Ionisation effects on the permeation of pharmaceutical compounds through silicone membrane

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Abstract

Silicone membrane is frequently used as an *in vitro* skin mimic whereby experiments incorporate a range of buffered media which may vary in pH. As a consequence of such variability in pH there is a corresponding variability in the degree of ionisation which in turn, could influence permeation through the mainly hydrophobic-rich membrane structure. This study reports the effect of pH on the permeation of five model compounds (benzoic acid, benzotriazole, ibuprofen, ketoprofen and lidocaine). For the five compounds analysed, each at three distinct percentages of ionisation, it was found that the greater extent of permeation was always for the more 'neutral', i.e. more greatly unionised, species rather than the anionic or cationic species. These findings fit with the theory that the hydrophobic membrane encourages permeation of 'lipid-like' structures, i.e. the more unionised form of compounds. However, results obtained with an Inverse Gas Chromatography Surface Energy Analyser (iGC SEA) indicate the membrane surface to be an electron dense environment. In the knowledge that unionised forms of compounds permeate (rather than the charged species) this negatively charged surface was not anticipated, i.e. the basic membrane surface did not appear to affect permeation.

Keywords: silicone; PDMS; transdermal; permeation; ionisation; pKa

Introduction

Understanding the permeation of compounds through human skin is a complex issue and can be difficult to predict using *in vitro* methods currently available such as human skin equivalents, mathematical models and synthetic membranes [1]. Although limited in predictive ability, their development and application are encouraged as there is a general trend to move away from animal-based studies within the EU. Analytical techniques using synthetic membranes are particularly popular and are mainly concerned with the application of materials such as polymeric membranes, including polydimethylsiloxane (PDMS), more commonly referred to as silicone membrane [2]. PDMS is a material that has many industrial uses (for example, microfluidic devices [3], pervaporation [4, 5], separation [6, 7]) and is known to adsorb solvents [8]. In pharmaceutical analysis PDMS is used to mimic the skin layer in a system designed to incorporate a donor solution and receiver solution, the latter from which samples are taken for analysis to determine quantitatively the rate and extent of permeation within a pre-selected period of time [9]. From such data it is possible to predict the fate of a compound following application on to the skin surface which is essential for toxicological profiling and formulation development. Experiments based on these *in vitro* predictive systems use equipment known as Franz cells and are routinely used for analysing cosmetic, household and pharmaceutical compounds [10, 11]. Membrane materials such as PDMS are favourable amongst researchers for several reasons including their reproducible composition and thickness, simplicity of use and cost effectiveness [12]. The hydrophobic nature of the membrane allows a barrier effect to occur, as is seen *in vivo* and as long as certain criteria are met, i.e. permeation is through passive diffusion, the compound permeating is metabolically inert and the formulation does not contain a skin-specific permeability enhancer [13], then useful data relating to permeation can be attained.

When analysing compounds using skin (or skin mimics, such as PDMS) there is a vast array of donor phase compositions available. These range from simple water-based solutions of the compound under investigation [14] to compositions that replicate the complex formulations intended for market [15, 16]. For example, even when considering a model compound such as ibuprofen, previous studies have focussed on a basic aqueous solvent at a pre-selected pH through to the application of far more complex formulations, such as small amounts of ibuprofen gel, as would be applied to human skin [17] or the addition of surfactants [13]. It would appear that little attention has been paid to how, or why, particular solvents have been selected in the majority of cases for the donor phase although recent work within our group has begun to consider the importance of the presence of binary mixtures in the donor phase and effects of variation in the receptor phase [18]. From such work it is clear that the composition of the donor phase can, and does, influence permeation yet the extent to which this occurs is as yet unclear.

One particular aspect of all aqueous based formulations that can fundamentally dictate the physicochemical behaviour of the compound under investigation is the pH of the chosen solution. Through knowledge of the pKa of a compound it is possible to calculate the percentage ionised (with application of the Henderson-Hasselbalch equation (Eqtn. 1)) at any given pH where the unionised species (HA) is in equilibrium with the ionised species (A^-).

$$(1) \quad pH = pKa - \log([HA]/[A^-])$$

Thus, it is possible to manipulate the pH of a solution so that the ratio of the concentration unionised ([HA]) to ionised ([A^-]) can be controlled and known for any compound with a pre-determined pKa. Other physicochemical factors may also play a role in permeation, such as the octanol-water partition coefficient (logP) so for the compounds considered in this work a wide range of lipophilicities were considered to confirm the potential importance of this additional factor on permeation.

The ability of Franz cell-based experiments to predict the behaviour of a compound within a formulation has led some researchers to select donor phases with pH values similar to those found *in vivo*. For example, some studies have used an aqueous phase at a low pH to replicate the typical pH of skin or acne-prone skin [19] yet others have selected pH values such as 7.4 [20]. Despite such studies implying an appreciation of how important it can be to select and control the pH of the donor phase, little research has been conducted prior to this study to investigate the role the relationship between pH and percentage ionisation can have on the subsequent extent of permeation in Franz cell studies. One particular study has investigated ionisation and the effect of absorption enhancers on the transport of one compound, namely salicylic acid, through silastic rubber and human skin [21]. In this study it was found that permeant concentration was directly related to the degree of ionisation of the solute, i.e. permeation conformed to the pH-partition hypothesis. However, only this one specific compound was considered in the work. Whether a similar relationship would be observed for a range of compounds was not considered, particularly with a range of drugs that ionise to form cationic and anionic species such as those studied in this work.

Materials and Methods

Materials

Polydimethylsiloxane membrane (PDMS) was used as purchased (ATOS Medical, Sweden) with a standard thickness of 130 µm and cut to size as required.

The glassware used was of class B. Deionised water was used throughout the experiments. The flow-through diffusion cells were purchased from PermeGear Inc., USA.

Methods

Flow through permeation methodology

In all experiments the concentration of the model compounds in the donor solution was 1 mg/mL with a total volume of 0.8 mL per solution. PDMS membrane was employed as a permeability barrier. The membrane was soaked in buffer solution for 30 minutes prior to being mounted in the flow-through diffusion cells. After assembly the integrity of each cell was checked visually by inversion. Phosphate buffer solution was pumped through the diffusion cells at a rate of 2 mL/h and collected by means of a fraction collector at the predetermined time interval. Extracted samples were analysed by means of UV spectroscopy to quantify the model compounds over a period of 7 hours (benzoic acid at 226 nm, benzotriazole at 262 nm, ibuprofen at 230 nm, ketoprofen at 264 nm and lidocaine at 219

nm). All experiments were conducted in triplicate with the mean value shown with standard deviation based error limits. All flow-through cells used in this study had a diffusion area of 0.554 cm^2.

iGC methodology:

70 mg of PDMS membrane were packed into an iGC silanised glass column. The dispersive surface energy (γ_s^D) and the acid-base free energy (γ_s^{AB}) of adsorption were determined by running the sample at a series of surface coverage with alkanes and polar probe molecules. The sample column was pre-conditioned for 2 hours at 25 °C and 0 % RH with 10 mL/min helium carrier gas. The experiment was conducted at 25 °C with 10 mL/min total flow rate of helium, and using methane for dead volume correction.

Results and Discussion

Permeation dependence with ionisation

Five compounds were analysed to determine the amount permeated through silicone membrane, each at three specific percentages of ionisation (calculated using Equation 1) and pKa values of benzoic acid 4.2 [22], benzotriazole 8.2 [23], ibuprofen 4.9 [24], ketoprofen 4.0 [25] and lidocaine 7.8 [26]. These compounds were selected for their diverse range of both pKa values and lipophilicities, the latter ranging from a logP of 1.2 for benzotriazole [27] to 3.6 for ibuprofen [28].

Previous work [21] investigated salicylic acid permeation to determine if it conformed to the pH-hypothesis whereby observed permeability coefficients and steady-state flux increased with decreasing pH and a linear relationship was found between the fraction unionised and flux. The results suggested that the change in flux was a direct consequence of pH, which controlled the concentration of undissociated species. However, their study only investigated one specific compound, namely salicylic acid. This study had the aim of determining if a similar relationship would be observed for a range of compounds with differing pKa and logP values, i.e. to identify if the flux-pH relationship can be applied more generally. Figures 1-5 display the cumulative amount permeated for all five compounds as a function of percentage unionsed. Although it was not possible for all five compounds to achieve the same degrees of ionisation with their individual pKa values and the pH range available, the distribution of percentages allows a comparative study to be undertaken. It can be seen that in all cases there was a reduction in permeation as the percentage unionised decreased which would be expected based on the hydrophobic nature of the membrane. Through modifying the pH of the aqueous solution (for example from pH 8.5 for ketoprofen through to pH 4.5 for benzoic acid) it was interesting to observe a significant change in the degree of permeation over the experimental period of seven hours.

Considering the data in Figures 1-5, more specifically the relationship between the amount permeated after 7 hours and the percentage of compound unionsed for each, then it can be concluded they all display a generally linear relationship with increasing amount permeated with increasing percentage unionised. A plot of such data allows comparisons to be made between the gradients, i.e. how influential the percentage ionised is on permeation

(data not shown). The most dramatic change in amount permeated as a function of percentage unionised was observed for ibuprofen and the least change was seen for benzotriazole, possibly as a consequence of the pKa with a value of 8.2 for benzotriazole [23]. Alternatively, this may have been a consequence of the comparative logP values with ibuprofen displaying the greatest change in amount permeated and being the most hydrophobic of the compounds through to the least change observed for benzotriazole with this compound being the least hydrophobic. Benzoic acid did not show such a clearly linear relationship between the percentage unionised and amount permeated although the overall trend fitted with the data from the remaining compounds.

Flux values (μg cm^{-2} h^{-1}) were calculated for each compound and plotted against the fraction unionised to determine if a linear relationship was apparent for all compounds or, if permeation was a more complex process and was dependent upon other factors, such as logP. This analysis was conducted in a similar manner to that of Smith and Irwin [21] whereby they observed a linear relationship for the one compound analysed, namely salicylic acid. Data for all five compounds can be found in Table 1.

As expected, a similar trend in the data was observed for that discussed above, i.e. from comparative consideration of the amount permeated with percentage unionsed, as all experiments throughout the study were conducted for seven hours. Again, the relationship between flux and fraction unionsed was mainly linear with the one exception of benzoic acid where the intermediate fraction of unionised solution appeared to allow more compound to permeate than expected. This anomaly was unexpected although the general trend was similar to that of the remaining compounds, i.e. flux increased as the percentage of unionised compound increased. One factor that can certainly be removed from consideration is the membrane thickness as this was consistent throughout the experimental study, the importance of maintaining membrane thickness has been reported previously by others [29].

iGC membrane surface analysis

The BET Specific Surface Area (BET-SSA) of the membrane was measured with the physical adsorption of ethanol by DVS (Table 2).

Dispersive (γ_s^D), acid-base (γ_s^{AB}) and total surface energy (γ_s^T) profiles are shown in Figure 6 and indicate the sample is energetically heterogeneous, i.e. surface energy changed as a function of surface coverage with a major dispersive component contribution.

Heterogeneity was confirmed from the wide variation of surface active sites through plotting surface energy distributions (data not shown). Furthermore, the specific acid-base Gibbs free energy of adsorption (ΔG_{SP}) varied with surface coverage, confirming the heterogeneous nature of the sample. From analysing interactions with five polar probe molecules the rank order of decreasing ΔG_{SP} was found to be

acetonitrile>ethanol>dichloromethane>acetone>ethyl acetate although the sample showed only a relatively small degree of interaction with all probes. Gutmann acid (K_a) and base (K_b) values were calculated using ΔG_{SP} values with K_b values consistently higher than K_a. These results confirm that the surface of the sample to be basic in nature and present a high concentration of electron-donating surface functional groups. It can be assumed these are un-substituted hydroxyl groups based on the chemistry of the material. These findings can be linked with the previously discussed permeation data to understand why it was always the more unionised form of a compound that favoured permeation. This can be explained in terms of the iGC data which indicates the surface is basic which one might expect would repel the ionised form of basic compounds (such as lidocaine) and weakly bond the ionised form of the acid compounds (benzoic acid, benzotriazole, ibuprofen and ketoprofen). In either case, the ionised form is less inclined to permeate the negatively charged membrane surface compared with the unionised form.

Conclusion

From considering permeation results in cojunction with iGC data it can be seen that data presented based on permeation indicates there is a general preference for permeation for the most unionised species for all compounds. Based on these findings it can be concluded that the general hydrophobic nature of the membrane outweighs the effects of any surface groups that may be present. Alternatively, it may be the case that the existence of a peripheral layer of basic groups creates an electrostatic attraction or repulsion for the ionised forms of drugs, preventing permeation from occurring. In either case, it is clear that permeation is more favourable for the more unionised form of a compound despite the presence of a basic PDMS surface.

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