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A comparative study of chemical based
skin mimics with pharmaceutical
applications

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A thesis submitted in partial fulfilment of the requirements for the degree of
Doctorate of Philosophy

The University of Huddersfield
2016

Abstract

The prediction of percutaneous absorption is of enormous importance for the effective design, development and quality assessment of topical and transdermal formulations. *In vitro* diffusion experiments are widely carried out for such predictions and are of substantial interest across the pharmaceutical and cosmetic industries. Human or animal skin, usually excised, are often used in *in vitro* drug diffusion studies. However, difficulties in obtaining the mammalian skin and variation in their permeability directed researchers towards using synthetic membranes as skin mimics in preformulation screening experiments, where a large number of experiments are required. Polydimethylsiloxane (PDMS) membranes have been accepted as the most commonly used *in vitro* skin mimic because of their homogeneity, uniformity and skin-analogous rate-limiting permeation properties.

This thesis investigates the effects of ionisation and surfactants on the permeation of pharmaceutical compounds of varied physicochemical properties through PDMS membranes using a flow-through diffusion cell system. Data suggests that drug permeation had a dependency on the extent of its ionisation, with the permeation being more favourable for the more unionised form of a drug. All of the surfactants studied were found to reduce the permeation of the drugs, with an inverse relationship being observed between the surfactant concentration and the amount of drug permeated. DSC (differential scanning calorimetry), SEM (scanning electron microscopy), FTIR (Fourier transform infrared) and NMR (nuclear magnetic resonance) spectroscopy were employed to study the interactions between the membrane and the surfactants. Results indicated that the permeation effects of the surfactants are a consequence of the interactions between the drugs and surfactant micelles, and/or the membrane and the surfactants.

Air plasma treatment was used to modify the PDMS surfaces to become hydrophilic, which was confirmed by water contact angle (WCA) and SEM-EDX analysis. The permeation data for the modified membranes revealed that the plasma-induced hydrophilicity significantly reduced the fluxes of the hydrophobic compounds, while not affecting that of the hydrophilic drug. Aging studies of the plasma-treated membranes showed that the hydrophilic surfaces were maintained even after 8 weeks under airtight storage conditions.

In summary, ionisation and surfactant effects on drug permeation across PDMS were thoroughly investigated, and plasma treatment was found to be a stable, economic and convenient method of modifying PDMS to offer skin-like slower drug permeation i.e. to produce a potential *in vitro* skin mimic.

Dedication

This thesis is dedicated to my caring parents, for their relentless love, prayer and support.

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I sincerely thank Allah, the Most Merciful, for His countless blessings on every aspect of this thesis.

I would like to express my special thanks and gratitude to my supervisor, Dr Laura J. Waters, who gave me the faith and encouragement that kept my enthusiasm for this thesis, and whose persistent guidance, incredible support and tireless patience made my doctoral degree a wonderful and memorable learning experience.

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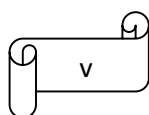
I would also like to sincerely thank all of my colleagues for creating a friendly environment and for encouraging me in this challenging journey. I also want to thank all of the technical staffs of the School of Applied Sciences for their laboratory assistance, which means a lot to me.

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List of Abbreviations

PDMS	Polydimethylsiloxane
SC	<i>Stratum corneum</i>
TDD	Transdermal drug delivery
ADHD	Attention deficit and hyperactivity disorder
CMC	Critical micelle concentration
SDS	Sodium dodecyl sulphate
CTAB	Cetyltrimethylammonium bromide
BZK	Benzalkonium chloride
PES	Polyethersulfone
ADME	Absorption, distribution, metabolism, elimination
LSE	Living skin equivalent
VOC	Volatile organic compound
QSPR	Quantitative structure-permeation relationship
PEG	Polyethylene glycol
EOF	Electroosmotic flow
CVD	Chemical vapour deposition
IGC SEA	Inverse gas chromatography – surface energy analyser
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared
NMR	Nuclear magnetic resonance
SEM	Scanning electron microscopy
EDX	Energy dispersive X-ray
DVS	Dynamic vapour sorption
HSPE	Human skin penetration effect
IPM	Isopropyl myristate
WCA	Water contact angle
ITC	Isothermal calorimetry
XPS	X-ray photoelectron spectroscopy

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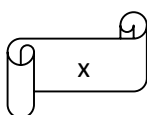


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Chapter 1: Introduction

1.1 Background

Topical and transdermal administration of drugs has received enormous interest as it fulfils a variety of medical and cosmetic purposes including the treatment of local skin disorders, therapy of local muscle injury, UV protection and systemic drug delivery. Such interest has prompted a significant research effort into the analysis of drug permeation through skin, and the prediction of such permeation (Anissimov & Roberts 2011). A number of *in vitro* methods have been established for the prediction of percutaneous permeation. Examples include mathematical models, cultured skin alternatives and synthetic membranes (Waters 2015). These predictive techniques are important not only for the design and development of novel formulations, but also for the quality assessment of products (Shah *et al.* 1999), and toxicity screening (Zorin *et al.* 1999). Amongst the predictive models, synthetic membranes have found extensive application as accepted *in vitro* skin mimics, based on their competitive advantages including better reproducibility, reduced cost (Ng *et al.* 2012), and simplicity of use. One such widely used skin mimic for studying transdermal permeation is poly(dimethylsiloxane) (PDMS) membrane (Waters *et al.* 2013), simply known as silicone membrane. The fact that PDMS membrane is a simplified model of skin, means that it has the advantage of producing greater reproducibility in data acquired yet has the disadvantage of behaving differently to skin under certain conditions. Several factors have already been reported to effect permeation through PDMS including membrane thickness (Lee *et al.* 2008), solvent selection (Dias *et al.* 2007; Shahzad *et al.* 2014) and temperature (Waters *et al.* 2013). Furthermore, surfactants are a group of compounds that are known to have the ability to alter skin permeation (Walters *et al.* 1993; Shokri *et al.* 2001), and have been studied, though to a very limited extent, to find their effect on the permeation

of compounds through PDMS membrane (Waters *et al.* 2013). The role of compound ionisation (as a result of formulation pH) on permeation across PDMS has also received very little attention, and is not well understood. Another influential factor on permeation is changing the PDMS surface, for example, by making the surface more hydrophilic, by means of chemical or physical treatment. Numerous applications have been reported for modified PDMS membrane (Hsu & Chang 2015; Ko *et al.* 2008), yet the impact of such modification on *in vitro* drug diffusion has received very little attention, and hence requires a thorough investigation. Understanding all of the above-mentioned aspects could further the suitability of PDMS membrane as an *in vitro* skin mimic, especially in the design and development of topical and transdermal formulations.

1.2 Human skin: structure and function

The skin is considered the most complex and largest organ in the human body (Ghafourian *et al.* 2010), and provides a multifunctional interface between the body and the external environment. The main purpose of the skin is to protect the body from the penetration of harmful chemicals (Gupta *et al.* 2016) *i.e.* an excellent biological barrier. The skin contributes to the body weight by about 4 %, and is $10^2 - 10^4$ times less permeable than a blood capillary wall (Cevc & Vierl 2010). It has a surface area of approximately $1.5 - 2 \text{ m}^2$, with a wide-ranging thickness according to gender and anatomical site (Pegoraro *et al.* 2012). Below are a few examples of the functions of the skin:

- Protects the internal body against foreign pathogens, chemicals, allergens, UV radiation.
- Prevents the loss of water, electrolytes and macromolecules.
- Helps in the synthesis of Vitamin D

- Forms an extensive sensory surface for sensing touch, pressure, heat, cold and pain (Moss *et al.* 2015).
- Plays a role in homeostasis by regulating body temperature and blood pressure (Benson & Watkinson 2012).

The skin is a multi-layered organ composed of three distinctive histological layers – the outer epidermis, the dermis and the hypodermis (also known as the subcutaneous tissue) (Fig. 1.1 A).

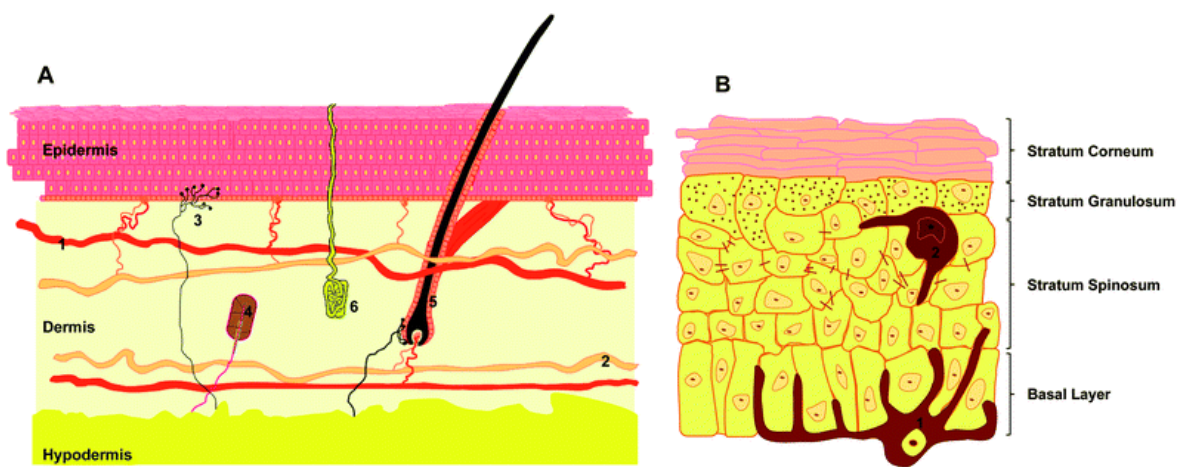


Fig. 1.1: (A) Cross-section of human skin (B) Human epidermis (Pegoraro *et al.* 2012)

The epidermis is the outermost, and also the thinnest, part of the skin. It is a multi-layered epithelium, consisting of four, or often five, separate layers – the *stratum corneum* (SC), the *stratum lucidum* (present in thick skin), the *stratum granulosum*, the *stratum spinosum* and the *stratum germinativum* (also known as the basal layer) (Fig.1.1. B). The outermost layer of the epidermis, and hence of the skin, is the *stratum corneum*, which is 10-20 μm thick. It is highly compacted, and acts as the primary barrier for the permeation of drugs. The SC consists of several layers of corneocytes with about 10 lipid bilayers compacted between two adjacent corneocyte layers. These lipid bilayers form the intercellular lipid matrix of the SC (Mitragotri 2003). The next layer of the skin is dermis (or corium), which lies immediately above the hypodermis. It contains blood vessels, lymphatic

vessels, nerve tissues, sense receptors, hair follicles, sweat glands and sebaceous glands (Fig. 1.1. A: numbered as 1, 2, 3, 4, 5, 6 respectively). It is usually 1 – 5 mm in thickness (Moss *et al.* 2015), and consists of collagen, elastin, glycosaminoglycan, salts and water. Underneath the dermis is the hypodermis, the deepest region of the skin. The hypodermis is rich in collagen and fat. Its main functions are insulation and shock-absorption (Dias *et al.* 2001; Pegoraro *et al.* 2012).

The skin, in recent years, has widely been accepted as a site for topical and systemic drug delivery. Topical application of drugs is useful when the target site is the skin. This type of drug delivery is usually intended to treat local skin disorders, or for cosmetic purposes. Examples of topical formulations include ointment, gel, cream, powder, foam, lotion and spray (Mugglestone *et al.* 2012). For systemic administration, drugs are delivered through the transdermal route using a vehicle such as a transdermal patch (Lenz & Gillespie 2011).

1.3 Transdermal drug delivery

In transdermal drug delivery or TDD, therapeutic compounds are administered continuously through the skin. The last few decades have resulted in significant development and success in the area of transdermal drug delivery (Hillery *et al.* 2001). The success of the TDD method can be evidenced by the fact that, currently in the USA, more than 35 transdermal products are approved for use to treat a number of conditions including severe pain, hypertension, nicotine dependence, female menopause and contraception (Thomas & Finnin 2004). There are also several products in the late-stage development phase that can potentially extend the use of TDD into complex therapeutic areas such as Parkinson's disease, female sexual dysfunction and attention deficit and hyperactivity disorder (ADHD). Another interesting application of the TDD method is the possibility of administering vaccines through the skin, to directly target the immune cells of the viable epidermis and initiate a

strong immune reaction (Pegoraro *et al.* 2012). Drug delivery via the transdermal route is considered a useful alternative to the intravenous or oral route, and the benefits have been well-documented. Below are several examples:

- TDD offers improved patient compliance (Thomas & Finnin 2004).
- No hospitalisation is required.
- The treatment mode is non-invasive.
- The affected area can be targeted selectively.
- First-pass effect can be avoided.
- Drug pharmacokinetics can be enhanced.
- The release of drugs can be controlled and sustained (Pegoraro *et al.* 2012).

1.4 Transdermal routes of drug permeation

Drugs can permeate through the skin by four possible routes (Mitragotri 2003):

- i. Free-volume diffusion across lipid bilayers
- ii. Lateral diffusion along lipid bilayers
- iii. Diffusion through pores
- iv. Diffusion through the transappendageal route

The transappendageal route is also known as the ‘shunt route’, for the fact that the skin has several natural openings, for example, sweat glands, hair follicles and wrinkles, which enable drug permeation through the skin. However, this route only constitutes 0.1 % of the total surface of the skin, and hence, is limited (Pegoraro *et al.* 2012). Transdermal transport of drugs, especially hydrophobic ones, is predominantly governed by the skin’s lipid bilayers (Mitragotri 2003), which create a lipophilic route of permeation. When permeating through the lipid bilayers of the intercellular lipid matrix, drugs can also diffuse through the corneocytes, though this hydrophilic route is unfavourable for most drugs. Hence,

the preferred pathway for transdermal permeation is the intercellular route – a continuous but twisted pathway across the intercellular lipid matrix (Fig. 1.2) (Pegoraro *et al.* 2012).

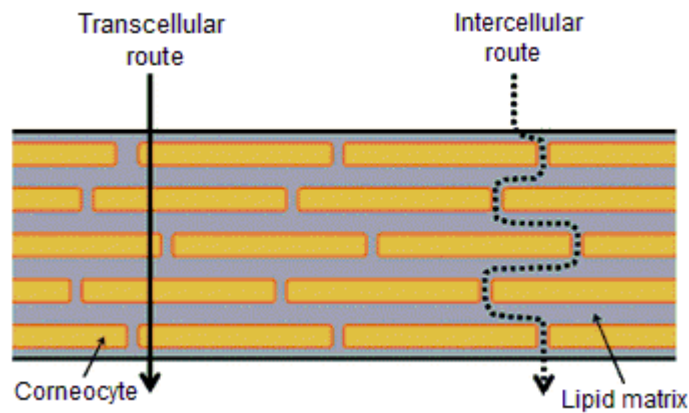


Fig. 1.2: Transepidermal routes of drug diffusion: transcellular and intercellular (Pegoraro *et al.* 2012).

1.5 Factors affecting drug permeation

Skin permeation of drug involves several processes including release of the drug from the formulation, followed by partitioning into, and diffusion through the *stratum corneum*, then uptake into other skin layers and penetration through to the deeper tissues or cutaneous circulation (Benson & Watkinson 2012). The rate and extent to which a drug permeates the skin depend not only on physiological factors but also on the physicochemical properties of the drug, and the formulation (Moss *et al.* 2015).

1.5.1 Physicochemical properties of penetrant

The permeation of drugs, into and across, the skin is known to be significantly affected by their physicochemical properties such as partition coefficient, molecular size and shape, aqueous solubility and ionisation. Le and Lippold found a linear relationship between permeability and partition coefficient for the permeation of homologous esters of nicotinic acid through the skin (Le & Lippold 1995). Moss *et al.* discussed that compounds with an

intermediate lipophilicity – having a log log P of between 1 and 3 – would permeate the skin via both lipophilic and hydrophilic routes but the intercellular pathway would predominate, and lipophilic compounds (those having a log P greater than 3) would traverse predominantly through the intercellular pathway (Moss *et al.* 2015). Molecular size and shape of a drug is also known to affect skin permeation. It has been shown that permeant size is inversely related to skin permeation (Idson 1975; Pugh *et al.* 2000). Scheuplein and Blank observed a decrease in the skin permeability of steroids when polar groups were incorporated in their structures (Scheuplein *et al.* 1969). Another important factor affecting transdermal permeation is the drug solubility in the lipid domain of the *stratum corneum*. The melting point (MP) of a drug is often used, in predictive models, to determine such solubility (Ostrenga *et al.* 1971). Drugs with a relatively low melting point, for example, nicotine (MP -79 °C) and nitroglycerine (13.5 °C), are very good skin penetrants (Benson & Watkinson 2012). Skin permeation can also be affected by the concentration of a drug in a topical formulation. Moss *et al.* mentioned that an increase in drug concentration would increase the amount of drug permeated through the skin (Moss *et al.* 2015). Permeation also depends on the ionisation state of a drug, in terms of its pK_a and the formulation pH. As the *stratum corneum* is predominantly lipophilic in nature, the unionised species are more likely to penetrate the skin than the ionised species. Therefore, the adjustment of formulation pH will alter the rate and extent of drug permeation across the skin (Moss *et al.* 2006).

1.5.2 Physiological factors

Besides the physicochemical properties of a penetrant, there are a number of physiological factors that can alter skin permeation. One of the important factors is skin condition. Generally, skin permeability relates to the permeation of penetrants across healthy and intact skin. Hence, the ‘quality’ of the skin is significant in experiments that are intended

to determine skin absorption. Diseases, such as eczema, or mechanical damage, such as cuts and abrasions, or chemical burns may disrupt the skin barrier and hence skin permeability (Moss *et al.* 2015). For example, one study found a 46-fold and 146-fold increase in the skin permeation of salicylic acid in mild dermatitis and severe dermatitis, respectively, relative to healthy skin (Benfeldt *et al.* 1999). There are some other physiological factors that can have a profound effect on skin permeation. These include skin hydration, skin age, anatomical site and skin temperature. Generally, permeation rate increases with an increase in skin temperature. It is known that skin permeation is initially a process of diffusion and therefore temperature dependant. Another factor that affects drug permeation is skin hydration, which is known to enhance the permeation of most chemicals – by elevating the rate of diffusion (Moss *et al.* 2015). With regards to skin age, the skin structure – in particular, the lipid composition – changes significantly as the skin ages which can alter the skin permeability of drugs (Benson & Watkinson 2012). For example, the skin permeation of topical steroids has been found to be greater in children than in adults (Idson 1975). However, not all penetrants show the age-related permeability pattern. One example is the permeation of sufentanil and fentanyl, where neither gender nor age were found to affect their permeation through skin (Roy & Flynn 1990). One widely studied area – with regards to percutaneous absorption – is skin permeability at different body sites (Benson & Watkinson 2012). Wide variations in skin permeation rates are generally observed across different anatomical sites in the same individual and between different individuals. Attempts have been made to rank body sites in terms of skin permeability. Below is an example of such rankings:

posterior aricular skin > scrotum > head and neck > abdomen > forearm > thigh > instep > heel > planter (Moss *et al.* 2015)

1.5.3 Formulation effects

As mentioned previously, skin permeation involves a series of steps – diffusion and partitioning from, and between, a number of compartments within the several skin layers. Formulations can be tailored to meet their goals – either enhancement/optimisation of percutaneous absorption, for example, in the case of pharmaceutical applications, or reduction of absorption into the physiologically active tissue in the case of cosmetic purposes (Moss *et al.* 2015). Formulations may include penetration retardants to discourage skin permeation, especially, to combat xenobiotic-related toxicity issues (Kaushik *et al.* 2008), or penetration enhancers to elevate drug permeation (Borrás-Blasco *et al.* 1997; Javadzadeh *et al.* 2010) and hence, to optimise transdermal drug delivery. Formulation selection can also enhance drug penetration through skin hydration and occlusion. For example, ointment and waxes are generally known to increase hydration through occlusion. However, the permeation profiles of drugs from different formulations can be significantly different. One example is the permeation of benzoic acid, caffeine and testosterone from three different vehicles (ethylene glycol, petroleum and an aqueous gel) through the skin (Moss *et al.* 2015). With regards to penetration enhancers, a number of excipients have been reported to have skin penetration enhancement effects. Examples include terpenes (Kang *et al.* 2007), fatty acid esters (Casiraghi *et al.* 2012), pluronic gels (Escobar-Chávez *et al.* 2005), cinnamic acid, cinnamic alcohol, cinnamaldehyde (Zhang *et al.* 2007), cyclodextrins (Sinha *et al.* 2003) and liposomes (Bouwstra & Honeywell-Nguyen 2002). One group of compounds that are known to have an effect on skin penetration are surfactants. They are known to intercalate with the continuous lipid domain of the *stratum corneum*, thus enhancing fluidity. The effects of surfactants on skin permeation have been investigated using a wide range of compounds. Surfactants have also been used in conjunction with physical penetration enhancement techniques such as iontophoresis to enhance transdermal drug delivery (Silva *et al.* 2012).

1.6 Surfactants

‘Surfactant’ is a diminutive form of ‘Surface Active Agent’. Many pharmaceutical, cosmetic and agro-chemical formulations contain surfactants where they are used as an emulsifier, or as a suspending, solubilising, wetting and stabilising agent (Borrás-Blasco *et al.* 1997). More importantly, recent years have seen a widespread use of surfactants as penetration enhancers to increase drug permeation through skin. The effects of surfactants on the skin is determined by their interactions with the skin – binding with the surface proteins, denaturing the surface proteins, solubilising the lipid bilayers, penetration through the lipid domain, and interaction with the living cells (Mohd. Yasir *et al.* 2012). The effects of surfactants can lead to an alteration in skin permeability. Many studies suggest an apparent concentration-dependent biphasic action of surfactant on skin permeability, such that skin permeability increases at low surfactant concentrations but decreases at higher values, usually above the critical micelle concentration (CMC). CMC is known as a narrow range of concentrations upon reaching which surfactant molecules form micelles (Walters *et al.* 1993). At concentrations below the CMC, surfactant exists as monomers and/or premicelles (Cui *et al.* 2008). Any surfactant-driven increase in skin permeability at these concentrations (i.e. below the CMC) is generally attributed to the ability of the monomers to penetrate the skin lipid domain and to increase its fluidity, thus disrupting the skin barrier function. At or above the CMC, a high level of solubilisation of penetrants into the micelles occurs, and this decreases the thermodynamic activity of the penetrants and hence, their permeation through the skin (Walters *et al.* 1993).

Surfactants are amphipathic molecules consisting of a hydrophilic (or polar) head group attached to a hydrophobic tail, which is a straight or branched hydrocarbon or fluorocarbon chain with 8 – 18 carbon atoms. The molecular weights of surfactants range from low to moderate. The uniqueness of their properties is evident in the fact that they tend

to concentrate at interfaces and reduce interfacial tension (Mohd. Yasir *et al.* 2012). The interfaces could be between solid/liquid, liquid/liquid, or gas/liquid pairs of phases. Surfactants can be well-differentiated from other compounds by two important properties – 1) adsorption at the surface, and 2) formation of micelles in solution (Porter 1994) (Fig. 1.3).

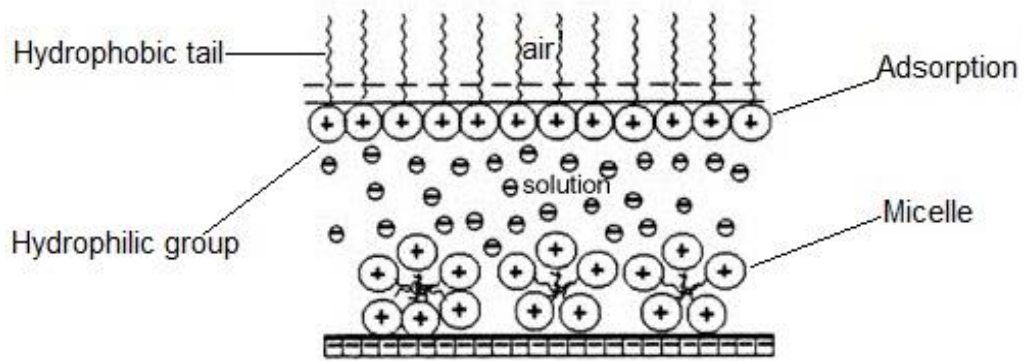


Fig. 1.3: Surfactant adsorption and micelle formation (Alexandrova 2007).

Micelles aggregate in a way that the hydrophobic tails are aligned towards the centre of the micelles, and the hydrophilic polar heads towards the aqueous bulk phase. To put simply, a micelle can be thought of as having two regions – a hydrophilic or polar region and a hydrophobic core. Compounds with predominantly lipophilic properties tend to partition into the hydrophobic core in a process called solubilisation (Walters *et al.* 1993), whereas hydrophilic or polar compounds prefer to stay in the aqueous bulk phase.

Surfactants are classified into four categories (Mohd. Yasir *et al.* 2012), based on the nature of their polar head groups:

- Anionic surfactant
- Cationic surfactant
- Zwitterionic or amphoteric surfactant
- Non-ionic surfactant

Anionic surfactants carry a negative charge in their head groups. Examples of this class include carboxylates (*e.g.* alkyl carboxylate-fatty acid salts), sulphonates (*e.g.* alkyl benzene sulphonates), phosphate esters (*e.g.* alkyl ether phosphates) and sulphates (*e.g.* sodium dodecyl sulphate). The penetration enhancement effects of anionic surfactants are associated with their ability to penetrate the skin and interact with keratin protein in the corneocytes, and the lipid bilayers. The interactions with keratin result in the unfolding of its coiled structure thus increasing the water binding sites, and hence enhancing skin hydration. The interactions with lipid bilayers induce a structural disorder of the lipid domain of the skin. Such disordering increases the fluidity of the *stratum corneum*, and therefore skin permeability (Mohd. Yasir *et al.* 2012). Several studies reported skin penetration enhancement effects of anionic surfactants, both *in vitro* and *in vivo*. In one study, sodium dodecyl sulphate (SDS) was found to increase the permeation of an antiviral agent, foscarnet, through rat skin (Piret *et al.* 2000). SDS has also been reported to enhance *in vitro* skin permeation of diazepam (Shokri *et al.* 2001) and lorazepam (Nokhodchi *et al.* 2003). Kitagawa and Ikarashi reported an increase in skin permeation of ketotifen induced by the presence of SDS (Kitagawa & Ikarashi 2003). Yamato *et al.* observed a higher skin permeation of propofol, both *in vitro* and *in vivo*, with the combined use of SDS and propylene glycol in the formulation (Yamato *et al.* 2009). SDS treatment of murine skin resulted in an enhancement of the transdermal permeation of polyethylene glycol (Tsai *et al.* 2003).

Unlike anionic surfactants, cationic surfactants contain a positive charge in their head groups. Most of the surfactants of this category correspond to nitrogenous compounds such as quaternary ammoniums and fatty amine salts. Examples of cationic surfactants include cetyltrimethylammonium bromide (CTAB), benzalkonium chloride (BZK) and cetrimide. Cationic surfactants are more damaging to the skin than anionic surfactants (Walker & Smith

1996). They alter skin permeability mainly by disrupting keratin fibrils of the skin corneocytes. The disruption results in the swelling of the *stratum corneum* and a subsequent increase in skin permeability (Mohd. Yasir *et al.* 2012). The cationic surfactant *n*-dodecyltrimethylammonium bromide has been reported to enhance drug permeation across skin (Kitagawa & Ikarashi 2003). Shokri *et al.* found that both benzalkonium chloride and CTAB induced a higher flux of diazepam through rat skin (Shokri *et al.* 2001). These two surfactants have also been reported to increase the percutaneous absorption of lorazepam (Nokhodchi *et al.* 2003).

Zwitterionic surfactants exhibit both cationic and anionic dissociations. The polar head groups of these surfactants contain a quaternary amine group and a carboxyl or sulfonic group. Examples are CHAPS, phospholipids (phosphatidylethanolamine, phosphatidylcholine), dodecylbetaine and hexadecylbetaine, and amino acids. Zwitterionic surfactants enhance skin permeability through the solubilisation of the *stratum corneum* lipids (Mohd. Yasir *et al.* 2012). Ridout *et al.* examined the effect of dodecylbetaine and hexadecylbetaine on the permeation of nicotinamide across excised skin and found an increment in nicotinamide flux owing to the presence of these surfactants (Ridout *et al.* 1991). CHAPS has been reported to increase the flux of mannitol through Caco-2 cells, an *in vitro* model of the intestinal mucosa (Nerurker *et al.* 1996).

Finally, non-ionic surfactants do not ionise in aqueous solution. Their head groups do not carry any charge and are of non-dissociable types, for example, alcohol, phenol, ester, amide and ether. Examples of non-ionic surfactants include Tween 80, polyoxyethylenes-2-oleyl ether, polysorbates, Span 20, poloxamer and Brij 35 (Shin *et al.* 2001). Non-ionic surfactants are less of an irritant than other surfactants and are most frequently used in drug delivery applications (Mohd. Yasir *et al.* 2012). Literature suggests that non-ionic surfactants enhance skin permeability by disrupting the intercellular lipids and/or keratin filaments of

the corneocytes (Nokhodchi *et al.* 2003). Limpongsa and Umprayn found Tween 80 to increase the skin permeation of diltiazem hydrochloride by three fold (Limpongsa & Umprayn 2008). Brij 35 has been reported to enhance the permeation of atenolol (Mohd. Yasir *et al.* 2012).

From the above discussion, it is clear that surfactants have widely been studied as penetration enhancers using both human and animal skin. However, the effects of surfactants on permeation have also been studied using artificial membranes such as polyethersulfone (PES) and polydimethylsiloxane (PDMS) membranes. Rahimpour *et al.* investigated the effects of CTAB, SDS and Triton X-100 on the flux of pure water and milk across PES membrane (Rahimpour *et al.* 2007). They observed an increase in water and milk flux, which they attributed to the surfactant-induced porosity of the membrane. Another study found Tween 80 to enhance water flux through PES membrane (Amirilargani *et al.* 2009). Waters *et al.* investigated the effects of two surfactants, namely, SDS and Brij 35, on the permeation of methylparaben and ethylparaben through PDMS membrane, and found a reduction in paraben permeation by SDS, whereas no significant effect was observed with Brij 35 (Waters *et al.* 2013).

1.7 *In vitro* drug diffusion

The assessment of skin permeation of drugs or other penetrants is vital for several reasons such as:

- Estimation of the potential of transdermal drug delivery
- Risk assessment of the contact of toxic substances with skin (Ottaviani *et al.* 2006)
- Measurement of the percutaneous absorption of materials used in cosmetics
- Risk assessment of the occupational exposure of materials used in several industries

(Moss *et al.* 2015)

There are a number of ways skin permeation can be measured or predicted such as *in vivo* (Pillai & Panchagnula 2003; Rosado *et al.* 2003; Godin & Touitou 2007), *ex vivo* (Schwingel *et al.* 2008; Minghetti *et al.* 2000), *in situ* (Plessis *et al.* 2001) and *in vitro* (Padula *et al.* 2008; Kwon *et al.* 2012; Fang *et al.* 2001) methods. Schwingel *et al.* used an *ex vivo* technique to measure the skin permeation of 3-*O*-methylquercetin, a potential antiviral flavonoid (Schwingel *et al.* 2008). In one study the skin permeability of doxylamine was investigated using both *in vitro* and *in situ* methods, and a good correlation was found between the two techniques within a specific period of time (Plessis *et al.* 2001). However, amongst all of the above-mentioned methods, *in vitro* and *in vivo* systems have been extensively used in skin permeation research.

In vivo techniques have been reported to provide useful information about the mechanism of skin permeation. They are generally non-invasive in a sense that skin responses, such as vasodilatation or skin blanching, are assessed in these techniques, rather than measuring blood samples or punch biopsies for subsequent analysis. However, they are limited in their applicability to those chemicals that do not produce any non-invasively quantifiable physiological changes. Moreover, the non-invasive monitoring of certain chemicals, such as those in cosmetics, does not provide any indication of cutaneous toxicity. *In vivo* methods are suitable, particularly, if the compound to be examined is an established material (such as ibuprofen), and its ADME (absorption, distribution, metabolism and elimination) are measured by analysing bodily fluids (Moss *et al.* 2015). Nevertheless, it can be significantly difficult to perform such experiments for a number of penetrants not only for toxicological aspects but also for logistical reasons, for example, the consistent availability of volunteers (Ansari *et al.* 2006). In addition to human volunteers, animals are also used to analyse skin permeation *in vivo*. However, a growing concern against animal testing and a ban on animal-tested cosmetics in the EU since 2013 has led researchers to alternative

approaches (Adler *et al.* 2011). Overall, because of the limits of *in vivo* methods in terms of feasibility, availability, or ethical concerns, *in vitro* techniques are being increasingly accepted.

In vitro techniques are often used prior to *in vivo* tests and in some instances, for example, the evaluation of new compound, are solely used to assess the potential toxicity to the human body. Consequently, *in vitro* models find a widespread application in the assessment of the risks and hazards associated with the skin exposure of exogenous chemicals. The vast majority of mathematical models have been developed based on the data obtained from *in vitro* experiments using excised human skin. *In vitro* methods for assessing percutaneous absorption are numerous and different in the details of their experimental protocols. The diversity of such methods is based on the selection of the diffusion membrane, nature of the experiments (*e.g.* occlusion, duration), type of diffusion cell (*i.e.* static or flow-through cells) and the composition of the donor, or receptor phases that are placed either side of the diffusion membrane (Moss *et al.* 2015). The temperature at which an *in vitro* permeation study is performed can also be varied. The majority of the published experiments were conducted at either 32 or 37 °C. However, a number of studies were also conducted at temperatures of 31-32, 22-30 and 25-31 °C (Moss *et al.* 2009).

The aim of an *in vitro* skin permeation study is to measure the amount of permeant that penetrates into and across skin. For this purpose, a diffusion apparatus is used which has two chambers – a donor chamber and a receptor chamber – separated by a membrane (generally human or animal skin, or artificial). The formulation containing the permeant of interest is introduced in the donor chamber at the start of an experiment. The receptor chamber is the chamber which is exposed to the membrane, and into which the permeant may diffuse, following transport into and across the membrane. A viable diffusion gradient must be maintained between donor and receptor solution to avoid equilibrium between both

chambers (Moss *et al.* 2015). The receptor chamber usually has a sampling port from which aliquots of samples are collected at pre-determined time intervals for quantification. Permeation across the membrane is generally measured by monitoring the rate of compound permeation into the receptor chamber, or the rate of compound loss from the donor chamber (Ottaviani *et al.* 2006).

There are two major types of diffusion cells: static and flow-through cells (Fig. 1.4). The static diffusion cells are commonly known as Franz cells (Franz 1975). In a Franz cell, the permeant penetrates into a “static” receptor chamber of a fixed volume that is kept at a controlled temperature and is continuously stirred to ensure homogenous mixing of the receptor solution throughout an experiment and to avoid any “dead” zones or diffusion gradients within the receptor chamber. Franz-type permeation cells can be found in a range of designs and sizes. These cells are upright and hence allow a range of pharmaceutical or cosmetic formulations such as solutions, creams or ointments to be investigated. One of the main limitations of Franz-type diffusion cells is the associated issue of “sink conditions”. In a static permeation cell, the concentration of penetrant in the receptor chamber needs to be no greater than 10 % of its saturated solution (Anissimov & Roberts 1999). An alternative way of solving issues related with “sink conditions” is to use flow-through type diffusion cells. Flow-through cells are also known as “Bronaugh” cells. In a flow-through diffusion cell, the receptor solution is continuously pumped through tubing that supplies a small compartment beneath the membrane and is collected into a receptacle for analysis, either offline or online. The advantage of a flow-through system is that the receptor phase does not have to be replaced, even for a permeant that rapidly penetrates. Despite the flexibility of flow-through cells, these cells are more complex and significantly more expensive than static Franz cells. The other commonly used diffusion cell is a side-by-side diffusion cell, which has the advantage of stirring and mixing in both donor and receiver chambers, but is limited

by the fact that a number of formulations can be difficult to apply in this system (Moss *et al.* 2015).

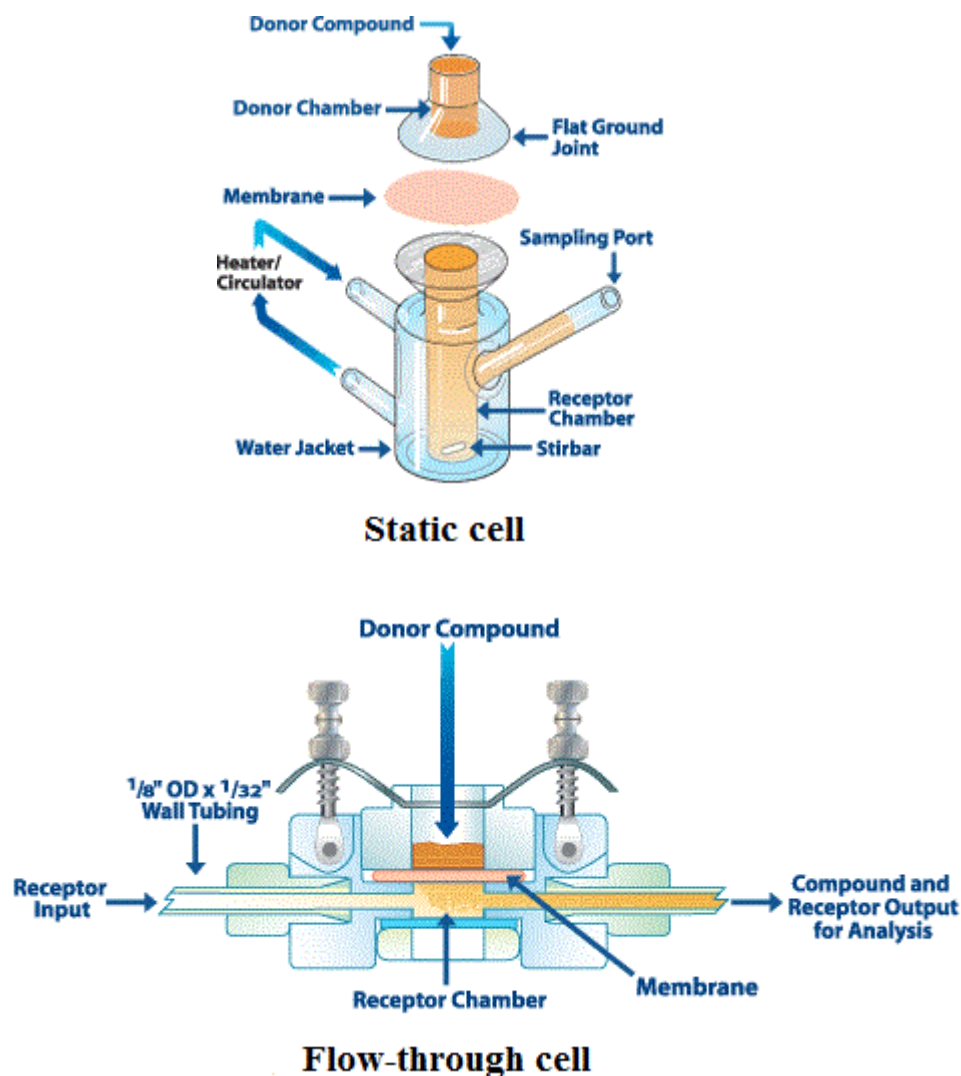


Fig. 1.4: Examples of major types of diffusion cells (PermeGear 2015)

In *in vitro* skin permeation studies, the donor phases generally involve aqueous or water-ethanol solvents. Other organic solvents such as propylene glycol may also be used to facilitate drug delivery across the skin. The majority of the receptor phases are based on buffer solutions, usually phosphate buffers with a pH of 7.4 (Moss *et al.* 2015). The receptor solution may also contain solubilising agents including various surfactants and proteins (e.g. bovine serum albumin). It is clear that there are significant variations in the composition of

formulations (donor or receptor solutions), yet only a few studies have considered formulation matters (Pugh *et al.* 2005; Ghafourian *et al.* 2010). In particular, ionisation of compound is not generally taken into consideration when modelling percutaneous absorption.

The widely used membrane in the assessment of *in vitro* skin permeation is human skin (Franz 1975). Skin from several animal species such as pigs, rats, snakes, monkeys and guinea pigs are also commonly used as a human skin replacement (Roberts & Mueller 1990; Barbero & Frasch 2009). Amongst animal skins, pig and rat skins have found widespread use, with the former being similar to the diffusion characteristics of human skin and hence a better surrogate for transdermal studies. Rat or mouse skin is more permeable (up to 10 times) than human skin (Roberts & Mueller 1990). However, rodent skin is also very popular, possibly because of its predominant use in pharmacological research. LSEs (living skin equivalents) have also been reported when studying *in vitro* skin permeation (Hager *et al.* 1994). Nevertheless, LSEs have so far failed to find widespread use, mainly because of the cost, reproducibility issues and their lack of robustness compared with human or animal skin. Moreover, they are generally known to overestimate the permeation rate through human skin (Moss *et al.* 2015). Studies have also reported the use of natural membranes such as egg, tomato, peach and onion membranes as skin mimics. One study compared the *in vitro* permeation of diclofenac across onion membrane and human skin, and found no significant difference in permeation ($p > 0.93$) (Ansari *et al.* 2006). However, these natural membranes are very limited in terms of applicability and robustness.

Human skin, usually excised, is the gold standard for *in vitro* drug diffusion studies, yet the use of excised skin is not without problems. Human skin is generally obtained from skin banks or tissue donors where surgical procedures including amputations are used to remove the skin. Surgical procedures for removing the skin may involve the use of alcohol-based disinfectants, which can compromise the barrier integrity of the skin and hence the skin

permeability. Therefore, the experimenter of *in vitro* permeation study has little control over the handling and quality of the skin. Moreover, the skin may be kept frozen prior to dispatch for use in experiments, and this can possibly cause the damage or degradation to the membrane (Moss *et al.* 2015). Furthermore, human or animal skin can be difficult to obtain, and there can be variation in skin permeability because of age, sex, race and anatomical site (Ansari *et al.* 2006). The above-mentioned difficulties and limitations of using mammalian skin have led researchers to consider artificial (or synthetic) membranes as *in vitro* skin mimics. Synthetic membranes are very useful when a large number of experiments, in particular, preformulation screening studies, need to be carried out (Moss *et al.* 2015).

1.8 Synthetic membranes as skin mimics

Synthetic membranes are composed of polymeric macromolecules, generally compacted in layers. They can be prepared using synthetic polymers (such as polysulfone or polycarbonate) or semi-synthetic polymer (such as cellulose acetate or regenerated cellulose etc.). Synthetic membranes have found their extensive applications, mainly, in industrial separation processes such as gas separation, pervaporation, reverse osmosis, electro dialysis, microfiltration and ultrafiltration (Ng *et al.* 2012). For *in vitro* drug diffusion studies, synthetic membranes are utilised, as mentioned earlier, to reduce the variation in skin permeability or when biological skins are not available. Numerous studies have considered the use of synthetic membranes to study drug diffusion *in vitro* (Parks *et al.* 1997; Feldstein *et al.* 1998; Iordanskii *et al.* 2000; Wasdo *et al.* 2008; Sugibayashi *et al.* 2010; Ng *et al.* 2010). Synthetic membranes for *in vitro* drug diffusion experiments are intended mainly for two purposes – either to simulate the skin, or to assess the quality of drug products. In 1989, Shah and co-researchers from the FDA demonstrated that synthetic membranes, along with Franz diffusion cells can be used for the quality assessment of semi-solid products (Shah *et*

al. 1989). Unlike skin simulation, synthetic membranes for quality assessment purposes should not be rate-limiting, and should only provide a means for separating the donor and receptor phases (Ng *et al.* 2012). Examples of synthetic membranes used for *in vitro* permeation studies include pure cellulose, cellulose acetate, cellulose ester, cellulose nitrate, carbosil (Feldstein *et al.* 1998), Celgard 3500, polycarbonate, polypropylene, polysulfone and silicone membranes (Ng *et al.* 2012). One study investigated the permeation of ibuprofen across a total of 14 synthetic membranes, namely, cellulose nitrate, Nuclepore, Celgard, Cyclopore, Tuffryn, Supor, AN69, cellulose ester, Biodyne B, Biodyne C, Cuprophan, benzoylated cellulose, Visking and PDMS membranes (Ng *et al.* 2010). The study suggested that when selecting a synthetic membrane, it would be important to consider the compatibility of the membrane with the donor and receptor solution, and the cost effectiveness of the membrane.

Synthetic membranes employed in *in vitro* drug diffusion studies can be broadly divided into two categories – cellulose-based and polymeric-based membranes. Cellulose-based membranes are made of regenerated cellulose and its derivatives, whereas polymer-based membranes are composed of non-cellulose polymers such as polysulfone, polycarbonates and polydimethylsiloxane (PDMS). The synthetic membranes used for topical formulation assessment are generally based on regenerated cellulose (Reid *et al.* 2008; Ng *et al.* 2012). Cellulose ester and polysulfone membranes are also used in the assessment of topical drug products (Shah *et al.* 1989; Wu *et al.* 1992). The common feature of the membranes used for topical formulation assessment is that they contain pores. Therefore, these membranes do not offer rate-limiting permeation properties, which are necessary, should the membranes be used to simulate human skin. One type of synthetic membrane that has a skin-like rate-limiting barrier property is PDMS membrane, commonly known as silicone membrane. Silicone membrane has been extensively used *in vitro* to

estimate or predict the skin permeability of drugs or cosmetic ingredients (McCarley & Bunge 2003; Ley & Bunge 2007; Santos *et al.* 2009; Oliveira *et al.* 2010; Santos *et al.* 2011; Oliveira *et al.* 2012).

1.9 Polydimethylsiloxane membrane

Polydimethylsiloxane (PDMS) (Fig. 1.5) membrane is a non-porous, amorphous and hydrophobic elastomer (Twist & Zatz 1986). The siloxane bond (-Si-O-Si-) that forms the backbone of the membrane is very highly stable. PDMS membrane can be immersed into water for a long period, with no effect on its mechanical strength or physicochemical properties. The physiological and chemical inertness of PDMS membrane broadened its applications in a number of fields including medical, electronic, automotive and aerospace industries (Simon *et al.* 2008). In particular, PDMS has found its widespread use in the fabrication of microfluidic devices, mainly because of its incredible properties such as gas permeability, transparency and ease of patterning with soft lithography. PDMS-containing microfluidic devices are used for several purposes including electrophoresis, long-term organotypic culture and cell culture (Markov *et al.* 2014).

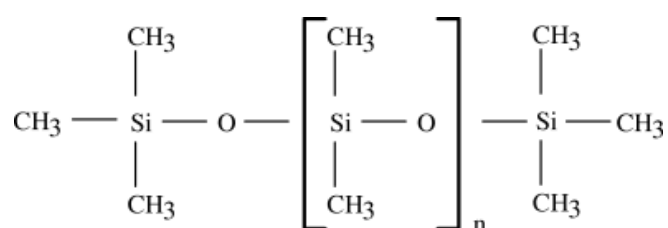


Fig. 1.5: Chemical structure of polydimethylsiloxane (Glombitza & Muller-Goymann 2001)

The non-porous nature of PDMS membrane made it suitable for the selective permeation of liquids and gases, and thus the membrane is used for water desalination, the separation of gases from liquids and many other purposes. Amongst many synthetic membranes, PDMS has shown excellent analytical performance for the fast and efficient

permeation of a range of volatile organic compounds (VOCs), with an exception to polar compounds such as alcohols (Boscaini *et al.* 2004). Moreover, PDMS membrane is widely used in *in vitro* drug diffusion studies to simulate the skin. The suitability of PDMS to predict drug permeation is generally attributed to the skin-analogous hydrophobicity and rate-limiting properties of the membrane. Drug permeation through PDMS membrane can be explained using a solution-diffusion model. According to this model, drug molecules first dissolve in the membrane and diffuse across the membrane down a concentration gradient. In this model, the steady-state flux (J) of drug is based on Fick's law of diffusion (Eq. 1.1) (Ng *et al.* 2012)

$$J = \frac{D \cdot K \cdot C_v}{h} \quad (1.1)$$

where, K is the partition coefficient of drug between the vehicle and the membrane, D is the diffusion coefficient of drug into the vehicle that fills in the membrane pores, C_v is drug solubility in the vehicle, and h is the membrane thickness. To permeate across a non-porous membrane, such as PDMS, drug molecules traverse through the tiny gaps between the polymer chains of the membrane, which generally results from the thermal motion of the polymer molecules (Ng *et al.* 2012).

A large number of studies have been performed on PDMS membrane, either to compare drug permeability profiles with an *in vivo* situation (Woolfson *et al.* 1998) or human skin *in vitro* (Romonchuk & Bunge 2006), or to establish QSPR (Quantitative Structure-Permeation Relationship) models (Geinoz *et al.* 2002), or to search for factors affecting drug permeation (Oliveira *et al.* 2010; Dias *et al.* 2007; Santos *et al.* 2011). Romonchuk and Bunge studied the permeation of two compounds, 4-cyanophenol and methyl paraben, from pure powder and saturated aqueous solution through human skin and PDMS membrane (Romonchuk & Bunge 2006). They found a faster permeation for both of the model

compounds across PDMS compared with the skin. Wasdo *et al.* found correlations ($r^2 = 0.743$) between hairless mouse skin and PDMS membrane for the permeation of 32 compounds from water (Wasdo *et al.* 2009). Woolfson *et al.* examined the permeation of tetracaine (a local anaesthetic agent) from a range of formulations through PDMS membrane, and found good *in vivo* correlations in cases where the lipophilicity of the permeant was the prime factor for drug permeation (Woolfson *et al.* 1998). With regards to a QSPR model, a major study for quantifying permeability across PDMS membrane was carried out by Chen *et al.* (Chen *et al.* 1996) where they developed a model (Eq. 1.2) using the permeation data of 103 compounds.

$$\log J_{mss} = -2.497 - 4.339 \sum e_+ - 1.531 \sum e_- + 4.065 \left(\sum e_+ \cdot \sum e_{p-} \right) \\ + 0.649 \log C_s - 0.00651 MW - 0.640 \text{imidazole} + 0.689 \text{amine} \\ [n = 103 \quad r^2 = 0.966 \quad s = 0.238 \quad F = 386.5] \quad (1.2)$$

where

J_{mss} is the maximum steady-state flux ($\mu \text{ mol/s/cm}^2$);

$\sum e_+$ is the sum of the charge values of hydrogen atoms with charge higher than 0.1 and the positive charge of a nitrogen atom in a nitro group;

$\sum e_-$ is the sum of the absolute charge values of all other heteroatoms with unshared electron pairs in the same molecule;

C_s is the solubility;

MW is the molecular weight (g/mol); and

Imidazole and amine are indicator variables for imidazole and aliphatic amine groups.

Using the data published by Chen *et al.* (1996), Agatonovic-Kustrin *et al.* developed a novel artificial neural network model, which indicated that molecular shape and size, hydrogen-bonding capacity of drugs, intermolecular interactions and conformational stability were important for drug penetration across PDMS membrane (Agatonovic-Kustrin *et al.* 2001).

Several studies have reported that drug permeation through PDMS membrane can be affected by a number of factors including vehicles, temperature and the physicochemical properties of the drug itself. Oliveira *et al.* studied the permeation of methyl paraben in the presence of butanol and heptanol across PDMS membrane at different temperatures, and found an enhancement of paraben permeation owing to both the vehicles and temperature (Oliveira *et al.* 2010). Dias *et al.* investigated the role of several solvents on the permeation of three model compounds, namely, caffeine, salicylic acid and benzoic acid, across silicone membrane, and suggested that the drug diffusion process was influenced by both the membrane-solvent interactions and solute characteristics such as size, shape and charge distribution (Dias *et al.* 2007). Two other factors that can affect permeation through PDMS membrane (though have only been studied to a limited extent), are ionisation (Smith & Irwin 2000) and surfactants (Waters *et al.* 2013).

One of the attractive features of PDMS-based materials is that their surface properties can be altered using a range of chemicals (Roman & Culbertson 2006), or physical treatment techniques (Markov *et al.* 2014). In particular, the surfaces of PDMS-based materials can be transformed from hydrophobic to hydrophilic, and then additional functional groups can be added to the modified surface to customise it for various applications, for example, making the materials more suitable for separation purposes. Several methods are utilised for the modification of PDMS surface, with each method having its own specifications, and purposes such as improving electroosmotic flow, making the membrane surface more hydrophilic and resistant to chemical absorption or adsorption (Roman & Culbertson 2006).

1.10 Methods for the modification of PDMS surface

PDMS surfaces can be modified by a number of methods. The purposes of such modifications range from improving the performance of PDMS-based microfluidic devices to optimising gas permeability through PDMS membrane. Various surface modification techniques can be found in literature:

- i. Generally, plasma discharge and/or UV radiation are used to oxidise PDMS surfaces. Such treatments result in the surface silane (Si-CH₃) groups of the membrane being replaced with silanol (Si-OH) groups, thus introducing hydrophilicity to the membrane surface (Sui *et al.* 2005). The silanol groups of the PDMS surface can then be used to attach a variety of functional groups using simple silanisation or free radical chemistry. For example, acrylamide can be attached onto the oxidised PDMS surface through a silanisation process. (Roman & Culbertson 2006). Polyethylene glycol (PEG) can also be attached onto the modified PDMS surface. The resulting PEG-grafted surface is relatively stable and shows protein-propelling characteristics, which is required for the development of microfluidic-based biosensing devices (Papra *et al.* 2001).
- ii. Covalent surface modification techniques involve the use of chemicals such as cerium and nitric acid to oxidise the PDMS surface. This technique does not use coronal discharge or plasma (Roman & Culbertson 2006). The oxidised PDMS surface can then be used for the attachment of acrylamide, which offers a reproducible and stable coating of the microfluidic device allowing highly efficient peptide separation (Slentz *et al.* 2002). Covalent surface modification can also be achieved, for example, in assembled PDMS channels, using graft photo-polymerisation. However, the requirement of multiple washings of the channels makes the procedure lengthier (Bauer *et al.* 2010).

- iii. Non-covalent methods involve the use of proteins, ionic surfactants and polyelectrolyte layers (Roman & Culbertson 2006). For example, Badal *et al.* used SDS and CTAB solutions in PDMS-coated microchip devices to control electroosmotic flow (EOF) (Youssef Badal *et al.* 2002). Yang *et al.* used a protein-bound phospholipid bilayer-coated PDMS microchannel for a rapid immunoassay (Yang *et al.* 2001). Polyelectrolyte layers are used to increase, decrease, or reverse the EOF (Roman *et al.* 2005).
- iv. Chemical vapour deposition (CVD) methods are used to coat the luminal surfaces of PDMS-based lab-on-a-chip devices with thin polymer films such as poly (*p*-xylene carboxylic acid pentafluorophenolester-*co-p*-xylene) (PPX-PPF) and poly (*p*-xylene-2,3-dicarboxylic acid anhydride). Such coatings are used to immobilise protein in PDMS-based microfluidic devices, and to screen pharmacologically active compounds in cell-based assays (Lahann *et al.* 2003).
- v. Sol-gel methods are generally used to modify cured PDMS surfaces. In these methods, inorganic compounds, for example, silica and the oxides of titanium, zirconium, aluminium and germanium, are imbedded within the PDMS surface. Sol-gel techniques are suitable for rapid and efficient inorganic coatings, which increase the separation efficiencies of PDMS-based microfluidic devices (Roman & Culbertson 2006).

The above-mentioned methods for PDMS surface modification have predominantly been used or studied with regards to PDMS-based microfluidic devices. Among these methods, plasma surface treatment has been accepted as an easy, economic and reliable technique. In some cases, plasma treatment acts as a first stage in a series of procedures to modify the PDMS surface (Yu *et al.* 2015). The purposes of modifying PDMS membrane

surface by plasma treatment have been limited – either to induce grafted polymerisation (Lee *et al.* 1996), investigate gas permeability (Markov *et al.* 2014), or analyse surface properties such as wettability (Bodas *et al.* 2008).

1.11 Plasma surface treatment

‘Plasma’ is regarded as the fourth state of matter. Plasma is generated when gases are excited by radio frequency (rf), microwave or electrons from a hot filament discharge. It contains highly excited gas atoms and molecules, ions, electrons, UV radiation, neutral gas atoms and molecules, and radical species. The high density of excited and ionised species of plasma can interact and alter material surfaces placed in contact with plasma. Plasma treatment can incorporate different functional groups on polymer surfaces. Examples include carboxyl, hydroxyl, hydroperoxide, carbonyl and amino groups (Tu *et al.* 2005). Plasma surface treatment is performed in an evacuated chamber. After pumping the air out from the chamber, a gas or mixture of gases is allowed to flow in at low pressure. Energy in the form of electrical power is then applied through the gas, and plasma is generated. The energy is produced and provided by an excitation source, known as a plasma source (Fig. 1.6). Active oxygen species and UV radiation from plasma break up the separating agents, impurities and oils from sample surface. These are pumped away by a vacuum system. Activated oxygen species (radicals) from plasma react and bind with the active surface sites all over the sample material, producing a highly chemically reactive surface.

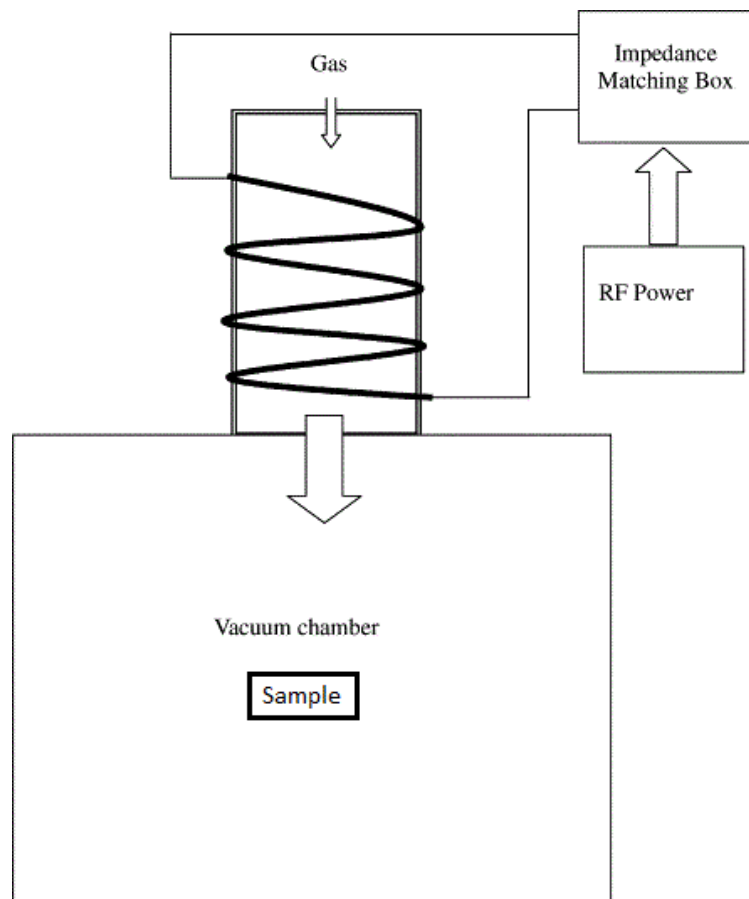


Fig. 1.6: Schematic diagram of a plasma source (Chu *et al.* 2002)

Through plasma surface treatment, it is possible to change the compositions and properties of a polymer such as wettability, chemical inertness, metal adhesion and biocompatibility. Consequently, plasma-based techniques have found extensive and diverse applications, mainly, in the biomedical field, for example, to treat surgical instruments and venous catheters to improve friction and biocompatibility (Chu *et al.* 2002). For biomedical, biotechnological and bio-diagnostic applications, plasma-based approaches are utilised in several strategies:

- i. Biologically active molecules, such as proteins, are immobilised on plasma-fabricated surfaces through conventional chemical reaction.
- ii. Protective surfaces or coatings are created to control bio-interfacial interactions.

- iii. Non-reactive surfaces are produced that do not possess attractive interfacial forces toward proteins and thus have non-fouling properties (Siow *et al.* 2015).

Plasma treatment can be of various types depending on the choice of gas or mixture of gases. Below are the few examples that have been reported in literature:

- Air plasma (Volkov *et al.* 2015)
- Nitrogen-based plasma such as N₂, NH₃ (Kull *et al.* 2005; Lai *et al.* 2008; Pang *et al.* 2012)
- Ar-plasma (Lee *et al.* 1996)
- O₂ plasma (Kim *et al.* 2002; Sartowska *et al.* 2003; Bodas *et al.* 2008)
- H₂O plasma (Steen *et al.* 2002)
- Acrylic acid plasma (Weibel *et al.* 2006)
- Mixed gas-based plasma such as Ar/NH₃ (Bryjak *et al.* 2002; Kull *et al.* 2005), O₂/NH₃ (Kull *et al.* 2005), C₂H₂/N₂ (Tu *et al.* 2005).

It can be seen from these examples that plasma types are diverse, and this diversity has enabled plasma treatment to be used for a wide range of purposes. For example, Zarshenas *et al.* utilised corona air plasma for modifying polyamide membrane to improve gas separation performance (Zarshenas *et al.* 2015). H₂O and N₂ plasmas were used to improve antifouling properties of polypropylene membrane (Yu *et al.* 2008). Weibel *et al.* modified polyurethane membrane using acrylic acid plasma to improve the efficiency for the separation of methanol from methyl-*t*-butyl ether (Weibel *et al.* 2006). H₂O plasma was used to increase the wettability of polyethersulfone and polyethylene membranes (Steen *et al.* 2002). Nitrogen plasma was used to create a permanent hydrophilic membrane surface (Kull *et al.* 2005). Markov *et al.* used low pressure air plasma to measure oxygen diffusivity

through PDMS membrane to understand normoxic and hypoxic oxygen conditions in microfluidic bioreactor systems (Markov *et al.* 2014). Despite the extensive research on plasma treatment methods for the modification of synthetic membranes, limited research, as mentioned in Section 1.10, has focused on such modification with regards to PDMS membrane. Moreover, no known study has considered the implication of plasma-modified PDMS membranes in *in vitro* drug diffusion studies, where PDMS membranes are often used as skin mimics.

1.12 Aim and objectives

The research presented in this thesis aims to understand the effect of several factors including formulation pH, excipients, and membrane surface modification on drug permeation through a chemical based skin mimic, namely silicone membrane.

The objectives of this research work are presented below:

1. To study the effect of the ionisation on the permeation of drugs through silicone membrane.
2. To study the effect of surfactants on the permeation of drugs across silicone membrane.
3. To predict the mechanism of surfactant effect on drug permeation through silicone membrane.
4. To investigate the effect of plasma surface modification of silicone membrane on the permeation of drugs.

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Chapter 2: Experimental

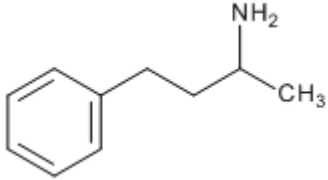
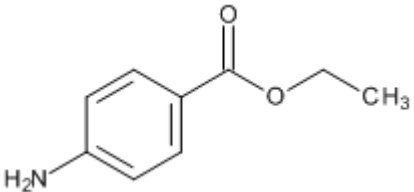
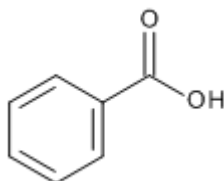
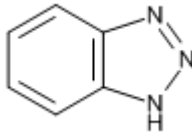
2.1 Materials

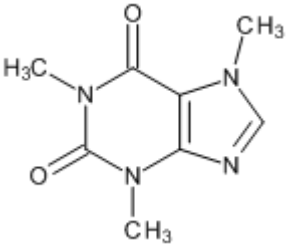
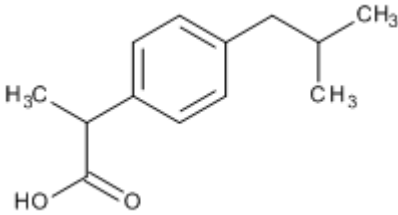
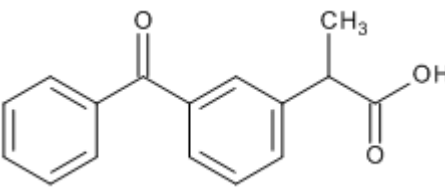
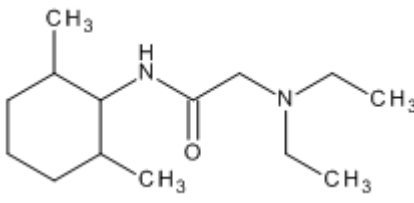
Polydimethylsiloxane (PDMS) membrane, with a standard thickness of 130 μm , was purchased from ATOS Medical (Hörby, Sweden). The flow-through diffusion cells were purchased from PermeGear Inc. (Hellertown, PA 18055 USA). De-ionised water was used throughout the experiments. Chemicals used are listed in Table 2.1, along with their physicochemical properties (Table 2.2).

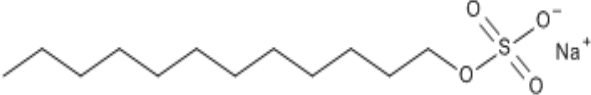
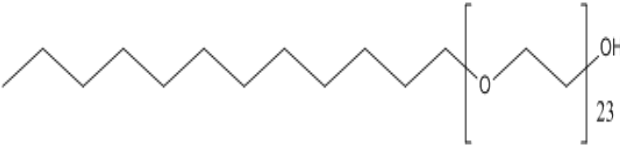
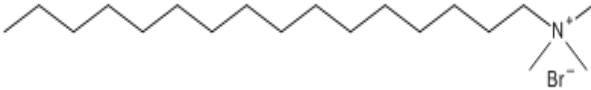
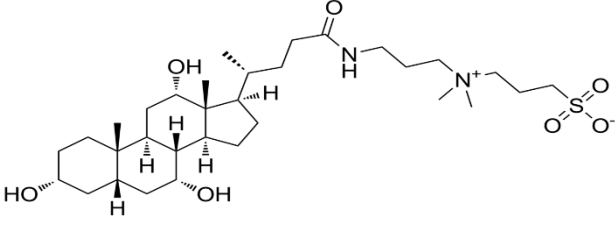
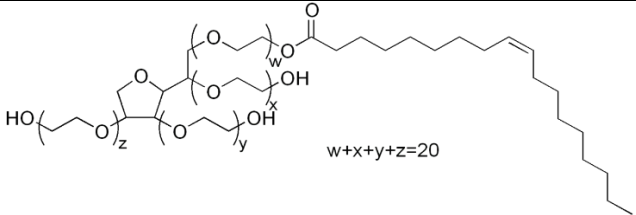
Table 2.1: The purity and supplier of the compounds analysed

Compound	Purity	Supplier
1-methyl-3-phenylpropylamine	98.0 %	Sigma-Aldrich
Benzocaine	≥ 99.0 %	Sigma-Aldrich
Benzoic acid	> 99.5 %	Sigma-Aldrich
Benzotriazole	99.0 %	Sigma-Aldrich
Caffeine	97.0 %	Sigma-Aldrich
Ibuprofen	> 97.0 %	BASF
Ketoprofen	> 98.0 %	TCI Europe
Lidocaine	> 98.0 %	Sigma-Aldrich
Sodium dodecyl sulfate	≥ 99.0 %	Sigma-Aldrich
Brij 35	Proteomics grade	BDH Laboratory
CTAB	≥ 98.0 %	Sigma-Aldrich
CHAPS	≥ 98.0 %	Fisher Scientific
Tween 80	Super refined	Croda International

Table 2.2: Physicochemical properties of chemicals (Molecular weight, polar surface area and log P were generated from ACD/labs, RSC, UK)

1-methyl-3-phenylpropylamine		
	Molecular weight	149.23 g/mol
	Polar surface area	26 Å ²
	log P	2.18
	pK _a (U.S National Library of Medicine 2016)	9.79
	CAS Number	22374-89-6
Benzocaine		
	Molecular weight	165.19 g/mol
	Polar surface area	52 Å ²
	log P	1.95
	pK _a (DrugBank 2016)	2.78
	CAS Number	94-09-7
Benzoic acid		
	Molecular weight	122.12 g/mol
	Polar surface area	37 Å ²
	log P	1.89
	pK _a (Wang <i>et al.</i> 2013)	4.2
	CAS Number	65-85-0
Benzotriazole		
	Molecular weight	119.12 g/mol
	Polar surface area	42 Å ²
	log P	1.34
	pK _a (Benitez <i>et al.</i> 2015)	8.2
	CAS Number	95-14-7

Caffeine		
	Molecular weight	194.19 g/mol
	Polar surface area	58 Å ²
	log P	-0.13
	pK _a (ACD Lab, RSC)	0.52
	CAS Number	58-08-2
Ibuprofen		
	Molecular weight	206.28 g/mol
	Polar surface area	37 Å ²
	log P	3.72
	pK _a (William & Pritchett 2014)	4.9
	CAS Number	15687-27-1
Ketoprofen		
	Molecular weight	254.28 g/mol
	Polar surface area	54 Å ²
	log P	2.81
	pK _a (Bechet 1999)	4.0
	CAS Number	22071-5-4
Lidocaine		
	Molecular weight	234.34 g/mol
	Polar surface area	36 Å ²
	log P	3.63
	pK _a (Liu <i>et al.</i> 2003)	7.8
	CAS Number	137-58-6

Sodium Dodecyl Sulphate		
	Molecular Weight	288.40 g/mol
	Charge	Anionic
	CMC	8 mM
	Micellar shape	Spherical
Brij 35		
	Molecular Weight	1199.54 g/mol
	Charge	Non-ionic
	CMC	0.09 mM
	Micellar shape	Spherical
CTAB		
	Molecular Weight	364.45 g/mol
	Charge	Cationic
	CMC	0.82 mM
	Micellar shape	Spherical
CHAPS		
	Molecular Weight	614.88 g/mol
	Charge	Zwitterionic
	CMC	4 mM
	Micellar shape	Rod-like
Tween 80		
	Molecular Weight	1310 g/mol
	Charge	Non-ionic
	CMC	5-50 μ M
	Micellar shape	Cylindrical

2.2 Methods

2.2.1 iGC SEA (Inverse gas chromatography – surface energy analyser) methodology

70 mg of PDMS membrane was cut into small pieces and packed into an individualised 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. The data were analysed using both standard and advanced surface energy analysis software (Surface Measurement Systems, UK).

2.2.2 Differential scanning calorimetry (DSC)

PDMS membrane was cut to an appropriate size for investigation and treated overnight in phosphate buffer (0.02 M PBS, pH 7.4) solution with or without the surfactants (SDS, Brij 35, CTAB, CHAPS and Tween 80) present in the buffer at 20 mM. The samples were then dried with soft tissue to remove excess liquid. DSC scans of the untreated and the treated samples were performed using a DSC 1 (Mettler-Toledo Ltd., Leicester, UK), at a heating rate of 1 °C/min over a range of -60 °C to -20 °C. All DSC thermograms were assessed with regard to the phase transition of PDMS membrane, which was reported to be -40 °C (Dias *et al.* 2007).

2.2.3 Fourier-transform infrared (FTIR) spectroscopy

FTIR analysis of the untreated and treated membranes (see Section 2.2.2 for treatment procedure) were performed using a Nicolet IR 380 spectrometer. The samples were cut into suitable sizes and placed in direct contact with the diamond crystal of the spectrometer. A

measurement range of 4000-400 cm^{-1} was used for all experiments. The data were obtained from, and analysed by a spectrometer-linked computer equipped with the Omnic software (version 7.2a).

2.2.4 Nuclear magnetic resonance (NMR) spectroscopy

SDS and Brij 35 were used to prepare the sample solutions, at concentrations of 4, 6, 8, 10 and 20 mM for the former, and 1 and 10 mM for the latter. When necessary, membranes were kept in the solutions for ~24 h. D_2O was used as solvent. In all ^1H NMR experiments, samples were analysed using a Bruker AV400 spectrometer (400 MHz) at 25 °C, and the spectra were then processed using Bruker TopSpin 3.1 software.

2.2.5 Plasma surface treatment process

The surface of PDMS membrane was modified using a standard plasma surface treatment method. The treatments were performed in a benchtop laboratory plasma unit (Henniker Scientific) (Fig. 2.1).



Fig. 2.1: A benchtop laboratory plasma unit.

PDMS membrane pieces were cut with a diffusional area of 0.554 cm² for flow-through permeation cells and placed in the plasma unit under low pressure on full power (40 kHz, 100 W). The same treatment was repeated on the alternate surface of the pieces under identical conditions with both surfaces exposed to air plasma for 90 seconds each. Contact angle measurement, SEM (Scanning Electron Microscopy), EDX (Energy Dispersive X-ray) analysis and permeation studies were conducted using the membrane immediately after the plasma treatment.

For aging studies, the treated PDMS samples were stored under air tight conditions for a maximum of 8 weeks, and permeation studies were performed using the stored samples aged for 1, 2, 4 and 8 weeks.

2.2.6 Contact angle measurement

Contact angle analysis was carried out to examine the alteration of hydrophilicity of PDMS membrane before and after the plasma treatment. The static contact angles of the treated and untreated PDMS samples were measured at room temperature using a sessile drop method. This method employs an optical goniometer with an attached precision syringe (FTA1000, Surface Science Instruments, USA). A droplet of deionised water was dispensed from the precision syringe onto PDMS surface (treated or untreated) and the resultant angles were measured over a period of 200 s.

2.2.7 Scanning electron microscopy (SEM)

SEM images of PDMS membrane were obtained on a FEI Quanta 250 equipped with backscattering and secondary electron detectors. The membrane samples were affixed to a standard aluminium stub by double-sided carbon tape and then sputter-coated with a thin layer of Au (gold) to prevent surface charging. After that the samples were loaded in the SEM

for imaging. For the study mentioned in Chapter 5, two categories of PDMS samples were analysed – untreated and treated (see Section 2.2.2 for treatment procedure). For the study mentioned in Chapter 6, three categories of PDMS samples were investigated – untreated, plasma-treated, and aged (maximum for 8 weeks) plasma-treated. In all analysis, the acceleration voltage was 5 keV. Everhart-Thornley detector (ETD) was used to detect the secondary electrons that are ejected from the k-shell of the sample atoms.



Fig. 2.2: A FEI Quanta 250 Scanning Electron Microscope.

2.2.8 Energy dispersive X-ray analysis (EDX)

EDX analysis of the PDMS membranes, both plasma-treated and aged plasma-treated, were performed using an Oxford detector attached to a FEI Quanta 250 ESEM. The procedure was analogous to that mentioned in Section 2.2.6.

2.2.9 Permeation experiments and data analysis

A system employing flow-through type diffusion cells with a diffusional area of 0.554 cm² was used for all permeation studies. PDMS membrane, with a measured thickness of 130 μm, was employed as a permeability barrier. In all cases, except for plasma-treated samples, the membrane was soaked in buffer solution for 30 minutes prior to being mounted in the diffusion cells. After assembly the cells were placed on a cell warmer for a temperature of 32 °C to be maintained. To start each permeation experiment, 0.8 mL of the donor solution containing model compound (and/or surfactant) was added to the diffusion cell. In all experiments the concentration of the model compounds in the donor solution was 1 mg/mL. In the cases whereby surfactants were involved (Chapter 4), the concentrations used were 0, 4, 8 or 20 mM for SDS, Brij 35, Tween 80, CTAB and 0, 2, 4 or 20 mM for CHAPS. Phosphate buffer saline (pH 7.4 for the study discussed in Chapter 4 and 6, and pH 4.5, 6.0, 6.5, 7.0, 7.4, 8.0 and 8.5 for the study discussed in Chapter 3) was pumped through the cells at 5 mL/h. The concentration of PBS used in Chapter 3 and 4 was 0.02 M, whereas for Chapter 6 it was 0.05 M. The samples were collected by means of a fraction collector at the predetermined time intervals.

Extracted samples were assayed by means of a validated UV spectroscopic method to quantify the model compounds (1-methyl-3-phenylpropylamine at 217 nm, benzocaine at 258 nm, benzoic acid at 226 nm, benzotriazole at 262 nm, caffeine at 273 nm, ibuprofen at 230 nm, ketoprofen at 264 and lidocaine at 219 nm). Calibration plots were constructed using at least five concentration points for all model compounds and found to be linear ($R^2 \geq 0.990$) in the range of 2-10 μg/mL.

The steady state flux (J) was determined from the slope of the best-fit linear plot of the cumulative amount of the drug permeated per unit area versus time. All values are expressed as the mean values of three replicates shown with standard deviation based error

limits. Statistical analysis was carried out using Minitab software (version 16). One-way ANOVA at 95 % confidence interval level ($p \leq 0.05$) was performed to test the significance in the values obtained, and post hoc comparison was executed using pairwise Tukey's test, where the difference in means was found to be significant.

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Chapter 3: Effect of ionisation on the permeation of compounds through poly(dimethylsiloxane) membrane

This chapter investigates the role of compound ionisation on their permeation across polydimethylsiloxane (PDMS) membrane. Six model compounds were analysed to determine the amount permeated across the membrane, each at three specific percentages of ionisation. In addition, iGC-SEA (Inverse Gas Chromatography Surface Energy Analyser) was utilised to determine the surface chemistry and surface energy profile of the membrane.

3.1 Introduction

The permeation of compounds through human skin is a complex process and can be difficult to predict using currently established *in vitro* methods such as mathematical models, cultured skin alternatives and artificial membranes (Waters 2015). Despite the limited ability of predicting percutaneous absorption, their development and use have gained widespread interest among researchers as there is an on-going trend to move away from an animal-based testing model in the EU. In particular, artificial membranes have found extensive application in early-stage assessment of percutaneous absorption (Moss *et al.* 2015). Analytical techniques using artificial membranes are mainly concerned with the use of polymeric materials such as polydimethylsiloxane (PDMS) membrane, generally known as silicone membrane (Oshima *et al.* 2012). PDMS is a commonly used polymer that has a wide range of industrial applications, for example, liquid and gas separation (Li *et al.* 2013; Alexander Stern 1994), pervaporation (Dong *et al.* 2014) and microfluidic devices (Fan *et al.* 2015). In pharmaceutical analysis, PDMS membrane is used to mimic human *stratum corneum* (the outermost layer of skin) in a system incorporating a donor solution and receptor solution, the latter from which samples are taken routinely for analysis to quantify the rate and extent of compound permeation within a pre-determined period of time (Sloan *et al.* 2013). Such data are useful to predict the fate of compounds following their application on to the skin surface

which is crucial for toxicological assessment and formulation development. Studies based on these *in vitro* predictive methods use diffusion apparatus known as Franz cells and are routinely carried out for analysing pharmaceutical, cosmetic and household products (Baert *et al.* 2010; Bartosova & Bajgar 2012). PDMS membranes are favourable (Zhu *et al.* 2010) amongst researchers for a number of reasons, for example, simplicity of their use, reproducibility of their composition and thickness, and cost effectiveness (Ng *et al.* 2012). Moreover, PDMS membrane has been reported to show good correlation with the *in vivo* situation in the case where the penetrant lipophilicity is the prime determinant of compound permeation across the membrane (Moss *et al.* 2015). The hydrophobic nature of the membrane creates a barrier effect, as is seen *in vivo* and provided that certain conditions are met, i.e. permeation is via passive diffusion, the penetrant is metabolically inert and no permeability enhancer is present in the formulation (Waters *et al.* 2013), then useful permeability data can be attained.

When analysing the permeation of compounds using skin (or skin mimics, such as PDMS) there exists a vast collection of donor phase compositions – ranging from water-based solutions of the model compound (Majumdar *et al.* 2007) to compositions that duplicate the complex formulations intended for market (Watkinson *et al.* 2010). For instance, even in the case of ibuprofen as a model compound, previous work has focused on a basic aqueous solvent at a pre-determined pH through to the use of far more complex formulations, such as ibuprofen gel, as would be applied on to human skin (Herkenne *et al.* 2007) or the addition of surfactants (Waters *et al.* 2013). However, the question – how, or why, certain solvents have been chosen for the donor solution in the majority of cases – has been paid very little attention although a recent study within our group has begun to consider the role of the binary mixtures present in the donor solution (Shahzad *et al.* 2014). Such

study clearly indicates that donor phase composition can, and does, play a role in compound permeation yet the extent to which it occurs has yet to be known.

For any aqueous based formulation, the physicochemical behaviour of the compound under investigation can be fundamentally dictated by the pH of the chosen solution. By knowing the pK_a of a compound the percentage ionised can be calculated using the Henderson-Hasselbalch equation (Eq. 3.1) at any given pH with the ionised species (A^-) being in equilibrium with the unionised species (HA).

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) \quad (3.1)$$

Hence, the pH of a solution can be manipulated so that the ratio of the concentration of unionised species to that of ionised species can be controlled and determined for any compound with a known pK_a . Compound permeation can also be influenced by other physicochemical factors, such as $\log P$ (the octanol-water partition coefficient). Therefore, this study selected a set of compounds with a wide range of lipophilicities to confirm the importance of this additional determinant on permeation.

For the prediction of the behaviour of a compound in a formulation using Franz-cell based experiments, researchers often choose donor solutions with pH values analogous to those found *in vivo*. For example, some research has considered a donor solution at low pH to mimic the typical skin pH (Guo *et al.* 2014) whereas others have chosen pH values such as 7.4 (Mertz & Sloan 2014). Although such studies focused on the importance of controlling and selecting the pH of the donor phase, little attention has been paid to understand the relationship between the extent of ionisation and permeation in Franz cell based studies. One particular study analysed only one compound, namely salicylic acid, to understand the effect of ionisation on the subsequent permeation across human skin and silicone membrane (Smith & Irwin 2000). The study found a direct relationship between permeation and the degree of ionisation of compound. However, only one specific compound was considered in the study.

This study aimed to understand whether a similar relationship would be observed for a range of compounds that, upon ionisation, form cationic and anionic species, and have a wide range of lipophilicities.

3.2 Results and discussion

3.2.1 Effect of ionisation on compound permeation across PDMS membrane

Six model compounds were selected to assess the permeation dependence with ionisation. The amounts of the compounds permeated through PDMS membrane were determined using a flow-through diffusion cell with each compound at three specific percentages of ionisation (calculated using Eq. 3.1 and pK_a values of 1-methyl-3-phenylpropylamine 9.79 (U.S National Library of Medicine 2016), benzoic acid 4.2 (Wang *et al.* 2013), benzotriazole 8.2 (Benitez *et al.* 2015), ibuprofen 4.9 (Willian & Pritchett 2014), ketoprofen 4.0 (Bechet 1999) and lidocaine 7.8 (Liu *et al.* 2003). These compounds were selected for their diverse range of pK_a values and lipophilicities, the latter ranging from a log P of 1.2 for benzotriazole (Hart *et al.* 2004) to 3.6 for ibuprofen (Waters *et al.* 2010).

A previous study (Smith & Irwin 2000) investigated the permeation of salicylic acid across PDMS membrane to determine if it followed the pH-hypothesis whereby steady-state flux and permeability coefficients increased with a decrease in pH and a linear relationship was found between the flux and fraction unionised. The results suggested that the change in salicylic acid flux was a direct consequence of pH, which regulated the concentration of unionised species. However, the study only considered salicylic acid i.e. one specific compound. The aim of this study was to determine if a similar relationship would be observed for a diverse range of compounds with different pK_a and log P values, i.e. to identify if the flux-pH relationship can be applied more generally. The permeation profiles of all six

compounds are displayed in Figs. 3.1-3.6 with the cumulative amount permeated shown for a period of seven hours.

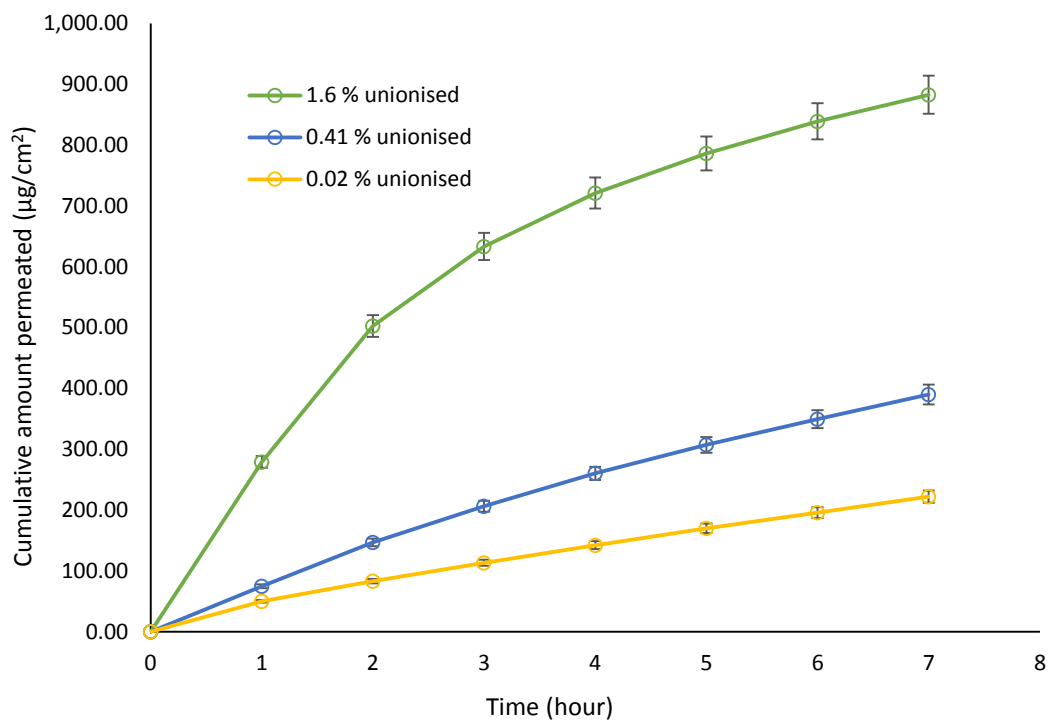


Fig. 3.1: Cumulative amount of 1-methyl-3-phenylpropylamine permeated with percentage unionised. Each data point represents the mean \pm SD ($n = 3$). The corresponding pH values to obtain 1.6 %, 0.41 % and 0.02 % of unionised compound were 8.0, 7.4 and 6.0, respectively.

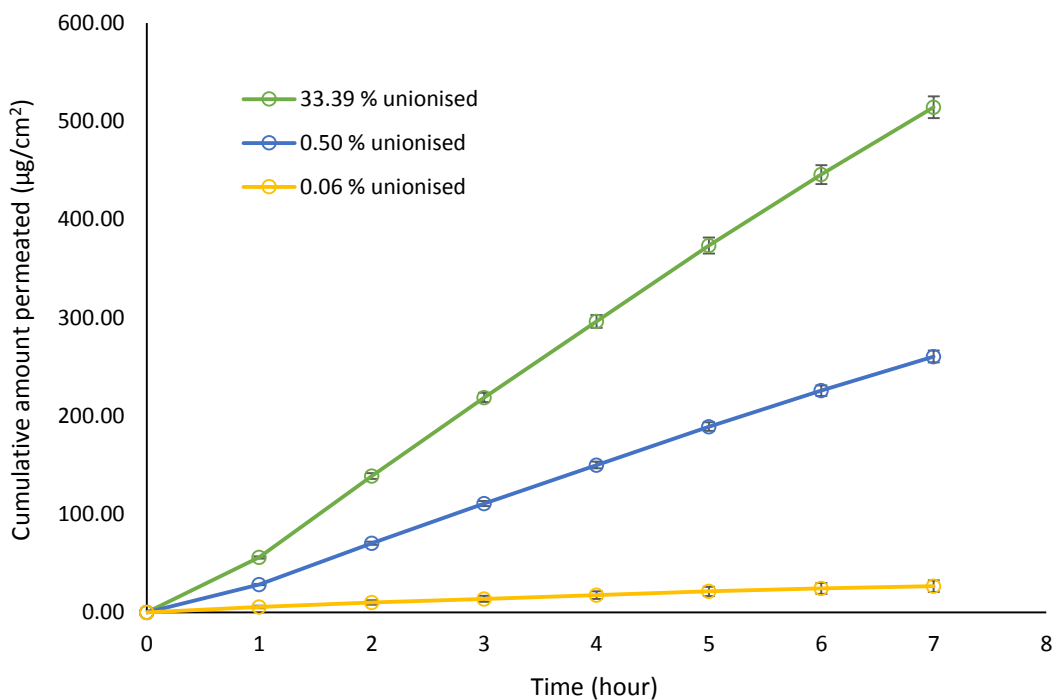


Fig. 3.2: Cumulative amount of benzoic acid permeated with percentage unionised. Each data point represents the mean \pm SD (n = 3). The corresponding pH values to obtain 33.39 %, 0.50 % and 0.06 % of unionised compound were 4.5, 6.5 and 7.4, respectively.

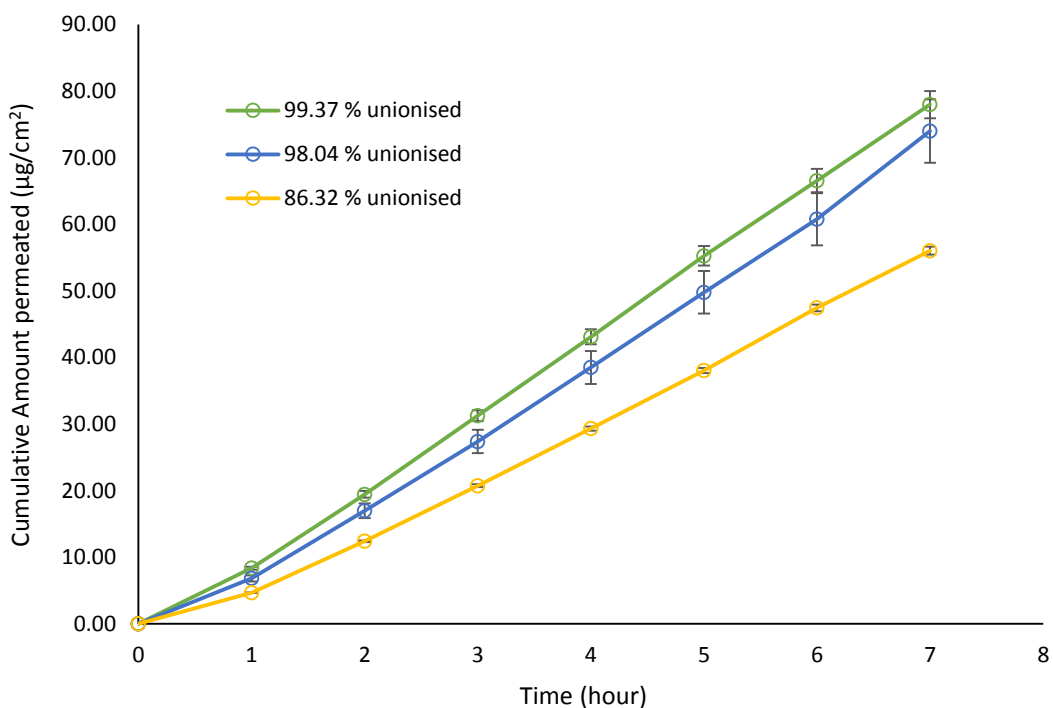


Fig. 3.3: Cumulative amount of benzotriazole permeated with percentage unionised. Each data point represents the mean \pm SD (n = 3). The corresponding pH values to obtain 99.37 %, 98.04 % and 86.32 % of unionised compound were 6.0, 6.5 and 7.4, respectively.

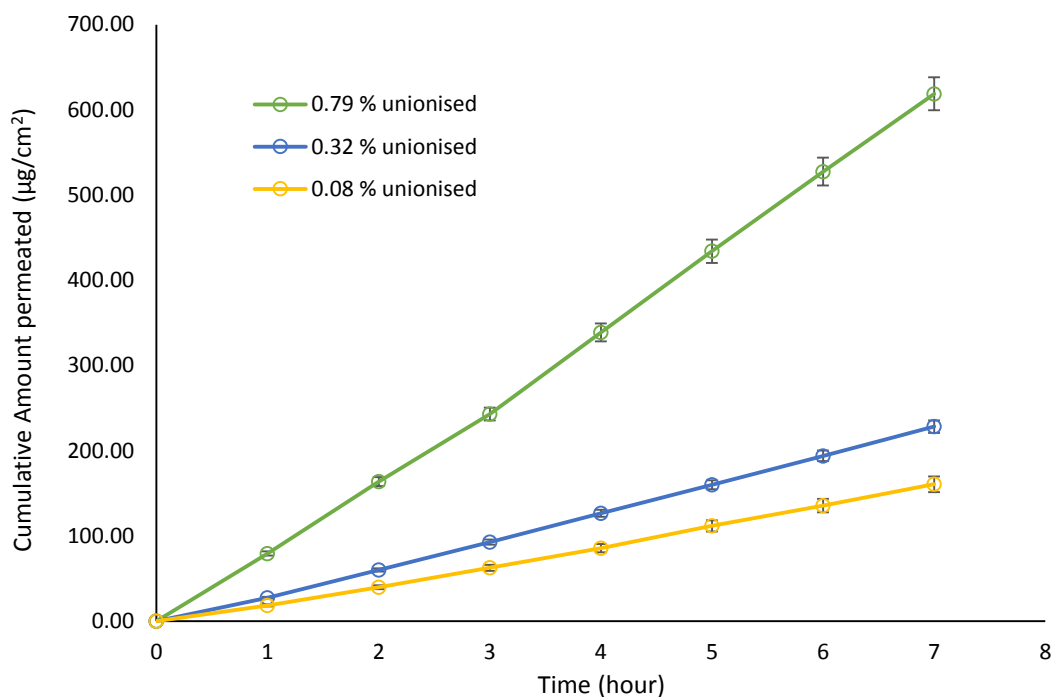


Fig. 3.4: Cumulative amount of ibuprofen permeated with percentage unionised. Each data point represents the mean \pm SD ($n = 3$). The corresponding pH values to obtain 0.79 %, 0.32 % and 0.08 % of unionised compound were 7.0, 7.4 and 8.0, respectively.

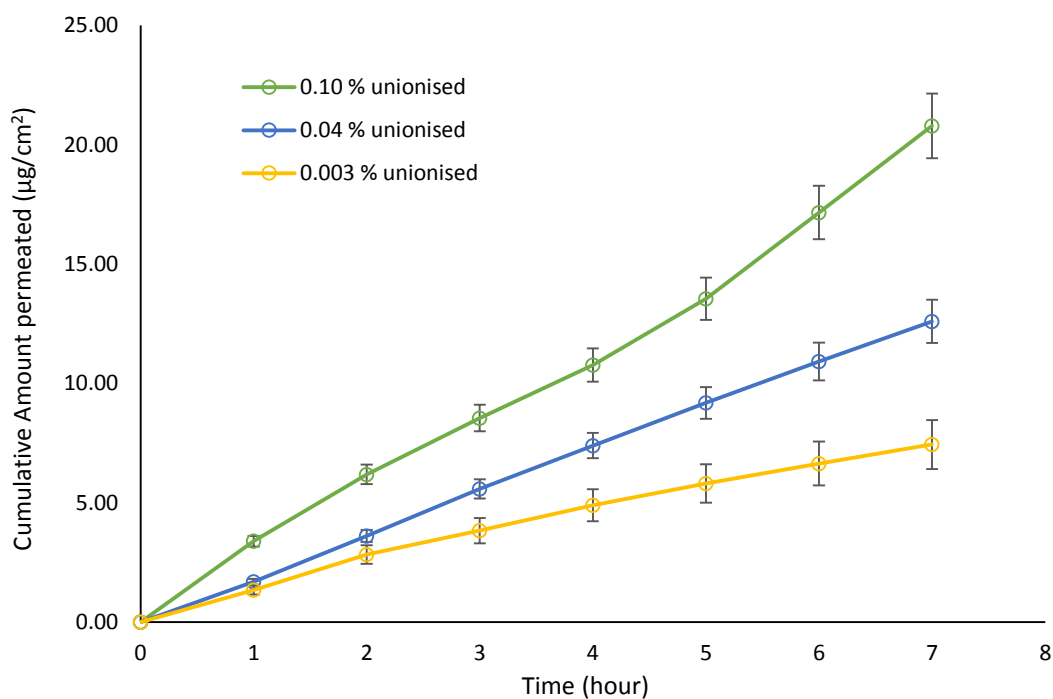


Fig. 3.5: Cumulative amount of ketoprofen permeated with percentage unionised. Each data point represents the mean \pm SD ($n = 3$). The corresponding pH values to obtain 0.10 %, 0.04 % and 0.003 % of unionised compound were 7.0, 7.4 and 8.5, respectively.

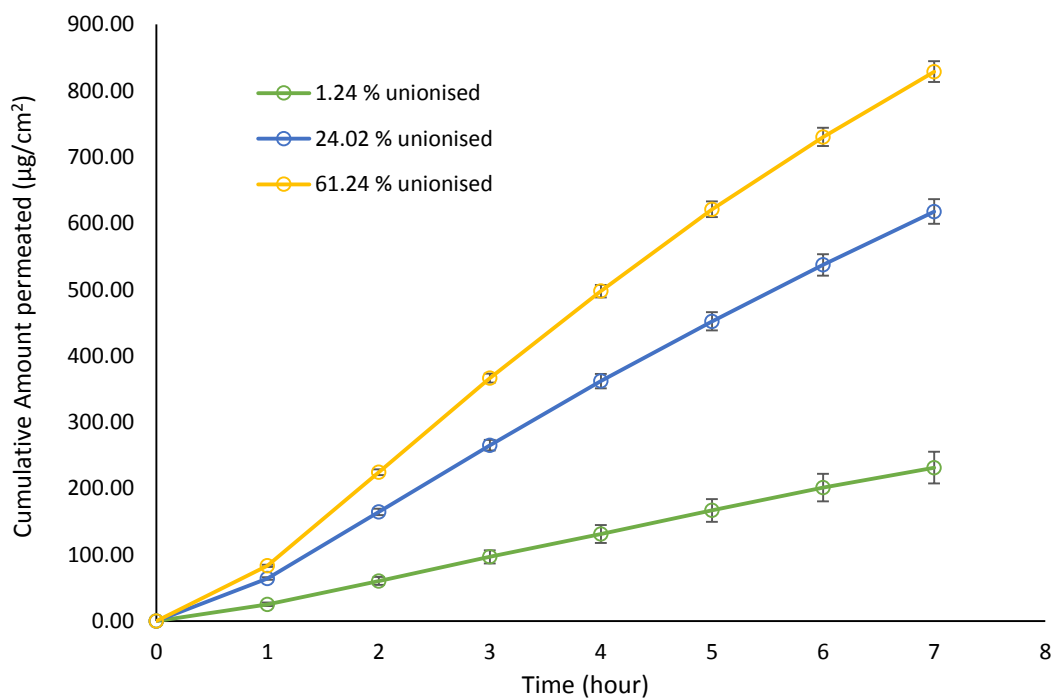


Fig. 3.6: Cumulative amount of lidocaine permeated with percentage unionised. Each data point represents the mean \pm SD ($n = 3$). The corresponding pH values to obtain 1.24 %, 24.02 % and 61.24 % of unionised compound were 6.0, 7.4 and 8.0, respectively.

Although it was not possible for all six compounds to achieve the same degrees of ionisation with their individual pK_a values and the pH range available, the distribution of percentages allowed a comparative study to be undertaken. It is to be noted that a specific set of pH values were chosen to achieve the complete dissolution of the compounds in the buffer solutions. It can be seen that in all cases (Figs. 3.1-3.6) there was a reduction in compound permeation as the percentage of unionised species decreased which would be anticipated based on the hydrophobic characteristic of PDMS membrane. Through modifying the pH of the donor 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 compound permeation across the membrane.

Based on the data in Figs. 3.1-3.6, it can be concluded that they all show a generally linear relationship i.e. the more the percentage unionised, the more the amount of compound

permeated. A clearer picture of this phenomenon can be drawn by plotting Q_7 (the amount permeated after 7 h) values against the percentage of compound unionised (Figs. 3.7-3.12). A plot of such data allows comparison to be made between the gradients i.e. how influential the percentage ionised is on compound permeation across PDMS membrane.

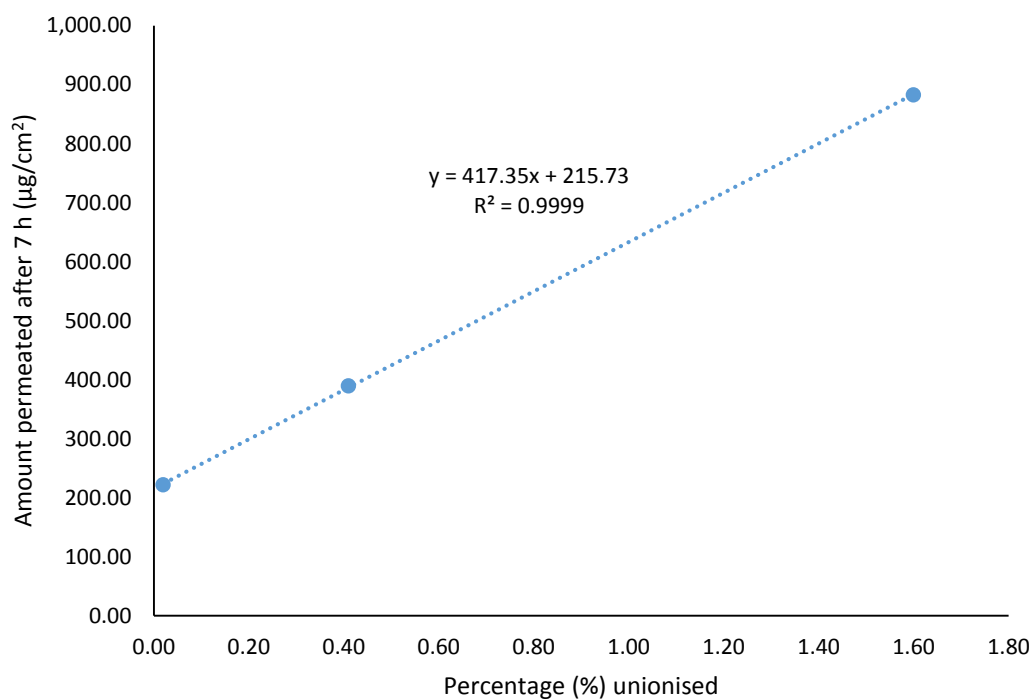


Fig. 3.7: Amount of 1-methyl-3-phenylpropylamine permeated after 7 h at three specific percentages of ionisation

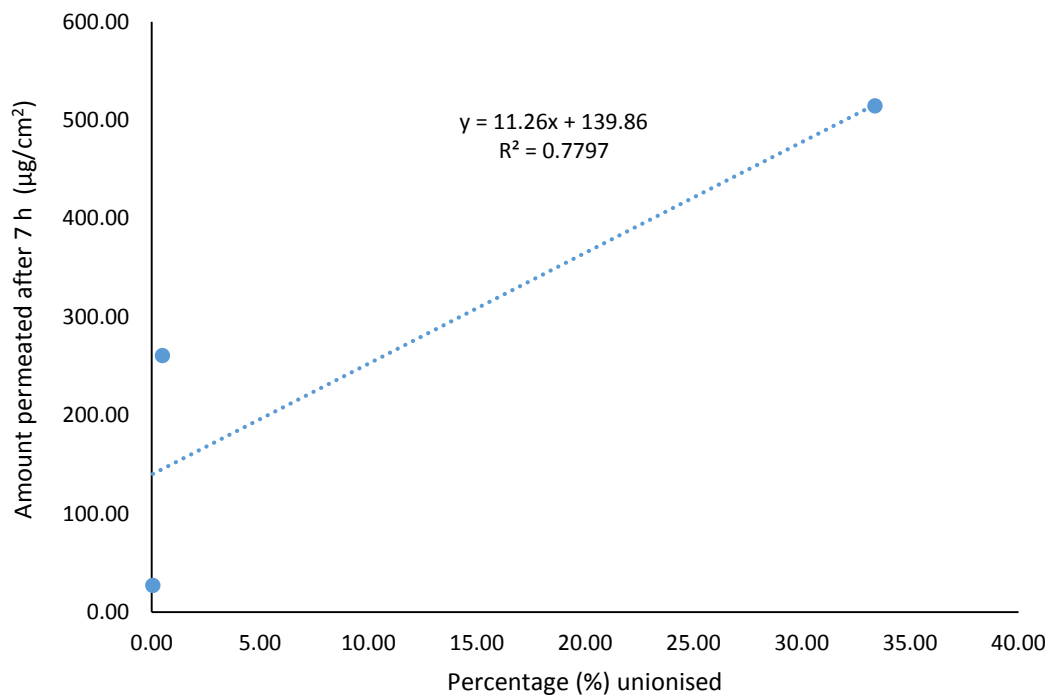


Fig. 3.8: Amount of benzoic acid permeated after 7 h at three specific percentages of ionisation

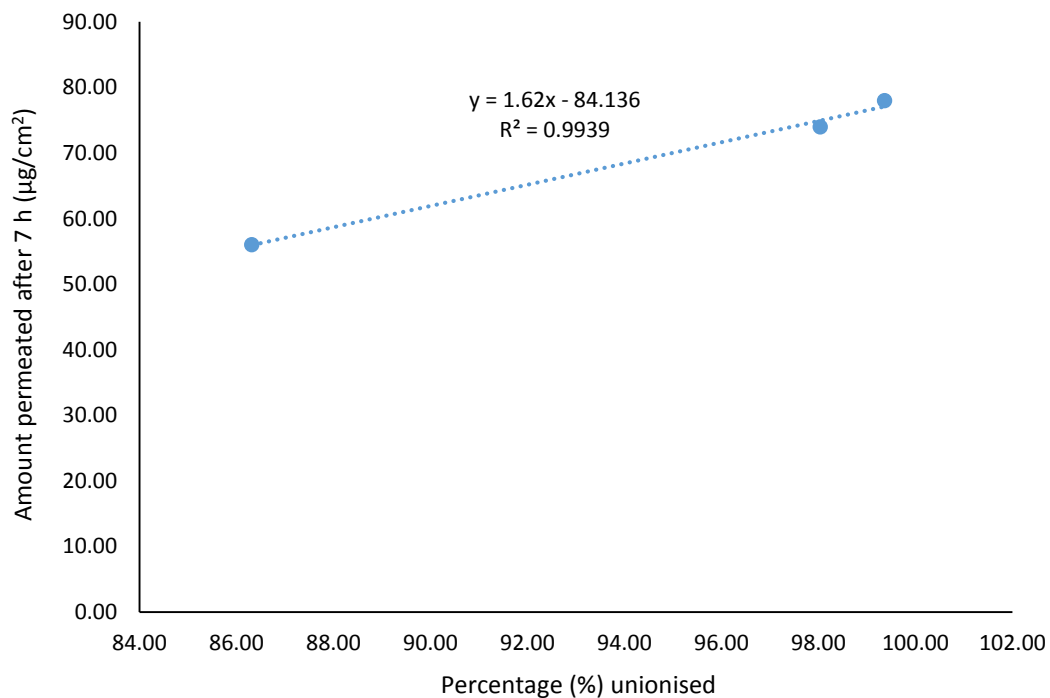


Fig. 3.9: Amount of benzotriazole permeated after 7 h at three specific percentages of ionisation

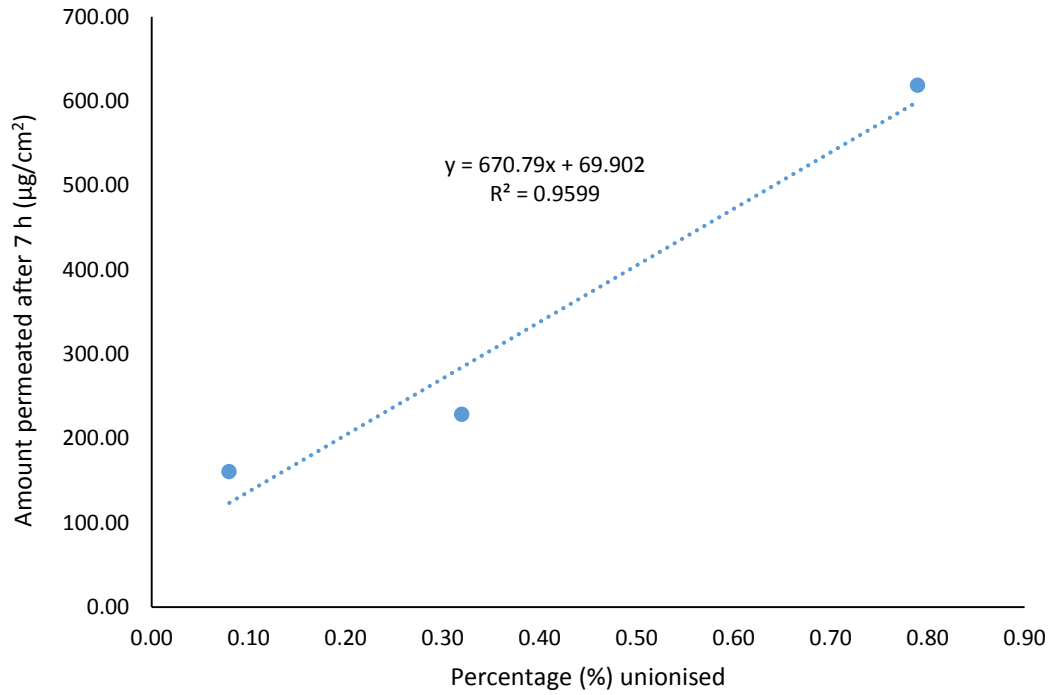


Fig. 3.10: Amount of ibuprofen permeated after 7 h at three specific percentages of ionisation

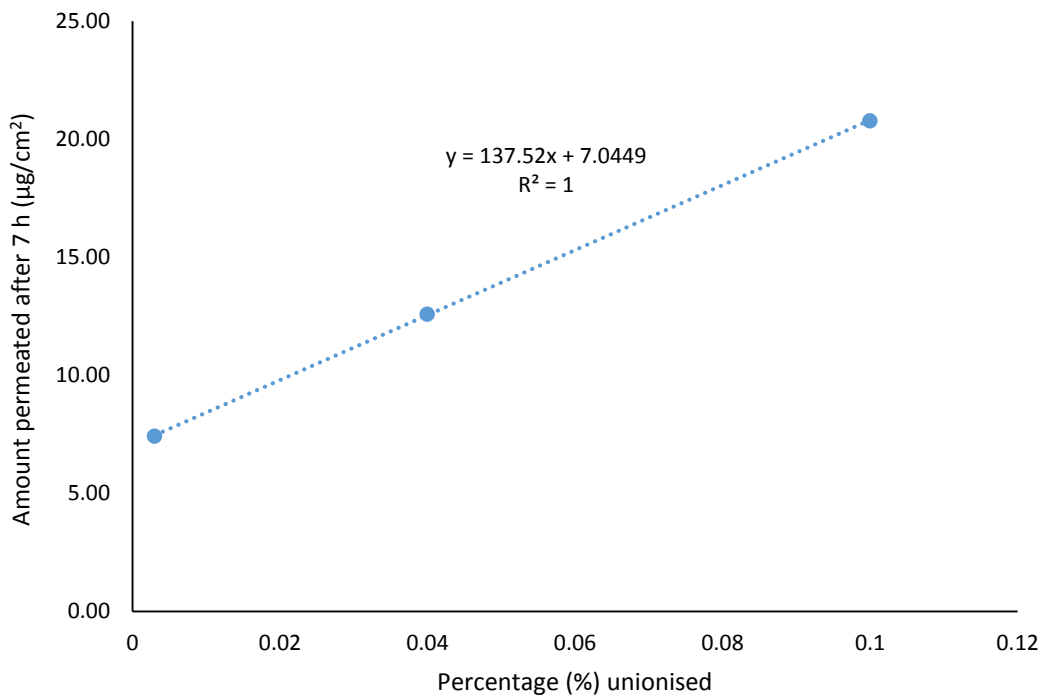


Fig. 3.11: Amount of ketoprofen permeated after 7 h at three specific percentages of ionisation

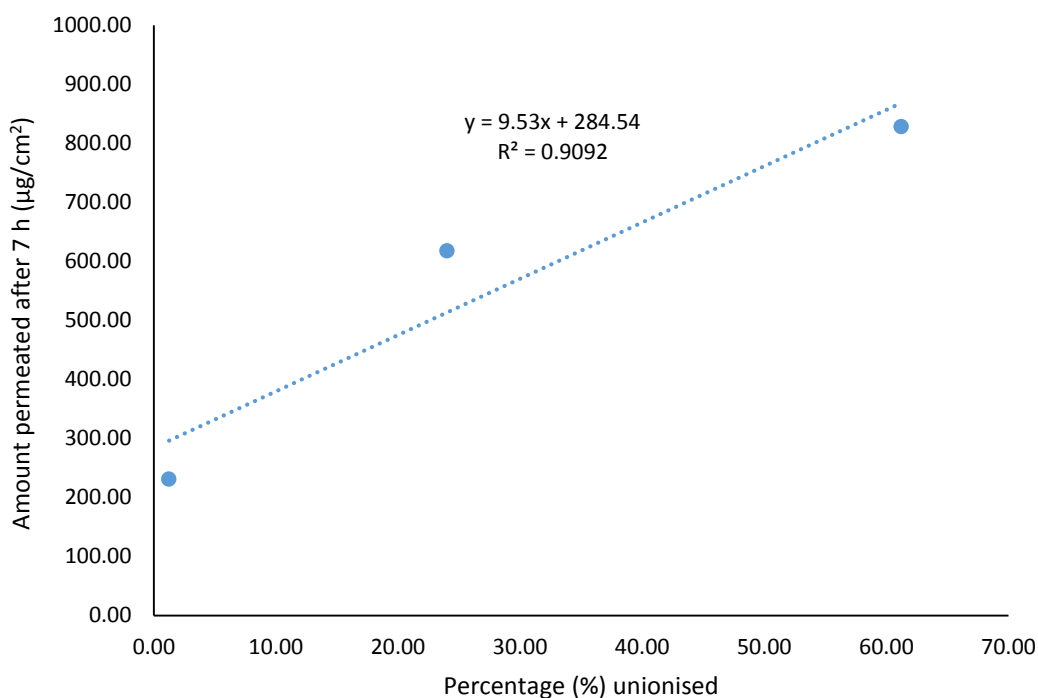


Fig. 3.12: Amount of lidocaine permeated after 7 h at three specific percentages of ionisation

A closer look at the gradient (m) values in Figs. 3.7-3.12 reveals that 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 pK_a with a value of 8.2 for benzotriazole (Benitez *et al.* 2015). Alternatively, this might have been a consequence of the comparative $\log P$ values with ibuprofen showing the greatest change ($m = 670.79$) in amount permeated and being the most hydrophobic of the compounds, and benzotriazole displaying the least change ($m = 1.62$) and being the least hydrophobic. The R^2 values in Figs. 3.7-3.12 show that the overall relationship between the amount permeated and the percentage unionised is linear, though benzoic acid ($R^2 = 0.7797$) did not show such a clearly linear relationship.

Of additional interest, steady-state flux values ($\mu\text{g}/\text{cm}^2/\text{h}$) 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 depended

upon factors, such as log P. This analysis was performed in a similar manner to that of Smith and Irwin (Smith & Irwin 2000) whereby they found a linear relationship for the one compound studied, namely salicylic acid. Data for all six compounds are shown in Table 3.1.

Table 3.1: Permeation data for six model compounds across silicone membrane over a series of percentages/fractions unionised

Compound	% Unionised	Fraction Unionised	Steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$)
1-methyl-3-phenylpropylamine	0.02	2×10^{-4}	30.61 ± 0.96
	0.41	4×10^{-3}	55.21 ± 2.56
	1.60	0.02	118.09 ± 4.96
Benzoic acid	0.06	6×10^{-4}	3.80 ± 0.95
	0.50	5×10^{-3}	31.91 ± 0.53
	33.39	0.33	75.36 ± 1.21
Benzotriazole	86.32	0.86	8.23 ± 0.03
	98.04	0.98	10.68 ± 0.65
	99.37	0.99	11.38 ± 0.23
Ibuprofen	0.08	8×10^{-4}	23.22 ± 0.10
	0.32	3×10^{-3}	32.91 ± 0.82
	0.79	8×10^{-3}	89.03 ± 2.30
Ketoprofen	0.003	3×10^{-5}	1.05 ± 0.10
	0.04	4×10^{-4}	1.82 ± 0.11
	0.10	1×10^{-3}	2.84 ± 0.07
Lidocaine	1.24	0.01	33.99 ± 2.05
	24.02	0.24	91.09 ± 3.14
	61.24	0.61	123.30 ± 1.89

Moreover, the flux values were statistically analysed using One-way ANOVA, and the calculated p -values for each compound are displayed in Table 3.2.

Table 3.2: p -values calculated using ANOVA test for each compound

Drug	p-values
1-methyl-3-phenylpropylamine	0.000
Benzoic acid	0.000
Benzotriazole	0.000
Ibuprofen	0.000
Ketoprofen	0.000
Lidocaine	0.000

The p -values (Table 3.2) demonstrate that for all compounds a change in fraction unionised resulted in a significant change ($p < 0.05$) in their flux across PDMS membrane. Considering the data in Table 3.1, a trend similar to Smith and Irwin 2000 was observed for that discussed above, i.e. from comparative consideration of the amount permeated with percentage unionised, as all experiments of the study were conducted for seven hours. Again, the relationship between fraction unionised and flux was predominantly linear with the one exception of benzoic acid where the intermediate fraction of unionised solution seemed to show greater flux than expected. This anomaly was unexpected although the general trend was similar to that of the remaining compounds. One factor that can certainly be excluded from consideration is membrane thickness as this was consistent throughout the study, the importance of the consistency of membrane thickness has been mentioned by others (Firpo *et al.* 2015). Overall it can be concluded that steady-state flux increases as the percentage of unionised compound increases.

3.2.2 iGC SEA membrane surface analysis

The BET Specific Surface Area (BET-SSA) of PDMS membrane was determined with the adsorption of ethanol molecules by DVS (Dynamic Vapour Sorption) method. To calculate the BET specific surface area, the BET equation was employed (Eq. 3.1).

$$\frac{1}{n \left[\left(\frac{p_0}{p} \right) - 1 \right]} = \frac{c - 1}{n_m c} \left(\frac{p}{p_0} \right) + \frac{1}{n_m c} \dots \dots \dots (3.1)$$

where, p and p_0 are the equilibrium and saturation pressure of adsorbates at the temperature of adsorption, n is the adsorbed gas amount, n_m is the monolayer adsorbed gas amount, c is sorption constant. This equation is an adsorption isotherm and a straight line was taken by plotting p/p_0 versus $\frac{1}{n \left[\left(\frac{p_0}{p} \right) - 1 \right]}$ (data not shown). The sorption constant (c) and the monolayer capacity (n_m) were calculated from the slope and intercept of the line. The surface area was determined by the following equation:

$$S_{BET} = \frac{(n_m N_{Av} a)}{V_m} \dots \dots \dots (3.2)$$

where, n_m is the monolayer adsorbed gas amount, N_{Av} is the Avogadro's number, a is the adsorption cross section of the adsorbing species, V is the molar volume of adsorbed gas and m is the mass of adsorbent (in g).

The results are presented in Table 3.3.

Table 3.3: Specific surface area data for PDMS membrane using iGC

Sorption constant	Monolayer capacity (cm ³ /g)	BET Specific Surface Area (m ² /g)	R ²
5.832	2.061	19.560	0.999

The dispersive (γ_s^d), acid-base (γ_s^{ab}) and total surface energy (γ_s^t) profiles of the membrane are shown in Fig. 3.13. The profiles show that the sample is energetically heterogeneous, *i.e.*

the surface energy changed as a function of surface coverage with a major contribution from the dispersive component.

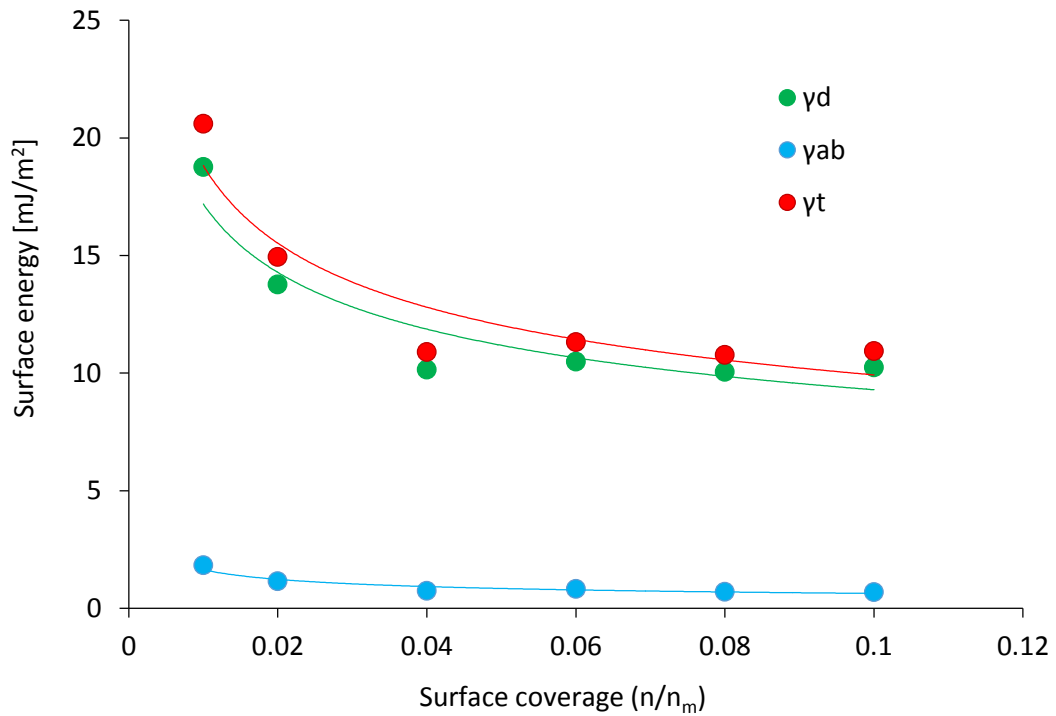


Fig. 3.13: Surface energy profiles (as a function of surface coverage) of PDMS membrane

To represent the heterogeneity of the sample in a more illustrative manner, the surface energy distributions were obtained by a point-by-point integration of the surface energy profiles, resulting in plots of γ_s^d , γ_s^{ab} and γ_s^t surface energy versus percentage of surface (area increment), as shown in Fig. 3.14. As expected, the energetically heterogeneous membrane has a wide variation of surface active sites. Fig. 3.14 shows that the membrane exhibits a relatively wide range of γ_s^d distribution, ranging from 10.12 to 30.19 mJ/m² and with a mean value of 13.88 mJ/m².

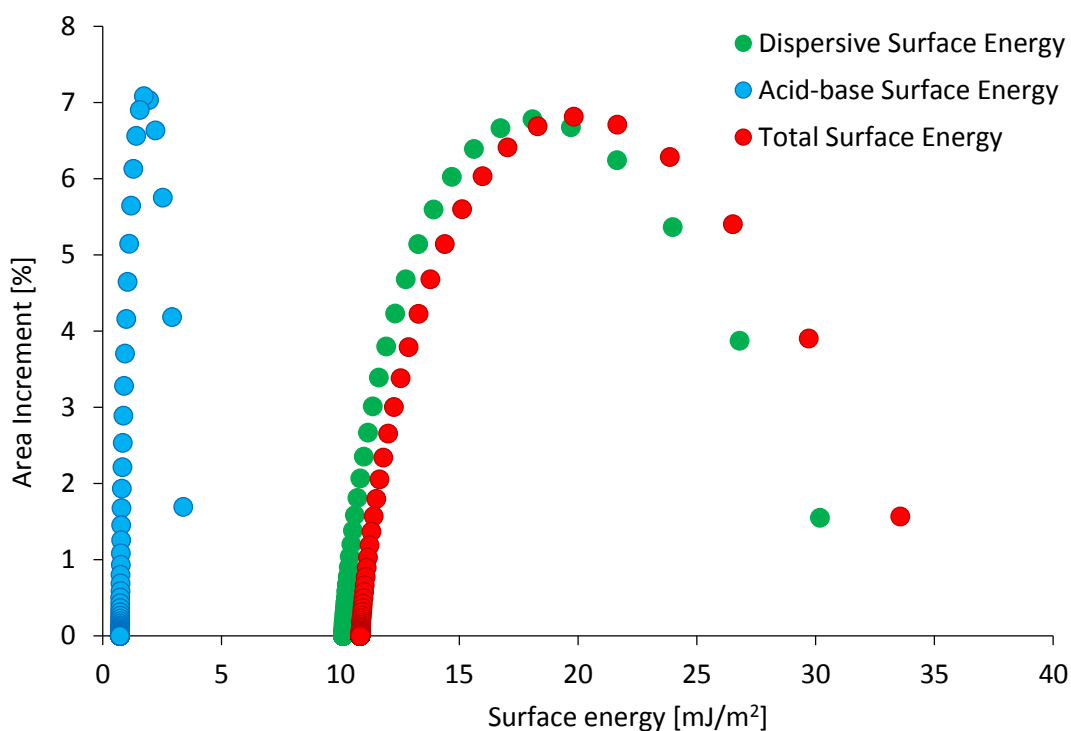


Fig. 3.14: Dispersive surface energy distribution for PDMS membrane

Furthermore, the specific (acid-base) Gibbs free energy of adsorption (ΔG_{sp}) changed with surface coverage, again confirming the heterogeneous nature of the membrane. The ΔG_{sp} profiles, resulted from the interactions of the membrane with five polar probe molecules, are shown in Fig. 3.15. From analysing the interactions with the polar probe molecules the rank order of decreasing ΔG_{sp} was found to be acetonitrile > ethanol > dichloromethane > acetone > ethyl acetate although the membrane showed only a relatively small degree of interactions with all five probes.

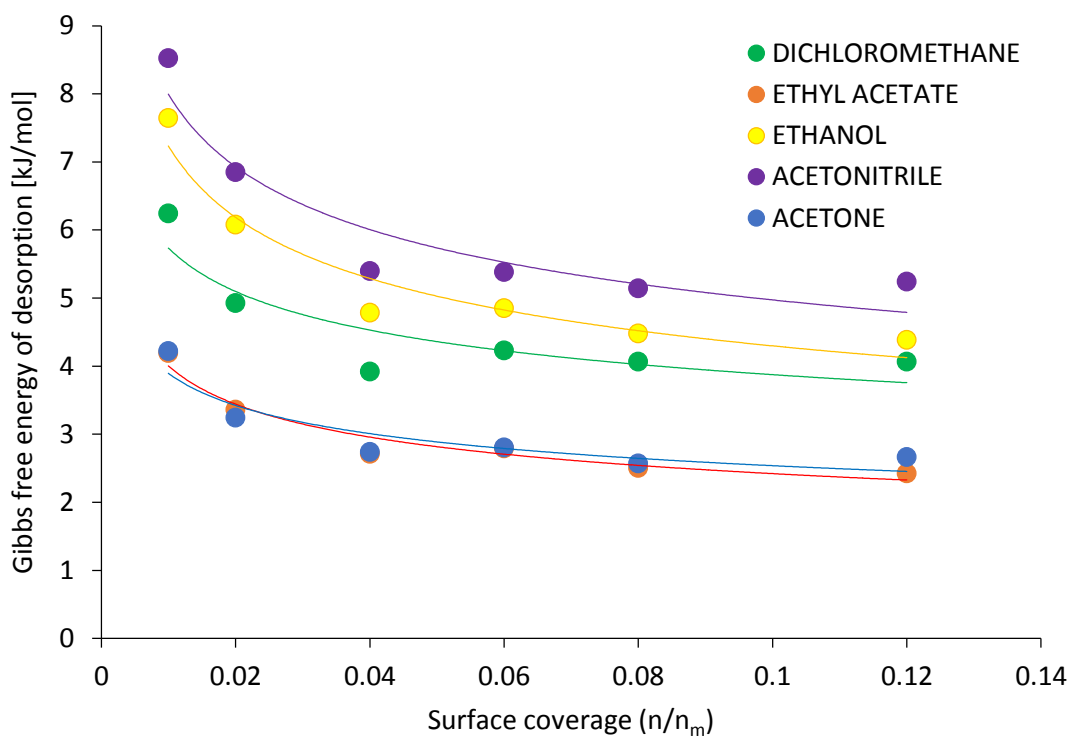


Fig. 3.15: Specific (acid-base) free energy of different solvents for PDMS membrane

The surface chemistry of the membrane was assessed by Gutmann acid (K_a) and base (K_b) numbers, determined using the following probes: dichloromethane, ethyl acetate, acetonitrile and chloroform. K_a and K_b values of the membrane were calculated using the ΔG_{sp} values of the polar probes at the particular surface coverage (Fig. 3.16). It can be seen that K_b values are consistently higher than K_a . These results indicate that the surface of PDMS membrane is basic in nature and possesses a high concentration of electron-donating surface functional groups. These can be the Lewis bases in the form of bridging oxygen atoms in Si-O-Si backbone. Moreover, there may be some residual un-substituted hydroxyl groups from the manufacturing process (based on the chemistry of the material).

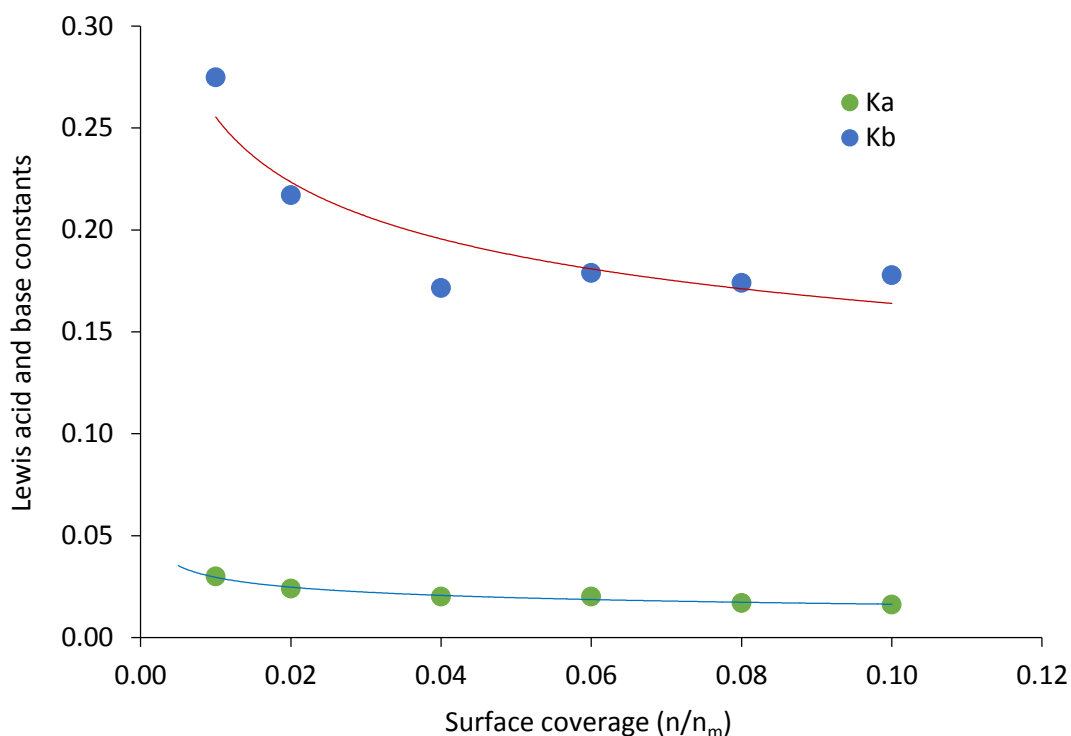


Fig. 3.16: Gutmann acid and base number profiles of PDMS membrane

The above-mentioned findings can be linked with the previously discussed permeation data to elucidate 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 indicated the basicity of the PDMS surface. Thus it can be expected that the basic surface would repel the ionised form of the acid compounds (benzoic acid, benzotriazole, ibuprofen and ketoprofen) and weakly bond the ionised form of basic compounds (1-methyl-3-phenylpropylamine and lidocaine). In either case, the ionised form of a compound would be less inclined to permeate through the basic membrane surface than the unionised form.

3.3 Conclusion

The results confirm that permeation has a dependence on compound ionisation. Permeation experiments of six compounds were carried out using PDMS membrane, with each compound at three specific percentages of ionisation. The surface energetics and surface chemistry of the membrane were determined using iGC SEA. The iGC data showed that the membrane is energetically heterogeneous and more basic in nature.

From considering the combined results of the permeation study and iGC SEA it can be seen that data presented based on permeation suggests there is a general preference for permeation for the most unionised forms for all compounds. Based on these findings it can be concluded that the overall hydrophobic nature of PDMS membrane outweighs the effects of any surface groups that might be present. Alternatively, there might be the existence of a peripheral layer of basic groups that creates an electrostatic attraction or repulsion for the ionised species of compounds, thus hindering their permeation. In either case, the permeation would be more favourable for the more unionised form of a compound notwithstanding the presence of a basic membrane surface.

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Chapter 4: Effect of surfactant type on the permeation of pharmaceutical compounds through silicone membrane

This chapter examines the effects of different surfactants on drug permeation across polydimethylsiloxane (PDMS) membrane. Four model compounds were investigated to determine their permeation through PDMS in the presence of four types of surfactants, namely, anionic, cationic, non-ionic and zwitterionic surfactants.

4.1 Introduction

Pharmaceutical formulations often include excipients that are known to have human skin penetration effects (HSPE). Some examples of these excipients are terpenes (Kang *et al.* 2007), pluronic gels (Escobar-Chávez *et al.* 2005), fatty acid esters (Casiraghi *et al.* 2012), nanoemulsions (Barakat *et al.* 2011) and liposomes (Bouwstra & Honeywell-Nguyen 2002). Another class of compounds that are known to alter skin permeation is surfactants (Walters *et al.* 1993; Shokri *et al.* 2001) where they intercalate with the continuous lipid region of *stratum corneum*, thus facilitating fluidity. A number of studies has reported their use to enhance transdermal permeation of compounds. One of the studies was carried out by Borrás-Blasco *et al.* (1997) which estimated the influence of an anionic surfactant, namely sodium lauryl sulphate (SLS), on the penetration of seven model compounds of a wide range of lipophilicities across rat skin, and they found SLS to be a penetration enhancer depending on the lipophilicities of the compounds. Some other studies were carried out to examine the penetration effect of cationic surfactant such as CTAB (cetyltrimethylammonium bromide) and non-ionic surfactant such as Tween 80. For example, Nokhodchi *et al.* (2003) investigated the effect of CTAB and Tween 80 on Lorazepam permeation across rat skin and observed a significant enhancement caused by both of the surfactants. Zwitterionic

surfactants such as CHAPS have been reported to enhance mannitol permeability across Caco-2 cells, an *in vitro* model for mimicking intestinal mucosa (Nerurker *et al.* 1996). Nevertheless in some circumstances surfactants were observed to initiate only a minimal enhancement. One such study approached an attempted enhancement of methotrexate permeation using a variety of surfactants, where SLS did not produce a substantial enhancement effect (Javadzadeh & Hamishehkar 2011). To summarise, the presence of surfactants, along with other factors, are known to alter compound permeation across biological membranes, though little research has investigated the impact of such changes with respect to silicone membrane – a widely accepted artificial *in vitro* skin mimic. One particular study has investigated the effect of two surfactants, namely sodium dodecyl sulfate and Brij 35, on the permeation of methylparaben and ethylparaben through silicone membrane (Waters *et al.* 2013). The paraben derivatives, considered in the study, are widely used excipients in cosmetic and pharmaceutical products. However, the study found a reduction in paraben permeation in the presence of SDS (an anionic surfactant) whereas Brij 35 (a non-ionic surfactant) did not show any significant effect. Whether a similar relationship would be observed for a range of surfactants such as cationic or zwitterionic types, or a range of therapeutic compounds was not considered. Hence, this study investigates the permeation of a range of therapeutic compounds through silicone membrane in the presence of four types of surfactants.

4.2 Results and discussion

A set of four model compounds were examined to assess the permeation effect of different surfactants across silicone (PDMS) membrane. The model compounds were benzocaine, benzotriazole, ibuprofen and lidocaine. These compounds were selected because of their diverse range of lipophilicities ranging from a log P of 1.2 for benzotriazole (Hart *et*

al. 2004) to 3.6 for ibuprofen (Waters & Bhuiyan 2016). The surfactants selected for this study were SDS (an anionic surfactant), CTAB (a cationic surfactant), CHAPS (a zwitterionic surfactant), Brij 35 and Tween 80 (non-ionic surfactants) i.e. to encompass all four types.

As a control, the permeation of the model compounds through silicone membrane were assessed at 32 °C with no surfactant present in the donor solution over a period of 6 hours. Three additional solutions were then prepared containing the surfactants at three different concentrations (4, 8 and 20 mM for SDS, CTAB, Brij 35 and Tween 80, and 2, 4 and 20 mM for CHAPS) and the permeation of the model compounds were measured. In all cases, the donor solution pH was 7.4. The concentrations of the surfactants were chosen to be, where suitable, either below, equal or above the critical micellar concentration (CMC). The CMCs of the surfactants considered for this study were 8 mM for SDS (Waters *et al.* 2013), 0.83 mM for CTAB (Prazeres *et al.* 2012), 0.09 mM for Brij 35, 0.012 mM for Tween 80 (Chou *et al.* 2005) and 4-6 mM for CHAPS (Chattopadhyay & Harikumar 1996). Two permeation parameters i.e. steady-state flux (J) and the cumulative amount of compound permeated after 6 hours (Q_6) were calculated from the data obtained using a flow-through diffusion cell system and are summarised in Table 4.1, 4.2, 4.5 and 4.6. The steady-state flux (J) values of the compounds were analysed statistically using One-way ANOVA, and the calculated p -values for each compound and surfactant are presented in Table 4.3 and 4.4. The p -values in Table 4.3 reflect whether there is a difference in compound permeation owing to a change in surfactant concentration, and that in Table 4.4 shows whether the variability in surfactant types caused a significant difference in compound permeability.

Table 4.1: Steady-state flux values of four model compounds in the presence of SDS, CTAB, Brij 35 and Tween 80 across silicone membrane

Compound	Surfactant	Steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$) of compound at three different surfactant concentrations			
		0 mM (control)	4 mM	8 mM	20 mM
Benzocaine	SDS	97.92 ± 2.22	89.80 ± 1.70	89.16 ± 0.85	62.87 ± 1.84
	CTAB	104.59 ± 3.22	70.77 ± 6.79	56.71 ± 2.94	38.82 ± 5.48
	Brij 35	102.07 ± 6.88	77.54 ± 5.67	63.29 ± 2.61	43.36 ± 1.15
	Tween 80	106.66 ± 3.15	78.50 ± 0.55	65.33 ± 1.72	43.34 ± 0.40
Benzotriazole *	SDS	18.33 ± 0.80	17.94 ± 0.43	13.75 ± 0.23	12.21 ± 0.26
	CTAB	9.96 ± 0.58	9.51 ± 0.27	8.00 ± 0.25	6.88 ± 0.23
	Brij 35	13.30 ± 0.09	13.04 ± 0.73	10.62 ± 0.43	9.58 ± 0.37
Ibuprofen	SDS	26.25 ± 1.95	27.53 ± 1.40	23.37 ± 1.27	21.29 ± 1.55
	CTAB	21.15 ± 1.46	9.82 ± 0.55	5.12 ± 0.75	2.37 ± 0.31
	Brij 35	31.00 ± 1.83	26.50 ± 1.69	17.49 ± 0.12	12.29 ± 0.33
	Tween 80	28.20 ± 1.48	21.53 ± 1.25	14.76 ± 0.55	9.96 ± 0.17
Lidocaine	SDS	69.70 ± 1.12	43.07 ± 1.70	31.69 ± 3.10	13.54 ± 1.08
	CTAB	56.98 ± 6.64	52.93 ± 4.63	47.77 ± 6.77	37.66 ± 3.23
	Brij 35	64.84 ± 3.66	66.96 ± 3.09	60.48 ± 4.07	57.44 ± 2.57
	Tween 80	60.05 ± 0.92	65.14 ± 8.52	61.23 ± 5.74	44.45 ± 1.00

* To examine the permeation effect of Tween 80, only three model compounds, namely, benzocaine, ibuprofen and lidocaine, were studied. Benzotriazole was not studied in this instance. This is because the purpose of using Tween 80 was only to confirm the phenomenon associated with Brij 35 (discussed later in this chapter).

Table 4.2: Steady-state flux values of the model compounds in the presence of CHAPS across silicone membrane

Compound	Surfactant	Steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$) of compound at three different concentrations of CHAPS			
		0 mM (control)	2 mM	4 mM	20 mM
Benzocaine	CHAPS	107.95 ± 3.99	105.10 ± 6.75	106.75 ± 5.42	87.53 ± 4.10
Benzotriazole	CHAPS	10.46 ± 0.53	10.14 ± 0.51	9.45 ± 0.26	9.47 ± 0.18
Ibuprofen	CHAPS	32.13 ± 1.12	32.48 ± 1.76	18.50 ± 0.39	9.90 ± 1.93
Lidocaine	CHAPS	55.28 ± 6.64	54.68 ± 3.73	52.62 ± 3.05	49.94 ± 4.01

In an ideal condition, all donor solutions of the same penetrant should yield an identical steady-state flux across a membrane, not depending on the composition of the vehicle, provided that the formulation components do not interact with the membrane (Dias *et al.* 2007). Therefore, the steady-state flux of a compound from donor solutions from any of the surfactant-containing vehicles would be anticipated to be same. However, the data presented in Table 4.1 and 4.2 demonstrate that the flux values of the penetrants are not identical. This indicates that the situations were not ideal and, approximately in all cases, interactions between either surfactant and membrane, or drug and surfactant were observed that could possibly alter the compound flux across the membrane. These types of interactions can be affected by the choice of surfactant concentration and surfactant types. The *p*-values in Table 4.3 show the evidence of a significant difference in permeation owing to a change in surfactant concentration whereas Table 4.4 indicates that compound permeation can also be affected by different surfactant types.

Table 4.3: *p*-values calculated using ANOVA test for each compound and surfactant

Compound	Surfactant	<i>p</i>-values
Benzocaine	SDS	0.000
	CTAB	0.000
	Brij 35	0.000
	CHAPS	0.004
	Tween 80	0.000
Benzotriazole	SDS	0.000
	CTAB	0.000
	Brij 35	0.000
	CHAPS	0.035
Ibuprofen	SDS	0.005
	CTAB	0.000
	Brij 35	0.000
	CHAPS	0.000
	Tween 80	0.000
Lidocaine	SDS	0.000
	CTAB	0.012
	Brij 35	0.034
	CHAPS	0.510
	Tween 80	0.006

Table 4.4: *p*-values calculated using ANOVA test for each compound and surfactant (20 mM)

	SDS	CTAB	CHAPS	Brij 35	Tween 80
SDS		B: 0.002 BT: 0.000 I: 0.000 L: 0.000	B: 0.001 BT: 0.000 I: 0.001 L: 0.000	B: 0.000 BT: 0.001 I: 0.001 L: 0.000	B: 0.000 I: 0.000 L: 0.000
CTAB	B: 0.002 BT: 0.000 I: 0.000 L: 0.000		B: 0.000 BT: 0.000 I: 0.003 L: 0.015	B: 0.233 BT: 0.000 I: 0.000 L: 0.001	B: 0.228 I: 0.000 L: 0.025
CHAPS	B: 0.001 BT: 0.000 I: 0.001 L: 0.000	B: 0.000 BT: 0.000 I: 0.003 L: 0.015		B: 0.000 BT: 0.686 I: 0.102 L: 0.053	B: 0.000 I: 0.962 L: 0.083
Brij 35	B: 0.000 BT: 0.001 I: 0.001 L: 0.000	B: 0.233 BT: 0.000 I: 0.000 L: 0.001	B: 0.000 BT: 0.686 I: 0.102 L: 0.053		B: 0.972 I: 0.000 L: 0.001
Tween 80	B: 0.000 I: 0.000 L: 0.000	B: 0.228 I: 0.000 L: 0.025	B: 0.000 I: 0.962 L: 0.083	B: 0.972 I: 0.000 L: 0.001	

B, Benzocaine; BT, Benzotriazole; I, Ibuprofen; L, Lidocaine.

To understand the effect of individual surfactant type and concentration, the cumulative amount of compound permeated after 6 h were tabulated in Table 4.5 and 4.6. It can be seen from these tables that the amount of the model compounds permeated after 6 hours varies with a change in surfactant concentration and type. Moreover, permeability profiles were shown as percentage permeated after 6 h, graphically, in Figs. 4.1 – 4.4 in an attempt to provide a comprehensive understanding of the relationship between the surfactant concentration and the reduction in the amount permeated. In all of the figures (Figs. 4.1 – 4.4) the amount permeated after 6 h for the control solution was considered 100 %, and then the calculations for other solutions were performed accordingly. The data from all calculations are shown in Tables 4.7 and 4.8. Such presentations offer a convenient way of

comparing different active compounds in terms of the effect on their permeation by a surfactant.

Table 4.5: The values of cumulative amount permeated after 6 hours (Q_6) of four model compounds in the presence of various surfactants across PDMS membrane

Compound	Surfactant	Q_6 values ($\mu\text{g}/\text{cm}^2$) of compound at three different surfactant concentrations			
		0 mM (control)	4 mM	8 mM	20 mM
Benzocaine	SDS	570.65 ± 13.00	526.98 ± 11.19	520.29 ± 4.56	370.01 ± 10.93
	CTAB	611.95 ± 20.24	412.35 ± 37.75	336.94 ± 17.46	229.99 ± 31.91
	Brij 35	600.99 ± 39.63	456.40 ± 32.33	372.96 ± 14.80	257.46 ± 6.52
	Tween 80	627.31 ± 19.04	461.77 ± 3.21	386.27 ± 10.18	257.13 ± 2.28
Benzotriazole	SDS	110.80 ± 3.90	108.55 ± 2.60	83.72 ± 1.33	74.63 ± 1.24
	CTAB	60.06 ± 3.23	57.66 ± 2.03	48.92 ± 1.40	41.99 ± 1.41
	Brij 35	80.90 ± 0.64	79.45 ± 4.24	64.79 ± 2.29	58.10 ± 2.22
Ibuprofen	SDS	155.67 ± 10.95	163.60 ± 8.24	138.18 ± 6.53	126.20 ± 9.45
	CTAB	126.09 ± 8.67	60.67 ± 3.51	31.88 ± 4.27	15.23 ± 1.80
	Brij 35	185.47 ± 10.62	158.84 ± 10.30	105.09 ± 0.51	74.88 ± 2.15
	Tween 80	167.61 ± 8.62	127.94 ± 7.75	87.54 ± 3.07	59.84 ± 1.10
Lidocaine	SDS	410.35 ± 8.29	253.74 ± 11.36	187.76 ± 17.99	81.17 ± 6.68
	CTAB	333.97 ± 37.25	314.68 ± 27.91	283.63 ± 41.67	221.73 ± 20.32
	Brij 35	380.52 ± 22.63	394.04 ± 18.87	354.49 ± 24.16	337.36 ± 15.73
	Tween 80	352.27 ± 5.62	383.75 ± 51.90	359.09 ± 34.18	258.94 ± 5.46

Table 4.6: The values of cumulative amount permeated after 6 hours (Q_6) of four model compounds in the presence of CHAPS across PDMS membrane

Compound	Surfactant	Q_6 values ($\mu\text{g}/\text{cm}^2$) of compound at three different concentrations of CHAPS			
		0 mM (control)	2 mM	4 mM	20 mM
Benzocaine	CHAPS	635.17 ± 23.38	617.92 ± 41.17	630.04 ± 31.97	517.98 ± 24.85
Benzotriazole	CHAPS	62.59 ± 3.57	61.18 ± 3.07	56.85 ± 1.67	56.72 ± 1.16
Ibuprofen	CHAPS	188.30 ± 7.40	194.57 ± 10.60	109.94 ± 1.93	59.05 ± 11.19
Lidocaine	CHAPS	322.81 ± 39.99	318.98 ± 21.29	308.78 ± 19.19	293.14 ± 24.37

Table 4.7: The values of percentage (%) permeated after 6 h of four model compounds in the presence of various surfactants across PDMS membrane

Compound	Surfactant	% permeated of compound after 6 h at three different surfactant concentrations			
		0 mM (control)	4 mM	8 mM	20 mM
Benzocaine	SDS	100 ± 2.28	92.35 ± 1.96	91.17 ± 0.80	64.84 ± 1.92
	CTAB	100 ± 3.31	67.38 ± 6.17	55.06 ± 2.85	37.58 ± 5.21
	Brij 35	100 ± 6.59	75.94 ± 5.38	62.06 ± 2.46	42.84 ± 1.08
	Tween 80	100 ± 3.04	73.61 ± 0.51	61.58 ± 1.62	40.99 ± 0.36
Benzotriazole	SDS	100 ± 3.52	97.97 ± 2.35	75.56 ± 1.20	67.36 ± 1.12
	CTAB	100 ± 5.38	96.00 ± 3.38	81.45 ± 2.33	69.91 ± 2.35
	Brij 35	100 ± 0.79	98.21 ± 5.24	80.09 ± 2.83	71.82 ± 2.74
Ibuprofen	SDS	100 ± 7.03	105.09 ± 5.29	88.76 ± 4.19	81.07 ± 6.07
	CTAB	100 ± 6.88	48.12 ± 2.78	25.28 ± 3.39	12.08 ± 1.43
	Brij 35	100 ± 5.73	85.64 ± 5.55	56.66 ± 0.27	40.37 ± 1.16
	Tween 80	100 ± 5.14	76.33 ± 4.62	52.23 ± 1.83	35.70 ± 0.66
Lidocaine	SDS	100 ± 2.02	61.84 ± 2.77	45.76 ± 4.38	19.78 ± 1.63
	CTAB	100 ± 11.15	94.22 ± 8.36	84.93 ± 12.48	66.39 ± 6.08
	Brij 35	100 ± 5.95	103.55 ± 4.96	93.16 ± 6.35	88.66 ± 4.13
	Tween 80	100 ± 1.60	108.94 ± 14.73	101.94 ± 9.70	73.22 ± 1.55

Table 4.8: The values of % permeated after 6 hours of four model compound in the presence of CHAPS across PDMS membrane

Compound	Surfactant	% permeated of compounds after 6 h at three different concentrations of CHAPS			
		0 mM (control)	2 mM	4 mM	20 mM
Benzocaine	CHAPS	100 ± 3.68	97.28 ± 6.48	99.19 ± 5.03	81.55 ± 3.91
Benzotriazole	CHAPS	100 ± 5.70	97.75 ± 4.90	90.83 ± 2.67	90.62 ± 1.85
Ibuprofen	CHAPS	100 ± 3.93	103.33 ± 5.63	58.39 ± 1.02	31.36 ± 5.94
Lidocaine	CHAPS	100 ± 12.39	98.81 ± 6.60	95.65 ± 5.94	90.81 ± 7.55

In the first set of experiments, permeation of benzocaine, benzotriazole, ibuprofen and lidocaine through silicone membrane from the donor solutions containing SDS (an anionic surfactant) at three different concentrations (4, 8 & 20 mM) were evaluated. It can be seen in Fig. 4.1, and also in Table 4.3, that the presence of the anionic surfactant significantly ($p < 0.05$) affected the transport of all compounds over a period of 6 h with the lowest percentage permeated observed at the highest concentration of surfactant examined.

Overall, the results here would indicate that the reduction in the amount permeated is directly related to the concentration of surfactant. These results are similar to the findings of a recent study where Waters and co-researchers reported a decrease in the permeation of paraben derivatives with an increase in SDS concentration in the donor solution (Waters *et al.* 2013). It can be seen in Fig. 4.1 that the maximum reduction in permeation of each compound resulted from 20 mM SDS being present in the donor compartment, with lidocaine experiencing a reduction of 80.22 %, being the highest when compared with other model compounds, and ibuprofen having a reduction of 18.93 %, being the lowest.

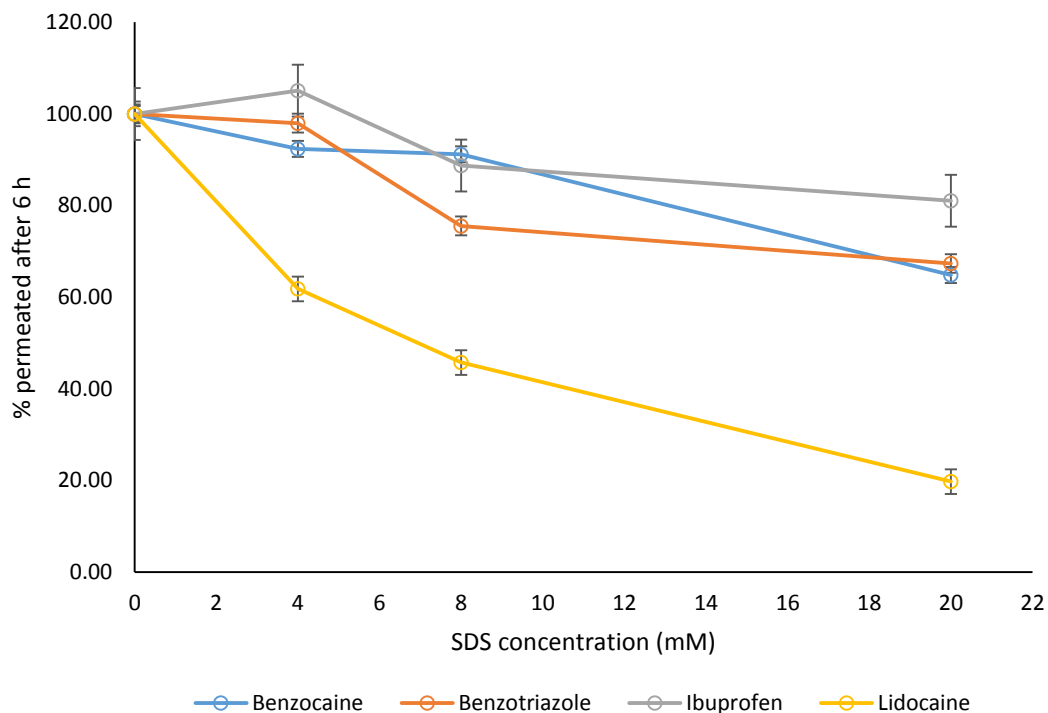


Fig. 4.1: Effect of the presence of SDS on compound permeation across PDMS membrane

The other noticeable phenomenon in Fig. 4.1 is that the permeability profiles of benzocaine, benzotriazole, and ibuprofen, position themselves, more likely, to be part of a group whereas lidocaine is very distinctive in this regard. Therefore, the scenario suggests that SDS is behaving differently to two groups which can be based on their physicochemical properties. From the physicochemical perspective, lidocaine is basic in nature whereas the other three compounds are acidic. Thus, upon ionisation in buffer solution, lidocaine produces cations while benzocaine, benzotriazole, and ibuprofen, produce anions. Hence, the compounds, in donor solutions, would exist as ionised (charged) species and unionised (neutral) species. As PDMS membrane is predominantly hydrophobic in nature, only the neutral species can pass through the membrane while the charged species stay in the donor solution. Although both the neutral and charged (anionic and cationic) species can interact with SDS, the interaction of SDS with an anion could not be the same to that with a cation, and this variation might result in the compounds experiencing a dissimilar effect from SDS.

From the above discussion, it is clear that the influence on compound permeability can result from a multidimensional interaction or a mixture of interactions, such as, surfactant-membrane, and/or surfactant-drug interaction. One study suggested surfactant-membrane interaction to be a triggering factor in the reduction of compound permeation (Waters *et al.* 2013). Their study assumed that the hydrophobic tail of SDS was submerged within PDMS membrane, thus, resulting in the charged head group exposed to the donor solution. Therefore, they proposed that the SDS impregnated membrane surface create a negatively charged environment which would, in turn, repel the neutral species of compound. Their study also, similar to this study, found 20 mM SDS to produce a greater hindrance in permeation than all others (0, 4 and 8 mM SDS) which, they suggested, is because of the coexistence of free monomer, monomer-membrane surface interactions and micellisation. It is noticeable that the above-mentioned mechanisms offer a comprehensive explanation of SDS effect on the overall reduction in compound permeation. However, the fact that SDS produces a dissimilar effect for different compounds cannot be addressed by applying these mechanisms. Therefore, a detailed look at surfactant-drug or surfactant-membrane interactions would be necessary.

As mentioned above, only the unionised form of compound can permeate through PDMS membrane. Thus, the extent of permeation depends on the availability of compounds in their unionised form in the donor compartment of the diffusion cell. In a solution, an equilibrium exists between unionised and ionised forms while maintaining a specific ratio between two forms depending on the pH of the solution. For example, in a buffer solution of pH 7.4, ibuprofen would have 0.32 % of total as neutral (unionised) and 91.68 % as anionic (ionised) species whereas lidocaine would have 24.02 % as neutral and 75.98 % as cationic species. This ratio gives the actual percentage of species in the donor solution, provided that they do not interact with other components such as surfactant. However, this might not be

the case for lidocaine. As lidocaine produces cations in the solution, a portion of these ions might weakly bond the anionic head groups of SDS. In other words, a portion of cationic lidocaine molecules, from the bulk solution, will migrate to the SDS-submerged membrane surface. Therefore, to maintain the equilibrium ratio between two species (ionised and unionised) in the bulk solution a certain number of unionised species would be converted to the ionised form which, in turn, decrease the number of neutral (unionised) lidocaine molecules available to diffuse through the membrane. In the case of a micellar surfactant solution, an additional interaction can happen where cationic lidocaine species interact with SDS head groups in the micelles thus further decreasing the number of neutral lidocaine molecules that would pass through the membrane. In both cases, the permeation of lidocaine would be further reduced. These scenarios might not be observed for benzocaine, benzotriazole and ibuprofen, as upon ionisation they produce anions which would be repelled by the SDS head group, and stay in the bulk solution i.e. the equilibrium ratio of ionised and unionised forms would not be affected.

A second type of surfactant was investigated in this study, namely a cationic surfactant, cetyltrimethylammonium bromide (CTAB). Fig. 4.2 shows the permeability profiles of the compounds in the presence of CTAB. Fig. 4.2, along with the *p*-values (< 0.05) from Table 4.3, clearly indicate that the compound fluxes were significantly influenced by the cationic surfactant being present in the donor solution.

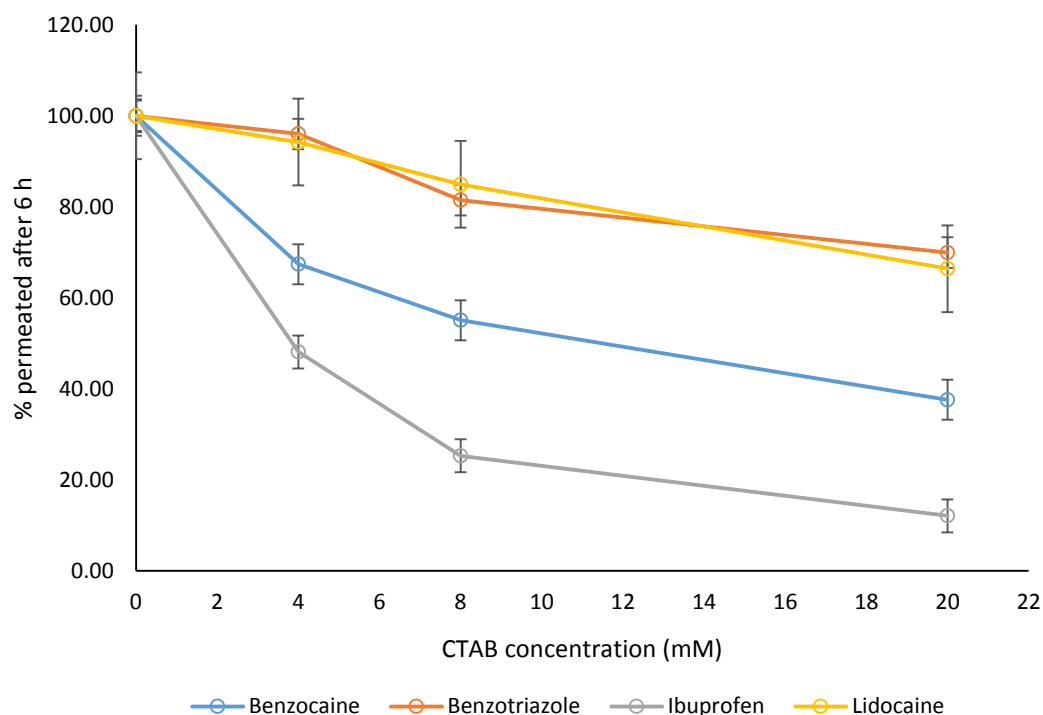


Fig. 4.2: Effect of the presence of CTAB on compound permeation across PDMS membrane

Such an effect of CTAB was hypothesised in a study by Waters and co-researchers (Waters *et al.* 2013) where they assumed that CTAB would reduce the transport of paraben derivatives (the model compounds considered in their study) across PDMS membrane. Their hypothesis stated that CTAB would create a positively charged membrane surface i.e. the hydrophobic tail of CTAB would be submerged within PDMS membrane thus exposing the cationic head group to the donor solution, and consequently, this would reduce the likelihood of the permeation of neutral paraben molecules through the membrane. The same mechanism could be observed in this study. In other words, the positively charged CTAB-submerged membrane surface could repel the compound molecules away from the membrane resulting in an overall reduction in permeation. As mentioned earlier (in the case of SDS), though this mechanism may explain the reduction of compound permeation in general, it cannot clarify the inter-difference amongst the compounds in terms of percentage reduced. It can be seen

from Fig. 4.2 that the percentage of the amount reduced by CTAB is different for each compound.

Although both SDS and CTAB create a barrier effect in compound permeability, the overall trend they follow is different. From Fig. 4.1 and 4.2, if the percentages of overall reduction are placed in an order, then for CTAB the order appears as ibuprofen > benzocaine > lidocaine > benzotriazole whereas, for SDS it becomes lidocaine > benzocaine > benzotriazole > ibuprofen. In general, the reduction effect of both these surfactants on compound permeation is different for each drug. Previously, it was mentioned that the difference produced by SDS was due to the interaction between its anionic head groups and ionised compound species in the donor solution. In the case of CTAB, the difference in compound reduction can be the result of the interaction between its cationic head groups and ionised species of the compounds. To explain, it can be assumed that the hydrophobic regions of CTAB are submerged in PDMS membrane thus exposing the cationic head groups to the donor solution and making a positively charged membrane surface. A portion of anionic species, which are formed upon ionisation of acid compounds, may migrate to the positively charged membrane surface, and weakly bond the cationic head groups of CTAB. Consequently, to maintain the equilibrium ratio between ionised and unionised forms of acid compounds in the bulk solution, a number of unionised species are converted to the ionised (anionic) species, thus, decreasing the total available number of neutral molecules to be transported across the membrane. In the case of a micellar solution, the number of neutral molecules can be further decreased because of the interaction between the anionic form of the compound and the cationic head group of CTAB. In both scenarios, the compound would experience a reduction in transport through membrane. However, the aforementioned circumstances may not be observed for lidocaine as it forms a cation upon ionisation which is repelled by the cationic CTAB head. Unexpectedly, though benzotriazole forms an anion

upon ionisation, it was not affected by the scenarios mentioned above. This may be the result of a complex chemical interaction which is currently unclear and the focus of current study.

The third type of surfactant, investigated in this study, was a zwitterionic surfactant, namely CHAPS. The effect of CHAPS on compound permeation is shown in Fig. 4.3.

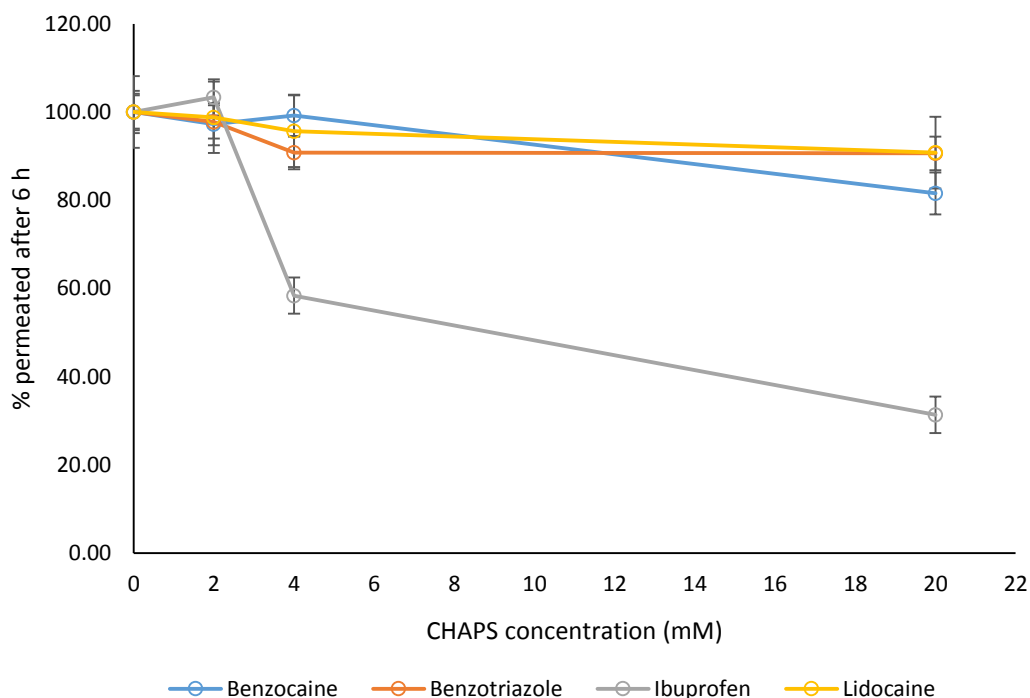


Fig. 4.3: Effect of the presence of CHAPS on compound permeation across PDMS membrane

It appears that the overall permeation of compounds, except for ibuprofen, was not significantly affected by CHAPS. It is also observable that the permeation of ibuprofen was reduced only in the presence of CHAPS being present at, and above its CMC which is 4 mM. At 2 mM, i.e. below the CMC, CHAPS did not affect ibuprofen permeation. This may be the result of an interaction between the ibuprofen molecules and CHAPS micelles as upon reaching the CMC, the surfactant forms micelles. The formation of surfactant micelles creates a hydrophobic core which contains the hydrophobic regions of surfactant. One study reported that the hydrophobic core of micelles can strongly interact with hydrophobic molecules and entrap them inside the core (Tehrani-Bagha & Holmberg 2013). A similar mechanism can be

observed in this study where ibuprofen, with a log P value of 3.6, strongly interacted with the hydrophobic core of CHAPS micelles and became trapped inside them thus reducing the number of ibuprofen molecules available to cross through PDMS membrane. Consequently, there would be a reduction in ibuprofen permeation. As the other three compounds are relatively less hydrophobic, they might not as strongly interact with CHAPS micelles and hence, their fluxes would not be significantly affected.

This study also investigated the effect of a non-ionic surfactant, namely Brij 35, on drug transport across PDMS membrane. The results (Fig. 4.4 and Table 4.8) indicate that the presence of this non-ionic surfactant significantly retarded the overall transport of all compounds except for lidocaine. It can also be seen that the fluxes of lidocaine and benzotriazole remain unaffected in the case of 4 mM Brij 35.

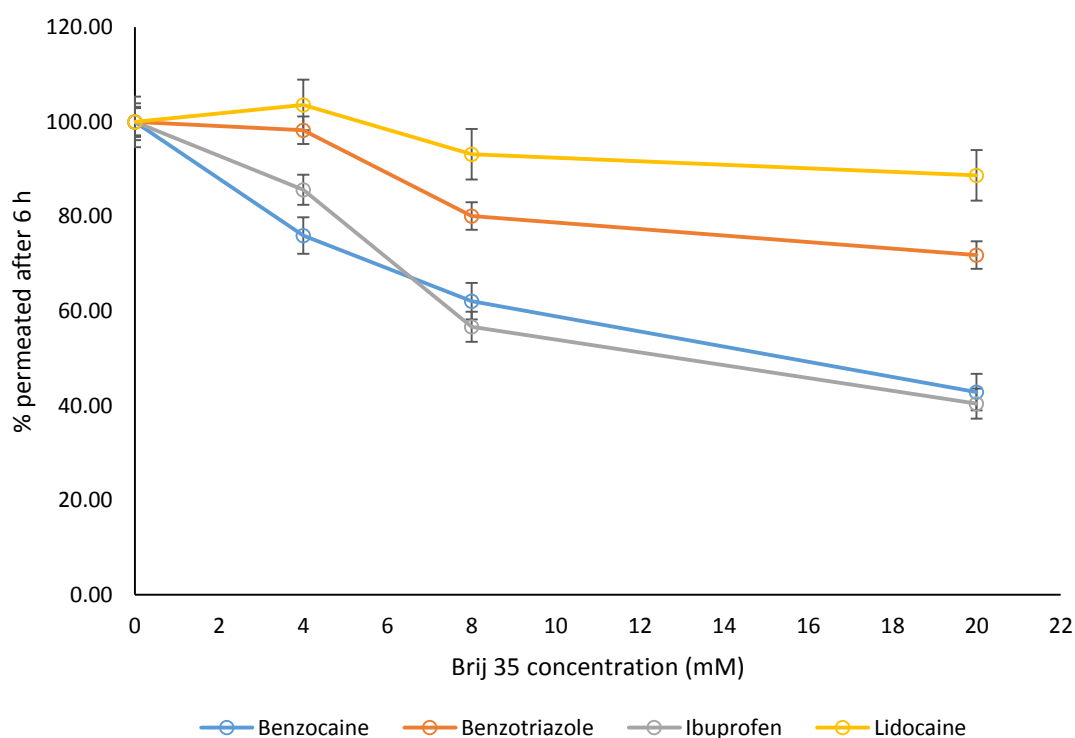


Fig. 4.4: Effect of the presence of Brij 35 on compound permeation across PDMS membrane

Nevertheless, in general, an increase in the concentration of Brij 35 resulted in a decrease in the flux of the compounds. Interestingly, this finding appears to be different than

that observed in a recent study (Waters et al. 2013). In that study, Brij 35 was reported not to have a significant effect on compound permeation through PDMS membrane. The study considered paraben derivatives, namely, methylparaben and ethylparaben as model compounds. However, to investigate whether such an anomaly of Brij 35 effect results from its particular interaction with different model compounds based on their physicochemical properties, some relevant properties of all compounds from both studies, were tabulated and compared (Table 4.9).

Table 4.9: The physicochemical properties¹ of the model compounds

Compound	Log P	Polar surface area (Å ²)	Free rotating bonds	H bond acceptors	H bond donors
Methylparaben	1.87	47	2	3	1
Ethylparaben	2.40	47	3	3	1
Benzocaine	1.95	52	3	3	2
Benzotriazole	1.34	42	0	3	1
Ibuprofen	3.72	37	4	2	1
Lidocaine	3.63	36	5	3	1

¹The physicochemical properties were generated from ACD/Labs, RSC, UK

A closer look at Table 4.9 reveals that there is no observable pattern in the physicochemical characteristics of the compounds which could explain the anomaly in Brij 35 effect on their permeation. Hence, further investigation, such as a calorimetric study of surfactant-compound interaction, could be useful and remains the focus of current study.

However, to confirm if this phenomenon is a result of Brij 35 in particular (or a more broadly observed trend of non-ionic surfactant) a further study was carried out focusing on the permeation of three model compounds (benzocaine, ibuprofen and lidocaine) in the presence of another non-ionic surfactant, namely Tween 80.

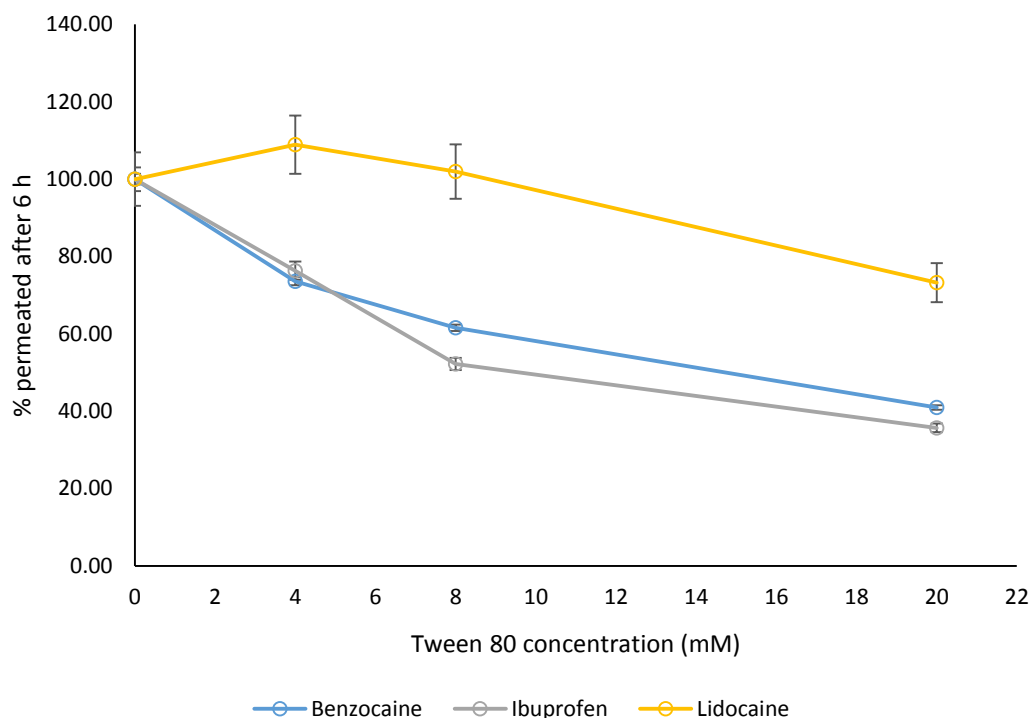


Fig. 4.5: Effect of the presence of Tween 80 on compound permeation across PDMS membrane

Fig. 4.5 clearly shows that the presence of this non-ionic surfactant retards the permeation of the compounds in a similar trend to that observed for Brij 35. Therefore, it can be inferred that this non-ionic surfactant does affect compound permeation.

In summary, the current study demonstrates that all five surfactants investigated here had a significant effect on compound permeation. Comparing different concentrations of various surfactants, it is obvious from Table 4.1 that the solution containing 20 mM surfactant leads to the lowest flux of compound across PDMS membrane. However, while the surfactants show the greatest reduction effect at 20 mM, clear differences can be found in their effect at this concentration (Fig. 4.6). It also appears from Fig. 4.6 that among the four surfactants tested, CTAB facilitates the lowest flux in the case of all compounds, except for lidocaine – the lowest flux of lidocaine was obtained in the presence of SDS. The same trend was observed for the surfactants being present in the donor solution at a concentration of 4 mM (figure not shown).

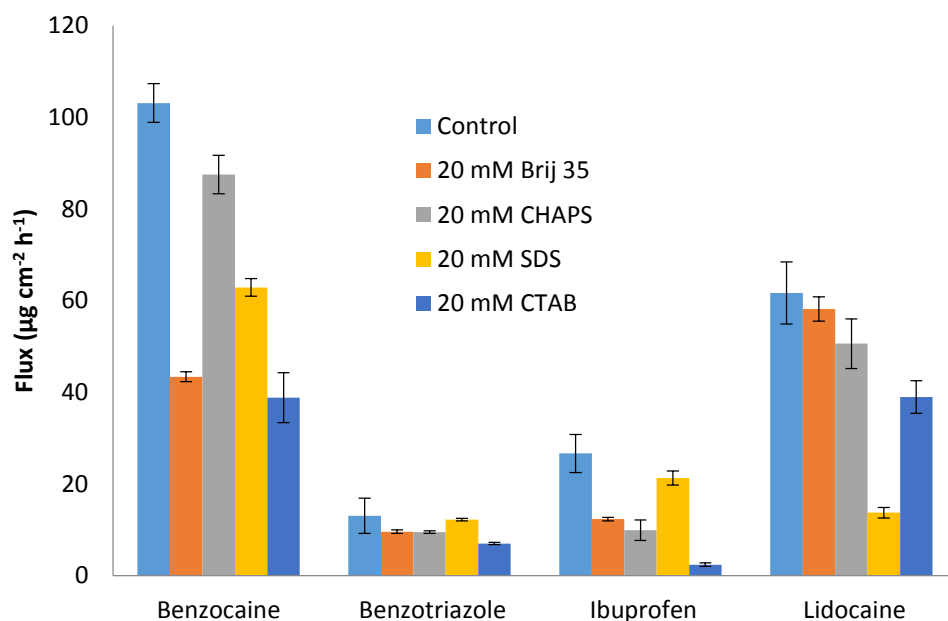


Fig. 4.6: Effect of the presence of surfactant (20 mM) on compound permeation across PDMS membrane

While the studies reported here demonstrate a phenomenon common to all surfactants, there was a clear difference in their effect on compound permeation through silicone membrane. Creating a detailed mechanistic picture of this difference would require an in-depth investigation of surfactant- compound -membrane interactions using techniques such as calorimetric techniques, and is the focus of a current study.

4.3 Conclusion

The current study exhibits a wider perspective of surfactant effect on compound permeation across silicone membrane. The surfactants examined in this study appear to reduce the transport of four compounds through the membrane. Overall, there was an inverse relationship between surfactant present and the amount of compound permeated. It was also observable that the effect of surfactant on compound permeation was different for different surfactant types, and also for different compounds. This variance was thought to result from a variation in the interaction of the charged and neutral compound species with the surfactant head group, and/or the surface and core of the surfactant micelle. Comparing all four surfactants, CTAB appeared to facilitate the lowest flux of compound through silicone membrane.

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Chapter 5: Prediction of the mechanism of the effect of surfactant on compound permeation through silicone membrane.

This chapter aims to predict the mechanism behind the effect of surfactant on drug permeation through silicone membrane. Analytical techniques such as differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) were used to investigate the physicochemical properties of the untreated and surfactant-treated membrane whereas nuclear magnetic resonance (NMR) spectroscopy was used to examine the behaviour of two surfactants in the solution with or without the membrane being present.

5.1 Introduction

As mentioned in Chapter 4, a previous study in our laboratory investigated the effects of two surfactants, namely, sodium dodecyl sulphate (SDS) and Brij 35, on the permeation of two paraben derivatives through PDMS membrane (Waters *et al.* 2013). The study found that the presence of SDS significantly reduced the permeation of the parabens, whereas the presence of Brij 35 had no substantial effect on their permeation. The authors postulated that SDS molecules were impregnated within the membrane creating a charged barrier, thus repelling the paraben molecules attempting to permeate, although the exact mechanism was yet to be confirmed using further analytical techniques.

Surfactants are known to self-assemble into micelles upon reaching the critical micelle concentration (CMC) with a mixture of monomers and pre-micelles at concentrations lower than this value. A study by Khossravi proposed that surfactant reduced drug permeation by entrapping drug molecules within its micelles (Khossravi 1997), which may be analogous to that observed here, yet the mechanism was not confirmed by further investigation. In this study, a number of analytical techniques, namely, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) and nuclear

magnetic resonance (NMR) spectroscopies were used to investigate the interactions between PDMS membrane and different surfactants to enhance our understanding of how some surfactants alter drug permeation through the membrane, yet some do not.

5.2 Results and discussion

5.2.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was employed to investigate whether the surfactant-treatment of PDMS membrane induced any structural change of the membrane. The surfactants were selected to encompass all four types – SDS (anionic), Brij 35 and Tween 80 (non-ionic), CTAB (cationic), and CHAPS (zwitterionic). Fig. 5.1 shows the DSC thermograms of PDMS membranes, either untreated or pre-treated in the surfactant solutions.

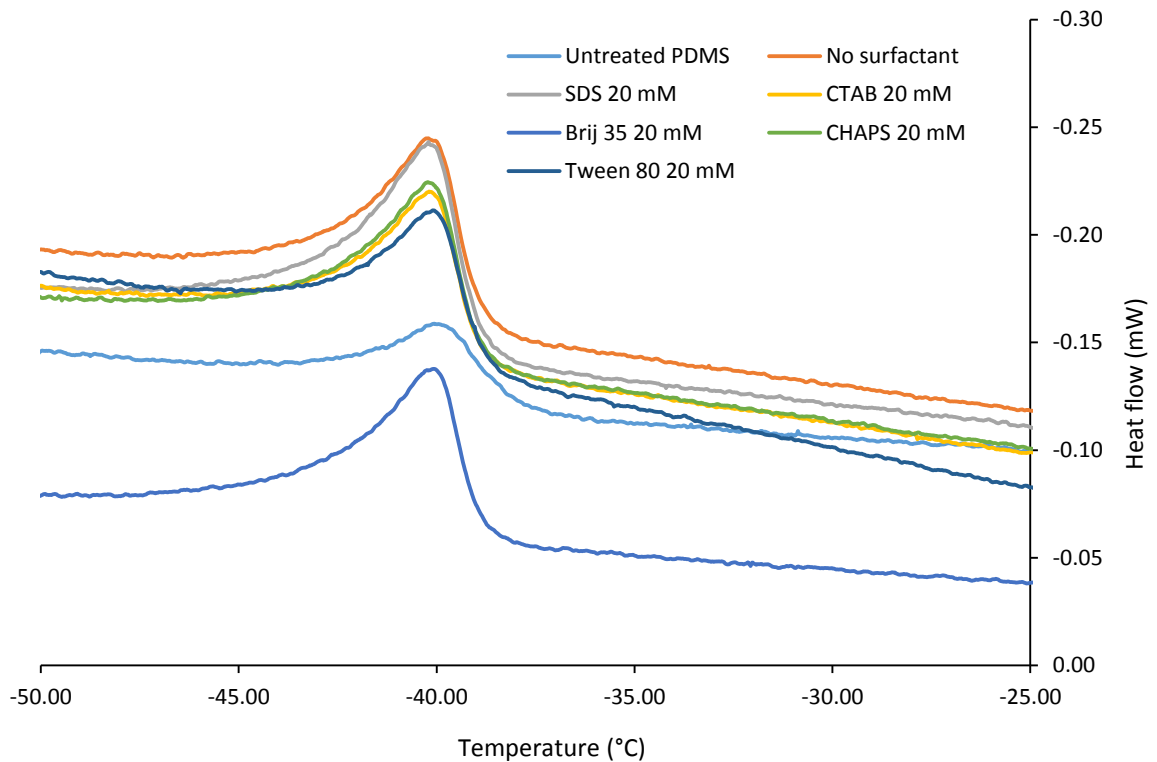


Fig. 5.1: DSC thermograms of PDMS membrane – untreated or pre-treated

The onset, and crystalline melting point temperatures for the samples are given in Table 5.1. For the untreated membrane the crystalline melting point was at -39.94 °C, which is consistent with the literature value of -40.00 °C (Dias *et al.* 2007). The DSC curves for the pre-treated samples were compared with that of the untreated membrane to observe any characteristic change that might result from the treatment with the surfactant solutions.

Table 5.1: Values of onset and crystalline melting temperatures for PDMS membranes either untreated or pre-treated in different solutions

Treatment type	Onset temperature (°C)	Crystalline melting point (°C)
Untreated membrane	- 41.83 °C	- 39.94 °C
No surfactant	- 42.68 °C	- 40.20 °C
SDS 20 mM	- 42.79 °C	- 40.20 °C
CTAB 20 mM	- 42.50 °C	- 40.19 °C
Brij 35 20 mM	- 42.62 °C	- 40.05 °C
CHAPS 20 mM	- 42.75 °C	- 40.22 °C
Tween 80 20 mM	- 42.49 °C	- 40.05 °C

From Fig. 5.1 and Table 5.1 it is clear that none of the surfactants appeared to produce a significant shift in the crystalline melting point of the membrane, and the DSC thermograms for all of the treated samples are very similar to that for the untreated sample. Moreover, there was no additional peak formed in any of the DSC curves for the treated membranes. These findings suggest that the surfactants under investigation do not interact with the PDMS structure. This is because any interaction with the membrane structure would result in a clear shift of the crystalline melting point of the membrane, as stated in a previous study, whereby, upon treatment with IPM (isopropyl myristate), PDMS showed a shift in its crystalline melting point to a lower temperature, whereas no shift was observed for the treatment with

propylene glycol and decanol (Dias *et al.* 2007). With regards to the permeation effect of the surfactants discussed in Chapter 4, the DSC data indicates that the surfactants do not reduce the permeation of the compounds across PDMS membrane by interacting with or altering the bulk structure of the membrane.

5.2.2 FTIR (Fourier transformed infrared) spectroscopy

Previously, DSC results suggested that the surfactants did not alter the physicochemical properties of PDMS membrane. To further confirm the DSC findings, FTIR spectroscopy was utilised to investigate any possible structural change in PDMS membrane owing to its treatment in the surfactant solutions. This technique is frequently used to characterise chemical structure or to identify any structural change (Chen *et al.* 2009). Fig. 5.2 shows the FTIR spectra of the untreated and pre-treated samples, with the latter being soaked overnight in the surfactant solutions.

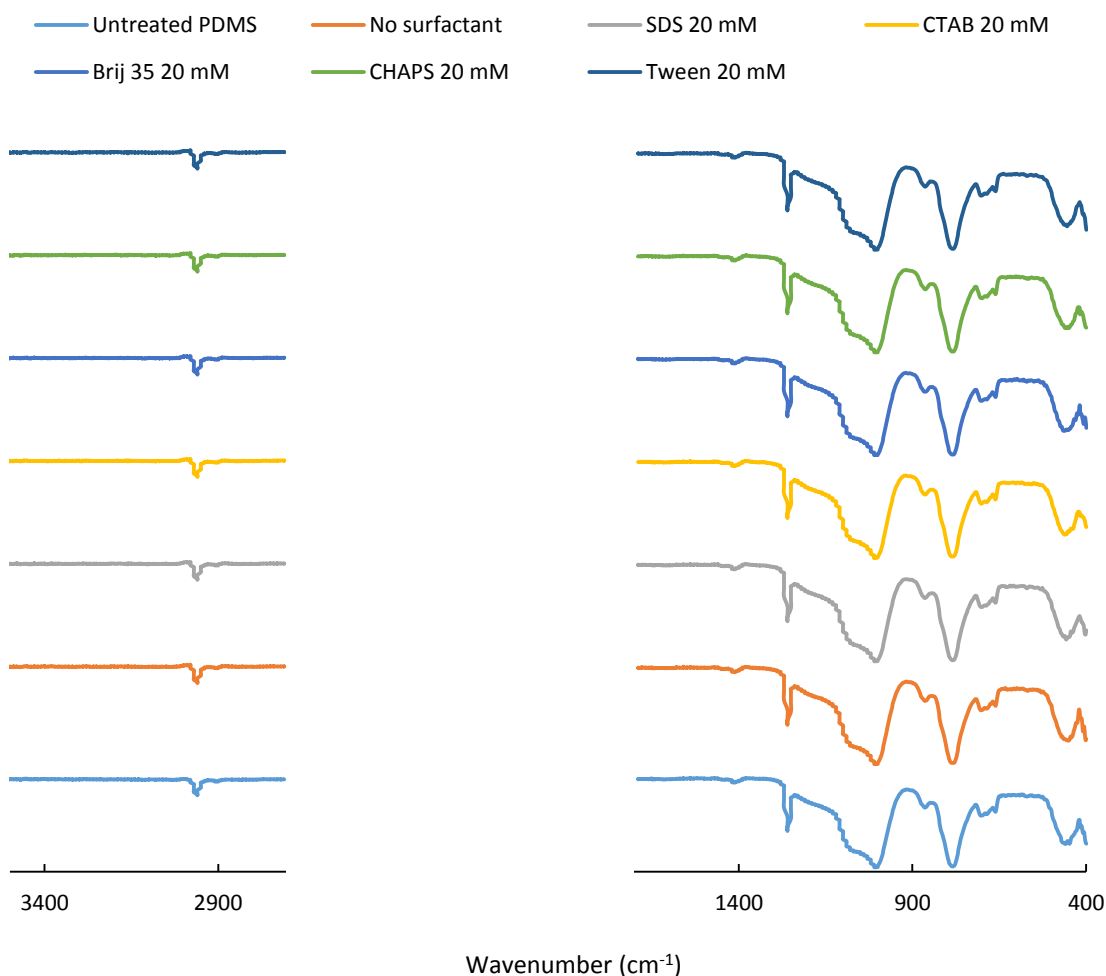


Fig. 5.2: FTIR spectra of PDMS samples – untreated, and pre-treated

It is apparent from Fig. 5.2 that all of the spectra are very similar, with the intensities of the peaks also being identical. It is also to be noted that no appreciable water content was found by FTIR spectroscopy. Moreover, no further peak was formed except the peaks for the untreated PDMS membrane, and no band from the surfactants was observed. These findings can further be confirmed by looking at the values of the major peaks of the FTIR spectra for all samples (Table 5.2). The peak values for the untreated sample were used as reference to evaluate other spectra. For the untreated PDMS, the characteristic peak at 2962.0 cm^{-1} was assigned to the asymmetric and symmetric stretching vibrations of C-H bonds of the methyl group (Juárez-moreno *et al.* 2015). The peaks at 1258.1 cm^{-1} and 784.2 cm^{-1} are associated

with Si-(CH₃)₂ (Zhang *et al.* 2011), whereas the peak at 1006.3 cm⁻¹ is attributed to the stretching vibration of Si-O bond of the backbone of PDMS structure (Paschoal *et al.* 2011). When comparing the absorption bands for all spectra, it is obvious from Table 5.2 that there is no significant variation in their values.

Table 5.2: The major peak values of the typical FTIR spectra of PDMS membranes – untreated or pre-treated in surfactant solutions

Treatment type	Values of absorption bands (cm ⁻¹) for different bonds present in PDMS structure			
	C-H	Si-(CH ₃) ₂	Si-O	Si-C
Untreated membrane	2962.0	1258.1	1006.3	784.2
No surfactant	2961.8	1258.1	1006.2	785.9
20 mM SDS	2961.7	1258.1	1006.7	783.7
20 mM CTAB	2962.0	1258.1	1007.8	783.4
20 mM Brij 35	2961.7	1258.1	1008.2	783.1
20 mM CHAPS	2961.9	1258.1	1007.7	784.0
20 mM Tween 80	2961.8	1258.1	1006.5	783.5

Using FTIR spectroscopy it is possible to identify any change in a chemical structure by looking at the intensity of absorbance signal or the formation of a new peak. For example, the intensity of an absorbance signal may become weak because of a cross-linking modification (Han *et al.* 2011), or new peaks can be formed as a consequence of the change in PDMS structure by a chemical, for example, tetraethylorthosilicate (Chen *et al.* 2009). None of the above-mentioned phenomena was observed in this study. Therefore, it can be said that the structure of PDMS membrane remains intact in the presence of the surfactants. Again, in relation to the surfactant effect on compound permeation discussed in Chapter 4,

the FTIR results conform to the DSC findings whereby the mechanisms for reducing compound permeation were not associated with the structural modification of PDMS membrane.

5.2.3 Scanning electron microscopy (SEM)

The surfaces of the untreated, and pre-treated PDMS membranes were examined by scanning electron microscopy. Fig. 5.3 shows the microscopic images for different membrane samples. All of the images illustrate a certain degree of asymmetry and patterned surface associated with these membranes. It is known that polymeric membranes such as PDMS, often have patterned surfaces that are advantageous to compound permeation, and any change in the pattern can alter permeability across the membrane (Redondo *et al.* 2001). To investigate whether the presence of surfactant induces any change in the patterned PDMS surface, the morphology of the pre-treated membranes was compared with that of the untreated sample. It is apparent from Fig. 5.3 that the surfactant-treatment does not alter the morphology of PDMS membrane, with all of the images showing a significant similarity. Therefore, with regards to the permeation effect of the surfactant (discussed in Chapter 4), SEM data suggests that the mechanism behind the barrier effect is not associated with the morphological changes of PDMS membrane.

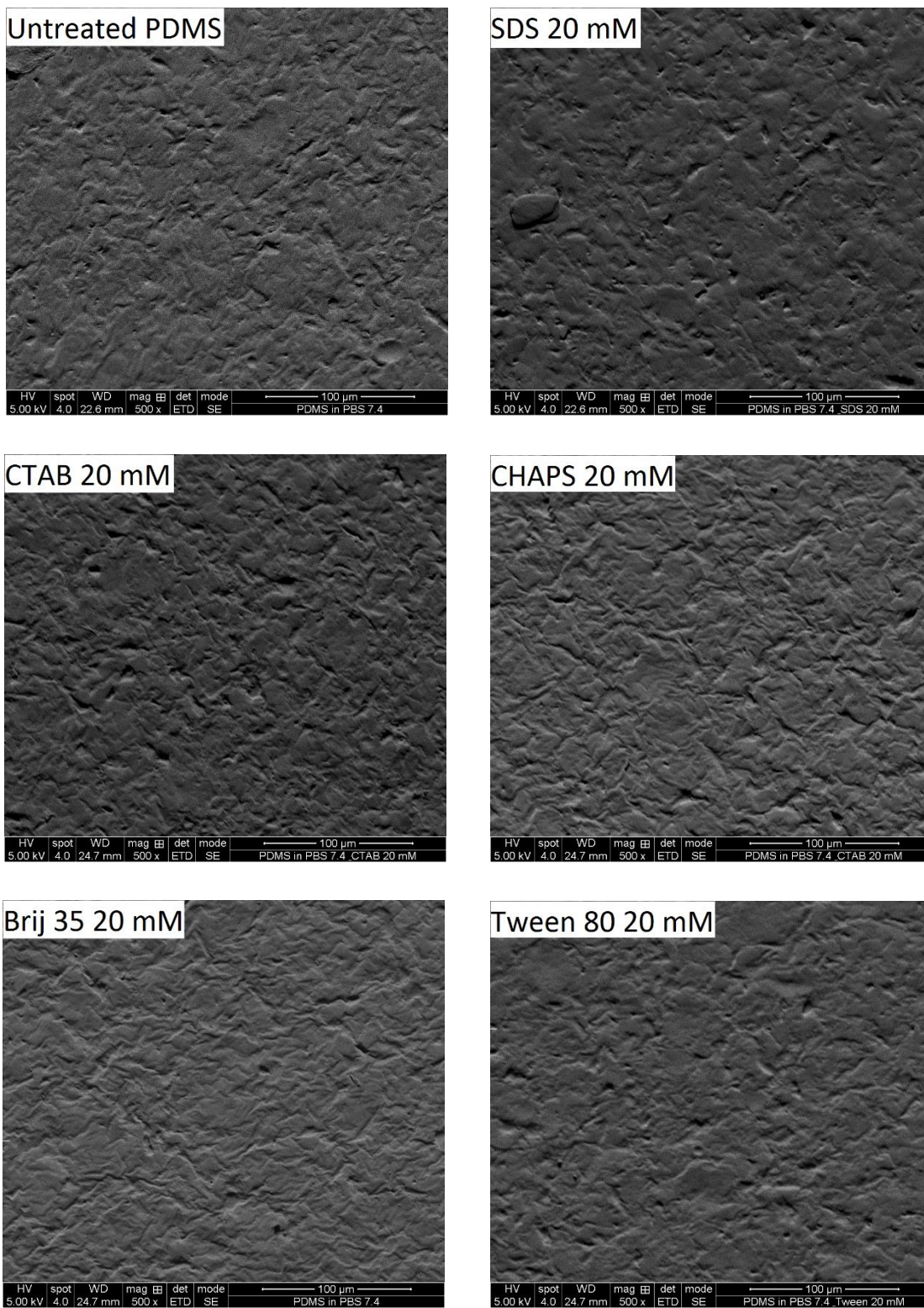


Fig. 5.3: SEM micrographs of the untreated, and pre-treated PDMS surfaces

5.2.4 Nuclear magnetic resonance (NMR) spectroscopy

Previously, the morphology and chemical structure of PDMS membrane were analysed both in the presence and absence of surfactants to predict the mechanism behind their effect on compound permeation across the membrane, and based on DSC, FTIR and SEM, it was found that the mechanism is not associated with the morphology or structure of the membrane. In this section, the behaviour of surfactant in aqueous solution was examined using ^1H NMR spectroscopy. Primarily, two surfactants were selected – SDS and Brij 35. These surfactants were reported in a previous permeation study (Waters *et al.* 2013) whereby SDS was found to reduce compound permeation across PDMS membrane with Brij 35 showing no appreciable effect. However, in this study, both of the surfactants appeared to reduce the permeation of compounds across the membrane. To investigate the anomaly with regards to Brij 35, and also to predict the mechanism of the surfactant effect on compound permeation, NMR studies of the surfactants were conducted.

In the first set of experiments, the chemical shifts for H1 and H5 for SDS were measured at five different concentrations both in the presence and absence of PDMS membrane. The results are shown in Table 5.3. The proton numbering of the chemical structure of SDS is shown in Fig. 5.4.

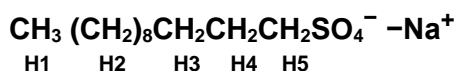


Fig. 5.4: Chemical formula of SDS with proton numbering (Cui *et al.* 2008)

Table 5.3: Chemical shifts (ppm) for H1 and H5 protons of SDS in the presence and absence of PDMS membrane

	Chemical Shift (ppm) H1	Chemical Shift (ppm) H5
4 mM SDS	0.7447	3.9151
4 mM SDS + membrane	0.7429	3.9143
6 mM SDS	0.7448	3.9152
6 mM SDS + membrane	0.7451	3.9152
8 mM SDS	0.7466	3.9134
8 mM SDS + membrane	0.7462	3.9137
10 mM SDS	0.7545	3.9083
10 mM SDS + membrane	0.7582	3.9129
20 mM SDS	0.7576	3.8955
20 mM SDS + membrane	0.7554	3.9089

The aim of the chemical shift measurements was to find out if there were any significant changes in the chemical shifts of SDS before and after the immersion of PDMS membrane in the SDS solutions. Any changes in the chemical shifts would indicate a change in SDS concentration, based on a study by Cui *et al.* (Cui *et al.* 2008). The study found that a decrease in SDS concentration resulted in a decrease in the chemical shift for H1 proton accompanied by an increase in that for H5 proton, and vice versa. The study also mentioned that the chemical shifts move to the high-frequency side as the concentration increased, and when the surfactant concentration reached its CMC, the chemical shift changed significantly with the increase in concentration, which implied micelle formation. In this study, it was hypothesised that the immersion of PDMS membrane would change the chemical shifts of

SDS. To comprehend this phenomenon, the experimental procedure can be considered. The sample solution was kept in a small volumetric flask with or without the pieces of PDMS membrane placed at the bottom of the flask. The samples for NMR were taken from the upper regions of the solutions *i.e.* considerably far from the membrane. It was assumed that a number of SDS molecules would be adsorbed on the membrane surface (PDMS membrane is hydrophobic in nature, and hence the hydrophobic tail of the surfactant can be impregnated into the membrane surface), subsequently decreasing the number of the molecules in the rest of the solution *i.e.* a decrease in SDS concentration. In such a scenario, a decrease in SDS concentration would happen in the upper part of the solution, which is not adjacent to the membrane. Therefore, it was expected that there would be significant changes in the chemical shifts of SDS upon immersing PDMS membrane in the solution which would in turn indicate a decrease in SDS concentration. However, it is apparent from Table 5.3 that the variations in chemical shifts that occurred upon the addition of PDMS membrane for all concentrations studied are not significant. For all cases, the changes in the chemical shifts are in the third or fourth decimal places. Overall, it can be said that the NMR data does not show any meaningful change when comparing the SDS solutions with those which have had PDMS membrane immersed in them. It might also be that the NMR method used in this study is not sensitive enough to detect small changes in SDS concentration upon the immersion of PDMS membrane.

In the second set of experiments, the behaviour of Brij 35 was examined at two different concentrations both in the presence and absence of PDMS membrane. The chemical shifts for H2 and H7 protons of Brij 35 were measured and are presented in Table 5.4. The formula of the surfactant with proton numbering is shown in Fig. 5.5.

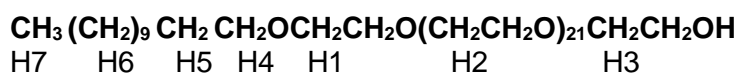


Fig. 5.5: Chemical formula of Brij 35 with proton numbering (Hongchang *et al.* 2002)

Table 5.4: Chemical shifts (ppm) for H7 and H2 protons of Brij 35 in the presence and absence of PDMS membrane

	Chemical Shift (ppm) H7	Chemical Shift (ppm) H2
1 mM Brij 35	0.7701	3.5267
1 mM Brij 35 + membrane	0.7708	3.5269
10 mM Brij 35	0.7798	3.5456
10 mM Brij 35 +membrane	0.7803	3.5461

It can be seen in Table 5.4 that for both samples in the presence of the membrane, there was no significant change in the chemical shifts of Brij 35, a scenario similar to SDS. Therefore, it can be said that the concentration of Brij 35 remained unchanged in the presence of the membrane, and there could be a tendency for Brij 35 molecules to stay in the solution rather than being adsorbed into the membrane surface. These findings can help explain the phenomenon observed in a previous study that compound permeation across silicone membrane is ‘blocked’ by the adsorption of SDS on the membrane surface resulting in a reduction in permeation, yet Brij 35 does not exhibit the same effect (Waters *et al.* 2013). However, in this study (Chapter 4) Brij 35 was found to reduce compound transport through silicone membrane. One explanation of this anomaly could be that in a micellar solution of Brij 35, the compounds that are considered in this study – benzocaine, benzotriazole, ibuprofen and lidocaine – interact with and become entrapped within the hydrophobic core of the micelles, thus reducing their permeability through the membrane whereas the compounds considered in the previous study, namely methylparaben and ethylparaben, do

not show such interaction thus not having any effect on their permeation. However, a calorimetric study of Brij 35 and the compounds would be useful to confirm the above-mentioned phenomena and hence remains the focus of a current study.

In the case of SDS, though the NMR data did not show any meaningful changes when comparing the SDS solutions with or without PDMS membranes, however, based on the DSC, FTIR and SEM data, and the findings by Waters *et al.* (Waters *et al.* 2013), two mechanisms can be suggested for the permeation effect of this surfactant. Firstly, the surfactant molecules are adsorbed on the membrane surface with their charged head group exposed to the donor solution of the diffusion cell thus preventing the permeation of the neutral drug molecules by repelling them away from the membrane. Secondly, the surfactant micelles can bind with, or entrap the drug molecules in their hydrophobic core (Tehrani-Bagha & Holmberg 2013) and thus stop them passing through the membrane. The aforementioned mechanisms are illustrated in Figs. 5.6 and 5.7. These mechanisms can also be related to the permeation effect of other surfactants that were discussed in Chapter 4, namely, CTAB, CHAPS and Tween 80.

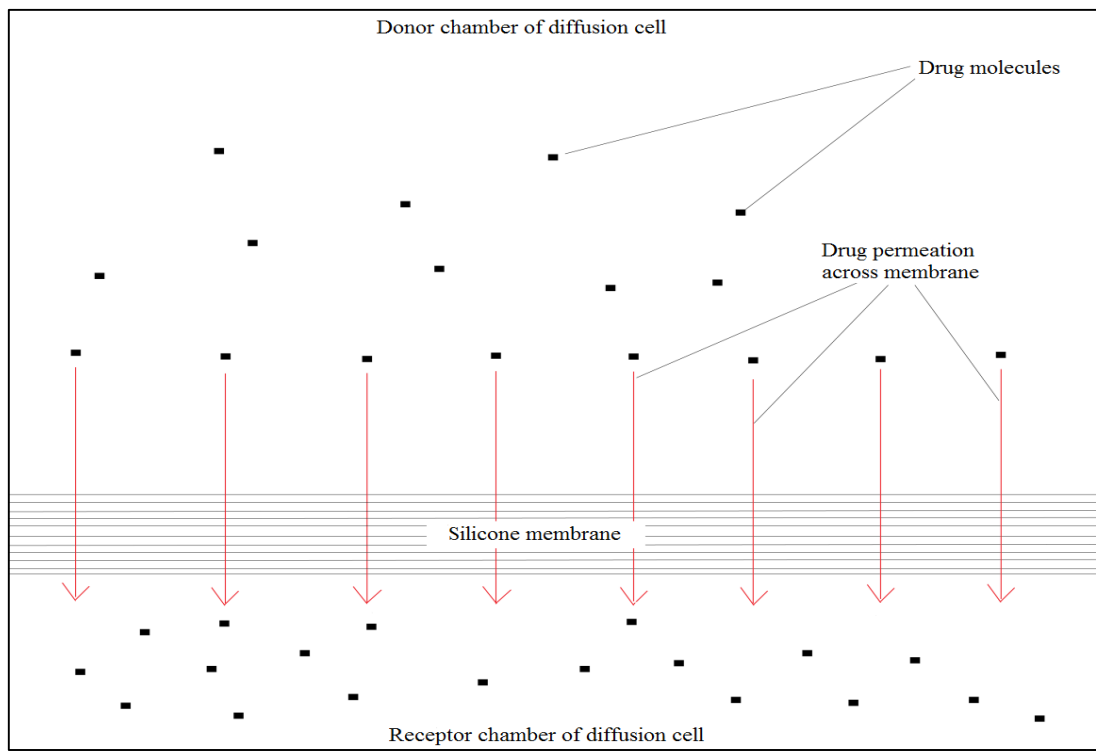


Fig. 5.6: Drug permeation across silicone membrane in the absence of the surfactant in the donor solution

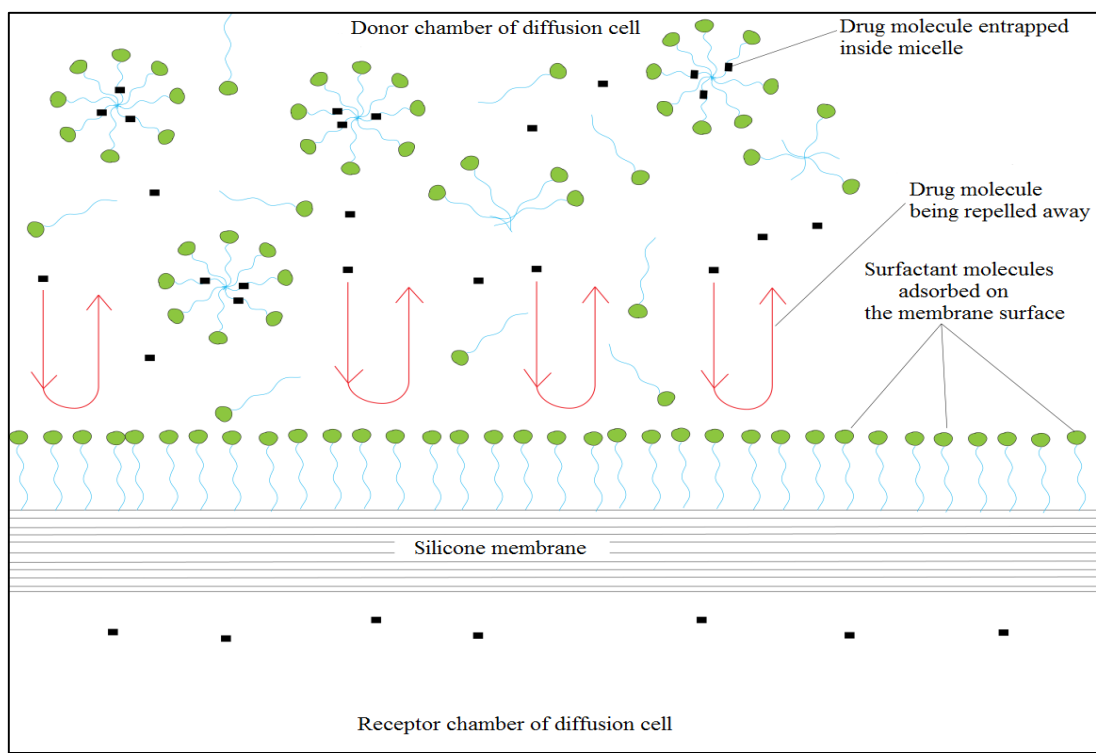


Fig. 5.7: Drug permeation across silicone membrane in the presence of the surfactant in the donor solution

5.3 Conclusion

DSC, SEM, FTIR and NMR spectroscopy were used to predict the mechanism behind the effect of the selected surfactants on drug permeation across silicone membrane. The DSC, FTIR and SEM studies of the untreated and surfactant-treated membrane indicate that the surfactants do not reduce the drug transport across the membrane by altering either the chemical structure or the surface morphology of silicone membrane. NMR studies of two surfactants, namely, SDS and Brij 35, did not show significant differences when comparing the surfactant solutions with or without PDMS membrane. However, from DSC, SEM, FTIR and permeation data it can be suggested that the drug molecules are entrapped within the surfactant micelle and/or repelled, or blocked by the surfactant-impregnated membrane surface, with both of the cases resulting in a reduced permeation of drugs across the membrane. However, additional studies such as calorimetric investigation of drug-surfactant interaction would be helpful to further characterise the system under investigation.

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Chapter 6: Plasma surface modification of polydimethylsiloxane membrane and its effect on the permeation of pharmaceutical compounds

Previous chapters investigated the effects of several factors, namely the ionisation of compounds, the presence of surfactants in the donor solution, on the compound permeation through polydimethylsiloxane (PDMS) membrane. This chapter highlights the modification of PDMS membrane using plasma treatment, and its implication in the permeation study of five model compounds. Moreover, the stability of the treatment will be examined through aging studies of the modified membranes.

6.1 Introduction

Polydimethylsiloxane (PDMS) is a commonly used attractive polymer based on many advantages it possesses such as high optical transparency, biocompatibility, thermal stability, low toxicity (Bacharouche *et al.* 2013) and gas permeability (Markov *et al.* 2014). The wide-ranging use of PDMS includes environmental control (Turner & Cheng 1998), air separation (Kujawska & Kujawski 2015; Li *et al.* 2013), liquid mixture separation (Dong *et al.* 2014), microfluidics (Fan *et al.* 2015), wound dressings and medical applications (Agarwal *et al.* 2012; Juárez-moreno *et al.* 2015), and biochemical sensing (Gu *et al.* 2013). In spite of many advantages, PDMS membrane often needs modification of its surface to enhance its suitability, particularly in the area of microfluidics. This is because of the easy and strong interaction of biological samples with the PDMS surface which is inherently hydrophobic. There are numerous applications of modified PDMS surface including cell culture, DNA hybridisation (Hsu & Chang 2015), biomolecule separation and immunoassay (Ko *et al.* 2008).

A number of techniques have been employed to modify the surface of PDMS including physical or chemical treatments, or a combination of both. In particular, plasma

treatment has widely been used, in recent years, for modification of the PDMS surface (Bodas *et al.* 2008). Plasma treatment is capable of altering the surface polarity and wettability of most polymers (Kull *et al.* 2005). It is generally accepted that during plasma treatment the methyl groups ($-\text{CH}_3$) of PDMS surface are removed and replaced with hydroxyl groups ($-\text{OH}$) (S. Bhattacharya, A. Datta, J. M. Berg 2005) resulting in an oxidised membrane surface. This creates a hydrophilic PDMS surface which can be observed through a significant reduction in the water contact angle (WCA) (Deshpande *et al.* 2012). As a consequence of this modification, the properties of the membrane transforms, for example, it has been reported that freshly treated (oxidised) PDMS showed a substantially smaller gas diffusion coefficient relative to untreated membrane (Markov *et al.* 2014). In some studies plasma treatment process is the first stage in a series of procedures to change the surface i.e. to create various complex products that again, possess different properties to the original PDMS or evade some disadvantages. For instance, following plasma treatment compounds can be incorporated onto the treated surface to minimise nonspecific protein adsorption thus expanding the use of PDMS-based microfluidic chips to carry out complex biological investigation (Yu *et al.* 2015).

One issue with plasma treatment is that the hydrophilicity of the treated surface may disappear during aging, and the surface can recover its hydrophobicity over time. One study investigated the hydrophobic recovery of plasma-treated PDMS surface stored either in air or under water (Markov *et al.* 2014). It was found that in the presence of air the treated surface recovered hydrophobicity after 3 days. Interestingly, storage under water delayed recovery for ~ 3 weeks. Moreover, the study compared two types of PDMS membrane – standard and highly cross-linked. It was observed that, in the presence of air, it took only 3 days for the standard membrane to recover whereas for the highly cross-linked membrane the surface was not restored even after a 3-week aging period. In another study,

polyethersulfone (PES) membrane, which is also hydrophobic in nature, was modified to have a hydrophilic surface using plasma treatment (Kull *et al.* 2005). Interestingly, their modified membrane did not show any hydrophobic recovery even 12 months after treatment *i.e.* the hydrophilicity of the membrane was stable for 12 months. It is important to mention that hydrophobic recovery needs to be eliminated or reduced to prolong the shelf life of modified membrane for practical applications.

One important area of topical and transdermal research is the use of PDMS membrane to predict the permeation of pharmaceutical compounds across skin. Consequently, PDMS has been suggested, in recent years, as a reliable, economic and ethical alternative for measuring compound permeation together with a variety of other techniques (Waters 2015). Although, PDMS has been utilised in a significant number of drug diffusion studies, no known study has previously focused on the implication of plasma-treated PDMS in drug diffusion experiments. In the current study, the surface of PDMS membrane was modified using plasma treatment. The treated membranes were characterised by a number of surface analytical techniques such as contact angle measurement, SEM (scanning electron microscopy), and EDX (energy dispersive X-ray) analysis. Moreover, we reported the first study aimed at understanding the impact of plasma surface modification on permeation of a set of model compounds with the intention of creating a more hydrophilic, and thus potentially more suitable, *in vitro* skin mimic. The hydrophilic stability of the treated membrane was examined using *in vitro* drug diffusion studies and EDX analysis.

6.2 Results and discussion

6.2.1 Surface modification of PDMS membrane

PDMS is known to have a hydrophobic surface which can be modified with the application of plasma surface treatment. In this study, the surface of PDMS membrane was

exposed to air plasma to make it more hydrophilic. Water contact angle (WCA) was used to assess the change in membrane hydrophilicity induced by plasma treatment. WCAs were measured for both treated and untreated PDMS membrane. The WCAs were plotted as a function of the age of the water drop (Fig. 6.1). Figure 6.1 shows that the contact angle was significantly reduced after plasma treatment of the membrane surface. Therefore, it can be inferred that the hydrophobicity of PDMS surface was reduced by the plasma treatment, as expected based on previous literature (Bodas *et al.* 2008).

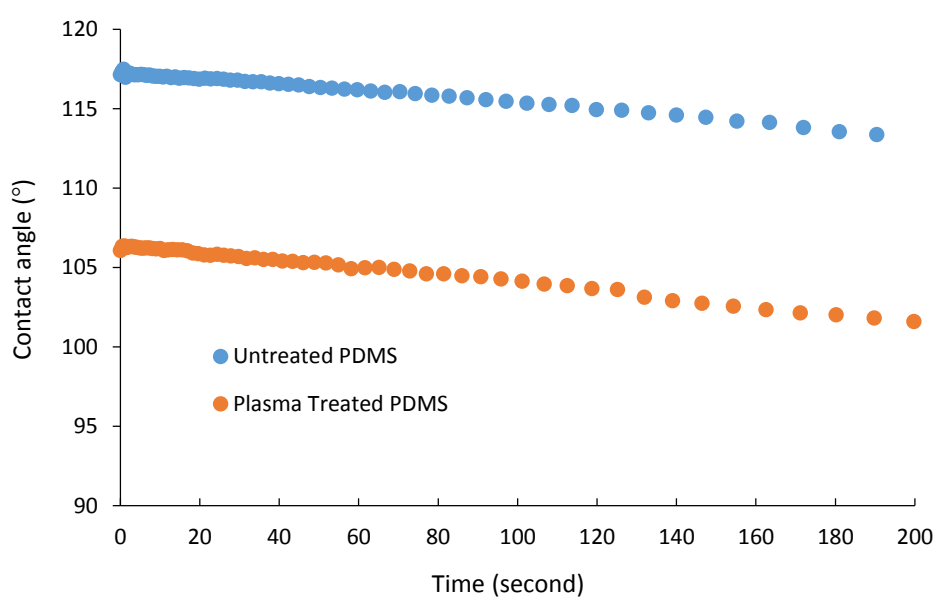


Fig. 6.1: Static contact angles for water on a PDMS membrane untreated or plasma-treated

In addition to the contact angle measurement, the reduction in the hydrophobicity of the PDMS surface was further evidenced by SEM-EDX analysis. The elemental composition (C, O & Si) of PDMS surface untreated, and plasma-treated, was measured using SEM-EDX, and the results are presented in Table 6.1.

Table 6.1: Percentage of weight of chemical elements at the surface of PDMS membrane before and after the plasma treatment. [The data are presented as mean \pm SD, (n = 3)]

Element	% of weight of elements at PDMS membrane surface	
	Untreated Membrane	Plasma Treated Membrane
Carbon (C)	46.33 \pm 2.69	30.68 \pm 2.26
Oxygen (O)	29.86 \pm 2.21	46.49 \pm 2.44
Silicone (Si)	23.82 \pm 2.09	22.84 \pm 2.20

The results in Table 6.1 clearly demonstrate that following the plasma treatment of PDMS surface there was an increase in oxygen content with a subsequent decrease in carbon content. More specifically, the oxygen content of the membrane surface increased by $\sim 17\%$ with a concomitant decrease in carbon content (by $\sim 16\%$). It is evident from literature that during plasma treatment the methyl groups ($-\text{CH}_3$) of PDMS membrane surface are removed and replaced with hydroxyl groups ($-\text{OH}$) (Markov *et al.* 2014). The same process could be observed here i.e. the plasma treatment resulted in an increase in the hydroxyl ($-\text{OH}$) groups with a concomitant decrease in methyl ($-\text{CH}_3$) groups at the membrane surface. In other words, the treatment incorporates a significant concentration of oxygen containing functional groups at the membrane surface. These functional groups are covalently bonded to the polymeric backbone with the formation of silanol groups ($\text{Si}-\text{OH}$). Thus, following plasma treatment, the surface of PDMS membrane became more hydrophilic.

6.2.2 Membrane integrity

Fig. 6.2 shows SEM images of PDMS membrane surfaces. Images for both treated and untreated membranes illustrate a certain degree of asymmetry associated with these membranes. It can also be seen that the plasma treatments did not cause any obvious damage

to the membrane surfaces. Therefore, the membrane integrity was not compromised by these treatments.

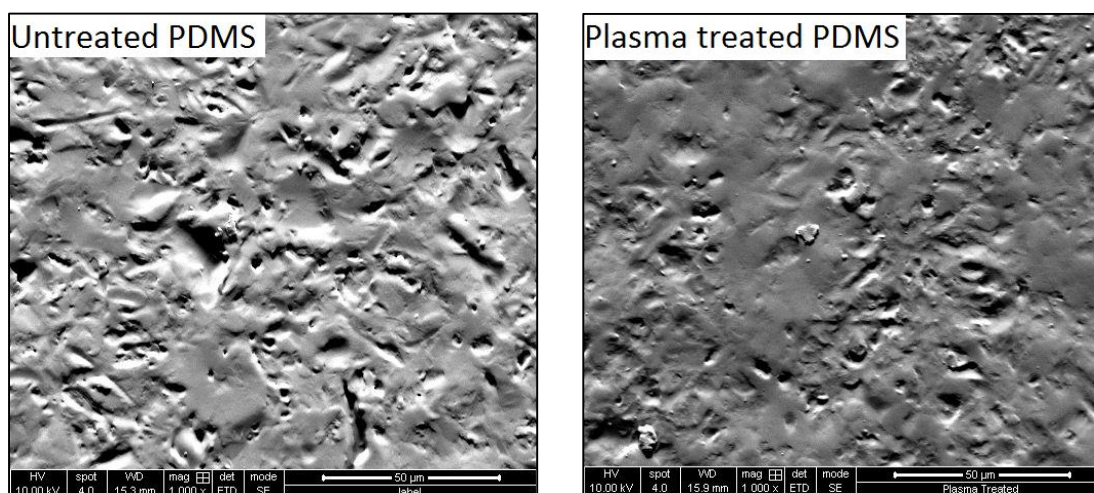


Fig. 6.2: SEM micrographs of the surface of untreated and plasma-treated PDMS membrane

6.2.3 Effect of surface modification of PDMS membrane on compound permeation

The permeation of five model compounds was carried out through silicone (PDMS) membrane prior to plasma treatment, and immediately after the treatment. The model compounds were benzocaine, benzotriazole, caffeine, ibuprofen and lidocaine. These compounds have a diverse range of lipophilicities ranging from a log P of -0.07 for caffeine (Waters *et al.* 2013) to 3.6 for ibuprofen (Waters & Bhuiyan 2016) i.e. to encompass both hydrophilic and hydrophobic types. For all compounds, the pH of the donor and receptor solutions was 7.4. The percentages of compounds unionised were 0.000024 %, 86.32 %, 0.00000013 %, 0.32 % and 24.02 % for benzocaine, benzotriazole, caffeine, ibuprofen and lidocaine, respectively.

The effects of PDMS surface modification on the permeation of the model compounds are graphically presented in Figs. 6.3 – 6.7. In addition to the graphical presentations, two permeation parameters *i.e.* J (steady-state flux) and Q_6 (cumulative amount permeated after 6 hours) were calculated, and the values are shown in Table 6.2 and 6.4. The flux values were statistically analysed using One-way ANOVA, and the calculated

p-values for each compound are shown in Table 6.3. The *p*-values were employed to identify any significant difference in compound permeation owing to the modified membrane surface.

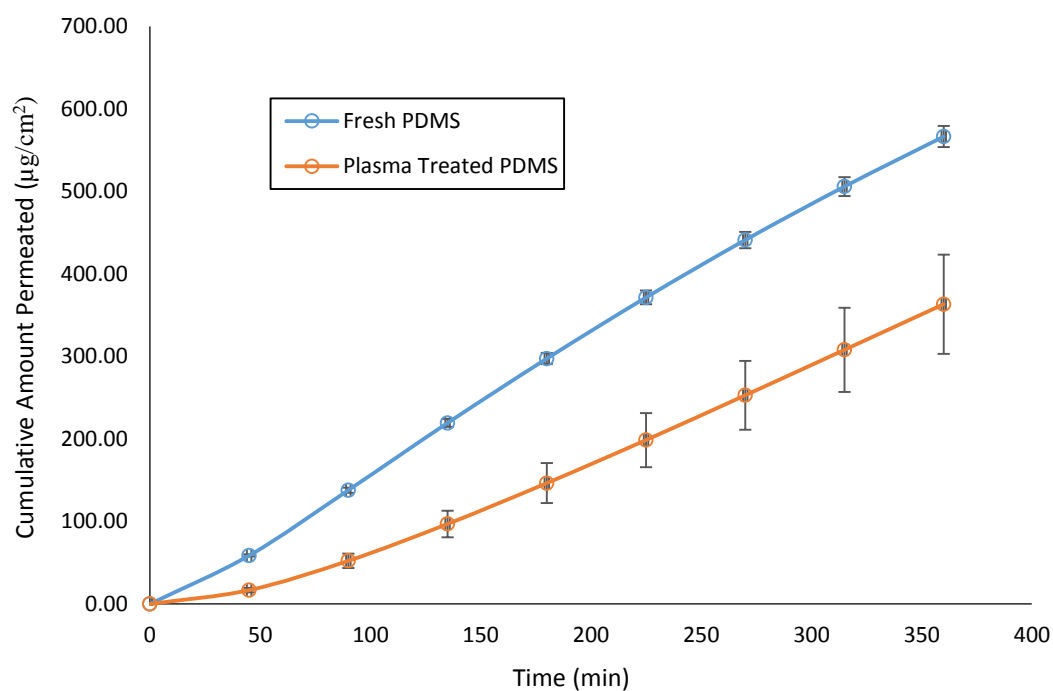


Fig. 6.3: Permeation profiles of benzocaine through PDMS membrane untreated or plasma-treated

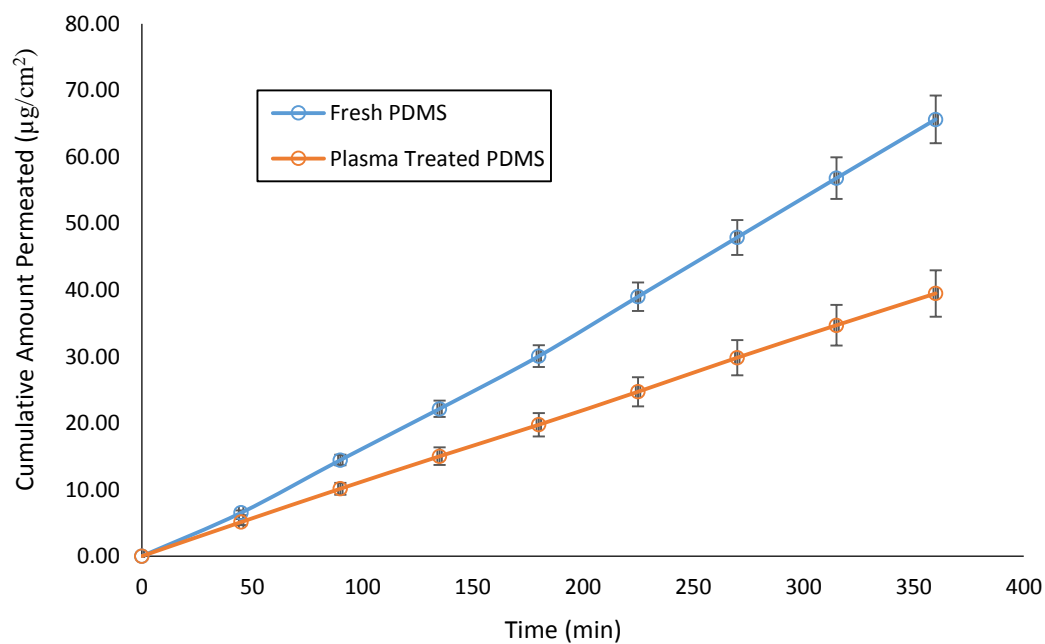


Fig. 6.4: Permeation profiles of benzotriazole across PDMS membrane untreated or plasma-treated

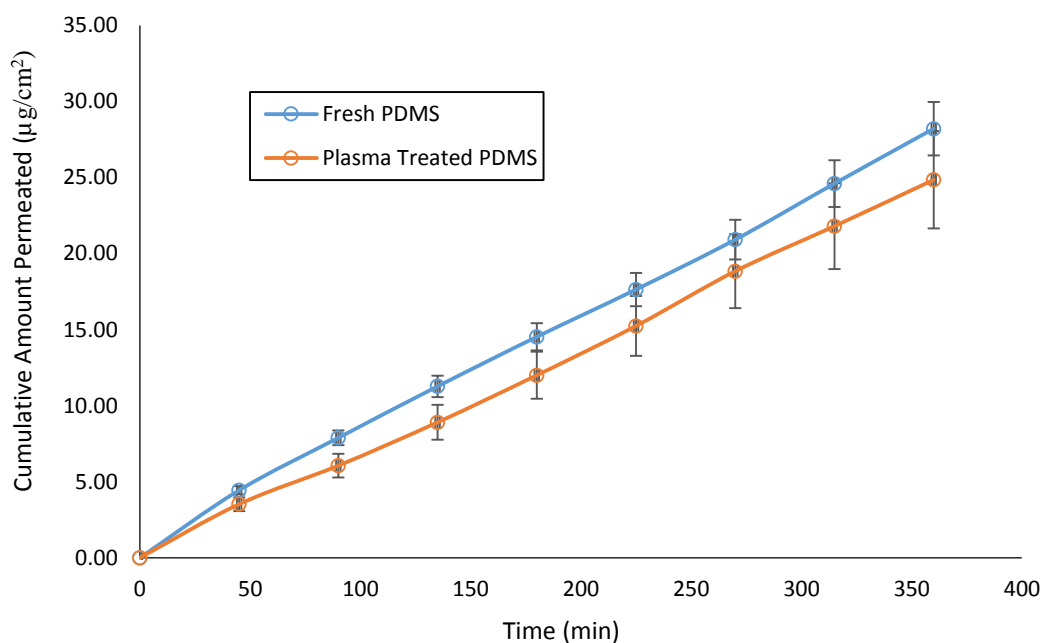


Fig. 6.5: Permeation profiles of caffeine across PDMS membrane untreated or plasma-treated

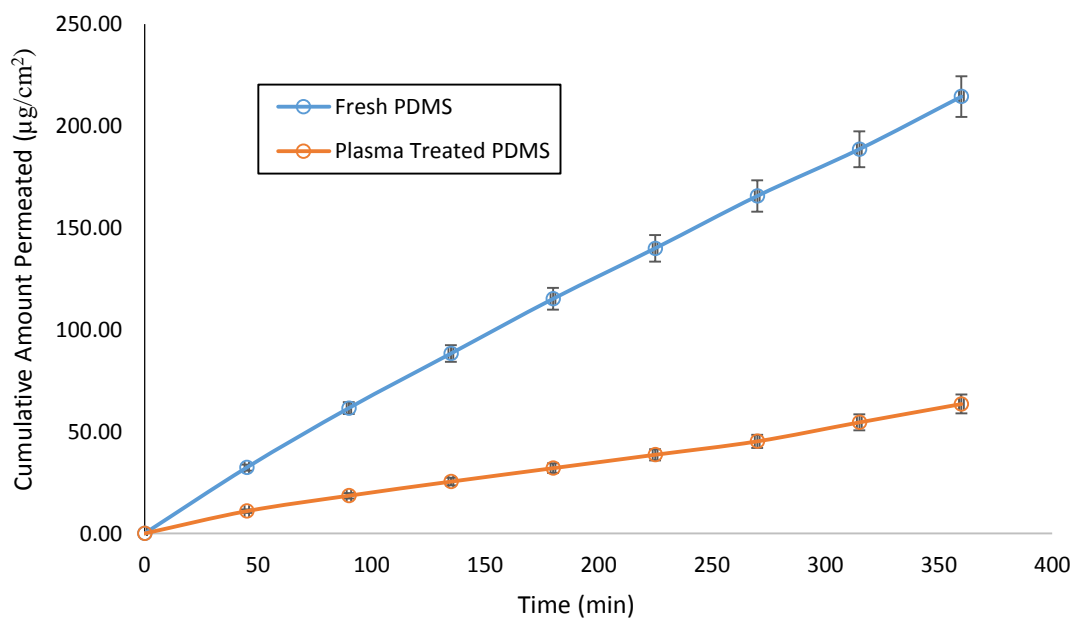


Fig. 6.6: Permeation profiles of ibuprofen across PDMS membrane untreated or plasma-treated

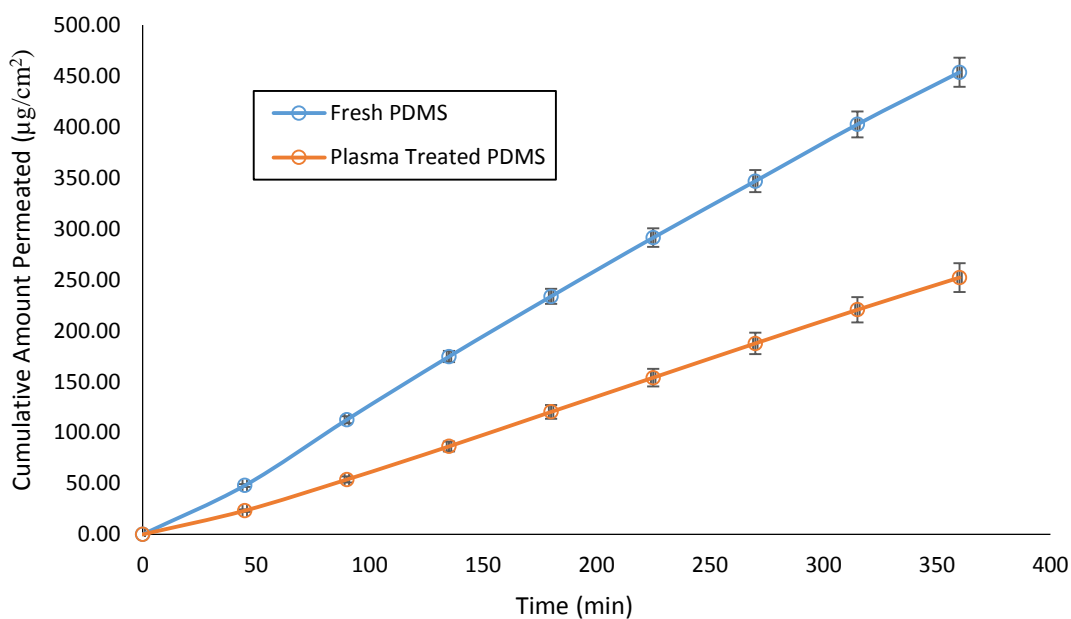


Fig. 6.7: Permeation profiles of lidocaine across PDMS membrane untreated or plasma-treated.

It can be seen in Figs. 6.3 – 6.7 that the permeation profiles of all compounds, except for caffeine, were significantly affected by the plasma-modified PDMS surface. A more specific and quantitative view of this phenomenon can be obtained from the data presented in Table 6.2, 6.3 and 6.4. The data in these tables indicate that the plasma modification of PDMS resulted in a significant change ($p < 0.05$) in the flux of all compounds across the membrane, with caffeine being the exception ($p > 0.05$). The lower flux values of benzocaine, benzotriazole, ibuprofen and lidocaine obtained with the plasma-modified PDMS surface show a clear interaction between these compounds and the modified membrane. However, caffeine was found not to have an observable interaction with the modified PDMS surface. It is obvious from the above-mentioned facts that the modification of membrane surface does play an important role in compound permeation.

Table 6.2: Steady-state flux values of five model compounds across untreated or plasma-treated silicone membrane

Compound	Steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$) of compounds across PDMS membrane	
	Untreated Membrane	Plasma Treated Membrane
Benzocaine	97.03 ± 2.52	62.89 ± 9.80
Benzotriazole	11.05 ± 0.71	6.57 ± 0.62
Caffeine	4.57 ± 0.32	4.14 ± 0.56
Ibuprofen	35.26 ± 1.30	10.03 ± 0.81
Lidocaine	76.97 ± 2.47	43.07 ± 2.50

Table 6.3: *p*-values calculated using ANOVA test for each drug

Drug	<i>p</i> -values
Benzocaine	0.004
Benzotriazole	0.001
Caffeine	0.308
Ibuprofen	0.000
Lidocaine	0.000

In addition to the flux values, this study also calculated the cumulative amount of compound permeated after 6 h and the results are presented in Table 6.4. The purpose of such calculation was to understand the extent of reduction in permeation owing to the plasma treatment of PDMS membrane. Using the data in Table 6.4, the percentage of compound permeated after 6 h was calculated and shown in Table 6.5 and Fig. 6.8. In Table 6.5 the amount of compound permeated after 6 h across untreated membrane was considered 100 %, and then the calculation for plasma-treated membrane was performed accordingly. Such

presentation provides a convenient way of comparing different model compounds with regards to the effect of a modified membrane on their permeation.

Table 6.4: The values of cumulative amount permeated after 6 hours (Q_6) of five model compounds across untreated or plasma-treated silicone membrane

Compound	Q_6 values ($\mu\text{g}/\text{cm}^2$) of compounds across PDMS membrane	
	Untreated Membrane	Plasma-treated Membrane
Benzocaine	566.59 ± 13.67	363.41 ± 55.81
Benzotriazole	65.65 ± 3.84	39.48 ± 3.63
Caffeine	28.22 ± 1.86	24.85 ± 3.54
Ibuprofen	214.48 ± 7.80	63.54 ± 4.90
Lidocaine	453.72 ± 15.41	252.16 ± 15.59

Table 6.5: The values of percentage (%) permeated after 6 hours of five model compounds across untreated or plasma-treated silicone membrane

Compound	% permeated of compounds after 6 hours across PDMS membrane	
	Fresh Membrane	Plasma-treated Membrane
Benzocaine	100 ± 2.41	64.14 ± 9.85
Benzotriazole	100 ± 5.85	60.14 ± 5.53
Caffeine	100 ± 6.59	88.06 ± 12.54
Ibuprofen	100 ± 3.64	29.63 ± 2.28
Lidocaine	100 ± 3.40	55.58 ± 3.44

It can be seen in Table 6.5 and Fig. 6.7 that the presence of the plasma-modified PDMS surface created a significant reduction in the permeation of benzocaine, benzotriazole, ibuprofen and lidocaine. This scenario, therefore, indicates that the modified membrane surface prevents these compounds passing across the membrane and passing into the receptor chamber of the flow-through diffusion cell. In contrast to this, the permeation of caffeine was not significantly affected by the modified membrane surface and hence no appreciable reduction in its permeation was observed. These findings portray that the plasma-treated PDMS surface is generating a barrier effect for the former four compounds, preventing their movement through the membrane – a situation not apparent with caffeine.

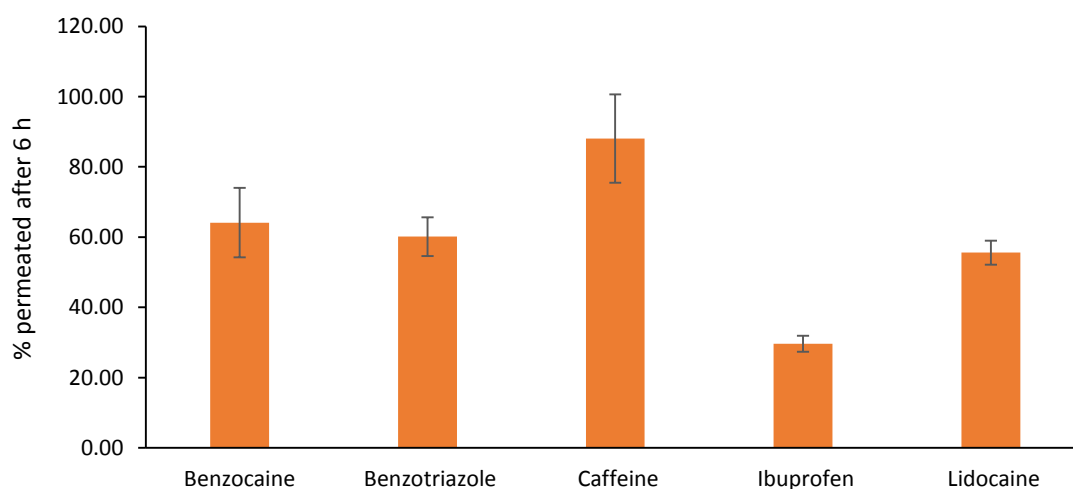


Fig. 6.8: The values of percentage of compound permeated after 6 h through plasma-treated PDMS membrane. The % permeated was defined with respect to the corresponding value for the untreated membrane.

As previously stated, plasma treatment replaces the methyl groups ($-\text{CH}_3$) on the PDMS surface with hydroxyl groups ($-\text{OH}$), resulting in a predominant presence of oxygen containing functional groups on the membrane surface. Therefore, it can be postulated that the treatment of the membrane surface results in a significant number of the hydroxyl groups ($-\text{OH}$) being present at the membrane surface exposed to the donor solution of the flow-

through diffusion cell, thus rendering the membrane surface hydrophilic. As benzocaine (log P of 1.9), benzotriazole (log P of 1.2), lidocaine (log P of 2.4) and ibuprofen (log P of 3.6) are lipophilic in nature, they could be reluctant to approach the plasma-treated membrane surface as it presents a hydrophilic environment thus repelling the compounds away from the membrane resulting in a reduced permeation through to the receptor chamber. However, although all four compounds experienced a reduction in permeation owing to the plasma treatment, the trend they follow is not the same. From Table 6.5, if the extent of overall reduction for these four compounds is positioned in an order, then it appears as ibuprofen > lidocaine > benzocaine > benzotriazole. Considering the log P values of these compound the order suggests that the higher the log P the greater the reduction in compound permeation. In other words, the compound with a higher lipophilicity faces a greater barrier effect from the plasma-modified hydrophilic PDMS surface, as hypothesised. Ibuprofen, with a log P value of 3.6, experienced a reduction of ~ 70 %, being the highest when compared with the other compounds, and benzocaine, with a log P value of 1.9, had a reduction of ~ 36 %, being the lowest. As caffeine is hydrophilic in nature, the same phenomenon i.e. the barrier effect from the plasma-treated hydrophilic surface would not occur to repel caffeine away from PDMS membrane, and hence no reduction in permeation would result. Thus, the permeation profile of caffeine through the untreated and treated silicone membrane would be similar. However, one might expect that, as the treated surface becomes hydrophilic, it should enhance caffeine permeation which was not observed in this study. This is because though the surface becomes hydrophilic after the plasma treatment, the core of the membrane remains hydrophobic in nature. In other words, the core remains unchanged for both the treated and untreated membranes as plasma treatment only works at surface level. As prior to transport into a receiver compartment of diffusion cell, caffeine molecules permeate through the hydrophobic

core, their permeation rate would be similar for both treated and untreated silicone membrane.

In addition to log P values, two other physicochemical properties, namely the number of hydrogen bond donors, and hydrogen bond acceptors, of the model compounds were also considered to investigate if there is an effect of these properties on compound permeation through plasma treated membrane. The properties are tabulated below (Table 6.6).

Table 6.6: The physicochemical properties¹ of the model compounds

	Benzocaine	Benzotriazole	Caffeine	Ibuprofen	Lidocaine
H bond acceptors	2	1	6	1	1
H bond donors	3	3	0	2	3

¹The physicochemical properties were generated from ACD/Labs, RSC, UK

Comparing the data in Table 6.5 and 6.6, it is apparent that there is no observable relationship between the percentages permeated of the compounds through the plasma-treated membranes and the number of hydrogen bond donors/acceptors of the compounds. Therefore, it can be inferred that, even though the hydrogen bond donors/acceptors of the compounds may interact with the hydroxyl groups on the surface of the plasma-treated membrane, such interactions would not significantly affect compound permeability through the plasma-treated membrane.

6.2.4 Stability studies of plasma treated PDMS membrane

It is clear from the above-mentioned discussion that plasma treatment does create a permeability barrier for compounds by creating a hydrophilic membrane surface. However, as mentioned earlier, the hydrophilic stability of the treated surface can be time-dependent (Kull *et al.* 2005). Studies suggest that the hydrophilic stability of plasma treated membrane varies depending on the type and composition of the membrane, and also the sample storage

condition (Markov *et al.* 2014). However, none of the above-mentioned studies considered the storage of membrane samples under airtight conditions. Moreover, the effect of aging of the plasma-treated silicone membrane on compound permeation is yet to be known. In this study, the plasma-treated PDMS membrane was aged for 1, 2, 4 and 8 weeks under airtight conditions at room temperature. The aged samples were then used in the permeation study of a model compound, namely lidocaine. The pH of the donor and receptor solutions used in the permeation study was 7.4.

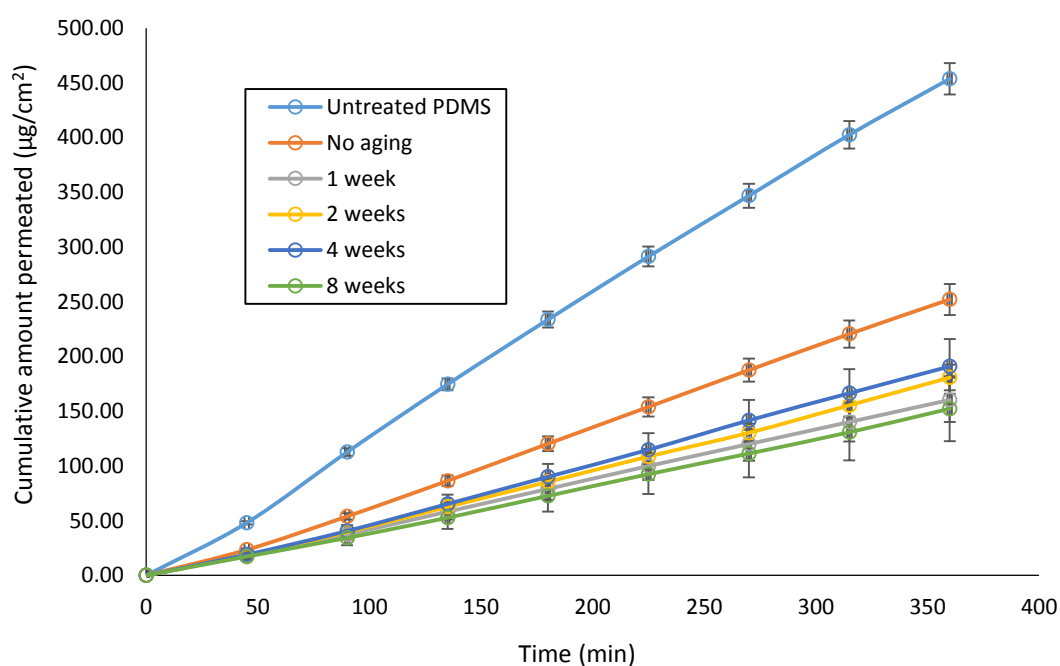


Fig. 6.9: Permeation profiles of lidocaine through PDMS membrane before and after the plasma-treatment. Note: the plasma-treated samples were used either immediately after the treatment or aged over a period of 1, 2, 4 and 8 weeks prior to the permeation experiments

The permeation of lidocaine through the aged samples was then compared with that through the untreated membrane to examine the hydrophilic stability of the treated membrane (Fig. 6.9). The data was also used to investigate the effect of aging of the treated membrane on lidocaine permeation. It can be seen in Fig. 6.9 that the permeation profiles of lidocaine across the untreated and the aged treated membranes are clearly distinctive. For all of the

aged treated membranes, there was a significant reduction in lidocaine permeation when compared with the untreated samples. A quantitative view of this phenomenon can be obtained by looking at the data presented in Table 6.7 and 6.8. Table 6.7 shows the steady-state flux values of lidocaine whereas Table 6.8 demonstrates the *p*-values calculated using ANOVA test.

Table 6.7: Steady-state flux values of lidocaine across plasma-treated PDMS membrane with or without aging

Length of aging	Steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$)
Untreated membrane	76.97 ± 2.47
No aging (freshly treated)	43.07 ± 2.50
1 week	26.99 ± 3.79
2 weeks	30.26 ± 2.08
4 weeks	32.4 ± 4.49
8 weeks	25.42 ± 5.46

Table 6.8: *p*-values calculated using ANOVA test

	Untreated	No aging	1 week	2 weeks	4 weeks	8 weeks
Untreated		0.000	0.000	0.000	0.000	0.000
No aging	0.000		0.004	0.002	0.023	0.007
1 week	0.000	0.004		0.260	0.186	0.704
2 weeks	0.000	0.002	0.260		0.496	0.225
4 weeks	0.000	0.023	0.186	0.496		0.163
8 weeks	0.000	0.007	0.704	0.225	0.163	

It is obvious from Table 6.7 that the flux values obtained for the untreated and the aged treated membranes are different, and the p -values in Table 6.8 show that the difference in lidocaine fluxes is significant ($p < 0.05$) when comparing the flux values through the untreated membrane with that through any of the aged treated membranes. Moreover, all of the aged treated samples drastically reduced lidocaine permeation (Table 6.7), a phenomenon also observed for the freshly treated membrane (no aging). It was mentioned in the previous section that the permeation of the hydrophobic lidocaine was reduced because of the presence of a hydrophilic membrane surface resulting from the plasma treatment. Therefore, these findings imply that the plasma-treated membranes retain their hydrophilic surfaces even after 8 weeks of storage under airtight condition, thus producing a permeability barrier for the hydrophobic lidocaine.

However, it is known from literature that the hydrophilic surface of plasma-treated PDMS membrane is thermodynamically unstable, and the treated membrane undergoes hydrophobic recovery with storage time (Bodas & Khan-malek 2007). Hydrophobic recovery is generally ascribed to the migration of unmodified low molecular weight chains from the bulk towards the surface and the diffusion of the oxidised species from the surface inside the bulk of PDMS polymer (Bacharouche *et al.* 2013). On the basis of these phenomena, one study mentioned that a polymeric membrane with a smaller amount of low molecular weight species would remain hydrophilic longer (Bodas *et al.* 2008). Another study, previously mentioned, found that the hydrophobic recovery could be delayed by using a highly cross-linked PDMS membrane rather than a standard version (Markov *et al.* 2014). Thus, it can be postulated that the PDMS membranes, employed in this study, are highly cross-linked, and contain fewer low molecular weight species, thus showing a hydrophilic stability upon aging following the plasma treatment. Storage under airtight conditions can also contribute to the longer hydrophilic stability by preventing contamination of the treated surface from air

exposure. It is evident from literature that this decreases the hydrophilicity of the treated membrane surface (Bacharouche *et al.* 2013).

In addition to the permeation data, the hydrophilic stability of the plasma-treated membrane was also assessed by SEM-EDX analysis. For this purpose, the elemental composition (C, O and Si) of the treated surface, aged for 8 weeks, was determined using SEM-EDX and the data were then compared with the untreated surface (Table 6.9).

Table 6.9: Percentage of weight of chemical elements at the surface of PDMS membrane, either untreated, or treated also with 8 weeks of aging

Element	% of weight of elements at PDMS membrane surface		
	Untreated membrane	Treated membrane with no aging	Treated membrane with aging for 8 weeks under airtight condition
Carbon (C)	46.33 ± 2.69	30.68 ± 2.26	31.90 ± 2.37
Oxygen (O)	29.86 ± 2.21	46.49 ± 2.44	43.01 ± 2.67
Silicone (Si)	23.82 ± 2.09	22.84 ± 2.20	25.09 ± 2.46

It can be seen in Table 6.9 that for the plasma-treated surfaces, the oxygen and carbon content remain similar before and after the aging period. Therefore, it is obvious that aging under airtight conditions did not affect the hydrophilic stability of the treated surface. Overall, from the above-mentioned discussion it can be suggested that the hydrophilic stability of the plasma-treated PDMS surface can be maintained for a moderately long period (> 8 weeks) by storing the samples under airtight conditions.

The other interesting phenomenon is that the flux of lidocaine across any of the aged samples is significantly lower than that across the non-aged sample (Table 6.7), thus indicating that upon aging, even for only 1 week, the plasma-treated surface becomes more hindering to lidocaine permeation. Such an increase in hindrance might be because of the reorientation of the polar surface groups upon aging. However, the exact mechanism is yet

to be known, and is the focus of a current study. It can also be seen in Table 6.8 that the fluxes of lidocaine through the treated samples, aged for 1, 2, 4, and 8 weeks, are not significantly different from each other ($p > 0.05$). Therefore, it can be postulated that the above-mentioned surface reorientation occurs within 1 week of aging, and the reoriented surface remains unchanged for any further period of aging i.e. 2, 4 and 8 weeks. Thus, all of the aged samples offer a similar flux of lidocaine.

6.3 Conclusion

Hydrophilic PDMS membranes were created by air plasma treatment which resulted in the incorporation of oxygen containing polar functional groups at the membrane surface. The presence of these groups was confirmed by SEM-EDX analyses, which showed higher oxygen content in the treated membranes, compared with the untreated membrane. The hydrophilicity of the modified membrane was demonstrated by measuring water contact angle (WCA). The membrane integrity was examined using SEM images, which showed no physical damage of the treated membrane. The modified membranes appeared to reduce the permeation of the hydrophobic compounds, namely benzotriazole, benzocaine, ibuprofen and lidocaine, while not affecting the hydrophilic caffeine permeation. As the plasma treatment produced a hydrophilic surface, hydrophobic compounds would be reluctant to approach it. Moreover, it was found that the higher the log P of the compound, the greater the reduction in its permeation through the modified membrane.

With respect to stability studies, the combined results of lidocaine permeation through aged samples and EDX analysis revealed that the modified membranes retained their hydrophilic stability even after aging for 8 weeks under airtight conditions. The reasons for the hydrophilic stability were assumed to be – 1) the highly cross-linked nature of the membrane and 2) the prevention of organic contamination of the modified membrane surfaces by storing them under air tight conditions.

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Chapter 7: Conclusion and future work

Chemical based skin mimics, for example, silicone membranes, are widely used to simulate the *stratum corneum* of skin in *in vitro* drug diffusion studies. In particular, polydimethylsiloxane (PDMS) membrane, simply known as silicone membrane, is frequently employed as an *in vitro* skin mimic, to aid the prediction of percutaneous absorption, and to perform the qualitative assessment of compounds. A number of attempts have been made to investigate factors affecting drug permeation across PDMS membrane, including those reported in this thesis. This study, also, found plasma surface treatment to be an economic, convenient and stable method of modifying silicone membrane to make it, potentially, a more suitable *in vitro* skin mimic.

The objectives of this thesis, as mentioned in Chapter 1, were accomplished successfully, and are summarised below:

1. To study the effect of ionisation on the permeation of drugs through silicone membrane.

Chapter 3 investigated six model drugs, namely, 1-methyl-3-phenylpropylamine, benzoic acid, benzotriazole, ibuprofen, ketoprofen and lidocaine, to determine their permeation across PDMS membrane at three specific percentages of ionisation. Moreover, iGC SEA was employed to characterise the surface chemistry of the membrane. The data suggested that there was an overall linear relationship between the percentage unionised of the drugs and the amount permeated. In other words, permeation was preferable for the unionised forms for all drugs, though the membrane surface is basic in nature, as confirmed from the iGC SEA data, thus indicating that the overall hydrophobic nature of the membrane outweighs any possible surface effect. In summary, it can be said that formulation pH does play an important role in the permeation of drugs through silicone membrane.

2. To study the effect of surfactants on the permeation of drugs across silicone membrane.

Chapter 4 examined the permeation of four model drugs, namely, benzocaine, benzotriazole, ibuprofen and lidocaine, through PDMS membrane in the presence of four types of surfactants – anionic (SDS), cationic (CTAB), non-ionic (Brij 35 and Tween 80) and zwitterionic (CHAPS). All of the surfactants were found to reduce the permeation of the drugs through the membrane, though the extent of the reduction varied for different surfactants. The variations in reduction effects were assumed to result from the variability of the interactions of drug species with the surfactant head groups and/or micelles. Overall, there was an inverse relationship between surfactant concentration and the amount of drug permeated over a period of 6 h. For benzocaine, benzotriazole and ibuprofen, the highest permeation-hindering effect came from CTAB, whereas for lidocaine, that was from SDS. CHAPS appeared not to have a significant effect on drug permeation, except for ibuprofen.

3. To predict the mechanism of surfactant effect on drug permeation through silicone membrane.

Chapter 5 aimed at predicting the mechanism of surfactant effects on drug permeation through PDMS membrane. DSC, FTIR spectroscopy and SEM were used to analyse the surfactant-treated PDMS membrane, whereas NMR was used to study the behaviour of two surfactants, namely, SDS and Brij 35, in solutions with or without PDMS membrane being present. The data from DSC, FTIR and SEM suggested that the surfactants do not alter either the physicochemical properties or chemical structure or morphology of the membrane, and therefore, the mechanisms for permeation-reduction effects by the surfactants were not associated with these. Although NMR data did not show any significant change, based on the data from DSC, SEM, FTIR and permeation studies, two mechanisms can be suggested – 1)

the binding or entrapment of the drug molecules within the surfactant micelles, and 2) the repulsion and/or obstruction of the drug molecules by the surfactant-impregnated membrane surface. Both of these mechanisms, alone or in combination, could result in a decrease in drug permeation across PDMS membrane.

4. To investigate the effect of plasma surface modification of silicone membrane on the permeation of drugs.

In Chapter 6, PDMS membrane surfaces were modified by air plasma treatment, and the permeation studies of five model drugs, namely, benzocaine, benzotriazole, caffeine, ibuprofen and lidocaine, were conducted using the modified membranes. The plasma-induced hydrophilicities of the modified membranes were confirmed by the WCA measurements and SEM-EDX analysis. Moreover, SEM images of the modified membrane surfaces showed no apparent physical alteration following the plasma treatment. The permeation data revealed that plasma-induced hydrophilicity produced a significant reduction in the fluxes of the hydrophobic compounds (benzocaine, benzotriazole, ibuprofen and lidocaine), whereas the permeation of caffeine, which is hydrophilic, was unaffected. It was also observed that the higher the log P of the drug, the lower the flux through the plasma-treated membrane.

The stability of the plasma-modified PDMS membrane was analysed using SEM-EDX and *in vitro* permeation studies. The modified membranes were aged for 1, 2, 4 and 8 weeks under airtight conditions, and the permeation of lidocaine was performed using the aged membranes. The combined data from EDX analysis and permeation studies suggested that the hydrophilicities of the modified membranes lasted even after 8 weeks, which could be because of the highly cross-linked nature of PDMS membrane, and the absence of organic contamination of the membranes under airtight storage conditions.

Future work

This work could be expanded through several avenues, including:

a) Expansion of analytical techniques

The magnitude of the reduction in drug permeation were dissimilar for different surfactants. To explain these phenomena, it would be useful to investigate the interactions between the drugs and the surfactants by isothermal calorimetry (ITC). Moreover, ITC could provide a detailed mechanistic picture of the surfactant effects.

The NMR studies were conducted only for SDS and Brij 35. Further NMR studies involving CTAB, CHAPS and Tween 80 would be helpful to enhance the understanding of the surfactant effects on drug permeation through PDMS membrane.

The elemental composition of the PDMS surface was analysed by SEM-EDX method. A better accuracy of such analysis could be obtained by X-ray photoelectron spectroscopy (XPS).

b) Use of different plasma treatments and conditions

This study used air plasma to modify the PDMS surface to have silanol (Si-OH) groups, thus rendering it hydrophilic. There is scope for using additional plasmas such as nitrogen-based plasmas (N_2 , NH_3), which can incorporate amine functionalities on the PDMS surface, which can then be chemically reacted with suitable acyl chlorides to produce amide groups analogous to that present in the skin ceramides, thus broadening the possibility of using the membrane as a skin surrogate in *in vitro* drug diffusion studies.

The plasma-treated membranes were stored under airtight conditions for the stability studies. Several other storage conditions, for example, under water, phosphate buffers and liquid nitrogen, could be explored to determine their effect on stability. There is also need to

investigate different treatment times and powers for the purpose of optimising experimental conditions.

With regards to drug permeation through the modified membrane, more compounds and the calculation of more parameters, such as permeability coefficients (K_p), are needed to allow comparison of data with human or animal skin, or the *in vivo* situation. Such a comparison could be useful for the development of an effective *in vitro* skin mimic in the assessment of topical and transdermal products.

Appendix

Peer reviewed publications

1. Ionisation effects on the permeation of pharmaceutical compounds through silicone membrane,
Colloids and Surfaces B: Biointerfaces, Vol. 141, pp. 553–557, 2016.
2. The effect of surfactant charge on the permeation of pharmaceutical compounds through silicone membrane,
Colloids and Surfaces A: Physicochemical and Engineering Aspects, Vol. 516, pp. 121-128, 2017.

Oral presentations

1. Effects of surfactant on drug permeation through skin mimic systems,
20th International Symposium on Surfactants in Solution, Coimbra, Portugal, 2014.
2. Characterisation of silicone membrane using iGC SEA,
UKPharmSci, Nottingham, UK, 2015.

Poster presentations

1. Analysing the effects of surfactants on skin mimic systems,
UKPharmSci, Edinburgh, UK, 2013.
2. Application of the novel iGC-SEA to characterize drug permeation profile through skin mimic systems,
Inverse Gas Chromatography Symposium, Newark, USA, 2015.
3. Surface modification of a skin mimic by plasma treatment, and its implication,
UKPharmSci, Nottingham, UK, 2015.
4. Investigation of surfactant tail length effect on *in vitro* drug permeation,
UKPharmSci, Nottingham, UK, 2015.