NOVEL ANTISEPTIC FORMULATIONS FOR SKIN AND SOFT TISSUE INFECTIONS

THI PHUONG NGA HOANG

A thesis submitted in partial fulfilment of the requirements for the degree of MSc Pharmaceutical Sciences (Research)

The University of Huddersfield
2020

Abstract

Skin and soft tissue infections (SSTIs) often refer to acute conditions of inflammatory microbial occupation of the skin layers and underlying soft tissues. As one of the most frequent types of infections, SSTIs typically require medical intervention contributing to morbidity and mortality in both hospitalized patients and those in primary care. The present study aimed to formulate and characterize nanoemulsion as a carrier system to deliver triclosan and chlorhexidine digluconate (CHG) for the purpose of prevention and treatment of SSTIs, by targeting the layers within the skin. Four nanoemulsion formulations were developed successfully using an ultrasonication method. They were stable with a droplet size less than 115 nm along with polydispersity index from 0.15 to 0.41, the pH values in lightly acidic range (4.60-6.80) and the absolute zeta potential values higher than 20 mV. The degree of drug encapsulations ranged from around 77-85%. The result from in vitro permeation experiment exhibited that nanoemulsions made using eucalyptus oil transferred between 2.1 and 6.6 times greater amounts of triclosan through full thickness porcine skin than those formulated using olive oil or triclosan solution. They also lead to more drug being retained within the skin after 24 hours. These findings demonstrated that this nanoemulsion is a promising candidate for topical dermal delivery of a water-insoluble antiseptic agent to the skin. In contrast, although the skin permeation from CHG loaded nanoemulsions was lower than 2% CHG solution, eucalyptus oil nanoemulsion was more effective to withhold CHG in skin than olive oil nanoemulsion and solution, which may be advantageous for localized effect. Further investigations should be conducted to confirm the role of nanoemulsions in delivery of water-soluble antiseptics.

Copyright declaration

1. The author of this thesis (including any appendices and/or schedules to this thesis) owns any copyright in it (the "Copyright") and she has given The University of Huddersfield the right to use such Copyright for any administrative, promotional, educational and/or teaching purposes.

2. Copies of this thesis, either in full or in extracts, may be made only in accordance with the regulations of the University Library. Details of these regulations may be obtained from the Librarian. This page must form part of any such copies made.

3. The ownership of any patents, designs, trademarks and any and all other intellectual property rights except for the Copyright (the "Intellectual Property Rights") and any reproductions of copyright works, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property Rights and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property Rights and/or Reproductions.

Acknowledgments

This master thesis at University of Huddersfield will be unable to complete without the considerable support, encourage and valuable advice from my supervisors, technicians, my family, and friends. Thereby, this is a wonderful chance for me to express my sincere gratefulness to everyone.

Foremost, I would like to express my sincere appreciation to my supervisors Prof Barbara Conway and Dr Muhamad Usman Ghori, for their wholehearted guidance and massive encouragement. I would like to specially thank Prof Barbara for your helpful academic advice and excellent support for my thesis. Particularly, I am genuinely grateful to Dr Usman, who gave me not only the wonderful instructions about lab experiments but also encouragement regarding psychological aspects.

I must also honestly thank technicians and staff at School of Applied Science. I would like to thank Miss Hayley Markham for her enthusiastic support with regards to technical works. I also would like to thank Dr Richard Hughes because of his devoted guidance on HPLC technique within the thesis. Also, I want to send my appreciation to Mr. James Rooney and other staff as well as my lab-mates, who assisted me a lot and make my studying period much more memorable.

Importantly, I would like to convey my sincere gratitude to my family and my fiancé. From the bottom of my heart, I am so thankful for my father, my mother, and my sister. Without their encouragement, support, and belief in me, I cannot have enough strength and patience to finalize this thesis. Finally, I also would like to thank my beloved friend, who gave me enormous moral supports and useful advice that help me to overcome the tough times. Thank you for always beside me.

I appreciate all of your kindness and support. Thank you so much!

Table of Content

List of Figures

List of Tables

List of Abbreviations

µg	Microgram
µL	Microlitre
BSA	Bovine serum albumin
BZK	Benzalkonium chloride
BZT	Benzethonium chloride
CAR	Carbopol
CHG	Chlorhexidine digluconate
CPC	Cetylpyridinium chloride
DEE	Drug Entrapment Efficiency
DLS	Dynamic Light Scattering
EBOV	Ebola virus
EO	Eucalyptus oil
FDA	Food and drug administration
g	Gram
GB	Glyceryl behenate
GMO	Glyceryl monooleate
GP	Glyceryl palmitostearate
HLB	Hydrophilic−lipophilic balance
HPLC	High performance liquid chromatography
LCS	Liquid crystalline systems
LOD	Limit of detection
LOQ	Limit of quantitation
MBC	Minimum bactericidal concentration
MDRAB-Bs	Multidrug-resistant *Acinetobacter baumannii* with biofilms
MDS	Mean droplet size
MIC	Minimum inhibitory concentration
min	Minute
ml	Millilitre
mM	Millimole
MRSA	Methicillin-resistant *Staphylococcus aureus*

mV	Millivoltage
MVA	Modified vaccinia virus Ankara
Na-LS	Na-lauryl sulfate
NEs	Nanoemulsions
nm	Nanometre
NPs	Nanoparticles
O/W	Oil in water
^{o}C	Degree Celsius
OIAIs	Orthopedic implant associated infections
OO	Olive oil
OTC	Over-the-counter
PBS	Phosphate-buffered saline
PCL	Poly(epsilon-caprolactone)
PDI	Polydispersity index
PHMB	Polihexanide
PIC	Phase inversion composition
PIT	Phase inversion temperature
QACs	Quaternary ammonium compounds
rpm	Revolutions per minute
RSD	Relative standard deviation
S80	Span80
SC	Stratum corneum
SD	Standard deviation
SLS	Sodium Lauryl Sulfate
SSTIs	Skin and soft tissue infections
T20	Tween20
T80	Tween80
TCS	Triclosan
TLR-2	Toll-like receptor 2
TTO	Tea tree oil
UV	Ultraviolet
UV-B	Ultraviolet B-rays
v/v	Volume per volume

W/O	Water in oil
w/v	Weight per volume
w/w	Weight per weight
ZP	Zeta potential

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1. General introduction and literature review

1.1. Physiological and Function of Skin

Skin is a large, continuous and specialized organ, responsible for about 15% of the adult body weight [1-3]. Human skin has two appearances, which are glabrous skin on the soles, the glans penis, and the palms and hairy skin on the remaining areas of the body [4]. According to Mosteller, the human skin surface is around 2 m^2 in area [5]; however, due to the enormous number of skin appendages (approximately 5 million) like sweat ducts and hair follicles, human skin may offer an epithelial surface of larger than 30 m^2 for potential colonization with microorganisms [6]. Human skin thickness varies from less than 0.1 mm to more than 1 mm, and also differs considerably, depending on anatomical body regions, ethnic origin, age, and gender [7-9]. The normal cutaneous pH is found to be from 4 to 6.8 [9], this acidic pH range along with hydration and minerals provide an ideal environment for the growth of resident microorganisms, contributing to the formation of a physical, chemical and microbiological barrier [10,11]. Human skin has four major physiological functions, including sensation, thermoregulation, protection, and metabolism [12]. Each function corresponds to specific structures and properties of distinct skin areas [9,13].

1.2. Structure of skin

The epidermis, the dermis and the subcutaneous tissue make up the three main layers of skin [14].

(i) The epidermis is the outermost layer and is composed of four different layers, including the basal layer, spinous layer, granular layer and cornified layer, in the order from inner to outer [14]. The basal layer (stratum basale) contains small cuboidal cells that divide continually and migrate upward to repopulate the cells lost from the skin's surface [2]. Just above the basal

layer is the spinous layer (stratum spinosum), so-called due to the high density of desmosomes (intercellular connections) and keratin filaments (tonofilaments) surrounding each cell, bring the cells a “spiny” shape. The next layer is the granular layer in which keratohyalin granules are formed and then bind to the tonofilaments to form abundant electron-dense masses within cytoplasm that bring this layer its “granular” characteristic [14]. The cornified layer (stratum corneum) is the superficial, non-nucleated barrier where the organelles and nucleus of keratinocytes are removed. The keratin filaments and keratohyalin granules combine to create an amorphous mass within the keratinocytes prior to becoming the lengthy and level cells of the horny layer [2,14].

There are four cell types observed in the epidermis area [15], in which, keratinocytes account for the majority of the epidermal cells [15]. These cells derive from the stratum basale layer, undergo three stages of a keratinization process - germinative, differentiation, and protective - before formulating the keratin layer [9]. Keratinocytes are sloughed off constantly and undetectably from the skin’s surface every 24 hours [9]. This progress continues through life and it takes approximately 30-50 days for the epidermal turnover time of normal physiological skin from basal cells to exfoliation, depending on different sites [2]. The epidermis also contains a smaller number of melanocytes, Merkel cells and Langerhans cells [2]. Melanocytes are dendritic cells [9] responsible for the synthesis of the pigment melanin and play an important role in absorbing harmful solar ultraviolet radiation [15]. Melanin pigment ranges in colours from yellow, red to brown, black and are the key factors in determining the skin and hair colour [9]. Long-term light exposure increases the number of melanocytes surrounding the keratinocytes, this is the reason for the variation in the melanocyte numbers in different body regions, though the entire amount is equivalent among all individuals [15]. Ultraviolet light and hormones are considered as the elements affecting skin pigmentation process [9]. Langerhans cells originate in bone marrow and are mostly distributed in the spinous layer of the epidermis

[9,15]. These dendritic cells act as antigen representing cells and are very important in immunologic reactions by reactivating the lymphocytes [9]. Normally, there is only a small number produced in the skin but this amount increases in several inflammatory conditions, for example, allergic contact dermatitis [2]. The remaining specialized cell in the epidermis are the Merkel cells whose usual functions are not fully understood but are considered to take part in touch sensation due to the large concentration found in the basement membrane zone and connect with nerve endings [15].

(ii) The dermis lies directly underneath the epidermis, provides support and nourishment to the epidermis as well as taking part in the wound healing process and re-modelling [9]. The thin outward zone called the papillary dermis contains fine elastic fibres and collagen, capillaries and anchoring fibrils which help to adhere the epidermis to the dermis [2]. The bulk of the dermis is assembled by the reticular dermis which composed of type III collagen and a thinner matrix of elastic fibres [2,15]. Whilst the collagen fibres contribute to skin solidity, the elastic fibres confer elasticity to the skin [9]. Dermal proteoglycans (primarily hyaluronic acid) surround collagen and elastic fibres and have the primary contribution in impeding water evaporation [14]. Others constituents present within the dermis are lymphocytes, macrophages, mast cells and fibroblasts; fibroblasts are mainly responsible for producing collagen, elastin, and proteoglycans [2,14]. Additionally, blood vessels, lymphatic vessels, sensory nerve endings, pressure receptors, sweat glands, sebaceous glands and hair follicles also lie across the dermis [2]. The bundles of collagen in the form of ridges radiate horizontally around the body and are named cleavage lines. They are unique features of each individual, and can be used in genetical identification [16].

(iii) The subcutaneous tissue (also called the hypodermis) is the deepest layer of the skin which mainly consists of fat, connective tissues and capillaries [16]. The essential functions of

this area are insulating from extreme heat and cold and performing as a mechanical shock absorber. There is a regional difference in the thickness of the hypodermis layer and is completely absent from the penis, nipples, eyelids, areolae and scrotum, and the skin around the tibias. The distribution of subcutaneous tissue is influenced by secondary sex characteristics, genetics, age as well as caloric amount intake [9].

(iv) The skin appendages comprise glands, hair and nails [9]. There are three sorts of **gland**: eccrine sweat glands, apocrine sweat glands and sebaceous glands [9]. Eccrine glands are located in a widespread pattern across the skin surface with the highest density on the axillae, the soles of the feet, palms and the forehead [2]. The eccrine sweat gland is a part of the body temperature control system, achieved through secreting sweat which gives an evaporative cooling effect for the skin [2,9]. Sweat is conveyed in a spiral duct that is located upwards through the dermis and epidermis to the surface [2]. Apocrine glands principally exist in the anogenital areas, mammary areolae, axillae, and external auditory canal [2,9]. The apocrine duct empties into the superficial layer which is above the sebaceous glands. The substance released from the apocrine gland is a viscous material that contributes to body scent [9]. The sebaceous gland is a holocrine gland and they are distributed across all areas of the skin's surface but predominantly on the scalp, face, upper torso and genitalia but in contrast to eccrine glands, they are absent from the palms and soles [9]. Their function is producing sebum – a lipid-based combination of wax esters, squalene, cholesterol esters and triglycerides [14]- which makes up a moist, oily acidic film that has antibacterial and antifungal properties as well as waterproofing properties and protecting the hair shaft and the epidermis [2,3]. **Hair** is a structure of the epidermis derived from keratin, present in all anatomical sites of the body, apart from the palms, soles and muco-cutaneous junctions [9]. The hair follicle is a tubular epithelial structure [2] and is the result of the downward growth of the epidermal cells invaginating into the dermis [9]. The deepest part of the hair follicle is the germinative hair bulb which situated

in the dermis or subcutaneous fat layer [2]. The hair shaft, which begins from the hair bulb, is a complex multi-layered structure with an outer cuticle- cortex and an inner medulla [15]. Melanocytes in the hair bulb contribute to hair colour determination [2]. **Nails** are made from a specialized type of keratin and are sited at the ends of the distal surface of fingers and toes. The typical functions of nails are to protect the digit endings and serve as a grasping tool [9]. The nail is shaped by the hard plate (nail plate) which is surrounded on three sides by the cuticle (or nail fold). The nail matrix lies below the proximal nail fold and is the site of the nail plate growth. The crescent-shaped, white zone at the end of the proximal nail fold is called the lunula which implies the mitosis position [15].

1.3. Penetration of drug molecule into the skin

Intrinsically, skin penetration is a diffusional process that obeys a passive transport mechanism. It is influenced by solubility of the active substance, the partitioning of drugs in the carriers and in the skin and the diffusive ability of actives in the skin [17,18]. The fundamental barrier with regard to the physical skin structure that restricts the percutaneous absorption of substances is the stratum corneum (SC), which is the most outward 10-20 μm layer of the epidermis [19]. Owing to the specific structure and composition of corneocytes and intercellular bilayer lipids that make up the "Brick-and-Mortar" structure, the stratum corneum offers a protection against the pervasion of external chemical and biological toxicants as well as preventing transdermal dehydration [20,21]. As active molecules come into expose with the skin surface, they can diffuse through three different pathways, i.e., (1) the appendageal or shunt route through sebaceous glands, sweat glands and/or hair follicles, (2) the intercellular route across lipid organizations between the corneocytes, and (3) the intracellular route passing the corneocytes (Figure 1.1) [19,22].

Most permeants are able to move through the SC by both the intracellular and intercellular

pathways, although hydrophilic substances tend to follow the former pathway and lipophilic compounds the latter route. The predominant permeation route for the majority of actives is through the intercellular lipid spaces [23,24]. Physicochemical characteristics of any xenobiotic are the critical factors that govern their diffusion into the skin, are molecular weight, polarity, and hydrophobicity [25]. It is generally accepted that only a small number of drugs with specific physicochemical features can adequately transfer through the skin, such as low molecular weight, adequate solubility in water and oil or high lipophilicity [24,26]. For large polar molecules and ions that are difficult to diffuse across the SC, the shunt route through hair follicles is the major pathway [27,28]. Yet, the appendages only made up approximately 0.1% of the entire skin volume so that their contribution to the drug penetration progress is often considered trivial [29].

Penetration enhancers, which are chemical substances with the ability to change the skin properties, are used to promote active permeation. They can act by the following mechanisms: (1) disrupting the intercellular lipid matrix; (2) increasing the skin moisturization; (3) improving the solubility and partitioning of drugs in the SC; and (4) interacting with keratin filaments that leads to disruption of corneocyte organization [30]. For instances, terpenes (cineole, menthol, eugenol, etc) are well-known penetration enhancer as they can influence the non-polar penetration pathway by interact with intercellular lipid. They may also increase partition coefficient, lipid extraction and actives solubility [31].

In addition to the chemical approach, electrical methods that apply external energy can enhance penetration of a topical therapeutic agent, for instance, electroporation, iontophoresis, ultrasound or laser radiation and photomechanical waves [22,32]. Other mechanical methods as tape stripping or dermal scraping can thin the cutaneous upper layers thus enabling penetration, whilst massages typically soften the skin barricade and can facilitate the appendageal transport

[33].

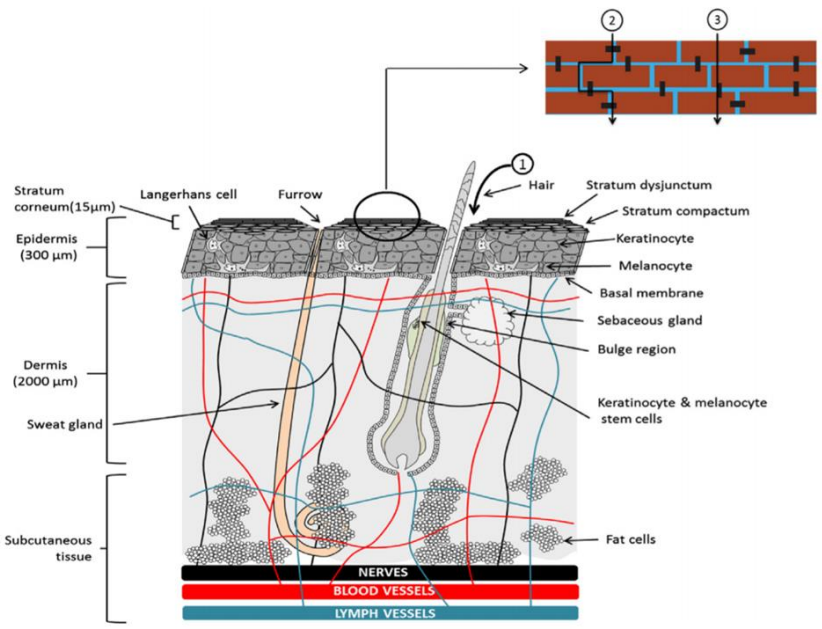


Figure 1.1 Structure of skin and penetration pathways, adapted from [22].

1.4. Skin microbiota

The community of microorganisms (bacteria, yeasts, and viruses) found on the human skin is diverse on account of number, types as well as colonizing sites on the body. Such variety arises from differences between the structure and constituents of the skin, which are its thickness, pH, temperature, hydration, and distribution of secretory glands and hair follicles [34]. The estimated number of microorganisms on the skin is 3.8×10^{13}; this figure is much greater than the number of human cells [35]. The skin microbiome has traditionally been divided into two main genres, namely resident and transient floras. Skin resident flora (also called "microflora") include miscellaneous species of *Corynebacterium, Propionibacterium, Staphylococcus and Micrococcus* and its colonization varies with anatomical areas. Drier skin regions tend to have higher levels of gram-positive cocci, including *S. epidermidis* and *Micrococcus* species, while moister regions present more gram-positive rods, like *Corynebacterium* and *Propionibacterium* species [34,36]. Transient flora (or 'contaminant flora'), which establish

colonies in the outermost skin layers, mainly consist of *Staphylococcus aureus*, and some Gram-negative bacteria of the *Enterobacteriaceae* and *Pseudomonaceae* groups [37].

Skin microflora communities play a crucial role in reinforcing the cutaneous defence of the host against harmful microorganisms through a range of different mechanisms. Particularly, they potentially inhibit the flourishing of cutaneous pathogens by excreting antibacterial substances. *S. epidermidis*, for instance, produces a serine protease (called *Esp*) which can interfere with biofilm formation of *S. aureus*, and phenol-soluble modulins which are able to disrupt lipid membranes of some microorganisms such as *S. aureus* and Group A *Streptococcus*. Furthermore, several studies indicated that *S. epidermidis* bolsters the expression of antimicrobial peptides in keratinocytes such as β-defensins 2,3 via impacting on Toll-like receptor 2 (TLR-2), contributing to the prohibition of *S. aureus*-induced infections. Activating TLR-2 by lipoteichoic acid (LTA, a TLR-2 ligand) from *S. epidermidis* also can increase the number of both mast cells being gathered in the site of viral colony and antimicrobial peptides (AMPs) released from these mast cells [38,39]. Furthermore, staphylococcal LTAs mediated via TLR-2 on keratinocytes indirectly inhibit the release of TLR3-dependent inflammatory cytokines, thus limiting inflammatory responses subsequent to skin injury [40].

It can be generally accepted that the breakdown of the balance of microbial community on human skin brings opportunity for the overgrowth of pathogenic microorganisms, as a result causing a variety of skin diseases [39], such as seborrheic dermatitis typically emerging on oily areas like the scalp, nose, upper back where is dominantly cultured by *Malassezia spp*.; atopic dermatitis (AD) mostly caused by *S. aureus* or teenage acne that mainly correlates with *Propionibacterium acnes* [38].

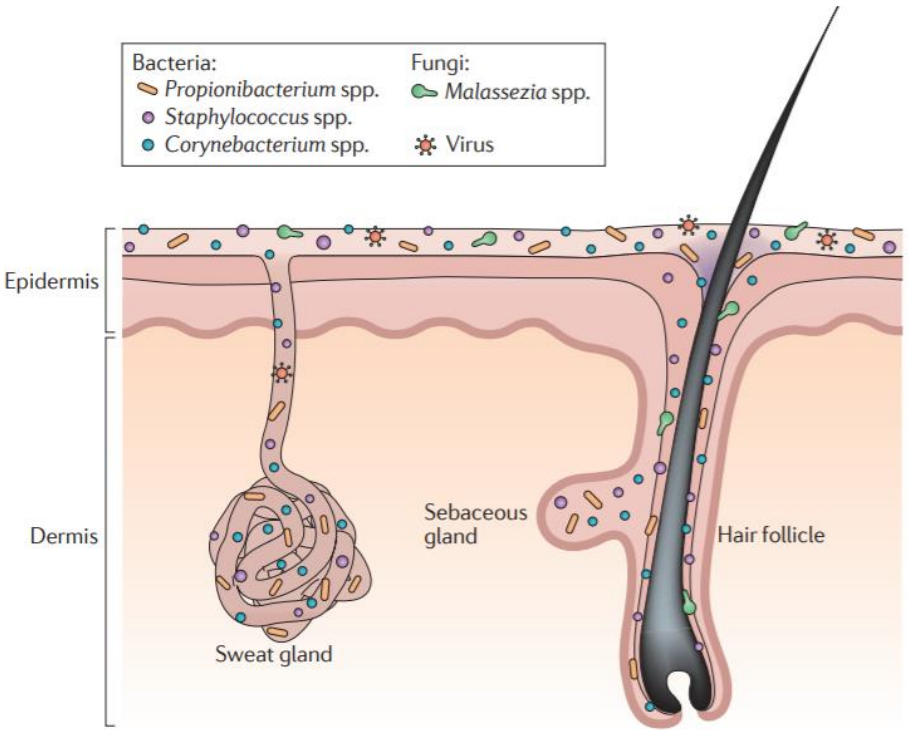


Figure 1.2 The distribution of human skin microbial communities, adapted from [36].

1.5. Skin and soft tissue infections

Skin and soft tissue infections (SSTIs) often refer to acute conditions of inflammatory microbial occupation of the skin layers and underlying soft tissues [37,41]. The consequences have implications on healthcare not only in developing countries with poor sanitary conditions but also in developed ones with higher hygiene standards [42]. As one of the most frequent types of infections, SSTIs typically require medical intervention contributing to morbidity and mortality in both hospitalized patients and those in primary care [37]. The estimated percentage of 7-10% of entire hospital administrations in 2005 in North America was made up by skin and soft tissue infections [43]. A national investigation conducted in the United States between 1997 and 2005 indicated that there was an elevation of 65% of SSTIs patients admitted indifferent hospital departments, from 32.1 visits per 1000 population in 1997 to 48.1 visits per 1000

population in 2005 [44]. Likewise, Lee and co-workers surveyed SSTIs tendency in the US from 2000 to 2012 and they recovered that the total prevalence of SSTIs rose from 2.4 million to 3.3 million (nearly 40%) during this period [45]. In 2013, nearly a third of the American population asked for medical advice related to skin conditions [46]. The incidence of SSTIs has increased, possibly as a result of an ageing population, the escalation of multidrug-resistant strains and the increasing numbers of immunocompromised patients related to immunosuppressive therapy, cancer, transplant interventions or HIV/AIDS [37,47]. Global Health Metrics published findings in 2017 regarding the prevalence, incidence, and years lived with disability covering 354 diseases in 195 countries; accordingly, there were nearly 4.2 billion new cases of skin and subcutaneous diseases worldwide. Around 50% of those were fungal skin diseases (with more than 2.1 billion), whereas the incident cases linked with bacterial and viral pathogens were 0.27 and 0.12 billion, respectively [48].

Pathophysiology of SSTIs is related to an interruption of the balance of between the immune barrier of the host and the pathogenicity of microbial population colonizing human skin [37]. Cellulitis, as an example, is caused by pathogens disrupting skin integrity, and is more prevalent in patients with comorbidities [49]. Disruption of the protective cutaneous layers can be caused by numerous chemical and physical impacts such as ulceration, trauma, bites or surgical wounds, thermal injury or previous inflammation [37,49]. Both the patient and the environment are key factors contributing to the risk of developing an SSTI. Patients with advancing age or with long-term conditions such as critical illness, obesity, cardiovascular diseases, chronic kidney disease failure will be at higher risk of skin breakdown. Patients with spinal cord injury and paralysis that result in the alteration of skin perfusion and temperature control also should be considered higher risk. The external factors which are likely to impair the skin barrier function can be scratching, pressure, shear and friction, UV exposure or radiation contact in cancer patients [37,50]. Additionally, biofilm formation, which is modality

for microbe to survive and adapt to unfavourable conditions, has become a severe problem in the healthcare fields as it was responsible for 65% of nosocomial infections. The biofilm is produced by attaching cell to a surface, multiplying, maturating, then creating an extracellular polymeric matrix which resists environmental impacts such as mechanical forces and antibiotics. This structure is detachable, making opportunities for microorganisms to transmit into new sites and spread infections. The biofilms of poly-microbial were observed in medical devices such as intravenous and urinary catheters, stents, implants, ventilator tubes, or heart valves, contributing to the accumulating antimicrobial resistance [51].

In children, bacterial skin infections are more prevalent than fungal, parasitic and viral infections [52]. The major causative pathogens associated with skin and soft tissue infections are Gram-positive microorganisms, typically *Staphylococcus aureus* (including methicillin-resistant *S. aureus*/MRSA strains) and *beta-hemolytic streptococci* [41]. The most frequent Gram-negative strain isolated was *Klebsiella sp*. [53]. *S. aureus* was responsible for more than 40% of total SSTIs cases in 2003, and was a frequent cause of cellulitis, abscesses and wound infections [37]. The incidence of *S. aureus*-related skin and soft tissue infections increased two-fold from 2001 to 2009 [54]. However, in the next 5 years, it was reported that the proportion of hospital administrations caused by MRSA-related skin and soft tissue infections (SSTIs) declined by 29% [55].

Patients with dermatologic conditions often withstand intensive physiological, psychological as well as financial issues; not only that, many cutaneous concerns can lead to systemic diseases [56]. Moreover, comorbidity factors, such as diabetes, immune-compromisation, obesity, liver and kidney failure, and cardiovascular diseases, have repercussions on treatment costs and prolong the length of stay in hospital [57]. Suaya et al. determined the cost of SSTI hospitalizations in relation to *S. aureus* in 2009 was $4.50 billion which was 34% higher than in 2001 [54]. According to the Global Burden of Disease Study, 15 different dermatologic

concerns account for 1.79% of the total global burden of disease in 2013. This was calculated through disability-adjusted life years index, of which cellulitis, viral skin diseases and fungal skin diseases accounted for 0.04%, 0.16% and 0.15% respectively. Skin and subcutaneous conditions, next to iron deficiency anaemia, tuberculosis, and sensory organ diseases were the leading reasons inducing disability in the world [48,56].

The management of SSTIs often depends on the relative severity. Uncomplicated SSTIs located in superficial layers typically can be controlled with a topical antimicrobial agent, heat packs or minor incision and wound exudate draining, while more complicated cases with involvement of deeper layers with high-risk factors often require systemic antibiotic therapy and hospital administration [37]. With regards to the emergence of resistant bacteria and antimicrobial stewardship, there is an overall drive to reduce any unnecessary and inappropriate use of antibiotics. Owing to the broad-spectrum of antimicrobial activity alongside with the varying inhibitory mechanisms, topical antiseptics are advocated as a potential alternative to topical antibiotics in the treatment of minor skin infections [58-60]. Although the safety and clinical effectiveness of many antiseptic agents have been insufficient so far [61], they bring potential benefits in the prevention of infections in wounds [58] and are still commonly recommended during pre- and per-operative processes which are documented in many global practical guidelines [62]. Also, a wide range of antiseptics are used mainly as simple dosage forms like solutions and semisolid, but there have been numerous studies to implement formulation strategies in order to potentially influence therapeutic efficacy in recent years [63].

1.6. Antiseptics

Antiseptics are biocidal products that can kill or impact the growth of disease-causing bacteria in or on living tissue, e.g. on the skin. Ideal properties include widespread and rapid bioactivity against bacteria, fungi and viruses, no toxicity or damage to the healthy tissue and insignificant

absorption into the systemic circulation following external application [34]. Antiseptic products may contain one or more active ingredients and are presented in various formulations and preparations, for example, antimicrobial hand washes, surgical scrubs, preoperative preparations, tinctures, ointments, creams, mouth-rinses, and toothpaste. They are commonly used as pre-operative skin preparations for prevention of surgical site infections [64], as routine skin hygiene such as hand-washes and hand rub products or for treating skin and wound diseases [34]. For skin and wound infections in deeper skin layers, antibiotics are popularly selected as the treatment of the specific skin pathogens; in contrast, topical antiseptics are preferred in the cases of infections in the outermost surface. In such cases, the aim is to minimize any microbial colonization in a wound or skin surface infection without causing any serious effects on the living tissue or impeding the healing process [34,65]. In the following section commonly used antiseptics are discussed and their chemical structure are illustrated in Figure 1.3.

1.6.1. Chlorhexidine

Chlorhexidine is a cationic polybiguanide (bisbiguanide) [66]. It primarily used as salt forms because of its insolubility in water. Chlorhexidine gluconate, known as CHG, and other salts like chlorhexidine diacetate, dihydrochloride and dihydrobromide are used as surficial disinfectants, in cosmetics (added ingredient in creams, hair care products, deodorants, and antiperspirants), and health-care preparations (e.g., preservative in eye drops, wound dressings and mouth-rinse) [34]. Chlorhexidine is supplied typically in solution from 0.5 to 4% w/v. Chlorhexidine gluconate 2% w/v (CHG) in 70% v/v isopropyl alcohol (IPA) is particularly recommended in order to clean skin before surgical procedures by several organizations, such as The Health Protection Scotland (2013), Centre for Disease Prevention and Control (2017), National Institute for Health and Clinical Excellence (2013) and Word Health Organization (2017) [67,68]. Chlorhexidine solutions at concentrations 0.5% and above with alcohol are

employed to prepare skin prior to peripheral venous catheter insertion to prevent catheter-related bloodstream infections [69]. Chlorhexidine is a broad-spectrum antibacterial active against both gram-positive and gram-negative bacteria, exhibiting some restricted activity on yeasts, dermatophytes, and some lipid-enveloped viruses [34]. Furthermore, Macias *et al* concluded that CHG in IPA is the preferred antiseptic for prolonged medical interventions because of its long-lasting residual effect, in comparison with 1% w/v triclosan in 70% IPA [70]. Gels containing 2% w/v CHG also demonstrated a higher fungicidal activity than those containing silver nanoparticles [71]. Alcoholic CHG solutions at both 0.5% and 1.0% w/v concentrations were better than 10% w/v aqueous povidone-iodine (PVP-I) in minimizing microbial colony formation related to intravascular catheters [72].

The mechanism of antimicrobial activity of chlorhexidine is that the positively charged molecular binds to the negatively charged lipid bacterial cell surface, thus weakening the cell membrane integrity followed by leakage of cytoplasm and precipitation of proteins and nucleic acids at lower concentrations and membrane disruption at higher concentrations [34,73]. Due to this non-specific mechanism of action, chlorhexidine use is widespread. However, there are some issues with its use, such as potential toxicity in the eyes, ears and brain, it become inactive quickly in the presence of non-ionic surfactants, and it may cause dry skin [34,74]. Recently, the Food and Drug Administration (FDA) released a warning regarding the increasing occurrence of rare but severe allergic reactions to CHG. According to the FDA, healthcare specialists should take into account the patient's allergy history prior to prescribing CHG-based products [75]. Furthermore, some current studies indicated that the increased use of CHG may responsible for cross-resistance to colistin and daptomycin and the reduced susceptibility (manifested by the higher of CHG minimum inhibitory concentrations) in several skin pathogens such as *Klebsiella pneumoniae*, multidrug-resistant *Acinetobacter baumannii* , *S. epidermidis, S. aureus* and vancomycin-resistant enterococci (VRE) [76-79].

1.6.2. Triclosan

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is a phenoxyphenol compound that has been principally considered as an antibacterial and antifungal agent [34]. Triclosan has a very low aqueous solubility of 0.012 g/L at 20^{o}C [80]. It is one of the most common ingredients used in various antiseptic products, especially in antimicrobial soap, body and hand washes, toothpaste. The concentrations of 0.1 to 2 % w/v are regularly applied, with or without other active antimicrobials such as alcohols, to bring about a long-lasting activity on the skin. Triclosan is active against gram-positive bacteria, including *Staphylococcus* species. Moreover, it may also have an effect on gram-negative bacteria and yeast, with some weaker activity against enveloped viruses, pseudomonads, and fungi [34]. Originally, triclosan was thought to target the cell membrane in a non-specific modality. However, recent studies have found a specific bacteriostatic action for triclosan on bacteria through inhibiting the bacterial fatty acid biosynthetic pathway. At the higher concentrations found in antiseptics preparations (2-20 mg/ml), there is a hypothesis that triclosan acts as a biocide with multiple actions on lipid, RNA and protein synthesis, leading to the cell lysis [79,81]. The antimicrobial property of triclosan-containing antiseptics can be influenced by formulation effects, for example, there is a synergistic activity with chelating agents (e.g. EDTA) in destroying the gram-negative bacterial cell wall thereby improving uptake into cells. Triclosan shows negligible irritation and allergy skin reactions and it can retain persistent on the skin surface [34]. Yet, because of the lack of the scientific literature regarding the safety and effectiveness of triclosan for human health, in December 2017, the FDA issued a final rule related to the prohibition use of triclosan in certain over-the-counter antiseptic preparations [61].

1.6.3. Povidone-Iodine (PVP-I)

Povidone-Iodine (also known as PVP-I), which is a complex of elemental iodine loosely bound to the carrier polyvinylpyrrolidone, is used as a broad-spectrum antimicrobial agent against bacteria, virus, fungi and protozoa at relatively low concentrations [34,82]. Typically, PVP-I is widely applied as a topical antiseptic and disinfectant for skin and wound infections mostly in solution, dry powder and lotion formulations. Use as an iodophor improves both solubility and stability while releasing the active iodine gradually from the polymer network over time. Therefore, its residual antimicrobial activity is maintained stably while side effects associated with iodine such as irritation and brown staining on the skin and mucous membranes are reduced. The precise mechanism of action is still unknown but it is believed that the active iodine species plays a role as an oxidizing agent which reacts with cell walls and membranes as well as cytoplasm by exchanging and inactivating functional groups of amino acids (e.g. lysine, histidine, cysteine and arginine). The consequence is the loss of cell structure and function [34].

1.6.4. Alcohol

Alcohols offer rapid and broad-ranging activity against bacteria, fungi and viruses although less is known about their activity against protozoa and bacterial spores but they are sporistatic. Isopropanol (isopropyl alcohol), ethanol and *n*-propanol are the most popular alcohols used as antiseptics and disinfectants. The exact mechanism of action on microorganisms is not clear but they are able to cause denaturing and precipitation of proteins thus destroying cell membranes and leading to cell lysis. Concentrations ranging from 60% to 80% v/v are recommended for maximum antimicrobial activity because, in more concentrated solutions, alcohol quickly coagulates protein molecular external the cell wall and interferes with the penetration into the cell, therefore limiting further effects on protein-based inner cell compositions. Other potential attributes are relative stability, low toxicity, less odour and low

cost. Alcohols are also used as preservatives and common solvents for other biocides such as chlorhexidine [34].

1.6.5. Essential oils

Essential oils are secondary metabolic products found in various parts of plants (such as flowers, seeds, leaves, peels, buds, barks, wood or roots), and can be extracted by hydro-distillation and steam distillation, mechanical processes, or by "dry" distillation for some woods [34,83]. They are complex mixtures containing hundreds of compounds and the exact chemical composition depends on extraction processes and specific conditions. For example, dry vapor steam distillation is used when there is a requirement to minimize ester hydrolysis (e.g., linalyl acetate), or cohobating is proposed to improve the quantity of particular compounds such as sulfur compounds [83]. Essential oils and their components have been used in a wide range of products, from fragrances, toothpastes, cosmetics, to aromatherapy and phytomedicine, with tea tree oil and eugenol, being combined in many commercial antiseptic preparations, such as Ord River Tea Tree Antiseptic Cream®, Australian Tea Tree Antiseptic Cream® or Manuka Doctor ApiRevive Manuka & Tea Tree Antiseptic Gel® [34]. In dermatology, essential oils are primarily used for treating skin infections (62% of the total cases), followed by skin inflammatory and general skin maintenance with 20% and 18%, respectively [84]. Relative bioactivity varies between the different oils. In particular, tea tree oil demonstrates bactericidal activity (at 0.25 to 0.5% v/v), fungicidal activity (at 0.06-1% v/v), fungistatic activity (within 0.03-0.5% v/v) as well as activity against yeasts and dermatophytes (including *Candida* and *Trichophyton*). Tea tree oil, amongst others, presents persistent and long-lasting activity on the skin after application. Despite most essential oils presenting antimicrobial effectiveness at low concentrations, they have been reported to generate irritancy and allergenicity following application to skin and mucous membranes [34,84]. Almost 1.8% of

patients tested with 5% and 10% tea tree oil patches experienced allergic contact dermatitis [85].

1.6.6. Silver compounds

The active element is the silver ion (Ag^{2+}) in silver nitrate ($AgNO_3$) and silver sulfadiazine (AgSD). Generally, topical silver antiseptics are applied for prevention of skin and wound infections mostly caused by *S. aureus* and *Pseudomonas* in cream or solution forms and used in eye drop preparations for eye bacterial infections in neonates [34]. There are a number of studies indicating the valuable role of silver in wound care [86]. Additionally, silver compounds are also commonly used to cover surfaces prone to bacterial colonization such as catheters or dental instruments. Many commercial silver-based products are now available in many forms such as Atrauman Ag® Wound Dressing, Urgotul® SSD Antibacterial Contact Layer, Flamazine® Antibacterial Cream, Colloidal Silver Spray®, Silver Solution® Antimicrobial Wound Gel, MSM+Silver® Water Drops or Natural Sense Colloidal Silver ® Eye Drops.

In term of spectrum of activity, silver compounds exhibit bacteriostatic and bactericidal activity at fairly low concentrations, especially on gram-positive bacteria. Regarding the mechanism of action, active silver ions bind to sulfhydryl, amino and carboxyl groups of amino acids on microorganism surfaces, thus denaturing proteins, and disrupting the cell wall and membrane functions. Silver also specifically inhibits cell wall metabolism, electron transport as well as the respiration chain [34,87]. Through applications of topical antiseptic, respiratory sprays, implanted medical devices or wound dressings, silver may be absorbed into the systemic circulation, mostly in conjugation with protein and then deposited in human tissues, with higher levels in skin, kidneys, eyes, brain, liver, and bone marrow [88]. Argyria is a rare cutaneous condition resulted by excessive or chronic use of preparations containing silver, with the most characteristic symptom is the discolouration of skin into blue or blue-grey, especially in

sunlight-exposed areas [89].

1.6.7. Other antiseptic agents

(i) Quaternary ammonium compounds (QACs) are cationic surfactants which have both hydrophobic and hydrophilic groups in molecular [34]. QACs target cell walls and membranes. They are quickly absorbed into cell walls, interact with membrane lipids, thus disrupting cell structure and function or cause denaturing essential cell proteins, and leaking cytoplasmic material [34]. The antimicrobial activities of QACs remarkably rely on their specific types and formulations and can be impacted by fatty substances or anionic surfactants. For example, benzethonium chloride (BZT) is used as a topical anti-infective and an antiseptic effective against bacteria, fungi, moulds and viruses [90]. Also, benzalkonium chloride (BZK) is used widely as antimicrobial preservative or biocide surfactant, and is especially commonly found in ophthalmic solutions [91]. BZK displays broad-spectrum activities against bacteria, fungi, virus, algal, but not endospores [92]. The widespread use of BZK may contribute to the increase in antibiotic resistance concern [93]. Cetylpyridinium chloride (CPC) demonstrates antiseptic activities against gram-positive pathogens and yeasts, but has no effect on gram-negative microorganisms and mycobacteria. CPC is commonly found as active ingredient in mouthwashes, toothpastes, lozenges or mouth sprays for treating minor mouth and throat infections [94,95].

(ii) Octenidine dihydrochloride (OCT) is also a cationic surfactant, belongs to the bipyridine group [96] and has offered a wide range of applications such as preoperative skin preparations, prevention and treatment skin and wound infections [97]. Its spectrum of activity covers both gram-positive and gram-negative pathogen including MRSA [97]. Octenidine reduced more than $7\log_{10}$ of high-level mupirocin-resistant *Staphylococcus aureus* isolates *in vitro* at concentrations as low as 0.001% w/w within only 30 seconds [98]. Similar findings were seen

for multidrug-resistant gram-negative bacteria [99]. Octenidine dihydrochloride (0.1%) with 30% v/v 1-propanol and 45% v/v 2-propanol was more effective than 74% v/v ethanol with 10% v/v 2-propanol for eradication of skin colonization in central venous catheter sites over 24h [100]. Moreover, octenidine was highly effective in reduction of infections associated with biofilm formation on orthopaedic implants infections, compared to gentamicin [101].

(iii) Polihexanide (PHMB) is a biguanide antiseptic whose chemical structure is similar to chlorhexidine [102]. The positively charged molecular species interacts electrostatically with the negative-charged lipopolysaccharide compounds of bacterial cell membrane, leading to the leakage of intracellular components, therefore, PHMB can be effective on both gram-negative and gram-positive pathogens [103]. Octenidol® and ProntOral® mouthwashes which contain octenidine and polyhexamethylene, respectively displayed indistinguishable antimicrobial potency with 0.2% chlorhexidine digluconate in eliminating *Streptococcus sanguinis, Streptococcus mutans, Candida albicans and Fusobacterium nucleatum* [104]. Additionally, both 0.02% PHMB and 0.05% OCT were superior than NaCl 0.09% w/v solution in removal of biofilms of *Pseudomonas aeruginosa* [105]. PHMB is well- tolerant when applied to both skin, and wounds [106].

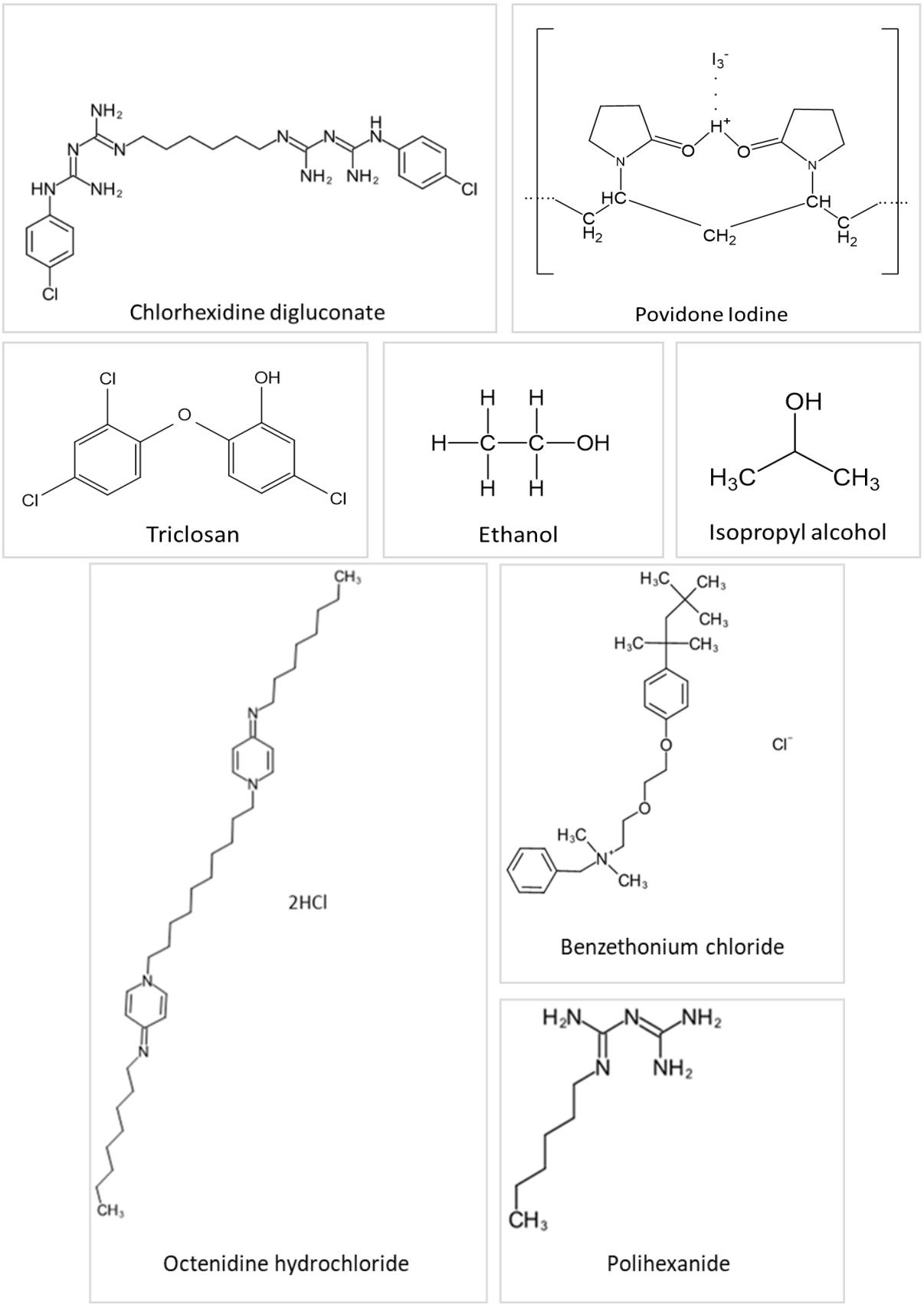


Figure 1.3 Chemical structure of several antiseptic agents

1.7. Traditional pharmaceutical formulations

1.7.1. Solutions

According to the US Pharmacopeia-National Formulary, solutions are defined as aqueous homogeneous systems which comprise of solvents and one or more solutes [107].

Comparisons of antiseptic performance of solution formulations are relatively well reported. Particularly, in a 2-step study, 2% w/v chlorhexidine gluconate in 70% v/v isopropyl alcohol was proven to have more substantive efficacy compared to 10% w/v sodium hypochlorite and 10% w/v povidone-iodine [108]. Similarly, it has demonstrated a longer-lasting residual effect than triclosan (1% w/v) in 70% v/v IPA, making it more suitable for long procedures such as catheter insertion or surgery than other antiseptics [70]. Chlorhexidine gluconate 2% w/v in 70% v/v ethanol was effective in eradicating multidrug-resistant *Acinetobacter baumannii* with biofilms (MDRAB-Bs) as no MDRAB-Bs detected after only 1 minute of contact [109]. On the other hand, according to Koburger et al. (2010), taking into account minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) values, the antimicrobial effect of polyhexanide and octenidine were deemed to be greater than chlorhexidine digluconate, PVP-iodine and triclosan against the tested microorganisms. In the quantitative suspension test (to determine the minimal concentrations to achieve at least a reduction of 3.8 log cycles for *C. albicans* and 4.8 logs for *S. aureus and P. aeruginosa*), octenidine showed the most effective bactericidal and fungicidal efficacy at all of time points, in contrast to triclosan [110]. Another recent clinical trial found that 70% isopropyl alcohol solution offered an equivalent effect with 2% chlorhexidine gluconate in 70% IPA for skin antisepsis [111], supporting the use of cheaper antiseptics like alcohol [111]. Furthermore, it was found that the simultaneous application of 10% w/v PVP-I and a topical antiseptic Alkosol® (96% ethanol, 30g isopropanol and 0.1g ortophenilphenol) in a 2-step pre-operative procedure

reduced the extent of surgical site infections as only 6% of included patients had at least one symptom of inflammation after 24 hours of surgery, whereas this figure for the use of 10% povidone iodine alone was up to 40% [112].

Bashir *et al* reported that using a film-forming polymer such as an acrylate in a pre-operative solution preparation comprised of 2% chlorhexidine in 70% isopropyl alcohol to sustain CHG at the skin surface effectively reduced bacterial colonization in an *ex vivo* model, corresponding to greater antimicrobial activity in preventing surgical site infections [113].

A topical povidone-iodine solution was employed in a phase 2 trial to treat cancer therapy-related paronychia – an acute nail infection caused by targeted and cytotoxic remedies. The outcomes were a positive effect with twice daily application of 2% PVP-I solution on clinical outcomes and quality of life [114].

1.7.2. Patch formulations

A novel mucoadhesive buccal patch which consisted of 4 mg triclosan and used matrix-forming polymers low methoxy amidated pectin (AMP) and 20% w/w Carbopol (CAR). The authors estimated the impact of β-cyclodextrin-epichlorohydrin polymer (EPIβCD) and anionic carboxymethylated β-cyclodextrin-epichlorohydrin polymer (CMEPIβCD) in raising TCS solubility, as well as its release, from the patch, compared to that of parent β-cyclodextrin. The TCS-EPIβCD complex did improve solubility, although the presence of 1% (w/v) AMP compromised the complexation and solubilizing properties of both polymeric β-cyclodextrin derivatives (CMEPIβCD and EPIβCD). In addition, the buccal patches formulated with TCS-EPIβCD in combination with AMP-CAR 80:20 (w/w) provided immediate and stable drug release and efficacy against *Streptococcus mutans*, which was isolated from the oral cavity [115].

In 2015, a similar study investigated the role of polysaccharide psyllium in controlling the

releasing rate of chlorhexidine from a buccal muco-adhesive patch. Combining semi-synthetic polymers including sodium carboxymethyl cellulose and hydroxypropyl methyl cellulose with psyllium had the advantages of zero-order kinetics of drug release and effective antimicrobial activity against Gram (+) and Gram (-) bacteria [116]. Recently, Eudragit® RL 100 was used as the gel-forming agent in chlorhexidine-based medicated dermal patches. Eudragit® RL 100 is a complex made up of "ethyl acrylate, methyl methacrylate and low content of methacrylic acid ester with quaternary ammonium groups" [117]. The amount of quaternary ammonium groups in the RL type is greater than other ones of Eudragit polymers, making it becomes more permeable [118,119]. It is widely used as drug vehicle, controlled release agent, film former, bioadhesive material or suspending agent [120]. Typically, the dermal patches using Eudragit® RL 100 exhibited efficacious activity against the tested microorganisms [73].

1.7.3. Gels

Gels can be described as semisolid systems for topical usage comprising of colloidal globules distributed in an aqueous liquid vehicle [121]. Gels, along with creams and ointments are common semisolid formulations used for dermal applications [122]. Gels may be spread easily and offer a cooling effect as a result of solvent volatilization after application [121]. They can be categorized into hydrogels and organogels; hydrogels mainly include water in the liquid phases, while organogels comprise organic solvents [123]. Furthermore, the term "emugels" (as emulsified gels) is used to refer to biphasic systems which encompass a dispersed aqueous gel and a lipid base. Emugels were developed in order to enhance the occlusive characteristics of gels [122].

A thiolated povidine-iodine complex was developed with the intention of improving mucoadhesive properties. The gel-forming ability of thiolated PVP and thiolated PVP-I on contacting with mucosal surface and the mucoadhesion features were assessed. Based on the

results of this study, the thiolated PVP and thiolated PVP-I complex demonstrated merits, such as increasing viscosity, improving the mucoadhesion as well as controlling iodine release from systems, compared to unprocessed PVP and PVP-I complex. [124].

A topical alginate gel containing povidone-iodine and vancomycin-loaded chitosan nanoparticles was developed in order to impede and treat orthopedic implant associated infections (OIAIs) [125]. This formulation displayed sustained release of active compounds at the specific sites as well as good biocompatibility and hemocompatibility. Furthermore, this study indicated potential benefits to antibiofilm and antibacterial activity on *Staphylococcus aureus*, which is the key cause of OIAIs [125].

1.7.4. Lotions

The term "Lotion" refers to low- to medium viscous dosage forms which are used for both medicated and non-medicated applications [123]. Lotions are utilized particularly (but not popularly in clinical applications) as topical formulations of active substances (i.e., antibiotics, antiseptics, corticosteroids), intended for treatment of localized cutaneous disorders [121,123]. Also, lotions are more easily applied to sizeable skin areas than more viscous creams or ointments [123].

An aqueous antiseptic lotion containing benzethonium chloride (BZT) at 0.2% was reported to have a rapid and wide -spectrum antimicrobial efficacy equivalent to 76% v/v ethanol [126] when was tested according to standard Time-Kill protocols [127]. Combined with its known persistence and low propensity for skin irritation, BZT-based antiseptic product may furnish remarkable clinical applications over alcohol-based ones [126,128].

1.7.5. Ointments

Ointments are formed by the dispersion of active substances into an ointment base and are used on skin and mucous membranes, particularly in the treatment of infection and inflammatory

conditions. They are often selected for their tenacity on the skin to extend a drug's therapeutic activity over a long time as well as producing a protective layer covering the sites of application. However, they can be associated with irritation due to their occlusive nature arising from their tallowy characteristics [121].

The combination of ointment and body wash containing tea tree oil at 4% and 5%, respectively was reported to be better than the conventional regime consisting of 2% mupirocin nasal ointment and triclosan body wash for prevention of MRSA-reduced infections [129].

An *in vitro* study was designed to test the ability of PVP-I ointment at numerous concentrations (both standard and diluted concentrations) versus six others antiseptic preparations and a silver-based wound dressing, in terms of eliminating biofilms of *Pseudomonas aeruginosa*, *Candida albicans*, and MRSA. Following treatment with PVP-I ointment at all concentrations, there were no viable biofilms of *P. aeruginosa* detected after 4 and 24 hours. Additionally, PVP-I ointment containing 10% w/v active PVP-I was deemed effective at eradiating biofilm materials *of C. albicans* and MRSA after both 4 and 24h of applications and performed better than the other tested antimicrobial agents [130].

1.7.6. Creams

Creams or emulsions comprise two immiscible liquid phases, of dispersed globules within a continuous medium [121,122]. There are two main types of cream, oil in water or water in oil cream, of which, o/w cream is more popularly utilized to produce a local effect in case of external disorders, for instance, skin and wound infections [121].

A therapeutic regime of tea tree oil comprising tea tree oil 10% cream and tea tree oil 5% body wash was proposed for eradicating MRSA colonization. There was no significant difference with the standard therapy of 2% mupirocin nasal ointment, 4% chlorhexidine gluconate soap and 1% silver sulfadiazine cream [131].

1.7.7. Washes/ rubs

The FDA defined antiseptic washes, also known as antibacterial soaps, as products used with water and are rinsed off after use, including hand washes, hand soaps and body washes [132]. Antiseptic rubs (also called hand "sanitizers," or antiseptic wipes) are substituted when soap and water are inconvenient; they are left on and there is no need to rinse with water [132].

Four different hand-wash and hand rub formulations of PVP-I, including 4% PVP-I skin cleanser, 10% PVP-I solution, 3.2% PVP-I in 78% alcohol and 7.5% PVP-I surgical scrub were tested in a suspension test against Ebola virus (EBOV) and modified vaccinia virus Ankara (MVA) *in vitro*. Viral titres of MVA and EBOV were reduced by more than 99.99% under both clean environments (0.3 g/L bovine serum albumin; BSA) and contaminated environments (3.0 g/L BSA with 3.0 ml/L erythrocytes) within 15 seconds of exposure. Among those products, PVP-I solution in an alcohol mixture of 2-propanol and ethanol was the most efficacious at early timepoints. PVP-I could have an important role in limiting diseases related with Ebola, especially in combination with alcohol [133].

Glycerol, which often used as a humectant, can restrict the clinical effect of pre-operative hand rubs of isopropanol. After a 3h period of application, a hand rub preparation based on isopropanol without glycerol, which in this case was a combination of ethylhexylglycerin, dexpanthenol and a fatty alcohol, was more effective in eradiating skin pathogens than the product containing glycerol [134].

Triclosan is one of the most popular antimicrobial agents used in soaps. However, a systematic literature review indicated that triclosan based soaps used at the concentrations commonly found commercially (0.1% - 0.45% w/v) were not more efficacious in preventing infections than non-antimicrobial soaps [135]. The effectiveness of triclosan in antibacterial soaps was tested against twenty isolated strains proposed by FDA [136] either *in vitro* or *in vivo*. It was found that the use of antibacterial soaps containing 0.3% w/w triclosan did not show a superior

effect, compared to plain soaps under experimental conditions. This could be a consequence of a short exposure time, or the impact of surfactants in soaps like sodium laureth sulphate in diminishing the bactericidal activity of triclosan [137]. This result led to an US FDA ruling issued in 2013, which stated that all consumer antiseptic wash products need to have demonstrable clinical benefit prior commercialization, in comparison to plain soap and water [136]. Moreover, the latest FDA ruling released at the end of 2019 announced that three active antiseptic ingredients, benzalkonium chloride, alcohol (ethanol or ethyl alcohol), and isopropyl alcohol are not suitable for use as consumer antiseptic rubs [138].

In contrast, a medical triclosan-based shampoo was used to assess the antifungal and antibacterial effects against five isolated microorganisms. Based on the inhibition zones, the results indicated that, at all concentrations diluted from original concentration of 0.3% w/w (from 10% to 90%), the shampoo had efficacious antimicrobial activity against all three fungal species and one bacterial species (*E. coli*), but no effect on *Staphylococcus aureus*. Generally, antimicrobial shampoos, (e.g. triclosan), have shown efficacy in preventing and treating skin and scalp disorders, such as dandruff whose major cause is *Malassezia globose* [139]. Antiseptic soap with tea tree oil at 0.3% exhibited the similar efficacy in eliminating *E. coli* load on hand as the one containing triclosan at 0.5% [140].

Among all of conventional topical dosage forms, solutions are most mentioned in current clinical practice guidelines. Formulations including gels, creams, ointments and lotions are still the most common topical forms available commercially. Washes and rubs have been much studied recently, particularly focusing on comparing the difference of antimicrobial and non-antimicrobial washes/rubs products. Although patches have not been widely studied they are a promising alternative to other dosage forms, with regards to patient compliance and convenience. In terms of antiseptic agents, chlorhexidine has received the most favourable reports to date.

1.8. Advanced pharmaceutical formulations

Nanocarriers are colloidal drug delivery systems comprising dispersed particles with diameter less than 500 nm [141]. Nanocarriers have potential applications for parental, oral, dermal and transdermal administration routes [141]. They have been reported to present some merits over conventional preparations such as ameliorated bio-distribution and pharmacokinetics, enhanced therapeutic potency, minimized toxicity, controlled release, increased bioavailability, or drug delivery to target destinations [142,143].

These following sections summarizes the application of nanotechnology for delivery of antiseptic agents.

1.8.1. Nanoemulsions

Nanoemulsions are transparent or translucent emulsion systems with droplet sizes below 500 nm [142]. These colloidal systems can carry effectively both hydrophilic and hydrophobic drugs into the skin [142]. Compared to other traditional topical preparations like gels, creams and ointments, nanoemulsions have been reported to enhance permeation through the skin [144].

The fungistatic of a topical o/w nanoemulsion containing cetylpyridinium chloride was demonstrated for its effect against a range of pathogenic fungus, including *T. mentagrophytes, T. rubrum, E. floccosum, Trichophyton tonsurans,* and *Microsporum spp* as well as 12 species of hyphaes. Furthermore, it was more active against azole-resistant *C. albicans*, and azole-susceptible yeast, compared to other tested antifungal agents [145]. A benzalkonium chloride loaded nanoemulsion formulation demonstrated efficacious action against methicillin-resistant *Staphylococcus aureus in vitro* in mouse and porcine infected wound models. It promoted wound healing as a consequence of reducing inflammation within deep dermal layers and proinflammatory cytokine levels [146]. The formulation had previously been shown to reduce both bacterial colony and inflammation symptoms in burn wounds [147].

Triclosan based nanoemulsions (NEs) were prepared using a range of different concentrations of olive oil (OO) and eucalyptus oil (EO) to dissolve TCS. TCS-loaded NEs containing EO showed more merits than that of OO or solutions, in terms of both physicochemical properties and skin permeation ability. Similar results were found with nanoemulsions of CHG, as the inclusion of EO increased penetration into the skin, consequently improving drug retention for localised action. Thus, there are opportunities for nanoemulsions for both dermal hydrophilic and hydrophobic drug delivery [148]. A nanoemulsion of tea tree oil TTO, prepared via a high energy emulsification method, produced wider zones of growth inhibition against all isolated microbes than that of the available gel products with no observed skin irritation [149].

It was reported that there was no serious toxicity caused by a tea tree oil nanoemulsion incorporating with silver nanoparticles detected on human cells. TTO NE combined with Ag NPs presented antibacterial activity against selected microorganisms (from 90-95%) at the highest concentration tested of 14μg/ml. Besides, blending Ag NPs into a nanoemulsion (Figure 1.4) led to synergistic activity against clindamycin-resistant *E. coli* and an additive influence on *S. aureus* [150].

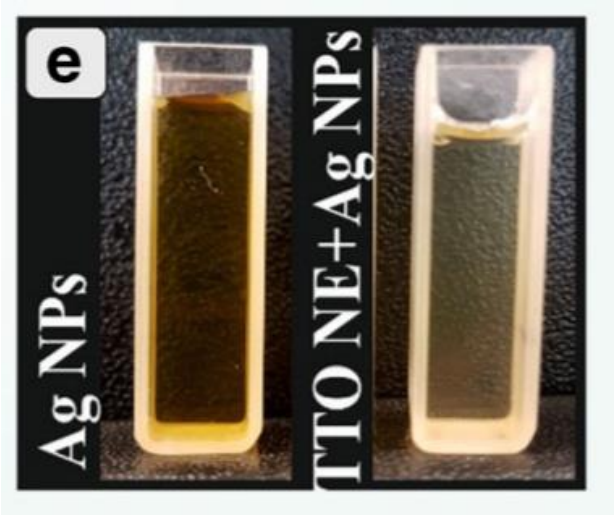


Figure 1.4 The images of silver nanoparticles and nanoemulsions containing tea tree oil in associated with silver nanoparticles, reprinted from [150].

1.8.2. Nanogels

Nanogels are nanoscale three-dimensional hydrogel globules made up of physically or chemically cross-linked hydrophilic polymer networks [151]. When nanogels are applied as dermatological preparations, the entrapment of nanoparticles in the gel matrix will extend exposure times on the skin and as a result, the therapeutic potency of the active compounds is lengthened [144].

A chlorhexidine-loaded nanogel was synthesized using poly (methyl methacrylate) (PMMA) as the colloidal matrix-forming constitute, which was cross-linked with α-, β-, or γ-cyclodextrin methacrylate (CD-MA) in organic medium. This study found higher levels of chlorhexidine base fixed on nanogel networks. Moreover, owing to the presence of CD-MA, the drug was released more slowly and less from the particle surface into PBS buffer solution than into water. The inhibitory activity of chlorhexidine base on the growth of *S. aureus* emanated from not only the nanogel surface, but also the aqueous environment [152].

Magnetic nanogels containing cobalt iron oxide nanoparticles were developed for the purpose of controlling pH-related release of chlorhexidine gluconate. It was found that that the magnetic nanogel was pH-responsive and its electroactivity increased at alkaline pH values. Besides, chlorhexidine was most active and is optimally released at pHs from 6-7, at which it is ionized. Therefore, it was proposed that these nanogels would be useful in burns treatment as the pH of the environment is higher than normal [153].

1.8.3. Nanoparticles

The inhibitory effects on autotrophic and heterotrophic microbial growth by silver nanoparticles (Ag NPs), silver ions and silver chloride colloids were assessed by Choi et al. (2008). According to the results of a short-term existent respirometry appraisal, at 1mg/L silver, silver nanoparticles had a much greater influence on prohibiting nitrifying microbe growth than

other forms. Based on an automatic microtiter appraisal, at silver content of 4.2 μM, Ag ions inhibited completely the growth of *E. coli*. None of three silver forms caused cell membrane lysis at 1mg/L Ag [154]. Colloidal silver formulations encompassing silver nanoparticles were effective against both Gram-positive and Gram-negative pathogens and excellent fungistatic properties were also reported after 7-14 days contact with the silver colloids, especially in case of systems using poly (N-vinylpyrrolidone) and Na-lauryl sulfate as stabilizers [155].

The antiseptic efficacy of an oil-in water emulsion containing nanoparticles of poly-hexamethylene biguanide hydrochloride (PHMB) was found to be more immediate and long-lasting on human skin colonies with durations of up to 150 min, in comparison with PHMB solutions [156].

Nanoparticles containing TCS for the treatment of acne were found to penetrate rapidly into hair follicles and provided a controlled and targeted transport of active. Permeation studies found that nanoparticles and emulsions has similar permeation ability but this was less than that of a solution, but the retention in the skin was similar for solution and nanoparticles and highest for emulsion formulations [157].

Solid lipid nanoparticles of triclosan were prepared for topical skin application by adopting glyceryl behenate (GB) and glyceryl palmitostearate (GP) [148]. Solid lipid nanoparticles provide a hydrophobic lipid network for drugs with low aqueous solubility [143]. Overall, solid lipid nanoparticles prepared with GP presented more advantages than with GB, such as smaller size, higher TCS loading, better permeation ability through skin (at 5% concentration of GP), and a greater amount of drug retained within the skin [148].

The issue with the relative hydrophobicity of triclosan has also been addressed by using branched deblock copolymers as stabilizers in nanoencapsulation. Three different amphiphilic branched di-block copolymers were synthesized via the copolymerization of a vinyl monomer (butyl methacrylate, styrene, or N-isopropylacrylamide) and a covalently cross-linker core. The

obtained triclosan nanoparticles presented a six-fold higher antimicrobial efficacy against *Candida albicans* than triclosan solution [158].

Polymeric nanoparticles (PNPs) are solid, nanostructure colloidal particles with the size of 10-100nm and produced by applying biodegradable polymers such as polylactide-polyglycolide copolymers, and polycaprolactones, or natural polymers, like gelatine, albumin and collagen [159]. PNPs are generally classified into two types: nanospheres and nanocapsules. Nanocapsules are composed of an outer solid polymeric membrane encapsulating an inner liquid core of oil or water in which the drug is dispersed whereas in nanospheres, actives are enmeshed within the polymer matrix structure [143].

Triclosan in poly L-Lactide (PLLA) nanoparticles (at loadings of 10%, 30% and 50% w/w) developed by the emulsification–diffusion method demonstrated the potency of inhibition of bacteria growth, that proposed that these nanoparticles showed the potential applications in the personal care and surgical implant products, drug delivery systems and wound dressing [160].

Polymeric nanoparticles containing PVP-I were fabricated using a surfactant-free emulsion copolymerization followed by iodination procedure, which nanoparticles eliminated 100% of the isolated organisms, including *E. coli* and *S. aureus,* and *P. aeruginosa* and the decreased hydrophobicity enabled a wider range of uses when it was amalgamated into conventional products like glue, ink or dye [161].

1.8.4. Nanocapsules

Chlorhexidine base was encapsulated into poly(epsilon-caprolactone) (PCL) nanocapsules. In an *ex vivo* study, after 8h of contamination, the number of colony forming units (CFUs) from skin treated for 3 min with chlorhexidine nanocapsules was notably less than that of CHG solution. Furthermore, the residual content of chlorhexidine from nanocapsule remaining in the SC was three-times greater, compared to solution control. Specifically, nanocapsules were

found in porcine skin follicles, this resulted in sustained action against *Staphylococcus epidermidis* [162].

Nanoemulsions and nanocapsules containing 10mg/mL TTO were prepared to test with two different infectious nail models. Generally, the nanosystems were effective at reducing the growth of *T. rubrum* which was presented through the significant diminution of microorganism count as well as the smallest zones of *T. rubrum* growth after exposure. Particularly, compared to nanoemulsion, the tea tree oil nanocapsules were more efficacious against fungi [163]. Further studies incorporated these TTO loaded nanosystems into hydrogel preparations. Based on the results of *in vivo* studies, hydrogels comprised of TTO nanocarriers reduced inflammation caused by UV-B radiation and in the wound healing process, with the most effective being TTO nanocapsule hydrogels [164].

Nanocapsule formulations have been proposed to address increasing antimicrobial resistance. Triclosan nanocapsules were formulated by interfacial deposition and used chitosan as a coating layer and α-bisabolol as the oil core. Positively charged chitosan was included to optimize interaction with negative charged microorganism membranes and α-bisabolol was selected for its ability to disperse lipophilic drugs such as triclosan. The MIC of nanocapsule coated chitosan was the lowest value compared to other samples. The chitosan-coated nanocapsules were incorporated into wound dressings, the effect of eradiation and inhibition on selected microbes was confirmed to be significantly increased and prolonged [165].

1.8.5. Other modern pharmaceutical formulations

A novel formulation comprising phospholipid and octenidine dihydrochloride was studied for the purpose of substituting for phenoxyethanol, which is often added as solubility enhancer of octenidine but may cause irritation, especially on mucosa and open wounds. The selected lipid form was Phospholipon90G (Phosphatydylocholine). According to the antiseptic efficacy test,

the studied lipid based formulation displayed the indistinguishable inhibitory potency with the available Octanisept® product, but fewer side effects due to lack of phenoxyethanol [166].

Liquid crystalline systems (LCS) of glyceryl monooleate (GMO) and water were developed as delivery systems for PHMB and cetylpyridinium chloride (CPC). The authors found that the inclusion of the active drugs into LCS affected the drug release, but not the creation of the liquid crystalline phases. Because of the interaction between CPC and GMO, the drug was trapped in the matrix and not likely to release into the medium, leading to the undetectable bactericidal effect of CPC associated with LCS. In contrast, it was reported that PHMB was released at a constant rate, thus having prolonged antibacterial activity against tested pathogens. In general, the evidence from this study suggests that the liquid crystalline systems can used as PHMB carrier [134].

Advanced drug delivery systems have been increasingly investigated for topical administration, primarily applying numerous forms of nano-technology. These formulations demonstrated superior therapeutic activities in prevention and treatment skin and wound infections, compared to conventional dosage forms. However, there is still scope for further development of optimised nanoparticulate dosage forms for topical delivery of antiseptics.

1.9. Thesis motivation

Being the outermost cover of the body, human skin is susceptible to various extraneous traumas such as temperature, UV-radiation, chemicals, and pathogens, which are likely to result in the disordering of the regulation and recovery mechanisms of normal skin and lead to dermatological skin concerns [167]. Skin and soft tissue infections are the most common infectious disease associated with hospitalization in developed countries, which have notable impacts on both the physical and mental health of patients [56,168]. In recent years, topical skin antisepsis has been regarded as an alternative for antibiotics for the purposes of prevention and treatment of skin and soft tissue infections, especially in the emergence of antibiotic resistance over the world [59,60].

To exert their optimum antiseptic activity, the agents need to target pathogenic microorganisms localizing below the outermost skin layer [169]. Nevertheless, because of the unique structure of the *stratum corneum* layer which made up the skin protective barrier, the cutaneous and transcutaneous transport of active substances from topical preparations remains a challenge, especially for water-insoluble drugs such as triclosan [170-172]. With the intention of addressing this, a promising approach is incorporating drugs into nano-carrier systems such as nanoemulsions [173]. Nanoemulsions are colloidal systems designed to appropriately carry both hydrophilic and lipophilic compounds with high drug loading capacity, that ensure the constant transfer of solutes from dispersed phase to external phase and thus stimulating skin absorption [173]. Also, emulsions with extremely small globules easily pass through the skin absorption membrane and offer large contact surfaces between the membrane and the formulation, thus enhance the bioavailability of very poorly soluble substances. In addition, it has been reported that nanoemulsions can be effective in minimizing toxicities of excipients for external uses [174,175]. Nanoemulsions can be prepared by high-energy or low-energy processes [142] and

ultrasonication has become the most common and effective technique fortheir preparation at a laboratory scale. Its operation is based on the effect of the acoustic cavitation phenomenon, which is defined as the formation, development, and subsequent disruption of micro-bubbles ascribed to acoustic waves. Nanoemulsions created by this method are extensively stable as droplet size can be as small as 0.2 nm [142,176,177].

Moreover, the oil phase in an emulsion is used not only to dissolve actives, but also it can contain some components that act as penetration enhancers and contribute to improving drug penetration into the skin [178]. Several studies were conducted to investigate the proficiency of eucalyptol (EO), and oleic acid (OA) in reinforcing the penetration of both hydrophilic and lipophilic drugs, especially as EO and OA were amalgamated into nanoemulsion structures in order to produce a synergistic effect, also acting as antimicrobials [178,179]. Eucalyptol (1,8-cineol) a natural water-soluble terpene, accounts for 75.7% of eucalyptus oil and is believed to be responsible for the biological activities (anti antibacterial, antifungal, and insecticidal) of this oil [180,181]. Oleic acid is a monounsaturated fatty acid. It makes up approximately 80% of olive oil, which is a common ingredient used in the pharmaceutical and cosmetic industry [182-184]. Based on the aforementioned advantages, nanoemulsions incorporating suitable excipients can be considered as a candidate formulation for the delivery of actives through dermal or transdermal administration, offering advantages compared to conventional topical dosage forms [185,186]. In the present study, the role of nanoemulsions as a drug carrier for chlorhexidine digluconate and triclosan compounds intended for topical applications was investigated.

1.10. The aim and objectives of the thesis

1.10.1. Aim

To explore nanoemulsions as a potential delivery system for topical application of antiseptics

1.10.2. Objectives

- To prepare nanoemulsion formulations of two antiseptic agents: chlorhexidine digluconate and triclosan by ultrasonication method.

- To evaluate the impact of oil type (olive oil and eucalyptus oil) on the stability and cutaneous permeation ability of developed nanoemulsions.

- To assess the stability of developed nanoemulsions by characterizing physicochemical properties.

- To evaluate the *in vitro* skin permeation ability of CHG and TCS loaded nanoemulsions and the amount of drug retained within skin after the application.

CHAPTER 2
MATERIALS AND METHODS

2. Materials and methods

2.1. Materials

Chlorhexidine digluconate (20 % w/v in water) solution (CAS: 18472-51-0), polyethylene glycol sorbitan monooleate (Tween® 80, HLB: 15); polyethylene glycol sorbitan monolaurate (Tween®20, HLB: 16.7); sorbitan monooleate (Span® 80, HLB: 4.3), olive oil (CAS: 8001 25-0), eucalyptus oil (CAS: 8000-48-4) obtained from Sigma-Aldrich (UK). Triclosan (CAS: 3380-34-5) was from Tokyo Chemical Industry Co., Tokyo (Japan). Ultrapure water, HPLC grade methanol, acetonitrile, diethylamine, sodium heptane sulfonate was used for HPLC analysis. All of other chemicals and solvents were of analytical reagent grade.

2.2. Methods

2.2.1. High performance liquid chromatography (HPLC) methods

2.2.1.1. HPLC method for triclosan

The HPLC method described by Kakadia (2016) [148] was adopted. The Shimadzu HPLC system (Shimadzu Corporation Ltd. Buckinghamshire, UK) equipped with isocratic delivery pump (LC-20AT), auto sampler (SIL-20A) and UV-VIS detector (SPD-20AV). The XTERRA® MS C18 from Waters (UK) was selected as a chromatography column. Acetonitrile and water at the ratio of 60:40 (% v/v) was used as a mobile phase with a constant flow rate of 1 ml/min and the maximum absorption wavelength was set at and 285 nm. All the HPLC conditions are summarised in Table 2.1. The standard solutions of TCS were prepared at concentration of 2.5, 5, 10, 20, 30, 40, and 50 µg/ml and their response were determined using HPLC. Figure 2.1 displays a typical standard calibration curve which was constructed by plotting the peak area against the concentration. The linear regression equation was employed for the quantification of TCS.

Table 2.1 HPLC conditions and validation parameters for TCS

Injection Volume	**20 µl**
Wavelength	285 nm
Flow rate	1 ml/min
Run time	10 min
Tailing factor	0.88
Retention time	7.15 min
LOD	0.45 µg/ml
LOQ	1.35 µg/ml
Precision and accuracy	RSD < 4%

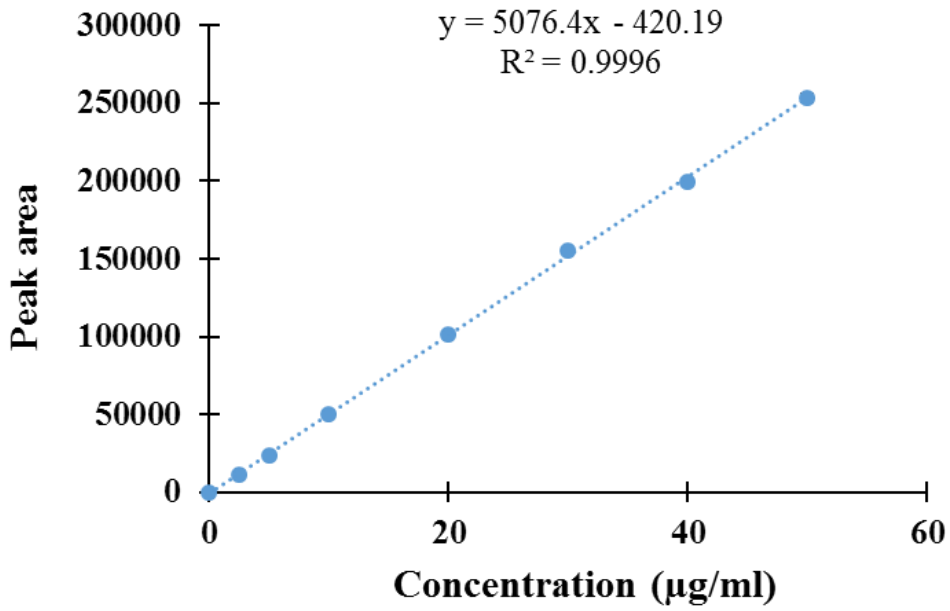


Figure 2.1 Standard calibration curve for TCS

2.2.1.2. HPLC method for chlorhexidine digluconate

The HPLC method described by Kakadia (2016) [148] was adopted. The employed HPLC system was same as described in section 2.2.1. The mobile phase was a methanol: water mixture (75:25, % v/v) with 0.1 % (v/v) diethylamine and 0.005 M sodium heptane sulphonate, modified to pH 4 by glacial acetic acid. A constant flow rate and the maximum absorption

wavelength was set at 1 ml/min and 260 nm, respectively. All the HPLC conditions are summarised in Table 2.2. The standard solutions of CHG were prepared at concentrations of 2.5, 5, 10, 20, 30, and 40 µg/ml and their response were determined using HPLC. Figure 2.2 displays a typical standard calibration curve which was constructed by plotting the peak area against the concentration. The linear regression equation was employed for the quantification of CHG.

Table 2.2 HPLC conditions and validation parameters of CHG.

Injection Volume	**20 µl**
Wavelength	260 nm
Flow rate	1 ml/min
Run time	10 min
Tailing factor	1.33
Retention time	5.13 min
LOD	0.18 µg/ml
LOQ	0.53 µg/ml
Precision and accuracy	RSD < 4%

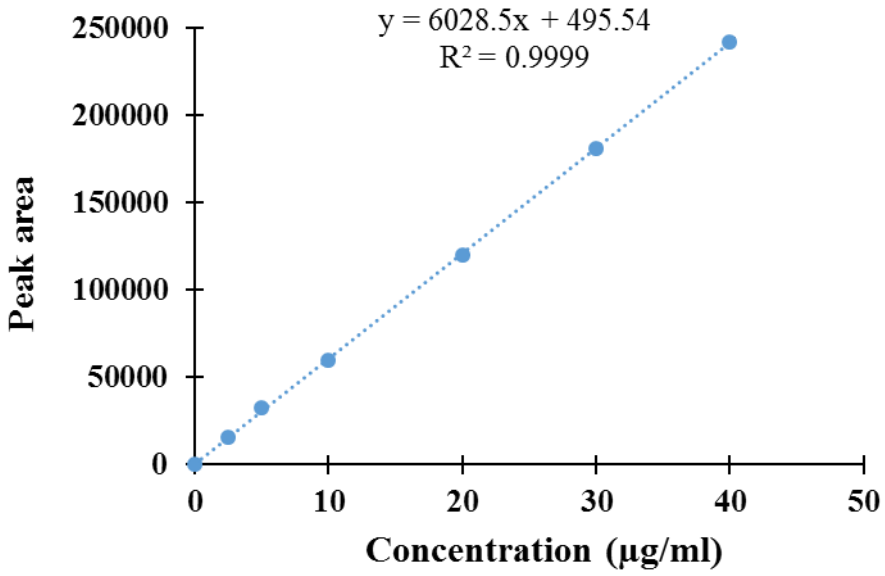


Figure 2.2 Standard calibration curve for CHG

2.2.2. Preparation of triclosan nanoemulsions

Triclosan nanoemulsion formulations were prepared using an ultrasonic homogenization method and the constitution of each formulation has been described in Table 2.3. Briefly, the method involved the separate preparation of oil and water phases.

- Water phase contained 0.5 % w/w of triclosan and 6 % w/w of emulsifier mixture dissolved in 90.5 % w/w of deionized water using a magnetic stirrer at 400 rpm for 15 min.
- Oil phase comprised of 3 % w/w of oil component (eucalyptus oil or olive oil).

Initially, the coarse emulsion was formed by adding slowly oil phase into water phase under magnetic stirring at 800 rpm for 20 minutes. The combination was subsequently processed using a Model 3000MP Ultrasonic Homogenizer (Biologics, Inc, UK) with ½" (12.7 mm) diameter solid titanium tip for 25 minutes at 40 % of frequency amplitude, each cycle comprises of 30s pulse on and 5s pulse off. The sample was placed in an ice bath in order to control temperature during the process. Moreover, 0.5% w/w triclosan solution in tween 80 was also prepared to act as a control. A schematic illustration of triclosan nanoemulsion formulation is shown in Figure 2.3.

Table 2.3 Formulations of TCS nanoemulsion

Formulation	TCS (% w/w)	Oil		S80: T20 (1:1) (% w/w)	Water (% w/w)
		Type	Concentration (% w/w)		
T1	0.5	EO	3	6	90.5
T2	0.5	OO	3	6	90.5

TCS: triclosan; EO: eucalyptus oil; OO; olive oil

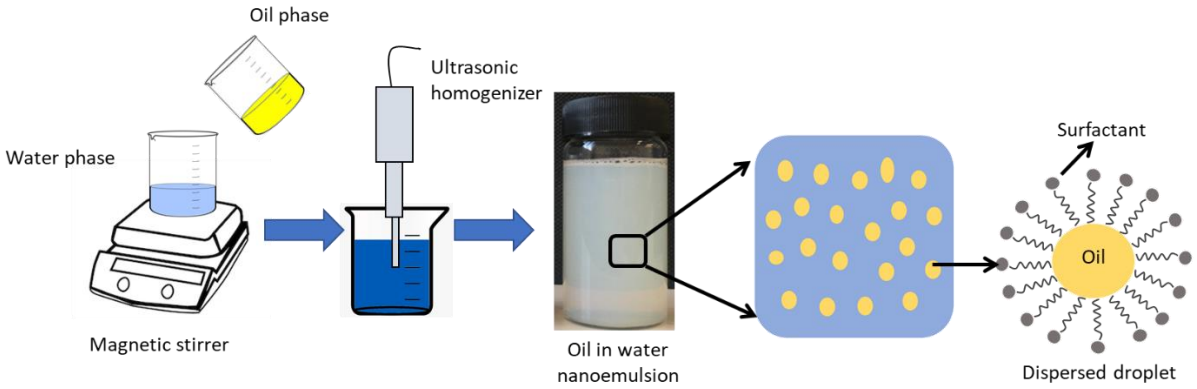


Figure 2.3 Schematic illustration showing the formation of TCS nanoemulsions.

2.2.3. Preparation of chlorhexidine digluconate nanoemulsions

Chlorhexidine digluconate nanoemulsion formulations were prepared using ultrasonic homogenization method and of the constitution of each formulation has been described in Table 2.4. Briefly, the method involved the separate preparation of oil and water phases.

- Water phase contained 10% w/w of aqueous chlorhexidine digluconate 20% w/v solution.
- Oil phase was formed by mixing 30 % w/w of the surfactant mixture (Span 80 and Tween 80) and 60 % w/w of oil (eucalyptus oil or olive oil) using a magnetic stirrer at (40 °C, 500 rpm) for 15 min.

Afterwards, the water phase was added gradually into the oil phase while stirring at 800 rpm for 20 minutes to form the coarse emulsion. The combination was subsequently processed by adopting Model 3000MP Ultrasonic Homogenizer (Biologics, Inc, UK) with ½" (12.7 mm) diameter solid titanium tip for 30 minutes at 50 % of frequency amplitude, each cycle comprised 30s pulse on and 5s pulse off. The sample beaker was placed in an ice bath to prevent over heating during the process. Moreover, 20 % w/v chlorhexidine digluconate solution was diluted 10 times with deionized water to develop a solution to act as a control. A schematic illustration of triclosan nanoemulsion formulation is summarised in Figure 2.4.

Table 2.4 Formulations scheme of CHG nanoemulsion

Formulation	20% w/v CHG (% w/w)	Oil		S80:T80 (2:1) (% w/w)
		Type	Concentration (% w/w)	
C1	10	EO	60	30
C2	10	OO	60	30

CHG: chlorhexidine digluconate; EO: eucalyptus oil; OO; olive oil

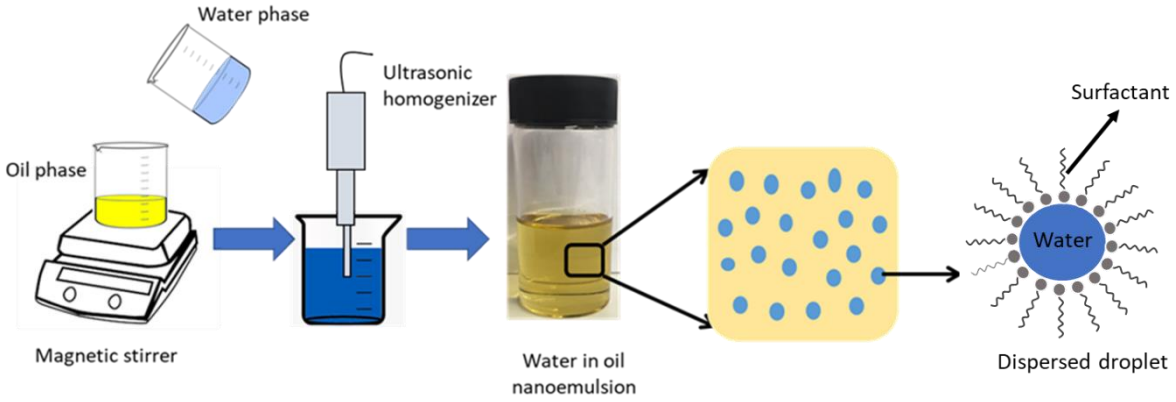


Figure 2.4 Schematic illustration showing the formation of CHG nanoemulsion.

2.2.4. Physicochemical characterisation of nanoemulsions

2.2.4.1. Content uniformity test

The drug content in prepared nanoemulsions was quantified by completely dissolving 0.05 ml of each sample in sodium phosphate buffer to achieve 10 ml solutions using an ultrasonic bath for 15 minutes, followed by TCS and CHG analysis utilizing the HPLC methods described as in section 2.2.1.1 and 2.2.1.2, respectively.

2.2.4.2. Determination of pH

The pH of each nanoemulsion formulation was determined using a digital pH meter (Seven Compact S220 Benchtop pH/Ion Meter, Cole-Parmer Instrument Company Ltd. Neots, United Kingdom). Each formulation was tested in triplicate (n=3).

2.2.4.3. Determination of droplet size and polydispersity index

The mean droplet size and polydispersity index of formed nanoemulsions were determined by using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The system was equilibrated for 60 seconds before each measurement at 25 °C. In order to warrant that the light scattering signals must be in the sensitivity range of the instrument, the samples were diluted using deionised water. The measurement performed at 90° scattering and were taken in triplicate (n=3).

2.2.4.4. Determination of zeta-potential charge

Zeta potential of prepared nanoemulsion samples were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) which is based on the principle of electrophoresis. The samples were placed in the zeta dip cell for 2 minutes prior to readings in order for the system reach to equilibrium. All measurements were made in triplicate (n=3).

2.2.5. *In-vitro* permeation studies using vertical Franz diffusion cell

2.2.5.1. Preparation of permeation media

Sodium phosphate buffer solution, pH 7.4, was prepared using disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate salts. Moreover, 1% sodium lauryl sulfate (SLS) was added before the commencement of triclosan nanoemulsion permeation experiments in order to ensure solubility of the drug in the solution. For chlorhexidine nanoemulsion, sodium phosphate buffer solution, pH 7.4, was used without SLS.

2.2.5.2. *In-vitro* permeation studies using Franz diffusion cells

Prior to the beginning of each experiment, frozen excised full-thickness porcine ear skin was defrosted for 30 min and then hydrated by immersing in phosphate buffer saline (PBS) pH 7.4 solution for 60 min. The skin was precisely cut in sections and mounted on the vertical diffusion

cell with *stratum corneum* facing upward toward the donor compartment and dermis facing downward towards the receiver compartment. The formulation was applied in the donor compartment and the receiver compartment contained permeation media as described in section 2.2.5.1. To temperature was maintained at 32°C using a circulating water which was maintained at 37 °C for 240 min with a stirring speed of 200 rpm. Nanoemulsion formulations of TCS (10 mg/g equivalent) and CHG (20 mg/g equivalent) or similar concentration of control solutions were loaded in the donor compartment and in order to avoid evaporation of nanoemulsions the compartments were sealed with Parafilm. After specified times (30, 60, 120, 180, 240 and 360 min) 500 µl aliquot was taken from the receiver compartment and replaced with respective fresh permeation media. All the samples of analysed using HPLC and cumulative permeated amount of TCS and CHG were quantified using HPLC methods described in section 2.2.1.1 and 2.2.1.2, respectively. At the end of the permeation experiments the skin samples were carefully removed and were soaked overnight in ethanol at room temperature. The amount of TCS and CHG retained in the skin were quantified using HPLC.

The flux and permeability coefficient were measured to support permeation profiles. The mass flux (J_{ss}) is a parameter indicating permeation rate and can be found through the slope achieved by plotting the cumulative amount of drug permeated through cross-sectional area of membrane versus time [187]. The permeability coefficient can be calculated by the following equation:

$$K_p = J_{ss}/C_0$$

Which K_p is the permeation coefficient of the solute, *Jss* is the flux and C_0 is the initial concentration of tested drug in the donor department [188].

CHAPTER 3

RESULTS AND DISCUSSION

3. Results and discussion

3.1. Preparation and optimisation of nanoemulsions

The coarse emulsions containing chlorhexidine digluconate (C1 and C2) were milky yellow in colour. They exhibited phase separation within a few minutes which is indicative of kinetic instability. After emulsification, the appearance of both C1 and C2-coded samples became yellow transparent, clear and homogenous. Likewise, following the ultrasonication, triclosan loading emulsions (T1 and T2 formulations) were changed from milky white to bluish and translucent in performance with no sign of phase separation was visually detected (Figure 3.1). The homogenous and pellucid visual appearance stems from the Rayleigh scattering effect resulted by nanosized particles [189,190].

Figure 3.1 Triclosan emulsion appearance before and after homogenization

Factors that impact the ultrasonication process were taken into account when preparing the nanoemulsions. Precisely, based on the experiments of Sugumar et al. (2013) [191], the processing time was up to 30 min and the amplitude was set up at 40 -50%. Combining one hydrophilic surfactant (Tween) with another lipophilic one (Span) demonstrated to favorably achieve nanoscale and more stable emulsion than single-used surfactant [192]. Precisely, the hydrocarbon chains of Span molecules show affinity with oil part, while the sorbitan rings tend

to connect with the aqueous part. The width of sorbitan ring prevent hydrocarbon chains from moving close together. In contrast, Tween composes of very strong hydrophilic polyoxyethylene chains so that a part of hydrocarbon chain lies in the water phase. As Tween with Span are blended, the part of hydrocarbon chains of Tween which lies in oil phase will penetrate between Span molecules, thus reducing distance between the adjacent hydrocarbon chains [193]. Different oil and surfactant contents were also tested to select optimum formulations for further assessments. For triclosan nanoemulsions, a lower concentration of surfactants was used, but the oil phase inadequately dispersed into water phase. In case of chlorhexidine nanoemulsion, the relatively low content of water phase led to the formulation become too viscous to homogenize. Finally, the formulations of triclosan nanoemulsion which comprised 3% of surfactant mixtures and 6% of oil and the one of chlorhexidine with 30% of emulsifiers and 60% oil were selected for further study based on visual inspection of relative stability.

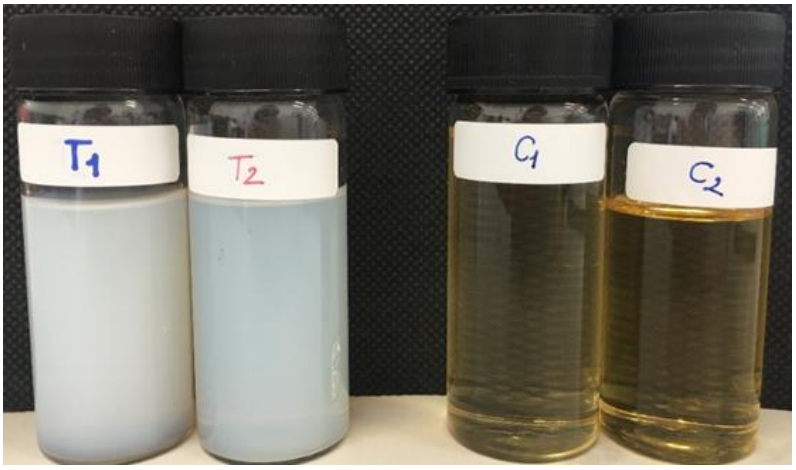


Figure 3.2 Four obtained nanoemulsion formulations of chlorhexidine digluconate and triclosan (*From left to right: T1, T1, C1 and C2*)

3.2. Physicochemical characterisation of nanoemulsions

3.2.1. Content uniformity of nanoemulsions

The drug content of four nanoemulsion samples are shown in Figure 3.3. Drug entrapment efficiency (DEE) is parameter used to measure the capacity of nano-structured delivery systems to preserve active compound and to secure transport sufficient content of drugs to desired targets [194].

In the present study, DEE for C1, C2, T1 and T2 formulations are 78.56 ± 1.49 %, 82.18 ± 7.87 %, 77.20 ± 4.00 %, 80.58 ± 7.87 %, respectively. There was no significant difference between the % DEE of EO-nanoemulsions and OO-nanoemulsions ($p > 0.05$). The loss of drug may be due to degradation during the homogenisation process.

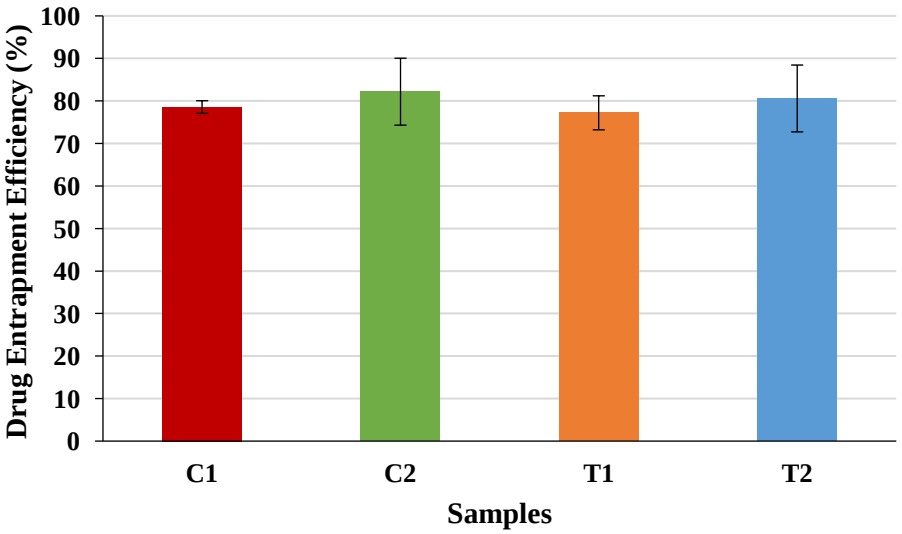


Figure 3.3 The percentage of drug encapsulation within each nanoemulsion system (Mean ± SD, n=3)

3.2.2. pH of nanoemulsions

The natural slightly acidic pH of human skin surface plays a crucial role in the maintenance of cutaneous protection, particularly in controlling the enzymatic activities in lipid metabolism as well as the anti-microbial properties on the skin surface [195]. The disruption of skin pH barrier may irritate the skin leading to, for instance, atopic dermatitis and psoriasis. Therefore, the pH

of topical preparations should be compatible with the physiological acidic mantle on the *stratum corneum*, which is around 5.5, in order to protect the natural skin defense mechanism as well as not causing any irritations [11,196].

The pHs of the formulations are shown in Figure 3.4, indicating that the prepared formulations would be suitable for external applications.

The acidic pH of obtained nanoemulsions may attributed to the free fatty acids content presented in formulations, which are arisen from the hydrolysis of the surfactant molecules and triglycerides in oil component [197]. Similarly, the oil in water nanoemulsion developed by de Godoi et al. (2017), which comprised 5% *Eucalyptus globulus oil*, 2% Span 80 and 2% Tween 80 had a pH of 4.68 [181].

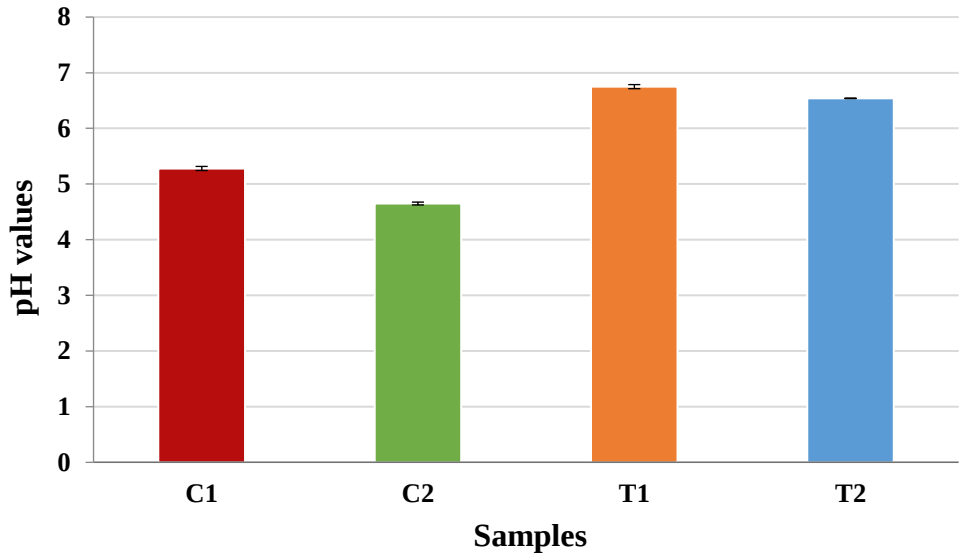


Figure 3.4 pH of nanoemulsion formulations (Mean ± SD, n=3).

3.2.3. Droplet size and PDI of nanoemulsions

The mean droplet size (MDS) along with polydispersity index (PDI) are particularly deemed important indicators used to describe the stability, homogeneity and dispersity of nanoemulsions [198,199]. The emulsion systems with the size in nano-range and well distributed

particles indicated a homogeneous structure, leading to kinetic stability resisting flocculation, gravitational separation, Ostwald ripening and coalescence [200-202].

All of four nanoemulsion represented droplet sizes below 115 nm, which accounts for the transparent appearance of obtained formulations due to the Rayleigh scattering effect as the size of droplets smaller than the wavelength of incident light [190]. There was insignificant difference ($p>0.05$) in droplet size of T1 and T2 nanoemulsion formulations, which are 52.09 ± 0.648nm and 50.92 ± 1.088 nm, respectively. In the other hand, the CHG based nanoemulsion formulation containing EO have significantly smaller size diameter of globules than the one containing OO, which are 11.42 ± 3.427 and 112.40 ± 6.185 nm, respectively ($p<0.05$). It also can be seen that droplet size of T1 and T2 were smaller than that of C2, which may be due to the less amount of oil content in T1 and T2 formulations, leading to lower viscosity, thus increasing the cavitation intensity and shear forces during ultrasonic homogenization and escalating the droplet breakdown [203]. Moreover, the size distribution graphs in Figure 3.6 of all the developed formulations presents only one peak, that pointed out the uniformity of droplet size distribution within each formula [204]. Tahir and colleagues optimized emulsifying conditions in order to attain and optimized nanoemulsion formulation containing 4% olive oil and 2.08 % surfactant, which had a droplet size of 151.68 nm [203]. Droplets with the size in nano-range not only ensure kinetic stability of formulations due to the preponderance of Brownian motion against gravitational force, but also provide a broader interface area for interactions and allow active substances to diffuse favorably through the skin [205].

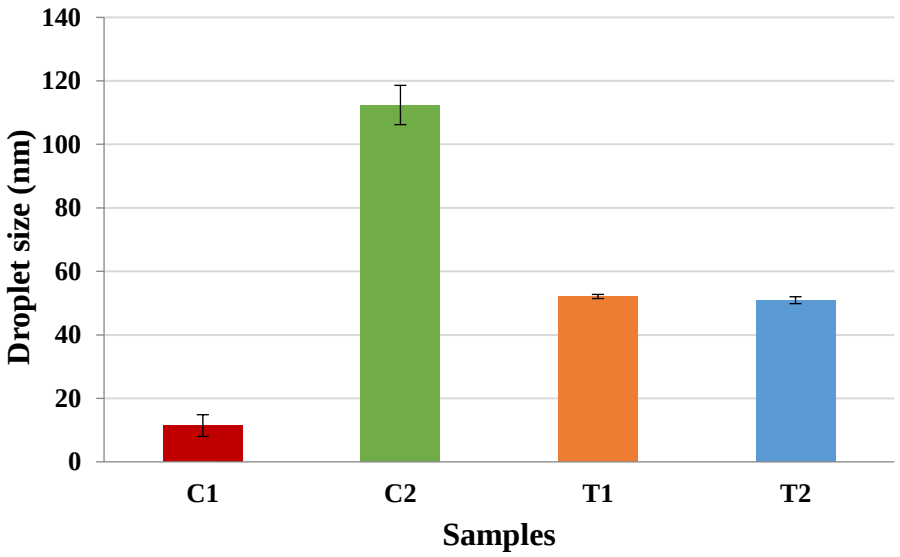
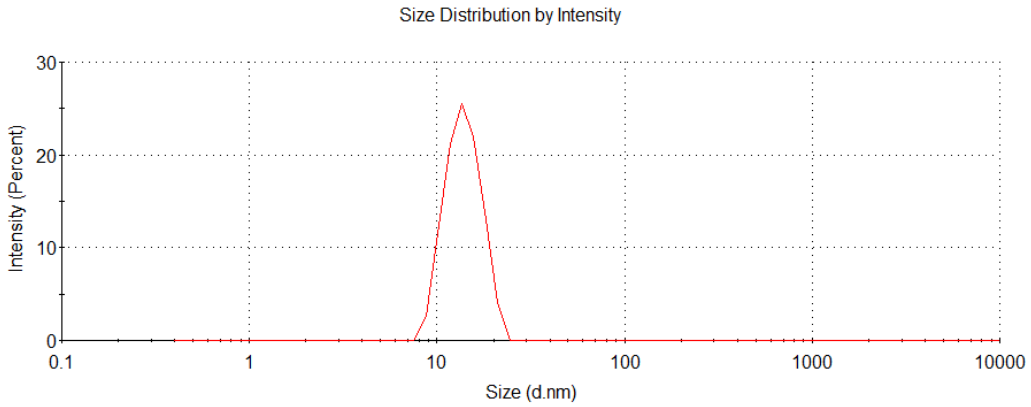
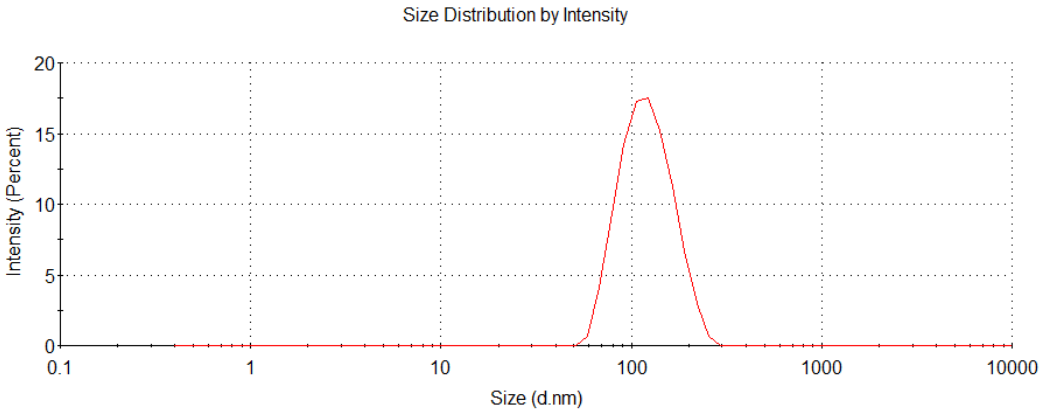


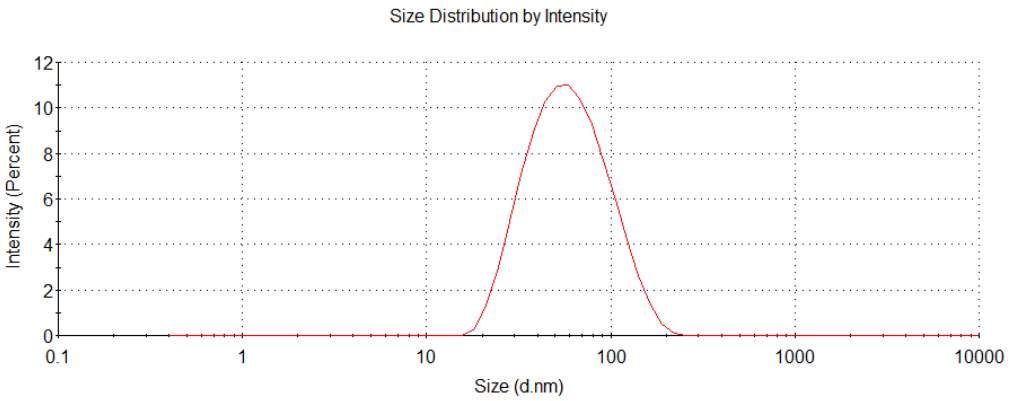
Figure 3.5 Droplet size of prepared nanoemulsion formulations (Mean ± SD, n=3)



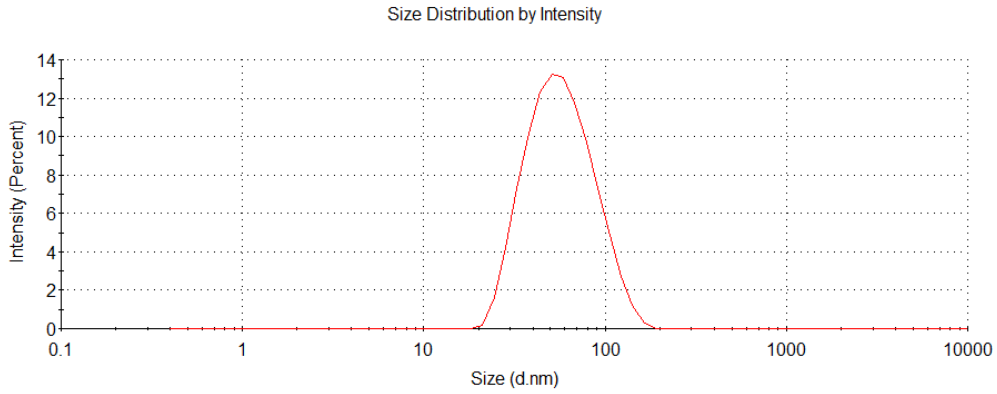
(a)



(b)



(c)



(d)

Figure 3.6 Size distribution of prepared nanoemulsions, *(a):* C1; *(b):* C2; *(c):* T1; *(d):* T2.

In addition, the polydispersity index of the prepared formulations was found to range from 0.15 to 0.41, which reflected the narrow difference in the average size between droplets. The PDI is a parameter inversely proportional to the stability and homogeneity of colloidal systems. A lower PDI is generally indicates a more stable nanoemulsion [206]. It can be seen from figure 3.7 that T2 formulation is deemed to be the most homogeneous and potentially the most stable with the lowest PDI value of 0.156. Quatrin *et al*. (2017) developed nanoemulsion using 5% of eucalyptus oil which showed the droplet size below 100 nm and the PDI of 0.22 [197]. Nanoemulsion containing chlorhexidine hydrochloride was formulated as potential root canal irrigant and perform the droplet size of 12.18 nm and the PDI was found to be lower than 0.2

[207].

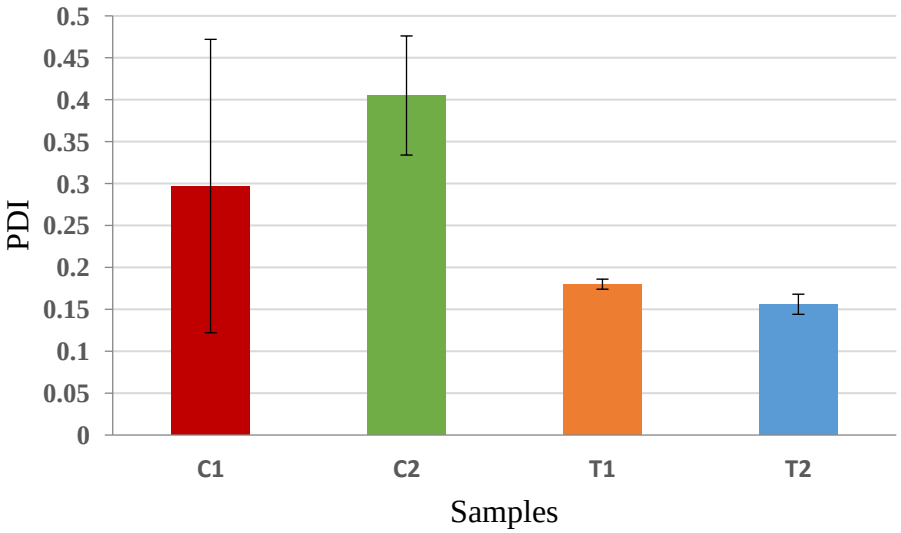


Figure 3.7 Polydispersity index of prepared nanoemulsions (Mean ± SD, n=3)

3.2.4. Zeta potential of nanoemulsions

Zeta-potential (ζ-potential) (ZP) test is used to measure the electrical surface charge of particles [208]. The magnitude of ζ-potential is one of the most crucial indicators of the physical stability of the colloidal dispersion systems through providing information about the interactions between droplets [192]. The higher ζ-potential value, the stronger repulsive forces between droplets against natural tendency of aggregation [201].

In this study, the T1, T2 nanoemulsions manifested negative ZP values, which were -34.3 ± 0.90 mV and -36.4 ± 0.93 mV, respectively. Nanoemulsion systems with zeta potentials lower than -30 mV tend to be stable and resistant to aggregation and flocculation as attractive interactions seem to be weaker than repulsive interactions [209]. Similar zeta charge of -33.3 mV was observed with the nanoemulsion using tween 20 as emulsifier and olive oil as oil phase [183].

Maruno and Rocha-Filho also signified the negative ZP of oil in water nanoemulsions yielded with a combination of nonionic emulsifiers. The negative surface charge on the emulsion

particles are probably attributed to hydrogen bonding at the ether-oxygen of the polyoxyethylene chain in nonionic surfactant molecular, subsequently to form oxonium ions [210].

In contrast, the C1 and C2 coded chlorhexidine digluconate formulations showed positive surface charge of +24.01 ± 6.35 mV and +20.95 ± 9.11 mV, respectively. According to Honary *et al.* (2013), as incorporating with non-ionic emulsifiers which mainly stabilized the colloidal systems by steric effects, the absolute zeta potential values approximate 20 mV are sufficient for the stability of the nano-suspensions [211]. The positive charge of CHG molecule may contribute to the positive charged nanoemulsion droplet [148].

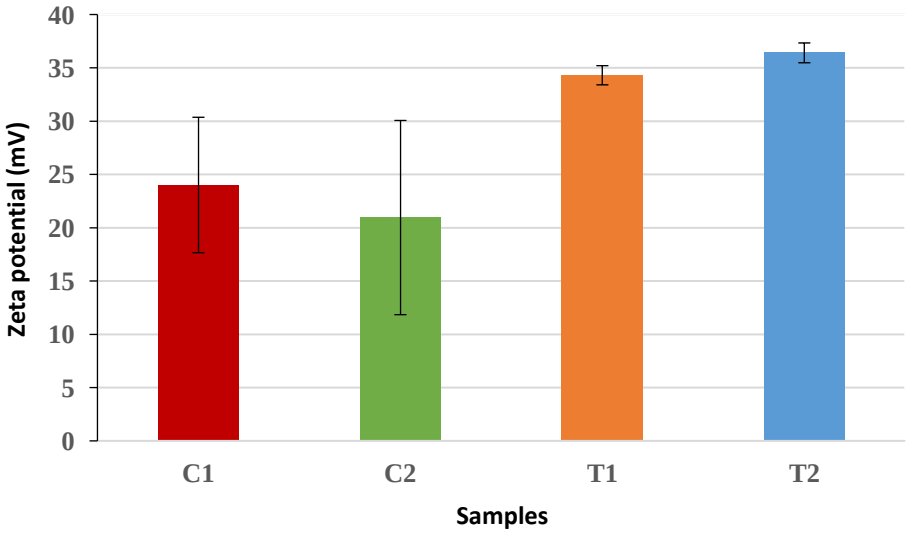


Figure 3.8 The absolute value of surface electrical charge of nanoemulsions (Mean ± SD, n=3)

3.3. *In vitro* permeation studies

The evaluation of percutaneous permeation is an essential criterion not only for the success of design and development of novel dermal and transdermal formulations, but also for the establishment of drug bioavailability. In the pharmaceutical industry, the skin permeation test

is also a useful tool for quality-control assessment to secure batch-to-batch uniformity [212-214]. In the present study, *in vitro* skin permeation experiments were performed to evaluate the influence of nano-structure and oil type used in emulsion on diffusion ability of both hydrophilic and hydrophobic drugs through skin, compared to conventional dosage forms. Also, full-thickness porcine ear skin was selected as diffusion cell membrane not only due to its availability but also its compatibility with physiological and structural characteristics of human skin, in terms of hair follicles; the distribution of Langerhans cells; keratinized, multilayered and stratified epidermis; stratum corneum layer thickness (10 to 50 µm in human and 15 µm in pigs); the viable epidermis thickness (80 µm of human skin and 60 µm of porcine skin) and collagen bundles organization in the dermis [215-217].

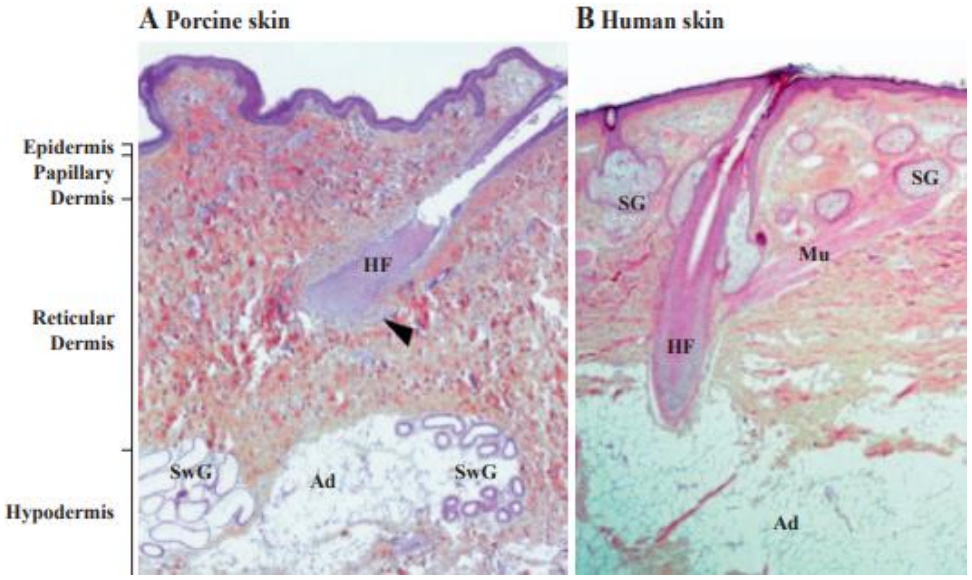


Figure 3.9 Histological similarity of porcine (A) and human (B) skin. **HF**: hair follicle, **Mu** and arrowhead: arrector pili muscle, **SwG**: Sweat gland, **SG**: sebaceous gland, **Ad**: Adipocytes (hypodermis), reprinted from [217].

The amount of triclosan permeated through full thickness porcine ear skin from two nanoemulsions (T1, T2) and a control solution are described in figure 3.10. The flux and permeability coefficient are summarized in table 3.1. It can be seen that extensively higher amount of triclosan from eucalyptus oil nanoemulsion detected in the receiver medium at all points of analysis time, compared to that from olive oil nanoemulsion and 0.5% TCS solution.

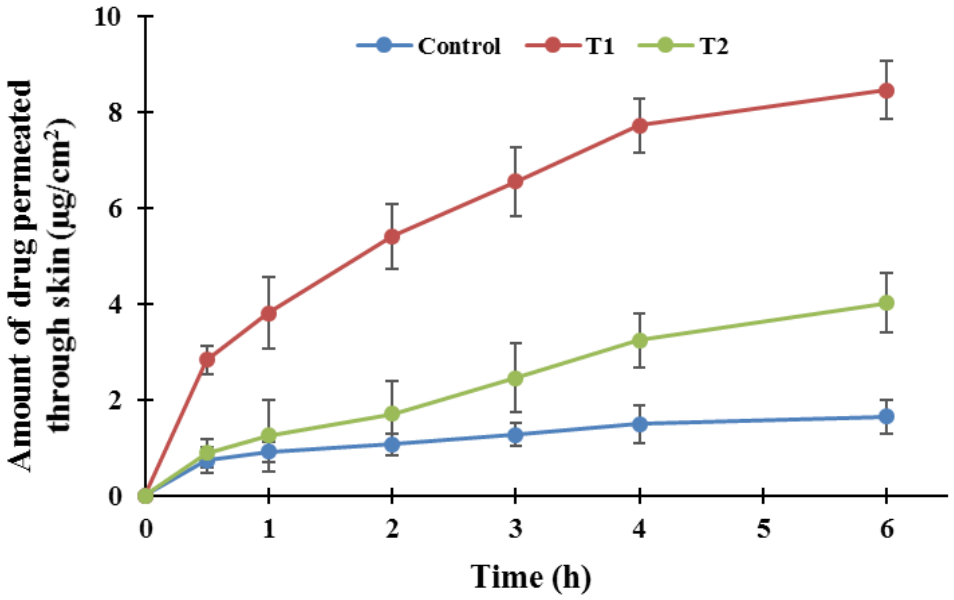


Figure 3.10 The amount of triclosan permeated through skin qualified in specific intervals (Mean± SD, n=3)

The J_{ss} of T1 was 6.6 times and 2.1 times higher than that of control and T2, respectively. Compared to the J_{ss} of control, that of T2 was increased by about 3.16-fold. This indicates that EO nanoemulsion was more effective at transferring lipophilic drugs through dermal route than other forms. This findings is in agreement with the study of Kakadia (2016), which showed that EO-NEs exhibited 2.6 times higher of the skin permeation of TCS than OO-NEs under the same conditions, which could be due to lower viscosity and smaller droplet size [148]. Additionally, Chen Liu *et al*. (2017) demonstrated that nanoemulsion using terpene mixture (mentol-camphor) intensified the percutaneous permeation of poorly-water soluble glabridin by approximately 7.4 times, compared to solution [218].

There are several available explanations proposed for skin permeation improvement of hydrophobic drugs using nanoemulsions, The NE components (like fatty acids, surfactants, terpenes) can act as penetration enhancers, which potentially disrupt the bilayer lipid domains of the SC barrier, and create greatly passable pathways for drug diffusion [219]. Nanoemulsions using eucalyptol and oleic acid as penetration enhancers significantly increased skin

penetration of caffeine and naproxen, in comparison to control solutions. [178] The author proposed several mechanisms for that effect, including increased solubility of actives in the stratum corneum, enhanced the fluidization of the phospholipid bilayer as well as improved the distribution of drug carrier into the SC. The oil droplet in nano-size is advantageous to provide a large available surface between active molecules with membrane [220]. A nanoemulsion gel formulation was demonstrated to increase permeation ability of glibenclamide through skin, which was attributed to the very small droplet size [221]. Furthermore, it was reported that nanoemulsions play an important role in changing the partition of drug into skin sections due to small droplets, thus raise effectively skin penetration and retention of active components [220].

The EO-NE was more effective in improving skin permeation of TCS than the OO-NE. This may be due to the higher solubility of TCS in EO as reported by Kakadia (2016). Eman Abd *et al.* also pointed out that the amount of hydrophilic minoxidil permeated through full thickness human skin from a nanoemulsion formulated with eucalyptol was significantly higher than that of the formulation using oleic acid [174]. Similarly, eucalyptol nanoemulsions were superior in the skin permeation enhancement of caffeine after 24 compared to oleic acid NEs, taking into account both the cumulative amount permeated through skin and the flux. Moreover, the amount of caffeine retained in skin as well as in hair follicle from eucalyptol NEs was also found to be greater [179]. Both eucalyptol and oleic acid are well-known penetration enhancers. Yet, compared to terpenes whose primary effect is through enhancing SC solubilization and disrupting the SC barrier, leading to the advanced diffusivity, oleic acid only demonstrated a moderate impact on improving the drug diffusion [222].

Table 3.1 Permeation parameters of triclosan nanoemulsions and 0.5% triclosan solution from *in vitro* permeation study (Mean± SD, n=3)

Formulation	**Flux (*Jss*) µg/cm^2/h**	**Permeability coefficient *Kp* × 10^{-5} cm/h**
T1	0.363 ± 0.1	3.63 ± 1.01
T2	0.174 ± 0.05	1.74 ± 0.50
Control	0.055 ± 0.001	0.55 ± 0.01

As described in Fig 3.11, after 30 min application, C1 produced a higher permeation of CHG than the control solution and C2, but after 1h, the accumulative amount of CHG permeated through skin from 2% CHG solution was higher than that from the nanoemulsions. Generally, this implies that nanoemulsion did not show any skin permeation enhancement over the aqueous solution, which may be attributed to the high content of oil in formulation which resulted in high viscosity. Furthermore, w/o formulation requires actives to transfer from inner phase to external phase, subsequently diffuse through external phase. This might lead ta slow drug release rate, and consequently poor skin permeation. A study investigated the skin permeation of hydrophilic 5-aminolevulinic acid (ALA) as incorporated in w/o or o/w naneoemulsions., The best skin permeation was achieved by a soybean oil in water system, whilst w/o nanoemulsions also presented the lower *in vitro* flux than control solution [223]. In another work, it was reported that the w/o type nanoemulsion carrying metronidazole represented approximately 24.7-fold and 21.14-fold inferior of the flux value than optimum o/w type and gel form, respectively [224].

The permeability parameters of CHG from C1, C2 and solution are displayed in table 3.2. The *Kp* of control solution (13.01 × 10^{-5} cm/h) was about 2.3-fold and 3.5-fold higher than that of C1 (5.70 × 10^{-5} cm/h) and C2 (3.71 × 10^{-5} cm/h), respectively. The C1 exhibited 1.5 times higher skin permeability than that of C2, which could be due to smaller droplet size of C1.

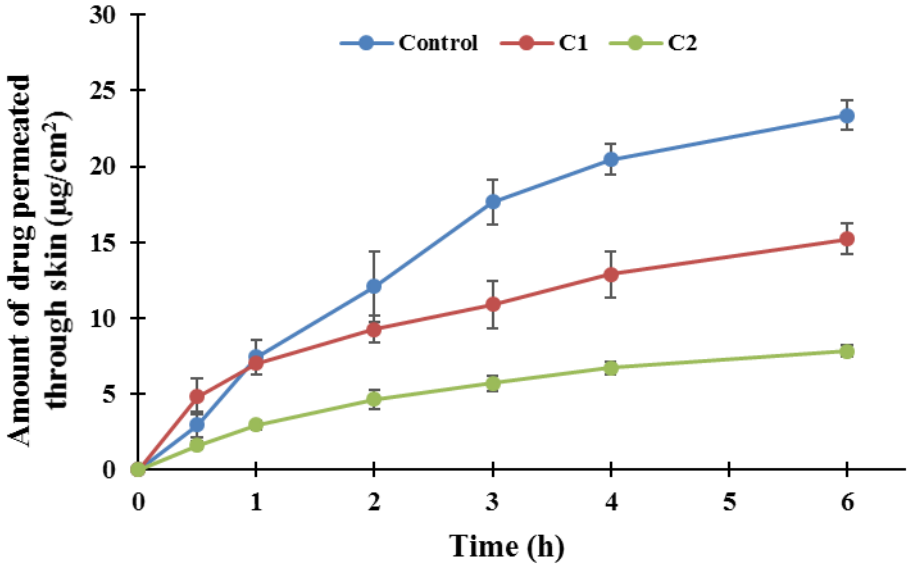


Figure 3.11 The amount of chlorhexidine permeated through skin qualified in specific intervals (Mean± SD, n=3)

Table 3.2 The *in vitro* permeability coefficient and flux of chlorhexidine digluconate nanoemulsions and 2% CHG solution (Mean± SD, n=3)

Formulation	Flux (*Jss*) µg/cm^2/h	Permeability coefficient *Kp* × 10^{-5} cm/h
Control	1.30 ± 0.12	13.01 ± 1.21
C1	0.57 ± 0.07	5.70 ± 0.72
C2	0.37 ± 0.01	3.71 ± 1.04

The results of drug retention analysis are presented in Fig. 3.12 and 3.13. After 24h, nanoemulsion formulations resulted in more drug being retained within skin than the drug solution, which indicate that nanoemulsion system has positive role on the retention of drug in skin. This would enable the active antiseptics to exert localised antimicrobial activity. EO-nanoemulsions provide a more efficacious skin retention enhancement than OO-nanoemulsion. The amount of TCS detained within skin were found to be 5.63 ± 1.63, 2.34 ± 0.86, 0.88 ± 0.30 µg/cm^2 for T1, T2 and solution, respectively.

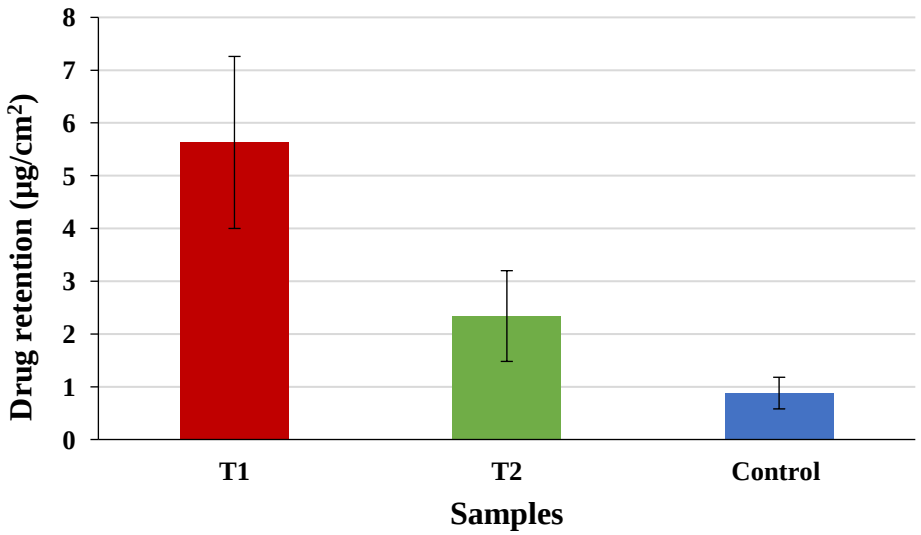


Figure 3.12 The amount of triclosan retained within skin after 24h (Mean ± SD, n=3).

Eucalyptus oil nanoemulsion was able to preserve 4.32 ± 1.15 µg/cm^2 CHG within skin, which is about 2.4-fold and 3.4-fold higher than that of olive oil nanoemulsion (1.73 ± 0.58 µg/cm^2) and 2% CHG solution (1.26 ± 0.26 µg/cm^2), respectively.

Nanoemulsions increase drug retention in skin layers, which may be attributed to improving drug solubilisation and partitioning of drug between the carrier and epidermal layer. Nanoemulsions with very small droplet size can provide a larger surface for interaction with skin tissues as well as persistent drug release, thus producing continuous therapeutic activity in a localized site within skin. The interesting results from skin retention studies of Lucca *et al*. (2015) displayed that for a crude copaiba oil alone, the hydrophobic drug β-caryophyllene was only concentrated in the SC section. In contrast, using nanoemulsion incorporating the copaiba oil and using Span 80 and Tween 20 enabled the active to penetrate into deeper layers including the SC, epidermis and dermis, indicating that NEs are effective in enhancing drug retention within skin layers [225]. Another study also found that a nanoemulsion comprised of Tween 80 and PEG 400 as surfactant and co-surfactant deposited 45.65 ± 4.76% of resveratrol in rat skin, compared to 22.42 ± 1.32% from conventional aqueous dispersion [226]. Also, Yu *et al.*

developed successfully a nanoemulsion formulation to delivery metronidazole into target sites in skin. The *in vitro* and *in vivo* skin retention showed that after 24h, the amount of drug retained in skin was 164.75 µg/cm^2 for NEs and 75.08 µg/cm^2 for gel form, and metronidazole from NEs was detected in epidermis and dermis layers, which confirmed the merits of NEs over gel [224].

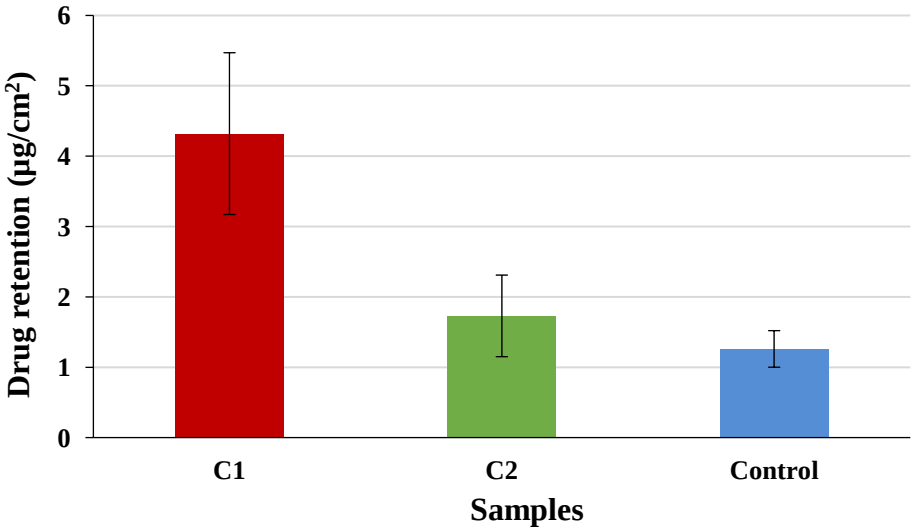


Figure 3.13 The amount of chlorhexidine retained within skin after 24h (Mean ± SD, n=3)

Overall, it can be concluded that after exposure to the cutaneous surface, hydrophilic or lipophilic drug molecules will gradually diffuse from the nanoemulsion system through the superficial layer of the SC. A part of drug will be distributed into skin layer and will be maintained to exert continuous localized antimicrobial activity. Some of the drug may pass into blood vessels under skin and then enter the systemic circulation. The remaining active will still remain at the applied site on the skin surface. The potential diffusion pathways of actives is shown in Fig 3.14.

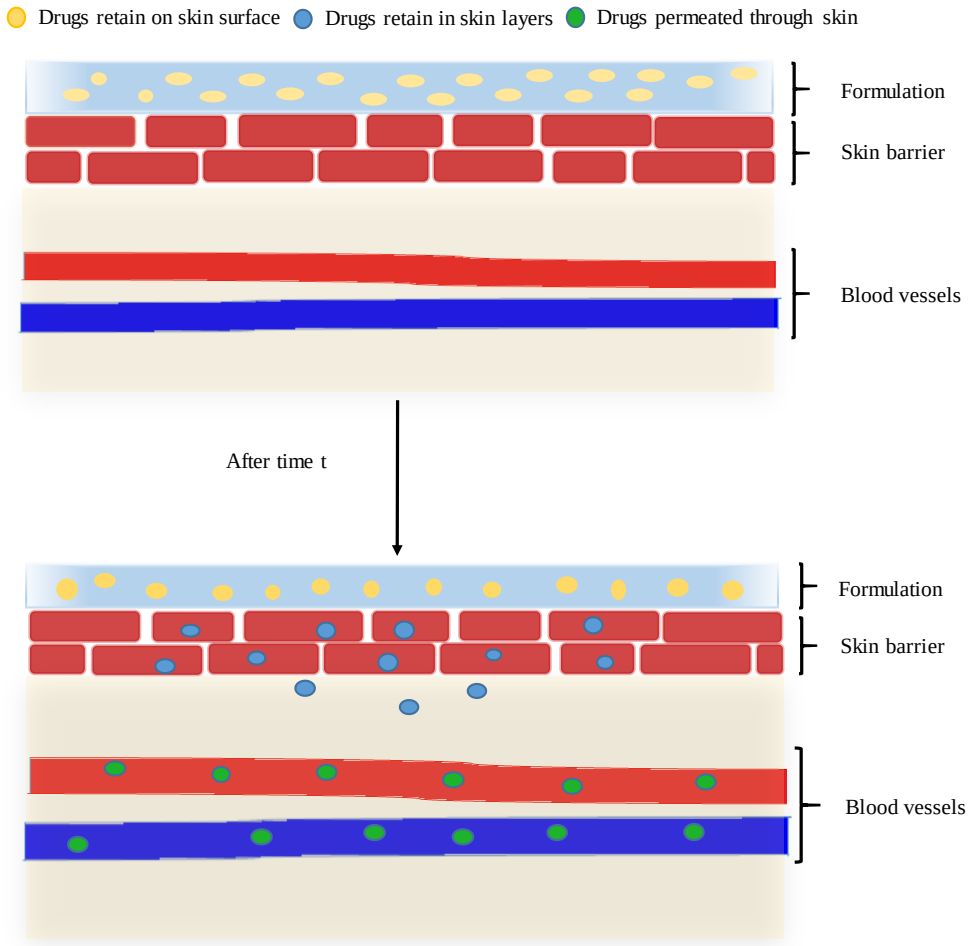


Figure 3.14 The schematic distribution of drugs after application as a function of time

CHAPTER 4

CONCLUSIONS

4. Conclusions

In the present contribution, nanoemulsions formulations carrying triclosan and chlorhexidine digluconate were formulated by using ultrasonic homogenization. Eucalyptus oil and olive oil were used as the oil phases. Both o/w TCS-containing nanoemulsions comprised of 3% oil and 6% surfactants (Span 80 and Tween 20) as well as w/o CHG based nanoemulsion including 60% oil and 30% Span 80 and Tween 80 mixture showed excellent visual transparent and homogeneous appearance. Based on the physicochemical characterization experiments, it was observed that all NEs formulation had droplet sizes smaller than 115 nm along with PDI from 0.15 to 0.41, the pH values in lightly acidic range (4.60-6.80) and the absolute zeta potential values higher than 20 mV, indicating that all developed formulations could be stable and adaptable for topical application.

According to the *in vitro* permeation test, the nanoemulsion which consisted of EO transferred the greatest amount of TCS through full thickness porcine skin than the one using OO or triclosan solution. Similarly, EO-NEs also appeared to be superior for drug retention within the skin after 24h, compared to OO-NEs and solution. These findings demonstrated that nanoemulsions are a promising candidate for topical dermal delivery of water-insoluble antiseptic agent.

On the other hand, although the skin permeation from CHG loaded nanoemulsions were lower than 2% CHG solution, EO-OO was more effective at leading to CHG retention in skin than OO-NEs and solution, which may be advantageous for localized effect.

CHAPTER 5
FUTURE WORK

5. Future work

From the results achieved in this work, there are a number of investigations that could be carried out to which potentially extend the current work. Particularly, in order to strengthen the stability study, morphological evaluation should be performed, such as using transmission electron microscopy to view if particle is spherical shape. In addition, to support for permeation study, the drug distribution in skin layers should be visualized by using some techniques like micro computed tomography, or confocal laser scanning microscope. Based on the permeation findings, *in vitro* and *in vivo* antiseptic studies should be conducted to investigate antimicrobial effects of obtained nanoemulsions. These should be performed simultaneously with available marketing products of chlorhexidine and triclosan to compare their therapeutic efficacy.

References

1. Kanitakis, J. Anatomy, histology and immunohistochemistry of normal human skin. *European Journal of Dermatology* **2002**, *12*, 390-401.
2. English, J.S.C. *General dermatology: an atlas of diagnosis and management*; Clinical Publishing: Oxford, 2007; p.1-4.
3. Waugh, A.; Grant, A. *Ross and Wilson Anatomy and Physiology in Health and Illness*; 2018; p.393-399.
4. Démarchez M. Normal human skin; 2011. Available online https://biologiedelapeau.fr/spip.php?article4 (acessed on 27 November 2020).
5. Mosteller, R. Simplified Calculation of Body-Surface Area. *New England Journal of Medicine* **1987**, *317*, 1098-1098, doi:10.1056/NEJM198710223171717.
6. Gallo, R.L. Human Skin Is the Largest Epithelial Surface for Interaction with Microbes. *Journal of Investigative Dermatology* **2017**, *137*, 1213-1214, doi:10.1016/j.jid.2016.11.045.
7. Seidenari, S.; Pagnoni, A.; Nardo, A.; Giannetti, A. Echographic Evaluation with Image Analysis of Normal Skin: Variations according to Age and Sex. *Skin pharmacology : the official journal of the Skin Pharmacology Society* **1994**, *7*, 201-209, doi:10.1159/000211295.
8. Sandby-Moller, J.; Poulsen, T.; Wulf, H.C. Epidermal Thickness at Different Body Sites: Relationship to Age, Gender, Pigmentation, Blood Content, Skin Type and Smoking Habits. *Acta Derm Venereol* **2003**, *83*, 410-413, doi:10.1080/00015550310015419
9. Hill, M.J. *Skin disorders*; Mosby: London; St. Louis, 1994.
10. Lambers, H.; Piessens, S.; Bloem, A.; Pronk, H.; Finkel, P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science* **2006**, *28*, 359-370, doi:10.1111/j.1467-2494.2006.00344.x.
11. Wohlrab, J.; Gebert, A. pH and Buffer Capacity of Topical Formulations. *Current Problems in Dermatology* **2018**, *54*, 123-131, doi:10.1159/000489526.
12. Sahle, F.F.; Gebre-Mariam, T.; Dobner, B.; Wohlrab, J.; Neubert, R.H. Skin diseases associated with the depletion of stratum corneum lipids and stratum corneum lipid substitution therapy. *Skin Pharmacol Physiol* **2015**, *28*, 42-55, doi:10.1159/000360009.
13. Hossler, F. *Ultrastructure atlas of human tissues*; 2014.

14. Fitzpatrick, J.E.; Aeling, J.L. *Dermatology secrets*; Hanley & Belfus: St. Louis; Philadelphia, 1996.
15. M.MacKie, R. *Clinical Dermatology*; Oxford University Press: 2003; p.21-38.
16. Peate, I. Skin Cancer. *British Journal of Healthcare Assistants* **2019**, *13*, 78-84, doi:10.12968/bjha.2019.13.2.78.
17. Pathan, I.B.; Setty, C.M. Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Tropical Journal of Pharmaceutical Research* **2009**, *8*, doi:10.4314/tjpr.v8i2.44527.
18. Karpanen, T. Studies on skin antiseptics and enhanced penetration of chlorhexidine. Aston University, 2008; p.34.
19. Saurabh V. R; Lalita S. N; Maya T. D; Shailejkumar D. B; Shweta U. D. Chemical penetration enhancers: For transdermal drug delivery systems. *International Journal of Pharmacy Review & Research*, **2014**, *4*, 33-40.
20. Fox, L.T.; Gerber, M.; Plessis, J.D.; Hamman, J.H. Transdermal Drug Delivery Enhancement by Compounds of Natural Origin. *Molecules* **2011**, *16*, 10507-10540, doi:10.3390/molecules161210507.
21. Potts, R.O.; Francoeur, M.L. The influence of stratum corneum morphology on water permeability. *J Invest Dermatol* **1991**, *96*, 495-499, doi:10.1111/1523-1747.ep12470197.
22. Machado, A.C.H.R.; Lopes, P.; Raffier, C.P.; Haridass, I.; Roberts, M.; Grice, J.; Leite-Silva, V. *Skin Penetration*; 2017; 10.1016/B978-0-12-802005-0.00046-Xpp. 741-755.
23. Fartasch, M.; Bassukas, I.D.; Diepgen, T.L. Structural relationship between epidermal lipid lamellae, lamellar bodies and desmosomes in human epidermis: an ultrastructural study. *Br J Dermatol* **1993**, *128*, 1-9, doi:10.1111/j.1365-2133.1993.tb00138.x.
24. Herman, A.; Herman, A.P. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. *Journal of Pharmacy and Pharmacology* **2015**, *67*, 473-485, doi:10.1111/jphp.12334.
25. Hiren J. Patel; Darshan G. Trivedi; Anand K. Bhandari; Dushyant A. Shah. Penetration enhancers for transdermal drug delivery system: A review. *IJPI's Journal of Pharmaceutics and Cosmetology* **2011**, *1*, 67-80.
26. Kim, B.; Cho, H.-E.; Moon, S.H.; Ahn, H.-J.; Bae, S.; Cho, H.-D.; An, S. Transdermal delivery systems in cosmetics. *Biomedical Dermatology* **2020**, *4*, doi:10.1186/s41702-020-0058-7.
27. Scheuplein, R.J.; Blank, I.H.; Brauner, G.J.; MacFarlane, D.J. Percutaneous absorption

of steroids. *J Invest Dermatol* **1969**, *52*, 63-70, doi:10.1038/jid.1969.9.

28. Scheuplein, R.J.; Blank, I.H. Permeability of the skin. *Physiol Rev* **1971**, *51*, 702-747, doi:10.1152/physrev.1971.51.4.702.
29. Higuchi, W.I. Analysis of data on the medicament release from ointments. *J Pharm Sci* **1962**, *51*, 802-804, doi:10.1002/jps.2600510825.
30. Benson, H.A. Transdermal drug delivery: penetration enhancement techniques. *Curr Drug Deliv* **2005**, *2*, 23-33, doi:10.2174/1567201052772915.
31. Cornwell, P.A.; Barry, B.W. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. *J Pharm Pharmacol* **1994**, *46*, 261-269, doi:10.1111/j.2042-7158.1994.tb03791.x.
32. Mathur, V.; Satrawala, Y.; Rajput, M. Physical and chemical penetration enhancers in transdermal drug delivery system. *Asian Journal of Pharmaceutics* **2010**, *4*, doi:10.4103/0973-8398.72115.
33. Trauer, S.; Richter, H.; Kuntsche, J.; Büttemeyer, R.; Liebsch, M.; Linscheid, M.; Fahr, A.; Schäfer-Korting, M.; Lademann, J.; Patzelt, A. Influence of massage and occlusion on the ex vivo skin penetration of rigid liposomes and invasomes. *European Journal of Pharmaceutics and Biopharmaceutics* **2014**, *86*, 301-306, doi:10.1016/j.ejpb.2013.11.004.
34. McDonnell, G.E. *Antisepsis, disinfection, and sterilization: types, action, and resistance*; ASM Press: Washington, D.C, 2007.
35. Wenzel, R.P. Surgical site infections and the microbiome: An updated perspective. *Infection Control & Hospital Epidemiology* **2019**, *40*, 590-596, doi:10.1017/ice.2018.363.
36. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The human skin microbiome. *Nat Rev Microbiol* **2018**, *16*, 143-155, doi:10.1038/nrmicro.2017.157.
37. Tognetti, L.; Martinelli, C.; Berti, S.; Hercogova, J.; Lotti, T.; Leoncini, F.; Moretti, S. Bacterial skin and soft tissue infections: review of the epidemiology, microbiology, aetiopathogenesis and treatment. *Journal of the European Academy of Dermatology and Venereology* **2012**, *26*, 931-941, doi:10.1111/j.1468-3083.2011.04416.x.
38. Grice, E.A.; Segre, J.A. The skin microbiome. *Nature reviews. Microbiology* **2011**, *9*, 244-253, doi:10.1038/nrmicro2537.
39. Sanford, J.A.; Gallo, R.L. Functions of the skin microbiota in health and disease. *Seminars in Immunology* **2013**, *25*, 370-377, doi:10.1016/j.smim.2013.09.005.
40. Lai, Y.; Di Nardo, A.; Nakatsuji, T.; Leichtle, A.; Yang, Y.; Cogen, A.L.; Wu, Z.-R.;

Hooper, L.V.; Schmidt, R.R.; von Aulock, S., et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nature medicine* **2009**, *15*, 1377-1382, doi:10.1038/nm.2062.

41. Dryden, M.S. Skin and soft tissue infection: microbiology and epidemiology. *International Journal of Antimicrobial Agents* **2009**, *34*, S2-S7.
42. Selcuk Mıstık, A.U., Demet Kartal, Salih Levent Çınar. Bacterial Skin Infections: Epidemiology and Latest Research. *Turkish Journal of Family Medicine & Primary Care* **2015**, *9*, 65-74, doi:10.5455/tjfmpc.177379.
43. Vinh, D.C.; Embil, J.M. Rapidly progressive soft tissue infections. *The Lancet Infectious Diseases* **2005**, *5*, 501-513, doi:10.1016/S1473-3099(05)70191-2.
44. Hersh, A.L.; Chambers, H.F.; Maselli, J.H.; Gonzales, R. National Trends in Ambulatory Visits and Antibiotic Prescribing for Skin and Soft-Tissue Infections. *Archives of Internal Medicine* **2008**, *168*, 1585-1591, doi:10.1001/archinte.168.14.1585
45. Lee, G.C.; Boyd, N.K.; Lawson, K.A.; Frei, C.R. Incidence and Cost of Skin and soft Tissue Infections In the united States. *Value In Health* **2015**, *18*, A245, doi:10.1016/j.jval.2015.03.1424.
46. Lim, H.W.; Collins, S.A.B.; Resneck, J.S., Jr.; Bolognia, J.L.; Hodge, J.A.; Rohrer, T.A.; Van Beek, M.J.; Margolis, D.J.; Sober, A.J.; Weinstock, M.A., et al. The burden of skin disease in the United States. *Journal of the American Academy of Dermatology* **2017**, *76*, 958-972, doi:10.1016/j.jaad.2016.12.043.
47. Esposito, S.; Noviello, S.; Leone, S. Epidemiology and microbiology of skin and soft tissue infections. *Current Opinion in Infectious Diseases* **2016**, *29*, 109-115, doi:10.1097/QCO.0000000000000239.
48. James, S.L.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A., et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* **2018**, *392*, 1789-1858, doi:10.1016/S0140-6736(18)32279-7.
49. Moffarah, A.S.; Al Mohajer, M.; Hurwitz, B.L.; Armstrong, D.G. Skin and Soft Tissue Infections. **2016**, *4*, doi:10.1128/microbiolspec.DMIH2-0014-2015.
50. Wounds UK. Best Practice Statement: Maintaining Skin Integrity. Availabe online: https://www.wounds-uk.com/resources/details/maintaining-skin-integrity (accessed on

19 December 2019).

51. Malheiro, J.; Simões, M. 4 - Antimicrobial resistance of biofilms in medical devices. In *Biofilms and Implantable Medical Devices*, Deng, Y., Lv, W., Eds. Woodhead Publishing: 2017; 10.1016/B978-0-08-100382-4.00004-6pp. 97-113.
52. World Health Organization. Epidemiology and Management of Common Skin Diseases in Children in Developing Countries. Availabe online: https://apps.who.int/iris/handle/10665/69229 (accessed on 15 December 2019).
53. Poulakou, G.; Lagou, S.; Tsiodras, S. What's new in the epidemiology of skin and soft tissue infections in 2018? **2019**, *32*, 77-86, doi:10.1097/qco.0000000000000527.
54. Suaya, J.A.; Mera, R.M.; Cassidy, A.; O'Hara, P.; Amrine-Madsen, H.; Burstin, S.; Miller, L.G. Incidence and cost of hospitalizations associated with Staphylococcus aureus skin and soft tissue infections in the United States from 2001 through 2009. *BMC infectious diseases* **2014**, *14*, 296-296, doi:10.1186/1471-2334-14-296.
55. Klein, E.Y.; Mojica, N.; Jiang, W.; Cosgrove, S.E.; Septimus, E.; Morgan, D.J.; Laxminarayan, R. Trends in Methicillin-Resistant Staphylococcus aureus Hospitalizations in the United States, 2010-2014. *Clinical Infectious Diseases* **2017**, *65*, 1921-1923, doi:10.1093/cid/cix640.
56. Karimkhani, C.; Dellavalle, R.P.; Coffeng, L.E.; Flohr, C.; Hay, R.J.; Langan, S.M.; Nsoesie, E.O.; Ferrari, A.J.; Erskine, H.E.; Silverberg, J.I., et al. Global Skin Disease Morbidity and Mortality: An Update From the Global Burden of Disease Study 2013. *JAMA Dermatol* **2017**, *153*, 406-412, doi:10.1001/jamadermatol.2016.5538.
57. Kaye, K.S.; Petty, L.A.; Shorr, A.F.; Zilberberg, M.D. Current Epidemiology, Etiology, and Burden of Acute Skin Infections in the United States. *Clinical Infectious Diseases* **2019**, *68*, S193-S199, doi:10.1093/cid/ciz002
58. Drosou, A.; Falabella, A.; Kirsner, R.S. Antiseptics on Wounds: An Area of Controversy. Availabe online: https://www.woundsresearch.com/article/1585 (accessed on 27 November 2019).
59. Leaper, D. Topical antiseptics in wound care: time for reflection. *Int Wound J* **2011**, *8*, 547-549, doi:10.1111/j.1742-481X.2011.00872.x.
60. Leaper, D.J.; Schultz, G.; Carville, K.; Fletcher, J.; Swanson, T.; Drake, R. Extending the TIME concept: what have we learned in the past 10 years?(*). *Int Wound J* **2012**, *9 Suppl 2*, 1-19, doi:10.1111/j.1742-481X.2012.01097.x.
61. The U.S. Food and Drug Administration (FDA). Q&A for Consumers: Health Care Antiseptics. Availabe online: https://www.fda.gov/drugs/information-drug-class/qa-

consumers-health-care-antiseptics (accessed on 21 Decmber 2019).

62. World Health Organization. Summary of a systematic literature review on surgical site preparation. Availabe online: https://www.who.int/gpsc/appendix8.pdf (accessed on 12 November 2019).
63. Chang, R.K.; Raw, A.; Lionberger, R.; Yu, L. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. *AAPS J* **2013**, *15*, 41-52, doi:10.1208/s12248-012-9411-0.
64. Berríos-Torres, S.I.; Umscheid, C.A.; Bratzler, D.W.; Leas, B.; Stone, E.C.; Kelz, R.R.; Reinke, C.E.; Morgan, S.; Solomkin, J.S.; Mazuski, J.E., et al. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. *JAMA Surgery* **2017**, *152*, 784-791, doi:10.1001/jamasurg.2017.0904.
65. Claesen, J. Topical Antiseptics and the Skin Microbiota. *Journal of Investigative Dermatology* **2018**, *138*, 2106-2107, doi:10.1016/j.jid.2018.06.001.
66. Tanzer, J.M.; Slee, A.M.; Kamay, B.A. Structural requirements of guanide, biguanide, and bisbiguanide agents for antiplaque activity. *Antimicrobial agents and chemotherapy* **1977**, *12*, 721-729, doi:10.1128/aac.12.6.721.
67. World Health Organization. Global guidelines on the prevention of surgical site infection. Availabe online: https://apps.who.int/iris/bitstream/handle/10665/250680/9789241549882-eng.pdf?sequence=8 (accessed on December 11, 2019).
68. Privitera, G.P.; Costa, A.L.; Brusaferro, S.; Chirletti, P.; Crosasso, P.; Massimetti, G.; Nespoli, A.; Petrosillo, N.; Pittiruti, M.; Scoppettuolo, G., et al. Skin antisepsis with chlorhexidine versus iodine for the prevention of surgical site infection: A systematic review and meta-analysis. *American Journal of Infection Control* **2017**, *45*, 180-189, doi:10.1016/j.ajic.2016.09.017.
69. O'Grady, N.P.; Alexander, M.; Burns, L.A.; Dellinger, E.P.; Garland, J.; Heard, S.O.; Lipsett, P.A.; Masur, H.; Mermel, L.A.; Pearson, M.L., et al. Guidelines for the prevention of intravascular catheter-related infections. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2011**, *52*, e162-e193, doi:10.1093/cid/cir257.
70. Macias, J.H.; Alvarez, M.F.; Arreguin, V.; Muñoz, J.M.; Macias, A.E.; Alvarez, J.A. Chlorhexidine avoids skin bacteria recolonization more than triclosan. *American Journal of Infection Control* **2016**, *44*, 1530-1534, doi:10.1016/j.ajic.2016.04.235.

71. Mozayeni, M.A.; Hadian, A.; Bakhshaei, P.; Dianat, O. Comparison of Antifungal Activity of 2% Chlorhexidine, Calcium Hydroxide, and Nanosilver gels against Candida Albicans. *J Dent (Tehran)* **2015**, *12*, 109-117.
72. Yasuda, H.; Sanui, M.; Abe, T.; Shime, N.; Komuro, T.; Hatakeyama, J.; Matsukubo, S.; Kawano, S.; Yamamoto, H.; Andoh, K., et al. Comparison of the efficacy of three topical antiseptic solutions for the prevention of catheter colonization: a multicenter randomized controlled study. *Critical care (London, England)* **2017**, *21*, 320-320, doi:10.1186/s13054-017-1890-z.
73. Auda, S.H.; Mahrous, G.M.; Ibrahim, M.A.; Shazly, G.A.; Salem-Bekhit, M.M. Novel chlorhexidine dermal patches, preparation characterization and antimicrobial evaluation. *Polymer Bulletin* **2017**, *74*, 3995-4007, doi:10.1007/s00289-017-1935-0.
74. Zinn, J.; Jenkins, J.B.; Swofford, V.; Harrelson, B.; McCarter, S. Intraoperative Patient Skin Prep Agents: Is There a Difference? *AORN J* **2010**, *92*, 662-674, doi:10.1016/j.aorn.2010.07.016.
75. Boyce, J.M. Best products for skin antisepsis. *American Journal of Infection Control* **2019**, *47*, A17-A22, doi:10.1016/j.ajic.2019.03.012.
76. Hayashi, M.; Kawamura, K.; Matsui, M.; Suzuki, M.; Suzuki, S.; Shibayama, K.; Arakawa, Y. Reduction in chlorhexidine efficacy against multi-drug-resistant Acinetobacter baumannii international clone II. *Journal of Hospital Infection* **2017**, *95*, 318-323, doi:10.1016/j.jhin.2016.12.004.
77. Bhardwaj, P.; Ziegler, E.; Palmer, K.L. Chlorhexidine Induces VanA-Type Vancomycin Resistance Genes in Enterococci. *Antimicrobial Agents and Chemotherapy* **2016**, *60*, 2209, doi:10.1128/AAC.02595-15.
78. Wand, M.E.; Bock, L.J.; Bonney, L.C.; Sutton, J.M. Mechanisms of Increased Resistance to Chlorhexidine and Cross-Resistance to Colistin following Exposure of Klebsiella pneumoniae Clinical Isolates to Chlorhexidine. *Antimicrobial agents and chemotherapy* **2016**, *61*, e01162-01116, doi:10.1128/AAC.01162-16.
79. Williamson, D.A.; Carter, G.P.; Howden, B.P. Current and Emerging Topical Antibacterials and Antiseptics: Agents, Action, and Resistance Patterns. *Clinical microbiology reviews* **2017**, *30*, 827-860, doi:10.1128/CMR.00112-16.
80. Lee, J.; Lee, J.; Kwack, S.; Shin, C.; Jang, H.-J.; Kim, H.; Kim, M.; Seo, D.-W.; Lee, B.; Kim, K.-B. Risk Assessment of Triclosan, a Cosmetic Preservative. *Toxicological Research* **2019**, *35*, 137-154, doi:10.5487/TR.2019.35.2.137.
81. Schweizer, H.P. Triclosan: a widely used biocide and its link to antibiotics. *FEMS*

Microbiology Letters **2001**, *202*, 1-7, doi:10.1111/j.1574-6968.2001.tb10772.x.

82. Günther Hierholzer, E.R.W., Karen Reimer. Topische Infektionstherapie und Prophylaxe. Aktueller Stellenwert von PVP-Jod. *Stuttgart: Thieme* **1996**.
83. Do, T.K.T.; Hadji-Minaglou, F.; Antoniotti, S.; Fernandez, X. Authenticity of essential oils. *Trends in Analytical Chemistry* **2015**, *66*, 146-157, doi:10.1016/j.trac.2014.10.007.
84. Orchard, A.; van Vuuren, S. Commercial Essential Oils as Potential Antimicrobials to Treat Skin Diseases. *Evidence-based complementary and alternative medicine : eCAM* **2017**, *2017*, 4517971-4517971, doi:10.1155/2017/4517971.
85. Rutherford, T.; Nixon, R.; Tam, M.; Tate, B. Allergy to tea tree oil: Retrospective review of 41 cases with positive patch tests over 4.5 years. **2007**, *48*, 83-87, doi:10.1111/j.1440-0960.2007.00341.x.
86. Graham, C. The role of silver in wound healing. **2005**, *14*, S22-S28, doi:10.12968/bjon.2005.14.Sup5.19954.
87. Jung, W.K.; Koo, H.C.; Kim, K.W.; Shin, S.; Kim, S.H.; Park, Y.H. Antibacterial Activity and Mechanism of Action of the Silver Ion in Staphylococcus aureus and Escherichia coli. **2008**, *74*, 2171-2178, doi:10.1128/AEM.02001-07
88. Lansdown, A. Silver in Health Care: Antimicrobial Effects and Safety in Use. *Current Problems in Dermatology* **2006**, *33*, 17-34, doi:10.1159/000093928.
89. Weedon, D. 14 - Cutaneous deposits. In *Weedon's Skin Pathology (Third Edition)*, Weedon, D., Ed. Churchill Livingstone: Edinburgh, 2010; 10.1016/B978-0-7020-3485-5.00015-2pp. 369-396.e320.
90. National Center for Biotechnology Information. Benzethonium chloride, CID=8478. Availabe online: https://pubchem.ncbi.nlm.nih.gov/compound/Benzethonium-chloride (accessed on 20 December 2019).
91. Magee, P. 24 - Antiseptic drugs and disinfectants. In *Side Effects of Drugs Annual*, Aronson, J.K., Ed. Elsevier: 2010; Vol. 32, pp. 437-443.
92. McKeen, L. 1 - Introduction to Food Irradiation and Medical Sterilization. In *The Effect of Sterilization on Plastics and Elastomers (Third Edition)*, McKeen, L., Ed. William Andrew Publishing: Boston, 2012; 10.1016/B978-1-4557-2598-4.00001-0pp. 1-40.
93. Kim, M.; Weigand, M.R.; Oh, S.; Hatt, J.K.; Krishnan, R.; Tezel, U.; Pavlostathis, S.G.; Konstantinidis, K.T. Widely Used Benzalkonium Chloride Disinfectants Can Promote Antibiotic Resistance. **2018**, *84*, doi:10.1128/AEM.01201-18
94. Pitten, F.-A.; Kramer, A. Efficacy of Cetylpyridinium Chloride Used as Oropharyngeal

Antiseptic. *Arzneimittelforschung* **2001**, *51*, 588-595, doi:10.1055/s-0031-1300084.

95. National Center for Biotechnology Information. Cetylpyridinium chloride, CID=31239. Availabe online: https://pubchem.ncbi.nlm.nih.gov/compound/Cetylpyridinium-chloride (accessed on 15 December 2019).
96. Al-Doori, Z.; Goroncy-Bermes, P.; Gemmell, C.G.; Morrison, D. Low-level exposure of MRSA to octenidine dihydrochloride does not select for resistance. *Journal of Antimicrobial Chemotherapy* **2007**, *59*, 1280-1281, doi:10.1093/jac/dkm092
97. Hübner, N.O.; Siebert, J.; Kramer, A. Octenidine Dihydrochloride, a Modern Antiseptic for Skin, Mucous Membranes and Wounds. *Skin Pharmacology and Physiology* **2010**, *23*, 244-258, doi:10.1159/000314699.
98. Conceição, T.; de Lencastre, H.; Aires-de-Sousa, M. Efficacy of octenidine against antibiotic-resistant Staphylococcus aureus epidemic clones. *Journal of Antimicrobial Chemotherapy* **2016**, *71*, 2991-2994, doi:10.1093/jac/dkw241.
99. Alvarez-Marin, R.; Aires-de-Sousa, M.; Nordmann, P.; Kieffer, N.; Poirel, L.J.E.J.o.C.M.; Diseases, I. Antimicrobial activity of octenidine against multidrug-resistant Gram-negative pathogens. **2017**, *36*, 2379-2383, doi:10.1007/s10096-017-3070-0.
100. Dettenkofer, M.; Jonas, D.; Wiechmann, C.; Rossner, R.; Frank, U.; Zentner, J.; Daschner, F.D. Effect of Skin Disinfection with Octenidine Dihydrochloride on Insertion Site Colonization of Intravascular Catheters. *Infection* **2002**, *30*, 282-285, doi:10.1007/s15010-002-2182-2.
101. Marzenna B.; Anna R.; Marek K.; Anna P. Penetration of a selected antibiotic and antiseptic into a biofilm formed on orthopedic steel implants. *Orthop Traumatol Rehabil* **2007**, *9*, 310-318.
102. Fjeld, H.; Lingaas, E. Polyhexanide – safety and efficacy as an antiseptic. *Tidsskr Nor Legeforen* **2016**, *136*, 707-711, doi:10.4045/tidsskr.14.1041.
103. Yanai, R.; Ueda, K.; Nishida, T.; Toyohara, M.; Mori, O. Effects of Ionic and Surfactant Agents on the Antimicrobial Activity of Polyhexamethylene Biguanide. **2011**, *37*, 85-89, doi:10.1097/ICL.0b013e31820cebc3.
104. Rohrer, N.; Widmer, A.F.; Waltimo, T.; Kulik, E.M.; Weiger, R.; Filipuzzi-Jenny, E.; Walter, C. Antimicrobial Efficacy of 3 Oral Antiseptics Containing Octenidine, Polyhexamethylene Biguanide, or Citroxx: Can Chlorhexidine Be Replaced? *Infection Control & Hospital Epidemiology* **2010**, *31*, 733-739, doi:10.1086/653822.

105. Harbs, N.; Siebert, J. In vitro efficacy of octenidine and polihexanide against biofilms composed of Pseudomonas aeruginosa. *GMS Krankenhaushygiene Interdisziplinär*, **2007**, *2*, Doc45.
106. Hübner, N.O.; Kramer, A. Review on the Efficacy, Safety and Clinical Applications of Polihexanide, a Modern Wound Antiseptic. *Skin Pharmacology and Physiology* **2010**, *23(suppl 1)*, 17-27, doi:10.1159/000318264.
107. United States Pharmacopeial Convention. *United States Pharmacopeia-National Formulary*; 2018.
108. Macias, J.H.M.D.; Arreguin, V.M.D.; Munoz, J.M.M.D.; Alvarez, J.A.M.D.; Mosqueda, J.L.M.D.; Macias, A.E.M.D. Chlorhexidine is a better antiseptic than povidone iodine and sodium hypochlorite because of its substantive effect. *AJIC: American Journal of Infection Control* **2013**, *41*, 634-637, doi:10.1016/j.ajic.2012.10.002.
109. Chiang, S.-R.; Jung, F.; Tang, H.-J.; Chen, C.-C.; Chen, C.-H.; Chou, H.-Y.; Chuang, Y.-C. Desiccation and ethanol resistances of multidrug resistant Acinetobacter baumannii embedded in biofilm: The favorable antiseptic efficacy of combination chlorhexidine gluconate and ethanol. *Journal of Microbiology, Immunology and Infection* **2018**, *51*, 770-777, doi:10.1016/j.jmii.2017.02.003.
110. Koburger, T.; Hübner, N.-O.; Braun, M.; Siebert, J.; Kramer, A. Standardized comparison of antiseptic efficacy of triclosan, PVP–iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. *Journal of Antimicrobial Chemotherapy* **2010**, *65*, 1712-1719, doi:10.1093/jac/dkq212.
111. Martínez, J.M.D.; Macías, J.H.M.D.; Arreguín, V.M.D.; Álvarez, J.A.M.D.; Macías, A.E.M.D.; Mosqueda-Gómez, J.L.M.D. Isopropyl alcohol is as efficient as chlorhexidine to prevent contamination of blood cultures. *AJIC: American Journal of Infection Control* **2017**, *45*, 350-353, doi:10.1016/j.ajic.2016.11.027.
112. Djozic, H.; Pandza, H.; Hasukic, S.; Custovic, S.; Pandza, B.; Krupalija, A.; Beciragic, E. Efficiency of Local Antiseptic Alkosol (Ethanol, Isopropanol-30g and Ortophenilphenol) and Povidone Iodide on the Incidence Of Surgical Site Infection After Inguinal Hernioplasty. *Medical archives (Sarajevo, Bosnia and Herzegovina)* **2016**, *70*, 108-111, doi:10.5455/medarh.2016.70.108-111.
113. Bashir, M.H.; Hollingsworth, A.; Schwab, D.; Prinsen, K.S.; Paulson, J.E.; Morse, D.J.; Bernatchez, S.F. Ex vivo and in vivo evaluation of residual chlorhexidine gluconate on skin following repetitive exposure to saline and wiping with 2% chlorhexidine

gluconate/70% isopropyl alcohol pre-operative skin preparations. *Journal of Hospital Infection* **2019**, *102*, 256-261, doi:10.1016/j.jhin.2018.10.004.

114. Capriotti, K.D.; Anadkat, M.; Choi, J.; Kaffenberger, B.; McLellan, B.; Barone, S.; Kukoyi, O.; Goldfarb, S.; Lacouture, M. A randomized phase 2 trial of the efficacy and safety of a novel topical povidone-iodine formulation for Cancer therapy-associated Paronychia. *Investigational New Drugs* **2019**, *37*, 1247-1256, doi:10.1007/s10637-019-00825-0.
115. Jug, M.; Kosalec, I.; Maestrelli, F.; Mura, P. Development of low methoxy amidated pectin-based mucoadhesive patches for buccal delivery of triclosan: Effect of cyclodextrin complexation. *Carbohydrate Polymers* **2012**, *90*, 1794-1803, doi:10.1016/j.carbpol.2012.07.074.
116. Cavallari, C.; Brigidi, P.; Fini, A. Ex-vivoand in-vitro assessment of mucoadhesive patches containing the gel-forming polysaccharide psyllium for buccal delivery of chlorhexidine base. *International Journal of Pharmaceutics* **2015**, *496*, 593-600, doi:10.1016/j.ijpharm.2015.10.077.
117. Sonje, A.; Chandra, A. Comprehencive review on eudragit polymers. *International Research Journal of Pharmacy* **2013**, *4*, 71-74, doi:10.7897/2230-8407.04515.
118. Kaur, G.; Grewal, J.; Jyoti, K.; Jain, U.K.; Chandra, R.; Madan, J. Chapter 15 - Oral controlled and sustained drug delivery systems: Concepts, advances, preclinical, and clinical status. In *Drug Targeting and Stimuli Sensitive Drug Delivery Systems*, Grumezescu, A.M., Ed. William Andrew Publishing: 2018; 10.1016/B978-0-12-813689-8.00015-Xpp. 567-626.
119. Brady, J.; Dürig, T.; Lee, P.I.; Li, J.X. Chapter 7 - Polymer Properties and Characterization. In *Developing Solid Oral Dosage Forms (Second Edition)*, Qiu, Y., Chen, Y., Zhang, G.G.Z., Yu, L., Mantri, R.V., Eds. Academic Press: Boston, 2017; 10.1016/B978-0-12-802447-8.00007-8pp. 181-223.
120. Patra, C.N.; Priya, R.; Swain, S.; Kumar Jena, G.; Panigrahi, K.C.; Ghose, D. Pharmaceutical significance of Eudragit: A review. *Future Journal of Pharmaceutical Sciences* **2017**, *3*, 33-45, doi:10.1016/j.fjps.2017.02.001.
121. Jones, D.S. *Pharmaceutics: dosage form and design*, Second ed.; Pharmaceutical Press: London, 2016.
122. Benson, H.A.E.; Watkinson, A.C. *Topical and transdermal drug delivery: principles and practice*, 1. Aufl.;1st; ed.; Wiley: Hoboken, N.J, 2011.
123. Mahato, R.I.; Narang, A.S. *Pharmaceutical dosage forms and drug delivery*, 2nd ed.;

CRC Press: Boca Raton, Florida;New York;London, [England];, 2012.

124. Jalil, A.; Matuszczak, B.; Nguyen Le, N.-M.; Mahmood, A.; Laffleur, F.; Bernkop-Schnürch, A. Synthesis and Characterization of Thiolated PVP-Iodine Complexes: Key to Highly Mucoadhesive Antimicrobial Gels. *Molecular pharmaceutics* **2018**, *15*, 3527-3534, doi:10.1021/acs.molpharmaceut.8b00503.

125. Pawar, V.; Topkar, H.; Srivastava, R. Chitosan nanoparticles and povidone iodine containing alginate gel for prevention and treatment of orthopedic implant associated infections. *International Journal of Biological Macromolecules* **2018**, *115*, 1131-1141, doi:10.1016/j.ijbiomac.2018.04.166.

126. Czerwinski, S.E.; Cozean, J.; Cozean, C. Novel water-based antiseptic lotion demonstrates rapid, broad-spectrum kill compared with alcohol antiseptic. *Journal of Infection and Public Health* **2014**, *7*, 199-204, doi:10.1016/j.jiph.2014.01.002.

127. FDA-Tentative Final Monograph. Topical antimicrobial products for over-the-counter-use, 21 CFR 333 and 369. *Fred Regist* **1994**, *59*.

128. Erasmus, V.; Daha, T.J.; Brug, H.; Richardus, J.H.; Behrendt, M.D.; Vos, M.C.; van Beeck, E.F. Systematic Review of Studies on Compliance with Hand Hygiene Guidelines in Hospital Care. *Infection Control & Hospital Epidemiology* **2010**, *31*, 283-294, doi:10.1086/650451.

129. Caelli, M.; Porteous, J.; Carson, C.F.; Heller, R.; Riley, T.V. Tea tree oil as an alternative topical decolonization agent for methicillin-resistant Staphylococcus aureus. *Journal of Hospital Infection* **2000**, *46*, 236-237, doi:10.1053/jhin.2000.0830.

130. Hoekstra, M.J.; Westgate, S.J.; Mueller, S. Povidone-iodine ointment demonstrates in vitro efficacy against biofilm formation. *International Wound Journal* **2017**, *14*, 172-179, doi:10.1111/iwj.12578.

131. Dryden, M.S.; Dailly, S.; Crouch, M. A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. *Journal of Hospital Infection* **2004**, *56*, 283-286, doi:10.1016/j.jhin.2004.01.008.

132. US FDA. Topical Antiseptic Products: Hand Sanitizers and Antibacterial Soaps. Availabe online: https://www.fda.gov/drugs/information-drug-class/topical-antiseptic-products-hand-sanitizers-and-antibacterial-soaps (accessed on 12 December 2019).

133. Eggers, M.; Eickmann, M.; Kowalski, K.; Zorn, J.; Reimer, K. Povidone-iodine hand wash and hand rub products demonstrated excellent in vitro virucidal efficacy against Ebola virus and modified vaccinia virus Ankara, the new European test virus for enveloped viruses. *BMC infectious diseases* **2015**, *15*, 375-375, doi:10.1186/s12879-

015-1111-9.

134. Souza, C.; Watanabe, E.; Aires, C.P.; Lara, M.G. Effect of Liquid Crystalline Systems Containing Antimicrobial Compounds on Infectious Skin Bacteria. *AAPS PharmSciTech* **2017**, *18*, 2110-2119, doi:10.1208/s12249-016-0690-0.

135. Aiello, A.E.; Larson, E.L.; Levy, S.B. Consumer Antibacterial Soaps: Effective or Just Risky? *Clinical Infectious Diseases* **2007**, *45*, S137-S147, doi:10.1086/519255.

136. US FDA. Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record. Availabe online: https://www.federalregister.gov/documents/2013/12/17/2013-29814/safety-and-effectiveness-of-consumer-antiseptics-topical-antimicrobial-drug-products-for (accessed on 25 December 2019).

137. Kim, S.A.; Moon, H.; Lee, K.; Rhee, M.S. Bactericidal effects of triclosan in soap both in vitro and in vivo. *Journal of Antimicrobial Chemother* **2015**, *70*, 3345–3352 doi:10.1093/jac/dkv275.

138. US FDA. Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. Availabe online: https://www.federalregister.gov/documents/2019/04/12/2019-06791/safety-and-effectiveness-of-consumer-antiseptic-rubs-topical-antimicrobial-drug-products-for (accessed on 25 December 2019).

139. Igwebike-ossi, C.D.; Francis, O.O.; Oke, B. Formulation and Antimicrobial Activity of Triclosan Based Conditioning Medicated Shampoo. *International Journal of Applied Chemistry* **2017**, *13*, 787-798

140. Gnatta, J.R.; Pinto, F.M.; Bruna, C.Q.; Souza, R.Q.; Graziano, K.U.; Silva, M.J. Comparison of hand hygiene antimicrobial efficacy: Melaleuca alternifolia essential oil versus triclosan. *Rev Lat Am Enfermagem* **2013**, *21*, 1212-1219, doi:10.1590/0104-1169.2957.2356.

141. Neubert, R.H. Potentials of new nanocarriers for dermal and transdermal drug delivery. *Eur J Pharm Biopharm* **2011**, *77*, 1-2, doi:10.1016/j.ejpb.2010.11.003.

142. Liang, X.-J. *Nanopharmaceutics: the potential application of nanomaterials*; World Scientific: New Jersey, 2013.

143. Din, F.U.; Aman, W.; Ullah, I.; Qureshi, O.S.; Mustapha, O.; Shafique, S.; Zeb, A. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International journal of nanomedicine* **2017**, *12*, 7291-7309,

doi:10.2147/IJN.S146315.

144. Javadzadeh, Y.; Azharshekoufeh Bahari, L. Chapter 8 - Therapeutic Nanostructures for Dermal and Transdermal Drug Delivery. In *Nano- and Microscale Drug Delivery Systems*, Grumezescu, A.M., Ed. Elsevier: 2017; 10.1016/B978-0-323-52727-9.00008-Xpp. 131-146.

145. Pannu, J.; McCarthy, A.; Martin, A.; Hamouda, T.; Ciotti, S.; Fothergill, A.; Sutcliffe, J. NB-002, a Novel Nanoemulsion with Broad Antifungal Activity against Dermatophytes, Other Filamentous Fungi, and Candida albicans. *Antimicrobial Agents and Chemotherapy* **2009**, *53*, 3273-3279, doi:10.1128/AAC.00218-09.

146. Cao, Z.; Spilker, T.; Fan, Y.; Kalikin, L.M.; Ciotti, S.; LiPuma, J.J.; Makidon, P.E.; Wilkinson, J.E.; Baker, J.R., Jr.; Wang, S.H. Nanoemulsion is an effective antimicrobial for methicillin-resistant Staphylococcus aureus in infected wounds. *Nanomedicine* **2017**, *12*, 1177-1185, doi:10.2217/nnm-2017-0025.

147. Hemmila, M.R.; Mattar, A.; Taddonio, M.A.; Arbabi, S.; Hamouda, T.; Ward, P.A.; Wang, S.C.; Baker, J.R., Jr. Topical nanoemulsion therapy reduces bacterial wound infection and inflammation after burn injury. *Surgery* **2010**, *148*, 499-509, doi:10.1016/j.surg.2010.01.001.

148. Kakadia, P.G. Formulation and Evaluation of Nanoencapsulated Antimicrobial Agents for Dermal Delivery. 2016.

149. Sinha, P.; Srivastava, S.; Mishra, N.; Singh, D.K.; Luqman, S.; Chanda, D.; Yadav, N.P. Development, optimization, and characterization of a novel tea tree oil nanogel using response surface methodology. *Drug Development and Industrial Pharmacy* **2016**, *42*, 1434-1445, doi:10.3109/03639045.2016.1141931.

150. Najafi-taher, R.; Ghaemi, B.; Kharazi, S.; Rasoulikoohi, S.; Amani, A. Promising Antibacterial Effects of Silver Nanoparticle-Loaded Tea Tree Oil Nanoemulsion: a Synergistic Combination Against Resistance Threat. *AAPS PharmSciTech* **2018**, *19*, 1133-1140, doi:10.1208/s12249-017-0922-y.

151. Zhang, H.; Zhai, Y.; Wang, J.; Zhai, G. New progress and prospects: The application of nanogel in drug delivery. *Materials Science and Engineering: C* **2015**, *60*, doi:10.1016/j.msec.2015.11.041.

152. Kettel, M.J.; Heine, E.; Schaefer, K.; Moeller, M. Chlorhexidine Loaded Cyclodextrin Containing PMMA Nanogels as Antimicrobial Coating and Delivery Systems. *Macromolecular Bioscience* **2017**, *17*, 1600230-n/a, doi:10.1002/mabi.201600230.

153. Khan, M.A.; Mujahid, M.; Gul, I.H.; Hussain, A. Electrochemical study of magnetic

nanogel designed for controlled release of chlorhexidine gluconate. *Electrochimica Acta* **2019**, *295*, 113-123, doi:10.1016/j.electacta.2018.10.098.

154. Choi, O.; Deng, K.K.; Kim, N.-J.; Ross, L.; Surampalli, R.Y.; Hu, Z. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Research* **2008**, *42*, 3066-3074, doi:10.1016/j.watres.2008.02.021.
155. Petica, A.; Gavriliu, S.; Lungu, M.; Buruntea, N.; Panzaru, C. Colloidal silver solutions with antimicrobial properties. *Materials Science & Engineering B* **2008**, *152*, 22-27, doi:10.1016/j.mseb.2008.06.021.
156. Ulmer, M.; Patzelt, A.; Vergou, T.; Richter, H.; Müller, G.; Kramer, A.; Sterry, W.; Czaika, V.; Lademann, J. In vivo investigation of the efficiency of a nanoparticle-emulsion containing polihexanide on the human skin. *European Journal of Pharmaceutics and Biopharmaceutics* **2013**, *84*, 325-329, doi:10.1016/j.ejpb.2012.11.011.
157. Domínguez-Delgado, C.L.; Rodríguez-Cruz, I.M.; Escobar-Chávez, J.J.; Calderón-Lojero, I.O.; Quintanar-Guerrero, D.; Ganem, A. Preparation and characterization of triclosan nanoparticles intended to be used for the treatment of acne. *European Journal of Pharmaceutics and Biopharmaceutics* **2011**, *79*, 102-107, doi:10.1016/j.ejpb.2011.01.017.
158. Wais, U.; Nawrath, M.M.; Jackson, A.W.; Zhang, H. Triclosan nanoparticles via emulsion-freeze-drying for enhanced antimicrobial activity. *Colloid and Polymer Science* **2018**, *296*, 951-960, doi:10.1007/s00396-018-4312-0.
159. Bamrungsap, S.; Zhao, Z.; Chen, T.; Wang, L.; Li, C.; Fu, T.; Tan, W. Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. *Nanomedicine* **2012**, *7*, 1253-1271, doi:10.2217/nnm.12.87.
160. Davachi, S.M.; Kaffashi, B. Preparation and Characterization of Poly L-Lactide/Triclosan Nanoparticles for Specific Antibacterial and Medical Applications. *International Journal of Polymeric Materials and Polymeric Biomaterials* **2015**, *64*, 497-508, doi:10.1080/00914037.2014.977897.
161. Gao, T.; Fan, H.; Wang, X.; Gao, Y.; Liu, W.; Chen, W.; Dong, A.; Wang, Y.-J. Povidone-Iodine-Based Polymeric Nanoparticles for Antibacterial Applications. *ACS applied materials & interfaces* **2017**, *9*, 25738-25746, doi:10.1021/acsami.7b05622.
162. Lboutounne, H.; Chaulet, J.-F.; Ploton, C.; Falson, F.; Pirot, F. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(ϵ-caprolactone) nanocapsule encapsulated form and as a digluconate. *Journal of Controlled Release* **2002**, *82*, 319-334,

doi:10.1016/S0168-3659(02)00142-6.

163. Flores, F.C.; de Lima, J.A.; Ribeiro, R.F.; Alves, S.H.; Rolim, C.M.B.; Beck, R.C.R.; da Silva, C.B. Antifungal Activity of Nanocapsule Suspensions Containing Tea Tree Oil on the Growth of Trichophyton rubrum. *Mycopathologia* **2013**, *175*, 281-286, doi:10.1007/s11046-013-9622-7.

164. Flores, F.C.; De Lima, J.A.; Da Silva, C.R.; Benvegnu, D.; Ferreira, J.; Burger, M.E.; Beck, R.C.; Rolim, C.M.; Rocha, M.I.; Da Veiga, M.L., et al. Hydrogels Containing Nanocapsules and Nanoemulsions of Tea Tree Oil Provide Antiedematogenic Effect and Improved Skin Wound Healing. *J Nanosci Nanotechnol* **2015**, *15(1)*, 800-809, doi:10.1166/jnn.2015.9176.

165. De Marchi, J.G.B.; Jornada, D.S.; Silva, F.K.; Freitas, A.L.; Fuentefria, A.M.; Pohlmann, A.R.; Guterres, S.S. Triclosan resistance reversion by encapsulation in chitosan-coated-nanocapsule containing α-bisabolol as core: development of wound dressing. *International Journal of Nanomedicine* **2017**, *12*, 7855—7868, doi:10.2147/IJN.S143324.

166. Szostak, K.; Czogalla, A.; Przybyło, M.; Langner, M. New lipid formulation of octenidine dihydrochloride. *Journal of Liposome Research* **2018**, *28*, 106-111, doi:10.1080/08982104.2016.1275678.

167. Mahler, V. Umweltassoziierte dermatologische Erkrankungen. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* **2017**, *60*, 605-617, doi:10.1007/s00103-017-2543-8.

168. Poulakou, G.; Giannitsioti, E.; Tsiodras, S. What is new in the management of skin and soft tissue infections in 2016? *Curr Opin Infect Dis* **2017**, *30*, 158-171, doi:10.1097/QCO.0000000000000360.

169. Judd, A.M.; Scurr, D.J.; Heylings, J.R.; Wan, K.-W.; Moss, G.P. Distribution and Visualisation of Chlorhexidine Within the Skin Using ToF-SIMS: A Potential Platform for the Design of More Efficacious Skin Antiseptic Formulations. *Pharmaceutical Research* **2013**, *30*, 1896-1905, doi:10.1007/s11095-013-1032-5.

170. Zsikó, S.; Csányi; Kovács, A.; Budai, S.; Gácsi, A.; Berkó, S. Methods to Evaluate Skin Penetration In Vitro. *Scientia Pharmaceutica* **2019**, *87*, 19, doi:10.3390/scipharm87030019.

171. Münch, S.; Wohlrab, J.; Neubert, R.H.H. Dermal and transdermal delivery of pharmaceutically relevant macromolecules. *European Journal of Pharmaceutics and Biopharmaceutics* **2017**, *119*, 235-242, doi:10.1016/j.ejpb.2017.06.019.

172. Lane, M.E. Skin penetration enhancers. *International Journal of Pharmaceutics* **2013**, *447*, 12-21, doi:10.1016/j.ijpharm.2013.02.040.
173. Nikolic, I.; Mitsou, E.; Pantelic, I.; Randjelovic, D.; Markovic, B.; Papadimitriou, V.; Xenakis, A.; Lunter, D.J.; Zugic, A.; Savic, S. Microstructure and biopharmaceutical performances of curcumin-loaded low-energy nanoemulsions containing eucalyptol and pinene: Terpenes' role overcome penetration enhancement effect? *Eur J Pharm Sci* **2020**, *142*, 105135, doi:10.1016/j.ejps.2019.105135.
174. Abd, E.; Benson, H.A.E.; Roberts, M.S.; Grice, J.E. Minoxidil Skin Delivery from Nanoemulsion Formulations Containing Eucalyptol or Oleic Acid: Enhanced Diffusivity and Follicular Targeting. *Pharmaceutics* **2018**, *10*, doi:10.3390/pharmaceutics10010019.
175. Salome Amarachi, C.; Attama, A.; Kenechukwu, F. Nanoemulsions — Advances in Formulation, Characterization and Applications in Drug Delivery. **2014**, 10.5772/58673, 76-126, doi:10.5772/58673.
176. Leong, T.S.H.; Wooster, T.J.; Kentish, S.E.; Ashokkumar, M. Minimising oil droplet size using ultrasonic emulsification. *Ultrasonics Sonochemistry* **2009**, *16*, 721-727, doi:10.1016/j.ultsonch.2009.02.008.
177. Jaiswal, M.; Dudhe, R.; Sharma, P.K. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech* **2015**, *5*, 123-127, doi:10.1007/s13205-014-0214-0.
178. Abd, E.; Namjoshi, S.; Mohammed, Y.H.; Roberts, M.S.; Grice, J.E. Synergistic Skin Penetration Enhancer and Nanoemulsion Formulations Promote the Human Epidermal Permeation of Caffeine and Naproxen. *J Pharm Sci* **2016**, *105*, 212-220, doi:10.1002/jps.24699.
179. Abd, E.; Benson, H.A.E.; Roberts, M.S.; Grice, J.E. Follicular Penetration of Caffeine from Topically Applied Nanoemulsion Formulations Containing Penetration Enhancers: In vitro Human Skin Studies. *Skin Pharmacol Physiol* **2018**, *31*, 252-260, doi:10.1159/000489857.
180. Tyagi, A.K.; Malik, A. Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms. *Food Chemistry* **2011**, *126*, 228-235, doi:10.1016/j.foodchem.2010.11.002.
181. de Godoi, S.N.; Quatrin, P.M.; Sagrillo, M.R.; Nascimento, K.; Wagner, R.; Klein, B.; Santos, R.C.V.; Ourique, A.F. Evaluation of Stability and In Vitro Security of Nanoemulsions Containing Eucalyptus globulus Oil. *Biomed Res Int* **2017**, *2017*,

2723418, doi:10.1155/2017/2723418.

182. Yukuyama, M.N.; Kato, E.T.M.; de Araujo, G.L.B.; Löbenberg, R.; Monteiro, L.M.; Lourenço, F.R.; Bou-Chacra, N.A. Olive oil nanoemulsion preparation using high-pressure homogenization and d-phase emulsification – A design space approach. *Journal of Drug Delivery Science and Technology* **2019**, *49*, 622-631, doi:10.1016/j.jddst.2018.12.029.

183. Mohamadi Saani, S.; Abdolalizadeh, J.; Zeinali Heris, S. Ultrasonic/sonochemical synthesis and evaluation of nanostructured oil in water emulsions for topical delivery of protein drugs. *Ultrason Sonochem* **2019**, *55*, 86-95, doi:10.1016/j.ultsonch.2019.03.018.

184. Choi, S.-G.; Won, S.-R.; Rhee, H.-I. Chapter 153 - Oleic Acid and Inhibition of Glucosyltransferase. In *Olives and Olive Oil in Health and Disease Prevention*, Preedy, V.R., Watson, R.R., Eds. Academic Press: San Diego, 2010; 10.1016/B978-0-12-374420-3.00153-4pp. 1375-1383.

185. Golden, G.M.; McKie, J.E.; Potts, R.O. Role of stratum corneum lipid fluidity in transdermal drug flux. *J Pharm Sci* **1987**, *76*, 25-28, doi:10.1002/jps.2600760108.

186. El Maghraby, G.M. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: effects of cosurfactants. *Int J Pharm* **2008**, *355*, 285-292, doi:10.1016/j.ijpharm.2007.12.022.

187. Brodin, B.; Bente, S.; Carsten, U.N. Passive diffusion of drug substances: the concepts of flux and permeability. In *Molecular biopharmaceutics: Aspects of drug characterisation, drug delivery and dosage form evaluation*, PharmaPress Ltd.: 2010; pp. 135-151.

188. Seo, J.E.; Kim, S.; Kim, B.H. In vitro skin absorption tests of three types of parabens using a Franz diffusion cell. *J Expo Sci Environ Epidemiol* **2017**, *27*, 320-325, doi:10.1038/jes.2016.33.

189. McClements, D. Edible nanoemulsions: Fabrication, properties, and functional performance. *Soft Matter* **2011**, *7*, 2297-2316, doi:10.1039/C0SM00549E.

190. Mason, T.G.; Wilking, J.N.; Meleson, K.; Chang, C.B.; Graves, S.M. Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter* **2006**, *18*, R635-R666, doi:10.1088/0953-8984/18/41/r01.

191. Sugumar, S.; Ghosh, V.; Nirmala, M.J.; Mukherjee, A.; Chandrasekaran, N. Ultrasonic emulsification of eucalyptus oil nanoemulsion: antibacterial activity against Staphylococcus aureus and wound healing activity in Wistar rats. *Ultrason Sonochem*

2014, *21*, 1044-1049, doi:10.1016/j.ultsonch.2013.10.021.

192. Aswathanarayan, J.B.; Vittal, R.R. Nanoemulsions and Their Potential Applications in Food Industry. **2019**, *3*, doi:10.3389/fsufs.2019.00095.
193. Takamura, A.; Minowa, T.; Noro, S.; Kubo, T. Effects of tween and span group emulsifiers on the stability of o/w emulsions. *Chemical & Pharmaceutical Bulletin* **1979**, *27*, 2921-2926, doi:10.1248/cpb.27.2921.
194. da Rocha Neto, A.C.; de Oliveira da Rocha, A.B.; Maraschin, M.; Di Piero, R.M.; Almenar, E. Factors affecting the entrapment efficiency of β-cyclodextrins and their effects on the formation of inclusion complexes containing essential oils. *Food Hydrocolloids* **2018**, *77*, 509-523, doi:10.1016/j.foodhyd.2017.10.029.
195. Schmid-Wendtner, M.H.; Korting, H.C. The pH of the Skin Surface and Its Impact on the Barrier Function. *Skin Pharmacology and Physiology* **2006**, *19*, 296-302, doi:10.1159/000094670.
196. Biju, S.S.; Ahuja, A.; Khar, R.K.; Chaudhry, R. Formulation and evaluation of an effective pH balanced topical antimicrobial product containing tea tree oil. *Die Pharmazie - An International Journal of Pharmaceutical Sciences* **2005**, *60*, 208-211.
197. Quatrin, P.M.; Verdi, C.M.; de Souza, M.E.; de Godoi, S.N.; Klein, B.; Gundel, A.; Wagner, R.; de Almeida Vaucher, R.; Ourique, A.F.; Santos, R.C.V. Antimicrobial and antibiofilm activities of nanoemulsions containing Eucalyptus globulus oil against Pseudomonas aeruginosa and Candida spp. *Microbial Pathogenesis* **2017**, *112*, 230-242, doi:10.1016/j.micpath.2017.09.062.
198. Danaei, M.; Dehghankhold, M.; Ataei, S.; Hasanzadeh Davarani, F.; Javanmard, R.; Dokhani, A.; Khorasani, S.; Mozafari, R.M. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics* **2018**, *10*, doi:10.3390/pharmaceutics10020057.
199. Polychniatou, V.; Tzia, C. Study of Formulation and Stability of Co-surfactant Free Water-in-Olive Oil Nano- and Submicron Emulsions with Food Grade Non-ionic Surfactants. *Journal of the American Oil Chemists' Society* **2013**, *91*, 79-88, doi:10.1007/s11746-013-2356-3.
200. de Oca-Ávalos, J.M.M.; Candal, R.J.; Herrera, M.L. Nanoemulsions: stability and physical properties. *Current Opinion in Food Science* **2017**, *16*, 1-6, doi:10.1016/j.cofs.2017.06.003.
201. Kumar, N.; Mandal, A. Surfactant Stabilized Oil-in-Water Nanoemulsion: Stability, Interfacial Tension, and Rheology Study for Enhanced Oil Recovery Application.

Energy & Fuels **2018**, *32*, 6452-6466, doi:10.1021/acs.energyfuels.8b00043.

202. Moghaddasi, F.; Housaindokht, M.R.; Darroudi, M.; Bozorgmehr, M.R.; Sadeghi, A. Synthesis of nano curcumin using black pepper oil by O/W Nanoemulsion Technique and investigation of their biological activities. *LWT* **2018**, *92*, 92-100, doi:10.1016/j.lwt.2018.02.023.
203. Mehmood, T.; Ahmad, A.; Ahmed, A.; Ahmed, Z. Optimization of olive oil based O/W nanoemulsions prepared through ultrasonic homogenization: A response surface methodology approach. *Food Chem* **2017**, *229*, 790-796, doi:10.1016/j.foodchem.2017.03.023.
204. Munawiroh, S.Z.; Nabila, A.N.; Chabib, L. Development of Water in Olive Oil (W/O) Nanoemulsions as Lipstick Base Formulation. *International Journal of Pharma Medicine and Biological Sciences* **2017**, *6*, 37-42, doi:10.18178/ijpmbs.6.2.37-42.
205. Tadros, T.; Izquierdo, P.; Esquena, J.; Solans, C. Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science* **2004**, *108-109*, 303-318, doi:10.1016/j.cis.2003.10.023.
206. Shinoda, K.; Saito, H. The Stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: The emulsification by PIT-method. *Journal of Colloid and Interface Science* **1969**, *30*, 258-263, doi:10.1016/S0021-9797(69)80012-3.
207. Abdelmonem, R.; Younis, M.K.; Hassan, D.H.; El-Sayed Ahmed, M.A.E.; Hassanein, E.; El-Batouty, K.; Elfaham, A. Formulation and characterization of chlorhexidine HCl nanoemulsion as a promising antibacterial root canal irrigant: in-vitro and ex-vivo studies. *Int J Nanomedicine* **2019**, *14*, 4697-4708, doi:10.2147/IJN.S204550.
208. Yilmaz, E.; Borchert, H.-H. Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. *European Journal of Pharmaceutics and Biopharmaceutics* **2005**, *60*, 91-98, doi:10.1016/j.ejpb.2004.11.009.
209. Che Marzuki, N.H.; Wahab, R.A.; Abdul Hamid, M. An overview of nanoemulsion: concepts of development and cosmeceutical applications. *Biotechnology & Biotechnological Equipment* **2019**, *33*, 779-797, doi:10.1080/13102818.2019.1620124.
210. Maruno, M.; Rocha-Filho, P.A.d. O/W Nanoemulsion After 15 Years of Preparation: A Suitable Vehicle for Pharmaceutical and Cosmetic Applications. *Journal of Dispersion Science and Technology* **2009**, *31*, 17-22, doi:10.1080/01932690903123775.
211. Honary, S.; Zahir, F. Effect of Zeta Potential on the Properties of Nano-Drug Delivery

Systems - A Review (Part 2). *Tropical Journal of Pharmaceutical Research* **2013**, *12*, doi:10.4314/tjpr.v12i2.20.

212. Ng, S.-F.; Rouse, J.J.; Sanderson, F.D.; Meidan, V.; Eccleston, G.M. Validation of a Static Franz Diffusion Cell System for In Vitro Permeation Studies. *AAPS PharmSciTech* **2010**, *11*, 1432-1441, doi:10.1208/s12249-010-9522-9.
213. Abd, E.; Yousef, S.A.; Pastore, M.N.; Telaprolu, K.; Mohammed, Y.H.; Namjoshi, S.; Grice, J.E.; Roberts, M.S. Skin models for the testing of transdermal drugs. *Clin Pharmacol* **2016**, *8*, 163-176, doi:10.2147/CPAA.S64788.
214. Salamanca, C.H.; Barrera-Ocampo, A.; Lasso, J.C.; Camacho, N.; Yarce, C.J. Franz Diffusion Cell Approach for Pre-Formulation Characterisation of Ketoprofen Semi-Solid Dosage Forms. *Pharmaceutics* **2018**, *10*, doi:10.3390/pharmaceutics10030148.
215. Baynes, R.E. Porcine skin flow-through diffusion cell system. *Curr Protoc Toxicol* **2001**, *Chapter 5*, Unit5 5, doi:10.1002/0471140856.tx0505s09.
216. Neupane, R.; Boddu, S.H.S.; Renukuntla, J.; Babu, R.J.; Tiwari, A.K. Alternatives to Biological Skin in Permeation Studies: Current Trends and Possibilities. *Pharmaceutics* **2020**, *12*, doi:10.3390/pharmaceutics12020152.
217. Debeer, S.; Le Luduec, J.B.; Kaiserlian, D.; Laurent, P.; Nicolas, J.F.; Dubois, B.; Kanitakis, J. Comparative histology and immunohistochemistry of porcine versus human skin. *Eur J Dermatol* **2013**, *23*, 456-466, doi:10.1684/ejd.2013.2060.
218. Liu, C.; Hu, J.; Sui, H.; Zhao, Q.; Zhang, X.; Wang, W. Enhanced skin permeation of glabridin using eutectic mixture-based nanoemulsion. *Drug Deliv Transl Res* **2017**, *7*, 325-332, doi:10.1007/s13346-017-0359-6.
219. Kogan, A.; Garti, N. Microemulsions as transdermal drug delivery vehicles. *Adv Colloid Interface Sci* **2006**, *123-126*, 369-385, doi:10.1016/j.cis.2006.05.014.
220. Shaker, D.S.; Ishak, R.A.H.; Ghoneim, A.; Elhuoni, M.A. Nanoemulsion: A Review on Mechanisms for the Transdermal Delivery of Hydrophobic and Hydrophilic Drugs. *Scientia Pharmaceutica* **2019**, *87*, 17, doi:10.3390/scipharm87030017.
221. Wais, M.; Samad, A.; Nazish, I.; Khale, A.; Aqil, M.; Khan, M. Formulation development Ex-vivo and in-vivo evaluation of nanoemulsion for transdermal delivery of glibenclamide. *International Journal of Pharmacy and Pharmaceutical Sciences* **2013**, *5*, 747-754.
222. Koyama, Y.; Bando, H.; Yamashita, F.; Takakura, Y.; Sezaki, H.; Hashida, M. Comparative analysis of percutaneous absorption enhancement by d-limonene and oleic acid based on a skin diffusion model. *Pharm Res* **1994**, *11*, 377-383,

doi:10.1023/a:1018904802566.

223. Zhang, L.W.; Al-Suwayeh, S.A.; Hung, C.F.; Chen, C.C.; Fang, J.Y. Oil components modulate the skin delivery of 5-aminolevulinic acid and its ester prodrug from oil-in-water and water-in-oil nanoemulsions. *Int J Nanomedicine* **2011**, *6*, 693-704, doi:10.2147/IJN.S17524.

224. Yu, M.; Ma, H.; Lei, M.; Li, N.; Tan, F. In vitro/in vivo characterization of nanoemulsion formulation of metronidazole with improved skin targeting and anti-rosacea properties. *Eur J Pharm Biopharm* **2014**, *88*, 92-103, doi:10.1016/j.ejpb.2014.03.019.

225. Lucca, L.G.; de Matos, S.P.; Borille, B.T.; de, O.D.D.; Teixeira, H.F.; Veiga, V.F., Jr.; Limberger, R.P.; Koester, L.S. Determination of beta-caryophyllene skin permeation/retention from crude copaiba oil (Copaifera multijuga Hayne) and respective oil-based nanoemulsion using a novel HS-GC/MS method. *J Pharm Biomed Anal* **2015**, *104*, 144-148, doi:10.1016/j.jpba.2014.11.013.

226. Sharma, B.; Iqbal, B.; Kumar, S.; Ali, J.; Baboota, S. Resveratrol-loaded nanoemulsion gel system to ameliorate UV-induced oxidative skin damage: from in vitro to in vivo investigation of antioxidant activity enhancement. *Arch Dermatol Res* **2019**, *311*, 773-793, doi:10.1007/s00403-019-01964-3.

227. Suchomel, M.; Weinlich, M.; Kundi, M. Influence of glycerol and an alternative humectant on the immediate and 3-hours bactericidal efficacies of two isopropanolbased antiseptics in laboratory experiments in vivo according to EN 12791 *Antimicrob Resist Infect Control* **2017**, *6*, 72, doi:10.1186/s13756-017-0229-5.

Appendix 1 Summarised characteristics of traditional skin antiseptic formulations.

Drug	Concentration	Formulation type	Combination	Carrier polymer	Manufacturing technique	Study characteristics	Reference
Chlorhexidine gluconate		Dermal polymeric patch		Eudragit RL100		To characterize properties of developed patches regarding their drug release and antimicrobial activity.	[73]
Chlorhexidine gluconate	2% CHG in 70% IPA	Solution	Acrylate copolymer			To test the effectiveness of a film-forming acrylate copolymer to a topical CHG-based preparation on minimizing CHG loss, compared to a CHG solution preparation available on the market.	[113]
Chlorhexidine gluconate	2% CHG in 70% IPA	Solution				To contrast the residual effects of 2% CHG in 70% IPA v/v and 1 % triclosan in 70 % IPA v/v on skin bacterial communities	[70]
Chlorhexidine gluconate	2% CHG in 70% IPA	Solution				To compare the antiseptic activity between 10% sodium hypochlorite and 2% CHG in 70% IPA	[108]
Chlorhexidine gluconate	2% CHG in 70% ethanol	Solution				To appraise the desiccation and ethanol	[109]

						resistances of MDAR-Bs. To compare the antiseptic activities of a combination of CHG and 70% ethanol with the 70% ethanol disinfectants used for MDRAB-Bs.	
Chlorhexidine base		Mucoadhesive polymer patches		Psyllium and three types of semi-synthetic HPMC	A casting-solvent evaporation technique	To test the effect of polysaccharide psyllium in the mucoadhesive patches for controlling the chlorhexidine release	[116]
Triclosan		Methoxy amidated pectin- based mucoadhesive buccal patch.		β-cyclodextrin		To develop buccal patches and determine drug release, antimicrobial and *in vitro* absorption of patches	[115]
Triclosan	0.3%	Soap				To study the *in vitro* and *in vivo* antibacterial activity of triclosan in soap.	[137]
Triclosan	0.3%	Shampoo				To assess the antimicrobial efficacy of the shampoo against bacteria and fungi	[139]
Povidone-iodine	10%	Ointment				To compare the *in vitro* antibiofilm effect of diluted PVP-I ointment with other six tested	[130]

						products against *P. aeruginosa* and multi-species biofilms of *C. albicans* and MRSA.	
Povidone-iodine	4% PVP-I skin cleanser, 7.5% PVP-I surgical scrub, 10% PVP-I solution and 3.2% PVP-I/alcohol solution.	Hand wash and hand rub				To study t *in vitro* potency of four different hand hygiene formulations of povidone iodine against EBOV	[133]
Povidone-Iodine		Alginate hydrogels	Vancomycin	Vancomycin loaded chitosan nanoparticles (CNPs) by ionic gelation method	Modified ionic gelation method	To assess *in vitro* the release ability of vancomycin and PVP-I from the hydrogel To assess the bactericidal and antibiofilm efficacy of hydrogels	[125]
Povidone-Iodine	1% and 2%	Solution				To analyse the effectiveness and safety of 1% or 2% PVP-I topical solution in patients with cancer therapy-associated paronychia during 6-8 weeks.	[114]
Thiolated PVP and Thiolated		Solution	2-(2-acryloyl – Ethyl disulfanyl)-			To test *in vitro* mucoadhesive properties and the	[124]

PVP-iodine complex			nicotinic acid (ACENA)			release of iodine from thiolated PVP-Iodine complexes	
Isopropanol	75% (w/w)	Hand rub	Glycerol 0.725% (w/w)			To investigate the role of glycerol in pre-surgical hand rub products, based on EN 12791, especially after 3 hours of application.	[227]
Isopropyl alcohol	70% (v/v)	Solution				To study the potency of isopropyl alcohol and chlorhexidine in the prevention of blood cultures impurities.	[111]
Ethanol	96%	Solution	Isopropanol-30g and ortophenilphenol-0.1g			To determine the effect of the combination of 96% ethanol, 30g isopropanol, 0.1g ortophenilphenol and PVP-I in minimizing surgical-site infections, compared to that of single use PVP-I.	[112]
Silver Chloride		Colloidal solution				To study the suspension potency on the autotrophic and heterotrophic growth	[154]
Benzethonium chloride (BZT)	0.2%	Lotion				To test the antimicrobial efficacy of an ethanol- based antiseptic and water-based antiseptic	[126]

						products containing 0.2% BZT	
Tea tree oil	3%	Soap				To assess the potency of 0.3% *Melaleuca alternifolia* essential oil versus 0.5% triclosan hand soap formulations	[140]
Tea tree oil		Tea tree 10% cream, tea tree 5% body wash				To compare the efficacy of the combination of tea tree 10% cream and tea tree 5% body wash with the standard theory in eliminating MRSA.	[131]
Triclosan	0.1%–0.45% w/v	Soap				To evaluate the efficacy of soaps with and without triclosan and investigate potential hazards in the emergence of antibiotic resistance	[135]
Tea tree oil		4% tea tree oil nasal ointment and 5% tea tree oil body wash				To compare the ability to eradicate MRSA between the combination of a 4% tea tree oil nasal ointment and 5% tea tree oil body wash with a standard theory of 2% mupirocin nasal ointment and triclosan body wash	

Appendix 2. Summarised characteristics of advanced skin antiseptic formulations.

Drug	Concentration	Formulation type	Combination	Carrier polymer	Manufacturing technique	Study characteristics	Reference
Chlorhexidine gluconate	0.2%	Nanogel containing magnetic Cobalt iron oxide nanoparticles		Chitosan and gelatin	Solution casting method	To investigate the release and pH-dependent response of chlorhexidine gluconate from a magnetic nanogel.	[153]
Chlorhexidine base		Poly(epsilon-caprolactone) nanocapsules		Poly(epsilon-caprolactone)	Solvent displacement method	To evaluate the antibacterial ability of poly(epsilon-caprolactone) nanocapsules containing chlorhexidine base and the absorption of active into the SC	[162]
Chlorhexidine base		α-, β-, and γ-cyclodextrin methacrylate (CD-MA) containing poly (methyl methacrylate) (PMMA) based nanogels			The radical precipitation polymerization technique.	To study the capacity of chlorhexidine base in PMMA nanogels. To assess the bactericidal against *Staphylococcus aureus* of CD-PA nanogels	[152]
Chlorhexidine digluconate		Nanoemulsions	Eucalyptus oil (EO) or Olive oil (OO)		HSH followed by probe ultrasonication	To investigate the drug release, skin permeation and retention of CHG from nanoemulsions.	[148]

						To valuate impact of methacrylate powder dressing in controlling the CHG release.	
Triclosan		Chitosan-coated nanocapsule		Poly(epsilon-caprolactone) (PCL)	Interfacial deposition of preformed polymers	To characterize properties of nanocapsule comprised of α-bisabolol and triclosan. To study the antimicrobial activity against tested pathogens To testify the compatibility as incorporating nanocapsule into wound dressings	[165]
Triclosan	10%, 30% and 50%	PLLA/triclosan nanoparticles		Poly-L-lactide (PLLA)	Emulsification – diffusion technique	To evaluate the release of triclosan from the PLLA nanoparticles and its antimicrobial activities	[160]
Triclosan	0.5 % w/w	Nanoparticles stabilized by branched diblock copolymers		Branched diblock copolymers: PEG-*b*-PNIPAM (BDP 1); PEG-*b*-PBMA (BDP 2); PEG-*b*-PSty (BDP 3)	Emulsion-freeze-drying technique	To assess fungicidal ability against *C. albicans* of triclosan nanoparticles	[158]

Triclosan		Nanoparticles		Eudragit E 100	Emulsification–diffusion by solvent displacement method	To compare *in vitro* percutaneous permeation of nanoparticles containing triclosan, with two commercial formulations used for treating acne, including a solution and an o/w emulsion	[157]
Triclosan		Solid lipid nanoparticles (SLNs)		Glyceryl behenate (GB) and Glyceryl palmitostearate (GP)	Hot HSH followed by probe ultrasonication	To investigate the impact of SLNs in delivery TCS to deeper skin layers and hair follicles and compare the permeation ability of GB-SLNs and GP-SLNs.	[148]
Triclosan		Nanoemulsions	Eucalyptus oil (EO) or Olive oil (OO)		HSH followed by probe ultrasonication method	To develop and characterise stable nanoemulsion formulations. To evaluate the ability of NEs in improving skin retention of TCS	[148]
Tea tree essential oil (TTO)	10.0 mg mL-1	Nanoemulsions (TTO-NE) and polymeric nanocapsules (TTO-NC)		Poly(e-caprolactone)	TTO-NE: spontaneous emulsification and TTO-NC: interfacial deposition of the	To investigate the *in vitro* fungicidal potency against *Trichophyton rubrum* of TTO-NE and TTO-NC systems.	[163]

					preformed polymer methods		
Tea tree essential oil (TTO)		Hydrogels containing Nanoemulsions and nanocapsules		Poly(e-caprolactone)	Nanoemulsion: spontaneous emulsification Nanocapsules: interfacial deposition of preformed polymer	To evaluate physicochemical properties of hydrogels and their efficacy in wound healing and protecting skin from UV-B rays	[164]
Tea tree oil (TTO)		Emulgel (EG) containing TTO-loaded nanoemulsion (NE)			Nanoemulsion: High energy emulsification	To evaluate the physicochemical properties, the *ex vivo* penetration, antimicrobial potency and safety of topical emulgel.	[149]
Tea tree oil (TTO)		Nanoemulsions (NE)	Silver nanoparticles (Ag-NPs)			To investigate cytotoxicity as well as antimicrobial ability of the prepared nanoemulsions against clindamycin-resistant *Escherichia coli* and *S. aureus* To appraise the synergistic effect of TTO NE and Ag NPs against tested microorganisms.	[150]

Silver		Silver nanoparticle (Ag NPs)		Polyvinyl alcohol (PVA)		To estimate the suspension efficacy on the autotrophic and heterotrophic growth To investigate silver species properties	[154]
Benzalkonium chloride (BZK)	0.6% BZK for *in vitro* studies and 0.2% BZK for *in vivo* studies.	Nanoemulsion		EDTA	High-energy homogenization using high shear conditions	To evaluate the *in vitro* and *in vivo* antimicrobial effect against isolated bacterial species.	[146]
Cetylpyridinium chloride (CPC)		Oil in water nanoemulsions				To assess the fungicidal potency	[145]
Polyhexanide (PHMB)	0.05%	nanoparticle-emulsion		Lipofundin® MCT 20%		To contrast the effectiveness of a particle- and non-particle antiseptic formula	[156]
Poly-hexamethylene biguanide hydrochloride (PHMB) **And** **cetylpyridinium chloride (CPC)**	0.2 and 2.0% (w/w) of PHMB 0.05 and 2.5% (w/w) of CPC	Liquid crystalline systems (LCS)		glyceryl monooleate (GMO)		To investigate the release of PHMB from liquid crystalline systems, and its antimicrobial activity as incorporated into these systems	[134]
Octenidine dihydrochloride	0.1%	Phosphatidylcholine formulation	Soybean phosphatidylcholine (Phospholipon 90G)			To assess the antimicrobial potency of octenidine formulations	[166]