A comparative study of the *in vitro* permeation of ibuprofen in mammalian skin, the PAMPA model and silicone membrane

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Abstract

Human skin remains the membrane of choice when conducting *in vitro* studies to determine dermal penetration of active pharmaceutical ingredients or xenobiotics. However there are ethical and safety issues associated with obtaining human tissue. For these reasons synthetic membranes, cell culture models or *in silico* predictive algorithms have been researched intensively as alternative approaches to predict dermal exposure in man. Porcine skin has also been recommended as an acceptable surrogate for topical or transdermal delivery research. Here we examine the *in vitro* permeation of a model active, ibuprofen, using human or porcine skin, as well as the Parallel Artificial Membrane Permeation Assay (PAMPA) model and silicone membrane. Finite dose studies were conducted in all models using commercial ibuprofen formulations and simple volatile ibuprofen solutions. The dose applied in the PAMPA model was also varied in order to determine the amount of applied formulation which best simulates typical amounts of topical products applied by patients or consumers. Permeation studies were conducted up to 6 h for PAMPA and silicone and up to 48 h for human and porcine skin. Cumulative amounts permeated at 6 h were comparable for PAMPA and silicone, ranging from 91–136 $\mu g/cm^2$ across the range of formulations studied. At 48 h, maximum ibuprofen permeation in human skin ranged from 11–38 $\mu g/cm^2$ and corresponding values in porcine skin were 59–81 $\mu g/cm^2$. A dose of 1 $\mu l/cm^2$ was confirmed as appropriate for finite dose studies in the PAMPA model. The formulation which delivered the greatest amount of ibuprofen in human skin was also significantly more efficient than other formulations when evaluated in the PAMPA model. The PAMPA model also discriminated between different formulation types (i.e. gel versus solution) compared with other models. Overall, the results confirm the more permeable nature of the PAMPA, silicone membrane and porcine tissue models to ibuprofen compared with human skin. Further finite dose studies to elucidate the effects of individual excipients on the barrier properties of the PAMPA model are needed to expand the applications of this model. The range of actives that are suitable for study using the model also needs to be delineated.

Key words: Human skin, porcine skin, silicone, PAMPA, ibuprofen, permeation

Introduction

Assessment of skin penetration of actives is of critical importance in a number of fields. Effective active pharmaceutical ingredient (API) permeation is required for therapeutic benefits, knowledge of the skin disposition of pesticides is important for human health and quantitation of delivery of cosmetic actives to the skin provides confidence for product claim support. Many different models of human skin have been proposed in order to quantify and predict percutaneous penetration. This reflects the difficulties in sourcing tissue as well as ethical issues and safety concerns associated with biological membranes. Early models of mass transfer in skin focussed on apparatus such as the rotating diffusion cell which employed isopropyl myristate (IPM) impregnated in filter paper as a surrogate skin lipid phase (Albery et al., 1976). Other lipids which have been used to model skin penetration include tetradecane, linoleic acid and dispersions of phospholipids in IPM (Guy and Fleming, 1979). Interestingly, eggshell membranes impregnated with IPM were considered by Washitake et al., (1980); removal of the shell with hydrochloric acid leaves a predominantly keratin rich membrane. With advances in knowledge of the composition of skin lipids more realistic mixtures of phosphatidyl choline, dipalmitoyl phosphatidly choline, ceramides, cholesterol, cholesterol palmitate, linoleic acid and tristearin were later investigated (Firestone and Guy, 1985).

In vitro permeation studies have also been conducted with simple polymeric membranes such as polydimethylsiloxane (Dias et al., 2007; Santos et al., 2009; Oliveira et al., 2012). While these studies are useful to probe thermodynamic activity of actives in specific formulations, they cannot provide any insight into specific excipient interactions with skin. The advent of tissue culture models for research applications stimulated much interest in the development of human skin equivalents (HSEs). Subsequently a number of models have become available including Epiderm™, Episkin™ and Labskin™. Typically these HSEs are based on cultures of normal human keratinocytes and/or fibroblasts and are metabolically and mitotically active. Although HSEs are reported to over-estimate likely permeation in human skin (Schmook et al., 2001; Basketter et al., 2007; Thakoersing et al., 2012; Labouta et al., 2013;) they are routinely used for toxicity and/or irritation testing (Spielmann et al., 2007; Alépée et al., 2014).

Recently, the Parallel Artificial Membrane Permeation Assay (PAMPA) has been proposed as a high throughput screening system that may be suitable to study skin permeation (Sinkó et al., 2012). Previously the PAMPA model was investigated for prediction of gastrointestinal absorption (Avdeef and Tsinman, 2006; Avdeef et al., 2007) and as a potential model of the blood-brain barrier (Tsinman et al., 2011). This model consists of a mixture of synthetic ceramides (certamides), cholesterol and free fatty acids mounted in 96-well plates (Sinkó et al., 2012). With respect to skin permeation the number of molecules and formulations evaluated in PAMPA to date remains low. Accordingly we set out to examine the permeation of a model API, ibuprofen, from two commercial preparations and two simple solutions using the PAMPA model. The data are compared with results from studies conducted with an artificial membrane (silicone) as well as with porcine and human skin. A further objective was to identify optimal dosing in the PAMPA model which best simulates typical amounts applied on skin by patients and consumers.

Ibuprofen was a gift from Wyeth (Haversham, Hants., UK). Polyethylene glycol (PEG) 300, propylene glycol (PG), HPLC grade isopropyl alcohol and trifluoroacetic acid (HPLC grade) were supplied by Fisher Scientific (UK). HPLC grade solvents (methanol and water) were provided by Sigma-Aldrich (UK). Phosphate buffered saline (pH 7.4) was prepared using Dulbecco A tablets (Oxoid, UK). Silicone membrane (250 μm) was obtained from Samco (Nuneaton, UK). This grade and thickness of silicone was selected because we have used it previously to examine the effects of a range of hydrophilic and lipophilic vehicles on ibuprofen permeation (Watkinson et al., 2009a,b; Watkinson et al., 2011). The pre-coated Skin PAMPA Sandwich stirring disks, hydration solution, and Gut-Box™ were obtained from pION Inc. (Billerica, USA). Porcine tissue was obtained from a local abattoir. Excised abdominal human skin was obtained from the UK Human Tissue Bank and was stored in a freezer at -20^{o}C until required (Research Ethics Committee reference 06/MRE04/37). The commercial formulations selected for evaluation were IBUGEL™ (Ibuprofen 5% w/w) and IBULEVE™ Speed Relief 5% Spray (Dermal Laboratories, Hitchin, Hertfordshire, UK). Two other formulations of ibuprofen were prepared as 5% w/w solutions in isopropyl alcohol and either PEG 300 or PG.

Silicone membrane was pre-treated as reported previously (Oliveira et al., 2012). Full-thickness porcine ear skin was prepared as described by Caon et al. (2010) and stored at -20°C until required. Heat-separated epidermis was obtained from human skin samples (Oliveira et al., 2012). Silicone and tissues were mounted between donor and receptor compartments of Franz cells (effective diffusion area ~1 cm^2). Assembled Franz cells were filled with PBS pH 7.4 which served as the receptor phase and 0.02% sodium azide (w/v) was also included in the receptor phase for studies that lasted for 48 h. All permeation studies were conducted at 32±1°C as confirmed with a Digitron TM-22 Differential Digital Thermometer, (RS Components, Corby, UK). Formulations were dosed at volumes of either 3.6 µL (solutions) or 4 µL (gel) in each Franz cell. Samples (200 µL) were removed from the receptor compartment at regular intervals over the duration of the permeation studies and replaced with fresh receptor phase. Experiments were conducted in silicone membrane and PAMPA for 6 h as preliminary studies had confirmed that most of the API had permeated by this time point.

The Skin PAMPA membrane was hydrated overnight by placing 200 µL of the hydration solution in each well. For these studies, three different doses were investigated in order to determine the amount of formulation which best represented finite dose conditions. Following removal of the hydration solution, 1, 3, or 30 µL of the tested formulations were applied by a Multipette® plus pipette (Eppendorf AG, Germany) on the membrane surface in each well of the top (donor) compartment of the PAMPA Sandwich. This corresponds to a dose of 3.3, 9.9 and 99 µL/cm^2. The corresponding wells in each bottom (receptor) plate were prefilled with 180 µL of PBS pH 7.4 and a stirring disk was also placed in each well. Subsequently, the PAMPA Sandwich was incubated on the stirring unit or "Gut-Box™" at 32±1°C. At 0.5, 1, 2, 3, 4 and 6 h, the bottom (receptor) plate was replaced with a plate prefilled with fresh receptor phase and stirrer disks. Replacing the entire receptor phase and a shorter interval between sample times compared with Franz cell studies was necessary in order to maintain sink conditions.

PAMPA samples were analysed using a Hewlett-Packard HPLC 1100 series equipped with a diode array detector. Separation was conducted at 30°C using a Luna C_{18} column (250×4.6 mm, 5 µm

stationary phase) fitted with a 4 mm C_{18} guard cartridge (Phenomenex Ltd., USA). The mobile phase consisted of methanol:water (80:20) with 0.1% (v/v) trifluoroacetic acid (TFA) and the flow rate was 1 mL/min. The wavelength employed was 222 nm and each sample was analysed for 10 min. The ibuprofen peak eluted at 7 min under these analytical conditions. For studies conducted with silicone membrane, porcine or human skin a Luna C_{18} column (200×4.6 mm, 5 µm stationary phase) fitted with two C_{18} 4×3 mm, 5 µm guard cartridges (Phenomenex Ltd., USA) was used. Analysis was conducted at 35°C, a flow rate of 1 ml/ min and a detection wavelength of 222 nm; the retention time of ibuprofen under these conditions was 6 min.

All the data were recorded by MS Excel® (Microsoft Corp., USA). The results are shown as mean ± standard deviation (SD). Statistical analysis was performed using MS Excel® and OriginPro® (OriginLab Corp., USA). One way analysis of variance (ANOVA) followed by a Tukey test was conducted (OriginPro®) for multiple comparison between the groups (at 5% significance level), $p<0.05$ was considered as the statistical significance.

Cumulative amounts of ibuprofen permeated from the various formulations for the four different models and the corresponding percentages permeated are shown in Figures 1 and 2, respectively.

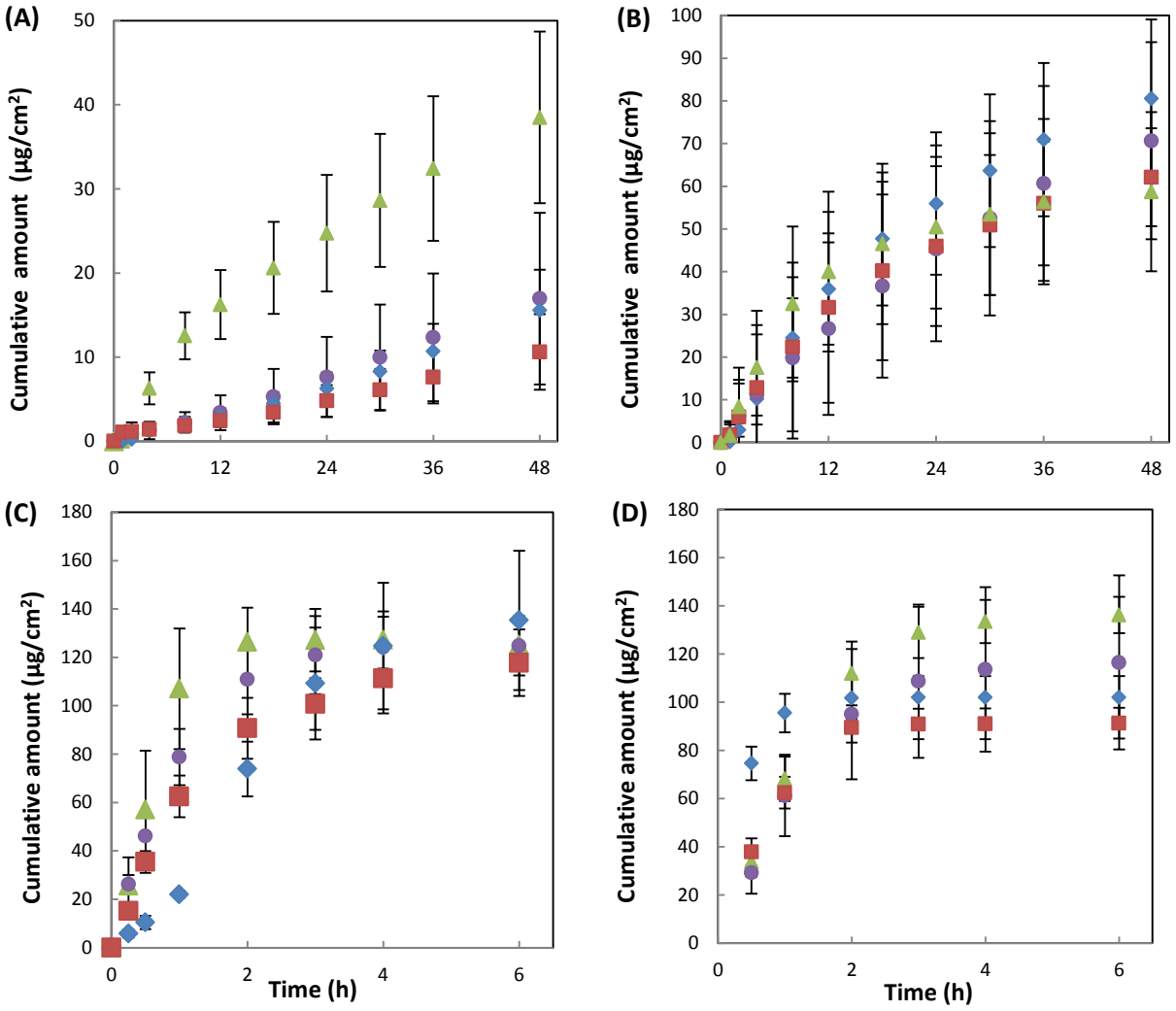

Figure 1 Cumulative amounts of ibuprofen permeated from IBUGEL® (◆), IBULEVE® (■), PG (▲) and PEG 300 (●) for: Human skin (A), Porcine skin (B), Silicone membrane (C) and Skin PAMPA dosed at 1 µl/cm^2 (D). Each data point represents the mean±SD (n≥5).

After 6 h, similar amounts of ibuprofen had permeated in silicone membrane and in PAMPA (~140 µg/cm^2). For porcine and human skin maximum amounts of permeation were ~80 µg/cm^2 and ~40 µg/cm^2 respectively. Typical curvilinear permeation profiles for all formulations were observed with all the membranes studied (Figure 1). Significantly higher permeation was observed in human skin for the PG formulation ($p<0.05$). In Skin PAMPA, significant differences were also found after 6 h when comparing the PG formulation with IBUGEL® and IBULEVE®. These differences were not observed for the amounts permeated in porcine skin at any time point nor in silicone membrane at 6 h. The higher ibuprofen permeation in porcine skin compared with human skin is consistent with data from other researchers (Dick and Scott, 1992; Singh et al., 2002; Barbero and Frasch, 2009). Both mammalian tissues had been stored in a freezer (-20°C) until use but a comparison of permeation behaviour in fresh

human skin and porcine skin would provide a useful insight into whether this storage process has contributed to the permeation results observed here.

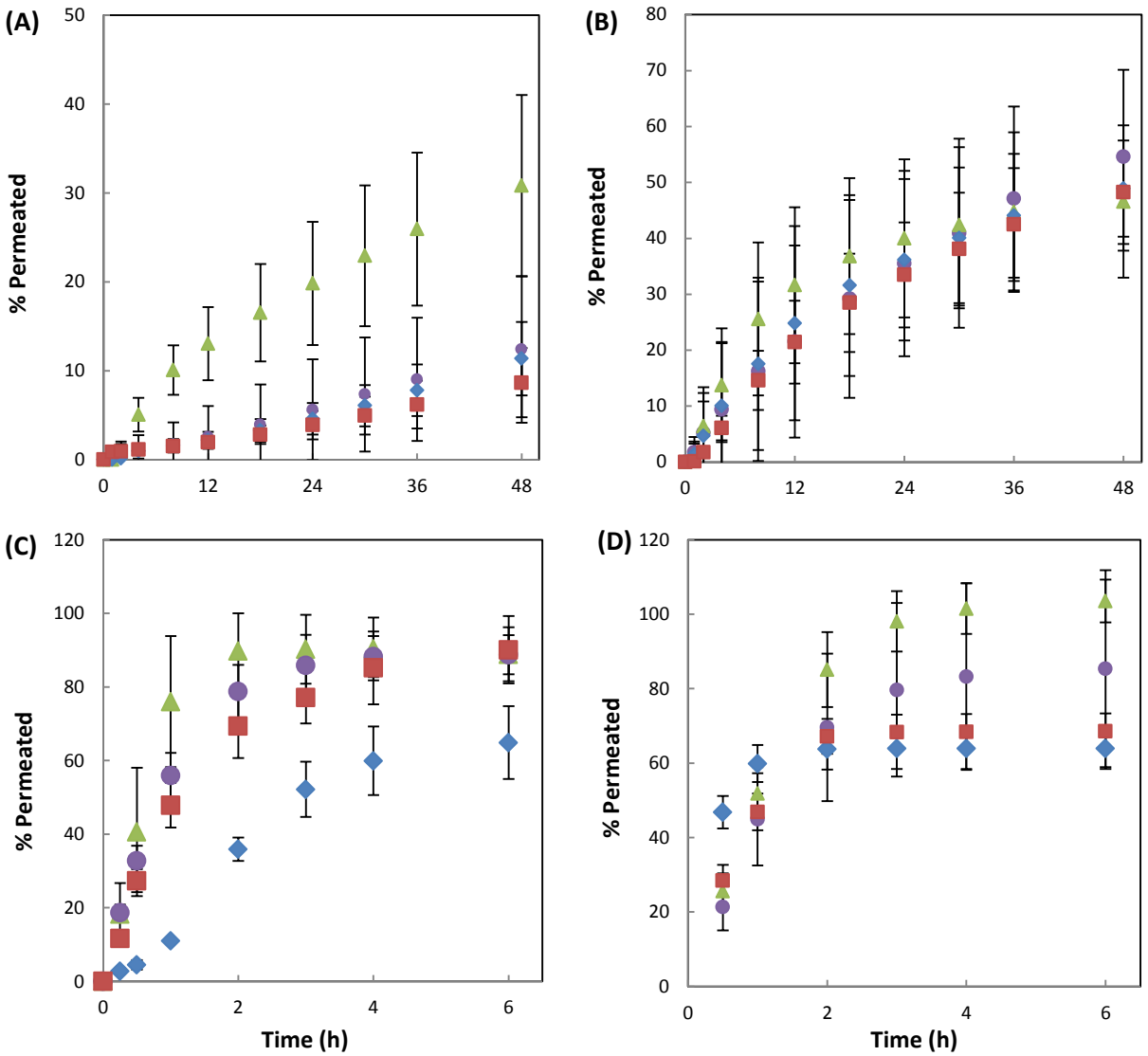

Figure 2 Percentages of ibuprofen permeated from IBUGEL® (◆), IBULEVE® (■), PG (▲) and PEG 300 (●) for: Human skin (A), Porcine skin (B), Silicone membrane (C) and Skin PAMPA dosed at 1 µl/cm^2 (D). Each data point represents the mean±SD (n≥5).

At 24 h the maximum average percentage permeation of ibuprofen was ~40% in porcine skin compared with ~4% in human skin, with the exception of the PG formulation in human skin where 20% of the dose permeated (Figure 2). Although PG is also present in IBUGEL ™, the amount used in the volatile PG solution has been adjusted to ensure optimal thermodynamic activity of ibuprofen. This likely explains the superior permeation of ibuprofen from this vehicle and will be reported in a separate publication (Patel et al., In Press). Comparatively higher percentages of active permeated at 6 h for the PAMPA and silicone membrane modes (Figure 2) with values ranging from 60-100%. Significantly lower

amounts of ibuprofen permeated through silicone membrane from the commercial gel formulation at 4 and 6 h compared with all other formulations ($p<0.05$).

Figure 3 shows cumulative amounts of drug and percentages permeated for three different doses (1, 3 and 30 μL) of all formulations evaluated in the PAMPA model.

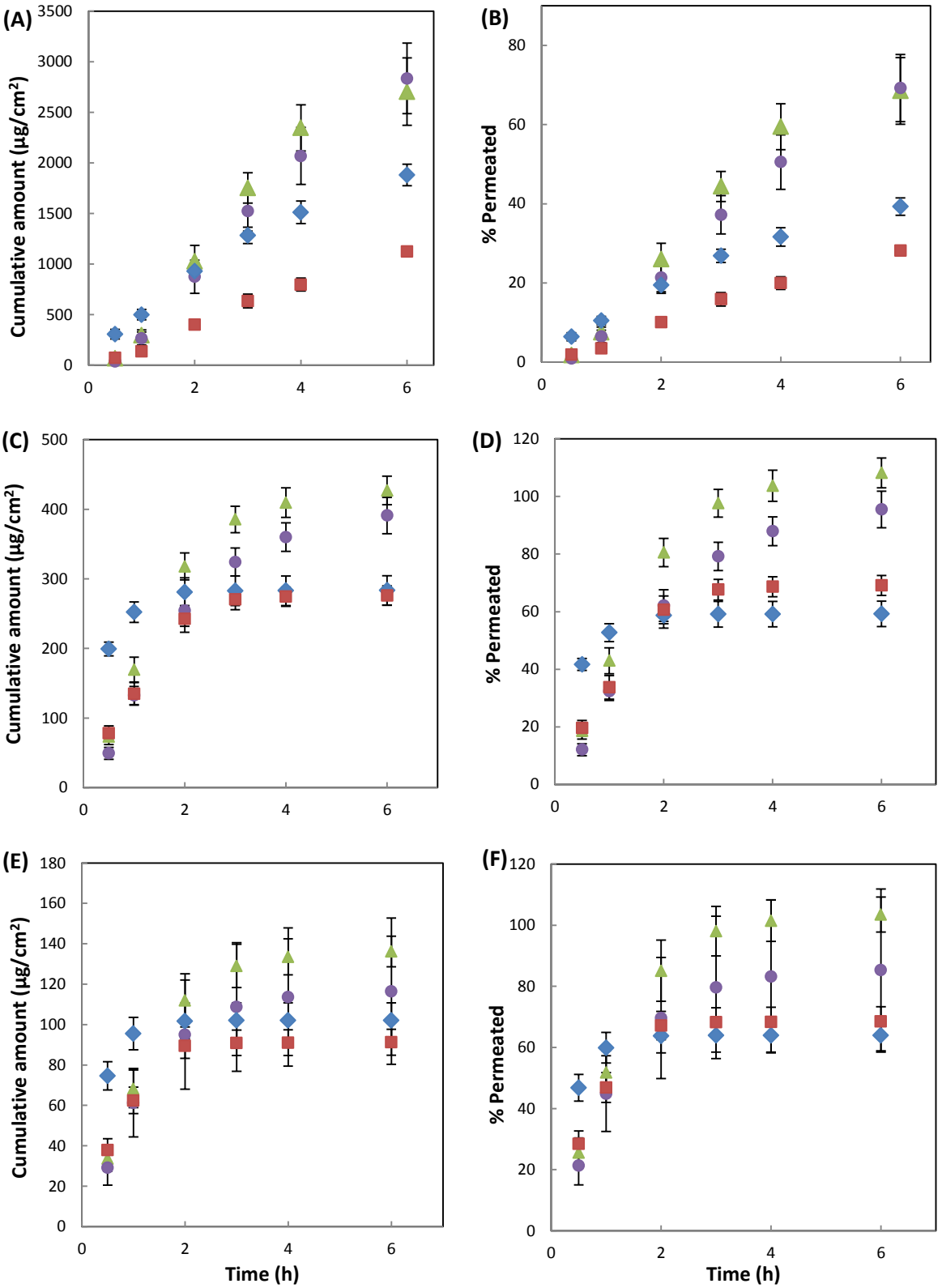

Figure 3 Cumulative amounts of ibuprofen and percentages permeated from IBUGEL® (◆), IBULEVE® (■), PG (▲) and PEG 300 (●) in PAMPA following application of 30 µL (A) and (B), 3 µL (C) and (D), and 1 µL (E) and (F) per well. Each data point represents the mean±SD (n=6).

For the 30 µl applications (Figures 3A, 3B), the profiles are generally linear, consistent with these amounts representing infinite doses. At 6 h, there are significant differences in ibuprofen permeation from the two commercial formulations and between the gel and all other formulations ($p<0.05$), but not between the PG and PEG 300 formulations. This is in contrast to the finite dose studies conducted in human skin where no differences in permeation from the commercial formulations were determined. Overall ibuprofen permeation from the PG and PEG 300 alcoholic solutions is higher than for the commercial formulations in PAMPA ($p<0.05$). For human skin studies significantly higher permeation was only evident for the PG formulation compared with all other formulations. For the commercial formulations the permeation differences may reflect the influence of various excipients on ibuprofen permeation. Both formulations contain industrial methylated spirit (IMS) or denatured alcohol. The gel contains PG whereas the spray does not. The volatile nature of the spray formulation should also result in a shorter residence time of this formulation on the PAMPA membrane compared with the gel. Differences between the commercial spray and the simple solutions may also reflect differences in the absolute content of the volatile components. However, further studies with individual excipients and the PAMPA lipid mixture will be needed to interpret these data. These specific excipients and the functions previously proposed for them are detailed in Table 1.

Table 1: Excipients included in commercial and experimental formulations and proposed functions reported in the literature

Excipient	**Functions**
IMS	Penetration enhancer*, solvent†
PEG 300	Solvent‡
PG	Penetration enhancer, solvent†

*Hadgraft et al., (2003); †Lane (2013); ‡Rowe et al., 2012

As expected cumulative amounts of ibuprofen which permeated drop for the 1 and 3 µL doses compared with the 30 µl application and there are no differences in the permeation between the commercial formulations at 6 h. This is consistent with an exaggerated influence of formulation excipients on membrane transport at the higher 30 µL dose. As we have previously noted data from infinite dose studies may not be extrapolated to finite dose conditions (Santos et al., 2011; Goh and Lane, 2014; Luo and Lane, 2015; Hadgraft and Lane, 2016). For the 3 µL dose, ibuprofen permeation is significantly ($p<0.05$) higher from the formulations containing PG and PEG 300 when compared with

both commercial formulations. However, after the application of 1 μL, only permeation from the PG formulation is statistically higher ($p<0.05$) than the commercial formulations. Overall, a greater percentage of each formulation permeates in the PAMPA model at these lower doses compared with the 30 μL dose. At 6 h approximately 280 $\mu g/cm^2$ of ibuprofen had permeated following application of 3 μL doses of the commercial formulations, accounting for 60–70% of the applied dose. For the 1 μl application the cumulative amounts permeated and percentages of ibuprofen delivered were 90–100 $\mu g/cm^2$ and 64-69% respectively. Clearly the amounts permeated for the 1 μL dose approach values for porcine skin and silicone membrane (Figure 1); however percentage permeation is closest to values for the silicone membrane.

In summary, a comparative study of ibuprofen permeation was conducted using human and porcine tissue, a skin PAMPA model and silicone membrane. After 6 h, ibuprofen was generally more permeable in PAMPA than human skin and PAMPA data were comparable to results in silicone membrane. For individual formulations permeation is also higher in PAMPA compared with porcine skin. Much like silicone membrane, the composition of the Skin PAMPA membrane is considered to be homogeneous and inert. The low variability of results obtained from PAMPA and silicone may be attributed to these membrane characteristics. With porcine and human skin, the results are naturally more variable due to the added complexity of biological membranes. Although the time for permeation studies in PAMPA was not varied, shorter experimental times may be more appropriate considering the relatively high percentage of ibuprofen permeation in PAMPA. Application conditions in PAMPA which approach realistic finite dose conditions were confirmed to be 1 μl (3.3 $\mu l/cm^2$) however it is important to note that this may be specific to a particular active. Interestingly, the formulation which demonstrated the highest delivery in human skin was also the best formulation which performed best for the 1 μl application conditions in PAMPA. The PAMPA model also appears to be more sensitive to differences in formulation composition i.e. gel versus solution. Further studies expanding the range of molecules and formulations which may be suitable for screening using PAMPA are currently underway. This should provide insight into which formulations are best suited to evaluation using this model.

Acknowledgement: AP is funded by an EPSRC Doctoral Training Grant reference EP/I01375X/1.

References

Albery WJ, Burke JF, Leffler EB, Hadgraft J. Interfacial transfer studied with a rotating diffusion cell. J. Chem. Soc., Faraday Trans. 1. 1976 72 1618-26.

Alépée N, Grandidier MH, Cotovio J; Organisation for Economic Co-operation and Development. Sub-categorisation of skin corrosive chemicals by the EpiSkin™ reconstructed human epidermis skin corrosion test method according to UN GHS: revision of OECD Test Guideline 431. Toxicol. In Vitro. 2014 28(2):131-45.

Avdeef A, Bendels S, Di L, Faller B, Kansy M, Sugano K, Yamauchi Y. PAMPA:critical factors for better prediction of absorption. J. Pharm. Sci. 2007 96 2893-2909.

Avdeef A, Tsinman O. PAMPA – a drug absorption in vitro model: 13. Chemical selectivity due to membrane hydrogen bonding: in combo comparison of HDC-, DOPC-, and DS-PAMPA models. Eur. J. Pharm. Sci. 2006 28 43-50.

Barbero AM , Frasch HF. Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. Toxicol. In Vitro. 2009 23(1) 1-13.

Basketter D, Pease C, Kasting G, Kimber I, Casati S, Cronin M, Diembeck W, Gerberick F, Hadgraft J, Hartung T, Marty JP, Nikolaidis E, Patlewicz G, Roberts D, Roggen E, Rovida C, van de Sandt J. Skin sensitisation and epidermal disposition: the relevance of epidermal disposition for sensitisation hazard identification and risk assessment. The report and recommendations of ECVAM workshop 59. Altern. Lab. Anim. 2007 35(1) 137-54.

Dias M, Hadgraft J, Lane ME. Influence of membrane-solvent-solute interactions on solute permeation in model membranes. Int. J. Pharm. 2007 336(1) 108-14.

Dick IP , Scott RC. Pig ear skin as an in-vitro model for human skin permeability. J. Pharm. Pharmacol. 1992 44(8) 640-5.

297

298 Firestone BA, Guy RH. Approaches to the prediction of dermal absorption and potential cutaneous
299 toxicity. In: Alternative Methods in Toxicology: In Vitro Toxicology (Ed. AM Goldberg). Mary Ann Liebert,
300 New York (1985) pp. 517-36.

301

302 Goh CF, Lane ME. Formulation of diclofenac for dermal delivery. Int. J. Pharm. 2014 473(1-2) 607-16.

303

304 Hadgraft J, Whitefield M, Rosher PH. Skin penetration of topical formulations of ibuprofen 5%: an in
305 vitro comparative study. Skin. Pharmacol. Appl. Skin Physiol. 2003 16(3) 137-42.
306
307 Guy RH, Fleming R. The estimation of diffusion coefficients using the rotating diffusion cell. Int. J. Pharm.
308 1979 3 143-9.
309
310 Hadgraft J, Lane ME. Drug crystallisation – implications for topical and transdermal delivery. Expert Opin.
311 Drug Delivery. 2016. **DOI:**10.1517/17425247.2016.1140146

312

313 Labouta HI, Thude S, Schneider M. Setup for investigating gold nanoparticle penetration through
314 reconstructed skin and comparison to published human skin data. J. Biomed. Opt. 2013 18(6) 061218.

315

316 Lane, ME. Skin penetration enhancers. Int. J. Pharm. 2013. 447(1-2) 12-21.

317

318 Luo L, Lane ME. Topical and transdermal delivery of caffeine. Int. J. Pharm. 2015 490(1-2) 155-64.

319

320 Oliveira G, Hadgraft J, Lane ME. The influence of volatile solvents on transport across model membranes
321 and human skin. Int. J. Pharm. 2012 435(1) 38-49.
322
323 Patel A, Bell M, Wibawa J, Hadgraft J, Lane ME. Rational design of ibuprofen formulations. In Press.
324

Rowe RC, Sheskey PJ, Cook WG, Fenton, ME. Handbook of Pharmaceutical Excipients. Seventh Edition. The Pharmaceutical Press, London, UK. The American Pharmacists Association, Washington DC, USA. 2012. 1033 pages.

Santos P, Machado M, Watkinson AC, Hadgraft J, Lane ME. The effect of drug concentration on solvent activity in silicone membranes. Int. J. Pharm. 2009 377(1-2) 70-5.

Santos P, Watkinson AC, Hadgraft J, Lane ME. Formulation issues associated with transdermal fentanyl delivery. Int. J. Pharm. 2011 416(1) 155-9.

Schmook FP, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skinin in-vitro percutaneous absorption. Int. J. Pharm. 2001 215(1-2) 51-6.

Singh S , Zhao K, Singh J. In vitro permeability and binding of hydrocarbons in pig ear and human abdominal skin. Drug Chem. Toxicol. 2002 25(1) 83-92.

Sinkó B, Garrigues TM, Balogh GT, Nagy ZK, Tsinman O, Avdeef A, Takács-Novák K. Skin-PAMPA: a new method for fast prediction of skin penetration. Eur. J. Pharm. Sci. 2012 45(5) 698-707.

Spielmann H, Hoffmann S, Liebsch M, Botham P, Fentem JH, Eskes C, Roguet R, Cotovio J, Cole T, Worth A, Heylings J, Jones P, Robles C, Kandárová H, Gamer A, Remmele M, Curren R, Raabe H, Cockshott A, Gerner I, Zuang V. The ECVAM international validation study on in vitro tests for acute skin irritation: report on the validity of the EPISKIN and EpiDerm assays and on the Skin Integrity Function Test. Altern Lab. Anim. 2007 35(6) 559-601.

Thakoersing VS, Gooris GS, Mulder A, Rietveld M, El Ghalbzouri A, Bouwstra JA. Unraveling barrier properties of three different in-house human skin equivalents.Tissue Eng. Part C Methods. 2012 18(1) 1-11.

354 Tsinman O, Tsinman K, Sun N, Avdeef A. Physicochemical selectivity of the BBB microenvironment
355 governing passive diffusion--matching with a porcine brain lipid extract artificial membrane permeability
356 model. Pharm. Res. 2011 28(2) 337-63.
357
358 Washitake M, Takashima Y, Tanaka S, Anmo T, Tanaka I. Drug permeation through eggshell membranes.
359 Chem. Pharm. Bull. 1980 28(10) 2855-61.
360
361 Watkinson RM, Herkenne C, Guy RH, Hadgraft J, Oliveira G, Lane ME. Influence of ethanol on the
362 solubility, ionization and permeation characteristics of ibuprofen in silicone and human skin.
363 Skin Pharmacol. Physiol. 2009 22(1) 15-21
364
365 Watkinson RM, Guy RH, Hadgraft J, Lane ME. Optimisation of cosolvent concentration for topical drug
366 delivery - II: influence of propylene glycol on ibuprofen permeation. Skin Pharmacol. Physiol. 2009 22(4)
367 225-30.
368
369 Watkinson RM, Guy RH, Oliveira G, Hadgraft J, Lane ME. Optimisation of cosolvent concentration for
370 topical drug delivery III--influence of lipophilic vehicles onibuprofen permeation. Skin Pharmacol Physiol.
371 2011 24(1) 22-6.
372
373