less is known about
291 transport beyond this stage, and about transport via the appendageal routes. When modelling skin
292 transport processes quantitatively, it is usual to consider the particular physiological regions of
293 interest as compartments (60). Drug levels within the compartments can be modelled as a single time-
294 dependent value, or as function of both position within that compartment (usually skin depth) as well
295 as time. For the latter choice, drug transport within the compartment may be modelled using partial
296 differential equations that describe the effects of drug diffusion, convection, elimination and
297 metabolism (61). Models usually concern the steady-state flux of drug into the skin and related
298 quantities such as the permeability coefficient and the maximum steady-state flux, the lag time
299 between drug application and attainment of the steady state; the clearance of drug through excretion;
300 and drug concentrations in the various skin layers, circulation, or other tissues (62). Theoretical
301 permeation models have become more and more complex over the past fifty years. For example,
302 ranging from simple models that consider the stratum corneum as a single compartment to those more
303 complex that consider the structural characteristics of skin, including contributions from the lipidic
304 components. Complexity may not always be necessary, for example, one particular study in 2011
305 found that only by using a two-compartment dermal clearance model that includes both diffusion and
306 transport by dermal blood vessels consistency was obtained between observed and previously
307 described *in vivo* literature data (63).

308 In general, two types of mathematical models have been developed to predict transdermal
309 permeation, i.e. those based on the properties of the compound permeating or those based on the
310 properties of the skin being permeated. Whereas the first is focused on predicting penetration through
311 the skin from a solute's physicochemical properties, the second type models transport processes in
312 skin layers using appropriate equations with the specific aim of predicting the concentration of a given
313 solute in viable skin tissues (64). In general, it has been found that models are an important tool for
314 accurate valuation of skin toxicity experiments, estimation of skin toxicity and for developing new
315 formulations for skin disease therapy. Being aware of toxicological responses is important for a
316 variety of reasons, for example when considering likely exposure of workers to certain compounds
317 (65, 66). However, more comprehensive mathematical models of drug transport in skin, especially
318 those based on more physiologically detailed mechanistic considerations of transport processes, are
319 required to further enhance their role in assessing skin toxicology.

320 Models of skin absorption have attracted significant interest in the last two decades following
321 the publication of two models for quantitative analysis (67, 68) in which permeability was related to
322 physicochemical properties of the permeant. Prior to that, the majority of studies tended to focus on
323 only small groups of compounds and the relationship with hydrophobicity where, unsurprisingly, it
324 was found an increase in hydrophobicity led to an increase in permeation. Expanding consideration to
325 a wider range of permeant characteristics, for example hydrogen bonding, melting point and
326 ionization properties, led to the development of more 'refined' models, summarized in references (35)
327 and (69).

328 Fitting experimental data to create statistically derived equations creates quantitative
329 structure-permeability relationship (QSPR) models. Most QSPR models consider skin permeation
330 through tortuous lipid pathways that may under-predict skin permeability of hydrophilic solutes, by
331 several orders of magnitude. As a result of this researchers have begun, in recent years, to consider
332 including aqueous pathways to improve the predictive abilities of permeation for hydrophilic solutes
333 (70). As a further complication, some compounds are known to undergo metabolism within the skin
334 thus several models also take into account contributions from metabolite permeation (71). As a variety
335 of physicochemical parameters are known to potentially impact on drug-release profiles, researchers
336 have developed mathematical models that take such parameters into account, for example taking into
337 consideration the effects of dosing level, the type of vehicle (i.e. formulation), concentration profiles
338 (72), solubility in particular solvents (73) and ionisation state of the permeant (74). Although these
339 models tend to be based on several assumptions (for example: that steady-state transport across the
340 skin is achieved even though a finite dose was applied) they have been shown to be successful for
341 predictive ability in certain situations. Combining calculations of skin concentrations within two
342 diffusion layers and results from silicone membrane permeations studies has led to precise predictions
343 of in-skin concentrations (75). A few studies have evaluated the correlation between skin permeability
344 predictive models and human *in vivo* data, with mixed findings, ranging from one study concluding
345 most models correlated well with the *in vivo* data (76), through to another study that declared models
346 were not suitable for accurately predicting permeation but were able to effectively rank the permeants
347 and could help to select candidate molecules for *in vitro* screening (77).

348 In summary, mathematical models of transdermal permeation play an essential role in the
349 investigation of epidermal and dermal transport of compounds, despite their limitations based on
350 assumptions introduced to simplify the process, they are useful tools for data analysis, and predictions
351 for dermal solute penetration (78).

352

353 **Human skin equivalent models**

354 As researchers have strived to create an *in vitro* model as close as possible to human skin,
355 many have viewed the development of three dimensional tissue models to be the ultimate goal. A
356 major drive towards this aim has arisen from the cosmetic industry with the necessity to replace
357 animal testing therefore creating a regulatory reason to provide human skin equivalents. This research
358 is now beginning to be adopted by pharmaceutical scientists as applications for measuring transdermal
359 permeation are realised. Although attempts have been made to produce a reliable model for over
360 twenty years, as recently as 2001 there was a belief that such models were not useful for *in vitro*
361 penetration studies compared with other existing methods. For example, one particular study found far
362 higher flux and skin concentration values using a living skin equivalent compared with human skin
363 (79). However, more recently, human skin equivalents have been described as an 'excellent' tool, for
364 example to study water distribution following application of moisturiser (80), and many view them as
365 the future of transdermal permeation studies *in vitro* (81-85).

366 Models formed using only one cell type, known as reconstructed human epidermis (RHE), are
367 used for their high level of reproducibility, yet can be viewed as too simplistic for consideration as a
368 complete human skin equivalent system. The European Centre for the Validation of Alternative
369 Methods has validated several RHE models for skin corrosion and irritancy studies confirming their
370 place as a viable replacement to other methods to obtain such data. To conform to the necessary
371 criteria the test systems must pass minimum standards relating to viability, barrier function,
372 morphology, reproducibility and quality control. Analytical techniques to verify these criteria include
373 infrared and Raman spectroscopy (86) and confocal laser scanning microscopy (87). Researchers
374 have attempted to apply RHE models to determine drug permeation and one such study found values
375 exceeded those for human epidermis yet, showed a tendency towards a lower level of variability (88).

376 Commercially available examples include SkinEthic®Rhe and Episkin®, both developed by L’Oreal,
377 France and more recently, oral epithelium models such as EpiOral™ (MatTek Corporation, USA).

378 Full thickness models consist of more complex systems, for example incorporating additional
379 cell types such as melanocytes and stem cells. By increasing the complexity of the models, through
380 incorporation of additional components, researchers believe they more truly replicate *in vivo* scenarios
381 yet it must also be remembered they decrease the reproducibility and can significantly increase
382 experimental costs. A detailed summary of the available state-of-the-art models can be found in
383 reference (89). Full thickness models are composed of both dermal and epidermal layers, i.e. creating
384 a bilayer structure similar to that found in human skin. More and more complex systems are
385 continually being created, such as by the creation of a viable adipose layer (90), allowing researchers
386 to resolve more complex dermatological issues (91). Commercially available examples include
387 RealSkin® (L’Oreal, France) and AST2000 (CellSystems Biotechnologie GmbH, Germany) (92).

388 Unsurprisingly, there are limitations even with three dimensional skin models which must be
389 addressed when considering the use of such systems for *in vitro* permeation determination. Firstly,
390 most relevant to cosmetic applications, is the inability of the models to generate stratum corneum, as
391 they consist of primary cells with a limited lifespan and, a lack of cells of the immune system. It has
392 been proposed that immortalised cell lines could improve the reproducibility and consistency of skin
393 models reducing intra and inter-laboratory variations (93). Secondly, some studies have reported a
394 dissimilar barrier function of human skin equivalents. For example, one such study found that the
395 models were able to mimic many aspects of human skin but, differed in their barrier properties (94),
396 implying they would not be suitable for permeation studies. Finally, the limited lifespan of these
397 living models (~ eight weeks), reduces their suitability for experimental study, although some work
398 has been conducted in this area to improve this situation, in one case increasing their availability for
399 up to twenty weeks (95).

400 With such a rapid expansion of work (and commercially available products) in this area it is
401 inevitable that the future of skin mimic systems incorporates aspects of RHE or full thickness models
402 to some extent.

403

404 **Chromatographic models**

405 High-performance liquid chromatography (HPLC) has been utilised and adapted to predict
406 skin permeability data, mainly in the last twenty years. Recently, the ability of a selection of
407 chromatographic systems to model human skin permeation has been evaluated and tested, by
408 correlating experimental data with *in vivo* data for a representative set of neutral solutes (96). It was
409 reported for the six systems (including the classic octanol-water partition system) that the HPLC
410 systems with C18 columns are the closest to the human skin permeation system whereas the micellar
411 electrokinetic chromatographic (MEKC) systems were most different – based on distance parameters.
412 However, the study also declared that error arising from the original skin permeability data is quite
413 significant and the variance from the C18 systems was possibly too high to provide precise
414 estimations of human skin permeability coefficients. This can be improved by introducing the solute’s
415 volume as an additional variable, resulting then in correlation models with good predictive abilities to
416 estimate permeation for untested solutes.

417 A great deal of interest has been shown in one particular type of column used in HPLC, the
418 so-called immobilised artificial membranes (IAMs). IAMs are synthesised by covalently binding
419 biologically relevant phospholipids to the surface of silica, such as attaching monolayers of
420 phosphatidylcholine to a propylamine silica support. For some years immobilised stationary-phase
421 liquid chromatography has been considered a potential *in vitro* technique (97), including studies to
422 examine its potential for predicting transdermal transport across neutral, basic, acidic and amphoteric

423 compounds (98). Interestingly, the findings indicated that IAM and partition coefficient values are
424 complementary and not alternative parameters whose combination yields more useful data than either
425 factor alone. Some researchers have taken the use of IAMs towards very specific applications, for
426 example through the physical immobilisation of keratin or collagen on the silica support, permitting a
427 comparison of the keratolytic properties of compounds (99, 100). Other forms of chromatography
428 have also been explored, for example liposome electrokinetic chromatography (LEKC) (101). LEKC
429 has been described as a promising simple method to predict drug penetration based on quantitative
430 retention-activity relationships (QRARs) constructed between skin permeability coefficients and
431 retention values.

432 Combining the desire to mimic the biological environment with a highly predictable
433 analytical technique has resulted in the development of biopartitioning micellar liquid
434 chromatography (MLC). In MLC, the mobile phase consists of surfactant molecules above the critical
435 micellar concentration, i.e. in micellar form. A compound is then injected into this mobile phase and
436 the retention time recorded over a series of concentrations. For some time, researchers have
437 appreciated the value of biopartitioning micellar separation methods for modelling drug absorption
438 (102), and more specifically, for predicting skin permeability (103). Within the last twelve months our
439 research group has developed, evaluated and published a method to measure the chromatographic
440 retention of drugs which can then be used to predict skin permeability using micellar chromatography,
441 achieving high levels of reliability (104). In our study, we looked at a series of model compounds and
442 found that the replacement of a traditional physicochemical parameter, namely the octanol-water
443 partition coefficient, with a chromatographically determined value resulted in a quantitative value that
444 was robust to variation, i.e. a suitable method to predict transdermal permeation. The advantages of
445 this method are numerous, and of particular benefit is the high level of predictive capability that has
446 not been seen in other studies.

447 In summary, chromatographic methods appear to show promise using a variety of
448 experimental conditions and, may make a positive contribution to the future prediction of transdermal
449 permeation.

450

451 **Conclusions**

452 When undertaking a study to investigate transdermal permeability, there are many options to
453 consider, and researchers tend to choose techniques that are most suited to their intended purpose,
454 availability, previous experience or economic constraints. The majority of the techniques currently
455 available have been shown to be suitable for ranking a series of compounds (or formulations), yet do
456 not provide an *in vitro-in vivo* correlation that suggests they can be used as a pure replacement. For
457 many reasons this is the ultimate aim of such studies and work continues to develop systems that can
458 either mimic skin to permit experimental data to be measured (i.e. using synthetic membranes or
459 human skin equivalents), or predict permeation (i.e. using chromatographic methods or mathematical
460 models). Development of the latter will undoubtedly lead to the availability of software that can
461 simulate absorption of dose into the skin, diffusion through the skin and clearance into blood which
462 can be used in the development of pharmaceuticals, cosmetics, household products and
463 agrochemicals. A very limited number of such packages are already available, such as the Skin-in-
464 Silico™ software (Xemet, Finland), yet it is highly anticipated that this is the likely direction of
465 transdermal studies in the future, thus completely replacing the need for animal testing. Clearly, it can
466 be seen that only through understanding the physicochemical properties of the compound under
467 investigation *and* the structure of the skin is it possible to quantify transdermal permeation. For this
468 reason, it will no doubt continue to be a combination of *in vitro* experimental measurement and
469 predictive techniques that yields the most valuable results.

470

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