DELIVERY OF AN ANTIMICROBIAL AGENT FROM TOPICAL DRUG DELIVERY SYSTEMS

PARVEEN MADHER

Master of Philosophy

ASTON UNIVERSITY

SEPTEMBER 2010

Aston University

DELIVERY OF AN ANTIMICROBIAL AGENT FROM TOPICAL DRUG DELIVERY SYSTEMS

PARVEEN MADHER

Master of Philosophy

2010

This research was focussed on improving and optimising the topical delivery of a specific antimicrobial from various drug delivery systems.

The drug of interest was triclosan. The main objective of this study was to deliver this drug to the lower layers of the skin over an extended period of time during catheter use. To attain continuous release of triclosan, topical drug-in-glue adhesives were formulated and tested. Permeation enhancers were incorporated into the adhesives to improve the permeation rates of triclosan across human epidermal skin. Permeation studies demonstrated that the inclusion of the enhancer oleic acid improved the permeability of triclosan over the other enhancers used. This finding was attributed to the mechanism of action of oleic acid, which is responsible for disrupting the lipid lamellae of the stratum corneum, thereby improving the triclosan permeation.

In order to improve the drug-in-adhesive formulation further, a novel patch system was developed, where instead of a backing liner, a silicone elastomer layer containing triclosan was utilised as part of the adhesive to generate further sustained release of triclosan. Investigations were undertaken to observe the release of triclosan from the silicone elastomer material which also contained different additives. It was found that linoleic acid was a suitable additive within the silicone matrix. Comparative studies were carried out to reveal which of the silicone/adhesive drug delivery systems provided the greatest permeation rate. The drug delivery system composed of linoleic acid in the silicone layer and oleic acid in the adhesive layer provided the greatest permeation rate of triclosan over 14 days compared with any other system tested within these studies, and reached concentrations higher than the necessary minimum inhibitory concentration and minimum bactericidal concentration values, suggesting this patch type system could potentially be used for disinfecting the skin during catheter use.

Key Words: Antimicrobial, Topical patch, Sustained release, Triclosan, Penetration enhancers, Silicone elastomer.

To my family, especially my brother

For all your love

Acknowledgements

I would like to thank my supervisor Professor Yvonne Perrie for her supervision, guidance and friendly support during the return to my studies. I am also grateful to Dr. Julian Smith my earlier supervisor.

I must also thank Aston University for their patience, understanding and confidence throughout my leave of absence and financial support whilst returning to my studies.

I must express my gratitude to Chris Bache and Jiteen Kansara for their excellent technical assistance.

A very special thank you to Habib Ali and Noosh Daneshpour for their support and friendship.

Thanks also to my friend Ruchi Khajuria for all her continual optimism, support and understanding.

List of Contents

List of Figures

List of Tables

List of Tables

List of Tables

List of Abbreviations

ANOVA	Analysis of variance
ACP	Enoyl-acyl carrier protein
aufs	Absorbance units full scale
Da	Daltons
DOC	Desoxycholate sodium
Ep	Elongation percentage
FAD	Flavin adenine dinucleotide
FCS	Fetal calf serum
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
L_b	Test length at break
L_o	Initial test length
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NaOH	Sodium hydroxide
NADH	Nicotinamide adenine dinucleotide dehydrogenase
PDMS	Polydimethylsiloxane

PSA	Pressure-sensitive adhesives
rpm	Revolutions per minute
SD	Standard deviation
t	Thickness of the test length
TC	Transcutol
TCS	Triclosan
THF	Tetrahydrofuran
Ts	Tensile strength
UV	Ultra-violet
W	Width of the narrow portion of the specimen

Chapter One:

General introduction

1.0 Introduction

1.1 Skin structure

The skin is the initial line of defence of an organism and the final barrier separating the organism from its hostile environment of toxic substances, viruses and pathogens. It is composed of a unique hierarchical structure of lipid rich matrix with embedded keratinocytes in the upper strata (15 μm) of skin, the stratum corneum is responsible for this barrier (Bouwstra, 1997). Thus, the skin acts as a physical and biological barrier. In addition to this function, it is also deemed a transport regulator allowing the flux of water molecules and a variety of small molecules, which are fairly lipophilic (log P > 1.5) and have a molecular weight less than 500 Da into and out of the organism (Bos et al., 2000). The natural selectivity of the skin membrane is therefore taken advantage of when designing a transdermal drug delivery system.

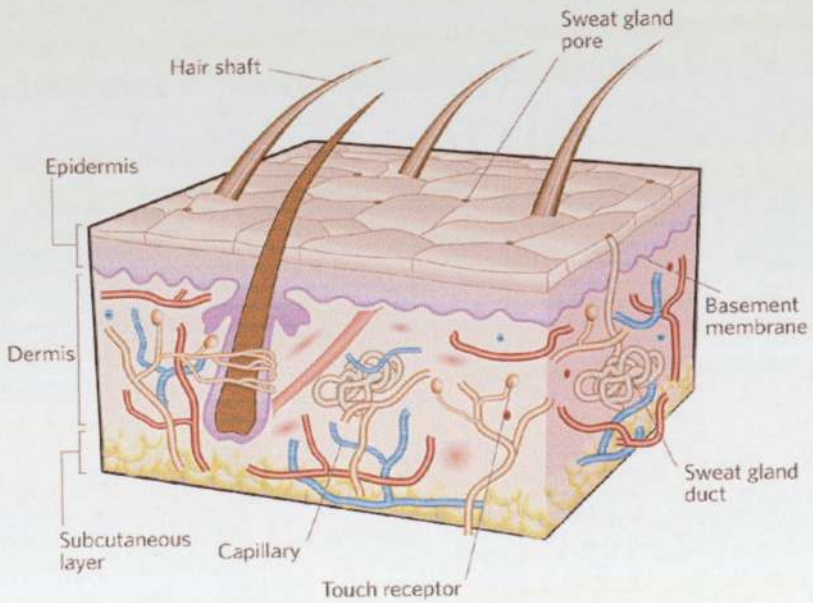


Figure 1.1 *The diagram of human skin shows the two main layers of the skin- the upper epidermal barrier layer and the lower dermis (taken from http://www.nature.com).*

1.2 Topical drug delivery

The composition of a dermatological base may exert a profound effect upon the release and the therapeutic activity of a topical product (Barry, 1987). Formulation has a major influence on bioavailability, and the physico-chemical properties of both the vehicle and drug are important in determining the rate of drug delivery. One of the most important functions of a vehicle is the control it exerts over the release and therefore the therapeutic activity of the medication which it carries. Although dermatological vehicles themselves may not penetrate the skin to any extent or actually carry the medicament through the epidermal barrier, the clinical effectiveness of a drug may vary when it is incorporated in different vehicles.

The physical and chemical properties of both the vehicle and of the drug are essential factors when considering drug release. Additionally, factors such as the condition of the skin, the site and duration of application also affect the percutaneous absorption. Percutaneous absorption refers to the penetration or movement of drug molecules into the skin from a cutaneous surface and to their fate after leaving the skin surface. It is generally recognised that the main barrier within the skin is located in the outer stratum corneum. This barrier is often the rate limiting factor, however, in some instances, the release of drug from the vehicle, rather than transport across the skin, may be rate-determining.

1.3 Strategic relevance of proposed work

Infections arising during surgical interventions have been a subject of debate for many years. The logical approach to the treatment of skin disease is the topical

application of medicaments. Current formulations are not able to deliver sufficient antiseptic to the reservoir sites of microbial colonisation in the skin. In particular, sepsis associated with intravascular catheters is a significant problem. Treatment of these infections is associated with high complication rates placing a burden on both the patient and healthcare resulting in prolonged hospital stay, increased morbidity and mortality, and serious economic sequelae being common consequences (Gollwitzer et al., 2003).

The situation is mainly due to the poor efficiency of current skin disinfection techniques which are able to kill surface-dwelling micro-organisms but do not target lower reservoir sites of bacteria. Surgical interventions which penetrate the epidermal and dermal layers risk mobilising these reservoirs of organisms, leading to the contamination of instruments and catheters. Consequently, this may lead to a potential source of infection.

In particular, the skin appears to be the main source of infection associated with catheters. The most frequent routes of infection are from the skin exit site, the tissue tunnel associated with the catheter and the catheter lumen (Figure 1.2). Exit site infection incidence has been cited as 0.11-0.14 episodes/patient/year (Thodis et al., 1998).There is also the issue associated with biofilm growth on the luminal surfaces of the catheter (Gorman et al., 1994). However, the importance of the migration of bacteria down the outer surface of the catheter is a significant problem (Read et al., 1989).

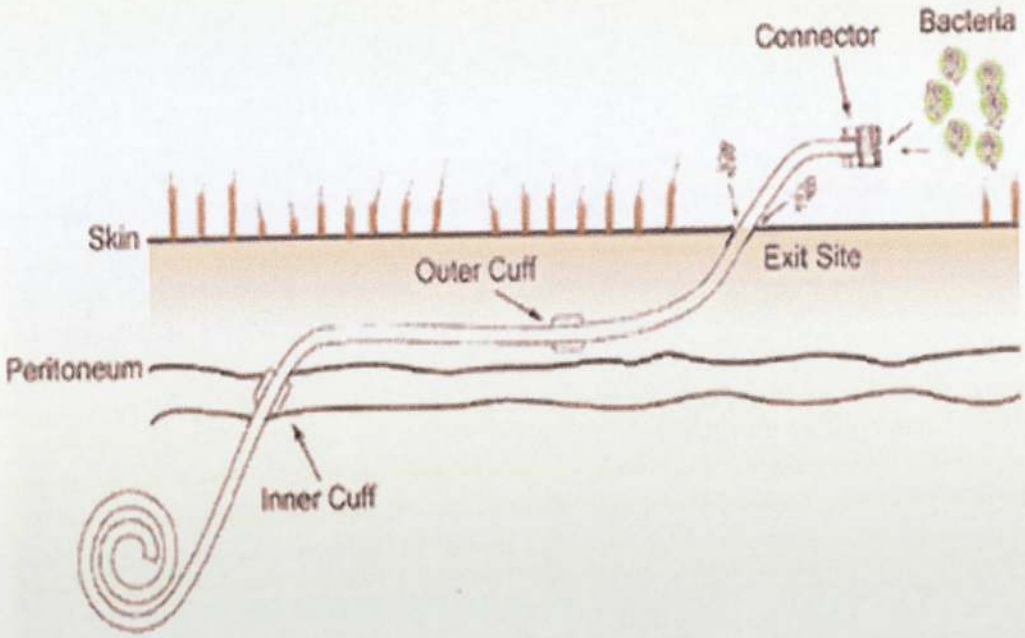


Figure 1.2 *Microbial routes of entry. Bacteria can gain access to the peritoneal cavity either by contaminating the connector and the catheter lumen, or by migration from the skin exit down the catheter track through the tissue (modified from Bayston et al., 2009).*

A study by Elliott and Moss (1997) found a close correlation between the culture results of insertion sites and catheters. It was demonstrated that similar micro-organisms associated with significant colonisation of catheters, were also present on the skin site. Additionally, a close relationship between heavy skin colonisation and central venous catheter-related infections and bacteraemia has also been reported (Elliott and Moss, 1997). This association may not only be due to organisms gaining access via the external route of the catheters, but also related to impaction at the time of insertion if this is the case then there is a clear need to develop techniques which will improve the effectiveness of skin disinfection procedures.

There are currently no effective procedures to overcome this problem. Potential microbial contamination of wounds from resident flora is compounded by the observation that despite surface disinfection with a range of topical medications, microbial repopulation occurs rapidly suggesting there is a reservoir of aerobic bacteria beneath the skin surface that is maintaining resident flora (Hendley and Ashe, 1991). Microbial resistance to topical disinfection may sometimes occur as significant proportions (20%) of bacteria are protected from follicles, crevices and lipids (Selwyn and Ellis, 1972).

The only reported treatment that largely prevented rapid regrowth was a triple antibiotic ointment, which not only eradicated organisms on the skin surface and in the stratum corneum, but prevented overnight repopulation of bacteria within the deeper reservoir. However, this formulation and vehicle was unsuitable for preoperative treatment (Hendley and Ashe, 1991). In addition, antibiotic-coated catheters have repeatedly been shown to decrease catheter related bloodstream infections compared to other catheters. The issue with use of antibiotics is microbial resistance, which is becoming a major problem and would be particularly inappropriate if a prolonged release of antibiotic was required. Although the post-antibiotic effects vary between antiseptics (Fuursted *et al.*, 1997), the main problem is due to poor access of disinfectants to the micro-organisms as opposed to use of ineffective antimicrobial agents. Additionally, the predicament could also be tackled by providing a continuous release of disinfectant.

1.3.1 Choice of antimicrobial agent

The physico-chemical properties of an agent may have a significant effect upon its activity profile. Certain parameters will control the release from the vehicles and target specific sites by interaction with cell surfaces. The route of penetration is mainly determined by:

- Solubility
- Lipophiliity
- Charge
- Molecular size

The partition coefficient also needs substantial consideration. In order to cross the stratum corneum, a permeant must first partition into the skin and this is often the rate-limiting step in the permeation process, dictating which pathway it will follow through the skin. It may be expected that a hydrophilic molecule will partition preferentially via a polar route through the hydrated keratin-filled keratinocytes rather than into the lipid bilayers, whereas lipophilic permeants will partition into the lipoidal areas such as the membrane. Consequently, hydrophilic molecules are expected to permeate via the intracellular route, whereas the intercellular route will dominate for lipophilic molecules. The vehicle used may be able to promote the transfollicular pathway, although it is the lipoidal area which is often the most difficult and crucial area to target.

Typical antiseptics available include:

1) Chlorhexidine dihydrochloride
2) Paraben esters
3) Triclosan
4) Mandelic and lactic acids
5) Mupirocin

1.3.1.1 Justification of choice of antimicrobial agent

Triclosan and Chlorhexidine are the main antiseptics of interest. Chlorhexidine is a highly active cationic compound which is available in a variety of salt forms with different water-solubilities. Chlorhexidine works by inhibiting membrane-bound ATP-ase thus causing physical injury to the membrane. It is an effective agent and one of the most frequently used skin disinfectants around central venous catheter insertion areas (Zing et al., 2008) (minimum inhibitory concentration (MIC) 5 mg/l, (Fuursted *et. al*, 1997)). However, it is absorbed poorly across whole skin (Lafforgue *et al.,* 1997). Triclosan has demonstrated immediate, persistent, broad-spectrum antimicrobial effectiveness in clinical health care settings (Jones et al., 2000).

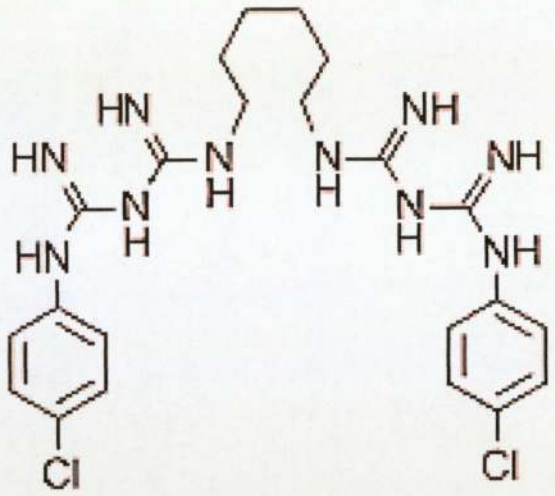


Figure 1.3 *Structure of chlorhexidine (free base structure) taken from www.the-medical-dictionary.com.*

1.4 Triclosan

1.4.1 Physico-chemical properties of triclosan

Triclosan (molecular weight = 289.5 gm/mol) is a tasteless and orally non-toxic bacteriostatic agent. The chemical formula is $C_{12}H_7CL_3O_2$. This organic chemical compound is a white powdered solid (at room temperature and pressure) with a melting point of about 55-57°C and a slight aromatic/phenolic odour. It is a chlorinated aromatic compound which has functional groups representative of both ethers and phenols. Phenols often show antibacterial properties. It is a slightly weak acid with a pKa of 7.9.

Triclosan has a high calculated log P of 4.76 or 4.89 (Moss *et al.*, 2000 and Chedzgoy *et al.*, 2002 respectively) making it a potential candidate for transdermal delivery.

1.4.2 Uses of triclosan

The phenylether or chlorinated bisphenol, triclosan (trade name Irgasan DP 300 or cloxifenol), is an antimicrobial agent that has been employed for a variety of purposes for more than 20 years.

Triclosan has been used in skincare products for some 30 years. It has also been employed as surgical scrubs, hand washes, body washes to control Methicillin-resistant *Staphylococcus aureus* and in dental hygiene products. It is currently used as an active ingredient in Colgate® toothpaste and Microban® plastic kitchen utensils. More recent uses include the suggestion that it could be used to control encrustation and blockage of Foley catheters caused by *Proteus mirabilis*, an organism that is highly susceptible to triclosan (Russell et al., 2004).

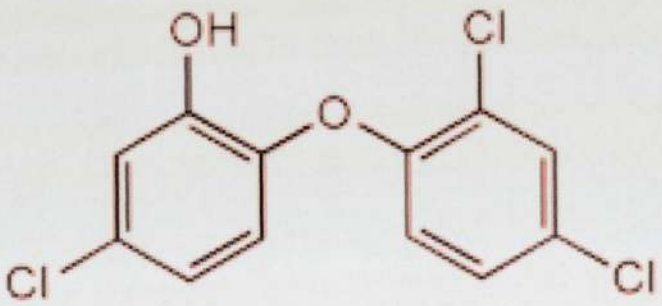


Figure 1.4 *Structure of triclosan (2, 4, 4'- trichloro-2'hydroxydiphenyl ether)(taken from www.sci-toys.com.)*

1.4.3 Triclosan as an antimicrobial

Triclosan is a broad spectrum antimicrobial compound that possesses a broad range of activity that encompasses many, but not all, types of Gram-positive and

Gram-negative non-sporulating bacteria, some fungi, *Plasmodium falciparum* and *Toxoplasma gondii* (Russell, 2004).

The simplest measure of antimicrobial activity is the Minimum Inhibitory Concentration (MIC). This is the lowest concentration required to inhibit the growth of micro-organisms. The MIC provides information about the bacteriostatic properties of a substance. Bacteriocidal effects occur at concentrations higher than the MIC. Triclosan's MIC against bacteria is very low (Chung et al., 2003). Its spectrum includes high activity against staphylococci, some streptococci, some mycobacteria, *Escherichia coli* and *Proteus* spp. Methicillin-resistant *Staphylcoccus aureus* (MRSA) strains may show an elevated triclosan MIC. Entercocci are much less susceptible than staphylococci, and *Pseudomonas aeruginosa* is highly resistant. Bacterial spores are unaffected. Triclosan is bacteriostatic at low concentrations but higher levels are bactericidal (Schweizer, 2001).

1.4.4 Mechanisms of action of triclosan

The mode of action of triclosan can be considered to be similar to other phenolic antimicrobial agents. In general, phenols target the bacterial cell wall resulting in lysis, inhibit active transport mechanisms in the cytoplasmic membrane and increase membrane permeability leading to leakage of the cytoplasmic constituents. At high concentrations, phenols cause coagulation of the cytoplasm (Hugo, 1992). The actions described will depend on various factors, including the concentration of the antimicrobial present as well as contact time with the bacterium. Due to the poor water solubility of triclosan, its bacteriostatic actions

are possibly restricted to cell wall lysis only, which occurs with low concentrations of disinfectants (Hugo, 1992).

Early studies suggest that the major target for triclosan was the cytoplasmic (inner) membrane (Russell, 2003). However, it was later demonstrated (Chedzgoy et al., 2002) in *Escherichia coli, Staphylcoccus aureus* and other triclosan-susceptible organisms that the growth-inhibitory activities of triclosan resulted from blocking the biosynthesis of Type II fatty acids by specifically inhibiting an NADH-dependent enoyl-acyl carrier protein (ACP) reductase, or FabI. Western immunoblotting demonstrated a strain of *Staphylcoccus aureus* overexpressing FabI, which gave rise to an increase in the MIC of triclosan, while susceptibilities to a range of unrelated antibacterials were unaffected. There are approximately 120,000 molecules of FabI per cell in mid-log phase growth. This number increased by approximately three to four fold in the *Staphylcoccus aureus* FabI overexpressor. Triclosan selectively inhibited the incorporation of [^{14}C] acetate into TCA-precipitable product, an indicator of fatty acid biosynthesis. Furthermore, it inhibited *de novo* fatty acid biosynthesis in this organism (Slater-Rodesti et al., 2001). The enoyl reductase, InhA, in *Mycobacterium smegmatis* was also found to be a target for triclosan action (Russell, 2004).

Studies (Hay *et al.,* 2001) involving the incorporation of radiolabelled substrates into fatty acids suggest that triclosan may reduce the oxidation of NADH and inhibit fatty acid synthesis by promoting the binding of NADP binding site of FabI. However, *Streptococcus pneumoniae*, which is also sensitive to triclosan,

does not contain a FabI homolog. Instead *Streptococcus pneumoniae* contains FabK, an isofunctional FAD-dependent enoyl-ACP reductase which is not sensitive to triclosan. This suggests that triclosan must have another target in *Streptococcus pneumoniae. Pseudomonoas aeruginosa*, which is highly resistant to triclosan, containing both FabI and FabK, in addition to a multidrug efflux pump which is able to transport triclosan out of the cell (Schweizer, 1998).

There still raises the question as to whether inhibition of a single enzyme by triclosan is responsible for its inhibitory and lethal actions. In practice, triclosan is normally utilised at higher concentrations than the above MIC against highly susceptible bacteria. Triclosan becomes rapidly bactericidal at these concentrations and this lethal activity extends to triclosan-resistant strains of *Escherichia Coli*. Triclosan-induced K+ leakage, indicative of membrane damage, takes place at bactericidal levels. A recent study (Russell, 2004) has also demonstrated membrane-destabilising effect. Triclosan shows a Z-pattern type of adsorption indicative of the breakdown of a structure, presumably the membrane, and the generation of new adsorbing sites.

Substantial evidence (Taylor et al., 2003) shows triclosan has multiple modes of action, including a non-specific mode involving gross membrane disruption, resulting in rapid cell death. The potency and speed of triclosan efficacy at high saturation states cannot be due to inhibition of enzymatic pathways, which would affect cellular metabolism, but not result in such catastrophic cell death in the time frames measured. As with other biocidal agents, triclosan possesses more than one type of action, and it is possible to delineate its growth-inhibitory and

lethal effects. It has recently been demonstrated that triclosan has anti-malarial and anti-inflammatory properties. Due to the presence of the FabI enzyme in the *Plasmodium parasite,* triclosan can inhibit the growth of *Plasmodium falciparum in vitro* (Chedzgoy et al., 2002; Loftsson et al., 2005 and Maestrelli et al., 2004).

1.4.5 Resistance Concerns

There is concern that the overuse of triclosan could cause resistant strains of bacteria to develop. Resistance may be due to overproduction of enoyl reductase, an insensitive form of enzyme or to changes in the cellular impermeability.

1.4.6 Side effects

Skin irritation was observed following administration of 3% triclosan in 3 % propylene glycol topically to rabbits for 13 weeks. Three cases of contact dermatitis have been reported following exposure to 2 % triclosan in petrolatum after use of a steroid /antibacterial treatment which contained 3% triclosan (Moss et al., 2000). 2% Triclosan appears to be the greatest concentration used within the clinical industry.

1.5 Co-solvents

Due to triclosan's low solubility a co-solvent system is generally used. Ethanol is commonly used in many transdermal formulations. It is also used as a co-solvent with water for ensuring sink conditions during *in vitro* permeation experiments. As with water, ethanol permeates rapidly through human skin with a steady state flux of approximately 1 $mg/cm^2/h$ (Berner et al., 1989). Such a rapid transport of the molecules through the membrane can lead to significant problems in

permeation study designs: If using an aqueous donor solution and an ethanol/water receptor phase, ethanol can permeate from the receptor to the donor phase and thus modify the formulation in terms of thermodynamic activity. In addition, as a solvent ethanol is capable of extracting lipids from stratum corneum membranes. Additionally, permeation of ethanol into the stratum corneum can alter the solubility properties of the tissue with a consequent improvement of drug partitioning into the membrane. Furthermore, it is feasible that the rapid permeation of ethanol or evaporative loss of the solvent from the donor phase modifies the thermodynamic activity of the drug in the formulation.

Ethanol can act as a permeation enhancer in a variety of ways. As a solvent it can increase the vehicular solubility of the drug, although at steady state the flux of a permeant from any saturated, non-enhancing vehicle should be equivalent. However, for poorly soluble permeants that are prone to deplete within the donor during a steady-state flux experiment, then ethanol can increase permeant solubility in the donor phase.

Fatty alcohols are usually applied to skin in a co-solvent (often propylene glycol) at between 1 and 10%. Propylene glycol permeates well through human stratum. Permeation of solvent through the tissue could alter the thermodynamic activity of the vehicle, which would in turn modify the driving force for diffusion. Solvent partitoning into the tissue could also change the solvent properties of the stratum corneum, thus facilitating uptake of the drug into skin. Studies have shown propylene glycol to be a very effective co-solvent for triclosan when used as an antimicrobial.

A recent study (Lee et al., 2003) has demonstrated the use of Transcutol (TC, diethylne glycol monoethyl ether) in hydrogel patches containing triclosan. TC is a potential transdermal permeation enhancer due to its non-toxicity, biocompatibility with skin and excellent solubilising properties.

1.6 Solubility and formulation approaches to maximise the effectiveness of triclosan

Triclosan is slightly soluble in water (0.6 mg/L at 25°C), but soluble in ethanol, diethyl ether, and stronger basic solutions such as 1M NaOH, like many other phenols. Very recent work involving cyclodextrin solubilisation of triclosan has shown it is possible to enhance the apparent intrinsic solubility of triclosan from about 1 ug/mL to about 7 mg/mL by fully ionising the compound in the aqueous complexation media by keeping the pH above 10 (Loftsson et al., 2005). At high pH, the increase in triclosan solubility is due to the hydrophilicity of the phenol anion. There has been very little literature exploring the triclosan efficacy. A great deal of previous literature has been limited to pH ranges that limit applicability.

A study by Kjarheim and co-workers (1994) found triclosan dissolved in alkali to have an insignificant antiplaque effect, whereas there was a marked effect with triclosan dissolved in propylene glycol. The hydrophilicity of the phenol anion had an unfavourable effect on the antibacterial activity. A recent study has also demonstrated the use of propylene glycol when producing supersaturated solutions of triclosan (Raghaven et al., 2003).

Solutions containing triclosan in the presence of surfactants have also

demonstrated reduced activity compared to the triclosan dissolved in propylene glycol. The triclosan molecules are trapped in surfactant micelles and less becomes available for an antibacterial effect (Kjarheim et al., 1994 and Taylor et al., 2004). Phenolic biocides are typically formulated with surfactants. Surfactants are excellent solubilisers for triclosan, however increasing the surfactant: TCS ratio causes a decrease in antibacterial efficacy. Such a high level of solubilisation implies that the biocide is highly partitioned to the micellar pseudophase, thus less triclosan is in the bioavailable state (Taylor et al., 2003).

A major factor of efficacy of a phenolic agent in surfactant solution was the % saturation of the phenolic in solution, and not simply its total concentration. The efficacy of an antimicrobial agent is related to the rate at which it has access to the biophase on the site at which it acts. The driving force that determines this rate of transport is the difference in chemical potential of the biocide between the site at which it acts and the external aqueous phase. Time dependence or kinetics is also an important aspect of antimicrobial activity. One factor influencing the kinetics of antimicrobial activity of triclosan is the saturation solubility of the biocide (Taylor et al., 2003).

Evidence has also demonstrated that the bacteriostatic efficacy of triclosan was significantly increased when solubilized with N-methylglucamine, L-arginine, and ethanolamine; increased solubilisation did not increase the effectiveness of triclosan for other solubilisers i.e sodium lauryl sulfate ,beta-cyclodextrin, sodium benzoate, sodium methyl 4-hydroxybenzoate, triethanolamine or diethanolamine (Grove et al., 2003). It therefore seems that the nature of the solvent is an

important consideration for the formulation of a suitable antimicrobial product, such that its clinical effect is retained.

1.7 Aims and objectives

There are currently numerous marketed products available for skin disinfection; however many of them have been shown to be ineffective often due to the insufficient delivery of antiseptic to the reservoir sites of microbial colonisation in the skin and the rapid, limited release of the antimicrobial drug. Therefore, the overall aim of this research is to formulate and test a drug delivery system which provides sustained release of an antimicrobial to the lower layers of the skin.

To achieve this, the objectives were:

- To establish a suitable method to accurately determine the rate of permeation of the model drug.
- To develop and prepare a drug in- adhesive patch system.
- To formulate and optimise the drug in- adhesive patch system.
- To characterise the release and permeation of the selected antimicrobial from these drug delivery systems.

Chapter Two:

Materials and methods

2.0 Materials and method

2.1 Materials

The materials used have been outlined in the table below.

Chemicals	Supplier
Acetic acid	Fisher Scientific, Loughborough, UK.
Buffer salts	Sigma, Dorset, UK.
DuroTak® 2677, 900a, 9301, 2852 and 2979 adhesives	Adhesives National Starch and Chemical Company, UK
Ethanol	Fisher Scientific, Loughborough, UK.
Excised human skin	Burns and Plastic Surgery Unit, Selly Oak Hospital
HPLC grade acetonitrile	Fisher Scientific, Loughborough, UK.
HPLC grade ethanol	Fisher Scientific, Loughborough, UK.
Hydrochloric acid	Fisher Scientific, Loughborough, UK.
Linoleic acid	Sigma, Dorset, UK.
Oleic acid	Fisher Scientific, Loughborough, UK.
Scotchpak PET film	3M, Loughborough, UK.
Silastic membrane	Advanced Bio-technologies, Inc, USA.
Sodium Hydroxide	Sigma, Dorset, UK.
Transcutol	Gattefosse, France.
Triclosan	Gift from Medicare ltd, Cannock, Ltd.
Virkon	Fisher Scientific, Loughborough, UK.
3.0 MIL Low adhesion release liner	3M, Loughborough, UK.

Table 2.1 *Materials and suppliers*

2.2 Method

This section outlines the method in detail. Quantitative assay of triclosan was achieved by high performance liquid chromatography (HPLC). Ultraviolet (UV) spectroscopy was used for the determination of a suitable wavelength for UV detection during HPLC analysis and validation of HPLC results.

2.2.1 High-Performance Liquid Chromatography

The predominant method of analysis used within this study was high-performance liquid chromatography (HPLC). The HPLC instrumentation used throughout this study was the Dionex HPLC (AS50 Autosampler, GP50 Gradient pump and UVD170U UV detector). The UV detector was set at 245 nm and the pump at 1.00 mL/min. The stationary phase was a Gemini reverse phase C18 5µm packed column (150 mm x 4.6 mm). The mobile phase was degassed immediately before use and the stationary phase was maintained at room temperature. The HPLC conditions used are summarized in Table 2.2.

The concentration of triclosan analysed was from10 ug/mL to50 ug/mL and was diluted with 40:60 ethanol: phosphate buffered saline solution (PBS) as this was the receiver composition. Concentrations were calculated from the area under the curve which was used to prepare a calibration curve (see section 3.1.7).

HPLC Conditions	
Mobile phase	**(65%)** Acetonitrile: Acetic acid (98:2), **(35%)** Water: Acetic acid (98:2).
Flow rate	1.0 mL/min
Column	C18 5µm packed column (150 mm x 4.6 mm)
Wavelength	245 nm
Sensitivity	0.005 AUFS
Injection Solvent	50:50 methanol :water mix
Injection Volume	200 µl (fixed loop)
Retention time	6.8 minutes

Table 2.2 *Finalised HPLC conditions and parameters for analysis of triclosan.*

2.2.2 Ultra-violet spectroscopy

The drug was also analysed by UV spectroscopy (Unicam Helios). The reference cell was filled with 40:60 ethanol: PBS (v/v) and set as background. A set of standard calibrated solutions of triclosan in ethanol: PBS ranging from 10 µg/mL to 50 µg/mL was prepared. Quantitative measurements were carried out by evaluating the optical densities at a specific wavelength. A suitable wavelength was determined by a wave-scan for optimum absorption peaks, 245nm was selected as an appropriate wavelength for analysis.

2.2.3 Donor Solutions and solubility determination

2.2.3.1 Donor solutions

Triclosan solutions were prepared with varying concentrations (v/v) of ethanol in water. Solutions of triclosan were prepared with the 70%, 40% and 50% concentration (v/v) of ethanol in water. The concentration of the donor sample was assayed (with appropriate dilutions). HPLC was used to quantify the concentrations. Saturated solutions were prepared by dissolving an excess of triclosan in the appropriate solvent in a water bath at 37 °C. The suspension was left for 24 hours to equilibrate. Various solvents were used.

2.2.3.2 Solubility

Saturated suspensions were prepared as described in 2.2.3.1. The saturated suspension was assayed by filtering an aliquot of supernatant through pre-saturated Whatman number 1 filter into a testube previously warmed to 37 °C. 1mL of filtrate was appropriately diluted in a volumetric flask using the correct solvent before analysis by HPLC.

2.2.4 Permeation procedure

2.2.4.1 Preparation of membranes

The skin samples were stored at -70 °C and thawed at room temperature. The subcutaneous fat was removed by blunt dissection using surgical scissors and tweezers. The skin was cut into approximately 4.0 cm^2 pieces and immersed in de-ionised water at 60 °C for 60 seconds. The epidermis was then carefully peeled away from the dermis. With the stratum corneum uppermost, the epidermis was floated onto aluminium foil and stored in a creasefree state at -20°C until required. With silicone, cuprophane and dialysis membrane, a piece of approximately 4.0cm^2 was cut for use. All membranes were refloated on de-ionised water. Excess water was removed from the surface of the membranes by gently dabbing with lint-free tissue paper.

2.2.4.2 Determination of model drug in Franz cell studies

The Hanson vertical diffusion cell system was used for permeation experiments. Experiments were mainly performed using either human skin or silastic membrane. Precision glass cell assemblies include receptor chamber, donor chamber, sampling and media replace ports, outside jacket for temperature control, and Helix™ stirrer.

The experimental procedure was essentially the same for all membrane types. 40:60 ethanol: PBS (v/v) was used as the receiver solvent which was maintained at 32 °C by the heater circulating bath (this also maintained controlled temperature in the media replacement beaker). The receptor solution was allowed to equilibrate overnight with the membrane in the diffusion cells. The solution was replaced approximately 16 hours later. However, prior to filling the receiver chamber, the receiver solvent was initially boiled and then sonicated. This procedure was essential to eliminate air bubbles. The Helix™ stirrer is initially set to 2200 *rpm* for 60 seconds to remove air bubbles and is then set to 550 *rpm* for the remaining experiment. The washed membrane is carefully mounted onto the donor cell with a small amount of silicone. The donor chamber is then secured onto the receptor compartment and held together with a clamp. 2 mL of the test sample when using donor solutions were added to the donor compartment and a lid was placed on the donor compartment to prevent evaporation of the solutions.

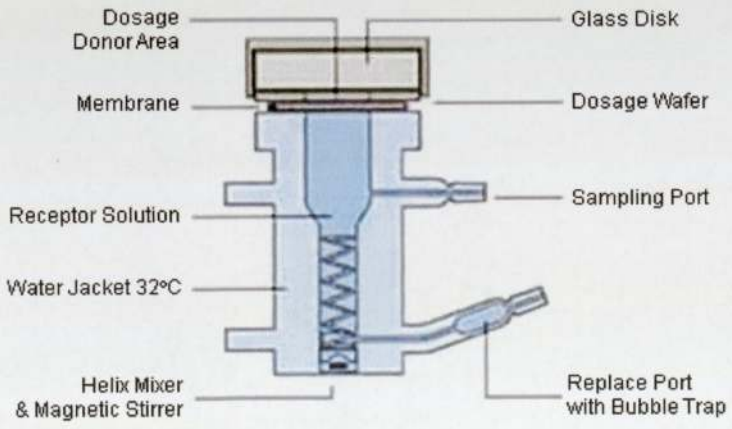


Figure 2.1 *Hanson vertical diffusion cell (modified from www.instrument.com.cn).*

At set time points (usually every 15 minutes up to an hour and then every half hour up to 5 hours in total for most experiments), 0.7 mL samples were slowly injected into the cell to prevent build up of air bubbles underneath the membrane. Simultaneously, a sample was collected from the receiver compartment and either placed in a HPLC vial to be analysed or placed in a quartz cuvette. The peak area or absorbance was then determined. Following UV assay, if the absorbance was greater than one, the receiver sample would be diluted appropriately.

This procedure produced a dilution of the receiver phase. It was necessary, therefore, to adjust each successive sample concentration for the dilution produced by all previous samples. This was performed by applying the following correction factor (Baker et al., 1990).

$$M_t[n] = V_r \cdot C[n] + V_s \cdot \sum_{m=1}^{n-1} C[m]$$

$M_t[n]$ - Current cumulative mass transported across the membrane at time t.

$C[n]$ - Current concentration in the receptor medium.

$\sum \{C[m]\}$ - Summed total of previous measured concentrations $\{(m = 1)$ to $(n - 1)\}$.

V_r - Volume of the receiver medium.

V_s - Volume of sample removed for analysis.

The available area for diffusion between the compartments was measured for each of the Franz cells in order for the flux per area to be determined. Six franz cells were set up simultaneously.

2.2.4.3 Determination of permeation results

The amount of drug penetration through the membrane barrier per unit area was calculated by taking into consideration the volume of the receptor solution and the area of membrane available for diffusion. The cumulative amount permeated per unit area was plotted against time and the linear portion of the graph represented the steady-state flux. The % of triclosan dosage permeated was calculated by dividing the initial dosage of triclosan placed in the donor by the amount of triclosan permeated within the receiver, this is then multiplied by 100 to provide a percentage format.

2.2.5 Adhesive studies

2.2.5.1 Formulation of triclosan adhesives

It was found that approximately 1 g of triclosan and 5 mL of adhesive mixture was required to produce a patch thickness of approximately 1 mm following evaporation of the solvent. Preliminary experiments performed to estimate the maximum loading dose of triclosan in the patches showed that adding in excess of 2 g to 5 mL of the adhesives produced a matrix which was excessively tacky and difficult to spread. 1g of triclosan dissolved in 5 mL of adhesive mixture gave the optimum consistency for

the patches. Various enhancers were then added (0.5 mL) and the mixture was stirred rapidly and spread onto a backing liner, the total area of the patches being 40 cm^2. The patches were covered and left for 72 h to allow the solvent to evaporate. Once the patches had dried, a release liner was applied. Patches were prepared at a loading dose of approximately 0.08 g per 3.141 cm^2.

2.2.5.2 Release of triclosan from the patches

Patches were prepared as above. Patches were removed from the release liner and inserted into dissolution baskets. Hanson dissolution apparatus were used. The dissolution baskets were placed into individual beakers containing 200 mL ethanol: PBS 40:60 (V/V), filtered and degassed prior to use. The apparatus was set to 100 rpm at 32.5°C. At appropriate time intervals 1 mL of dissolution medium was sampled and 1 mL was then replaced with fresh receptor medium. Samples were analysed by HPLC and six replicates for each adhesive were used in this experiment. The cumulative mass of triclosan released was determined using the equation in section 2.2.4.2.

2.2.5.3 Permeation of triclosan across human epidermal skin from model patches

In vitro permeation studies were conducted as described in section 2.2.4.2. Patches with 2 cm diameter were cut out using a cork borer and firmly affixed to the centre of the skin specimen.

2.2.6 Silicone formulations

2.2.6.1 Preparation and manufacture of silicone elastomer strips

The silicone elastomer is polydimethylsiloxane based (MED-6382) and is supplied as a two component kit (Part A and B). Part A is the base and part B the curing agent. The base consists of a mixture of high and low molecular weight hydroxy-terminated silicones (M_n = 10 000 and 2000, respectively) and 25% (w/w) of the micronized reinforcing filler diatomaceous earth. The elastomer mix was prepared by thoroughly mixing 100 parts by weight of the base with 0.5 parts of the curing agent. The required amount of triclosan was added and mixed together using a stirring rod for five minutes before sandwiching between two smooth glass plates (30 x 30 cm) separated by 1 mm thick spacers. The triclosan-loaded silicone elastomer mixes were cured within 24 h of preparation to produce silicone elastomer strips. The elastomeric silicone sheets produced were cut into strips of dimensions 10 x 30 mm using a scalpel. Preliminary experiments were carried out to determine a suitable quantity of further additives for some of the silicone formulations. It was found that 0.1mL was a suitable quantity for the additives PEG 200, linoleic acid and oleic acid within the silicone strips, as with these quantities the surface of the strip remained smooth, non-tacky and hydrophobic.

2.2.6.2 Release of triclosan from the silicone elastomer strips

Each of the silicone strips measuring 10 x 30 mm were placed in a stoppered 50 mL vial containing 10 mL of release media, and the vials were placed in a shaking orbital incubator maintained at 37 °C and set to 80 shakes per minute. The release medium was sampled and replenished with fresh release media every 24 h (± 0.5 h) over a period of 30 days and the amount of triclosan quantified by HPLC methods (section 2.2.1). The vials were returned to the incubator within 15 minutes. The release mediums were selected to maintain sink conditions over a 24-h release period. Release media for specific studies varied, however the general release media for most studies were composed of ethanol: PBS 40:60 (V/V), filtered and degassed prior to use.

2.2.6.3 Preparation and manufacture of silicone elastomer/adhesive systems

The silicone elastomer component was prepared as described in section 2.2.6.1, with or without additives depending on the formulation requirement. DuroTak® adhesive glues were prepared as described in section 2.2.5.1. Using the previous quantities of silicone elastomer (5 g), a sheeting area of approximately 10.5cm² was generated. Preliminary experiments were performed in order to form a layer of adhesive of approximately 1mm thick on the specific silicone sheeting area. 3 mL of DuroTak® adhesive was required to produce the appropriate thickness following evaporation of the solvent. Depending on the type of formulation required, 0.3 mL of oleic acid was added to the DuroTak® glue as an enhancer. The adhesive was covered and left for

72 h to allow the solvent to evaporate. Once the adhesive had dried, a release liner was applied. The DuroTak® adhesive component of the drug delivery system was prepared at a loading dose of approximately 0.08 g per 3.141 cm^2.

2.2.6.4 Permeation of triclosan across silastic membrane from silicone elastomer/adhesive systems

In vitro permeation studies were conducted as described in section 2.4.1. Patches with 2 cm diameter were cut out using a cork borer, release liners were removed and firmly affixed to the centre of the silastic membrane.

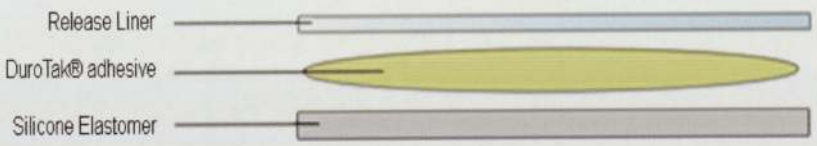


Figure 2.2 *Cross section of silicone/adhesive polymer system.*

2.2.6.5 Tensile properties

The tensile measurements were made using a Hounsefield tensile testing machine, with a separation of 20 mm between them. The depth of insertion was 20 mm, ensuring that the specimen is adequately gripped.

Prior to testing, the thickness of each specimen was measured at the centre and at each end of the test specimen, and the median thickness was used for the cross-sectional area calculations. Tensile strength (Ts) and elongation percentage (EP) were calculated automatically by the software using Equations (1) and (2), respectively. Six specimens of each formulation were tested.

(1) $$T_s = \frac{F_b}{W_t}$$

(2) $$EP = \frac{L_b - L_o}{L_o} \times 100\%$$

F_b - Force recorded at break (N)

W - width of the narrow portion of the specimen (mm)

t- thickness of the test length (mm)

L_o - initial test length (mm)

L_b - test length at break (mm)

2.2.6.6 Swelling studies

Swelling specimens were cut from the prepared sheets. Size changes in length and width of the strips were measured by using an electronic calliper; the average area of the silicone strip was reported.

2.2.6.7 Metallurgic microscopy

Silicone strips were examined at room temperature under a metallurgic microscope (Reichert – Jung Polyvar) fitted with an image caption JVC 5 megapixels camera at various magnifications, so as to examine the surface of the silicone strip and understand the nature of the elastomer structure. The different magnifications used were X 50, X 200 and X 500.

2.2.7 Statistical analysis

The treatment of the experimental results was based on the analysis average and the Analysis of variance (ANOVA). Thereafter, Bonferroni's multiple *t*-test was used to compare the difference between the various release and permeation profiles, tensile strength, elongation %, increase in swelling %. Differences were considered significant when $P < 0.05$. Each value was obtained from at least triplicate samples and expressed as mean ± S.D.

Chapter Three:

Delivery of an antimicrobial agent from topical drug delivery systems: preliminary investigations

3.0 Introduction

There are currently many effective antimicrobial agents; however one of the major drawbacks of current skin disinfectants is the inability of the drug to diffuse through to the lower (layers) of the skin over a sustained period of time. It is believed that there is a reservoir of aerobic bacteria beneath the skin surface which is maintaining resident flora (Hendley and Ashe, 1991). To target these reservoir sites it is necessary to ensure that the drug of interest can penetrate the lipid barriers under which the microorganisms are situated. Lipophilic molecules should be able to penetrate the lipid layers by diffusion and triclosan is an ideal candidate for investigation as it demonstrates a very low minimum inhibitory concentration (MIC) and it holds a high lipophilicity with a log P of 4.89 (Table 3.1).

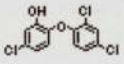
Property	Value
Molecular weight	289.5 Daltons
Chemical formula	$C_{12}H_7CL_3O_2$
Structure	
Physical state	Crystalline, white to off-white powder.
Melting point	55-57°C
pKa	8.14 at 20°C
Log P(octanol/water)	4.89
Water solubility	0.6 mg/mL at 25°C
MIC (ug/mL) for:	
Staphylococcus Aureus	0.1
Escherichia Coli	5

Table 3.1 *A table to show the properties of triclosan.*

In order to set up the protocols as described in chapter two, a number of experiments were carried out to determine appropriate experimental procedures. The objectives of the work outlined within this chapter were to devise and validate an appropriate *in vitro* method to measure the permeation of triclosan across a membrane. Consequently, a suitable method would be established which would serve as a model for further investigations. The intentions of the initial series of investigations were therefore to:

1) Investigate and optimise a suitable method to accurately quantify triclosan permeation.
2) Establish the use of diffusion cells as an appropriate method to determine the rate of permeation of triclosan across a model membrane.
3) Evaluate the various types of model membranes across a diffusion cell and their suitability when measuring the rate of permeation of triclosan.
4) Assess the solubility of triclosan in different vehicles.

3.1. HPLC method development

The use of high performance liquid chromoatography (HPLC) provides a rapid, specific, and sensitive assay procedure suitable for quantitative analysis. The ability of this method to efficiently separate and detect a wide range of molecules of various molecular weights, polarities and chemical properties renders it a versatile analytical technique widely applied in pharmaceutical analysis (Ahuja, 2003). In addition, this

technique is particularly suitable for the detection of triclosan due to the accuracy demonstrated when using low levels of permeant which are often apparent in topical drug studies.

3.1.1 HPLC mobile phase development

In reversed-phase HPLC, the mobile phase generally consists of water and a less polar organic solvent such as methanol or acetonitrile. Separations in these systems are due to different degrees of hydrophobicity of the solutes, the less polar solutes associating to a greater extent with the non-polar stationary phase and consequently being retained on the column longer than the more polar solutes. The rate of elution of the components is controlled by the proportion of the organic modifier in the mobile phase.

By manipulation of various parameters within an HPLC system, it is possible to achieve optimal detection and resolution of mixtures within a solution. The parameters that have the most significant effect upon analytical chromatography are the stationary phase, the selection of mobile phase and the wavelength of detection.

The HPLC instrumentation used throughout this study was the Dionex HPLC (AS50 Autosampler, GP50 Gradient pump and UVD170U UVdetector). The UV detector was set at 245 nm and the pump at 1.00 mL/min. The stationary phase was a Gemini reverse phase C18 5µm packed column (150 mm x 4.6 mm). A gemini column has

been designed to give good performance across compounds varying in the pH range from 2-11. This particular column was also selected due to its stability and increased column lifetime.

Table 3.2 below summarises the HPLC conditions found in various literature when assaying triclosan. This was used as a guide to select an appropriate mobile phase which would generate an optimum peak resolution whilst retaining a relatively short analysis time. The mobile phase conditions described by Moss et al., (1999) were immediately eliminated due to the retention time being too long. Additionally, this particular mobile phase was used to detect breakdown products of triclosan as it was being metabolized within the skin. HPLC conditions in the method of Lee et al., (2003), Chung et al., (2003) and Loftsson et al., (2005) consisted of water and the organic solvent as acetonitrile, in varying ratios.

Acetonitrile has a very good UV transparency and contributes rather little to the eluent viscosity and, thus, to the back pressure of the column. However, the HPLC conditions in the method of Chedzgoy et al.,(2002) consists of these solvents and acetic acid. Mobile phase pH should be selected so that it is at least ±1.5 pH units from the analytes pKa. This assures that the analytes are either 100% ionised or 100% non-ionised and should help run-run reproducibility. Organic acids are typically separated under ion suppression conditions where the pH is adjusted to 2 or 3.

HPLC conditions	Literature review of HPLC assays for triclosan
Mobile phase- (25%) water:acetic acid (98:2) (75%) acetrontrile:acetic acid (98:2) **Flow rate-**1.0 mL/min **Column-**Sphericlone 3µm ODS column (100 x 3.2 mm) **Wavelength-**281 nm **Retention time** -7.5 minutes	(Chedgzoy et al., 2002) Triclosan: release from transdermal adhesive formulations and *in vitro* permeation across human epidermal membranes.
Mobile phase- (75%) Acetonitrile (25%) Water **Flow rate-** 1.0 ul/min **Column-** Microsorb MV column **Wavelength-** 280 nm **Retention time-** not included	(Lee et al., 2003) Hydrogel patches containing Triclosan patches for acne treatment.
Mobile phase- (60%) methanol (40%) water with 0.002 M tetrabutylammonium acetate, adjusted to pH 3.0 with glacial acetic acid **Flow rate-** 1.5 mL/min **Column-** Spherisorb C18 column (150mm x 4.6mm) **Wavelength** – 240 nm **Retention time-** 14 minutes	(Moss et al., 1999) Percutaneous penetration and dermal metabolism of triclosan
Mobile phase- (60%) acetonitrile (40%) water **Flow rate-** 1.0 mL/min **Column-** Superisorb C18 column 3µm (100mm x 4.6mm) **Wavelength-** 254 nm **Retention time-** 8 minutes	(Chung et al. 2003) Evaluation of a polymer coating triclosan as the antimicrobial layer for packaging materials.
Mobile phase- (74%) acetonitrile (26%) water **Flow rate-** 1.5 mL/min **Column-** Phenomex ODS 5µm (150 mm x 4.6 mm) **Wavelength-** 283 nm **Retention time-** 2.9 minutes	(Loftsson et al., 2005) Cyclodextrin solubilisation of the antimicrobial agents triclosan and triclocarban: Effect of ionisation and polymers
Mobile phase – (80%) methanol (20%) water **Flow rate** – 1.0 mL/min **Column-** Not mentioned **Wavelength-** 245 nm **Retention time** – 6.8 minutes	(Chauan, 1995) Cyclodextrin complexes of antimicrobial agents

Table 3.2 *Table to show different HPLC conditions used to quantify triclosan.*

However, low pH decreases the solubility of organic acids in water and requires the use of a higher organic percentage in the mobile phase for practical elution under reverse phase conditions. The protocol outlined by Chauan et al., (1995) will also be considered.

3.1.2 Optimising the method of Chauan et al., (1995)

Initially a mobile phase made up of 50:50 methanol: water (v/v) was used to generate the following chromatogram:

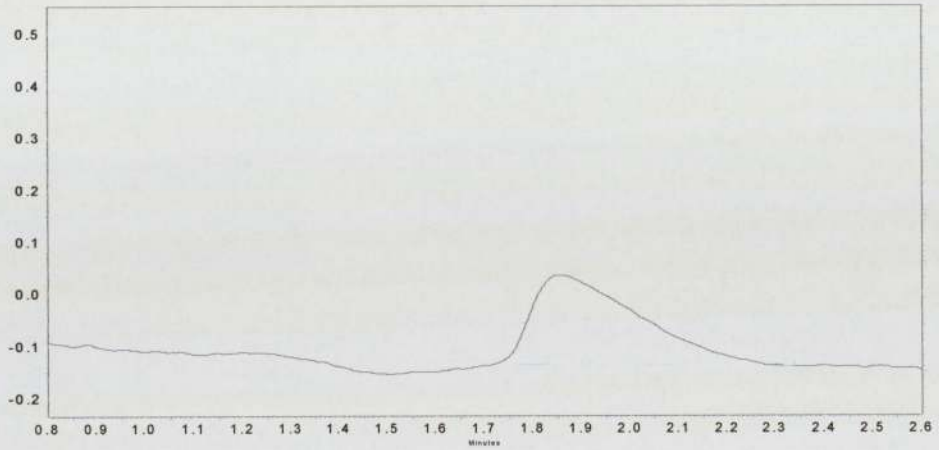


Figure 3.1. *HPLC chromatogram to show the triclosan peak generated when using 50:50 (v/v) methanol: water mobile phase.*

Figure 3.1 demonstrates a poor shape showing a broad tailing peak. This is probably due to the ratio of organic component in the mobile phase. Compounds stick to reverse phase HPLC columns in high aqueous mobile phase and are eluted from reverse phase HPLC columns with high organic mobile phase. Organic solvents are used to increase the eluting strength by decreasing the polar properties of the eluent.

In reverse phase chromatography HPLC, compounds are separated based on their hydrophobic character. Triclosan is very weakly hydrophilic, thus a higher percentage of an organic component would be required within the mobile phase.

By systemic and quantitative changes to the initial method it was found that a mobile phase in the ratio of 80:20 methanol: water (v/v) gave a relatively short analysis time, this was also demonstrated by Chauan et al., (1995). However, peak tailing was observed (Figure 3.2). 1% THF was added to the mobile phase to eliminate the tailing (Figure 3.3). Following this, some peak tailing still remained. All mobile phases were degassed by vacuum and filtration to eliminate dissolved gases. Dissolved gases may cause gassing out into the detector cell and also baseline drifts. Detection of the elutes was monitored at 245 nm. A sensitivity of 0.005 AUFS, the maximum possible with the instrumentation used.

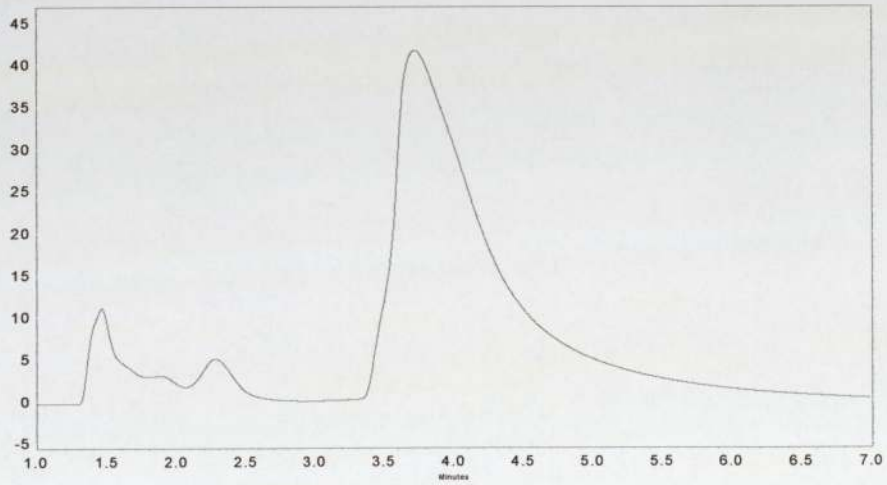


Figure 3.2 *HPLC chromatogram to show the triclosan peak generated when using 80:20 (v/v) methanol: water mobile phase.*

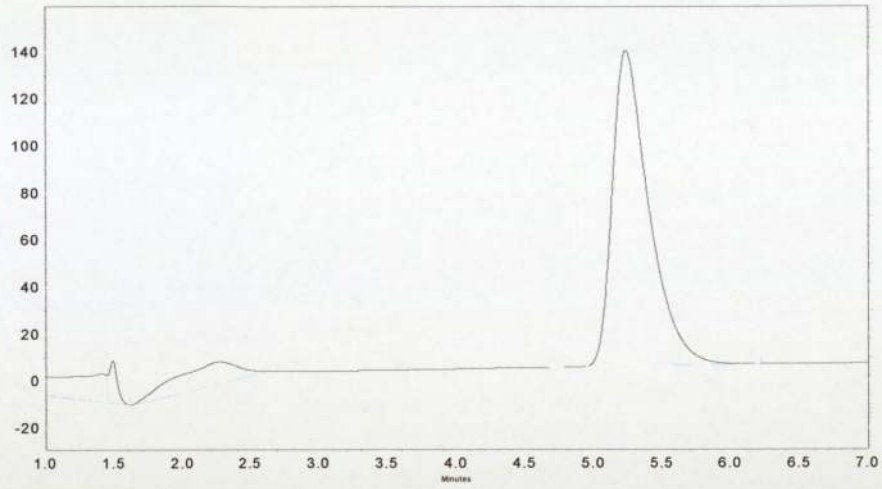


Figure 3.3 *HPLC chromatogram to show the triclosan peak generated when using 80:20 (v/v) methanol: water mobile phase with 1% THF.*

3.1.3 Optimising the final protocol using the method of Chedgzoy et al., (2002)

Changes were made to the original method and have been summarised in Table 3.3. These changes were necessary due to the differences in columns used and to shorten the original retention time from 7.5 minutes to 6.8 minutes. This method provided optimum peak resolution (Figure 3.5).

Mobile phase	**(65%)** Acetonitrile:Acetic acid (98:2), **(35%)** Water:Acetic acid (98:2).
Flow rate	1.0 mL/min
Column	C18 5µm packed column (150 mm x 4.6 mm)
Wavelength	245 nm
Sensitivity	0.005 AUFS
Injection Solvent	50:50 methanol:water mix
Injection Volume	200 ul

Table 3.3 *Finalised HPLC conditions for the quantitative assay of triclosan.*

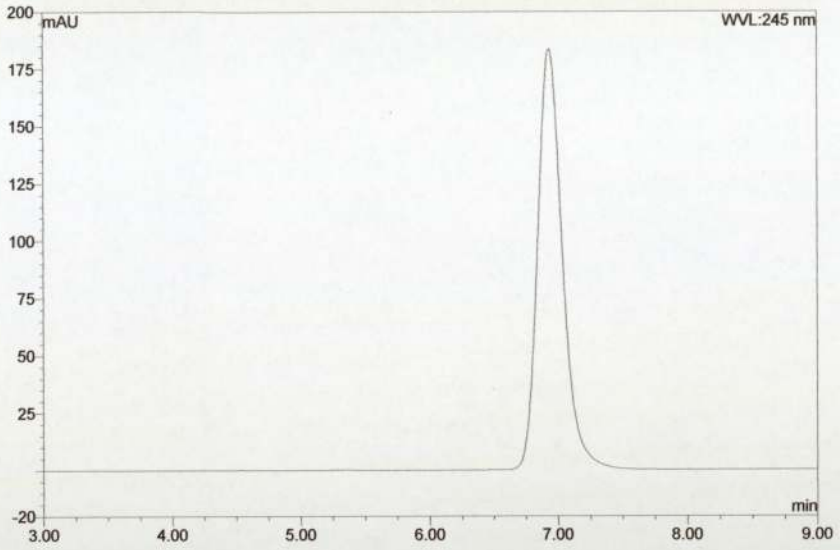


Figure 3.4 *Typical triclosan HPLC chromatogram (1% triclosan). Retention time 6.82 minutes. Symmetry factor 1.03.*

3.1.4 HPLC parameters

To measure HPLC optimisation, there are several mathematical parameters that can define chromatographic performance.

Parameter	Description	Acceptable range	Calculated value
K'	Capacity factor	1-10	4.6
N	Column efficiency	2500-10000	7541
As	Symmetry factor	0.8- 1.5	1.02

Table 3.4 *Efficiency and characteristics of HPLC method* (Kirkland, 1971).

The above table (Table 3.4) shows a typical HPLC trace obtained under the parameters listed. The acceptable range for the symmetry factor was obtained from

The British Pharmacopoeia volume II. The capacity factor K' (also known as the mass distribution ratio) represents the number of column volumes required to elute a given solute. The number of theoretical plates N, corresponds to a measure of column efficiency of a particular analyte. The symmetry factor is often called the tailing factor. A value of 1.0 signifies ideal symmetry. HPLC parameter values for a typical HPLC trace obtained with triclosan and the final proposed method have been calculated and they all lie within the acceptable range as shown in Table 3.4.

3.1.5 HPLC method validation

Assay procedures are intended to measure the analyte present in a given sample. The objective of the analytical procedure should be clearly understood, since this will govern the validation characteristics which need to be evaluated. The validation characteristics of interest here are shown in Table 3.5.

Validation characteristic	Notes
Accuracy	Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.
Precision	Expresses the closeness of agreement between a series of measurements. Obtained from multiple sampling of the same homogenous sample under the prescribed conditions.
Precision- Repeatability (Intra-assay precision)	Expresses the precision under the same operating conditions over a short interval of time.
Intermediate precision	Expresses within-laboratory variations: different days, different analysts, different equipment, etc
Reproducibility	Expresses the precision between laboratories.
Linearity	The ability within a range to obtain test results which are directly proportional to the concentration of analyte in the sample.
Range	The interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
Robustness	A measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

(Information taken from ICH harmonised tripartite guideline Q2A)

Table 3.5 *HPLC validation characteristics.*

Accuracy may be inferred once precision and linearity have been established.

The repeatability will be assessed using 6 determinations at 100% of the test concentration. It will be expressed as an estimated percentage relative standard deviation of a consecutive series of measurements of injections within a reference solution. This will determine the precision of the machine. The same procedure will

be carried out for six different solutions of the same concentration, thus the repeatability of actually preparing a standard will be assessed here. A mid-calibration standard of 20 ug/mL will be used. The intermediate precision does not really apply to the proposed method, as the analyst and equipment used will remain the same. Additionally, reproducibility is not applicable for this study.

The intra-day precision of the HPLC machine and of preparing a standard was satisfactory, as indicated by the relative standard deviation: (1.37% and 1.82%, respectively) of the peak area ratio, after replicate (n=6) injections of the same standard solution or 6 different standards of the same concentration.

3.1.6 Preparation of calibrators

Triclosan was accurately weighed, then dissolved in ethanol to form a stock-solution of 1mg/mL. Due to the high lipophilicity of triclosan, ethanol was required as a solvent. From the stock solution, a range of standard solutions (calibrators) were prepared by diluting with ethanol: phosphate buffer 40:60 (10-50 ug/mL) This composition was required as a diluent as this is what is used in the receptor solution in later franz cell studies

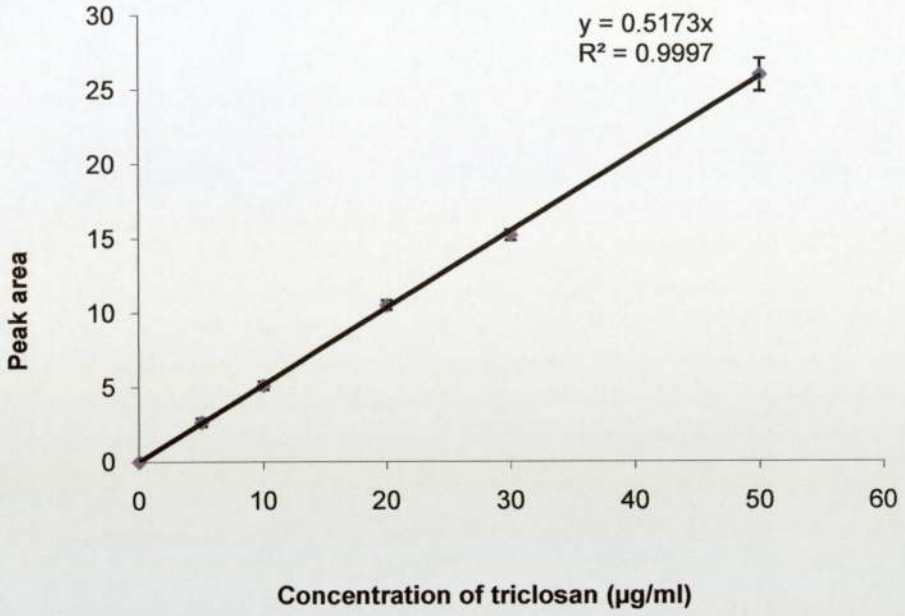


Figure 3.5 *Typical calibration curve for triclosan. Mean ± SD. n=5.*

The linearity can be shown on the calibration curve (Figure 3.4). The linearity is a measure of the range within which the analyte concentration is directly proportional to the analyte concentration in samples within a given range. The high correlation coefficient (0.999) represents good linearity between peak area and drug concentration.

A number of variations will be observed to evaluate the robustness:

1) Storage

2) Heat degradation

3) Acid degradation

4) Alkali degradation

5) Light degradation

3.1.7 Preparation of a standard calibration curve

Due to the vast number of samples generated in transdermal studies, storage overnight was required. A range of samples were prepared on day 1 (10 ug/mL to 50 ug/mL) and the peak areas were used to formulate a calibration curve. The samples were then stored in the fridge overnight and then analysed again on day 2. Additionally, on day 2 fresh standards were also be prepared (Table 3.6) to compare with the standards from day 1.

There was no significant difference in linearity between the standards prepared on day 1 and the following day even when compared with fresh standards on day 2. The linearity was assessed by comparing gradient and correlation coefficient values.

	Correlation coefficient	**Gradient**
Standards from Day 1	0.991	0.502
Standards from Day 2	0.989	0.504
Fresh standards Day 2	0.992	0.512

Table 3.6 *Correlation coefficients and gradients from different standards prepared on day 1 and day 2.*

The large number of samples being analysed meant that long HPLC run times would take place (~7hours). Thus, the peak area of a triplicate of 20 ug/mL standards were determined at the start of the run and at the end of the run and compared with a triplicate of freshly prepared standards of the same concentration to see if any degradation took place.

Initial Peak area	Final Peak area
10.41 ± 0.023	10.38 ± 0.012

Table 3.7 *Peak areas before and after run time Values denote mean ± S.D (n = 3).*

From the results in Table 3.7, it can be seen that there was no significant difference between the initial peak area compared with the peak area after the run time. For the following degradation studies an initial solution of 1% triclosan was used and tests were carried out in triplicate. The chromatogram in Figure 3.5 shows the original chromatogram before degradation took place.

3.1.8 Heat degradation

10 mL of the above triclosan solution was heated at 60 °C for 60 minutes. This was transferred to a 20 mL volumetric flask and made up to volume with water. Results show very little difference in peak areas, however the peak shape has become

broader (Figure 3.6). The tailing factor has increased from 1.03 to 1.42 and the retention time has also decreased by 0.1 minutes.

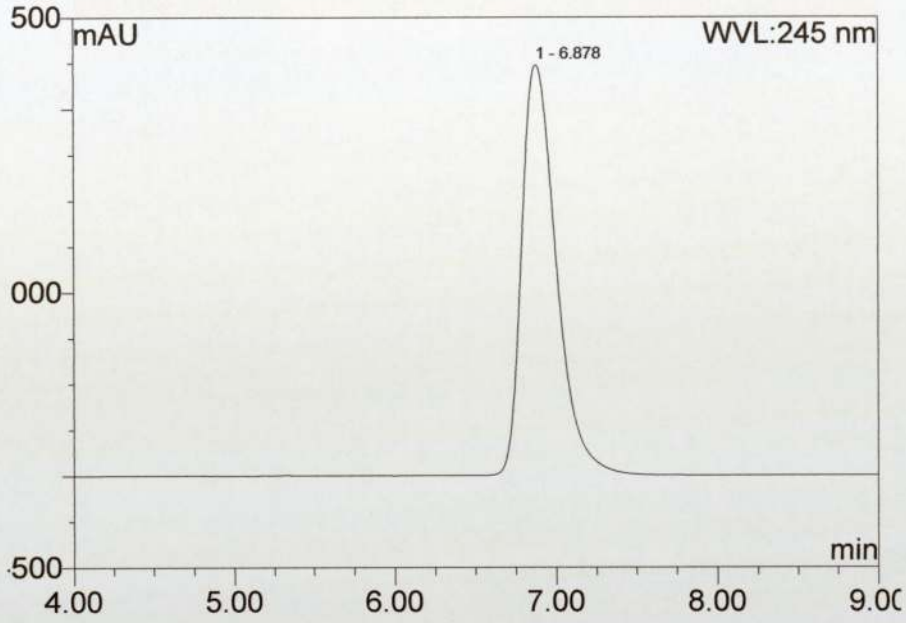


Figure 3.6 *Typical HPLC chromatogram when triclosan has been heated.*

3.1.9 Acid degradation

1mL of 1M HCl was added to 10 mL of the original solution and heated at 60 °C for 60 minutes. 1mL of 1M NaOH was added to a separate 20 mL volumetric rinse container and then made up to volume.

Results (Figure 3.7) showed a minimal decrease in peak area however the retention time decreased by 0.2 minutes and the tailing factor is now 1.39.

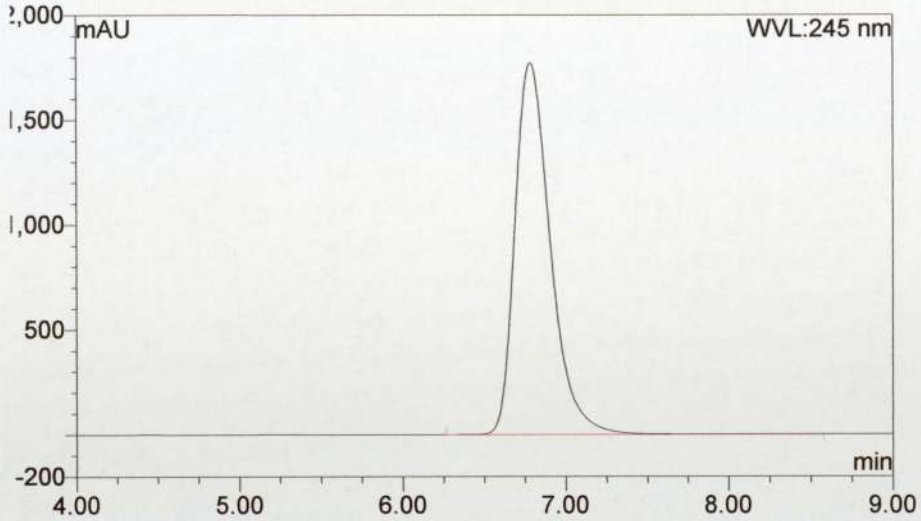


Figure 3.7*Typical HPLC chromatogram following acid degradation of triclosan*

3.1.10 Alkali degradation

1mL of 1M NaOH was added to 10 mL of the original solution and heated at 60 °C for 60 minutes. Following this, 1mL of 1M HCl was added to a separate 20 mL volumetric rinse container and then made up to volume. Results (Figure 3.8) show a similar trend to previous degradation studies; the retention time decreased by 0.15 minutes and the tailing factor is now 1.39.

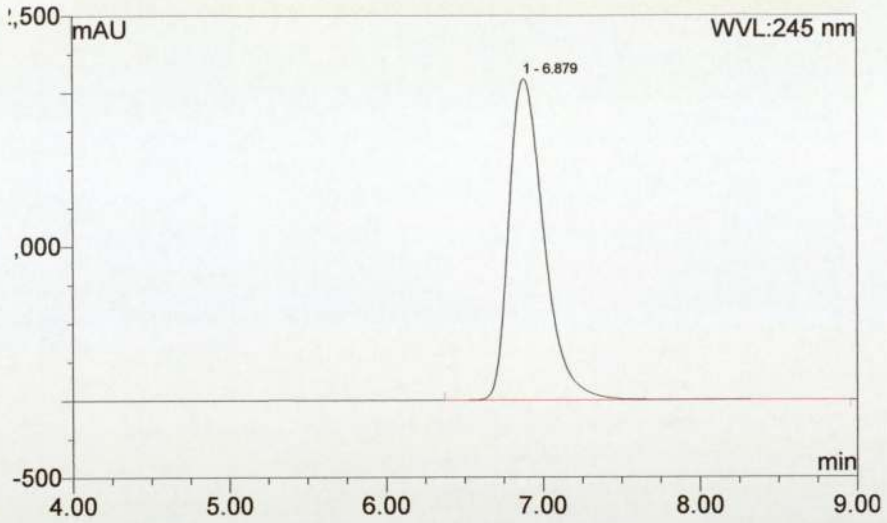


Figure 3.8 *Typical HPLC chromatogram following alkali degradation of triclosan*

3.1.11 Light degradation

Samples were compared when in amber vials and clear vials and placed on a window sill for 24 hours. There was no apparent difference in peak area or peak shape.

3.2 UV detection of triclosan

UV spectroscopy involves the measurement of the amount of ultraviolet radiation absorbed by a substance in solution. This method is rapid and was used to determine a suitable wavelength for UV detection during HPLC analysis and to validate the previous HPLC methods. The UV spectrum of triclosan was determined in the range 200-350 nm using a 10 ug/mL solution of triclosan prepared in 40:60 ethanol:phosphate buffer (v/v) solution. The λ_{max} of triclosan occurred at 283nm; however, the intensity of absorbance at this wavelength was relatively small. Absorbance was greater in the shoulder region of the spectrum, therefore, for greater

sensitivity, UV detection of triclosan during HPLC assaying was performed at 245 nm. To apply UV spectroscopy in quantitative analysis, Beer-Lambert's Law was used. The concentration of triclosan in solution was estimated according to Beer-Lambert's law:

$$A\lambda = \varepsilon\lambda bc$$

where $A\lambda$ is the absorbance at a wavelength λ , $\varepsilon\lambda$ the molar extinction coefficient (cm/mol), b the optical path (cm) of the cell and c the concentration (mol/l). The equation is linear for each wavelength, thus the concentration is proportional to the absorption. To confirm this, the absorbances of triclosan solutions of various concentrations were determined at 245 nm. Standard solutions of triclosan ranging from10 ug/mL to 50 ug/mL were prepared. A linear plot of absorbance versus concentration demonstrated R^2 values 0f 0.99, showing that Beer-Lambert's law was obeyed within this concentration range.

Although analysis with UV spectroscopy is rapid and easier to automate it is not as sensitive, accurate and specific as HPLC. Thus analysis with HPLC was the main technique utilised when assaying triclosan. However, as previously mentioned, UV analysis was carried out to validate HPLC methods.

3.3 Solubility of triclosan in different vehicles

3.3.1 *Varying ethanol water co-solvent systems*

Preliminary experiments were carried out as described in section 2.2.3.2 to determine the solubility of triclosan in various vehicles; this was used to assist in determining suitable donor and receiver composition when using franz cells.

Concentration of ethanol in water (%v/v)	**Triclosan solubility (ug/mL)**
Distilled water	0 ± 0
20	54.12 ± 1.81
25	108.20 ± 5.53
30	326.27 ± 13.17
40	1752.13 ± 3.63
50	21145.47 ± 11.23
70	48366.7 ± 12715.45

Table 3.8 *Table to show triclosan solubility with varying concentrations of ethanol in water (%V/V) (mean ± SD) n=3.*

The results show an increase in triclosan solubility with increasing ethanol concentration. This becomes highly significant when the concentration of ethanol in water is 60% (V/V) see appendix.

3.3.2 Varying propylene glycol-water co-solvent systems

The method described in section 2.2.3.2 was employed to test the solubility of triclosan in propylene glycol, however the samples were left in the waterbath for 48 hours. Additionally, the different solvents used were 10%, 20%, 30%, 40%, 50% and 60% propylene glycol.

Concentration (%v/v) of propylene glycol	**Triclosan solubility (ug/mL)**
10	5.65 ± 0.51
20	25.93 ± 0.77
30	76.33 ± 0.84
40	415.78 ± 4.37
50	2088.51 ± 12.98
60	8512.45 ± 56.12

Table 3.9 *Solubility of triclosan in propylene glycol/water vehicles (mean ± SD) n=3.*

The solubility of triclosan increases with increasing concentration of propylene glycol in water. However, the solubility of triclosan in corresponding concentration of ethanol (%v/v) in water is greater.

3.4 *In Vitro* experiments

Most information regarding the mechanisms of penetration has been gained from *in vitro* permeation studies, and from the 1940s to the 1980s there is a vast array of data. The objective of an *in vitro* experiment is to create an experimental method that can model the penetration kinetics of drugs into the human body *in vivo*. *In vitro*

investigations allow the control of the environment and simplify the complex variables present in *in vivo* experiments. However, these controls must still provide a fairly accurate model of the human body. Enzymatic metabolism may be compromised within excised skin and the absence of dermal vascularisation may modify the clearance thus, the flux of the permeant. Good correlation has been demonstrated between *in vitro* and *in vivo* data provided diffusant clearance from the distal surface of the barrier is not the rate limiting step to diffusion (Marzulli et al., 1969). *In vitro* investigation is particularly useful to compare and contrast relative drugs and vehicles to optimise formulation, determine absorption mechanisms and the role of enhancing mechanisms before exposing living organisms to potentially noxious chemicals.

3.4.1 In vitro diffusion Franz-cell models

To provide quantitative information on the transport of antimicrobials, an *in vitro* diffusion Franz-cell model was used. This system allowed the precise control of the composition of the donor and receiver compartments and it enabled one to assess the interaction (surface binding, penetration and transport) of permeants with skin. A disk of skin or artificial membrane allows isolation of the various interactions and forms the barrier between donor and receiver compartments. The donor surface is treated with the test formulation of the antiseptic agent. To assess flux, the appearance of antiseptic in the donor phase was monitored, with respect to time.

An *in vitro* diffusion cell essentially comprises of two compartments, with a membrane clamped between the donor and receptor sections. However, there are numerous different cell designs to determine percutaneous absorption *in vitro* and it is important to select a design where the transport is limited by the skin and not in any stagnant diffusion (non-stirred) layers adjacent to the skin surface (Hadgraft and Lane, 2005).

3.4.2 Static diffusion cells

The most simple static diffusion cells (Figure 3.9) tend to be upright with the donor compartment available for application of the permeant, and the receptor compartment of a fixed volume is kept at a controlled temperature (waterbath, usually at 37 °C to maintain surface skin temperature at 32 °C as an *in vivo* mimic). In the bottom, a buffer solution is kept in direct contact with the absorption barrier. The receptor fluid is agitated, most commonly with a magnetic bar stirrer, and a portal from the receptor compartment allows removal of receptor fluid at required time intervals. These cells are often termed 'Franz' -type diffusion cells (Franz, 1975) and are usually made of glass. They are versatile in that the diffusional area can be modified (typically from 0.5 to 5 cm^2) and the receptor volume can be changed to 'concentrate' the permeant to facilitate analysis (around 2 to 20 mL).

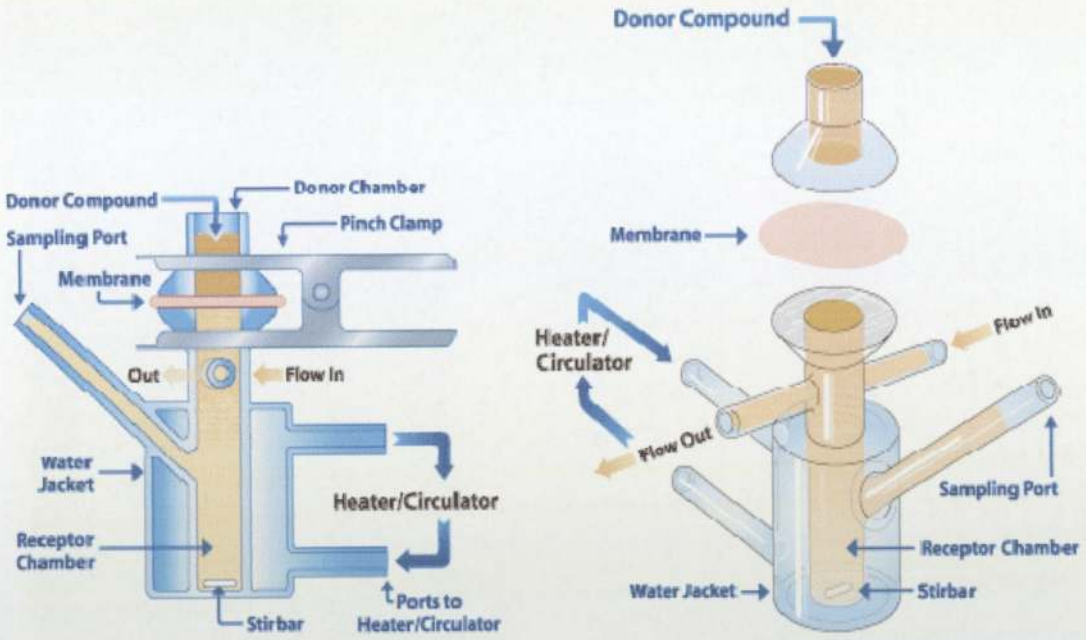


Figure 3.9 *A simple static diffusion cell modified from http://www.ses-analysesysteme.de/SESranz_Cell_Flow_Type_uk.htm.*

A variation on the above static design is a side-by-side cell which allows both donor and receptor compartments to be stirred at the same time (Figure 3.9). Horizontal diffusion cells have similar sized receptor and donor compartments positioned either side of the absorption barrier under assessment. This barrier is placed in the vertical plane. The design allows air bubbles to be removed relatively easily and agitation of both compartments ensures homogeneity of the formulation under investigation. A major disadvantage is that a large volume of donor solution is required to completely cover the barrier surface. This problem is exaggerated when investigating expensive donor substances or donor substances which are not readily available in large quantities, particularly when designing infinite dose experiments with saturated suspensions of a solute readily soluble in aqueous vehicles. Additionally, this type of

design requires large sections of membrane making them impractical for human skin investigations where availability is limited and it can be difficult to produce large sheets of intact membrane.

Pretreating membrane surfaces is unrealistic using horizontal cells; however, they are especially useful for assessing the intrinsic diffusivity of a molecule or the effect of certain variables (i.e. pH, partition coefficient, boundary layers) on diffusion. Identical donor and receptor solvents allow the study of drug diffusion, in the absence of any factors that may enhance permeation.

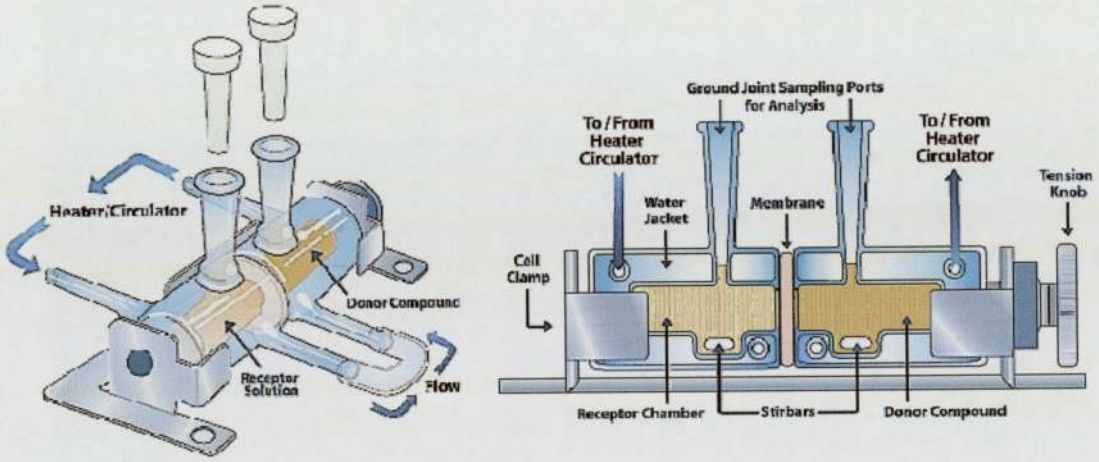


Figure 3.10 *Horizontal diffusion cell modified from http://www.ses-analysesysteme.de/SESFranz_Cell_Flow_Type_uk.htm.*

3.4.3 Flow-through diffusion cells

In contrast to static cells, flow through diffusion cells (Figure 3.11) attempt to model in-vivo conditions with a flow through receptor phase equating to the blood supply,

and with a non-agitated donor phase equivalent to drug formulation. With Flow-through diffusion cells the collection of receptor phase can be automated and the temperature is controlled. The main difference is that the continuous flow of receptor fluid beneath the diffusion cell ensures that sink conditions are maintained throughout the experiment, whereas the receptor fluid in a static permeation cell may need to be replaced. The flow of the receptor phase under the skin membrane causes turbulence that effectively stirs the receptor compartment and minimises the boundary layer underneath the membrane whereas with static cells there can be a stagnant layer underneath the membrane.

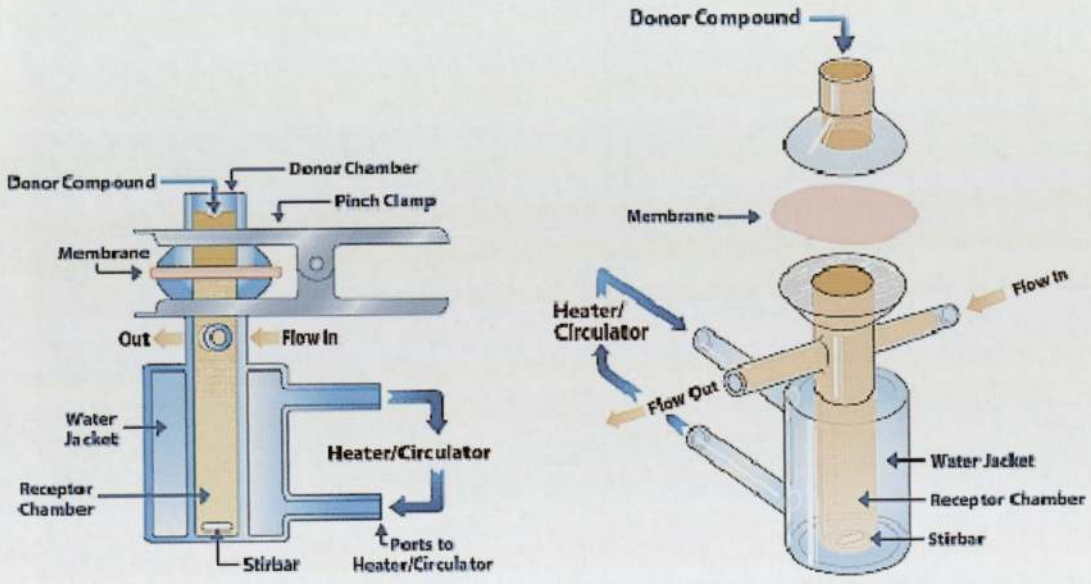


Figure 3.11 *Flow through Diffusion Cell modified from http://www.ses-analysesysteme.de/SESFranz_Cell_Flow_Type_uk.htm*

Various designs of flow-through apparatus exist. Automated systems allow easy overnight experimentation. It is now more usual to include a thermostated

temperature control and in some cases control the environment within the donor compartment. Additionally, increasingly lower volumes in the receptor compartment enable low levels of permeant to be detected with increasing accuracy. However, beyond the choice of cell, the experimental protocol and design is more important for ensuring that reliable and valid results are obtained.

3.4.4 Design aspects of in vitro diffusion cells

It is believed one of the most important design aspects of *in vitro* absorption cells is the degree of agitation within the compartments. The rate of agitation should be carefully monitored and maintained at a uniform rate to annul boundary layer effects (Ackerman and Flynn, 1987 and Ackerman et al., 1985). Agitation must be sufficient to minimise diffusion boundaries at the membrane surfaces. The depth of boundary layers decreases as compartment agitation increases, which results in a relative increase in drug permeation. The Helix™ stirrer was initially set to 2200 *rpm* for 60 seconds before the experiment was started, this helped eliminate air bubbles rapidly which had accumulated at the skin-receptor interface the stirrers were then set to 550 *rpm* for the remaining experiment. Additionally, the receptor phase is de-gassed prior to the experiment by warming to 32°C and sonicated to prevent accumulation of air bubbles at the skin receptor-interface. Experiments were performed using silastic membrane and human skin as the barrier between donor and receiver compartments, the reason for selecting these membranes has been explained in section 3.4.1 and 3.4.2.

3.4.5 Composition of donor and receiver phases

The composition of the donor and receiver phases must be carefully considered when designing an experimental protocol for *in vitro* experiments. The diffusion process will depend on the partitioning of the drug between the membrane and the receiver solvent. For hydrophobic compounds however, the membrane may become saturated with respect to the solute and thus aqueous diffusion layer transport will be dominant. Due to the hydrophobic nature of triclosan, its affinity for a purely aqueous receiver phase was expected to be very low and consequently triclosan may remain in the membrane. Additionally, if distilled water was used, saturation of the receiver phase, with respect to triclosan, may occur rapidly due to its limited water-solubility which would restrict further diffusion. Thus, it was vital to use co-solvents due to the high lipophilicity of the triclosan molecule.

In practice, ensuring that the concentration of the permeating species does not exceed 10% of its solubility in the receptor is normally sufficient to ensure that the flux of material through the skin is not significantly slowed by modification of the concentration gradient across the membrane. Solubility studies have been described in section 3.4 to determine the solubility of triclosan in various co-solvents. It was decided the co-solvent of interest would be ethanol, as ethanol itself is known to be an effective skin disinfectant and this would therefore further enhance the antimicrobial activity as well as act as a co-solvent. If the diffusant is very soluble in the donor vehicle, and is at a relatively low concentration, it will have little affinity

for the barrier membrane and will not readily partition into it (Smith and Haigh, 1989).

The receptor solution should mimic the in-vivo situation. However, the concentration of the permeant is equally important in the receiver solution as the donor solution and the use of a co-solvent was necessary. Receiver solutions containing solvents such as alcohols, may extract lipids from the skin membranes, potentially enhancing the permeation rate of many donor compounds. The receiver must however maintain a greater overall lipid solubility to enable the penetrant to overcome the lipophilic properties of the skin and partition adequately into the receiver. To allow transport of triclosan into the receiver phase, the polarity of the receiver solvent was adjusted by the addition of ethanol. Preliminary experiments with various concentrations of ethanol found 40% to be an optimum concentration. Lower concentrations were not appropriate as diffusion was too slow to be quantified appropriately. Higher concentrations lead to evaporation during the experiment causing problems in reproducibility. Silicone grease was smeared over the glass surfaces of the cell to prevent leakage and to also minimise evaporation. An ethanol: phosphate buffer solution 40:60 (V/V) was selected as the receiver solvent. Once the experiment was completed, the Franz cells used were rinsed three times with a solution of ethanol and water. This was necessary as any remaining traces of triclosan would contribute to the amount of triclosan permeated in future experiments, particularly as the amount of drug being permeated within Franz cells is already a small dose.

Preliminary experiments demonstrated that 3 rinses were required before all traces of triclosan within the cells were eliminated. Any traces of triclosan were determined using HPLC.

Franz cell number	Remaining concentration of triclosan following first rinse (ug/mL)	Remaining concentration of triclosan following second rinse (ug/mL)	Remaining concentration of triclosan following third rinse (ug/mL)
1	14.28	7.45	0
2	16.87	7.98	0
3	14.09	8.87	0
4	18.93	7.09	0
5	20.08	7.75	0
6	15.67	9.23	0

Table 3.10 *Table to show the rinses required to remove triclosan from diffusion cells.*

Clearly for formulation comparisons it is preferable to compare fluxes where the permeant is at the same thermodynamic activity in both formulations, most easily achieved by maintaining permeant saturation in both formulations, or selecting the same fraction of saturation. However, future experiments may focus on unsaturated solutions as well as saturated experiments.

3.5 In vitro absorption barriers

The most appropriate membrane to select for transdermal drug delivery is that for the species to be treated. In this case it would mean experimenting with human skin. However, this is often limited and only relatively small quantities are available. In addition, there are many problems associated with using human skin. The permeability of skin varies from site to site, furthermore the age, sex and hairiness of donors varies. These factors are known to influence penetration (Feldman and Maibach, 1967). Skin samples may also come from a various situation i.e. surgical amputations, burns patients and cadavers. It is also common for the barrier to become impaired during the separation process. Additionally, many donor samples are obtained from amputated limbs due to peripheral vascular disease, the skin may already be necrotic thus the overlying barrier is damaged. The above factors may therefore contribute to a lack of reproducibility.

A further drawback in using the skin itself within experiments was the lengthy process of preparing and storing the skin. Skin samples were stored at -70°C for preserving purposes and left to thaw when required. It was necessary to remove any subcutaneous fat and to remove the epidermis from the dermis in order that the flux of the permeant could be measured through the upper layers of the skin. Measuring the rate of permeation of triclosan through all the dermal layers would require a highly concentrated donor solution, moreover with triclosan being highly lipophilic the triclosan may have an affinity to reside within dermal layers.

Many different animal skins have been proposed as models for human skin *in vitro*. Data obtained from these membranes must be treated with caution in that results obtained from, i.e. animal skins may not transfer directly to human skin either *in vitro* or *in vivo*.

Mammalian skin has been used to model drug permeation through human tissue. Physiologically it is superior to rodent skin (Uno and Karata, 1993). Dog skin has also been evaluated as a model for the human membrane in comparison to rodent or reptile skin (Sato et al., 1991). However, the best 'natural' model membrane for permeation studies is pig skin. The stratum corneum of the pig is similar in thickness to the human membrane and the permeability properties are very close to that of human skin.

Artificial membranes offer the advantages of reproducibility and control. They are readily available and useful for comparing initial relative absorption rates from various vehicles. Simple inert polymer membranes are often used to study percutaneous absorption. Most commonly, polydimethylsiloxane (silastic or PDMS) membranes provide a 'non-porous' hydrophobic reproducible barrier. A simple membrane like this has been particularly useful for formulation optimisation studies and for testing physicochemical principles. Although it is often described as a hydrophobic non-porous membrane, it is important to note that membrane allows passage of water – equivalent to transepidermal water loss – during permeation

studies. It is useful for compounds that follow the intercellular route through the stratum corneum. There are obvious disadvantages; most importantly the skin is much more complex than simple hydrophobic/hydrophilic membranes of fixed pore size. Thus, the main role for artificial polymer membranes should be for comparative data for individual chemicals or for testing formulation variables. It would however be essential to fully validate permeation with a biological membrane, preferably human skin, before embarking on relative kinetic comparisons *in vivo*. Future studies will therefore be based on both human and artificial membranes.

3.5.1 Permeation of triclosan with different types of membranes

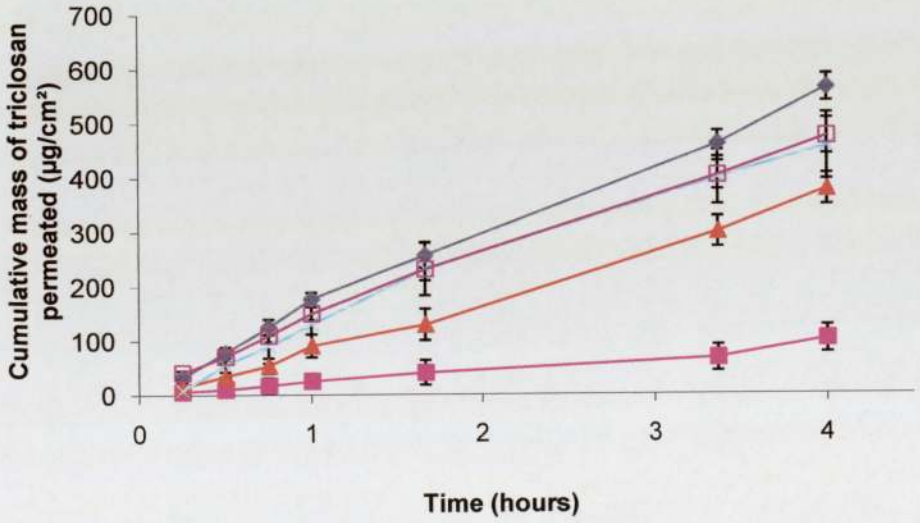


Figure 3.12 *The permeation of a 50% ethanolic triclosan solution 1% (w/v) across five different types of model membranes: silastic membrane with thickness 1.27 x 10^{-4} m" (navy blue); cuprophane membrane (light blue); human skin (light blue); silastic membrane with thickness 2.54 x 10^{-4} m (red); cellophane membrane (pink). Results are expressed as the means of three experiments ± S.D.*

Figure 3.12 demonstrates the permeation of an ethanolic solution across five different types of model membranes. The order of highest permeation profiles was silastic membrane with thickness of 1.27 x 10^{-4}m > cuprophane membrane>human skin> silastic membrane with thickness 2.54 x 10^{-4}m > dialysis membrane. As previously described human skin is the most appropriate model membrane to utilise as it fits the purpose of the research aims. Therefore, human skin will be used as the membrane barrier across diffusion cells where possible.

Silastic membrane of thickness 1.27 x 10^{-4}m will be selected as the main model membrane for the research aims of this thesis. This membrane demonstrates the greatest permeation rate and reflects the actual thickness of the epidermal layers well. The silastic membrane with greater thickness (2.54 x 10^{-4}m) is twice the thickness of the selected model membrane and shows a permeation rate of almost half that of the silastic membrane of 1.27 x 10^{-4}m. This clearly indicates that the thickness of the model membrane is proportional to the permeation rates of a drug and is expected as the permeating drug has a diffusional path which is twice the distance in the thicker membrane before it reaches the receiver component of the diffusion cells. The cuprophane membrane does demonstrate a good permeation rate however similar to cellophane membrane it is commonly used as a dialysis membrane. These membranes were mainly used as comparative purposes in this

study. The hydrophobic pores present within silastic membrane model human epidermal skin well.

3.6 Conclusion

Within this chapter a suitable HPLC method was developed by reviewing methods provided by other works to select an appropriate mobile phase which provided a suitable analysis time and optimum peak resolution. HPLC validation and parameters were also determined. Co-solvent systems were investigated in order to model receptor solutions for diffusion cell studies. *In vitro* methods were examined and model membranes were investigated to select a suitable experimental membrane for future studies.

Chapter Four:

Delivery of triclosan from topical adhesives

4.0 Introduction

In order to disinfect the skin effectively the release of an antimicrobial over an extended period of time would be highly advantageous. There are numerous topical formulations available which target the skin's surface, however microbial repopulation occurs rapidly indicating that multiplication of aerobic bacteria beneath the skin's surface is occurring (Hendley and Ashe, 1991). To achieve a sustained and constant release of the antimicrobial, patch type formulations may be used on the area of skin which requires disinfection. Topical patch formulations may be more preferable to ointments, creams, lotions and emulsions as they are more convenient for application, can prevent active components from being washed out and are released for a long period of time through zero-order delivery (Bian *et al.*, 2003).

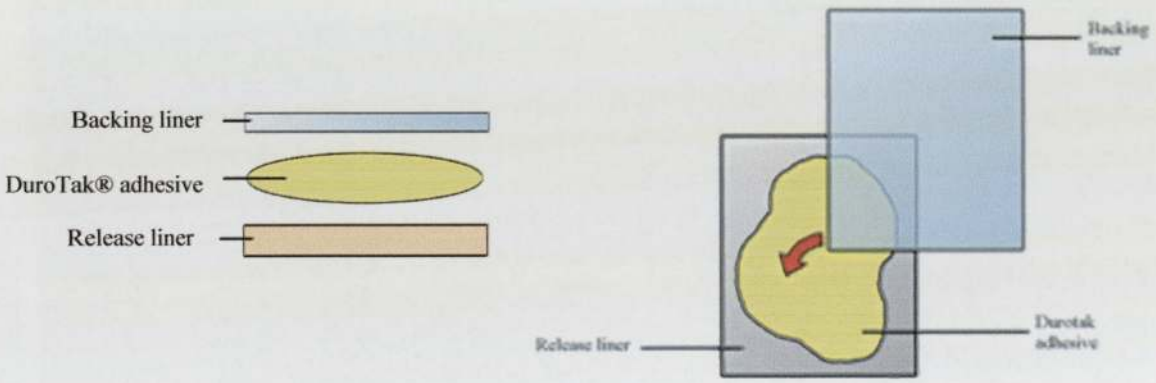


Figure 4.1 *(a) Cross-section of adhesive patch (b) Formulation of patches*

Transdermal patches are a form of topical drug delivery systems. They have developed and increased in complexity considerably over the past decade. Initial work with transdermal patches was limited by the number of molecules which

could be delivered by this route. For instance, the permeant needed to possess appropriate physico-chemical properties to allow delivery across the skin. In addition, the molecule had to be potent enough to function at relatively low doses (Williams, 2003). Transdermal patches containing nicotine for smoking cessation and estradiol for use in hormone replacement therapy have been particularly successful. This is mainly due to both molecules being small, their appropriate lipohilicity, solubility and potencies (Zhao et al., 2002; Valenta and Auner, 2004). However, recent technology focuses on the complexity of the patch design as well as selecting and modifying the permeant. Thus, a broader range of drugs can be delivered through the skin.

There are several types of patches currently available, these can be categorised into three main types depending on how the drug is incorporated into the transdermal system:

(i) Patches with the drug in the adhesive.

(ii) Systems with the drug in a matrix.

(iii) Adhesives with the drug as a reservoir.

There are however, variations depending on the use of the patch.

The adhesive is a vital component present within all transdermal patches. Transdermal systems mainly consist of pressure-sensitive adhesives (PSA). During adhesion there are two processes which occur. Firstly, the adhesive must wet the skin surface so that there is liquid flow of the adhesive on the skin. Once pressure is released from the patch it is vital that the adhesive must then fix onto

the skin (Williams, 2003).

Patches incorporating the drug in a matrix system are able to produce a more controlled release of the drug. Simple polymer mixtures are mixed with the drug. The proportions of the polymers vary the nature of the formulation and other excipients such as drug enhancers and plasticizers can be included in the matrix.

4.1 Aims and Objectives

Within this study the aim was to investigate a matrix-type adhesive delivery system (Figure 4.1 and 4.2). Once the PSA is placed in contact with the skin, the drug is released from the patch in a controlled rate and diffuses into the skin. Selecting a suitable PSA was critical when developing a topical delivery system since its adhesion, stability characteristics and compatibility with other patch components have a significant impact on the delivery efficacy of chemicals (Bian et al., 2003).

The main objectives of this work were:

(i) Develop the polymeric adhesive containing drug embedded in a solubilised form.

(ii) Prepare drug in-adhesive type patches using a polymeric adhesive layer.

(iii) Characterise the release and permeation of triclosan from these systems.

(iv) Incorporate enhancers into certain adhesive formulations and test the permeation of these systems.

Figure 4.2 *Photographic representation of DuroTak® adhesive*

4.2 Methods and Materials

Triclosan was the drug of interest within this study and the adhesives used (Table 4.1) were supplied from DuroTak®. Detailed formulation procedures of the adhesive patches, permeation and release protocols have been described in section 2.2.5.

DuroTak® Adhesive	Functional Group	Chemical Composition	Cross linker Added	Peel	Shear	Tack	Solvent Composition
900a	No functional groups	Acrylate copolymer	N/a	Relatively high	Extremely low	Relatively Low	Cyclohexane Ethyl acetate
9301	No functional groups	Acrylate Copolymer	N/a	Relatively high	Very low	Relatively High	Cyclohexane Ethylacetate
2979	-COOH/-OH	Acrylate-Vinylacaetate	Included	Relatively high	Relatively low	Relatively Low	Ethyl acetate Isopropyl alcohol Pentane - dione
2852	-COOH	Acrylate	N/a	Relatively High	Very High	Relatively High	Ethyl acetate Hexane Isopropyl alcohol Pentane-dione Toluene
2677	-COOH	Acrylate-Vinylacetate	Included	Relatively High	Very High	Very low	Ethyl acetate Heptane Isopropyl alcohol Pentane-dione Toluene

Table 4.1 Various DuroTak® adhesives and their properties

4.3. Release kinetics of the various DuroTak® adhesives

The mechanism of drug release from the five different patch types were determined by treating the release data according to three of the most familiar kinetic profiles; zero order, first order and Higuchi profiles. Zero-order (cumulative mass of drug released vs. time), first order (ln cumulative mass of drug released vs. time) and Higuchi's model (cumulative mass of drug released vs. square root of time). Results are shown in Figures 4.3 b, c and d respectively.

Plotting the results against each of the models it was found that Triclosan release from the systems correlated best with the Higuchi's model. This was based on the magnitude of the correlation coefficient which was greater than 0.94 for each of the systems when plotted using Higuchi's diffusion model modeled compared to zero-order and first-order ($R<0.82$ Table 4.2).

These results are in agreement with other published studies such as Zhao *et al.* (2005) where the release kinetics of testosterone from a DuroTak® 87-2510 patch were proportional to $t^{½}$ following the Higuchi principle. Qvist *et al.* (2002) also demonstrated that enhancers were released from DuroTak® adhesives 87-2287, 87-2516, 87-2051, 87-2052, 87-2676 and 87-4098 following the Higuchi model and Gwak *et al.* (2002) also demonstrated this trend when the release from DuroTak® 2196 and 2100 adhesives were also proportional to the square root of time. However not all studies have shown release of drugs from such adhesive to follow this model with studies carried out by Chedgzoy *et al.* (2002) demonstrating DuroTak® 87-2051, 87-2287 and 87-2516 indicating near zero

order release. Given that different adhesives were used in the above literature (Zhao et al., 2005; Gwak et al., 2002; Qvist et al., 2002) to the ones that have been used in this actual study, the type of adhesive may play a role in the type of release model determined.

In order for a drug to permeate across the skin, the drug should be appropriately released from the matrix, thus the patch should allow correct diffusion and release of triclosan. The Higuchi law demonstrates a matrix - controlled diffusion model ($Q' = K't^{½}$, Q': amount released, k': release rate constant) (Chien and Lambert, 1974). Roy *et al.* (1996) reported that the release rate of a drug from an adhesive matrix is dominated by the solubility of the drug and the diffusion coefficient in the polymer. The solubility of triclosan in the drug delivery device was above its solubility, a key requirement for a system to demonstrate diffusion-controlled release mechanism.

The release data demonstrates low correlation coefficients for zero order and first order models indicating that none of the formulations followed the type of release where constant rate of drug release or concentration dependent release is occurring. Although the R^2 values varied between 0.931 to 0.959 showing reasonably good linearity (Table 4.2); looking closer into the kinetics of triclosan release from the PSA's the profiles of all the formulations start to plateaux from approximately 8 hours or the square root of 3 hours onwards. This may be the result of a so-called depletion zone as suggested by Perrie and Rades (2010)

whereby a layer of drug-free matrix is apparent and the remaining drug has to diffuse over a longer distance to be released.

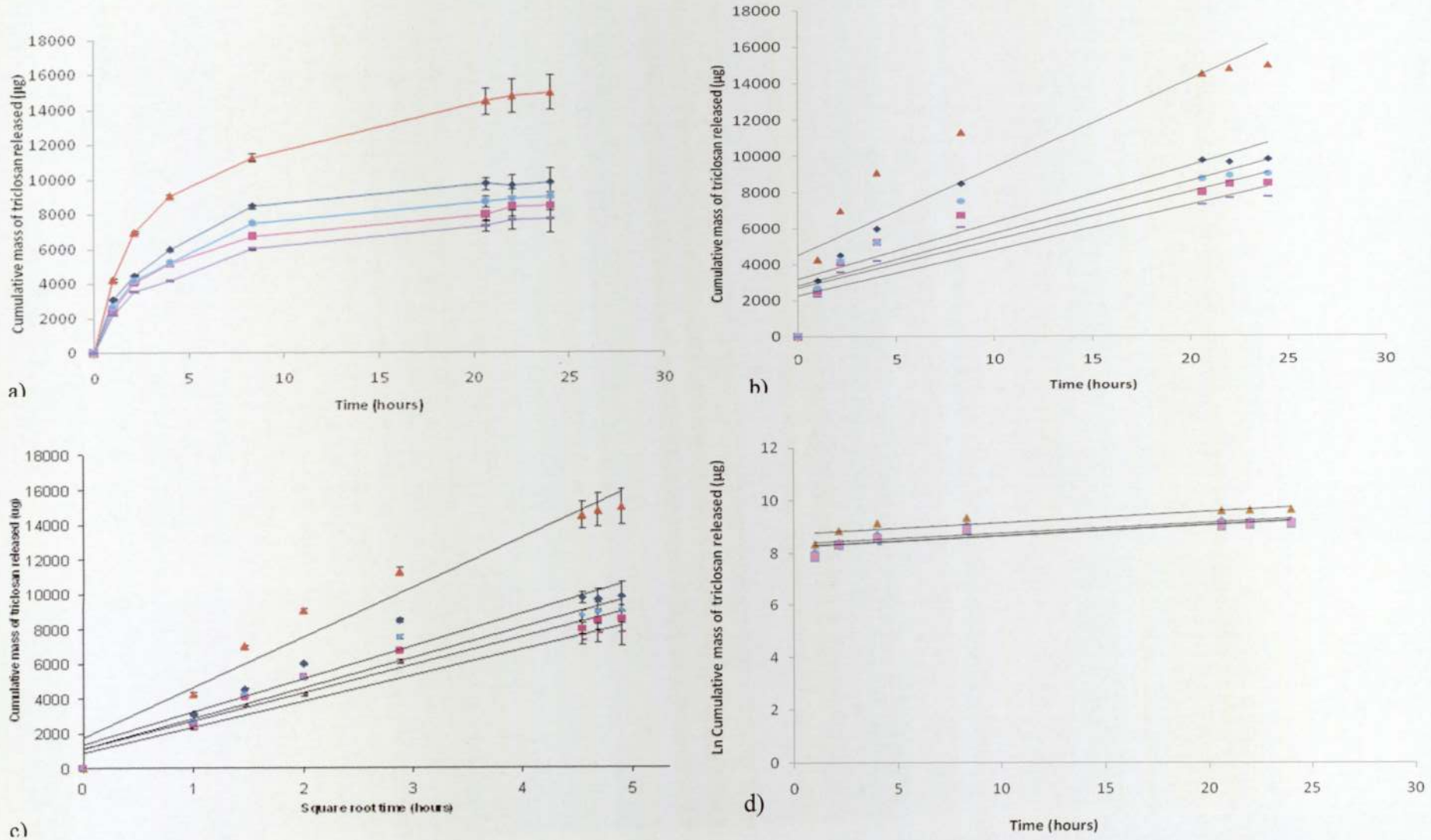


Figure 4.3 *Release of triclosan from five different pressure- sensitive adhesives.. The cumulative release profiles of triclosan in release media 1 in 10 60:40 PBS: ethanol at 37°C. Each patch of area 3.141 cm² contained (approximately) 80 mg of triclosan in DuroTak® 2677 (dark blue◆); DuroTak® 2979 (purple━); DuroTak®9301 (pink■); DuroTak® 2852 (red▲) and DuroTak® 900a (light blue●). The data are re-plotted according to zero-order (b)Higuchi (c) and first order (d) models. `Results are expressed as the means of six experiments ± S.D.*

	R^2		
DuroTak® Adhesive	**Zero order plot**	**First order plot**	**Higuchi's plot**
2852	0.816	0.769	0.956
2677	0.773	0.755	0.931
9301	0.789	0.768	0.941
900a	0.793	0.768	0.943
2979	0.823	0.768	0.959

Table 4.2 *Kinetic values of release of triclosan from five different DuroTak® adhesives using the correlation coefficient parameter (R^2).*

4.4 Effect of the various DuroTak® adhesives on the permeation and release of triclosan

4.4.1 Release of triclosan from the different DuroTak® adhesives

Cumulative release profiles of triclosan versus the square root of time from DuroTak® 2852, 2979, 9301, 2677 and 900a adhesives are shown in Figure 4.4. From the slope of the straight line the mean release rate was calculated and the % of total dose released is also shown in Table 4.3. There was no major initial burst of triclosan release apparent suggesting a homogeneous distribution of triclosan within the patch. Inter- patch variability was also low noted to be low as demonstrated by deviation in the samples from the mean (Figure 4.4). Over time the drug release rates for each of the patches decreased presumably due to the decrease in triclosan present within the patch.

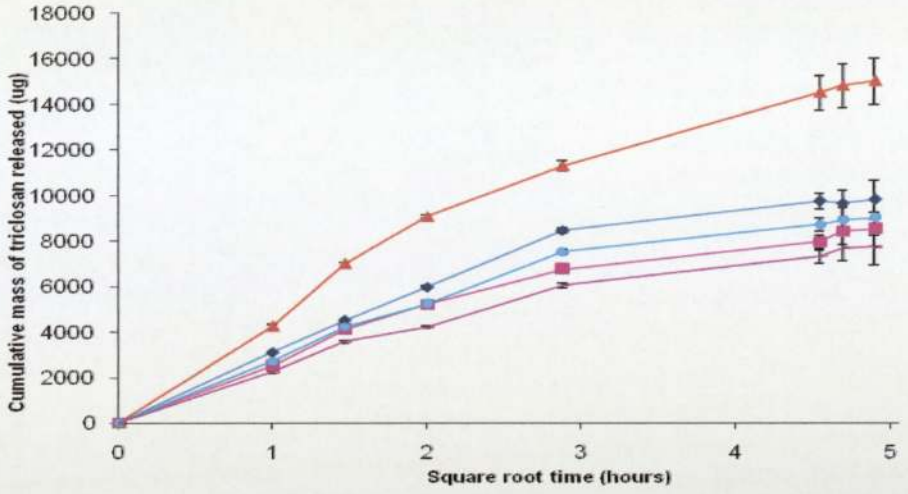


Figure 4.4 *Release of triclosan from five different type of DuroTak® pressure-sensitive adhesives into release media 1 in 10 60:40 PBS: ethanol. Each patch of area 3.141 cm^2 contained (approximately) 80 mg of triclosan in DuroTak® 2677 (navy blue ◆); DuroTak® 2979 (- purple); DuroTak® 9301 (pink■); DuroTak® 2852 (red▲) and DuroTak® 900a (light blue ●). The bars represent S.D., n = 6.*

Matrix (DuroTak®)	Release rate (ug/h)	% Total dose Released
2852	4222.73 ± 63.28	18.75
2677	2975.86 ± 60.12	12.26
9301	2479.52 ± 58.12	10.62
900a	2648.33 ± 58.56	11.27
2979	2156.62 ± 42.87	9.65

Table 4.3 *Release rate and % total dose released from various DuroTak® adhesives. Release media 1 in 10 60:40 PBS: ethanol. Each patch of area 3.141 cm^2 contained (approximately) 80 mg of triclosan.*

In order to optimise the formulation of triclosan patches, the effect of various acrylic PSA's on the release and permeation of triclosan were investigated (Table 4.3). Since the PSA's have different physicochemical properties they were expected to yield differing release and permeation rates. The relative order of the release rates for the different adhesives were DuroTak® 2852 >DuroTak® 2677

> DuroTak® 900a>DuroTak® 9301 and DuroTak® 2979. Among the five patches tested DuroTak®2852 demonstrated a significantly higher release rate ($P<0.05$) than the other patches. This may be attributed to DuroTak®2852 being the only adhesive which is predominately acrylate based; additionally there may be an association with the carboxyl group as the functional group, DuroTak® 2677 has the same functional group (COOH) and a slightly lower release rate than DuroTak®2852. DuroTak® 2979 demonstrated the lowest release rate and contained the functional group –COOH and –OH. Furthermore, Chedzgoy et al., (2002) also suggested that triclosan adhesives which have –COOH functionality demonstrate greater release rates than other patches which had –OH functionality, implying that triclosan is less strongly bound to the –COOH and therefore able to provide a higher release rate.

DuroTak® patches 9301 and 900a both had similar release rates (Table 4.3) and were not significantly different from each other; this may be attributed to their similar physicochemical properties and neither of them having a functional group. It has been postulated by Gwak et al., (2002) and Guyot et al., (2000) that similar release rates can be expected from patches which have the same functional groups. DuroTak® patch 2979 does however also contain a carboxyl group but it consists of a different solvent composition to DuroTak® patches 2852 and 2677. Thus, estimating the rate of release may not be as straightforward as observing the functional group.

Interestingly, a study carried out by Qvist *et al.*, (2002) demonstrated that there appeared to be no differences seen between release rates of enhancers despite the

adhesives having different physical characteristics such as functional groups and cross linkers. However, Zhao et al., (2002) speculated that the physicochemical properties of the adhesive affected the solubility of the drug in the DuroTak® matrix and thereby affected the rates of release of the drug. From the above studies it was not obvious as to why these different characteristics produce varying release rates; however it does seem that certain adhesives release triclosan at different release rates. Several studies agree that the release of drugs from a DuroTak® adhesive is probably due to compatibility and/or diffusion of the drug within the adhesive (Bian *et al.*, 2003; Zhao *et al.*, 2002; Gwak *et al.*, 2002).

It is also important to note that the pressure sensitive adhesive itself consists of various solvents (Table 4.1) which may also affect the drug solubility and can therefore be responsible for affecting release and permeation rates (Gwak et al.,2002; Zhao et al., 2002). As triclosan is a lipophilic drug it is readily soluble in the organic solvent compositions within the DuroTak® adhesives.

4.4.2 Permeation of triclosan from the various DuroTak® adhesives across silastic membrane

Permeation studies were undertaken across silastic membrane using diffusion cells. Figure 4.5 demonstrates the permeation of triclosan from the adhesives across silastic membrane. Permeation profiles are presented as cumulative mass of triclosan versus time for the five different adhesives. The permeation rate and % dose permeated was calculated (Table 4.4).

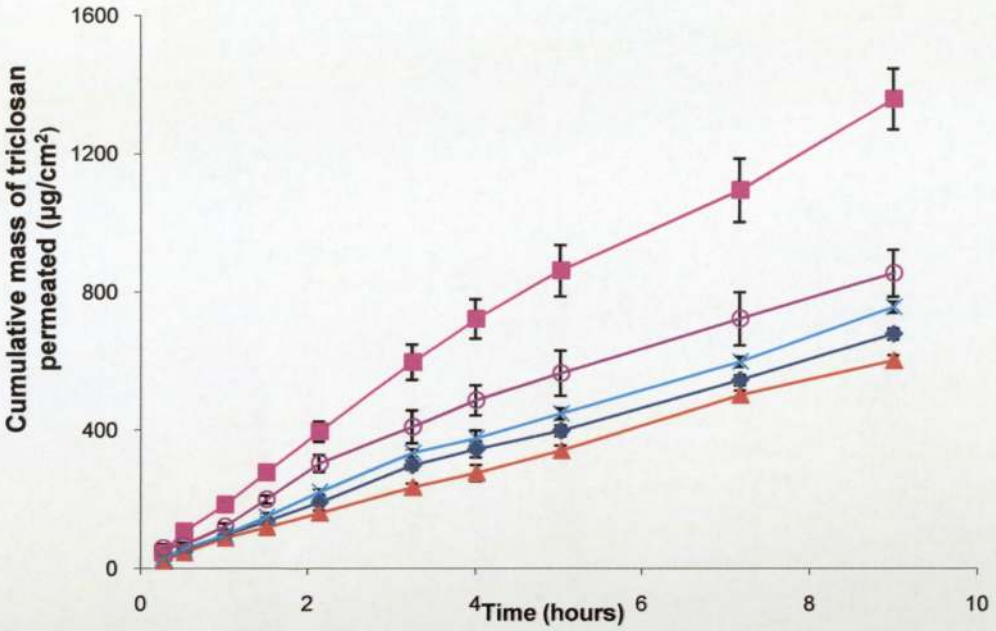


Figure 4.5 *Effect of various pressure-sensitive adhesives on permeation of triclosan across silastic membrane. Receiver media was composed of 1 in 10 60:40 PBS: ethanol. Each patch of area 3.141 cm² contained (approximately) 80 mg of triclosan in DuroTak® 2852 (pink ■); DuroTak® 2979 (red ▲); DuroTak® 9301 (navy blue ◆); DuroTak® 900a (light blue x): DuroTak® 2677 (purple ●). Results are expressed as the means of six experiments ± S.D.*

Matrix (DuroTak®)	Permeation rate (ug/cm²/h)	% Total dose Permeated
2852	159.76 ± 11.09	5.43
2677	104.53 ± 9.87	3.42
9301	78.48 ± 6.71	2.71
900a	87.12 ± 5.32	3.02
2979	68.64 ± 5.83	2.40

Table 4.4 *Permeation rate and % total dose permeated across silastic membrane from various DuroTak® adhesives of area 3.141 cm² contained (approximately) 80 mg of triclosan. Receiver media was composed of 1 in 10 60:40 PBS: ethanol.*

The profiles (Figure 4.5) demonstrate sink conditions during the experiment and the small error bars indicate good reproducibility between donors and maintenance of the silicone membrane barrier integrity. Good linearity is shown with r^2 values ranging between 0.9308 and 0.9592 for the adhesives. There are no lag times apparent within Figure 4.5 indicating that steady state flux is achieved rapidly. This is also consistent with findings by Chedzgoy et al., (2002) where lag times for the permeation of triclosan across the skin were relatively short. This is a desirable feature as it demonstrates rapid uptake from the patches followed by rapid permeation.

The release rate and the % total dose released (Table 4.3) is much greater than the results shown in the permeation data from Table 4.4. This is most likely due to the difference between triclosan being directly released into the release media and the use of silastic membrane across the diffusion cells. The silastic membrane is hydrophobic in nature and the triclosan itself is also hydrophobic, thus it is most likely that the triclosan readily partitions into the membrane lying within the silastic membrane and is not actually being released into the receiver media. The permeation rate and % of total dose permeated (Table 4.4) for each of the adhesives reflect the release rates (Table 4.3) in the same order 2852>2677>900a>9301>2979. Thus, DuroTak® 2852 displayed a significantly higher permeation rate compared with the other DuroTak® adhesives ($P<0.05$). This suggests that the release of triclosan from the patches is the governing factor when assessing the permeation of triclosan across the skin and has been demonstrated by several studies where DuroTak® adhesives were previously

used (Davaran et al., 2005; Chedzgoy et al., 2002). Before permeation of a drug occurs, it is necessary the drug should be suitably released from the patch, allowing diffusion and release of the drug (Gwak et al., 2002). Partitioning of the drug into the stratum corneum and/or membrane is often the rate limiting factor; however, in the above studies it does not appear to be a major rate limiting factor as there was an apparent absence of lag time.

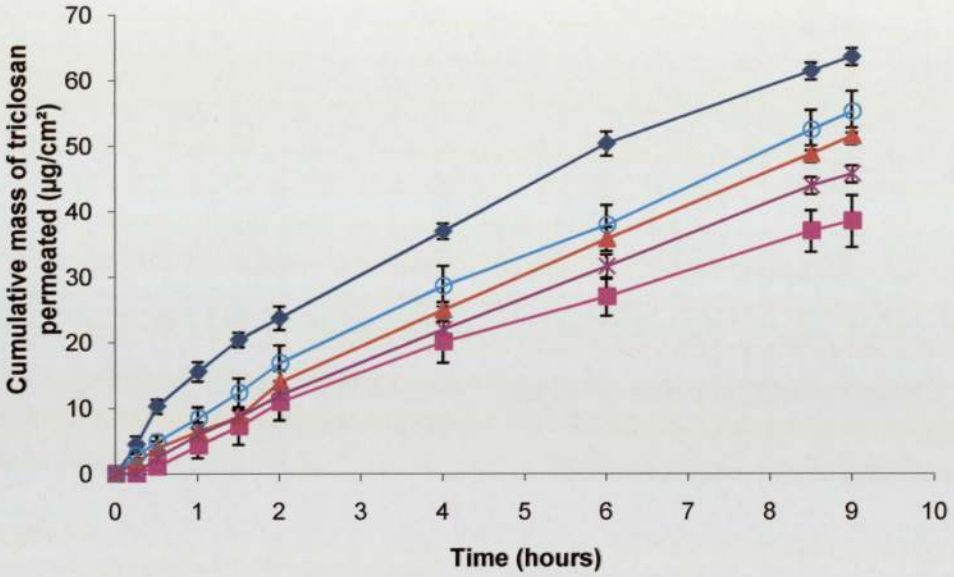


Figure 4.6 *Effect of various pressure-sensitive adhesives on the permeation of triclosan across human skin. Receiver media was composed of 1 in 10 60:40 PBS: ethanol. Each 3.141 cm² patch contained (approximately) 80 mg of triclosan in DuroTak® 2852 (navy blue ◆); DuroTak® 2979 (pink ■); DuroTak® 9301 (purple x); DuroTak® 900a (red ▲): DuroTak® 2677 (light blue ●). Results are expressed as the means of six experiments ± S.D.*

Matrix (Durotak)	Permeation rate (ug/cm²/h)	% Total dose permeated
2852	14.75 ± 0.17	0.25
2677	8.47 ± 0.21	0.22
9301	5.76 ± 0.12	0.18
900a	6.13 ± 0.10	0.21
2979	4.39 ± 0.28	0.15

Table 4.5 *Permeation rate and % total dose permeated across human membrane from various DuroTak® adhesives of size 3.141cm² containing approximately 80 mg of triclosan. Receiver media was composed of 1 in 10 60:40 PBS: ethanol.*

4.4.3 Permeation of triclosan from the various DuroTak® adhesives across human epidermal membrane

Heat separated epidermal membrane was the membrane barrier used in the following study. The permeation of triclosan from the adhesives across human epidermal membrane is shown in Figure 4.6. Generally, significant ($P<0.05$) changes in permeation rates were demonstrated for each of the adhesives with the order of permeation to be 2852>2677>900a>9301>2979 this parallels with results found from the permeation studies where silastic membrane was used as the membrane barrier (Figure 4.5), implying that the permeation rates are dictated by the release rates as previously mentioned. Figure 4.6 also demonstrates the evidence of a slight lag time for DuroTak® adhesive 2979, 9301 and 900a, suggesting triclosan is partitioning across the stratum corneum more slowly than across the silastic membrane. The permeation rate and % dose permeated (Table 4.5) was a great deal lower when human epidermal membranes were used

compared with silastic membrane (Table 4.4). This difference for each of the adhesives is demonstrated more clearly in Figure 4.7. There appears to be an approximate 10 fold increase from the permeation rate across silastic membrane when compared to the rate yielded across human epidermal membrane. These results show the difference between the complexity of the skin compared with silastic membrane. The skin is more complex than a simple hydrophobic membrane of fixed pore size and silastic membrane does not take into consideration enzyme action or dermal vascularisation. It is also believed triclosan is subject to considerable dermal metabolism, principally to the sulphate and glucuronide forms (Moss et al., 2000).

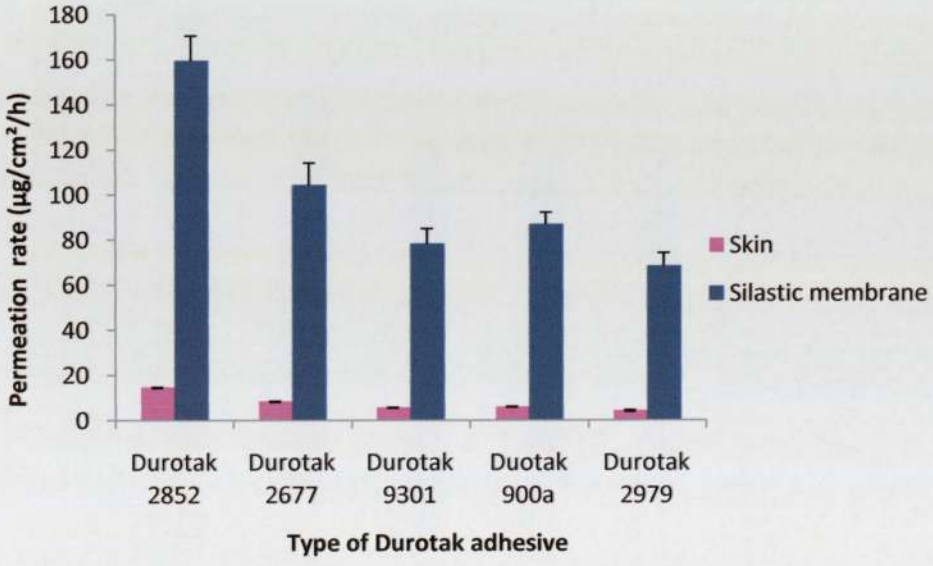


Figure 4.7. *The permeation rates of the various DuroTak® adhesives across skin and silastic membrane of size 3.14 cm² containing approximately 80 mg of triclosan. Receiver media was composed of 1 in 10 60:40 PBS: ethanol.*

4.5 Effect of loading doses on triclosan release

The effects of various loading doses were studied using DuroTak® adhesive 2852 and DuroTak® adhesive 2979.

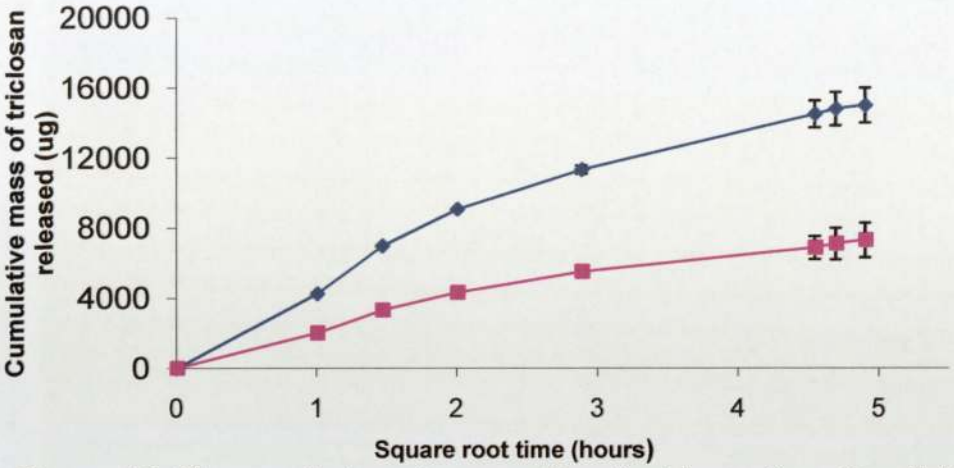


Figure 4.8 *The cumulative release profile of triclosan from DuroTak® patch 2852 with two different loading doses. Each patch was of area 3.141 cm^2 and contained 80 mg of triclosan (blue ◆) and 40 mg of triclosan (pink ■). Release media was composed of 1 in 10 60:40 PBS: ethanol. Results are expressed as the means of three experiments ± S.D.*

Figure 4.8 demonstrates the release of triclosan from DuroTak® patch 2852 with two different loading doses. The data was plotted according to Higuchi's Law (Table 4.6). Release rates and % total dose released were proportional to the increase in the loading dose. This was also shown in Figure 4.7 and Table 4.7 with the DuroTak® adhesive 2979.

Mass of triclosan in Durotak® in 2852 (mg)	Release Rate	% Total dose Released
80	4222.73	18.75
40	2027.12	18.25

Table 4.6 *Release rate and % total dose released from DuroTak® adhesive 2852 loaded with 80 mg and 40 mg of triclosan. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

Mass of triclosan in DuroTak® in 2979 (mg)	Release Rate	% Total dose Released
80	2156.62	9.65
40	1022.21	9.64

Table 4.7 *Release rate and % total dose released from DuroTak® adhesive 2979 loaded with 80 mg and 40 mg of triclosan. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

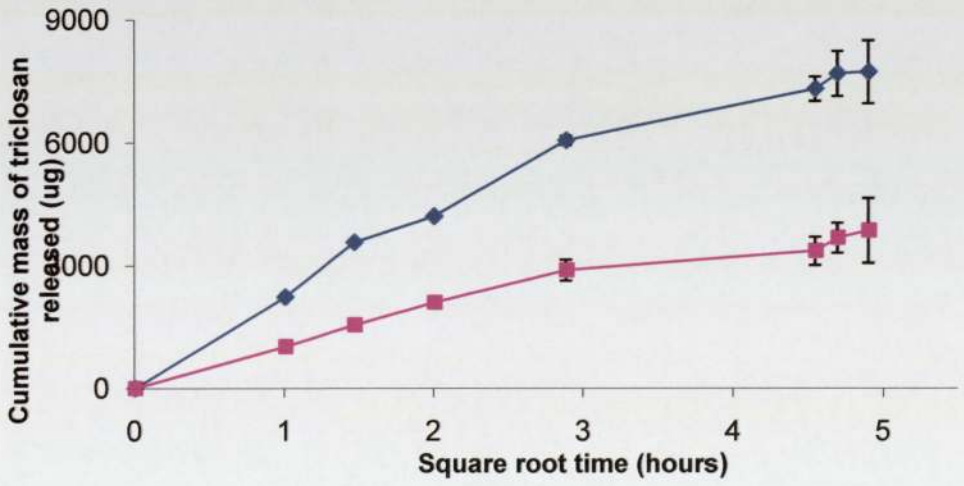


Figure 4.9 *The cumulative release profile of triclosan from DuroTak® patch 2979 with two different loading doses. Each patch was of area 3.141 cm^2 and contained 80 mg of triclosan (blue ◆) and 40 mg of triclosan (pink ■). Release media was composed of 1 in 10 60:40 PBS: ethanol. Results are expressed as the means of three experiments ± S.D.*

It is believed the loading dose may also reflect permeation rates (Bian et al., 2003; Gwak et al., 2002; Heo et al., 2008). Thus, it is likely that by increasing the loading dose with the patches used in these studies the permeation rates would also increase. However, it was noted that adding excess triclosan caused the adhesive mixture to become excessively tacky and difficult to spread, consequently it was not possible to make patches with very high doses of triclosan. Furthermore, crystals began to appear and triclosan reached its saturated solubility in the matrix, this problem was also discovered by Zhao et al., (2002) and Zhao et al., (2009); these studies demonstrated a decrease in permeation due to the alteration of polymer structures.

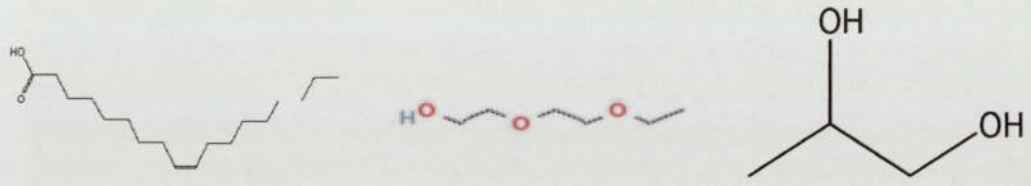
4.6 The effect of skin penetration enhancers on the release and permeation of triclosan from DuroTak® adhesive 2852

The human skin is an extremely efficient barrier for topical delivery of therapeutic agents. The intercellular lipid bilayer in the stratum corneum is responsible for this barrier property. Using skin permeation enhancers is a safe and effective method of reducing the barrier function of the skin (Zhao et al., 2009). Penetration enhancers interact with the skin to promote drug flux. There are a number of mechanisms whereby percutaneous absorption can be improved:

- Reduction of skin resistance as a permeability barrier by disruption of tightly packed lipid regions of stratum corneum. (Barry, 1987)
- Increased skin/vehicle partitioning of the drug (Green et al., 1988)

- Increased solvent transport into or across the skin (Yamada et al., 1987)
- Increased drug solubility in the vehicle (Aungst et al., 1990)

The three enhancers selected for further investigation were transcutol, propylene glycol and oleic acid. Oleic acid (Figure 4.10) is a long chain fatty acid with a *cis* configuration believed to disrupt the lipid domains of the stratum corneum. It is believed propylene glycol and transcutol (Figure 4.10) are solubilising agents which may alter the thermodynamic activity of the drug in the vehicle; this would vary the driving force for diffusion (Williams and Barry, 2004).



Oleic acid *Transcutol* *Propylene Glycol*

Figure 4.10 *The various skin penetration enhancers used within the patch.*

4.6.1. The effect of skin penetration enhancers on the release of triclosan from DuroTak® adhesive 2852.

In order to further optimize the permeation of triclosan across the human skin, DuroTak® 2852 was selected as the adhesive to be used in these studies since the highest release and permeation rates were achieved with this particular patch. Each patch was made up of one of the enhancers previously mentioned and the release profile plotted according to Higuchi's law (Figure 4.11). The release

profiles of all the patches begin to plateaux similar to previous release profiles observed.

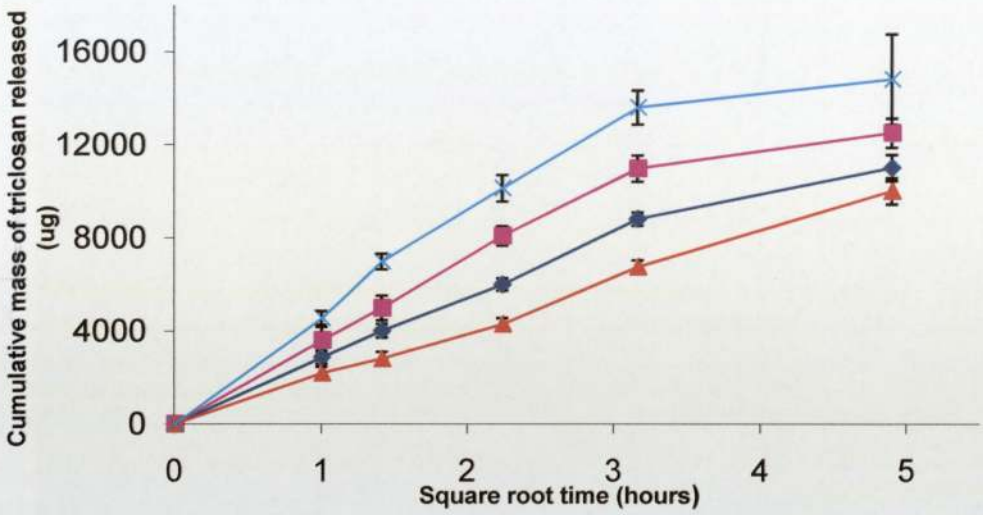


Fig. 4.11 *Effect of enhancers (8.0%, v/w) on release rate of triclosan patches. Each patch of area 3.141 cm^2 contained 80 mg triclosan and 0.5 mL enhancer with DuroTak® 2852 as an adhesive: (light blue x) without enhancer; (pink ■) oleic acid; (red▲) propylene glycol; (navy blue ◆) transcutol. The bars represent S.D., n = 6. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

Enhancer	**Release rate (ug/h)**	**% Total dose Released**
Without enhancer	4633.71 ± 301.17	18.51
Oleic acid	3589.21 ± 112.34	15.62
Transcutol	2742.22 ± 50.69	13.75
Propylene glycol	1969.26 ± 63.49	12.54

Table 4.8 *Release rate and % total dose released from DuroTak® adhesive 2852 with various enhancers. Each patch of area 3.141 cm^2 contained 80 mg triclosan and 0.5 mL enhancer. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

As shown in Figure 4.11 the release studies demonstrated that all formulations were significantly different ($P< 0.05$) from each other. Surprisingly, without the enhancers the release rate and % of total dose released was significantly higher than with any of the enhancers. The order of highest release rates was without enhancer>oleic acid>transcutol>propylene glycol. This trend may be explained by the fact that triclosan is highly soluble in the enhancers themselves. Previous work demonstrated that triclosan is highly soluble in propylene glycol and transcutol (Lee et al., 2003; Kjarheim et al., 2000) thus triclosan may have a higher affinity to stay within the adhesive and is released to a lesser extent. Gwak et al., (2002) also showed that the release rate of ondansetron hydrochloride from DuroTak® matrix 2100 and 2196 demonstrated a decrease; it was believed this was probably due to the addition of the co-solvent diethylene glycol monoethyl ether (DGME) which increased the solubility and thereby decreased the diffusion within the adhesive matrix. Cho and Choi (1998) also suggested that ketoprofen the drug of interest; demonstrated low fluxes from the DuroTak® matrix this was also believed to be due to decreased fluidity and high solubility in the acrylic adhesive matrix.

Oleic acid does not possess the same mechanism of action as transcutol and propylene glycol (Cooper, 1984; Williams and Barry, 1992; Alberti et al., 2001 and Valjakka-Koskela et al., 2000). Therefore, it is highly unlikely that the release rate is lower for the adhesive patch containing oleic acid compared without the enhancer due to the high solubility of triclosan in this enhancer. It was noted that the addition of enhancers to the adhesives appeared to visually

change the structure of the polymers within the adhesive. This finding was also observed in several other works; Bian et al., (2003) found that addition of more than 1% dodecylamine (enhancer) changed the polymer into a glue-like state which may possibly reduce the effect of enhancer activity. However, in the studies here it was difficult to measure whether there was a real change in polymer structure.

4.6.2 The effect of skin penetration enhancers on the permeation of triclosan from DuroTak® adhesive 2852 across human epidermal membrane.

Figure 4.12 demonstrates the permeation of triclosan from DuroTak® 2852 and the incorporated enhancers across human skin. In this study silastic membrane was not used as a model membrane as the mode of action of one of the enhancers is directly involved with the lipid structure of the skin.

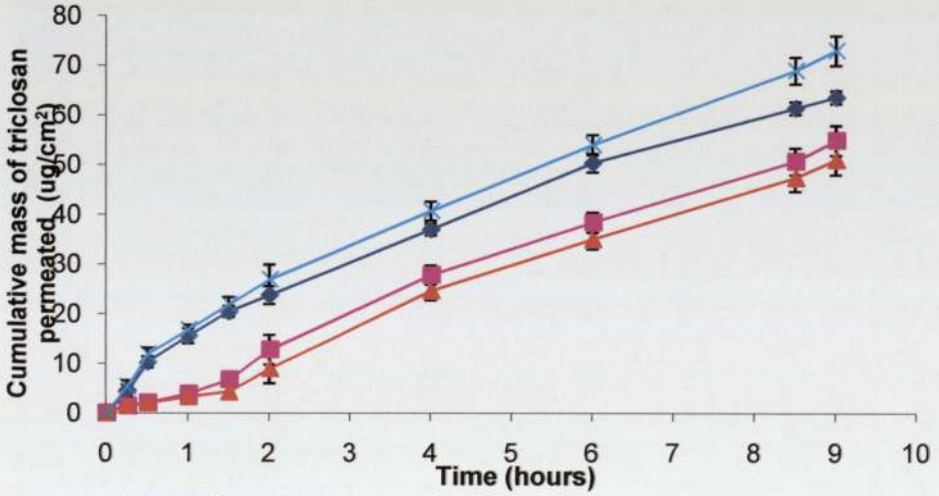


Figure. 4.12 *Effect of enhancers (8.0%, v/w) on human skin permeation of triclosan from pressure- sensitive adhesives. Each patch of area 3.141 cm^2 contained 80 mg triclosan and 0.5 mL enhancer with DuroTak 2852 as an adhesive: (navy blue ◆) without enhancer; (light blue x) oleic acid; (red▲) propylene glycol; (pink■) transcutol. Results are expressed as the means of six experiments ± S.D. Receiver media was composed of 1 in 10 60:40 PBS: ethanol.*

Enhancer	Permeation rate ($ug/cm^2/h$)	% Total dose permeated
Without enhancer	13.23 ± 0.17	0.25
Oleic acid	14.64 ± 0.24	0.29
Transcutol	5.39 ± 0.23	0.22
Propylene glycol	3.85 ± 0.21	0.20

Table 4.9 *Permeation rate and % total dose permeated across human membrane from DuroTak® adhesive 2852 with various enhancers. Receiver media was composed of 1 in 10 60:40 PBS: ethanol. Each patch of area 3.141 cm^2 contained 80 mg triclosan and 0.5 mL enhancer n=3.*

The linear profiles indicate sink conditions and the small error bars indicate good reproducibility between the patches and maintenance of the human skin integrity. There was no major burst release from the permeation profiles. There is a slight lag time with the adhesives containing propylene glycol and transcutol.

The results demonstrate that permeation rates are highest when patch 2852 is combined with the enhancer oleic acid, following that the adhesive without an enhancer provides a slightly lower permeation rate ($P<0.5$) and then propylene glycol and transcutol respectively. Thus, in this case the release rates are not entirely dependent on the permeation rates.

As previously noted in the release studies (Section 4.6.1) triclosan is highly soluble in transcutol and propylene glycol. Thus, the release of triclosan from the adhesive is lower when compared with the other adhesives and consequently the permeation rates are also lower. In contrast to my studies Zhao et al., (2002)

demonstrated that by adding solubilisers to DuroTak® adhesives the skin permeation rate increased. It was also found that without a solubilizer crystal formation in the matrix was rapid and severe.

It is assumed oleic acid does not dissolve triclosan as well as propylene glycol and transcutol. It is a fatty acid which has a potent skin permeation enhancing effect with various drugs (Cooper, 1984; Williams and Barry, 1992; Alberti et al., 2001; Valjakka-Koskela et al., 2000; Francouer et al., 1990; Santoyo et al., 1995; Gwak and Chun, 2002). It is believed the chain length of the saturated fatty acids affects the enhancement of skin permeation (Tanojo et al., 1997). This effect appears to involve the disruption of the lipid bilayer that fills the extracellular spaces of the stratum corneum to allow substances to travel through the skin more readily. Therefore, although the triclosan is still soluble in oleic acid the specific enhancer action increased the permeation activity when compared with studies without an enhancer. Conversely, Bian et al., (2003) found that the inclusion of oleic acid in DuroTak® patches tested did not show a potent increase in skin permeation of the drug being tested, however a different type of DuroTak® patch was used in this study. This was also observed by Gwak et al., (2002) where the addition of oleic acid did not affect the permeation flux of ondansetron hydrochloride from the transdermal system. Additionally, recent literatures suggest that oleic acid is not effectively released from the adhesive (Funke et al., 2002; Qvist et al., 2002).

4.7 Correlation between permeation and release rates from DuroTak® adhesives

The release rates of triclosan were compared with the permeation rates of the drug from the adhesives. Figure 4.13 demonstrates a good linear relationship between the release rates and skin permeation rates of each of the different types of adhesives when using both silastic and human skin membrane; implying that within this study the release rate is the main rate limiting process when looking at skin permeation. Similar findings were also demonstrated by Zhao et al., (2005) and Chedzgoy et al., (2002) suggesting that the efficiency of triclosan permeation from the adhesives is mainly determined by the release rate. The high lipophilicity of triclosan enables partition of triclosan into the stratum corneum and therefore the greater the release from the patch the larger is the concentration gradient across the skin and higher permeation rates are observed. There is however a study demonstrating that although the release of a certain DuroTak® adhesive was the highest from the range of adhesives tested the skin permeation rate was much lower than the other adhesives. This was however believed to be due to the low skin adhesion ability of this patch. This did not seem to be apparent with the patches used in the above studies (Zhao et al., 2005).

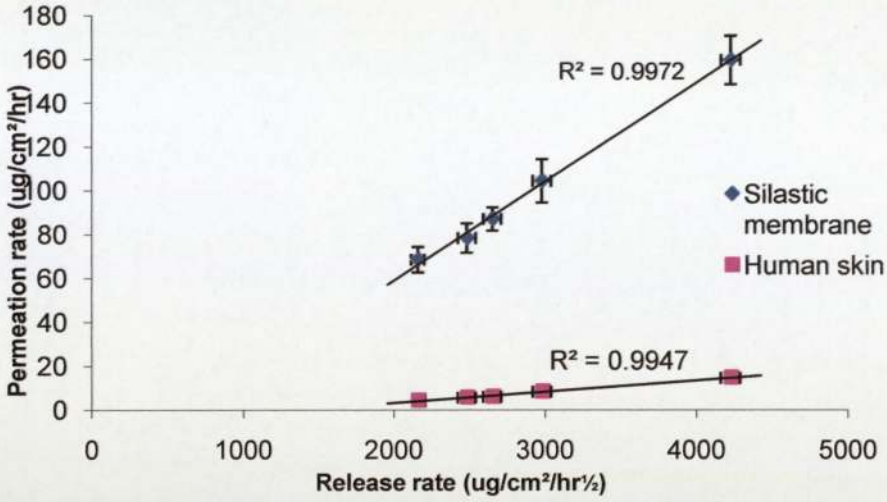


Figure 4.13 *Relationship between the release and the permeation rate of triclosan. each 3.141 cm^2 patch contained (approx) 80 mg of triclosan in each of the different type of adhesives (n=5, mean ± S.D).*

4.8 Conclusion

In order to overcome the problem associated with current skin disinfection techniques where microbial repopulation occurs rapidly, a formulation generating extended release of an antimicrobial was required. Topical adhesive patches were selected for investigation as they have the potential to release a drug over a long period of time and they are less likely to be washed away from the skin surface. Polymeric adhesives containing triclosan embedded in a solubilised form were developed and prepared, consequently release and permeation profiles were generated. The adhesives used were supplied from a company called DuroTak®. Various adhesives with different physicochemical properties were investigated and an appropriate adhesive was selected for optimisation of triclosan permeation by incorporating an enhancer to the matrix.

Release studies demonstrated that the adhesives being investigated followed Higuchi's law suggesting that the release rate of a drug from the matrix was dominated by the solubility of the drug and the diffusion coefficient of the polymer. Permeation studies show the absence of a lag time suggesting that the rate limiting factor is not due to the partitioning of triclosan into the model membrane as is prevalent in most permeation studies; this observation is probably due to the lipophilicity of triclosan. In addition the relative order of release from the different DuroTak® adhesives demonstrate parallel trends with the permeation rates of the same adhesives; this is also demonstrated in the form of a scatter graph (Figure 4.12) where a strong linear relationship was represented between the release and permeation rates of each of the different adhesives when using both silastic and human skin membrane. From this finding it was concluded that the release of triclosan from the adhesives is the key factor when considering the permeation of triclosan across the skin.

Permeation values were a lot lower across human epidermal membranes when compared with rates seen with silastic membrane. This finding is probably as a result of the complexity of the human skin when compared with the reproducible silastic membrane of fixed pore size.

Skin permeation enhancers were added to the adhesive which yielded the greatest permeation rate to improve the permeation of triclosan across the skin further. Oleic acid was selected as it reduces the barrier function of the skin by disrupting lipid domains in the stratum corneum. Propylene glycol and transcutol were selected as further enhancers to be examined as they are solubilising agents and

are therefore able to alter the thermodynamic properties of the drug within the matrix. Surprisingly, the matrix without an enhancer provided the greatest release rate followed by the adhesives containing oleic acid, transcutol and propylene glycol respectively. This pattern of release was thought to be due to the high solubility of triclosan in propylene glycol and transcutol. It was probable that due to the high solubility of triclosan in these enhancers the triclosan has a higher affinity to stay within the matrix and is released to a lesser extent.

The adhesives containing enhancers were then tested across human epidermal membrane to reveal the relative order of the permeation rates for the different adhesives as oleic acid>without enhancer>propylene glycol>transcutol. This trend did not reflect the release rate trend and it was concluded that the specific mechanism of action of oleic acid took place as the permeation process was occurring across the actual stratum corneum, thus it is likely that oleic acid causes the disruption of the lipid bilayer increasing the permeation rate of triclosan. As previously suggested with the release studies the solubilising enhancers are causing the triclosan to remain within the matrix subsequently the permeation rates are also lower.

From these studies it can be concluded that oleic acid was the most effective enhancer in allowing permeation of triclosan from a specific DuroTak® adhesive and this formulation could be used in future studies focused on topical disinfection.

Chapter Five:

Formulation of a drug delivery system using silicone elastomers

5.0 Introduction

There is a particular problem with sepsis and associated catheters (Bayston et al., 2009). It is believed organisms which live on the skin gain access via the external route of the catheters or at the time of insertion. Catheter material is also prone to surface formation of a microbial biofilm (Malcolm et al., 2004). Prevention of skin infection and biofilm formation may be overcome by the use of controlled delivery of an antimicrobial agent around the dermal area of catheter insertion or incorporated within catheter materials. Attempts have been made to develop catheters coated with antimicrobials; however they have only shown short term activity (Malcolm et al., 2004; Bayston et al., 2009). Thus, the incorporation of an antibacterial which provides a local drug delivery system ensuring high concentrations around the implant for long periods of time is required.

Owing to their excellent biocompatibility, relatively low encrustation rates compared to other polymeric biomaterials, and good thermal and mechanical stability, silicone elastomers are widely used in the manufacture of medical devices (McBride et al., 2009). Triclosan's lipophilicity renders it ideal to reach the lower lipidic regions of the skin. In addition the hydrophobic nature of triclosan (log P = 4.8) coupled with its low molecular weight (289.5 g/mol) suggests the potential of this to be incorporated into polydimethylsiloxane-based (PDMS) silicone elastomer materials to circumvent the above problem. The silicone elastomer being used in these studies was MED-6382 by Nusil® as this is a suitable silicone material for topical drug delivery. Given the high solubility in the hydrophobic silicone and relatively low molecular weight of triclosan is

thought to allow relatively rapid molecular diffusion based on findings previously published by Malcolm et al., 2004 (Figure 5.1) which allows the diffusion coefficient of any drug in the MED – 6382 silicone elastomer to be predicted based solely on the knowledge of its molecular weight (Malcolm et al., 2004).

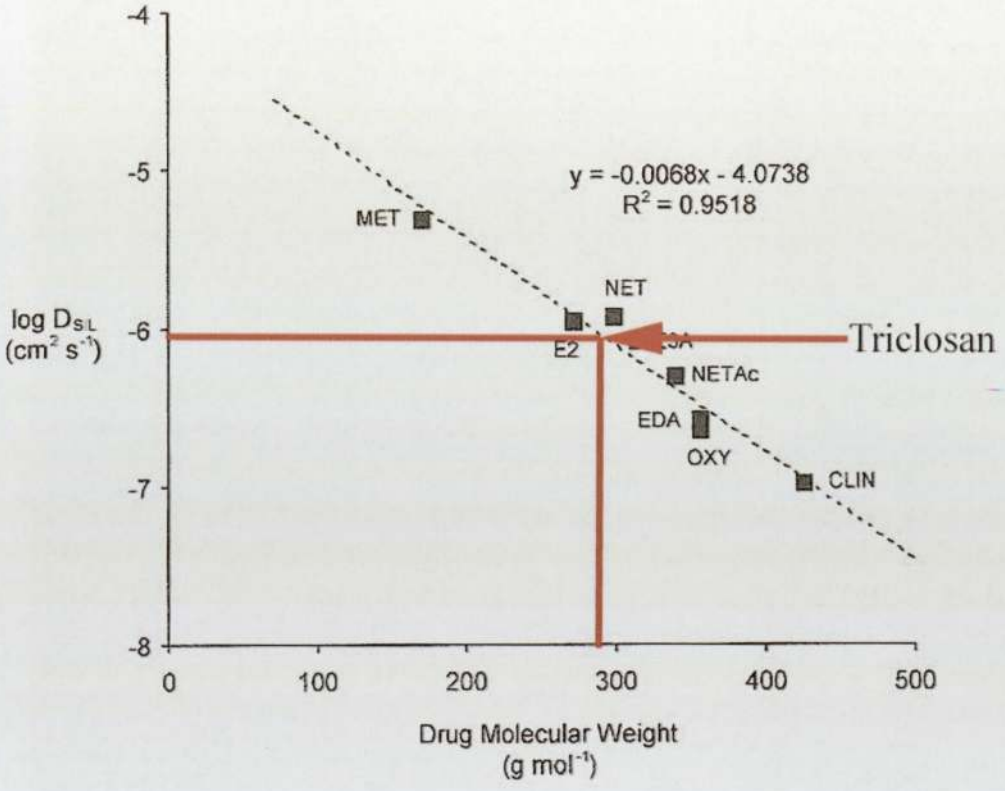


Figure 5.1 *Graph predicts the diffusion coefficient of any drug in MED – 6382 silicone elastomer from the molecular weight taken from Malcolm et al., (2004).*

Silicone was originally investigated as a carrier material for lipophilic drugs, however a range of drugs have been subsequently been incorporated and Figure 5.2 demonstrates a simplistic theory on the type of drug release mechanisms from silicone matrixes which are thought to be dependent upon the physicochemical properties of the drug. Depending on the release media it is more probable the test solution will diffuse into the matrix through both hydrophobic and

hydrophilic phases. As suggested by Kajihara et al., (2000) it is most likely the particles of drug powder existing on the surface of the silicone matrix are dissolved and released into the surrounding release media. The release media fills the pores from which particles have been eluted, leading to dissolution and release of the particles that exist near the pores. Repetitive occurrence of this process results in the formation of interconnecting channels and particles of the drug powder which exist inside the silicone formulation are sequentially dissolved and released. As the osmotic pressure is sufficiently increased by dissolution of the drug powder in the aqueous phase, small cracks are generated on the polymer walls. This cracking process contributes to further linking of the interconnecting channels; drug release is accelerated (Kajihara et al., 2001, Carelli et al., 1989, Kajihara et al., 2000 and Golomb et al., 1990).

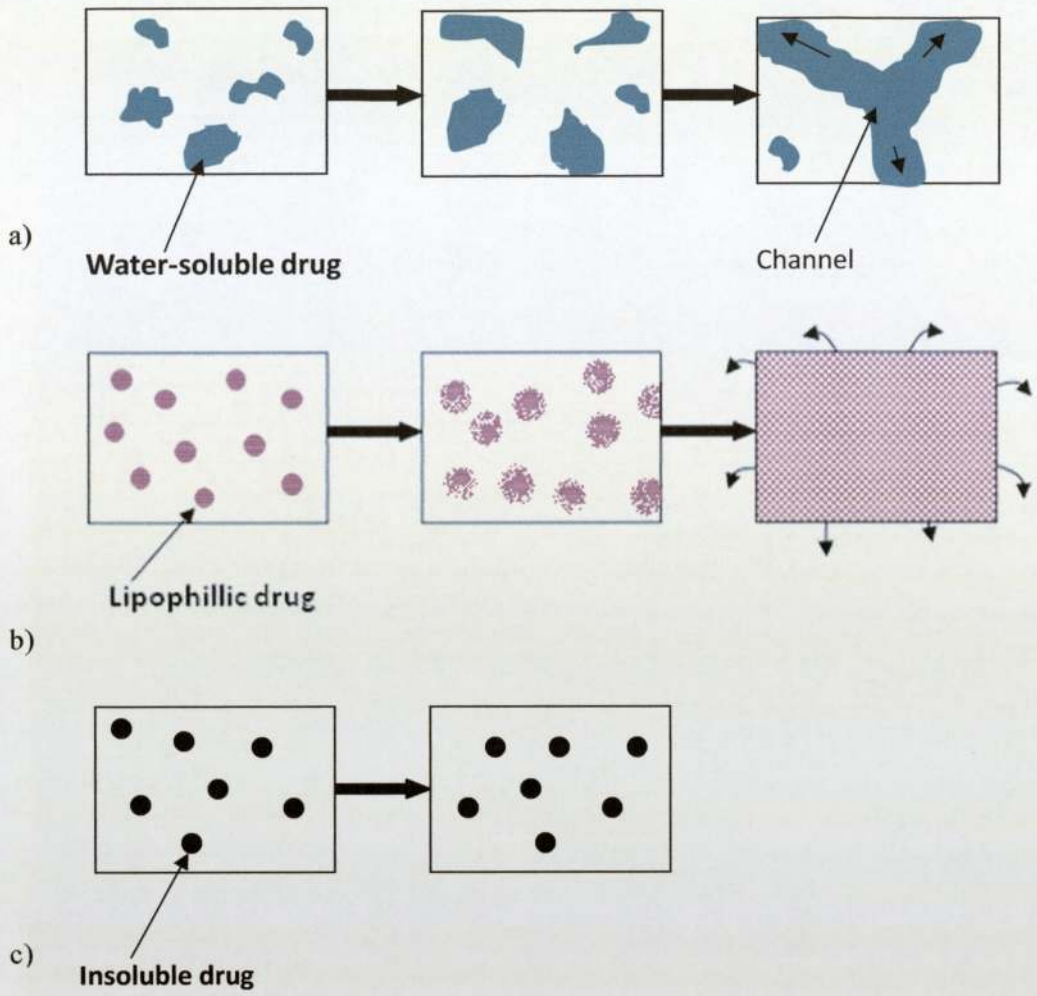


Figure 5.2 *Release mechanism of drugs with different physicochemical properties from a silicone carrier. (a) Water soluble drugs which exist on the surface dissolve into water and repetition of these processes lead to channel formation, allowing water soluble drugs in the bulk to be released. (b) Lipophilic drugs are solubilised in silicone therefore; they diffuse and are thought to be readily released. (c) Insoluble drugs neither dissolve nor diffuse in silicone and it is assumed they are not released due to their insolubility in water. Taken from Kajihara et al., (2003).*

5.1 Aims and objectives

Within this study the aim was to formulate and test a novel drug delivery system that enables a constant release of triclosan around the upper layers of the skin area where catheter insertions are made. The duration of catheterisation is the most important risk factor for development of catheter associated bacteriuria and catheters can be kept in place for approximately 14 days thus sustained delivery of an antimicrobial for this length of time is required. Due to the fact that catheter materials are normally composed of silicone, triclosan was incorporated into silicone elastomers and a drug delivery system was formulated whereby the adhesives experimented within chapter 4 form a lower layer which is attached to the skin and silicone will form the upper layer as shown in Figure 5.15. Both components will contain triclosan with the aim of providing adequate antimicrobial delivery due to the slower release of triclosan from the layer of silicone into the adhesive as well as the permeation of triclosan from the adhesive itself.

Therefore the objectives of this work were:

1. Prepare and incorporate triclosan into silicone elastomer strips.
2. Investigate the effects of additives on the release of triclosan.
3. Characterise the release of triclosan from silicone elastomer strips.
4. Develop and optimise a silicone/adhesive drug delivery system.
5. Characterise the permeation of triclosan from the new drug delivery system across a model membrane.

5.2 Method and materials

Materials, formulation of silicone elastomer systems, characterising silicone matrixes and release/permeation protocols are described in section 2.2.6.

5.3 Results and discussion

5. 3.1 Varying areas of triclosan

Initial studies of the release profile of silicone strips with different areas are shown in Figure 5.3. The final cumulative mass of triclosan for the silicone strip of area 10 cm^2 was 295.67 ug and the silicone strip of area 3cm^2 generated a total cumulative mass of 98.70 ug. These values are comfortably above MIC and MBC values of triclosan. Thus, the area of the silicone elastomer strip is approximately proportional to the final cumulative mass of the drug released. This finding was anticipated as the larger surface area of the elastomer strip is composed of an increased quantity of the drug therefore there is a greater amount of triclosan available for dissolution. Since the elastomer strip of area 3cm^2 provides a sufficient cumulative mass of triclosan to measure and this size poses a suitable area for a topical drug delivery system this area will be utilised in subsequent studies.

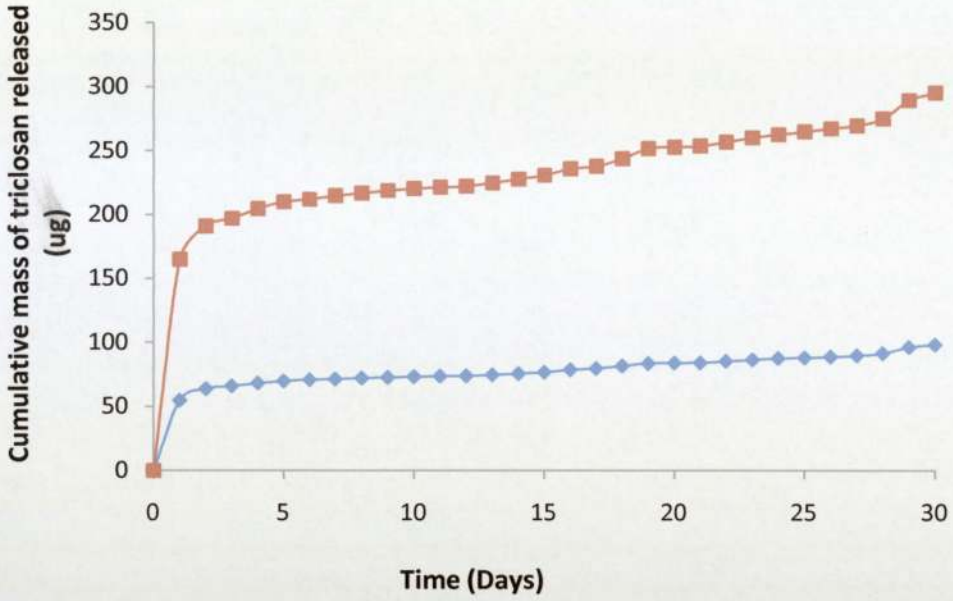


Figure 5.3 *Preliminary study - Release profile from silicone strips of two different areas containing 1.0%, w/w of triclosan in 60:40 PBS: ethanol release media: (red ■) 10cm² silicone strip area; (blue ◆) 3cm² silicone strip area. Release media was composed of 1 in 10 60:40 PBS: ethanol (n=1).*

5.3.2 Evaluation of release profiles in various test solutions

The release of triclosan from the silicone elastomers into various release media was investigated (Figure 5.4). These preliminary studies were mainly carried out for comparative purposes only. In addition, since the silicone strips are dry and non-fluid like it was useful to deduce whether release medium utilised in previous studies (60:40 PBS: ethanol) demonstrated sufficient quantities for further investigations.

Given that various test solutions are believed to affect release patterns due to differences in solubility of the released drug (Maeda et al., 2004 and Maeda et al., 2003), a range of media were tested and Fetal calf serum (FCS), 60:40 PBS: ethanol, and PBS were selected. Phosphate buffer solution is the simplest of basic salt solutions and is a commonly used release media. FCS is blood without cells

and clotting factors, the 60:40 PBS: ethanol composition has been selected due to its use in previous patch formulation studies in chapter 4. It is used to mimic the lipidic nature of the skin and the derivation of the exact composition is described in section 3.5.5.

These release media were investigated to maintain sink conditions over a 24 h release period for 30 days. Silicone elastomer strips cut into areas of 3cm² were immersed into 60: 40 PBS: Ethanol, FCS or PBS test solutions and incubated at 37°C. At a predetermined time points, the test solution was exchanged with fresh media and the triclosan concentration was measured by HPLC under conditions described in section 2.2.6.2.

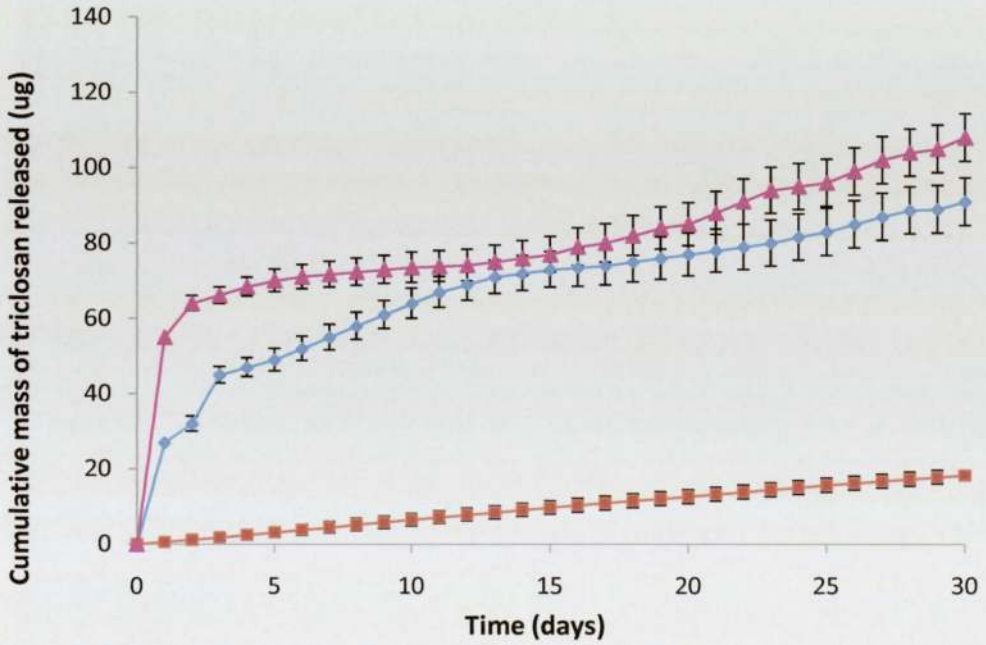


Figure 5.4 *Effect of various release media on the cumulative release profile of triclosan from MED-6382. Each silicone strip of area 3cm² contained 1.0% w/w of triclosan: (red■) 1 in 10 phosphate buffer solution (PBS); (light blue◆) fetal calf serum and (pink▲) 60:40 PBS: ethanol. Results are expressed as the means of four experiments ± S.D.*

Figure 5.4 shows the cumulative release versus time profiles for silicone elastomer strips in the three different release media described above. The average release rates over the first ten days are shown in Table 5.1. The release rate did not differ markedly between FCS and 60:40 PBS: Ethanol with an average release of 2.05ug/day and 1.49ug/day for each respectively. The release profiles also exhibited similar tendencies with an initial burst release over the first few days followed by a slower release rate for both 60:40 PBS: Ethanol and FCS as the release media, however the release rates did not plateau for either release media indicating a reservoir of drug was present over the time period measured.

The total cumulative mass of triclosan released over one month for each of the silicone strips was similar where the release media FCS (91 μg) and 60: 40 PBS: ethanol (98.7 μg) was investigated. FCS presents a complicated release media as it consists of numerous components. The undefined nature of the serum and batch to batch variability in composition also contribute to its complex nature however it was interesting to note that the release profile was similar in both media and this may be attributed to the lipophilic components within the FCS resulting in a similar release environment to that of 60:40 PBS: ethanol.

The initial burst release may be a result of the drug powder existing on the surface of the formulation which is then dissolved and released into the test solution instantaneously. Following this the release media fills the pores from where the particles have been eluted, leading to dissolution and release of particles that exist near the pores thus the release then occurs at a slower rate as suggested by (Kajihara et al., 2000).

In contrast, the release rate (Table 5.1) and the total cumulative mass of triclosan released into the PBS alone (18.32 μg) was significantly lower ($P<0.05$) compared with the release values obtained for FCS and 60:40 PBS: Ethanol. This is most likely due to the lipophilic nature of triclosan, its affinity for a purely aqueous test solution is expected to be very low. Thus, triclosan which has low solubility in PBS displays lower release rates into this media.

It has also been suggested by Maeda et al., (2004) that water does not permeate through silicone membranes therefore channelling does not occur with PBS as the release media which may also contribute to the above release profiles. The release profile of triclosan into PBS also demonstrates the absence of an initial burst release; instead a linear profile is generated and a constant rate of triclosan release is evident throughout the entire 30 day study demonstrating zero order kinetics. This may be attributed to the saturation of triclosan release within this media.

Maeda et al., (2004) examined a methanol/water mixture as a solvent for the release of the lipophilic drug indomethacin from a silicone elastomer. The results confirmed that by using a mixed solvent of methanol and water as a release test solution the overall drug release was accelerated and the period for evaluation of profiles of drug release was reduced. This study suggested that by using a methanol based release media rapid and accurate prediction of profiles of drug release in PBS- based solvent were obtained. This contrasts with the results where the release profiles of the silicone elastomer strips within the ethanol system compared with the PBS display different release kinetics. However,

although both triclosan and indomethacin are both lipophilic actual differences in release profiles may be attributed to differences in solubility of triclosan and indomethacin in the PBS test solution. Maeda et al., (2004) also used 0.3% Tween 20 in the PBS based test solution so as to increase the solubility of the drug. It must also be noted that Maeda et al., (2004) is investigating a covered rod type system where the inner layer is made up of a silicone matrix containing the drug and an outer silicone layer. Furthermore, the aim of the published study was focussed on modelling drug release into the systemic circulation and not across the skin.

Based on the studies in Figure 5.4, the 60:40 PBS: ethanol composition was selected as the appropriate release media for further studies.

Type of release media	**Average release rate over the first ten days (ug/day)**
60:40(1 in 10 PBS: Ethanol)	1. 49 ± 0.26
Fetal Calf Serum	2.05 ± 0.39
1 in 10 PBS	0.62 ± 0.09

Table 5.1 *The average release rate over the first ten days from MED-6382 silicone strips of 3cm^2 with release media* 60:40*(1 in 10 PBS: Ethanol), Fetal Calf Serum and 1 in 10 PBS . Each silicone strip contained 1.0% w/w of triclosan.*

5.3.3 Effect of different loading doses

Silicone elastomer strips containing different amounts of triclosan were prepared to assess whether a sufficient amount of triclosan was released from these strips. These formulations were immersed in release media as previously described to assay the effect of drug incorporation on release. The cumulative release versus time profiles for *in vitro* release of triclosan from silicone elastomers are presented in Figure 5.5 and show the significant effect ($P<0.05$) of triclosan loading upon release behaviour (0.5% - 1.0% w/w). The release rate for all 3 formulations was high during the first two days and characterised by a burst release. Following the burst release the overall release rate tends to decrease with time. Interestingly, both silicone elastomers containing 0.75% and 1.0 % w/w triclosan demonstrate a slight increase in release rate from approximately day 20 onwards. Furthermore, from the release profiles it can be concluded that triclosan is continuing to be released and drug exhaustion is not yet apparent over the time period tested. The release studies were not continued until the entire amount of triclosan was released as the main aim of my studies is to investigate the release of triclosan from a drug delivery system over 14 days. However, release studies took place over a time period of one month.

The total amount of triclosan released over 1 month for 1% w/w triclosan was 98.7μg, for 0.75% w/w the triclosan released was 80.04 μg and 0.5% w/w the total amount of triclosan released was 62.8 μg. The average triclosan release rate over the first ten days of the release study for each formulation is presented in

Table 5.2. The triclosan release rate and the final cumulative mass of triclosan released increased with increasing triclosan content.

As the drug dosage increases the release rate from the silicone elastomer strips is accelerated this finding is expected and in agreement with results collated by Malcolm et al., 2004; McBride et al., 2009 and Kajihara et al., 2000. This is most likely due to a greater quantity of drug available for release and as a greater quantity of triclosan is released further pores are developed possibly causing the development of a pore network allowing greater dissolution (Figure 5.2).

The plateaux type profile generated by the matrix containing 0.5 % triclosan w/w could be attributed to the exhaustion of triclosan release within the elastomer and/or interconnecting channels may not yet have been formed among the particles as the triclosan content is lower and particles are isolated from each other. A similar conclusion was published by Golomb et al., (1990) they also implied that a further reason for the increase in release rate throughout the release profile with higher dosage elastomers may be due to more pores being generated in matrices thus creating a network of canals which leads to relatively faster release rates.

The drug is not released at a constant rate regardless of time thus there is no presence of zero order release occurring. Although the release rate and final cumulative mass of triclosan has shown an increase with increasing loading dose a proportional trend has not been demonstrated therefore typical matrix-type-diffusion-controlled release characteristics was not evident, conversely Malcolm et al., (2004) demonstrated this type of release with silicone elastomer release

studies. It may be concluded that the dissolution rate from the silicone matrix is not entirely governed by the amount of drug powder it holds but may also be due to other factors such as the rate of pore development.

When comparing the release rates with patches formulation seen in chapter four the release values generated from the silicone elastomer strips are much lower. This is expected as the doses used are lower and silicone is a dry formulation whereas the patch formulations are tacky adhesives from which triclosan would readily dissolve from. Based on these release data, a loading of 1% w/w triclosan was selected for further study as this provided the greatest concentration of triclosan released.

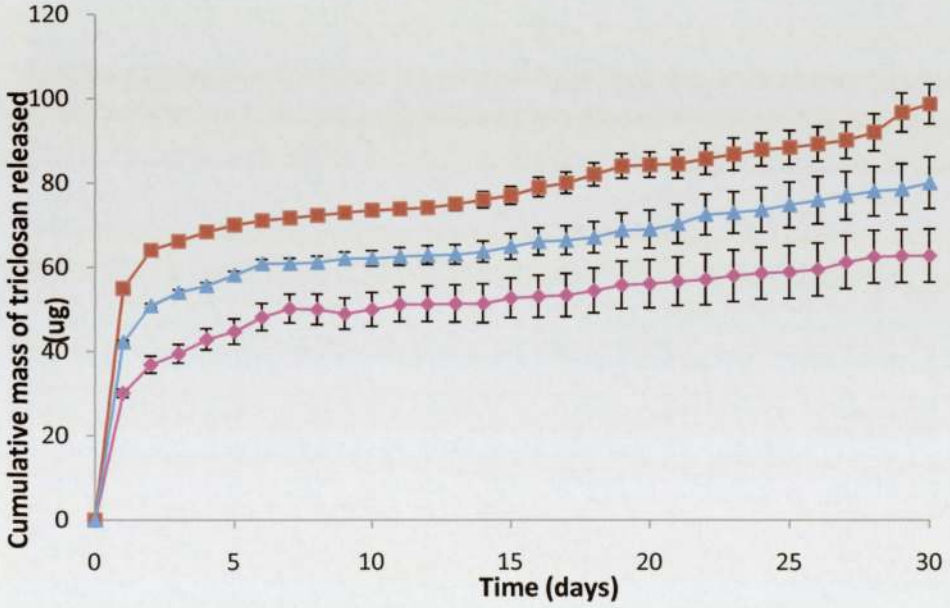


Figure 5.5 *The cumulative release profile of triclosan from MED-6382 with three different loading doses. Each silicone strip was of area 3cm^2 and contained 0.50% (pink ◆), 0.75% (blue ▲) and 1.0% (red■) w/w of triclosan. Release media was composed of 1 in 10 60:40 PBS: ethanol. Results are expressed as the means of four experiments ± S.D.*

Type of silicone strip	Average release rate over the first ten days (ug/day)
1% Triclosan	1.49 ± 0.09
0.75% Triclosan	1.27 ± 0.25
0.5% Triclosan	1.06 ± 0.32

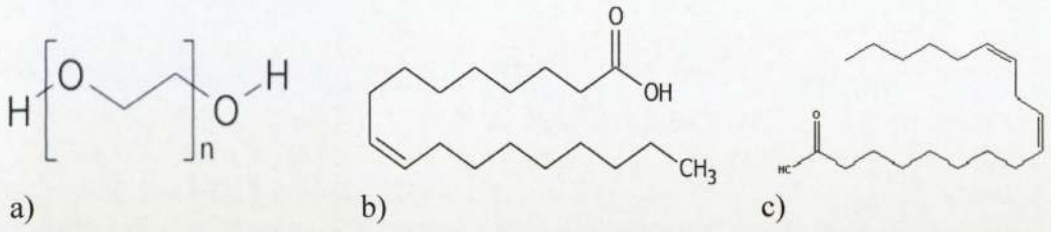
Table 5.2 *Average release rate over the first ten days from MED-6382 loaded with 0.50%, 0.75% and 1.0% w/w of triclosan. Each silicone strip was of area 3cm^2. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

5.4 Further studies

5.4.1 Influence of additive on release behaviour

When designing sustained release formulations information on factors controlling release characteristics play an important role in improving the efficiency of the formulation under study. The release rate can be controlled by changing parameters such as the inclusion of various additives: additives may alter the dispersion of a hydrophobic drug in the silicone film. For example Kajihara et al., (2001) showed that by adding glycine to a silicone elastomer the increase in osmotic pressure resulted in an increased release rate of interferon. Similarly, Maeda et al., (2003) demonstrated that the use of polyethylene glycol (4000) and desoxycholate sodium (DOC) also included the release rate of the model drug ivermectin. Based on these studies the three different additives selected to enhance triclosan release. The three different additives selected to enhance triclosan release:

1. Oleic acid
2. Linoleic acid
3. Polyethylene glycol 200 (PEG 200).



a) b) c)

Figure 5.6 *(a)PEG 200 (b) Oleic acid (c) Linoleic acid*

Oleic acid has previously been used with the topical pressure sensitive adhesives in Section 4.4 and it is thought to enhance the permeation of a drug across the skin via disruption of the lipid structure of the skin. PEG 200 was specifically selected as an additive in these studies as it would be interesting to investigate the effect of admixing a water carrier with the hydrophobic silicone matrix. In addition, the incorporation of PEG moieties is believed to reduce biofilm formation on polymeric medical devices via modification of the surface through chemical grafting. It is considered that the PEG chains fold over themselves and share water bound molecules between the folds, thereby creating an exclusion volume that can repel proteins, cells and microorganisms. Furthermore, it is believed that the high conformational mobility of the PEG molecules leads to decreased opportunities for substances to come into contact with the biomaterial surface (McBride et al., 2009). The final additive to be investigated was linoleic acid. A recent study has revealed that by including linoleic acid a change in the internal morphology of the silicone elastomer was observed and it also

demonstrated a more uniform distribution of drug throughout the silicone elastomer thereby reducing large burst release profiles (Brook et al., 2008).

Release tests were conducted for silicone elastomer strips containing various additives. Preliminary tests were carried out to determine an appropriate quantity of additive to the silicone elastomer mix as described in section 2.2.2.6.

The cumulative release profiles of triclosan from the various silicone strips and their additives are shown in Figure 5.7 The release of triclosan from the silicone elastomer strips increased in the order PEG>linoleic acid>oleic acid>without additive, with the total cumulative mass of triclosan on day 30 being 154.21 μg, 125.63 μg, 109.99 μg and 98.7 μg respectively. The average release rates of each of the matrices over the first ten days are shown in Table 5.3. It can be seen that with the inclusion of additives the release rates were accelerated for all of the formulations with each of the silicone strips release profiles characterised by a rapid initial burst release as previously seen in earlier silicone release studies.

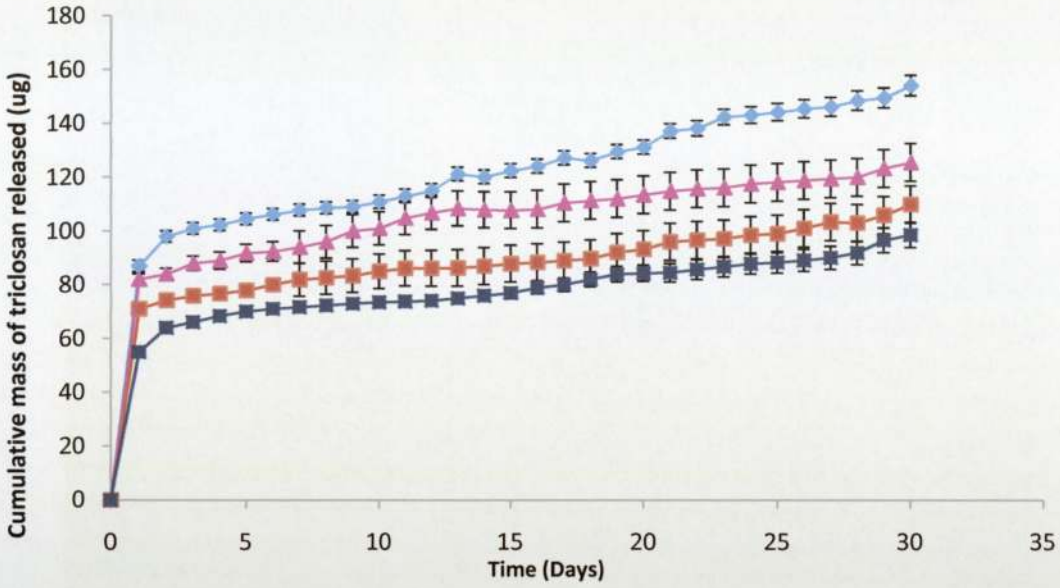


Figure 5.7 *Effect of enhancers (2.0 v/w) on the cumulative release profile of triclosan from MED-6382. Each silicone strip of area 3cm^2 contained 1.0% w/w of triclosan: (dark blue■) without enhancer; (light blue◆) linoleic acid; (pink▲) PEG and (red■) oleic acid. Release media was composed of 1 in 10 60:40 PBS: ethanol. Results are expressed as the means of four experiments ± S.D.*

Type of silicone strip	Average release rate over the first ten days (ug/day)
Linoleic acid	2.61 ± 0.09
PEG 200	1.87 ± 0.23
Oleic acid	1.56 ± 0.19
Without enhancer	1. 49 ± 0.08

Table 5.3 *The average release rate over the first ten days from MED-6382 silicone strips of 3cm^2 with various enhancers. Each silicone strip contained 1.0% w/w of triclosan and 2.0 v/w of enhancer. Release media was composed of 1 in 10 60:40 PBS: ethanol.*

McBride et al., (2009) incorporated PEG moieties into a silicone elastomer and the study demonstrated an overall decrease in release rates and an extended duration of release of triclosan as PEG concentrations increased. These trends did not appear to parallel with the results seen from the studies presented in Figure 5.7 whereby the release rates were accelerated with the use of PEG 200. It was believed the observations made by McBride et al., (2009) were the consequence of the concentration dependent influence of the hydrophilic PEG moieties on the hydrophobic character of the silicone elastomer materials which influences the solubility of triclosan within the elastomer and ultimately the permeation rate. On the other hand, Brook et al., (2008) revealed similar findings to the studies in Figure 5.7, demonstrating that by incorporating PEG 4000 into formulations of silicone elastomers containing ivermectin (hydrophobic drug) release rates accelerated by four-fold compared to silicone formulations without PEG. Carelli et al., (1989) also suggests that release from silicone polymers was promoted by admixing water carriers such as glycerol or PEG derivatives. This is further supported by conclusions reported by Brook et al., (2008) where the inclusion of the polar excipient poly (ethylene oxide) in a silicone elastomer system also led to a higher rate of drug release. It was postulated by Di Colo et al., (1986) that it is the formation of aqueous pores generated by the additive PEG 200 which enhances the release of drugs within the silicone matrices.

Riggs et al., (1997) investigated the influence of additives on the water uptake of hydrosilanised silicone rubbers. Hydrophilic agents formed solution droplets; it is believed the hygroscopic properties of the additive are responsible for the growth of droplets which consequently affects the elastomer elasticity causing internal

plastic deformation of the material. Once the osmotic force is too great to be restrained by the material crack formation occurs this may also give rise to a crack network within the material which can act as a pathway for diffusion within the material hence further raising the diffusion coefficient. Cracking also increases the rate of water uptake which in turn causes further swelling and strain on the internal structure of the silicone matrix.

The elastomer containing PEG 200 as the additive demonstrates the greatest release rate (Figure 5.7). Simultaneously, this matrix is the only elastomer containing a hydrophilic additive thus it is possible the formation of aqueous droplets causes the elastomer to swell which may involve formation of a crack network contributing to the high release rates.

In general drug release depends greatly on the solubility of the drug (Maeda et al., 2003). The process of permeation in silicone elastomer systems is governed by solubility and diffusional factors and release is enhanced by either increasing solubility of the drug or by decreasing the diffusional resistance in the system for the solubilised drug molecule or through a combination of both mechanisms (Malcolm et al., 2004; Maeda et al., 2003; Brook et al., 2008). The addition of certain excipients is therefore thought to alter the solubility of the drug and change the patterns of release. However, Malcolm et al., (2004) describes the solubility within silicone systems as a complex multicomponent process which may possibly combine to produce microenvironments with very different solubility/permeation characteristics. For example the drugs may be present as:

(1) Discrete solid drug particles dispersed throughout the silicone elastomer,

(2) Molecularly dissolved within the silicone elastomer,

(3) Dissolved within the silicone elastomer,

(4) Dissolved within the additive component that is dispersed within the silicone elastomer,

(5) Dissolved within an additive component which is itself dissolved within the silicone elastomer depending on the relative concentration of each component in the system.

The increase in solubility of triclosan within the elastomers containing oleic acid and linoleic acid compared to silicone alone is largely due to these additives being lipophilic therefore increasing the solubility of triclosan and for this reason release rates of these two elastomers are greater than silicone matrices without any additives. These high solubility additives may also cause internal plastic deformation of the material increasing the osmotic force leading to crack formation.

There was very little difference in release rates between silicone matrices containing linoleic acid and oleic acid. This is expected as the two additives possess very similar structures. However, the elastomer composed of linoleic acid yielded slightly higher release values compared with the elastomer made up of oleic acid. It is believed that both linoleic acid and oleic acid facilitate a more uniform distribution of drug throughout the elastomer (Brook et al., 2008). This

was not necessarily apparent with the findings presented as there was still evidence of a burst release for both of these elastomer strips.

It is not entirely clear why the elastomer containing linoleic acid as the additive provides a greater release rate compared with the matrix containing oleic acid. Brook et al., (2008) suggests that the combination of nicotine the drug being studied and linoleic acid led to completely different internal morphologies of the silicone elastomers. This may have affected the physical properties of the polymer i.e. hydrophobicity and elasticity. Additionally, the kinetic profile for the drug is changed such that the magnitude of the burst diminishes; and the release profiles were more extended. This was not apparent with the results generated for this study this may however be due the differences in nature between the two model drugs.

5.4.2 Swelling measurements

Swelling studies were undertaken where the various silicone elastomer strips were immersed in release media and their areas were measured to observe the effect of increasing triclosan doses and the inclusion of additives within the strips. The swelling properties of the strip may be responsible for an explanation of release rates of the various test specimens. Size changes of the silicone strips were measured and the areas of silicone strip were calculated. Figure5.8. shows the area of silicone strips with varying triclosan amounts over a specific time period. For each of the different loading doses, the greatest increase in swelling was seen between the time intervals 1 week and 1 month for each of the different loading doses (Figure 5.8). Figure 5 .9 demonstrates the area of the silicone strips

with the various additives (1% w/w triclosan) before and after swelling. The overall percentage increase in swelling for silicone strips with varying loading doses of triclosan and also various additives with 1% w/w of triclosan is represented in Figure 5.10.

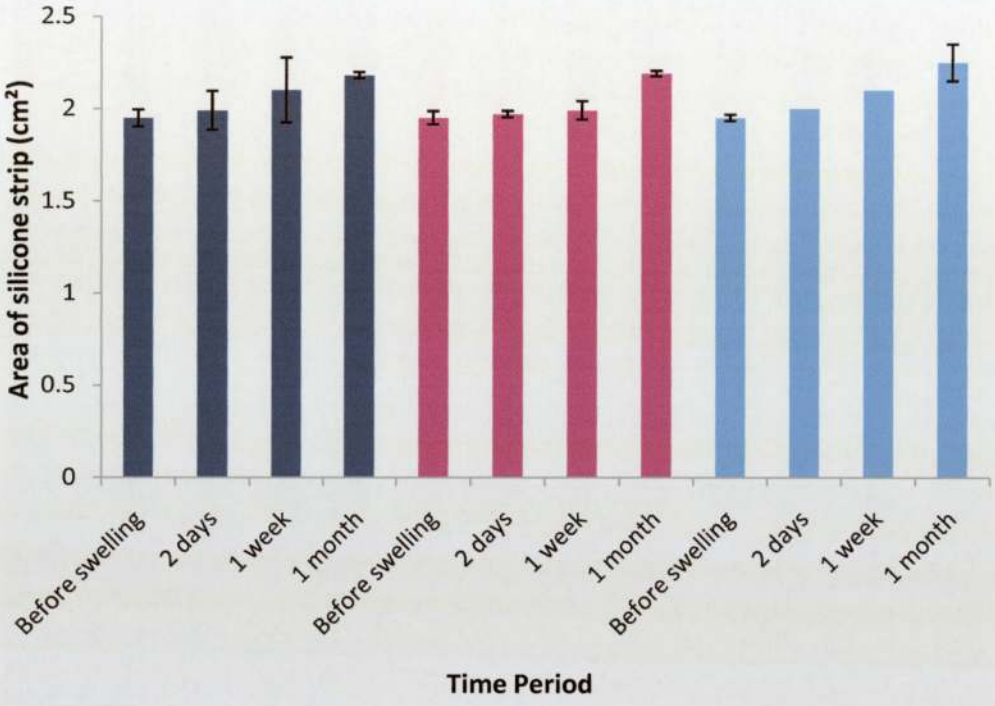


Figure 5.8 *Swelling of silicone strips before release test, 2 days, 1 week and 1 month after release test. No triclosan (navy blue), 0.5% w/w triclosan (pink) and 1% triclosan w/w (light blue). Results are expressed as the means of six experiments ± S.D.*

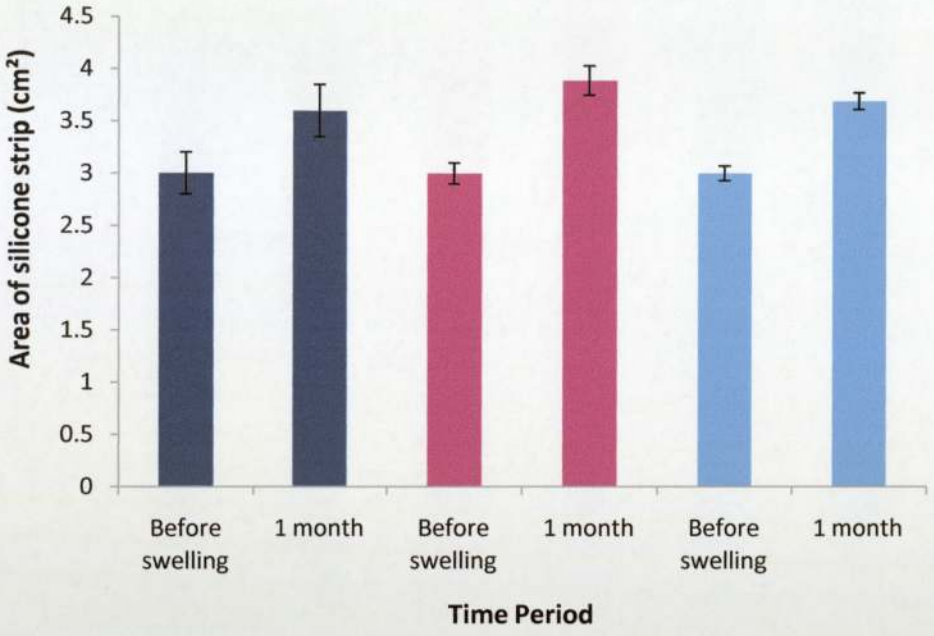


Figure 5.9 *Swelling of silicone strips containing enhancers before release test and 1 month after release test. Oleic acid (navy blue), PEG (pink) and linoleic acid (light blue). Results are expressed as the means of six experiments ± S.D.*

There appears to be a slight overall increase in swelling as the dosage of triclosan within the strip increases (Figure 5.8). The silicone strips containing additives demonstrated a greater increase in % swelling when compared with the elastomer strips without additives. Additionally, the elastomer composed of PEG 200 as an additive reveals a significantly higher ($P<0.05$) % increase in swelling compared with all of the elastomer strips investigated. The overall percentage increase in swelling increased in the order PEG 200> Linoleic acid> Oleic acid> 1% triclosan > 0.5% triclosan > No triclosan, this trend correlates with the average release rate of triclosan from the silicone elastomers (Figure 5.10).

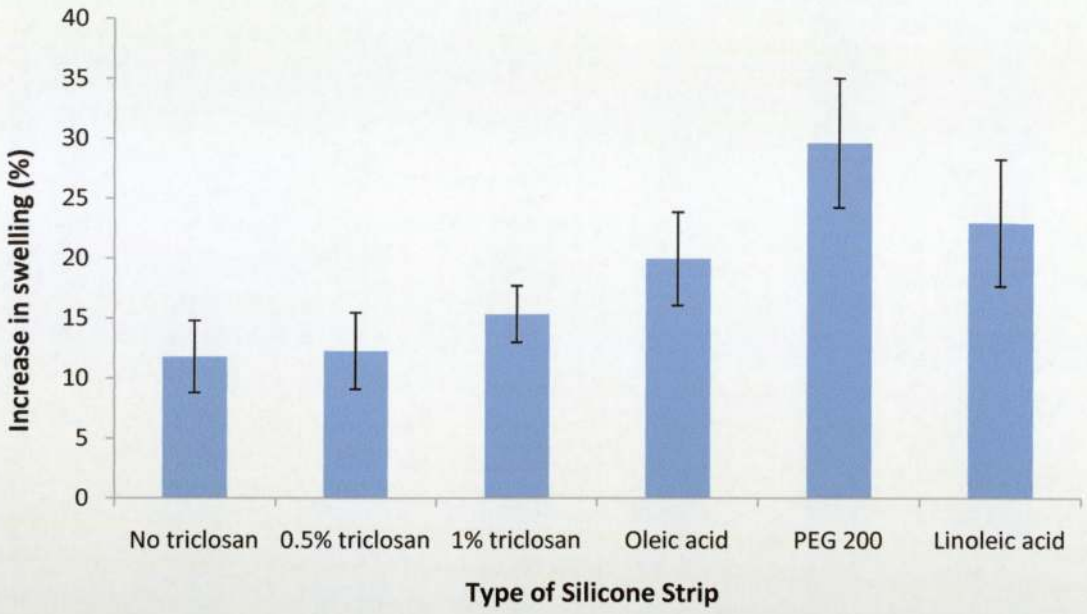


Figure 5.10 *Overall percentage increases in swelling for silicone strips with varying loading doses of triclosan and also various additives with 1% w/w of triclosan. Results are expressed as the means of six experiments ± S.D.*

From Figure 5.8 it is clear that swelling continues to occur for the silicone strips with various dosages of triclosan after one week to a month as the area of silicone strips show an increase in swelling. Thereby, suggesting that release media is continuing to diffuse into the silicone strips at 4 weeks.

Although swelling was evident with silicone elastomers, the overall % increase in swelling was relatively small. It is believed that plain silicone based elastomers undergo practically no swelling in water due to their high hydrophobicity Golomb et al., (1990). Thus, there was little % increase demonstrated with the elastomers without triclosan. Increasing the drug load demonstrated a higher degree of swelling. This observation is further demonstrated by Hsu and Langer (1985) it is cited that by increasing the drug load a higher degree of swelling is achieved. Thus, it is believed that as the quantity of solid drug particles within the

elastomers are increased swelling also increases as the entrapped drug particle is the driving force for the swelling (Golomb et al., 1990).

Silicone matrices containing additives demonstrated a greater % increase in swelling when compared with elastomers without additives. The hydrophilic additive PEG demonstrated the greatest % increase in swelling. This observation is expected due to the formation of solution droplets within the material. It may be possible that the ingress of water may be the greatest with this additive compared with other additives. The growth of these droplets are thought to be controlled by osmotic force to expand the droplet, which is developed due to the differences in osmotic pressure of internal droplet and the external release media. This is countered by physically restraining force of the material (Riggs et al., 1997). Thus, the driving force of water uptake causes osmotic pressure this coupled with elasticity are the two factors involved in the swelling process (Golomb et al., 1990).

Linoleic acid and oleic acid demonstrated similar % increase in swelling values. Both of these additives increased the solubility of triclosan, therefore causing the drug to dissolve creating more pores. This may allow the influx of release media to penetrate through the polymer causing increased swelling. PEG demonstrates the greatest % increase in swelling thus it is most likely that the formulation of solution droplets is the domineering factor which contributes to this increase in swelling over the increase in loading dose and additives such as linoleic acid and oleic acid which cause a general increase in solubility.

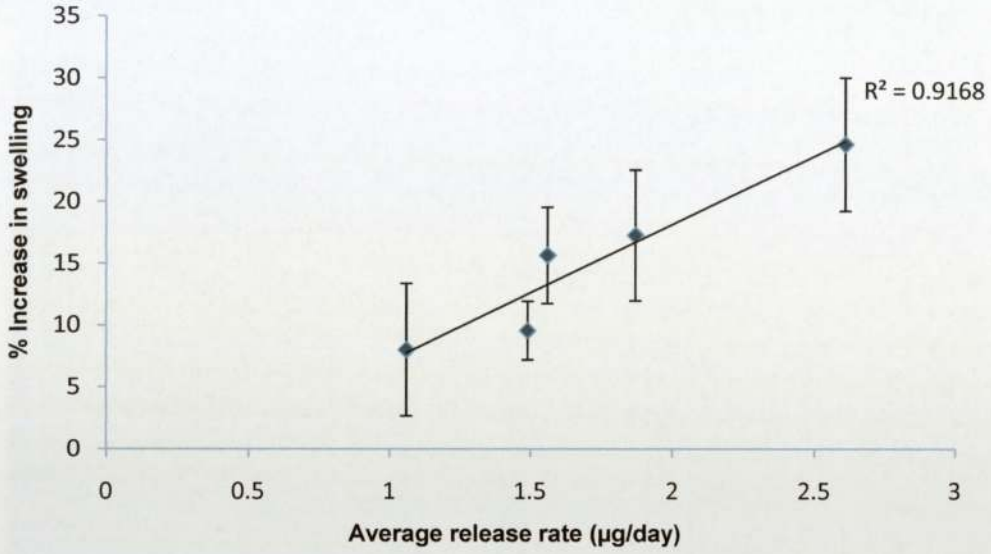


Figure 5.11 *Relationship between the % increase in swelling and the average release rate of triclosan from 3cm² silicone elastomer strips.*

Figure 5.11 indicates a strong correlation between the release rate of triclosan and the % increase in swelling. This is in agreement with the results seen by Golomb et al., (1990) whereby the release rate was directly proportional to the degree of swelling suggesting that there may be a greater release of triclosan occurring due to the generation of increased crack networks via increased osmotic pressure as previously mentioned in section 5.4.4.

5.4.3 Tensile properties

The tensile strength of a silicone elastomer is thought to indicate the overall strength and how far the material stretches before it breaks. The actual tensile test is a sensitive method and represents the tensile strength of the materials rather than their bonding to the substrate (HatamLeh and Watts, 2010). Figure 5.12 shows the tensile strengths and elongation percentage for elastomer strips with

varying doses of triclosan and silicone strips containing 1% w/w of triclosan with the recently mentioned additives.

An increase in percentage of triclosan incorporated into the strips resulted in both a general increase in elongation percentage and tensile strength (Figure 5.11). Inter-strip variability was minimal as demonstrated by the very small standard deviations. Silicone strips with 1% w/w of triclosan and various additives increased elongation percentage in the order linoleic acid>PEG>oleic acid although there was no statistical significance ($P>0.05$) between PEG and oleic acid for both elongation percentage and tensile strength values suggest the elastomer containing linoleic acid clearly demonstrate the highest tensile strength and elongation percentage compared with the other elastomers.

The increase in drug content of triclosan increased both the tensile strength and elongation therefore it is assumed the increase in drug powder must be modifying the morphology of the silicone polymer. The elongation percentage and tensile strength is clearly higher with the presence of additives particularly with linoleic acid as the additive. As previously mentioned Brook et al., (2008) demonstrated that the combination of nicotine and linoleic acid displayed a completely different internal structure of the elastomer thereby suggesting that linoleic acid is also modifying the structure of the silicone strip. Furthermore, it could be that the change in structure and consequently the mechanical properties of the elastomer strips containing additives may also be affecting the rate of cracking and release rate. Therefore, the increase in release rate of the elastomers could be

multifactorial and may be dependent on both the swelling increase and the change in morphology of the elastomer structure.

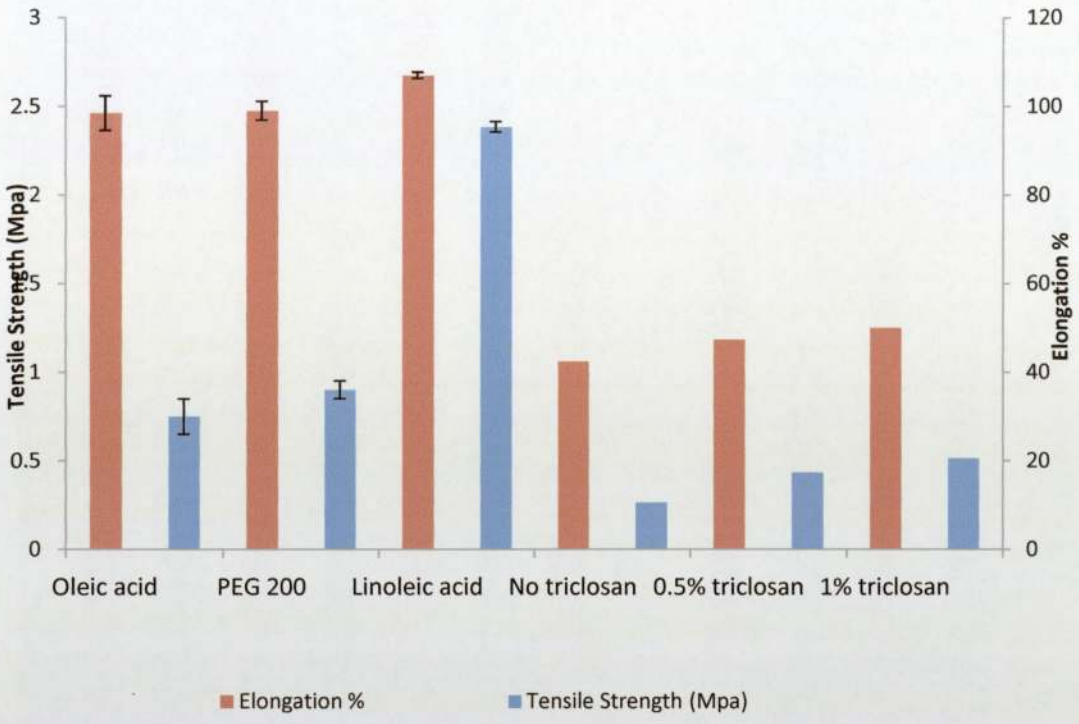


Figure 5.12 *Elongation % and tensile strength of silicone strips of 3cm² containing additives oleic acid, PEG 200 and linoleic acid (1% triclosan w/w). Strips without triclosan, 0.5% triclosan and 1% triclosan.*

5.4.4 Micrographs of silicone elastomer strips

Silicone elastomer strip surfaces were examined using optical micrographs in order to observe the effects of various triclosan doses and additives on the surface and/or structure of the elastomer strips. Figure 5.13 demonstrates micrographs with varying loading doses of triclosan. There appears to be very little visible difference between (a), (b) and (c) suggesting the % of triclosan within the strips does not affect the appearance and surface of the elastomer.

(a) 0.5% Triclosan X 200

(b) 1% Triclosan X 200

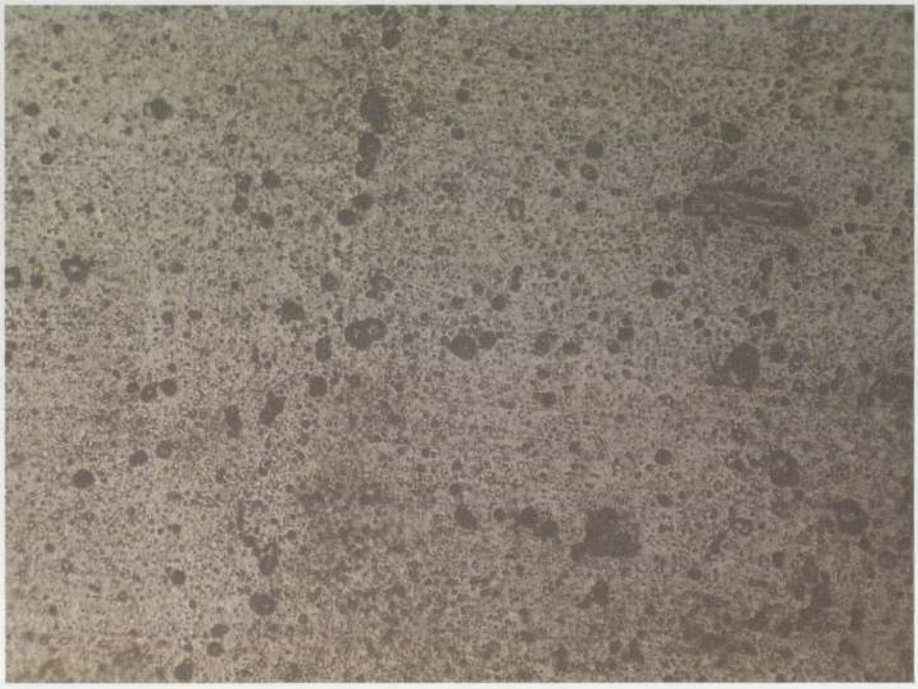
(c) No Triclosan X 200

Figure 5.13 *Metullargic microscope images of MED-6382 silicone strips before swelling, magnification X 200. (a) 0.5% Triclosan (w/w) (b) 1% Triclosan (w/w) and (c) No triclosan.*

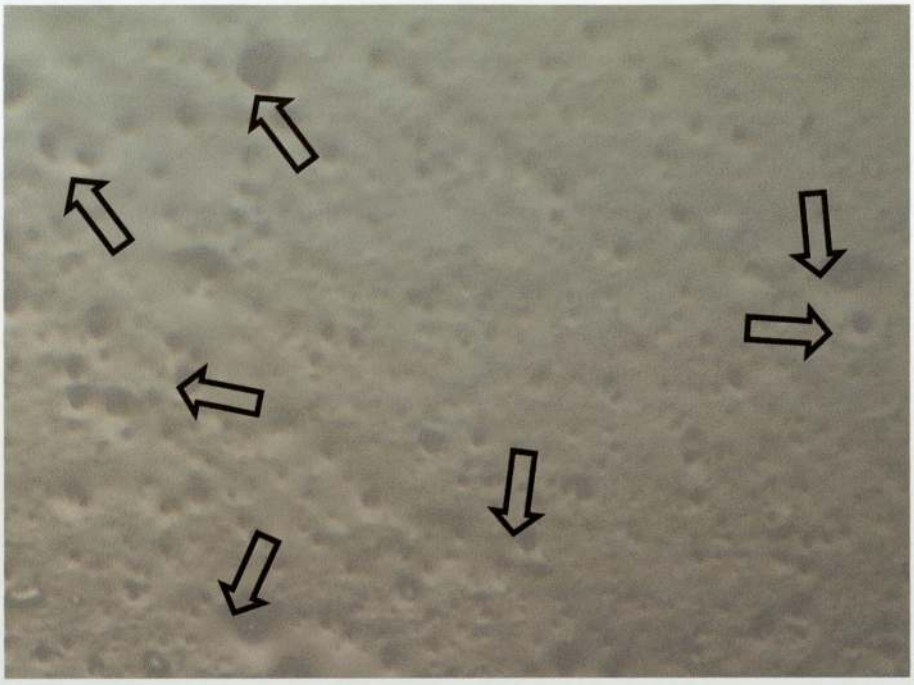
It is believed the specific morphology of the final polymers is dependent on the ingredients used (Brook et al., 2008).The presence of additives has shown considerable differences in appearance. Figure 5.14 (a) and (b) show micrographs of silicone elastomer strips containing oleic acid. Greater magnification of the silicone strips containing oleic acid are shown in (b) the micrographs show the presence of hole-like components (a) does not show this clearly as it is at a lower magnification. A study carried out by Kajihara et al., (2000) suggests the presence of holes in the elastomer may represent where the drug had been

dissolved and released from. This would make sense as with oleic acid the triclosan should demonstrate greater solubility dissolving the triclosan and allowing it to migrate into the release media thereby pores may have formed from where particles of the drug powder may have originally existed.

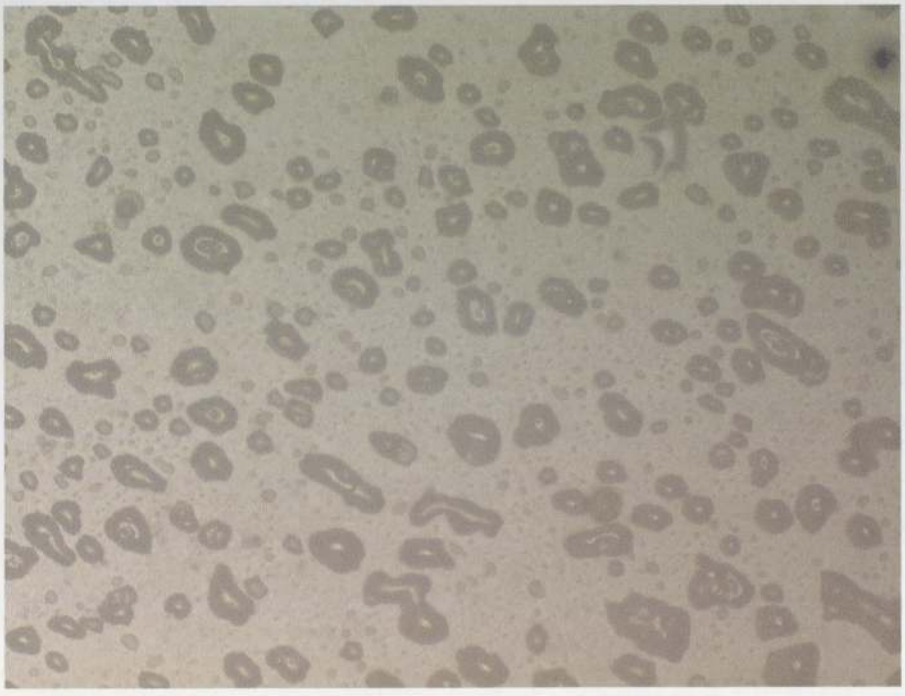
The silicone strips containing PEG 200 are shown at magnification x 200 (c) and x 500 (d). Greater magnification as shown in (a) and (b) demonstrate the presence of aqueous pores or possible water droplets. This is consistent with the theory that admixing of water carriers yields formation of aqueous pores (see section 5.4.4). Figure(c) represents a micrograph containing linoleic acid as the additive, there appears to be the presence of visible oil droplets although there are no holes clearly visible as there was with oleic acid.



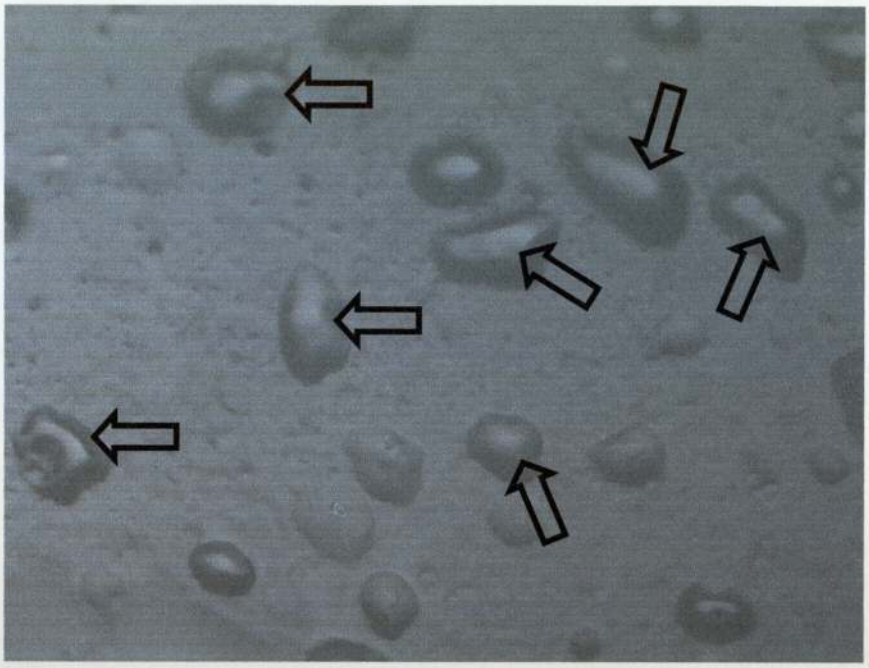
(a) Oleic acid x 50



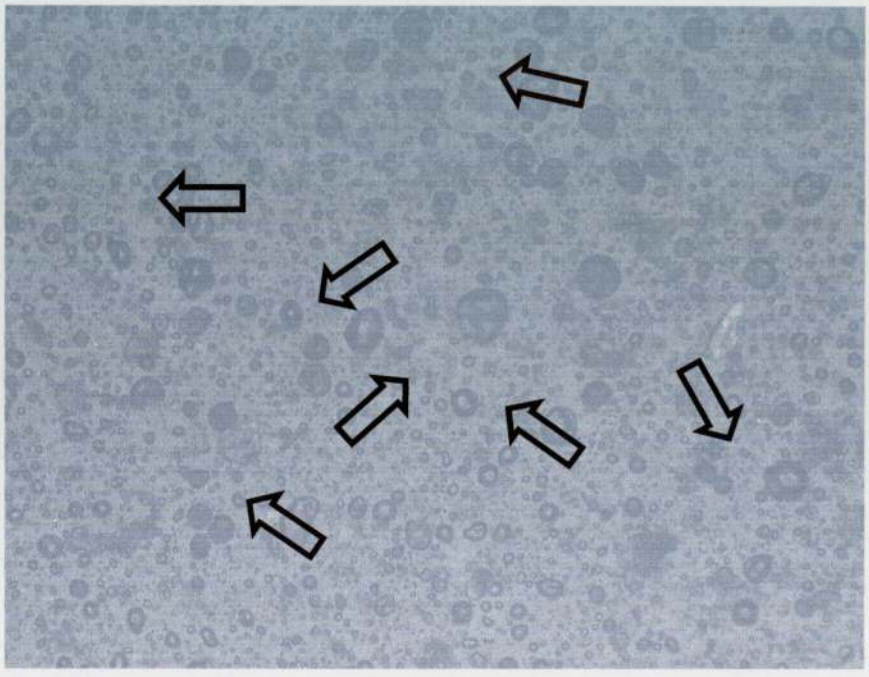
(b) Oleic acid x 200



(c) PEG X 200



(d) PEG X 500



(e)Linoleic acid X 200

Figure 5.14 *Metullargic microscope images of MED-6382 silicone strips containing oleic acid enhancer (before swelling) (a) oleic acid magnification X 50 (b) oleic acid magnification X 200 (c) PEG magnification X 200 (d) PEG magnification X 500 (e) Linoleic acid magnification X 200.*

5.4.5 Permeation of triclosan from a novel silicone/adhesive system

It was necessary to carry out preliminary studies in order to optimise the silicone/adhesive systems. The adhesive component of the drug delivery system remained the same as in section 4.4 whereby 8.0 % v/w of oleic acid was added to DuroTak® 2852. However, the adhesive layer was required to be of a uniform thickness of 1mm following evaporation. This was spread across a silicone elastomer sheet of an area 10.5 cm^2 from which the final patch systems were cut from. It was found that 3 mL of DuroTak® glue was required to be spread over the silicone sheet to provide the optimum consistency and thickness of the glue. The additive linoleic acid was added to the silicone mixture in the same quantities as in section 2.2.6.1.

It was necessary to deliver consistently high levels of triclosan across the skin for this length of time, permeation studies were carried out for this length of time and samples were taken daily. Within chapter 4 patch formulations were investigated however in order to ensure triclosan was permeated across the skin for this time period at a sustained release a novel drug delivery system was developed in which triclosan was incorporated into a silicone elastomer system which was attached to the adhesive (Figure 5.15).

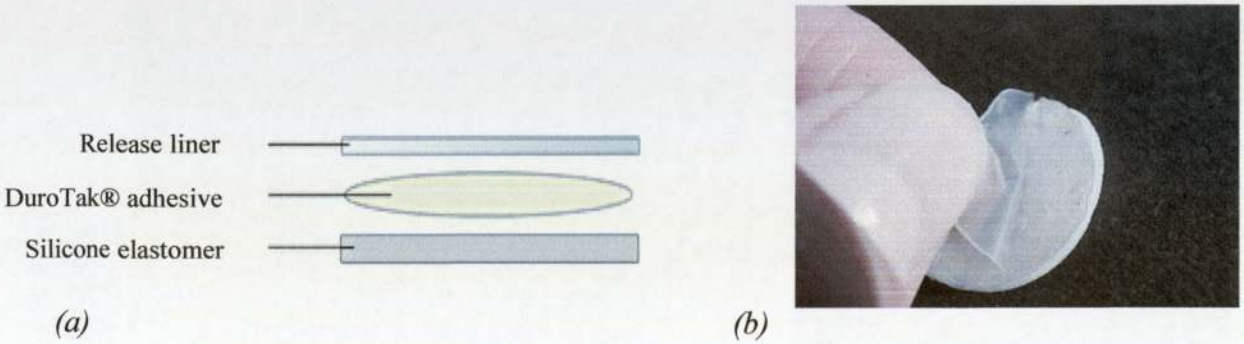


Figure 5.15 *(a) Photograph of silicone/adhesive system (b) Cross sectional representation of silicone/adhesive system.*

From the previous studies in chapter 4 it was concluded that the most appropriate patch type was DuroTak® 2852 containing the enhancer oleic acid. It was suggested that because oleic acid acts as a penetration enhancer across the skin by disrupting the lipid structure within the skin an increased permeation of triclosan was yielded. Thus, as part of this novel drug delivery system the adhesive component will be made up of DuroTak® 2852 composed of oleic acid. Two different drug delivery systems were formulated one of which the silicone component contains 1% w/w triclosan without an additive and the other will contain the same dosage of triclosan however the additive linoleic acid will be included in the silicone layer. The reason for selecting linoleic acid as the additive to increase the permeation rate of triclosan over PEG 200 was because it was believed that the release rate with this particular additive mainly increased due to its increase in swelling and rate of crack formation. However, the swelling is most likely to occur when the elastomer strip is fully immersed in release media. The patch system being developed has been arranged so that the silicone elastomer part is not in contact with any fluid like material only the adhesive glue therefore the type of swelling which brings about an increase of triclosan being released may not occur with the additive PEG 200. However, linoleic acid was selected as it acts as a solubiliser and also produces a higher release rate of triclosan compared with the other elastomers tested. Furthermore, although the silicone elastomer containing oleic acid did increase the release rate of triclosan when compared with without triclosan it may not be beneficial using oleic acid in the silicone layer as it would be part of the upper compartment of the patch which

does not have direct contact with the skin thus the enhancer action would not really be effective.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the two most dominant common pathogenic skin flora lie within the range 0.1 ug/mL – 1.56 ug/mL. Observing the skin permeation rates the concentration of triclosan released per hour for the drug delivery system containing linoleic acid as the additive was 114.44 ug/mL/h and the patch system without an additive 86.16 ug/mL/h. Thus, the concentrations are much higher than the required ranges. However, it must be noted that these experimental values have been obtained across silastic membrane. It must be taken into account that the permeation across silastic membrane was approximately 10 fold greater than across human skin membrane in section 4.4 thus the calculated concentration values are appropriate. Furthermore, it is believed that triclosan concentrations should be kept considerably higher than MIC and MBC values despite the concern that bacteria are unlikely to develop resistance. Moss et al., (2000) suggests that if bacteria are resistant to high concentrations some of these resistance mechanisms may be transferable to other bacteria. Furthermore, triclosan is extremely stable in the environment and low residual levels might encourage survival of triclosan resistant mutant. Thus, Moss et al., suggests this factor is particularly important in environments where bacteria encounter materials designed to slowly release triclosan as would be the case in these studies due to the catheter tubing.

Figure 5.16 shows the permeation profiles of triclosan from the two drug delivery systems. The permeation rate was calculated (Table 5.4). The permeation profiles are linear for both the silicone containing an additive and the system which does not contain an additive with r^2 values of 0.991 and 0.995 respectively. The permeation rates were higher for these drug delivery systems than the adhesive alone as seen in table 4.4 section 4.2. As with results seen in fig 4.10 there were no lag times present suggesting rapid uptake and permeation from the patches. This was expected as the same adhesive system previously used was in contact with the membrane. In comparison to the release profiles within this chapter there was no evidence of a burst release. Once again this was anticipated as it is the drug in glue component being permeated across the membrane. In addition, the triclosan is not being directly released into the media as the silastic membrane acts as a barrier. The hydrophobic membrane may also retain some of the triclosan.

The drug delivery system containing the additive linoleic acid within the silicone system yields a significantly higher ($P<0.05$) permeation rate than the drug delivery system without linoleic acid in the silicone component. The total cumulative mass of triclosan released from the system containing linoleic acid was 81995.5 ug and from the system without the additive 65505.41 ug. This was expected due to results seen in section 5.4.4 where release rates were higher for silicone strips with the additives than without additives. Thus, there must be some migration of triclosan from the silicone component into the adhesive which eventually permeates through to the skin and increases the permeation rate. From

the profiles it is also clear that triclosan release is continuing to occur suggesting that there is still some triclosan remaining within the system.

Drug delivery system	Permeation rate (ug/cm²/h)
Silicone layer containing linoleic acid and DuroTak® adhesive 2852 containing oleic acid	255.04
Silicone layer without additive adhesive component containing DuroTak® adhesive 2862 and oleic acid	192.02

Table 5.4 *Permeation rates of two different drug delivery systems.*

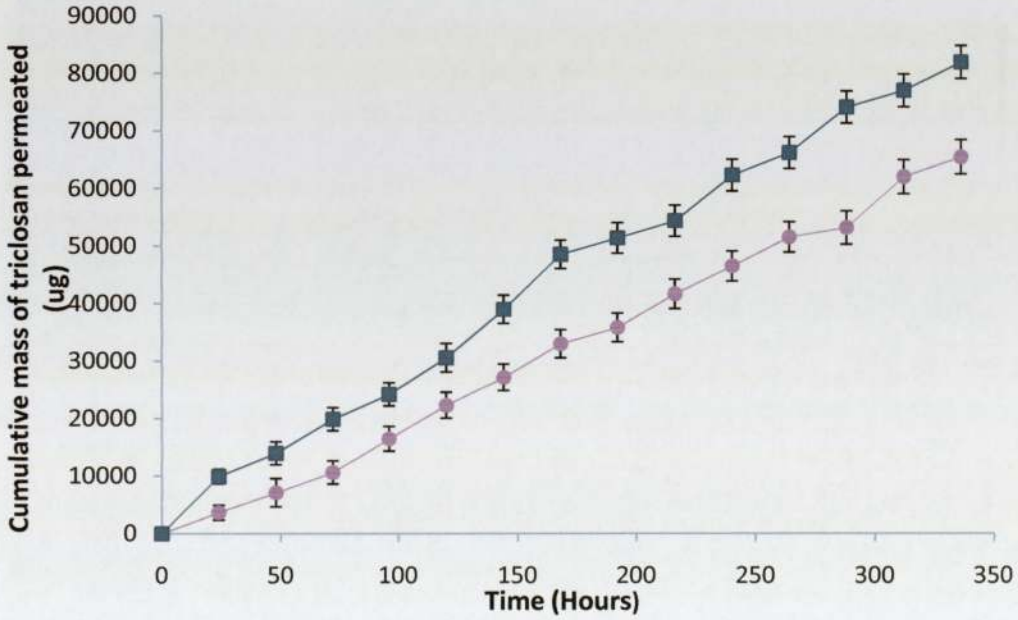


Figure 5.16 *Cumulative permeation profiles of two different silicone adhesive systems across silastic membrane. Each topical patch system consists of a silicone layer and an adhesive layer and is of area 3.141cm². Each system contained approximately 95000 ug of triclosan: (blue ■) silicone layer containing additive (2.0 % v/w) linoleic acid and DuroTak® adhesive layer containing (8.0 % v/w) oleic acid; (pink ●) silicone layer without additive and DuroTak® adhesive layer containing (8.0 % v/w) oleic acid. Receiver media contained 60:40 1 in 10 PBS: ethanol (v/v). Results are expressed as the means of five experiments ± S.D.*

5.5 Conclusions

A key predicament in the use of catheters is the risk of device-related infection caused by skin flora. Silicone elastomers are one of the commonest biomaterials used in the production of catheters. It is believed controlled delivery of an antimicrobial agent from the device is one means to overcome biofilm formation and infection. A drug delivery system was created consisting of a silicone layer and an adhesive type patch from where sustained delivery of triclosan was expected to occur for a period of 14 days.

Preliminary studies were carried out to discover appropriate sizes and release media for silicone elastomer strip release studies. Silicone strips of area $3cm^2$ and the release media 60:40 PBS: ethanol was deemed suitable for future investigations as it has previously been used with patch formulations and most importantly it represents the lipidic qualities of the skin.

Loading dose release studies demonstrated that as the drug dosage increased the release from the silicone elastomer strip is accelerated. Surprisingly, the loading dose was not proportional to the dissolution rate or the final cumulative mass of triclosan released suggesting that the release rate is not entirely dependent on the loading dose. A loading dose of 1% w/w triclosan was selected for future studies as it presented the highest release rates.

Various additives were incorporated into the elastomer strips aiming to optimise release rates of triclosan. Release of triclosan from the silicone elastomer strips increased in the order PEG>linoleic acid>oleic acid>without additive. The matrix

composed of PEG 200 displays the highest release possibly owing to the formation of aqueous droplets causing swelling which eventually may form a crack network. Both linoleic acid and oleic acid possess similar structures thus it was not surprising they demonstrated similar release rates. These additives were believed to increase the solubility of triclosan thereby accounting for the increase in release rate when compared with silicone matrices without additives.

Swelling studies demonstrated that release rates were directly proportional to the degree of swelling. It is therefore probable that the formation of aqueuos pores in the elastomer containing PEG 200 is the key factor which causes an increase in swelling over increasing loading doses and additives which increase solubility.

From tensile property studies higher doses of triclosan displayed greater tensile strength and elongation % values. This was also evident with the inclusion of additives, in particular linoleic acid. It could be postulated that these increases represent structural changes of the silicone elastomers.

Micrographs confirm the presence of aqueous droplets. Silicone elastomer strips containing oleic acid also revealed the presence of holes in the elastomer from where the drug had been dissolved and released from.

To fulfil the aim of this study a silicone /adhesive system was developed and tested. Two delivery systems were investigated. Both systems contained the skin permeation enhancer oleic acid in the adhesive layer and one of the systems included linoleic acid in the upper silicone layer whereas the other did not include any additive. Triclosan was added to both layers of the product in both systems.

Permeation profiles were compared showing that drug delivery system containing the additive linoleic acid within the silicone system generated a significantly higher ($P<0.05$) permeation rate compared with the drug delivery system without linoleic acid in the silicone component. The permeation rates for both systems were much higher than the required MIC and MBC values suggesting that this delivery system could potentially be used for skin disinfection during catheter use.

Chapter Six:

General discussion and conclusions

6.0 General discussion and conclusions

Current skin sterilisation procedures are believed to be insufficient for complete elimination of resident skin flora. Catheter related infections are thought to be caused by micro-organisms introduced from the skin surface into deeper tissue at the time of insertion. It is therefore necessary for a more thorough approach to intravenous catheter site disinfection for the prevention of catheter related sepsis. Several technological and medical innovations have been established in the hope of reducing infections caused by catheters; at present there have been few effective systems to deal with this predicament. In particular the core concern with recent skin disinfection techniques is the inability to target lower reservoir sites of bacteria and repopulation of skin microbes. Thus, the sustained delivery of an antimicrobial agent from the device is one means that may be used to overcome this problem.

This study investigated and optimised the delivery of a specific antibacterial from various drug delivery systems in an attempt to increase their antimicrobial efficacy. The stratum corneum located in the upper epidermis of the skin is often the rate limiting factor for release of drug from a vehicle. Skin penetration enhancers helped to promote drug flux and were incorporated into various drug delivery systems to maximise skin permeation. In order to target the reservoir site, it was essential the antiseptic could gain access to the depths of follicular sites and that it could also penetrate lipioidal areas protecting organisms. Triclosan was selected as an ideal drug candidate owing to its high calculated log

P of 4.89; low molecular weight and it is also a broad spectrum antimicrobial with low MIC and MBC values.

A systematic approach was undertaken commencing with optimisation investigations. Initial aims of the project were to assess techniques and methods to accurately quantify triclosan permeation. Although UV detection is a more rapid technique for quantifying analytes HPLC was selected as an appropriate tool due to the accuracy levels demonstrated, particularly as the drug of interest was likely to be of a small quantity as is often apparent in topical studies. Systemic and quantitative changes were made to cited HPLC methods due to differences in columns and to shorten retention times, generating HPLC conditions which provide an optimum peak resolution.

Subsequent preliminary investigations focused on modelling drug permeation across the skin. Diffusion cells were used to determine the rate of permeation of triclosan across a model membrane and preliminary investigations were undertaken to establish an appropriate receiver phase. It was necessary for an ethanolic receiver solution to be utilised due to the hydrophobic nature of triclosan. Permeation studies using various membranes were also carried out to select suitable membranes. Human skin was seen as the most appropriate model membrane as it was the actual membrane of interest. However, an artificial membrane was also chosen to experiment with as it was easier to obtain and it offers greater reproducibility and control.

In order to fulfil the aims of this study it was necessary to develop a formulation which effectively disinfects the skin over a prolonged period of time due to the

highlighted concern of rapid microbial repopulation and length of time the average catheter is applied. To attain a continuous release of triclosan the use of patch type formulations were studied. A drug in- adhesive type patch composed of solubilised triclosan in a polymeric adhesive layer was investigated and studies undertaken in Chapter 4 were designed to examine the release and permeation of triclosan from these systems. Various adhesives exhibiting altered physiochemical properties were explored as well as the addition of skin permeation enhancers in specific matrix formulations.

Release studies demonstrated that the adhesives displayed Higuchi based release kinetics implying that the release rate of triclosan from an adhesive matrix is governed by the solubility of the drug and the diffusion coefficient in the polymer (Roy et al., (1996)). Results obtained also verified that there was a parallel trend between release rates and permeation rates from the selected adhesives with varying physicochemical properties suggesting that the release of triclosan from these adhesives is the key factor when assessing the permeation of triclosan across a model membrane. This finding had also been confirmed in several studies (Davaran et al., 2005; Chedzgoy et al., 2002).

The adhesive which demonstrated the greatest permeation rate was selected for additional investigation in order to further optimise triclosan permeation from the adhesive system. Skin permeation enhancers with varying mechanisms of action were incorporated into the adhesives. Oleic acid is a long chain fatty acid which reduces the barrier function of the skin by disrupting the lipidic domains of the stratum corneum. Further enhancers tested were propylene glycol and transcutol.

These additives acted as solubilising agents modifying the thermodynamic activity of the drug in the vehicle which ultimately altered the driving force for diffusion.

Results collated from the release studies surprisingly demonstrated the order of highest release rates from the adhesives was without enhancer>oleic acid>transcutol>propylene glycol. It was therefore apparent that because triclosan was highly soluble in propylene glycol and transcutol it possesses a higher affinity to stay within the adhesive and was released to a lesser extent than the patches without any enhancer or oleic acid. However, permeation studies across human skin revealed that permeation rates were highest when oleic acid was the included enhancer; subsequently the patch without an enhancer displayed a slightly lower permeation rate and then propylene glycol and transcutol respectively. This outcome was thought to be due to the specific enhancer action of oleic acid across the actual human skin. It appears as though the use of human skin allowed the disruption of the lipid bilayer allowing triclosan to travel through the skin more rapidly. Thus, despite the fact that triclosan is still soluble in oleic acid the enhancer action was deemed responsible for the increase in permeation activity when compared with without an enhancer. The high solubility of triclosan in propylene glycol and transcutol most likely accounted for the lower permeation rates as described in the release studies.

Chapter 5 was more specifically focussed on designing a system which could be used to prevent skin infection developing around areas of catheter insertion caused by skin flora. A common biomaterial used in the production of catheters is

silicone elastomers. Attempts were made to formulate a system whereby triclosan was incorporated into a silicone elastomer system and combined with the drug in adhesive patch described above to create a layered patch system from where sustained delivery of triclosan was proposed to occur for a period of 14 days.

Preliminary investigations involving the silicone elastomer material itself were undertaken to measure the appropriate release of triclosan. Suitable sizes of elastomer strips, dosages of triclosan and release media were determined and at this stage only release studies were considered as the silicone elastomers are a dry material and intended to be part of the upper layer of the patch system thus it would not be in contact with the skin.

Additives were integrated into the elastomer strips to improve the release rates of triclosan. The following additives were used: PEG 200, linoleic acid and oleic acid. Oleic acid was used due to its skin permeation enhancing activity as previously described and linoleic acid was combined with the silicone elastomer system as Brook et al., (2008) had demonstrated that it yields a more uniform and sustained release of drug. In addition it was also seen to display morphological changes in the elastomer structure. PEG 200 was chosen as further additive as it is a water carrier and it was interesting to note its effect on the hydrophobic silicone material.

The matrix composed of PEG 200 provided the greatest release followed by linoleic acid, oleic acid and the elastomer without an additive respectively. As the elastomer containing PEG 200 was the only elastomer containing a water carrier and swelling studies demonstrated that this matrix generated the greatest

percentage increase in swelling it was assumed that the release rate was attributed to the formation of aqueous droplets which caused swelling and a subsequent crack network in the elastomer. Micrographs of the elastomer strips containing PEG 200 confirmed the presence of aqueous type droplet formation. Furthermore, swelling studies themselves revealed that release rates were directly proportional to the degree of swelling, thus swelling activity was a vital factor when comparing release rates. Elastomers made up of linoleic acid and oleic acid demonstrated similar release rates as would be expected due to their similar structures. It is probable their higher release rate compared with the elastomer without any additive was due to the increase in solubility of triclosan with linoleic acid and oleic acid.

Once results were collated from the release study with various additives, a final drug delivery device was developed. Two delivery systems were tested for comparative purposes. Both systems contained triclosan in both the adhesive and silicone layer, the skin permeation enhancer oleic acid in the adhesive layers and one of the systems included linoleic acid in the upper silicone layer whereas the other did not include any additive. The linoleic acid elastomer was selected over PEG 200 despite the fact that it showed the greater release as the reason for this high release rate was mainly believed to be due to the full immersion of the silicone elastomer within water. However, in this case the silicone elastomer is part of the upper layer of the full patch system and the elastomer containing linoleic acid provided the second greatest release values when compared with the other test specimens. Permeation profiles revealed that the system made up of linoleic acid within the silicone layer yielded the greatest permeation rate.

Permeation rates for both systems were a great deal higher than the necessary MIC and MBC values suggesting this integrated patch type system could potentially be used for disinfecting the skin during catheter use. Ideally the system containing linoleic acid would be the most appropriate due to its higher permeation rates.

Overall the objectives of the outlined research plan have been met; essentially the study has presented a potential solution to the problem of sufficient delivery of an antimicrobial around the dermal area of catheter insertion for a period of 14 days. The advantages of selecting this specific drug have also been discussed (Chapter 1). Toxicology studies cited in literature claims that triclosan demonstrates a suitable safety profile at the concentrations presented in these studies. The adhesive patch alone did not provide sufficient levels of antimicobial across the skin. By formulating a drug in glue adhesive and combining it with an upper silicone elastomer layer sustained release over 14 days was achieved. Further measures were taken to optimise and increase permeation rates of triclosan by incorporating suitable permeation enhancers and additives to the novel drug delivery system.

Further consideration for future work would be to assess the permeation of triclosan from the drug delivery system across human skin itself as the study had only tested the final system across silastic membrane. More importantly assessment of the microbiological activity would be required to test the overall efficacy of this system. Future work could also involve the addition of another antimicrobial which displays a different antimicrobial mechanism of action to

triclosan. This would allow the simultaneous permeation of two different antimicrobials thus it may counteract the common problem of microbial resistance.

References

Ackerman, C., Flynn, G.L. and Van Wyk, C.J., 1985. Percutaneous absorption of urea. *International Journal of Cosmetic Science* **7,** 251-264.

Ackerman, C. and Flynn, G.L., 1987. Ether-water partitioning and permeability through nude mouse skin in vitro. I. Urea, thiourea, glycerol and glucose. *International Journal of pharmaceutics* **36,** 61-66.

Ahuja, S., 2003. Chromatography and separation science. *Separation science and technology.* Volume **4**. Academic press, San Diego, California.

Alberti, I., Kalia, Y. N., Bonny, J.D., Guy, R.H., 2001. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. *Journal of Controlled Release* **71** (3), 319-327.

Aungst, B.J., Blake, J.A., and Hussain, M.A., 1990. Contributions of drug solubilisation, partitioning, barrier disruption, and solvent permeation to the enhancement of skin permeation of various compounds with fatty acids and amines. *Pharmaceutical. Research.*, **7**, 712-718.

Baker, N.D., Griffin, R.J. and Irwin W. J., 1990. The percutaneous absorption of m-azido-pyrimethamine: a soft antifolate for topical use. *International Journal of Pharmaceutics.*, **64** , 115-125.

Bayston,R., Fisher,L.E. and Weber,K., 2009. An antimicrobial modified silicone peritoneal catheter with activity against both Gram positive and Gram negative bacteria. *Biomaterials.*, **30**, 3167-3173.

Berner, S.L., Mazzenga, G.C., and Otte, J.H., 1989. Ethanol: water mutually enhanced transdermal therapeutic system II: skin permeation of ethanol and nitroglycerin. *Journal of Pharmaceutical science* **78,** 402-407.

Bian, S., Doh, H., Zheng, J., Kim, J.S., Lee, C., and Kim, D., 2003. In vitro evaluation of patch formulations for topical delivery of gentisic acid in rats. *European Journal of Pharmaceutical Sciences* **18,** 141-147.

Bos, J.D., 2000. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Experimental Dermatology*, **9**: 165–169.

Bouwstra, J.A., The skin, a well organised membrane. *Colloids and Surfaces. A,physicochemical and Engineering Aspects*, 1997. **123**: 403-413.

Brook, M.A., Holloway,A.C., Kenneth, K., Hrynyk,M., Moore,C. and Lall,R., 2008. Using a drug to structure its release matrix and release profile. *International Journal Of Pharmaceutics* **358,** 121-127.

Carelli,V., Di Colo,G., Guerrini,C. and Nannipieri, E., 1989. Drug release from silicone elastomer through controlled polymer cracking: an extention to macromolecular drugs. *International Journal Of Pharmaceutics* **50**, 181-188.

Chauan, N. 1995. Cyclodextrin complexes of antimicrobial agents (thesis).

Chedzgoy, P., Winckle, G. and Heard, C.M., 2002. Triclosan: release from transdermal adhesive formulations and in vitro permeation across human epidermal membranes. *International Journal of Pharmaceutics* **235**, 229-236.

Chien, Y. W. and Lambert, H, J., 1974. Controlled drug release from polymeric delivery devices II: differentiation between partition-controlled and matrix-controlled drug release mechanism. *Journal of Pharmaceutical science.* **63**, 515-519.

Cho, Y. J. and Choi, H. K., 1998. Enhancement of percutaneous absorption of ketoprofen: effect of vehicles and adhesive matrix. *International Journal of Pharmaceutics.*, **169**, 95-104.

Chung, D., Papadakis, S.E. and Yam, K.L., 2003. Evaluation of a polymer coating containing triclosan as the antimicrobial layer for packaging materials. *International Journal of Food Science and Technology* **38**, 165-169.

Cooper, E.R., 1984. Increased skin permeability for lipophilic molecules. *Journal of Pharmaceutical Science* **73**, 1153-1156.

Davaran, S., Rashidi, M., Khandaghi, R and Hashemi, M. 2005. Development of a novel prolonged-release nicotine transdermal patch. *Pharmacological Research.* **51**, 233-237.

Diagram taken from http://www.ses-analysesysteme.de/SESranz_Cell_Flow_Type_uk.htm.

Diagram taken from *http://www.ses-analysesysteme.de/SESFranz_Cell_Flow_Type_uk.htm.*

Diagram taken from *http://www.ses-analysesysteme.de/SESFranz_Cell_Flow_Type_uk.htm*

Di Colo,G., Carelli,V., Nannipieri,E., Serafini,M.F. and Vitale, D., 1986. Effect of water-soluble additives on drug release from silicone rubber matrices. II. Sustained release of prednisolne from non-swelling devices. *International Journal Of Pharmaceutics.*, **30**, 1-7.

Elliott, T.S.J., 1997. Intravascular catheter-associated infections and their prevention in Current Topics in Intensive Care, Burchardi, H. (Ed.), W.B. Saunders Co. Ltd., London **4**, 182-205.

Feldmann, R.J. and Maibach, H.I., 1967. Regional variation in percutaneous penetration of 14C hydrocortisone in man. *Journal of Investigative Dermatology* **48,** 181-183.

Francoeur, M.L., Golden, G.M., Potts, R.O., 1990. Oleic acid: its effects on stratum corneum in relation to (trans)dermal drug delivery. *Pharmaceutical Research* **7**, 621-627.

Franz, T.J., 1975. Percutaneous absorption on the relevance of in vitro data. *Journal of Investigative Dermatology* **64,** 190-195.

Franz cell diagram taken from - *www.instrument.com.cn*

Funke, A.P., Schiller, R., Motzkus, H.W., Gunther, C., Muller, R.H., Lipp, R., 2002. Transdermal delivery of highly lipopgilic drugs: in vitro fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulation. *Pharmaceutical Research* **19**, 661-668.

Fuursted, K., Hjort, A. and Knudsen , L., 1997. Evaluation of bactericidal activity and lag of regrowth (post antibiotic effect) of five antiseptics on nine bacterial pathogens, *Journal of Antimicrobial Chemotherapy* **40,** 221-226.

Gollwitzer, H., Ibrahim, K., Meyer, H., Mittelmeier, W., Busch, R. and Stemberger, A., 2003. Antibacterial poly (D, L-lactic acid) coating of medical implants using a biodegradable drug delivery technology. *Journal of Antimicrobial Chemotherapy* ***51,*** 585-591.

Golomb,G.,Fisher,P. and Rahamin, E., 1990. The relationship between drug release rate, particle size and swelling of silicone matrices. *Journal of Controlled Release* **12**, 121-132.

Gorman, S.P., Adair, C.G., Mawhinney, W.M., 1994. Incidence and nature of peritoneal catheter biofilm determined by electron and confocal laser laser scanning microscopy. Epidemiology Infection; **112**, 551-9.

Green., P.G., Guy, R. H., and Hadgraft, J., 1988. In vitro and in vivo enhancement of skin permeation with oleic and lauric acids. *International Journal of Pharmaceutics* **48**, 103-111.

Grove, C., Liebenberg, W., Du Preez, J.L., Yang, W. and De Villiers, M.M., 2003. Improving the aqueous solubility of triclosan by solubilisation, complexation and in situ salt formation. *Journal of Cosmetic Science* **6**, 537-550.

Guyot, M., Fawaz, F., 2000. Design and in vitro evaluation of adhesive matrix for transdermal delivery of propranolol. *International Journal of Pharmacy* **204**, 171- 182.

Guy, R.H., Hadgraft, J., 1989. Selection of Drug Candidates for Transdermal Delivery. In: Hadgraft, J., Guy, R.H. and Research Initiatives. Marcel Dekker, New York, pp. 59-81.

Gwak, H. S. And Chun, I. K., 2002. Effect of vehicles and penetration enhancers on the in vitro percutaneous absorption of tenoxicam through hairless mouse skin. *International Journal of Pharmaceutics* **236**, 57-64.

Hadgraft, J. and Lane, M.E., 2005. Skin permeation: The years of enlightenment- Historical perspective. *International Journal of Pharmaceutics* **305,** 1-2.

Hatamleh ,M.M. and Watts, D.C., 2010. Mechanical properties and bonding of maxillofacial silicone elastomers. *Dental Materials* **26**, 185-191.

Hay, A.G., Dees, P.M., Sayler, G.S., 2001. Growth of a bacterial consortium on triclosan. *FEMS Microbiology Ecology* **36**, 105-112.

Hendley, J.O. and Ashe, K.M., 1991. Effect of topical antimicrobial treatment on aerobic bacteria in the stratum corneum of human skin, *Antimicrobial agents chemotherapy* **35**, 627-631.

Heo, S., Cho, J., Cheon, J., Choi, M., Im, D., Kim, J., Choi, Y., Jeon, D., Chung, S., Shim, C and Kim, D., 2008. Pharmacokinetics and pharmacodynamics of Ketoprofen plasters. *Biopharmaceutics and Drug Disposition* **29**, 37-44.

Hugo W.B., 1992. Mode of action of non-antibiotic antibacterial agents. In: Hugo WB, Russell AD (eds), Pharmaceutical Microbiology, Fifth Edition, Blackwell Scientific Publications, London, 288-294.

Jones, R. D., Jampani, H. B., Newman, J. L and Lee, A. S., 2000. Triclosan: A review of effectiveness and safety in health care settings. *American Journal of Infection Control* **28**: 184-196.

Kajihara,M., Sugie, T., Hojo,T., Maeda,H., Sano,A., Fujioka,K., Sugawara,S. and Urabe, Y., 2001. Devolpment of a new drug delivery system for protein drugs using silicone (II). *Journal of Controlled Release* **73,** 279-291.

Kajihara, M., Sugie, T., Maeda, H., Sano, A., Fujioka, K., Urabe, Y., Tanihara, M. and Imanishi, Y., 2003. Novel drug delivery device using silicone: controlled release of insoluble drugs or two kinds of water-soluble drugs. *Chemistry Pharmaceutical Bulletin* **51**, 15-19.

Kajihara,M., Sugie, T., Mizuno, M., Tamura, N., Sano, A., Fujioka, K., Kashiwazaki, Y., Yamaoka,T., Sugawara, S. and Urabe, Y., 2000. Development of new drug delivery system for protein drugs using silicone (I). *Journal of Controlled Release* **66**, 49-61C

Kirkland, J.J., 1971. Modern practice of liquid chromatography, New York: Wiley.

Kjaerheim, V., Waaler, S.M., Rolla, G., 1994. Significance of choice of solvents for the clinical effect of triclosan-containing mouthwashes. *Scandanavian Journal of Dental Research* **102**, 202-205.

Lafforgue, C., Carret, L., Falson, F., Reverdy, M.E. and Freney, J., 1997. Percutaneous absorption of a chlorhexidine digluconate solution. *International Journal of Pharmaceutics* **147**, 243-246.

Langer, R and Hsu, T. 1985. Polymers for the controlled release of macromolecules: Effect of molecular weight of ethylene-vinyl acetate copolymer. *Journal of Biomedical Materials Research*, **19**, 445-460.

Lee, T., Kim, J. and Hwang, S., 2003. Hydrogel patches containing Triclosan for acne treatment. *European Journal of Pharmaceutics and Biopharmaceutics* **56,** 407-412**.**

Lofthouse,S.A., Kajihara,M., Nagahara,M., Nash,A., Barcham,G.J., Sedmen,B., Brandon,M.R. and Sano,A., 2002. Injectable silicone implants as vaccine delivery vehicles. *Vaccine* **20**, 1725-1732.

Loftsson, T., Ossurardottir, I.B., Thorsteinsson, T., Duan, M. and Masson, M., 2005. Cyclodextrin solubilisation of the antibacterial agents triclosan and triclocarban: Effect of

ionisation and polymers. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* **52,** 109-117.

Loth, H., 1991. Vehicular influence on transdermal drug penetration. *International Journal of Pharmaceutics* **68** (1-3): p.1-10.

Maeda,H., Sugie,T., Sano,A., Kawasaki,H. and Kurosaki,Y., 2004. Study on accelerated evaluation system for release profiles of covered-rod type silicone formulation using indomethacin as a model drug. *Journal of Controlled Release* **94,** 337-349

Maeda,H., Brandon, M. and Sano,A., 2003a. Design of controlled-release formulation for ivermectin using silicone. *International Journal of Pharmaceutics* **261**, 9-19.

Maeda,H., Ohashi,K., Sano,A., Kawaasaki,H. and Kurosaki,Y., 2003b. Investigation of the release of a covered-rod-type formulation using silicone. *Journal Of Controlled Release* **90**, 59-70.

Maestrelli, F., Mura, P. and Alonso, M.J., 2004. Formulation and chracterisation of triclosan sub-micron emulsions and nanocapsules. *Journal of Microencapsulation* **21**, 857-864.

Malcolm,R.K., McCullagh,S.D., Woolfson,A.D., Gorman,S.P., Jones,D.S. and Cuddy,J., 2004. Controlled release of a model antibacterial drug from a novel self-lubricating silicone biomaterial. *Journal Of Controlled Release* **97**, 313-320.

Marzulli, F.N., Brown, D.W.C. and Maibach, H.I., 1969. Techniques for studying skin penetration. *Toxicology Applied Pharmacology Supplement* **3**, 79-83.

McBride, M.C., Malcolm,R,K., Woolfson,A ,D. and Gorman,S,P., 2009. Persistence of elastomers. *Biomaterials* **30**, 6739-6747.

Moss, H. A. and Elliott, T.S.J., 1997. The cost of infection related to central venous catheters designed for long-term use. *British Journal of Medical Economics* **11**, 1-7.

Moss, T., Howes, D.,and Williams, F.M., 2000a. Percutaneous penetration and dermal metabolism of triclosan. *Journal of Food and chemical toxicology* **38**, 361-370.

Moss, T., Howes, D., Wlliams, F.M., 2000b. Percutaneous penetration and dermal metabolism of triclosan (2,4,4'- Trichloror-2'-hydroxyphenyl ether). *Food Chemical Toxicology* **38**, 361-370.

Nature, 2010 http://www.nature.com/

Perrie, Y and Rades, T., 2010. Pharmaceutics – Drug delivery and targeting. Pharmaceutical Press., Padstow. U.K.

Qvist, M. H., Hoeck, U., Kreilgaard, B., Madsen, F. and Frokjaer, S., 2002. Release of chemical permeation enhancers from drug-in adhesive transdermal patches. *International Journal of Pharmaceutics* **231**, 253-263.

Raghaven, S.L., Schuessel, K., Davis, A. and Hadgraft, J., 2003. Formation and stabilisation of triclosan colloidal suspensions using supersaturated systems. *International Journal of Pharmaceutics* **261**, 153-158.

Read, R.R., Eberwein, P., Dasgupta, M.K., Grant, S.K., Lam, K., Nickel, J.C., 1989. Peritonitis in peritoneal dialysis: bacterial colonisation by biofilm spread along the catheter surface. *Kidney International* **35**:614-21.

Riggs,P.D., Parker,S., Braden, M. and Kalachandra,S., 1997. Influence of additives on the water uptake of hydrosilanized silicone rubbers. *Biomaterials* **18**, 721-726.

Roy, S. D., Gutierrez, M., Flynn, G. L., and Cleary, G. W. N., 1996. Controlled transdermal delivery of fentanyl: characterisations of pressure- sensitive adhesives for matrix patch design. *Journal of Pharmaceutical Science* **85**: 491 – 495.

Russell, A. D., 2003. Similarities and differences in the responses of micro organisms to biocides. *Journal of antimicrobial chemotherapy* **52**, 750-63.

Russell, A. D., 2004. Whither triclosan? *Journal of Antimicrobial Chemotherapy* **53**, 693-695.

Santoyo, S., Arellano, A., Ygartua, P., and Martin, C., 1995. Penetration enhancer effects on the in vitro percutaneous absorption of piroxicam through rat skin. *International Journal of Pharmacy* **117**, 219-224.

Sato, K., Sugibayashi, K. and Morimoto, Y., 1991. Species difference in percutaneous absorption of nicorandil. *Journal of Pharmaceutical Science* **80,** 104-107.

Schweizer, H. P., 1998. Intrinsic resistance of inhibitors of fatty acid biosynthesis in *Pseudomonas aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. *Antimicrobial Agents Chemotherapy* **42,** 394-398.

Schweizer, H. P., 2001. Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiology Letters* **202**, 1-7.

Selwyn, S. and Ellis, H., 1972. Skin bacteria and skin disinfection reconsidered, *British Journal of, Medicine* **1**, 136-140

Slater-Radosti, C., Van Aller G., Greenwood, R., Nicholas, R., Keller, P. M., DeWolf, W. E., Fan, F., Payne, D. J., and Jaworski, D. D., 2001. Biochemical and genetic characterisation of the action of triclosan on Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy* **48**, 1-6.

Smith, E. W. and Haigh, J. H., 1989. In vitro systems for assessment of drug release from topical formulations and transmembrane permeation . In Percutaneous Absorption. Edited by Bronaugh, R.L. and Maibach, H.i. Marcel Dekker (1989) 2nd Edition 465-507.

Structure taken from - *www.the-medical-dictionary.com.*

Structure taken from - www.sci-toys.com.

Structure taken from - *www.instrument.com.cn.*

Table taken from - ICH harmonised tripartite guideline Q2A.

Tanojo, H., Bouwatra, J.A., Junginger, H.E., Bodde, H.E., 1997. In vitro human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. *Pharmaceutical Research* **14** (1), 42-49.

Taylor, T. J., Seitz, E. P., Fischler, G. E., Fuls, J. L. and Weldner, P. L., 2003. Physicochemical factors affecting the rapid bactericidal efficacy of the phenolic antibacterial triclosan. *International Journal of Cosmetic Science* **26,** 111-116.

Thodis, E., Bhaskaran, S., Pasadakis, P., Bargman, J.M., Vas, S.I., Oreopoulos, D.G., 1998. Decrease in Staphylococcus aureus exit site infections and peritonitis in CAPD patients by local application of mupirocin ointment at the catheter site. *Peritoneal Dialaysis International* **18,** 261-70.

Uno, H., and Kurata, S., 1993. Chemical agents and peptides affect hair growth. *Journal of Investigative Dermatology* **101**, 143S-147S.

Valenta, C. and Auner, B. G., 2004. The use of polymers for dermal and transdermal delivery *Europeon Journal of Pharmaceutical Science* **58**: 279-289.

Valjakka-Koskela, R., Hirvonen, J., Monkkonen, J., Kiesvaara, J., Antila, S., Lehtnen, L., Urtti, A., 2000. Transdermal delivery of levosimendan *Europeon Journal of Pharmaceutical Science* **11** (4) 343-350.

Williams, A.C. and Barry, B.W., 1992. Skin absorption enhancers. Critical Reviews in Therapeutic Dug Carrier Systems, **9**(3-4) p 305-353.

Williams, A. C., 2003. Transdermal and topical drug delivery. Pharmaceutical Press, TJ International, Cornwall, UK.

Williams, A.C. and Barry, B.W., 2004. Penetration enhancers. *Advanced Drug Delivery Reviews,* **56,** 603-618.

Yamada, M., Uda, Y., and Tanigawara, Y., 1987. Mechanism of enhancement of percutaneous absorption of moisidomine by oleic acid. *Chemical Pharmaceutical Bulletin*, **35**, 3399-3406.

Zhao, H., Park, D., Kim, S., Lee, C. and Kim, D., 2002. The effects of pressure sensitive adhesives and solubilisers on the skin permeation of testosterone from a matrix-type transdermal delivery system. *Drug development and Industrial Pharmacy*. **28**: 1125-1131.

Zhao, H., Lee, C., Chung, S., Shim, C., and Kim, D., 2005. In vitro and in vivo evaluation of a novel nonscrotal matrix-type transdermal delivery system of testosterone. *Drug development and Industrial Pharmacy,* **31**: 257-261.

Zhao, H., Choi, M., Kin, J., Yong, C., Choi, H., Chung, S., Shim, C and Kim, D., 2009. Synergic enhancing-effect of DEET and dodecylamine of the skin permeation of testosterone from a matrix-type transdermal delivery system. *Drug delivery*. **16**, 249-253.

Zing, W., Cartier-Fassler, V. and Walder, B., 2008. Central venous catheter-associated infections. Best practice and research clinical anaesthesiology. **22**, 407-421.

Appendix

Appendix

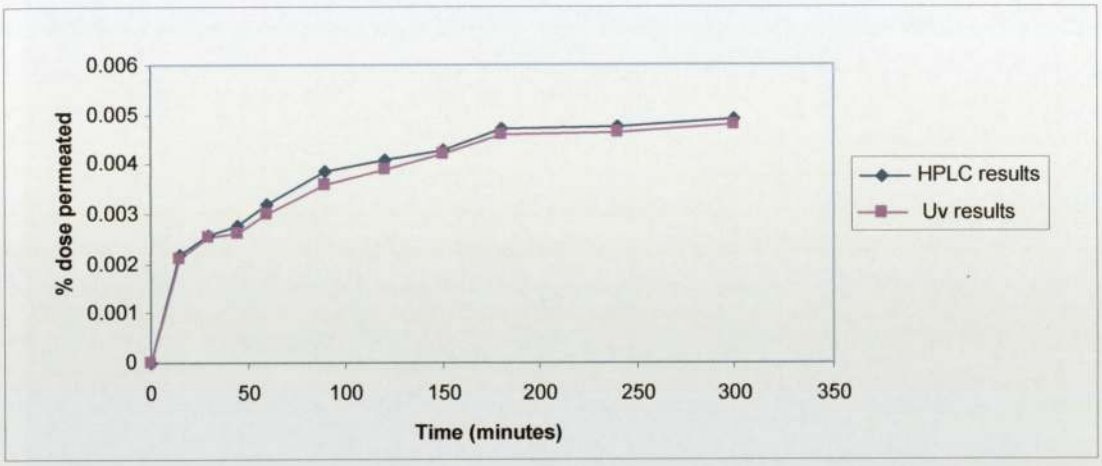


Figure 1 Permeation profile demonstrating the validation of HPLC results with UV results. % dose permeated of 1% triclosan formulation with 20 % propylene glycol (V/V) in water as vehicle.

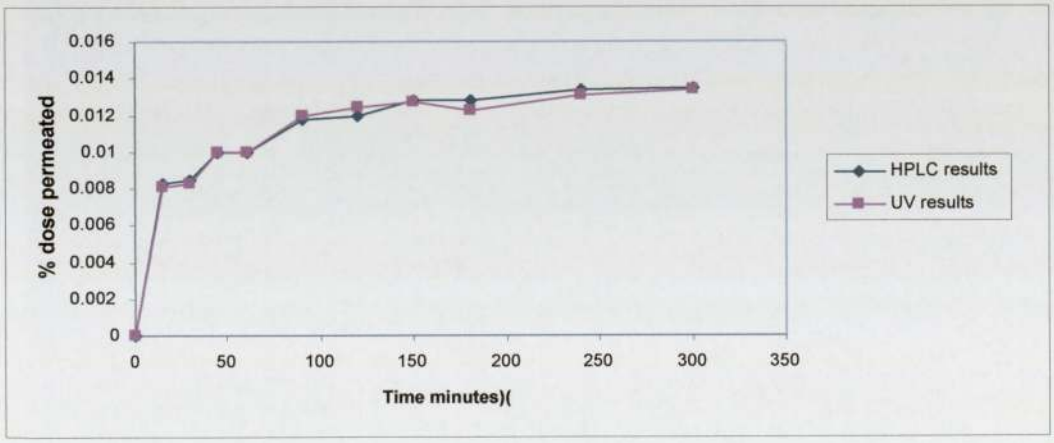


Figure 2 Permeation profile demonstrating the validation of HPLC results with UV results. % dose permeated of 1% triclosan formulation with 10 % propylene glycol (V/V) in water as vehicle.

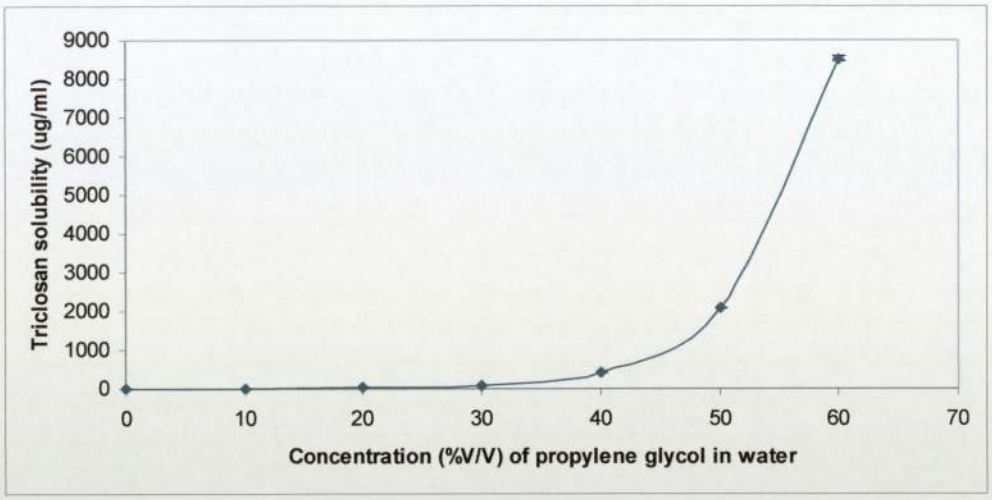


Figure 3 Solubility of triclosan in varying propylene glycol/water vehicles (mean ± SD) n=3.

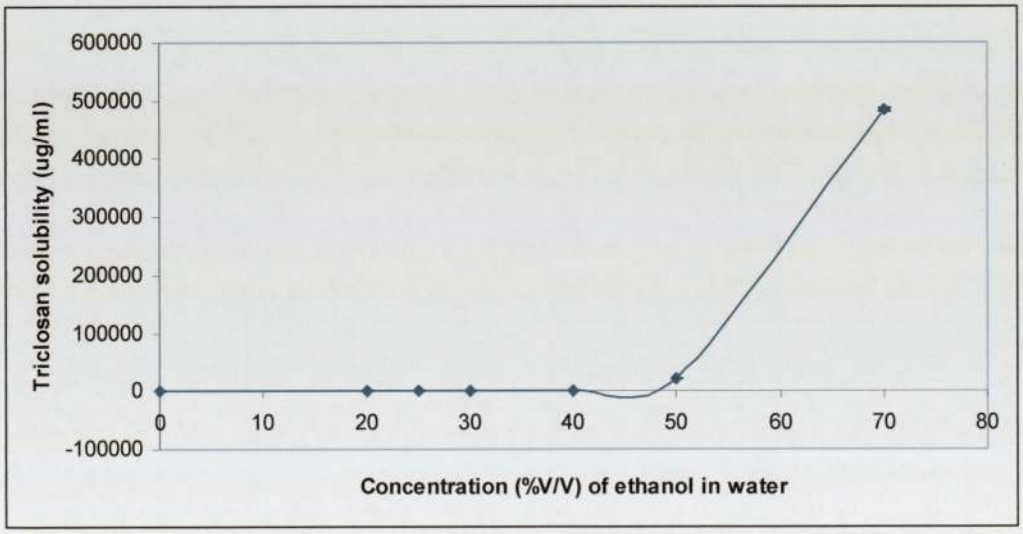


Figure 4 Solubility of triclosan in varying ethanol/water vehicles (mean ± SD) n=3.

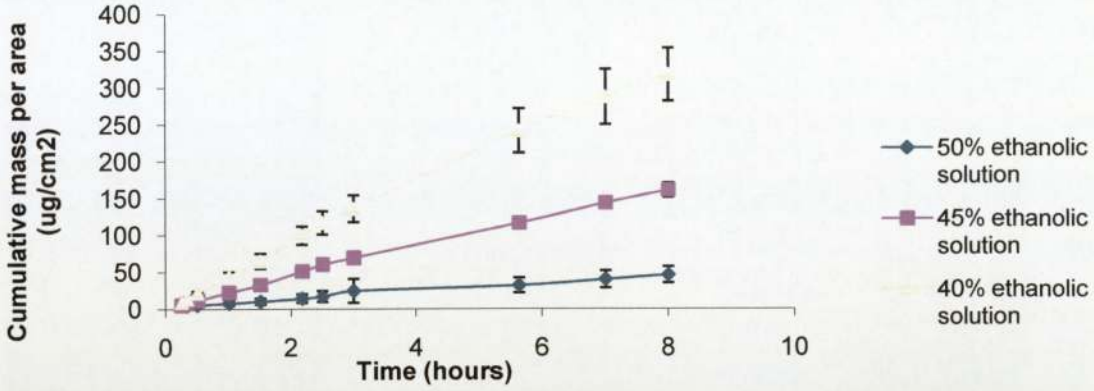


Figure 5Permeation profiles of 1% triclosan solutions (across silastic membrane) with 40, 45 and 50% V/V ethanolic concentrations. Points represent the means ± SD, n=2.

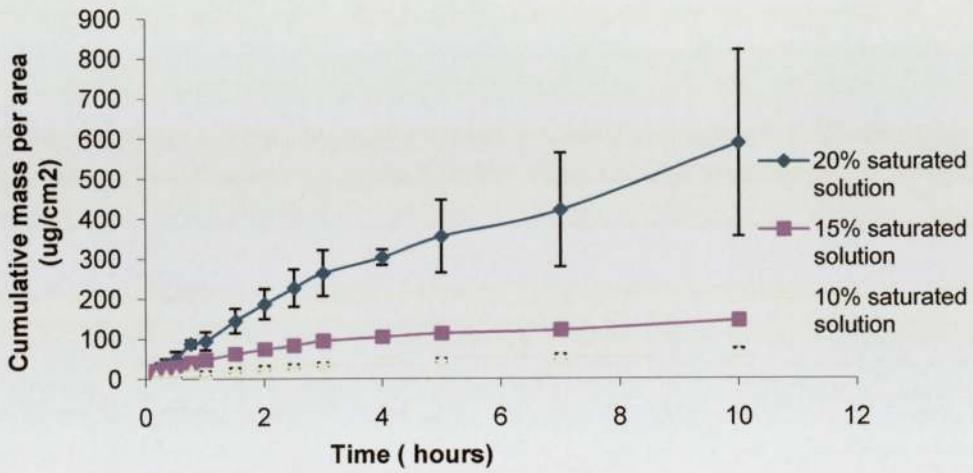


Figure 6 Permeation profiles of 1% triclosan (across silastic membrane) from saturated solutions with 10, 15 and 20% V/V ethanol concentrations. Points represent the means ± SD, n=2.