Formation of Ciprofloxacin Loaded Transethosomes to Check the Antibacterial Activity Against Skin Infections

Author

Huraira Tariq

Regn Number

364414

Supervisor

Dr. Nosheen Fatima Rana

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY

ISLAMABAD

JANUARY, 2024

Formation of Ciprofloxacin Loaded Transethosomes to Check the Antibacterial Activity Against Skin Infections

Author

Huraira Tariq

Regn Number

364414

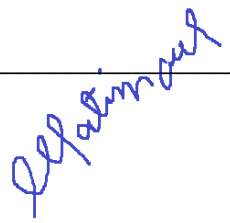
A thesis submitted in partial fulfillment of the requirements for the degree of

MS Biomedical Sciences

Thesis Supervisor:

Dr. Nosheen Fatima Rana

Thesis Supervisor's Signature: _____



DEPARTMENT BIOMEDICAL ENGINEERING & SCIENCES SCHOOL

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY,

ISLAMABAD

JANUARY, 2024

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS/MPhil thesis written by **Regn No. 00000364414 Huraira Tariq** of **School of Mechanical & Manufacturing Engineering (SMME) (SMME)** has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis titled. **Formulation of ciprofloxacin loaded trans-ethosomes to check the anti bacterial activity against skin infections**

Signature: 

Name (Supervisor): Nosheen Fatima Rana

Date: 11 - Jan - 2024

Signature (HOD): 

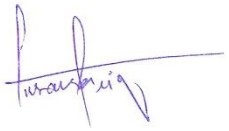
Date: 11 - Jan - 2024

Signature (DEAN): 

Date: 11 - Jan - 2024

DECLARATION

I certify that this research work titled "*Formation of Ciprofloxacin Loaded Transethosomes to Check the Antibacterial Activity Against Skin Infections"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged / referred.



Signature of Student

Huraira Tariq

2024-NUST-Ms-BMS-364414

COPYRIGHT STATEMENT

ACKNOWLEDGEMENTS

I am thankful to my Creator Allah Subhana-Watala to have guided me throughout this work at every step and for every new thought which you setup in my mind to improve it. I could have done nothing without your priceless help and guidance. Whosoever helped me throughout the course of my thesis, whether my parents or any other individual, was your will, so indeed none be worthy of praise but you.

I am profusely thankful to my beloved parents who raised me when I was not capable of walking and continued to support me throughout my life.

I would also like to express special thanks to my supervisor Dr. Nosheen Fatima for her help throughout my thesis and also for Molecular Nanotechnology and Nano and Micro Drug Delivery System courses which she has taught me. I can safely say that I haven't learned any other sciences subject in such depth as the ones which she has taught.

I would also like to pay special thanks to Ayesha Naeem for his tremendous support and cooperation. Each time I got stuck in something; she came up with the solution. Without her help I wouldn't have been able to complete my thesis. I appreciate her patience and guidance throughout the whole thesis.

I would also like to thank Dr. Aneeqa Noor, Dr. Asim Waris, and Dr. Jawad Khan for being on my thesis guidance and evaluation committee. I am also thankful to Afrah Nawaz and Iqra for their support and cooperation.

Finally, I would like to express my gratitude to all the individuals who have rendered valuable assistance to my study.

Dedicated to my exceptional parents and my brother whose tremendous support and cooperation led me to this wonderful accomplishment

ABSTRACT

One of the serious challenges in the treatment of infectious diseases is the presence of bacterial infections in subcutaneous wound tissue. *Staphylococcus epidermidis* (*S. epidermidis*) and *Propionibacterium acne* (*P. acne*) are resistant bacterial strains that cause severe disease in humans when penetrating the deeper layer of skin. Antibacterial drugs with a nonspecific target have more difficulty in penetrating the deeper layer of infected skin. Broad spectrum antibiotics are best to treat the infection but are not commonly used because bacteria's make resistance against them. To overcome these issues, a combined strategy of broad-spectrum antibacterial drug and nanoparticle was formulated for targeted delivery, enhanced penetration to the infection site specifically. The ciprofloxacin was entrapped in transethosomes and formulation was synthesized by using the cold method. Transethosomes are very small in size that can reach the deeper layer of skin to give potential effects. In the layers of skin, ciprofloxacin works by binding to the enzymes topoisomerase IV and DNA gyrase and inhibit the DNA replication in bacteria. The antibacterial activity of ciprofloxacin loaded transethosomes against skin infections was assessed using *Staphylococcus epidermidis* and *Propionibacterium acne* and the method used was well diffusion method. The characterization of ciprofloxacin loaded transethosomes was done through, zeta potential, particle size evaluation, and drug release efficiency. Loaded transethosomes displayed substantially have more potential effect than ciprofloxacin alone. The in-vitro studies show that ciprofloxacin loaded transethosomes boost the antibacterial activity of ciprofloxacin against gram positive.

Keywords: Ciprofloxacin, Transethosomes, Nanoparticle, Bacteria, Targeted drug delivery, Antibacterial activity

LIST OF ABBREVIATIONS

AFM	Atomic Force Spectroscopy
STEM	Scanning Tunneling Electron Microscopy
TET	Transethosomes
FTIR	Fourier Transform Infrared Spectroscopy
CIP	Ciprofloxacin
FQs	Fluoroquinolones
SEM	Scanning Electron Microscopy
MRO	Multidrug Resistance Organism
DDS	Drug Delivery System
UDVs	Ultra-Deformable Vesicle
PC	Phosphatidyl choline
UV	Ultraviolet
DLS	Dynamic Light Scattering
PBS	Phosphate Buffer Saline
NP	Nanoparticle
S. epidermidis	*Staphylococcus epidermidis*
p. acne	*Propionibacterium acne*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1 Objective

The research work is divided into two parts. The first part focuses on the formulation of ciprofloxacin loaded transethosomes and its characterization. The selected ciprofloxacin drug bearing versatile nature has been used against *Staphylococcus epidermidis* and *Propionibacterium acne*. But for the first time its ciprofloxacin loaded transethosomes are synthesized according to "cold method". Distinct aspects of loaded transethosomes are characterized by using different techniques and eventually made them for better antibacterial activity.

The second part focuses on the in-vitro analysis of antibacterial activity by agar well diffusion method. Our research was primarily focused on the analysis of anti-bacterial activity of ciprofloxacin loaded transethosomes will be considered as an important step toward advancing these encapsulated nanoparticles to preclinical trials level.

1.2 Bacterial Infections

Many different strains of bacteria can cause bacterial infections, which are a large group of illnesses with broad implications for human health. Single-celled germs called bacteria can live in a various environment, including the human body (Juhas, 2023). Many bacteria are necessary for healthy biological processes, but some species can also cause infections, which can result in a wide range of disorders. Almost every area of the body is susceptible to these diseases, from the skin and respiratory systems to the more intricate systems like the gastrointestinal and urinary tracts. Pathogenic bacteria can penetrate host tissues and interfere with normal physiological processes, which can result in a variety of disorders. While some bacteria cause direct injury to the host by competing with it for nutrition or inducing inflammatory reactions, others create toxins that can destroy cells and tissues. Food poisoning, pneumonia, strep throat, and urinary tract infections are a few examples of bacterial diseases. Due to the diversity of bacterial species, they can impact a variety of organ systems and cause a wide range of diseases that varied in severity and symptoms (Hou et al., 2022). Bacterial infections can range in intensity from minor,

self-limiting illnesses to serious, life-threatening diseases. The mechanisms by which bacteria penetrate, multiply, and cause host reactions is very important to understand for creating efficient preventive measures and treatments. Microbiology, immunology, and clinical medicine are all included in the wide range of research that is conducted in the field of bacterial infections as researchers work to understand the intricate processes involved in bacterial pathogenesis and develop novel strategies for treating these infections. In order to reduce the burden of bacterial illnesses on global public health, ongoing research efforts concentrate not only on treatment modalities but also on preventative measures, such as immunization and enhanced hygiene habits. This is because antibiotic resistance is becoming an increasingly important concern (Saeed et al., 2023).

1.2.1 Skin Infections

The body's largest and most adaptable barrier of defense is the skin, an essential organ. This amazing organ is essential for protecting the body from outside threats like infections, dangerous substances, and physical trauma. The skin, which is made up of several layers, including the dermis, epidermis, and subcutaneous tissue, also controls body temperature by sweating and dilation and constriction of blood vessels. Furthermore, the skin is essential to sensory perception because it contains a network of nerves that allow the sense of touch, pressure, and temperature, among other stimuli. Sunlight exposure enhances the skin's ability to synthesize vitamin D, underscoring the skin's critical function in preserving general health. In addition to its protective and regulating roles, the skin is a person's medium for self-expression and a window into their general health.

Bacterial skin infections provide a wide range of common and unique health challenges. The largest organ in the body, the skin acts as a vital defense against harmful microorganisms and other environmental aggressors (Rose et al., 2023). But some bacterial species have developed defense against this barrier, which can result in a variety of skin diseases. Among the most common species which are present in skin microbiota are *Staphylococcus epidermidis* and *Propionibacterium acne*, which can lead to illnesses including atopic dermatitis, and acne (Harris-Tryon & Grice, 2022). People with diabetes, weakened immune systems, or pre-existing skin disorders are more susceptible to skin infections (Dryden et al., 2015). Cuts, abrasions, and surgical wounds are examples of factors that can increase vulnerability by giving bacteria

entrance opportunities. Furthermore, because of its resistance to standard medicines, community-of skin microbiota like *Staphylococcus epidermidis* and *Propionibacterium acne* has become a noteworthy concern and complicates therapy (Ruchiatan et al., 2023). For bacterial skin infections to be appropriately managed, which usually involves antibiotic medication, an accurate and timely diagnosis is essential. But the increasing concern about antibiotic resistance calls for cautious consumption of these drugs and emphasizes the value of preventive measures including public health campaigns, good wound care, and hygiene habits. The goal of ongoing research is to improve patient outcomes and stop the emergence of antibiotic resistance in the field of dermatological health by investigating novel therapeutic pathways and creative approaches to meet the difficulties presented by bacterial skin infections.

1.2.2 Mechanism of Bacterial Skin Infection

Numerous complex systems that take advantage of weaknesses in the host's defense mechanisms allow germs to invade the skin. First, germs can come into direct touch with the skin or can enter through hair follicles, wounds, or abrasions. Certain bacteria have unique features, like pili or fimbriae that help them stick to the surface of the skin. The essential phase of adherence is what enables the bacteria to withstand removal by force and sidestep the host's immune system. After adhering, some bacterial species can break down the extracellular matrix of the skin and subcutaneous tissues by producing enzymes like collagenases or hyaluronidases. Bacteria can enter deeper levels with the help of this breakdown of barriers.

Additionally, bacteria frequently release toxins into the environment, such as endotoxins or exotoxins, which can harm host tissues and cells directly, causing inflammation and aiding in the spread of infection. The presence of these foreign invaders sets off the host's immunological response, which attracts immune cells to the infection site. Some bacteria have developed defenses against the immune system, which lets them survive and spread throughout the host. The course of the infection is ultimately determined by the intricate interactions between the host's defense mechanisms and the virulence factors of the bacteria. To effectively prevent and treat bacterial skin infections, it is imperative to comprehend these mechanisms (Chiller et al., 2001).

1.2.3 Skin Infection by Skin Microbiota

The complex population of microorganisms known as the skin microbiota resides on the skin and is essential to preserving skin health and preventing the colonization of diseases. On the other hand, opportunistic pathogens found in the skin microbiota can take advantage of weaknesses and cause infections. The process by which these microbes cause skin infections entails a fine balance between the host's defenses and the opportunistic pathogens' capacity to get past them. Pathogenic microbes may be able to flourish in the presence of certain conditions, such as alterations in the local microenvironment, immune system deficiencies, or abnormalities in the skin barrier. Adhesins and enzymes are examples of virulent factors that some skin bacteria may have. These traits allow the germs to cling to the skin's surface, elude the host's immune system, and penetrate deeper tissue layers. Dysregulation of the skin's microbial balance can also result from changes in the makeup of the skin microbiota, which can be caused by environmental variables or underlying medical diseases. This can create an environment that is favorable for infection. Deciphering the intricate relationship between the skin microbiota and the host's defenses is crucial to create focused preventative and treatment strategies and comprehend the intricacies of skin infection (Christensen & Brüggemann, 2014).

1.3 Nanotechnology; Introduction to New Era

The term "nanotechnology" refers to the science and technology of controlling and manipulating matter and devices on a scale of 1-100 nanometers. This field includes applied physics, chemistry, materials science, biology, and engineering (Mohan Bhagyaraj et al., 2018). At the level of nanoscale, the characteristics of matter are determined and there are fewer distinctions between scientific fields. In nanotechnology, there have been two primary methods employed. These are known as the “top-down” and “bottom-up” approaches. The first approach involves materials, electronics, and nanostructures are assembled by building up from atoms into molecules. The second approach is building devices and structures out of bigger things without atomic-level control. The invention and use of extremely sensitive equipment has speeded up progress in both directions in recent years. To advance research in this exciting new field, techniques like atomic force microscopy, electron beam lithography, scanning tunneling

microscopy, and molecular beam epitaxy, etc. are now accessible. These instruments make it possible to see and work with unique nanostructures (Subramani et al., 2019).

1.3.1 Development in nanotechnology

The first concept of nanotechnological strategies was presented by physicist Richard P. Feynman at the American Physical Society and won Nobel prize in 1959 in a talk titled "There's plenty of room at the bottom." (Feynman, 2012). Two instruments, atomic force microscope (AFM) (invented by Gerd Binnig, Calvin Quate and Christoph Gerber) (Sakai, 2019) and the scanning tunnelling microscope (STM) (invented by Gerd Binnig and Heinrich Rohrer) (Binnig & Rohrer, 1986) were the first steps towards the practical application of nanotechnologies. In fact, these discoveries allowed scientists from other disciplines to get access to the "nanoworld," providing them with the means to manipulate individual atoms and investigate picture surfaces with atomic precision (Schaming & Remita, 2015). Nanotechnologies have been used in a wide range of industries over time, including energy, food, electronics, cosmetics, agriculture, and health. Although nanotechnology-based systems and technologies are used on a daily basis by individuals all over the world, their use is still limited by concerns about safety and ethics.

1.3.2 Nanotechnology as a Therapeutic Weapon

Nowadays, nanotechnology has become a revolutionary force with a wide range of applications, establishing itself as a significant participant in science and medicine. Materials have special qualities at the nanoscale that can be used for creative purposes. Nanotechnology has enormous promise to improve drug delivery, targeted therapy, and diagnostics in the medical industry. Because of their tiny size, nanoparticles can be engineered to interact with certain tissues or cells, improving the accuracy and potency of medicinal treatments. Furthermore, nanotechnology makes it easier to construct complex imaging methods, which leads to a greater comprehension of biological processes at the molecular level. Nanomaterials are used in a wide range of scientific fields, including electronics, materials science, and environmental monitoring. Because nanotechnology is interdisciplinary, it encourages collaboration across scientific fields, which opens doors to novel discoveries and innovative technological advancements (Muhammad et al., 2019; J. Shi et al., 2010). A new world has been opened by the nanosized materials for

biomedical, industrial, and scientific purposes, which offering a set of technologies that allow the modification of functional and structural properties on a microscale (Tiwari et al., 2022). Nanotechnology is closely related to biomedicine, where nanoparticles (NPs) are utilized to create nanomaterial-based antiviral agents and disinfectants that prevent the virus from replicating inside the host or render it inactive outside of it. Additionally, Nano robots are applied to identify and repair tissue defects at the cellular level (Leon et al., 2019). Nanoparticles can be used as a targeted drug delivery vehicle to transport medication to a specific portion of the body, in contrast to conventional drug formulations that usually circulate throughout all body parts. The incorporation of nanotechnology into several fields has the potential to transform the way we tackle problems in science, technology, and medicine as the subject advances.

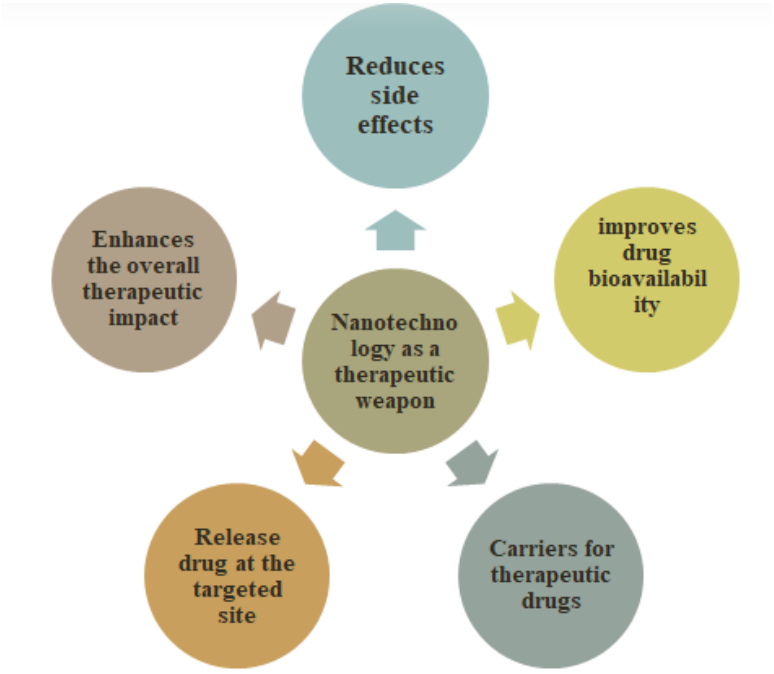


Figure 1: Nanotechnology as a therapeutic agent

1.4 Transethosomes a Type of Liposome

Liposomes are vesicular structures that have an aqueous interior environment that is enclosed by a bilayer of phospholipids that is created when phospholipids become dispersed in water.

It is form by the combination of two Greek words, “lipo” meaning fat and “soma”-meaning body. Liposomes are of five types ethosomes, transferosomes, menthosomes, invasomes and transethosomes. Ethosomes contain lipid and ethanol while Transferosomes contain lipid and

edge activator (Nayak & Tippavajhala, 2021). Menthosomes are ultra-deformable carrier contains edge activator and phospholipid, including cationic surfactants (Duangjit et al., 2014). Invasomes are vesicular drug delivery vehicle based on phospholipids that include ethanol and terpenes (Dragicevic-Curic et al., 2008). Transethosomes (TET) contain both edge activator and ethanol. Phospholipids (or nonionic surfactants) play the role of carrier for delivering drug molecules into the skin. They can easily interact with the stratum corneum, improve tissue hydration and merge with lipids of the stratum corneum. They contain a hydrophilic (polar) head as well as hydrophobic (nonpolar) tail (Bajaj et al., 2021). The edge activator in TET, increases the flexibility of vesicles by destabilizing the lipid bilayers (Ahad et al., 2018). This will enable the drug to be absorbed deeper into the skin (El Maghraby et al., 2004). Ethanol is another component in TET, interacts with the lipids in both vesicles and skin, making the layer more flexible, due to its natural capacity to provide fluidity to the lipid membranes (Moolakkadath et al., 2018). TET contained both edge activators and ethanol, thus offers the benefits of both deformable liposomes and ethosomes (C. K. Song et al., 2012). Furthermore, TET presented better deposition characteristics and penetration ability (Abdulbaqi et al., 2016).

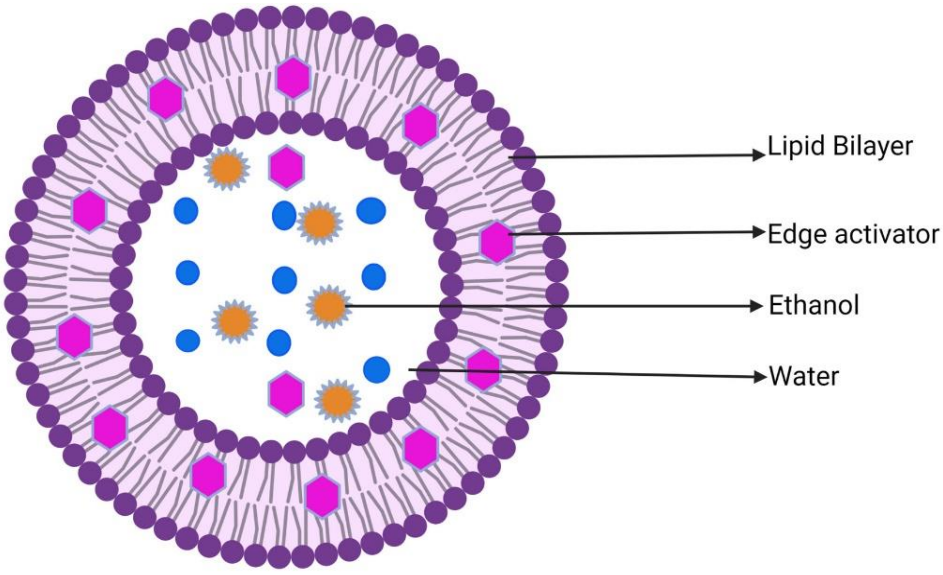


Figure 2: Structure of transethosomes

1.4.1 Transethosomes as a Carrier

Transethosomes an novel carrier system have drawn a lot of attention in the pharmaceutical and cosmetics industries due to its outstanding capacity to improve the delivery of active compounds especially drugs and chemicals used in skin care products and deliver through the skin. These specially designed vesicles provide a viable alternative for enhancing the bioavailability and skin penetration of different drugs by overcoming the drawbacks of conventional delivery methods (Nayak et al., 2020).

Transethosomes have a unique structure, primarily comprised of water, ethanol, phospholipids, and an edge activator typically a surfactant or a mixture of surfactants. The vesicles get flexibility from this edge activator, which is essential to their effectiveness as carriers. TET has better skin penetration than other delivery techniques because of their flexibility, which enables them to deform and fit through skin pores and follicles more successfully. TET are a great option for administering medications that have trouble passing through the skin barrier since the ethanol component helps to improve skin penetration even more (Yanhong Wang et al., 2021).

TET is used extensively in the pharmaceutical industry as a dependable means of drugs delivery. The drug is encapsulated by TET, which shields it and makes it easier for it to pass through the skin and reach its target site. Targeted drug administration reduces the possibility of side effects while simultaneously improving the drug's therapeutic impact. TET can also be modified to release drugs in a regulated and prolonged manner, which improves their efficacy as drug delivery vehicles (H. Song et al., 2019).

Using TET can improve the way these chemical substances are delivered, enabling them to penetrate to the skin's deeper layers, where they can release the drug. TET are now used in a variety of skin infection treatments (Ferrara et al., 2022).

TET have the important benefit of being quite versatile. It is possible to modify their composition to match the unique needs of the drugs they are delivering. Because of its flexibility, the carrier system may be precisely adjusted to ensure maximum performance and effectiveness. TET have a lot of potential as a carrier system, but it's important to remember that their efficacy might vary based on a number of variables, including the particular formulation, the characteristics of the encapsulated chemical, and the intended use (Chowdary et al., 2023). Therefore, while using TET in delivering drugs, it is essential to carry out in-depth research, testing, and assessment. This guarantees that safety and efficacy criteria are maintained while

achieving the intended results. In the fields of medicine and cosmetics, TET offer an advanced technology that creates new opportunities for the more efficient and focused administration of active ingredients.

1.5 *Propionibacterium acne* and *Staphylococcus epidermidis*

Propionibacterium acne and *Staphylococcus epidermidis*, are Gram-positive bacteria (Hung et al., n.d.), are essential constituents of skin microbiota and are widely distributed throughout the human body (Chen et al., 2021) according to the environmental conditions of skin, such as pH, range from 4.2 to 7.9 and temperature, ranges from 31.8 to 36.6 ˚C (Fournière et al., 2020). Staphylococci are common bacteria found on mucous membranes and skin of humans and other mammals in colonies. The most collected species from human epithelia is *S. epidermidis*, which mostly colonizes the head, nares (the nostrils), and axillae (armpits). Analysis of the *S. epidermidis* genome predict that the species is projected to be well-equipped with genes that offer protection against the severe environments seen in its natural habitat (Otto, 2009) and *S. epidermidis* quickly adjust to and flourish in any skin microenvironment (Brown & Horswill, 2020). *P. acnes* is an anaerobic bacteria that is primarily found in the follicles that produce sebaceous secretions (Moore et al., 2021). Predominant in sebaceous areas, *P. acnes* is essential for maintaining skin homeostasis and preventing the colonization of other dangerous infection (Dréno et al., 2018).

1.5.1 *Propionibacterium acne* and *Staphylococcus epidermidis* as a commensals

Since *S. epidermidis* and *P. acne* are benign in healthy environments and beneficial to the skin when it is untouched, they are typically regarded as commensal bacteria (Rahmdel & Götz, 2021). *P. acne* and *S. epidermidis* are skin hosts that are involved in host defense, innate immunity, and skin homeostasis. In healthy skin conditions, both bacteria helps the skin in combating harmful bacteria through a variety of mechanism and can directly inhibit the growth of organisms (Claudel et al., 2019). Both types of these commensal bacteria occupy the same biological niche and compete for nutrition; they are able to fight against pathogen invasion (Zhu et al., 2023).

1.5.2 *Propionibacterium acne* and *Staphylococcus epidermidis* Shift from Commensals to Pathogenicity

It is important to remember that "it's not all black and white" when discussing skin microbiota. A shift between commensalism and pathogenicity observed when disruption to the skin barrier frequently (drastic increase of skin pH, keratinocytes apoptosis water loss, skin flaking, and inflammation). *P. acne* and *S. epidermidis* strains are frequently detected in skin pathogenic situations in their biofilm forms, generating important virulence factors and linked to skin dysbiosis such as acne vulgaris (Da Silva et al., 2022) or atopic dermatitis (AD) (Gonzalez et al., 2021), respectively.

1.5.3 Diseases Caused by *Propionibacterium acne* and *Staphylococcus epidermidis*

P. acne overgrowth is linked to the inflammatory skin condition known as acne vulgaris (Yanhan Wang et al., 2014). One of the most prevalent skin conditions is acne, which affects 27% of women and 34% of men (Mustarichie et al., 2020). Increased sebum production leads to acne because microorganisms break down the sebum into free fatty acids, which promotes more microbial colonization. One well-known bacterium that plays a significant part in the development of acne is *P. acne*. Thick peptidoglycan covers the Gram-positive, anaerobic bacteria *P. acne*. *P. acne* bacterium uses sebum as a source of nutrition. By causing the release of inflammatory cytokines, *P. acne* can cause an inflammatory reaction that inflames the sebaceous follicle (Cong et al., 2019). Atopic dermatitis (AD) is a persistent, chronic inflammatory skin disease linked to a poor ability of skin barriers to function efficiently with immunity. The condition is now two to three times more common in children. Its symptoms, which include severe itching and eczematous skin lesions, significantly lower quality of life. (Brandwein et al., 2019). These conditions become severe if left untreated.

1.5.4 *Staphylococcus epidermidis* and *Propionibacterium acne* form Biofilms on the Surfaces of Medical Devices

Staphylococcus epidermidis and *Propionibacterium acnes* are two bacterial species that are well-known for their tendency to produce biofilms, especially on the surfaces of medical equipment. Normally a benign resident of human skin, *S. epidermidis* can change into an opportunistic

pathogen that causes infections frequently linked to implanted medical equipment like catheters and prosthetic implants (Moris et al., 2022). Similarly, *P. acnes*, a typical skin microbiota component, is known to create biofilms within sebaceous glands and hair follicles and is implicated in the development of acne vulgaris (Coenye et al., 2022). These bacteria can cause serious problems in clinical settings when they colonize medical device surfaces and produce biofilms, which makes infections more difficult to treat and more persistent. It is essential to understand these bacteria's capacity to build biofilms in order to create efficient plans for managing and preventing infections linked to medical equipment.

1.5.5 Available Antibacterial Drugs Against *Propionibacterium acne* and *Staphylococcus epidermidis*

There are various antibacterial drugs available in the market against the infections caused by *P. acne* and *S. epidermidis*. The tetracyclines, benzyl peroxide, retinoids, topical antibiotic, azelaic acid, and combined agents are frequently prescribed antibiotics and most widely studied for acne (Otlewska et al., 2020). Cloxacillin, imipenem, amoxicillin/clavulanic acid, cefpirome, erythromycin, clindamycin, roxithromycin, trimethoprim/sulfamethoxazole, doxycycline, gentamicin, fusidic acid, tobramycin, amikacin, netilmicin, isepamicin, ciprofloxacin ofloxacin, and daptomycin are affected against *S. epidermidis* (Singh et al., 2010).

CHAPTER 2: LITERATURE REVIEW

2.1 Ciprofloxacin: A Broad-Spectrum Antibiotic

Ciprofloxacin was patented by Bayer A.G. in 1983 and in 1987 approved by the US Food and Drug Administration (USFDA) (Babushkina et al., 2020). Ciprofloxacin belongs to fluoroquinolone class, is an antibiotic agent used to treat bacterial infections such as pneumonia and urinary tract infections (Sharma et al., 2010). Ciprofloxacin is approved by FDA for the treatment of sexually transmitted infections urinary tract infections, skin and soft tissue infection, bone, prostatitis, joint infections, pneumonia, gastrointestinal infections, typhoid fever, inhalation anthrax, lower respiratory tract infections, plague, and salmonellosis, and chronic bronchitis (Meyerhoff et al., 2004; Unemo et al., 2019). Ciprofloxacin is a recommended treatment for acute bacterial prostatitis by the American Academy of Family Physicians (Coker & Dierfeldt, 2016). Ciprofloxacin is also used to treat severe bacterial prostatitis (Heras-Cañas et al., 2017). Off-label usage of ciprofloxacin for the treatment of perianal fistula in Crohn's disease (Wu et al., 2015). The FDA has approved the use of ciprofloxacin ophthalmic solution to treat conjunctivitis and corneal ulcers caused by susceptible strains (McDonald et al., 2014).

The scientific community has also taken an interest in ciprofloxacin because of its apoptotic and anti-proliferative effects in a number of cancer cell lines. Researchers found that it is able to cause apoptosis and growth inhibition in several osteosarcoma, carcinoma, and leukaemia cell lines in a dose- and time-dependent manner. Ciprofloxacin may potentially be helpful in the treatment of bladder cancer. Tumor cells derived from bladder carcinomas with transitional cells were studied in vitro, and the results showed that the reduction of cell proliferation (Activity, 2023).

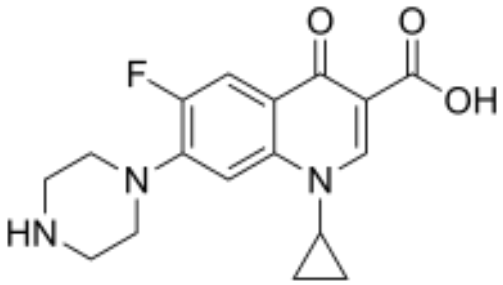


Figure 3: Structure of ciprofloxacin

2.1.1 Ciprofloxacin Antibacterial Mechanism

To treat bacterial infections many antibacterial drugs like cephalosporins, penicillin, fluoroquinolones, tetracyclines etc., are available in the market. Among these antibacterial drugs Ciprofloxacin (CIP) is a broad-spectrum antibacterial drug with good antibacterial activity and good efficacy. It is a second-generation fluoroquinolone (FQs), ciprofloxacin prevents the replication of bacterial DNA (Zhang et al., 2018). In bacteria, DNA gyrase is a crucial enzyme that causes DNA strands to supercoil. Many DNA functions, including transcription and replication, depend on this supercoiling. In order to prevent DNA gyrase from acting, ciprofloxacin binds to it. This puts too much tension and strain on the DNA molecule by causing positive supercoils to accumulate in front of the replication fork and negative supercoils to accumulate behind it. Topoisomerase IV is another bacterial enzyme that is inhibited by ciprofloxacin. During cell division, topoisomerase IV is essential for breaking apart double stranded DNA (Kokot et al., 2023). DNA gyrase consists of subunits A and B. Quinolones such as ciprofloxacin are believed to prevent subunit A from resealing the DNA double-strand; therefore, single-stranded DNA may result in exonucleolytic degradation.

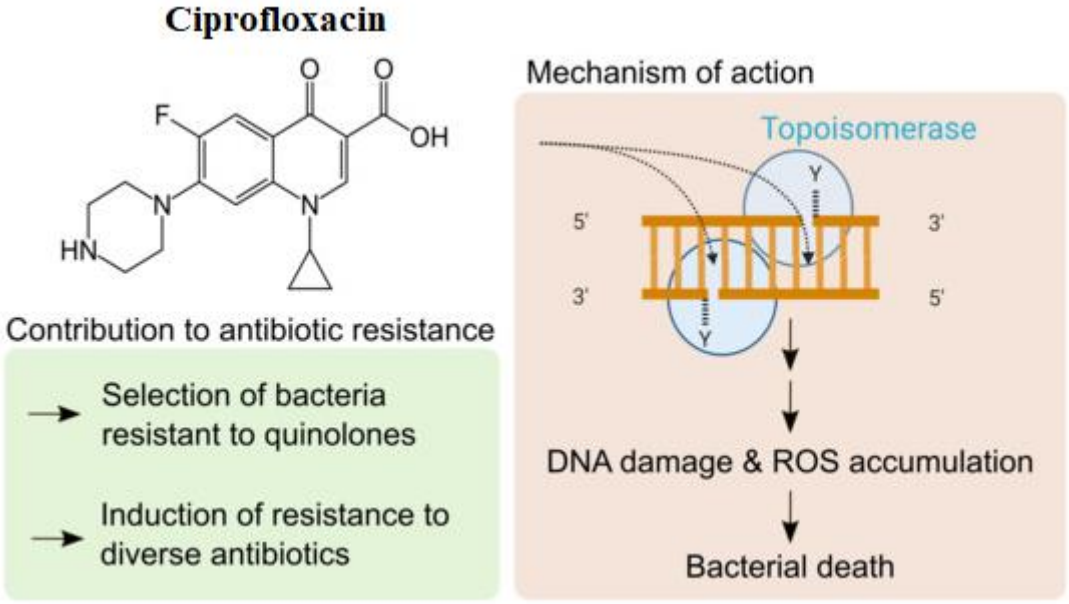


Figure 4: Mechanism of action of ciprofloxacin

2.2 Pathogens Antibacterial Resistance

Apart from their antibacterial properties, the pathogenic bacteria can develop new resistance mechanisms against the broad-spectrum antibacterial drugs to which they were once vulnerable, leading to the creation of multidrug-resistant organisms (MROs). The effectiveness of treatment significantly declines when a microorganism develops resistance to antibiotics, which restricts available therapeutic alternatives and worsens treatment results. MRO infections invariably correlate with increased death rates (Tan et al., 2022). Antibiotic resistance is a major problem, according to studies, particularly in tertiary care settings. Individuals are more vulnerable to serious nosocomial infections, which can result in more expensive medical care, longer hospital stays, and worse clinical results. But since the 1900s, there has been a decline in the development of new antibiotics despite the growth in antibiotic resistance. This decline is mostly attributable to technological challenges, regulatory obstacles, and low productivity when compared to long-term treatments for chronic conditions (Luepke et al., 2016).

2.3 Nanocarriers

Nanoparticles are defined as structures with a size in at least one dimension between 1 and 100 nm. The prefix "nano" is used to identify particles with sizes of several hundred nanometers or less. Cells are more easily able to accomplish the ideal physicochemical and biological properties than larger molecules, which makes using them as delivery systems for bioactive drugs already available on the market successful (Z. Shi et al., 2020). Examples of nanocarrier that are tested in drug delivery systems include liposomes, dendrimers, and polymers (Cheng et al., 2022). The type of lipid base nanocarrier is shown in figure 5.

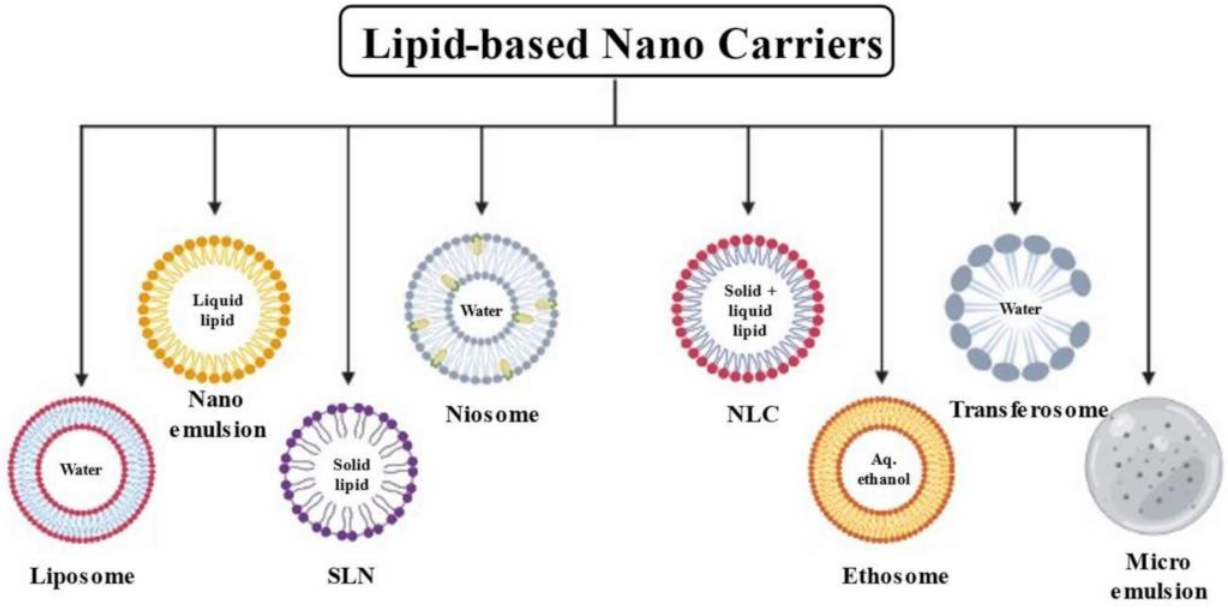


Figure 5: Types of lipid based nanocarrier

2.3.1 Nanocarriers Used for Medical Applications

In order to be used in the medical industry, nanocarriers need to be harmless and biocompatible. When something is biocompatible, it can interact with a biological system without causing harm. Nanoparticles' undesirable impacts are determined by their size, shape, quantity, and surface chemistry. The toxicity of nanoparticles is affected by many factors which makes their estimation difficult and thus each new DDS formulation focuses on toxicity of nanoparticle (Sala et al., 2018). It is generalized that nanoparticles which are small have greater surface area are more potential for as a therapeutic agent.

2.3.2 Different Types of Nano-carriers for Skin Infection

Nanoparticles have emerged as promising candidates for combating skin infections, offering diverse approaches to address microbial challenges. Metallic nanoparticles, such as silver and gold nanoparticles, exhibit potent antimicrobial properties by disrupting bacterial cell membranes and inhibiting microbial growth. Lipid-based nanoparticles, including liposomes, and solid lipid nanoparticles, facilitate controlled release of antimicrobial agents, enhancing their efficacy and minimizing side effects. Polymeric nanoparticles, composed of biocompatible polymers like chitosan or poly(lactic-co-glycolic acid), provide sustained release of antimicrobial

drugs and improved penetration into the skin layers. Additionally, quantum dots and carbon-based nanoparticles possess unique properties for imaging and targeted therapy, allowing for precise monitoring and treatment of skin infections. These diverse nanoparticle types showcase the versatility of nanotechnology in developing innovative solutions for combating skin infections, promising more effective and targeted treatments with reduced adverse effects. Nano-carriers and Nano-particles already made for skin infections are shown in Table 2.

Table 1: Nano-particles for skin infections

Nanoparticles	Type of nanoparticle	Antibacterial activity	Delivery	References
Metal nanoparticles	Silver nanoparticle	+	Topical	(Velmurugan et al., 2014)
	Gold nanoparticle	+	Topical	(Qiu et al., 2021)
	Copper nanoparticle	+	Topical	(Hayder et al., 2023)
Lipid nanoparticles	Liposomes	+	Topical	(Walduck et al., 2020)
	Ethosomes	+	Topical	(Paiva-Santos et al., 2021)
Polymeric nanoparticles	Polymeric nanoparticle	+	Topical	(Madawi et al., 2023)

2.3.3 Transethosomes as a Nanocarrier

Transethosomes represent a significant breakthrough in the field of nanocarrier systems, offering a novel approach to the efficient and accurate delivery of a variety of active substances such as drugs. These nanocarrier have attracted a lot of interest from the pharmaceutical industry because of their special qualities and the possibility of enhancing drug administration and bioavailability (Rodríguez-Luna et al., 2021).

Transethosomes has been shown to be a reliable drug delivery vehicle in the pharmaceutical industry. TET protects the drug from harm and makes it easier for it to travel through the skin to

the intended location by encasing it inside their vesicular structure. This targeted distribution method is an important tool for pharmaceutical formulations since it minimizes potential adverse effects while simultaneously optimizing the therapeutic effects of the medicine (Hesham et al., 2022).

TET controlled release properties can also be used to ensure constant delivery of drugs, which increases their usefulness in a range of therapeutic applications. TET versatility is one of their best features. This flexibility allows for accurate modifications to the carrier system, ensuring maximum efficiency and performance.

2.4 Transethosomes for a Topical Delivery

Lipid vesicles have been used more often in recent years to enhance the administration of topical medications. An alternative for more effective skin medication administration is offered by ultra-deformable vehicles (UDVs). Phospholipid-based drug delivery systems have proven to be a good choice for topical delivery due to their biocompatibility and biodegradability. The stratum corneum layer of the skin was shown to inhibit drug delivery through the skin because of its resistive nature. The researchers investigated several strategies for delivering drugs to the dermis through the stratum-corneum barrier. The cholesterol in the liposome gives it stiffness and reduces its flexibility, which hinders its ability to migrate over the stratum corneum (Haider et al., 2015). The issues related to the hard membrane composition of liposomes were resolved by Cevc and Grbauer's creation of flexible liposomes (Sabitha et al., 2013). In addition to phospholipids, edge activators in deformable liposomes cause the lipid bilayers disruption, increasing the vesicles flexibility. This will enable the drug to enter the skin more deeply (Ahad et al., 2018). TET, a novel class of lipid vesicular nanocarriers, was initially developed by Song et al. in 2012 and are based on transferosomes and ethosomes, a kind of liposome. Furthermore, TET shields the drug from metabolic degradation and has a high entrapment efficiency (greater than both ethosomes and transferosomes). TET have greater stability than other ultra-deformable vesicles and can change size and shape while travelling through narrow spaces without significantly losing drug (Allam et al., 2022).

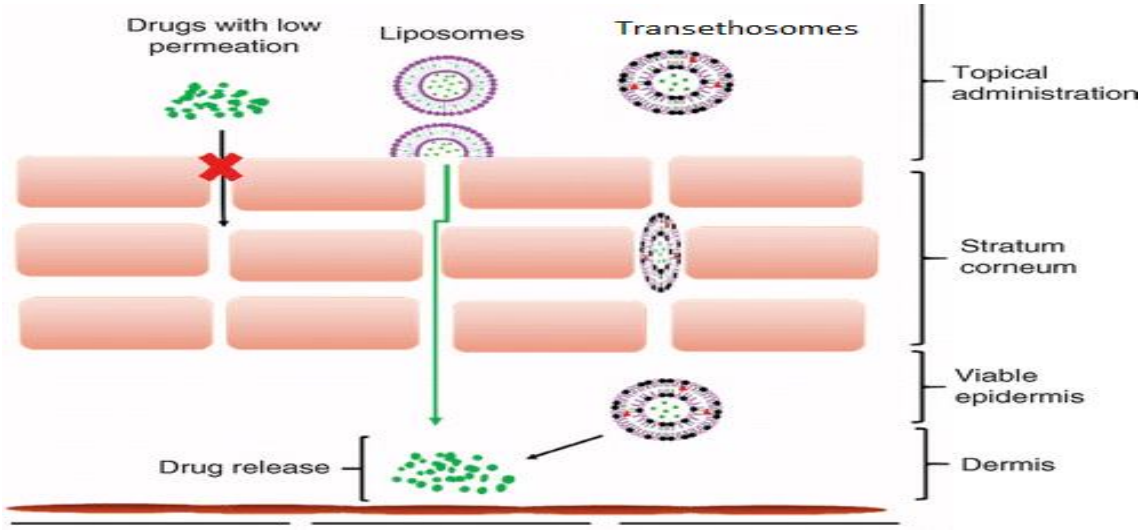


Figure 6: Transethosomes for topical targeted delivery

2.5 Ciprofloxacin Loaded Transethosomes Against *Propionibacterium acne* and *Staphylococcus epidermidis*

A new strategy based on nanotechnology has been developed to address every factor contributing to antibiotic resistance. Ciprofloxacin was entrapped in TET, which could decrease the drug's systemic bioavailability and distribution while potentially enhancing its effectiveness. The edge activator in TET, increases the flexibility of vesicles by destabilizing the lipid bilayers (Ahad et al., 2018). This will enable the drug to be absorbed deeper into the skin (El Maghraby et al., 2004). Ethanol is another component in TET, interacts with the lipids in both vesicles and skin, making the layer more flexible, due to its natural capacity to provide fluidity to the lipid membranes (Moolakkadath et al., 2018). TET contained both edge activators and ethanol, thus offers the benefits of both deformable liposomes and ethosomes (C. K. Song et al., 2012). Furthermore, TET presented better deposition characteristics and penetration ability (Abdulbaqi et al., 2016). In this research formation of ciprofloxacin loaded TET, their evaluation, characterization, and determination of their in-vitro antibacterial activity by well diffusion method had been studied.

2.6 Synthesis of Transethosomal Nanoparticle

Transethosomes are a particular class of lipid-based nanocarrier system that has drawn a lot of interest recently due to their potential uses in drug delivery. These vesicular nanoparticles

increase the penetration of drug into deeper tissues across the skin. TET are beneficial for drugs delivery through the topical route (Kahraman et al., 2017). TET are created using a number of techniques, such as reverse phase evaporation, thin film hydration, ethanol injection, and cold (Bajaj et al., 2021). In this study TET was synthesized by using the cold technique. These lipid-based nanocarrier systems, which are frequently used for transdermal drug delivery, can be prepared in a simple and gentle manner using the cold method of TET synthesis. The synthesis of TET by the cold method is efficient for transdermal drug delivery. In this process edge activators, lipid and cholesterol are dissolved in an organic solvent, usually ethanol. Simultaneously, an aqueous phase is often composed of buffer solution or distilled water. Water-in-oil (w/o) emulsion is the result of the mixing of two phases working together. These vesicles improve the absorption of drugs through the skin by acting as effective drug delivery vehicles. The cold approach is beneficial for sensitive drugs and heat-sensitive substances because it can stop lipid breakdown and phase change. Characterization ensure the stability and quality of the synthesized TET for pharmaceutical applications, particularly in transdermal drug delivery (Costanzo et al., 2021).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

Ciprofloxacin was purchased from Islamabad, Pakistan, phosphatidyl choline (PC), span 80, were purchased from Sigma Aldrich. PC 2%, Solvents ethanol of 30% was of HPLC grade and all other chemicals were of analytical grade. 70% Distilled water was also used during the synthesis of formulation.

3.2 Bacterial Strains and Growth Conditions

Gram-positive bacteria *S. epidermidis* and *p. acne* were adopted to assess the antibacterial activity of ciprofloxacin loaded TET. Luria–Bertani (LB) broth was used to form fresh inoculum for each bacterial strain and incubated at 37 ◦C for 20 h. Before antibacterial activity, the turbidity of the culture was adjusted to the 0.5 McFarland standard, equivalent to 1.5 × 108 CFU/mL, using LB broth.

3.3 Synthesis of ciprofloxacin loaded transethosome

Transethosomes were made using the "cold technique". PC was first dissolved in ethanol (30% w/v). 1mg/ml drug dissolved in ethanol. The drug solution mix with above PC solution and then surfactant is added. Following that, distilled water is added gradually up to ratio of 70:30% (v/v), and set the solution on magnetic stirring at 22-25 °C at 750 rpm for 30 min (Costanzo et al., 2021). Scanning electron microscopy, Fourier transform infrared spectroscopy, Ultraviolet visible spectroscopy, and zeta potential was used to characterize the nanoparticle.

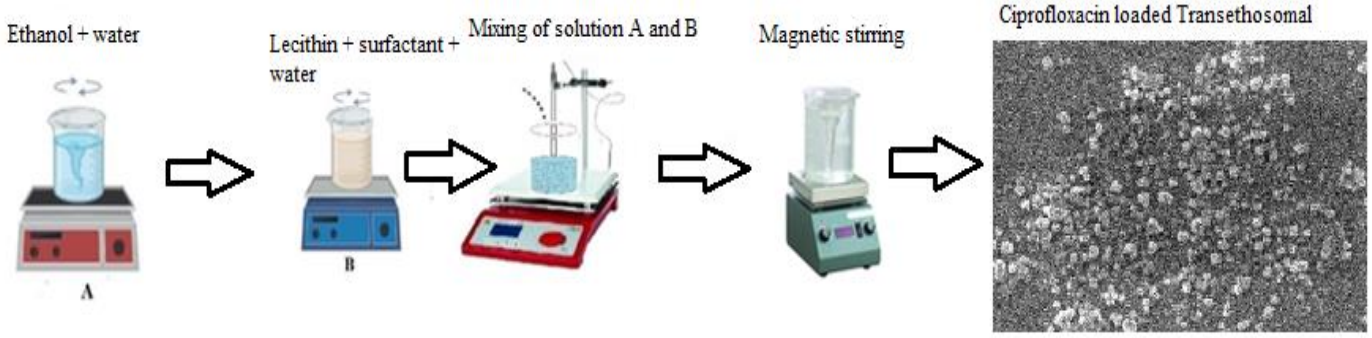


Figure 7: Synthesis of Ciprofloxacin loaded TET

Table 2**:** Composition of different component in TET

Chemicals	Composition
PC + Ethanol solution	850 µL
Span 80	150 µL
Ethanol	2 ml
Water	7 ml
Ciprofloxacin	10 mg

3.4 Characterization of Nanoparticle

Nanoparticles were characterized by different techniques including UV, FTIR, SEM, and ZETA.

3.4.1 Ultraviolet and Visible Absorption Spectroscopy (UV-Vis)

The technique of UV-Vis spectroscopy is mostly employed in clinical and chemical laboratories. When light beams travel through the sample and the absorption is measured from the reflected beam, it determines the amount of absorption in the sample. One half of the light beam is focused through the cuvette holding the measurement sample, and the other half is directed towards a cuvette that solely contains solvent as a control. A spectrum that plots the full wavelength range against its absorption at specific wavelengths can be created by measuring absorption at a desired range and a given wavelength. Lambda max refers to the maximum absorption at a particular wavelength and obeys the Beer Lambert Law theory. The sample absorbance is proportional to the sample molar concentration in the cuvette, molar absorptivity is used when comparing different compound spectra. Beer-Lambert Law says;

$$A = EcL$$

Molar absorptivity E= A/ cl (where A= absorbance, c= sample concentration in moles/ liter and L= length of light path through the cuvette in cm). This law enables UV-VIS spectroscopy as a useful tool for quantitative analysis. (Perkampus, 2013; Tomaszewska et al., 2013)

U.V-Vis spectra of ciprofloxacin loaded TET were determined by using UV-Vis 2800 spectrophotometer of BMS Scientific Technical Corporation (PVT.), from 200-450nm at a

resolution of 1nm. As a reference the de-ionized water and ethanol in ration of 70:30 was used. The UV spectra of ciprofloxacin, TET with and without drug were recorded.

3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transforming infrared spectroscopy (FTIR) is a technique used to identify functional group in organic and some inorganic molecule. The infrared absorption bands identify the molecular components and structures of the sample material, and this technique assesses the material by absorbing infrared radiation vs wavelength. When a substance is exposed to infrared radiation, the absorbed radiation often causes the molecules to vibrate at a greater frequency. The energy difference between the excited vibrational and resting states determines the wavelength of light that a single molecule absorbs. The sample's absorbed wavelengths reveal information about its molecular structure (Khan, Saeed, & Khan, 2019; Mohamed, Jaafar, Ismail, Othman, & Rahman, 2017). Samples were allowed to air dry before being processed with compressed KBr discs for FTIR analysis (Khan et al., 2019; Mohamed et al., 2017). FTIR spectra were examined using the NICOLET6700 FTIR spectrophotometer, with a range of 4000 to 350 cm^{-1}. FTIR of materials, including PC, span-80, ethanol, water, Blank TET, ciprofloxacin loaded TET was done.

3.4.3 Scanning electron microscopy (SEM)

It is used to analyze the practical size. Values for particle size are provided in the 0 to 'Infinity' range. In this region, particles with circularity values beyond the defined range will likewise be disregarded. Analyzed the 8-bit binary image of the best-fitting ellipse of the observed particle (cf. Edit. Range. Fit Ellipse; grey levels: Ellipses: 0; background: 255) (Goldstein et al., n.d.). Micro-pipette is used to place a small amount of the sample on a cover slip, both types of nanoparticles were photographed. Images were captured at the National University of Science and Technology, scanning electron microscope was used to analyze the size distribution and dispersity of both types of NPs using Dynamic Light Scattering (DLS).

3.4.4 Zeta Potential

It is the potential difference between the solids and liquids, across phase boundaries. It is a measure of the particle's electrical charge which is suspended in liquid. The potential difference at the surface of double layer is known as zeta potential or slipping plane. The magnitude of zeta potential shows potential stability of the colloidal system. Zeta potential is expressed in millivolts (mV) and is also known as electro kinetic potential. Zeta potential analyzer was known to have surface charge and zeta potential. Zeta potential tells about the nanoparticles' stability, surface charge and average size. Zeta potential in colloids is the difference of the electrical potential through the ionic layer around a charged colloid ion. Put another way; it is the potential at the slipping plane in the double layer interface (Karmakar, 2019). The colloids are least stable if zeta-potential equals zero. The zeta potential of particles more positive or negative than +30 mv or -30 are considered as stable (Saeb et al., 2014). The surface charge of TETs was analyzed by Zeta Sizer.

3.5 In-Vitro Testing of Nanoparticles

3.5.1 Drug Encapsulation

Different drug dilutions were synthesized and examined using a UV spectrophotometer to measure absorbance at 277 nm in order to determine the effectiveness of drug encapsulation. Unentrapped drug was determined by using this standard curve value. To find the drug fraction that was not entrapped, samples were centrifuged for 1 hour at 4500 rpm. The supernatants were then examined by using UV-visible spectrophotometry. The entrapment efficiency of the drug was calculated by using the formula described in Equ.(1) (Judy et al., 2021) as follows:

$$Entrapment\ efficiency = \frac{amount\ of\ encapsulated\ drug}{Total\ drug} \times 100 \qquad (1)$$

3.5.2 Drug Release

Drug release by TET was examined up to 48 hours along with the addition of specified volume of phosphate buffer saline (PBS). From both 25 ml solutions of TEs, 3ml samples were placed into separate centrifuge tube of 15 ml and allowed it for the centrifugation for 10 mins at 4500

Rpm and 25ºC. While on other hand 3ml of PBS was added to ciprofloxacin loaded TETs. After that, supernatant was analyzed by UV spectrophotometer. Procedure was repeated after 1, 2, 4, 6, 12, 24 and 48 hr. At 277nm of wavelength, absorbance values were taken and used as cumulative drug release.

3.5.3 Hemolytic assay

The hemolytic assay of ciprofloxacin and ciprofloxacin loaded TET was calculated by the proportion of hemolysis. Human female donors' blood samples taken in tubes with anticoagulant. 8 ml of PBS were added to 4 ml blood. After agitation, blood cells were suspended in PBS and tubes were centrifuged at 3000 g for 10 min. After repeating the procedure, blood cells were eventually suspended in the solution of PBS and fill up to their initial volume. For the hemolytic experiment, different ciprofloxacin, and ciprofloxacin nanoparticle concentrations (50, 100, 200, and 500 g/ml) were used. 100 µl of the RBC and 100 µl of every sample were incubated at 37 °C. Phosphate buffer saline was taken as negative control, and 0.5 % Triton X-100 was taken as positive control. The samples were combined and centrifuged one more time after 4 hours. A 96-well plate containing 100 µl of supernatant was used to measure the absorbance at 550 nm using a microplate reader. Negative and Positive controls produced respectively 0% and 100% absorbance (Laloy et al., 2014). The percentage hemolysis was determined by using the formula presented in Equ. (2) as mentioned below:

$$\text{Hemolysis } \% = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control} - \text{Absorbance of negative control}} \times 100 \quad (2)$$

3.5.4 Determination of the Inhibitory Activity Against *S. epidermidis* and *Propionibacterium acne* Biofilms

A microtiter plate spectroscopic experiment was used to quantitatively assess the anti-biofilm activity of *S. epidermidis* and *P. acne* (Batiha et al., 2020). It is among the most popular techniques for determining a compound's antimicrobial and anti-biofilm properties (Mulat et al., 2020). Briefly, 5 milliliters of TSB were used to generate fresh cultures of *S. epidermidis* and *P. acne*, which were then incubated at 37 °C for the entire night. Following incubation, 100 µL of each diluted culture *S. epidermidis* and *P. acne* was put to each well of a 96-well plate. The

cultures were then diluted (1:100) in TSB. Compounds were introduced to the test wells at MIC concentrations, and they were incubated for 24 hours at 37 °C. On the next day, autoclaved distilled water was used to gently wash the wells and dispose of the contents through aspiration. The wells were then stained with 250 µL of crystal violet (0.1% w/v) and left with the lid closed for 15 minutes at room temperature. Wells were incubated, cleaned with distilled water, and then allowed to dry. Then, each well was filled with 300 µL of 95% ethanol, and the lid was closed for 15 minutes. After carefully mixing the contents with a gentle pipette, 150 µL of the crystal violet and ethanol solution was transferred to a fresh 96-well plate, and the Multiskyskan Sky Microplate Spectrophotometer was used to detect the optical density at 630 nm. Three wells were used for the studies, with one well acting as the media control (blank). The following formula was used to determine the percentage of biofilm inhibitory activity:

$[(C - B) - (T - B)/(C - B)] * 100\%$, where C = absorbance of the control (biofilm, no treatment), B = absorbance of blank (only TSB), and T = absorbance of the test (biofilm and treatment) (Ali et al., 2021). In this assay, ethanol (96%) and distilled was used as a positive control.

3.5.5 Antibacterial Activity by Agar Well Diffusion Method

The antibacterial activity of many substances, such as medicines, plant extracts, and synthetic chemicals, can be evaluated using the widely used agar well diffusion method. An approach that is frequently used in microbiology to evaluate the antimicrobial activity of substances like antibiotics, plant extracts, or other chemicals is the well diffusion method, commonly referred to as the agar well diffusion method. Making wells or holes in a solid agar medium and inoculating it with a layer of microorganisms typically bacteria is the process. After that, the material to be tested is put into the wells and given time to permeate into the agar. If the material has antimicrobial qualities, it will stop bacteria from growing in the agar that surrounds the well. An indicator of the antibacterial potency of a substance is measured by the size of the inhibition zone, which is the clear area surrounding the well where microbial growth is restricted. A useful tool in microbiological research and the creation of antimicrobial drugs, the well diffusion method offers a straightforward but efficient way to screen and compare the antibacterial properties of various agents (Das et al., 2010).

In this method, a 100 µL of inoculum of microbes was obtained and a solid agar medium, typically tryptic soy agar is inoculated with a standardized solution of the target bacteria, evenly spread across the surface. Then, using a sterile cork tip, wells are made in the agar and various test compounds are introduced to them. Gradients of concentration are subsequently produced when the chemicals permeate into the surrounding agar. A distinct, circular zone of inhibition will appear around the well if the test material has antibacterial qualities, which will prevent bacterial growth in the area. The tested compound's antibacterial efficacy is ascertained by measuring the diameter of this zone. The agar well diffusion method is a widely used technique in research, clinical laboratories, and pharmaceutical development to evaluate the efficacy of antimicrobial agents against bacterial strains. It is a simple and economical tool for initial screening of potential antibacterial agents.

CHAPTER 4: RESULTS

4.1 Synthesis of Ciprofloxacin Loaded Transethosomes

Transethosomes have been widely utilized for transdermal or topical delivery because of their unique characteristics and diverse surface activities. Several techniques have been adopted for the synthesis of TET. In this study, we aimed to prepare ciprofloxacin loaded TET and evaluate their antibacterial efficacy against bacterial strains. Our results indicated that ciprofloxacin loaded TE efficiently acted as a antibacterial bacterial transdermal delivery. Herein, the synthesis of the ciprofloxacin loaded TE was confirmed by a gradual color change of the reaction solution to milky white from brown.

4.2 Characterization Ciprofloxacin Loaded Transethosomes

4.2.1 UV-Vis Absorption Spectroscopy

U.V-Vis spectra of ciprofloxacin loaded TET were determined by using UV-Vis 2800 spectrophotometer of BMS Scientific Technical Corporation (PVT.), from 200-450nm at a resolution of 1nm. As a reference the de-ionized water and ethanol in ration of 70:30 was used. The UV spectra of ciprofloxacin, TET with and without drug were recorded.

UV-Vis absorption spectroscopy of TET showed surface Plasmon resonance (SPR) peak at 277 nm, ciprofloxacin drug also show the peak at 277 while empty TET has no peak in this range (200-400 nm) that clearly determines that transethosoms with drug has formed (Figure 8).

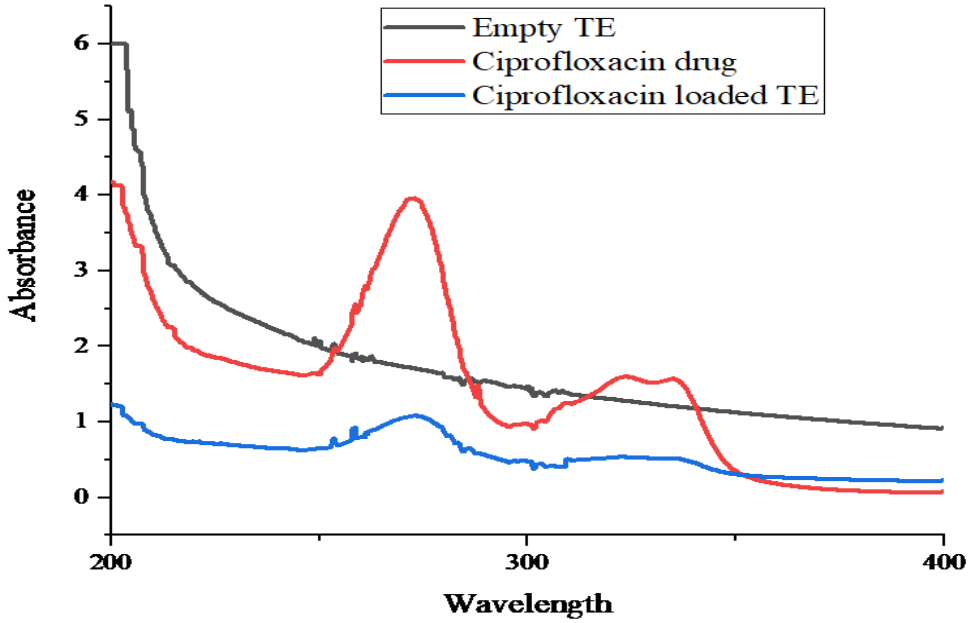


Figure 8: Comparative UV spectra of empty TET, Ciprofloxacin drug, and ciprofloxacin loaded TET

4.2.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrum of Lecithin indicated peaks or bands at 881/cm (CH, Alkanes), 1641/cm (C=C stretching, Alkenes) and at 3299/cm (OH stretch, Alcohol). Ethanol spectrum indicated peaks at 881/cm (CH, Alkanes), 1051/cm (C-O stretch, primary Alcohols), and 3299/cm (OH stretch, Alcohols). Ciprofloxacin exhibited peaks at 881/cm (C-H, Alkanes), 1088/cm (C-O stretch, ether). Ciprofloxacin loaded TETs show peaks at 1088/cm (C-O stretch, ether), 1641/cm (C=C stretching, Alkenes), 2985/cm (N-H, Amines). In distinction blank TETs exhibited peaks at 1641/cm (C=C stretch, Alkenes), 3299/cm (OH, Alcohol) and disappear the peak at 1088/cm (CO stretch, ether). The observed changes in infrared bands proven that transethosomes with ciprofloxacin drug were formed.

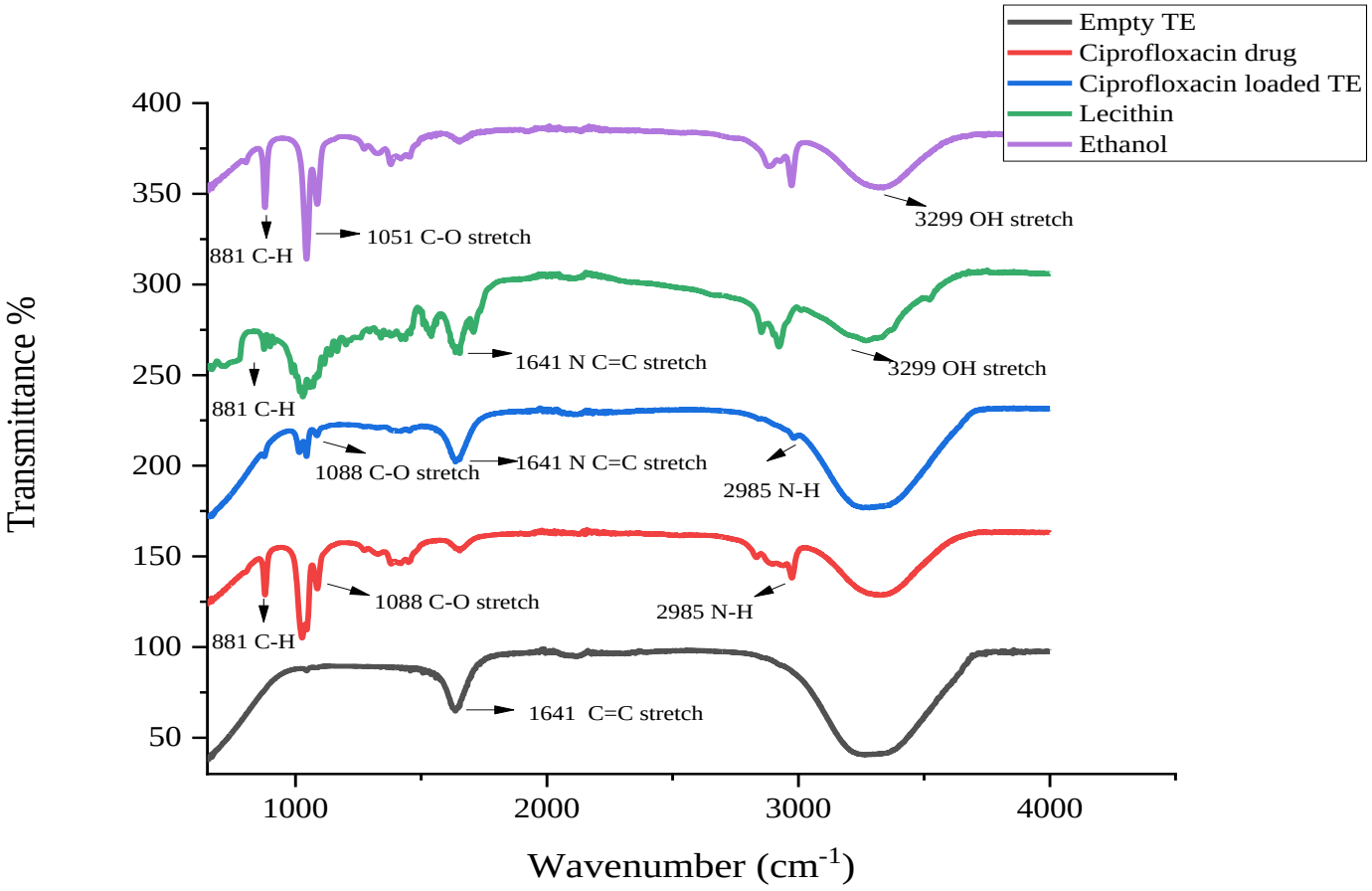


Figure 9: FTIR spectra of Lecithin, Ethanol, Ciprofloxacin drug, Empty TET and Ciprofloxacin loaded TET

4.2.3 Scanning Electron Microscopy

It is used to analyze the practical size. Values for particle size are provided in the 0 to 'Infinity' range. In this region, particles with circularity values beyond the defined range will likewise be disregarded. Analyzed the 8-bit binary image of the best-fitting ellipse of the observed particle (cf. Edit. Range. Fit Ellipse; grey levels: Ellipses: 0; background: 255) (Goldstein et al., n.d.). Micropipette is used to place a small amount of the sample on a cover slip, both types of TETs were photographed. Images were captured at the National University of Science and Technology.

Area distribution of the nanoparticles was determined by using image j software. Analysis was performed on a selected area. 8–bit binary image of each measured particle (gray levels: Background: 255; Ellipses: 0) was analyzed and particle size distribution was obtained. The results of this analysis corresponded with the SEM results, as the average particle was determined to be 90nm as shown in Figure 10.

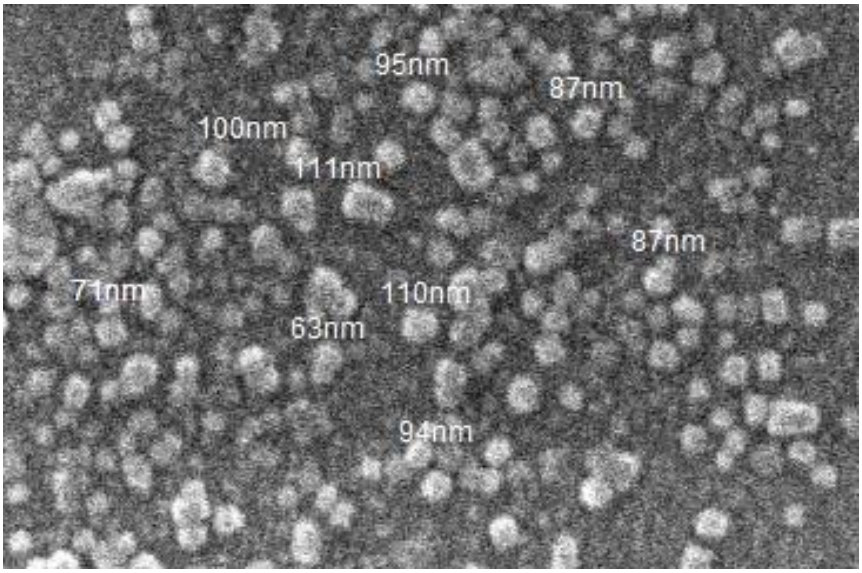


Figure 10: SEM image of ciprofloxacin loaded TET

4.2.4 Surface Charge or Zeta Potential

Zeta potential tells about the nanoparticles' stability, surface charge and average size. Zeta potential in colloids is the difference of the electrical potential through the ionic layer around a charged colloid ion. Put another way; it is the potential at the slipping plane in the double layer interface (Karmakar, 2019). The colloids are least stable if zeta-potential equals zero. The zeta potential of particles more positive or negative than +30 mv or -30 are considered as stable (Saeb et al., 2014). The magnitude of zeta potential suggests colloidal stability. The ciprofloxacin loaded TET formulation is stable because the zeta potential of ciprofloxacin loaded TET was determined to be -39.6 mV.

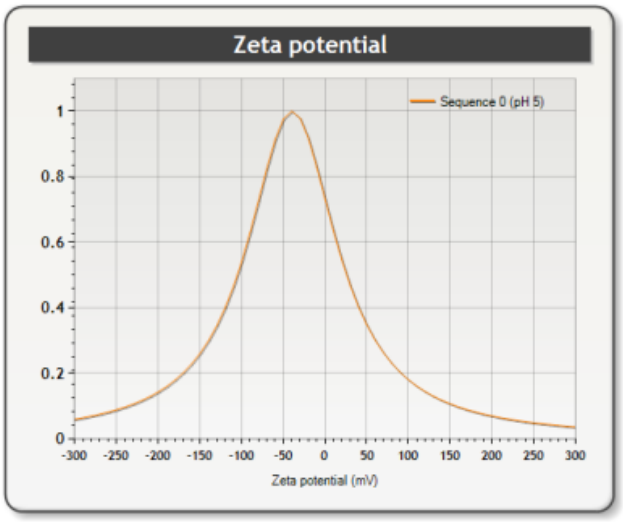


Figure 11: Zeta size analysis of ciprofloxacin loaded TET

4.3 In-Vitro Testing

4.3.1 Drug Encapsulation

The Encapsulation Efficiency was analyzed to be 67.5 %, that delineates the 67.5 % encapsulation of drug within Nanoparticles. Calculation of unknown concentration of drug with the help of standard curve;

$$Y = 0.0587\,x + 3.4553 \quad (3)$$

UV analysis of 1st supernatant from mini column centrifuge tube, gave the absorption at 277 nm for ciprofloxacin.

Abs (277 nm) = 3.5224

Y= Absorption value at specific point

X= Concentration of unknown

By putting value of abs (277 nm), the amount of non-entrapped drug = 1.143 mg/ml

Total drug was 1 mg/ml and absorbance of total drug at 277nm = 3.5427

The entrapment efficiency of drug was calculated by using following formula;

$$\text{Drug EE\%} = \frac{(\text{Total drug} - \text{Non-Entraped})}{\text{Total drug}} \times 100 \quad (4)$$

Total encapsulation efficiency is 67.5%

4.3.2 Drug Release Efficiency

The drug release analysis of nanoparticles was carried out using a UV spectrophotometer with empty nanoparticle solution used as control, over time duration of 32h. The results were tabulated, and a cumulative drug release percentage graph was obtained using graph pad prism 6, as shown in figure 12. This graph shows little burst effect, with a prolonged sustained release of ciprofloxacin from nanoparticles for 32 h. This is very helpful to eliminate the side effects of ciprofloxacin that are associated with its high doses and low retention time in the body. The in-vitro studies also show that free ciprofloxacin was released faster than loaded TET at the maintained pH values for blood. The delay of ciprofloxacin TET release was due to the binding of ciprofloxacin with the TETs, which accordingly improved the drug release as compared to free drug.

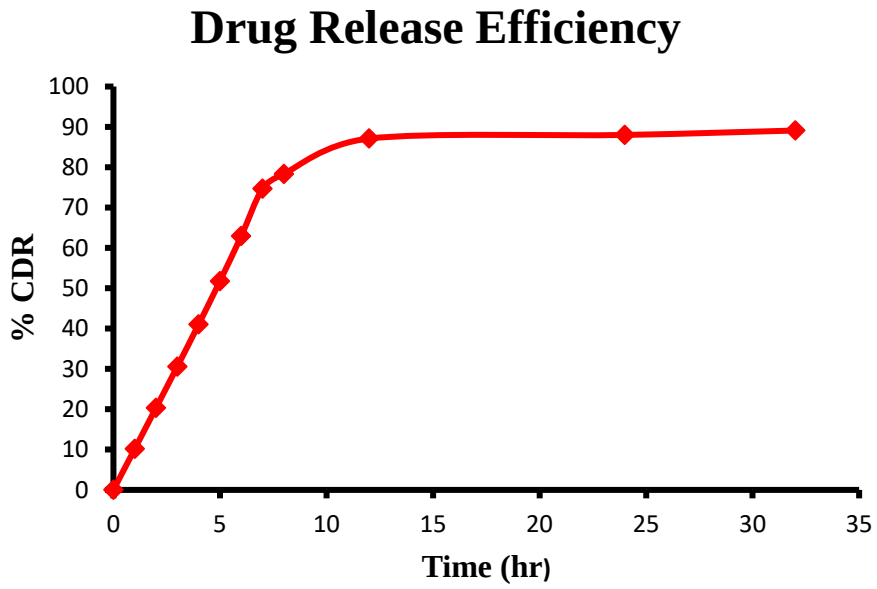


Figure 12: Drug release graph of ciprofloxacin loaded TET

4.3.3 Hemolytic Assay

To determine the cytotoxicity of the ciprofloxacin loaded TET, (%) cell viability was checked by the blood cells. The results in figure 7 show that % cell viability is greater with TET than the drug. The antibacterial activity of ciprofloxacin loaded transethosomes shows the efficient drug

delivery therapy. The percentage hemolysis was determined by using the formula presented in Equ. (2) as mentioned below:

$$\text{Hemolysis } \% = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control} - \text{Absorbance of negative control}} \times 100 \quad (5)$$

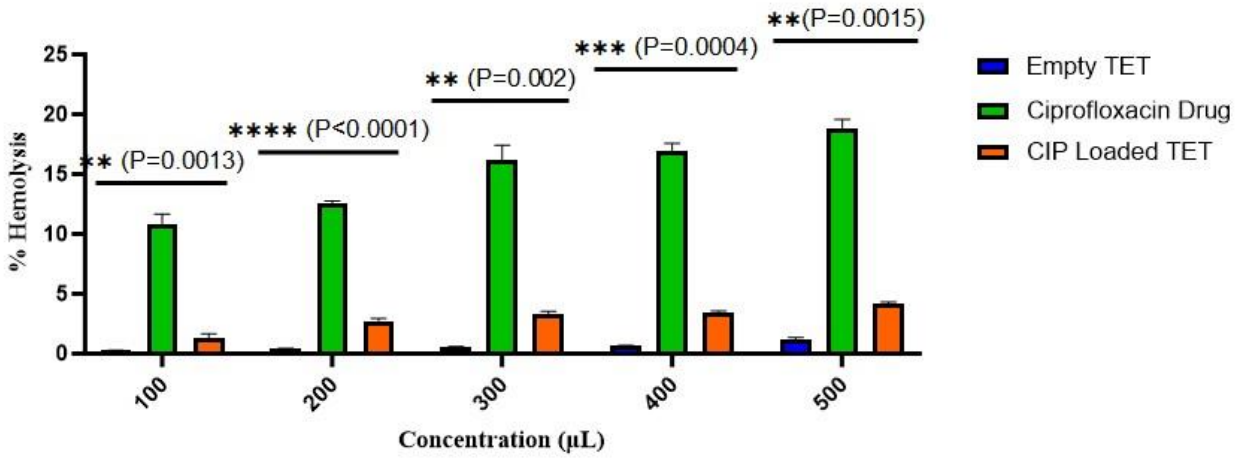


Figure 13: Blood cell viability to different concentration of Empty TET, Ciprofloxacin drug and Ciprofloxacin loaded TET

4.3.4 Determination of the Inhibitory Activity of the Test Compounds Against *S. epidermidis* and *Propionibacterium acne* Biofilms

A microtiter plate spectroscopic experiment was used to quantitatively assess the anti-biofilm activity of *S. epidermidis* and *P. acne* (Batiha et al., 2020). It is among the most popular techniques for determining a compound's antimicrobial and anti-biofilm properties (Mulat et al., 2020). The percentage of inhibitory activity of biofilm was calculated by the following formula: $[(C - B) - (T - B)/(C - B)] * 100\%$, where C = absorbance of the control (biofilm, no treatment), B = absorbance of blank (only TSB), and T = absorbance of the test (biofilm and treatment) [36]. Ciprofloxacin loaded TET exhibited 80-90% bacterial growth/biofilm inhibition as shown in figure 14.

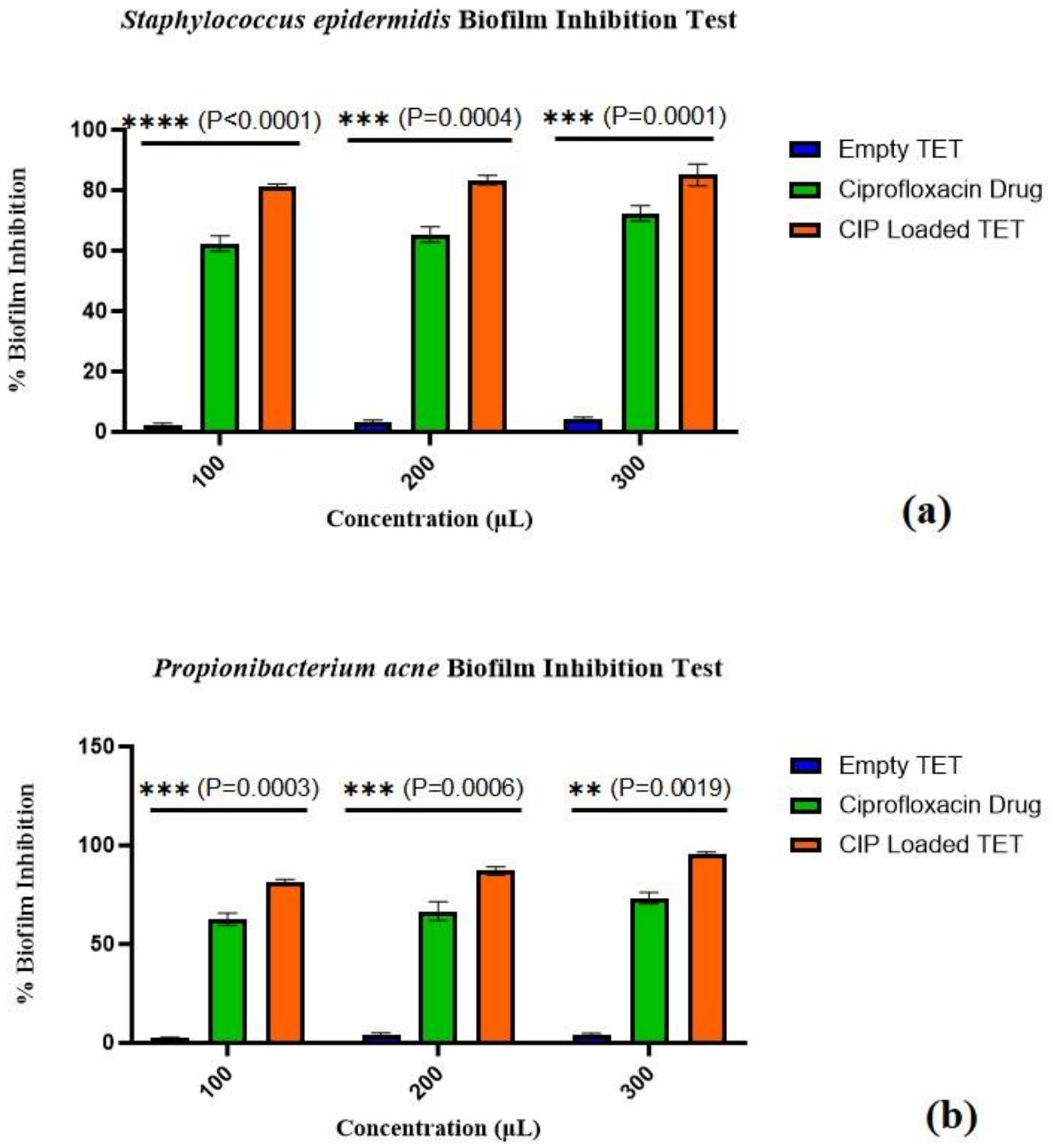


Figure 14: (a) Biofilm inhibitory test of Empty TET, Ciprofloxacin drug loaded TET against *S. epidermidis* (b) Biofilm inhibitory test of Empty TET, Ciprofloxacin drug loaded TET against *P. acne*

4.3.5 Antibacterial Activity Analysis of ciprofloxacin Loaded Transethosomes

The antibacterial activity of ciprofloxacin loaded TET, pure ciprofloxacin solution, and empty TETs (as control) were validated by testing them against Gram-positive (*S. epidermidis* and *P. acne*) bacterial strains. Following the experiment, it was noted that pure ciprofloxacin solution and ciprofloxacin loaded nanoparticle diffused into the agar and strongly suppressed bacterial growth. It is noteworthy that the concentration of empty nanoparticle and ciprofloxacin loaded nanoparticle was only 10 µg/well in comparison to the concentration of pure ciprofloxacin solution i.e., 20 µg/well. Thus, the data imply that using a very modest amount of ciprofloxacin loaded nanoparticle compared to pure ciprofloxacin solution can be equally efficient against the tested bacterial strains. Our primary findings established that CIP loaded TET shows better results than CIP alone. The results are shown in Figure 15 and 16 as a zone of inhibition.

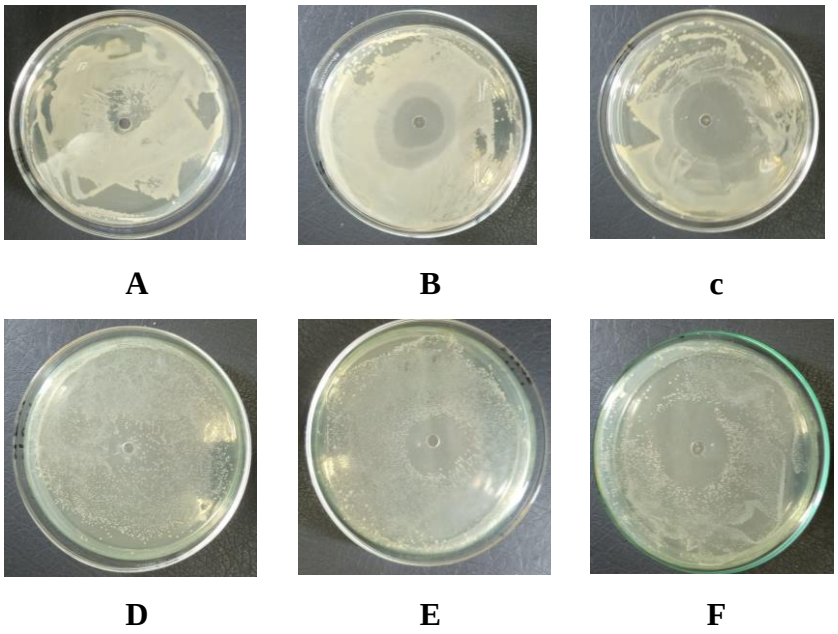


Figure 15: (a) Antibacterial activity of empty TET against *S. epidermidis* (b) Antibacterial activity of ciprofloxacin drug against *S. epidermidis* (c) Antibacterial activity of ciprofloxacin loaded TET against *S. epidermidis* (d) Antibacterial activity of empty TET against *P. acne* (b) Antibacterial activity of ciprofloxacin drug against *P. acne* (c) Antibacterial activity of ciprofloxacin loaded TET against *P. acne*.

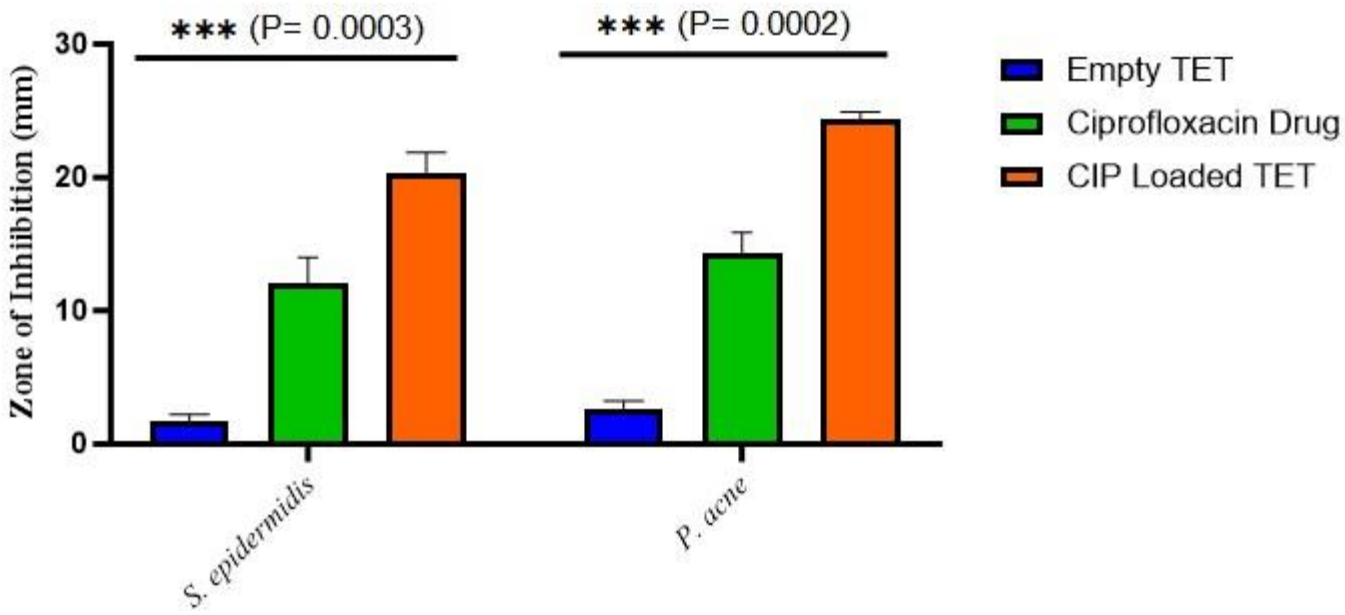


Figure 16: Antibacterial activity of Empty TET, Ciprofloxacin drug and Ciprofloxacin loaded TET against *S. epidermidis* and *P. acne*

CHAPTER 5: DISCUSSION

Bacterial infections can range in intensity from minor, self-limiting illnesses to serious, life-threatening diseases. The mechanisms by which bacteria penetrate, multiply, and cause host reactions are very important to understand for creating efficient preventive measures and treatments. . The essential phase of adherence is what enables the bacteria to withstand removal by force and sidestep the host's immune system. After adhering, some bacterial species can break down the extracellular matrix of the skin and subcutaneous tissues by producing enzymes like collagenases or hyaluronidases. Bacteria can enter deeper levels with the help of this breakdown of barriers. Additionally, bacteria frequently release toxins into the environment, such as endotoxins or exotoxins, which can harm host tissues and cells directly, causing inflammation and aiding in the spread of infection. The largest organ in the body, the skin acts as a vital defense against harmful microorganisms and other environmental aggressors. But some bacterial species have developed defense against this barrier, which can result in a variety of skin diseases. Among the most common species which are present in skin microbiota are *Staphylococcus epidermidis* and *Propionibacterium acne*, which can lead to illnesses including atopic dermatitis, and acne.

Furthermore, because of its resistance to standard medicines, community-of skin microbiota like *Staphylococcus epidermidis* and *Propionibacterium acne* has become a noteworthy concern and complicates therapy. For bacterial skin infections to be appropriately managed, which usually involves antibiotic medication, an accurate and timely diagnosis is essential. But the increasing concern about antibiotic resistance calls for cautious consumption of these drugs and emphasizes the value of preventive measures including public health campaigns, good wound care, and hygiene habits. In the current era of constantly evolving infectious diseases and rising resistance to antibiotics, finding novel drug delivery mechanisms to treat bacterial infections is critical. Using TET, a unique nanocarrier technology, to efficiently deliver quinolone antibiotics to target bacteria, is one potential strategy. Two of the most frequent organisms that cause a variety of diseases are *P. acne* and *S. epidermidis*. These bacteria are excellent subjects for studies because they frequently cause infections in the skin and soft tissues.

Transethosomes are a modern nanocarrier that is intended to improve the delivery of medicinal substances. They are a hybrid vesicular system. With special qualities including flexibility and compositional plasticity, these vesicles are an enhanced form of conventional liposomes.

Transethosomes are promising for applications in drug delivery due to their ability to encapsulate both hydrophobic and hydrophilic compounds, which is important when dealing with a wide range of pharmaceutical agents, like quinolones. In addition, their flexibility makes it simple for them to enter the stratum corneum, the skin's outermost layer, which makes them especially helpful for topical medication delivery. Antibiotics containing quinolone, such as ciprofloxacin, have long been known to be effective in treating a wide range of bacterial infections.

Ciprofloxacin is a second-generation fluoroquinolone (FQs), ciprofloxacin prevents the replication of bacterial DNA (Zhang et al., 2018). In bacteria, DNA gyrase is a crucial enzyme that causes DNA strands to supercoil. Many DNA functions, including transcription and replication, depend on this supercoiling. To prevent DNA gyrase from acting, ciprofloxacin binds to it. This puts too much tension and strain on the DNA molecule by causing positive supercoils to accumulate in front of the replication fork and negative supercoils to accumulate behind it. Topoisomerase IV is another bacterial enzyme that is inhibited by ciprofloxacin. During cell division, topoisomerase IV is essential for breaking apart double stranded DNA, (Kokot et al., 2023)DNA gyrase consist of subunits A and B. Quinolones such as ciprofloxacin are believed to prevent subunit A from resealing the DNA double-strand; therefore, single-stranded DNA may result in exonucleolytic degradation.

The ciprofloxacin loaded TET makes quinolones effective against a wide spectrum of Gram-positive and Gram-negative bacteria, including *S. epidermidis* and *P. acne*. The ability to target and destroy the diseases becomes even more attractive when combined with TET.

In this study we analyzed the antibacterial effects of ciprofloxacin by incorporating them to specific type of delivery systems which have the properties to deliver the lipid contents to the target site and maintain its originality. Human skin contains a variety of microorganisms that are divided into groups based on how they affect health and disease. *P. acne* and *S. epidermidis*, are Gram-positive, are essential constituents of skin microbiota and are widely distributed throughout the human body according to the environmental conditions of skin, such as pH, range from 4.2 to 7.9 and temperature, ranges from 31.8 to 36.6 ℃.

Propionibacterium and *S. epidermidis* are skin hosts that are involved in host defense, innate immunity, and skin homeostasis. In healthy skin conditions, both bacteria help the skin in combating harmful bacteria through a variety of mechanisms and can directly inhibit the growth

of organisms. When skin barrier is disturbed, these bacteria shift their property from commensals to pathogenicity and cause diseases like acne and atopic dermatitis.

P. acne overgrowth is linked to the inflammatory skin condition known as acne vulgaris. One of the most prevalent skin conditions is acne, which affects 27% of women and 34% of men. Atopic dermatitis (AD) is a persistent, chronic inflammatory skin disease linked to a poor ability of skin barriers to function efficiently with immunity. The condition is now two to three times more common in children. Its symptoms, which include severe itching and eczematous skin lesions, significantly lower quality of life. These conditions become severe if left untreated.

To treat these diseases many antibacterial drugs are available in the market to treat bacterial infections. Apart from their antibacterial properties, the pathogenic bacteria can develop new resistance mechanisms against the broad-spectrum antibacterial drugs to which they were once vulnerable, leading to the creation of multidrug-resistant organisms (MROs). The effectiveness of treatment significantly declines when a microorganism develops resistance to antibiotics, which restricts available therapeutic alternatives and worsens treatment results. MRO infections invariably correlate with increased death rates. To make antibiotic efficient against bacteria, ciprofloxacin loaded TET were made.

These vesicular nanoparticles increase the penetration of drug into deeper tissues across the skin. TET is beneficial for drugs delivery through the topical route. In this study TET was synthesized by using the cold technique. The synthesis of TET by the cold method is efficient for transdermal drug delivery. In this process edge activators, lipid is dissolved in a organic solvent, usually ethanol. Simultaneously, an aqueous phase is often composed of buffer solution or distilled water. Water-in-oil (w/o) emulsion is the result of the mixing of two phases working together. These vesicles improve the absorption of drugs through the skin by acting as effective drug delivery vehicles. Characterization ensures the stability and quality of the synthesized TET for pharmaceutical applications, particularly in transdermal drug delivery.

The antibacterial activity of many substances, such as medicines, plant extracts, and synthetic chemicals, can be evaluated using the widely used agar well diffusion method. In this method, a 100 µL of inoculum of microbes was obtained and a solid agar medium, typically tryptic soy agar is inoculated with a standardized solution of the target bacteria, evenly spread across the surface. Then, using a sterile cork tip, wells are made in the agar and various test compounds are introduced to them. Gradients of concentration are subsequently produced when the chemicals

permeate into the surrounding agar. A distinct, circular zone of inhibition will appear around the well if the test material has antibacterial qualities, which will prevent bacterial growth in the area. The tested compound's antibacterial efficacy is ascertained by measuring the diameter of this zone. The agar well diffusion method is a widely used technique in research, clinical laboratories, and pharmaceutical development to evaluate the efficacy of antimicrobial agents against particular bacterial strains. It is a simple and economical tool for initial screening of potential antibacterial agents. The experimental results proved that the designed formulation is potential against *S. epidermidis* and *P. acne*.

CONCLUSIONS AND FUTURE PERSPECTIVE

In summary, *Propionibacterium acne* and *Staphylococcus epidermidis*, are gram-positive bacteria are essential constituents of skin microbiota and are widely distributed throughout the human body according to the environmental conditions of skin, such as pH, range from 4.2 to 7.9 and temperature, ranges from 31.8 to 36.6 ˚C. Sometimes these bacterias shift their role to pathogenicity and cause diseases like acne and atopic dermatitis. To treat bacterial infections many antibacterial drugs like cephalosporins, penicillin, fluoroquinolones, tetracyclines etc., are available in the market. Among these antibacterial drugs Ciprofloxacin (CIP) is a broad-spectrum antibacterial drug with good antibacterial activity and good efficacy. Apart from their antibacterial properties, the pathogenic bacteria can develop new resistance mechanisms against the broad-spectrum antibacterial drugs to which they were once vulnerable, leading to the creation of multidrug-resistant organisms (MROs). To overcome this issue a novel therapy ciprofloxacin loaded transethosomes were made.

The use of transethosomes to treat bacterial skin infections is a novel and exciting development in the field of dermatology. It delivers antimicrobial drugs specifically to specific areas of the skin with improved penetration. Treatments for bacterial skin infections may be more effective if transethosomes are able to encapsulate and transfer therapeutic substances through the skin barrier. The problems with conventional topical therapies, like poor penetration, and possible resistance, may be solved by this method. Transethosome research and development show a dedication to improving therapeutic approaches for bacterial skin infections, opening the door to more efficient and user-friendly treatments.

In the last few years ciprofloxacin was incorporated in different nanoparticles to check the antibacterial activity. In this study, ciprofloxacin loaded transethosomes were synthesized and characterized. The main objective was to design a transethosomes that acts as vector for carrying an anti-bacterial drug ciprofloxacin and to check their antibacterial activity against *S. epidermidis* and *P. acne*, which would be more biocompatible than the drug alone. Ciprofloxacin loaded nanoparticles are biocompatible entities and are widely used in targeted drug delivery systems. Loaded TET contains a lower amount of drug with enhanced activity than when the drug is given alone thus reducing the side effects of to a much higher level. They also exhibit less cytotoxicity than the drug. The vector reduces opsonization by which nanoparticles are directly reaches to the skin. In the long run, Ciprofloxacin loaded TET may also increase the

circulation half time of the drug. Tailoring of loaded TET would make them more targets specific with enhanced activity. Thus, small amounts show more potential results. It is a simple and economical tool for initial screening of potential antibacterial agents. The experimental results proved that the designed formulation is potential against *S. epidermidis* and *P. acne*.

In the future landscape of dermatological therapeutics, ciprofloxacin loaded transethosomes emerge as a groundbreaking solution for combating bacterial skin infections. These advanced nano-carriers, characterized by their ability to penetrate the skin's layers effectively, hold immense promise in delivering targeted treatments for various dermatological conditions. The integration of transethosomes into skincare regimens offers a revolutionary approach, enabling precise and controlled release of antimicrobial agents at the site of infection. This not only enhances therapeutic efficacy but also minimizes systemic exposure, mitigating potential side effects. As technology continues to evolve, the customization of transethosomal formulations may become more tailored, allowing for personalized treatment strategies. The future holds the prospect of transethosomes revolutionizing the management of skin infections, fostering a new era of dermatological care that is not only highly effective but also remarkably patient-centric.

REFERENCES

Abdulbaqi, I. M., Darwis, Y., Khan, N. A. K., Assi, R. A., & Khan, A. A. (2016). Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. *International Journal of Nanomedicine*, *11*, 2279–2304. https://doi.org/10.2147/IJN.S105016

Activity, C. E. (2023). *Ciprofloxacin*. 1–9.

Ahad, A., Raish, M., Ahmad, A., Al-Jenoobi, F. I., & Al-Mohizea, A. M. (2018). Development and biological evaluation of vesicles containing bile salt of telmisartan for the treatment of diabetic nephropathy. *Artificial Cells, Nanomedicine and Biotechnology*, *46*(sup1), 532–539. https://doi.org/10.1080/21691401.2018.1430700

Ali, I. A. A., Matinlinna, J. P., Lévesque, C. M., & Neelakantan, P. (2021). Trans-cinnamaldehyde attenuates enterococcus faecalis virulence and inhibits biofilm formation. *Antibiotics*, *10*(6), 1–15. https://doi.org/10.3390/antibiotics10060702

Allam, A. A., Fathalla, D., Safwat, M. A., & Soliman, G. M. (2022). Transferosomes versus transethosomes for the dermal delivery for minoxidil: Preparation and in vitro/ex vivo appraisal. *Journal of Drug Delivery Science and Technology*, *76*(August), 103790. https://doi.org/10.1016/j.jddst.2022.103790

Babushkina, I. V., Mamonova, I. A., Ulyanov, V. Y., Shpinyak, S. P., & Bondarenko, A. S. (2020). Effects of Ciprofloxacin on the Dynamics of Biofilm Formation by Staphylococcus Epidermidis Strains Isolated from Implant-Associated Infection. *Journal of Experimental and Clinical Surgery*, *13*(3), 241–247. https://doi.org/10.18499/2070-478x-2020-13-3-241-247

Bajaj, K. J., Parab, B. S., & Shidhaye, S. S. (2021). Nano-transethosomes: A novel tool for drug delivery through skin. *Indian Journal of Pharmaceutical Education and Research*, *55*(1), s1–s10. https://doi.org/10.5530/ijper.55.1s.33

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2). https://doi.org/10.3390/biom10020202

Binnig, G., & Rohrer, H. (1986). Scanning Tunneling Microscopy. *IBM Journal of Research and Development*, *30*(4), 355–369. https://doi.org/10.2320/materia1962.25.821

Brandwein, M., Fuks, G., Israel, A., Sabbah, F., Hodak, E., Szitenberg, A., Harari, M., Steinberg, D., Bentwich, Z., Shental, N., & Meshner, S. (2019). Skin Microbiome Compositional Changes in Atopic Dermatitis Accompany Dead Sea Climatotherapy. *Photochemistry and Photobiology*, *95*(6), 1446–1453. https://doi.org/10.1111/php.13119

Brown, M. M., & Horswill, A. R. (2020). Staphylococcus epidermidis-Skin friend or foe? *PLoS Pathogens*, *16*(11), 1–6. https://doi.org/10.1371/JOURNAL.PPAT.1009026

Chen, P., He, G., Qian, J., Zhan, Y., & Xiao, R. (2021). Potential role of the skin microbiota in Inflammatory skin diseases. *Journal of Cosmetic Dermatology*, *20*(2), 400–409. https://doi.org/10.1111/jocd.13538

Cheng, X., Yan, H., Pang, S., Ya, M., Qiu, F., Qin, P., Zeng, C., & Lu, Y. (2022). Liposomes as Multifunctional Nano-Carriers for Medicinal Natural Products. *Frontiers in Chemistry*, *10*(August), 1–20. https://doi.org/10.3389/fchem.2022.963004

Chiller, K., Selkin, B. A., & Murakawa, G. J. (2001). Skin microflora and bacterial infections of the skin. *Journal of Investigative Dermatology Symposium Proceedings*, *6*(3), 170–174. https://doi.org/10.1046/j.0022-202x.2001.00043.x

Chowdary, P., Padmakumar, A., & Kumar, A. (2023). *Exploring the potential of transethosomes in therapeutic delivery : A comprehensive review*. *July*, 1–20. https://doi.org/10.1002/mba2.59

Christensen, G. J. M., & Brüggemann, H. (2014). Bacterial skin commensals and their role as host guardians. *Beneficial Microbes*, *5*(2), 201–215. https://doi.org/10.3920/BM2012.0062

Claudel, J. P., Auffret, N., Leccia, M. T., Poli, F., Corvec, S., & Dréno, B. (2019). Staphylococcus epidermidis: A Potential New Player in the Physiopathology of Acne? *Dermatology*, *235*(4), 287–294. https://doi.org/10.1159/000499858

Coenye, T., Spittaels, K. J., & Achermann, Y. (2022). The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of Cutibacterium acnes. *Biofilm*, *4*(December 2021), 100063. https://doi.org/10.1016/j.bioflm.2021.100063

Coker, T. J., & Dierfeldt, D. M. (2016). Acute bacterial prostatitis: Diagnosis and management. *American Family Physician*, *93*(2), 114–120.

Cong, T. X., Hao, D., Wen, X., Li, X. H., He, G., & Jiang, X. (2019). From pathogenesis of acne vulgaris to anti-acne agents. *Archives of Dermatological Research*, *311*(5), 337–349. https://doi.org/10.1007/s00403-019-01908-x

Costanzo, M., Esposito, E., Sguizzato, M., Lacavalla, M. A., Drechsler, M., Valacchi, G., Zancanaro, C., & Malatesta, M. (2021). Formulative study and intracellular fate evaluation of ethosomes and transethosomes for vitamin D3 delivery. *International Journal of Molecular Sciences*, *22*(10). https://doi.org/10.3390/ijms22105341

Da Silva, L. A., Ramos, L. P., Silva, T. A., De Lapena, S. A. B., Santos, C. E. R., Hasna, A. A., Bressane, A., & De oliveira, L. D. (2022). Effect of combining Zingiber officinale and Juglans regia extracts on Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis: antibiofilm action and low toxicity. *Anais Da Academia Brasileira de Ciencias*, *94*(4), 1–13. https://doi.org/10.1590/0001-3765202220201133

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, *4*(2), 104–111. https://doi.org/10.5897/JMPR09.030

Dragicevic-Curic, N., Gräfe, S., Albrecht, V., & Fahr, A. (2008). Topical application of temoporfin-loaded invasomes for photodynamic therapy of subcutaneously implanted tumours in mice: A pilot study. *Journal of Photochemistry and Photobiology B: Biology*, *91*(1), 41–50. https://doi.org/10.1016/j.jphotobiol.2008.01.009

Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A., & Roques, C. (2018). Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: a brief look at the latest updates. *Journal of the European Academy of Dermatology and Venereology*, *32*, 5–14. https://doi.org/10.1111/jdv.15043

Dryden, M., Baguneid, M., Eckmann, C., Corman, S., Stephens, J., Solem, C., Li, J., Charbonneau, C., Baillon-Plot, N., & Haider, S. (2015). Pathophysiology and burden of infection in patients with diabetes mellitus and peripheral vascular disease: focus on skin and soft-tissue infections. *Clinical Microbiology and Infection*, *21*, S27–S32. https://doi.org/10.1016/J.CMI.2015.03.024

Duangjit, S., Obata, Y., Sano, H., Onuki, Y., Opanasopit, P., Ngawhirunpat, T., Miyoshi, T., Kato, S., & Takayama, K. (2014). Comparative study of novel ultradeformable liposomes: Menthosomes, transfersomes and liposomes for enhancing skin permeation of meloxicam. *Biological and Pharmaceutical Bulletin*, *37*(2), 239–247. https://doi.org/10.1248/bpb.b13-00576

El Maghraby, G. M. M., Williams, A. C., & Barry, B. W. (2004). Interactions of surfactants

(edge activators) and skin penetration enhancers with liposomes. *International Journal of Pharmaceutics*, *276*(1–2), 143–161. https://doi.org/10.1016/j.ijpharm.2004.02.024

Ferrara, F., Benedusi, M., Sguizzato, M., Cortesi, R., Baldisserotto, A., Buzzi, R., Valacchi, G., & Esposito, E. (2022). Ethosomes and Transethosomes as Cutaneous Delivery Systems for Quercetin: A Preliminary Study on Melanoma Cells. *Pharmaceutics*, *14*(5). https://doi.org/10.3390/pharmaceutics14051038

Feynman, R. P. (2012). There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics*. In *Handbook of Nanoscience, Engineering, and Technology: Third Edition* (pp. 3–12). https://doi.org/10.1201/9781315217178-6

Fournière, M., Latire, T., Souak, D., Feuilloley, M. G. J., & Bedoux, G. (2020). Staphylococcus epidermidis and cutibacterium acnes: Two major sentinels of skin microbiota and the influence of cosmetics. *Microorganisms*, *8*(11), 1–31. https://doi.org/10.3390/microorganisms8111752

Goldstein, J., Newbury, D. E., Michael, J. R., Ritchie, N. W. M., Scott, J. H. J., & Joy, D. C. (n.d.). *Scanning electron microscopy and x-ray microanalysis*. 550.

Gonzalez, T., Stevens, M. L., Baatyrbek kyzy, A., Alarcon, R., He, H., Kroner, J. W., Spagna, D., Grashel, B., Sidler, E., Martin, L. J., Biagini Myers, J. M., Khurana Hershey, G. K., & Herr, A. B. (2021). Biofilm propensity of Staphylococcus aureus skin isolates is associated with increased atopic dermatitis severity and barrier dysfunction in the MPAACH pediatric cohort. *Allergy: European Journal of Allergy and Clinical Immunology*, *76*(1), 302–313. https://doi.org/10.1111/all.14489

Haider, M. F., Kanoujia, J., Tripathi, C. B., Arya, M., Kaithwas, G., & Saraf, S. A. (2015). Pioglitazone Loaded Vesicular Carriers for Anti-Diabetic Activity: Development and Optimization as Per Central Composite Design. *Journal of Pharmaceutical Sciences and Pharmacology*, *2*(1), 11–20. https://doi.org/10.1166/jpsp.2015.1042

Harris-Tryon, T. A., & Grice, E. A. (2022). Microbiota and maintenance of skin barrier function. *Science*, *376*(6596), 940–945. https://doi.org/10.1126/SCIENCE.ABO0693/ASSET/6844C5FF-EE47-4763-95D3-E99EBEC4FA93/ASSETS/IMAGES/LARGE/SCIENCE.ABO0693-F4.JPG

Hayder, T., Abusaiba, H., Hussein, A. A., Almahbob, T. F., & Hasan, T. H. (2023). The Efficacy of Copper Nanoparticles in Treating Viral Skin Infections: A Systematic Review and Meta-

Analysis. *Qeios*. https://doi.org/10.32388/DO0H1E

Heras-Cañas, V., Gutiérrez-Soto, B., Almonte-Fernández, H., Lara-Oya, A., Navarro-Marí, J. M., Garrido-Frenich, A., Vázquez-Alonso, F., & Gutiérrez-Fernández, J. (2017). Antibiotic activity and concentrations in clinical samples from patients with chronic bacterial prostatitis. *Actas Urológicas Españolas (English Edition)*, *41*(10), 631–638. https://doi.org/10.1016/j.acuroe.2017.10.007

Hesham, H., Rady, M., Hathout, R. M., Abdel-Halim, M., & Mansour, S. (2022). The skin delivery of tofacitinib citrate using transethosomes and hybridized ethosomes/nanostructured lipid carriers for vitiligo therapy: Dermatopharmacokinetics and in vivo assays. *International Journal of Pharmaceutics*, *629*, 122387. https://doi.org/10.1016/J.IJPHARM.2022.122387

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., & Chen, Z. S. (2022). Microbiota in health and diseases. *Signal Transduction and Targeted Therapy 2022 7:1*, *7*(1), 1–28. https://doi.org/10.1038/s41392-022-00974-4

Hung, S., Hong, Y., Lin, K., Hua, Y., Kuo, C., & Hu, A. (n.d.). *E ffi cient Photodynamic Killing of Gram-Positive Bacteria by Synthetic Curcuminoids*.

Judy, E., Lopus, M., & Kishore, N. (2021). Mechanistic insights into encapsulation and release of drugs in colloidal niosomal systems: Biophysical aspects. *RSC Advances*, *11*(56), 35110–35126. https://doi.org/10.1039/d1ra06057k

Juhas, M. (2023). The World of Microorganisms. *Brief Lessons in Microbiology*, 1–16. https://doi.org/10.1007/978-3-031-29544-7_1

Kahraman, E., Güngör, S., & Özsoy, Y. (2017). Potential enhancement and targeting strategies of polymeric and lipid-based nanocarriers in dermal drug delivery. *Therapeutic Delivery*, *8*(11), 967–985. https://doi.org/10.4155/tde-2017-0075

Karmakar, S. (2019). Particle Size Distribution and Zeta Potential Based on Dynamic Light Scattering: Techniques to Characterize Stability and Surface Charge Distribution of Charged Colloids. *Recent Trends in Materials Physics and Chemistry*, *January*, 117–159.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, *12*(7), 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011

Kokot, M., Novak, D., Zdovc, I., Anderluh, M., Hrast, M., & Minovski, N. (2023). Exploring Alternative Pathways to Target Bacterial Type II Topoisomerases Using NBTI

Antibacterials: Beyond Halogen-Bonding Interactions. *Antibiotics*, *12*(5). https://doi.org/10.3390/antibiotics12050930

Laloy, J., Minet, V., Alpan, L., Mullier, F., Beken, S., Toussaint, O., Lucas, S., & Dogné, J. M. (2014). Impact of Silver Nanoparticles on Haemolysis, Platelet Function and Coagulation. *Nanobiomedicine*, *1*, 1–9. https://doi.org/10.5772/59346

Leon, L., Chung, E. J., & Rinaldi, C. (2019). A brief history of nanotechnology and introduction to nanoparticles for biomedical applications. In *Nanoparticles for Biomedical Applications: Fundamental Concepts, Biological Interactions and Clinical Applications*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-816662-8.00001-1

Luepke, K. H., Suda, K. J., Boucher, H., Russo, R. L., Bonney, M. W., Hunt, T. D., & Mohr, J. F. (2016). Review on Antimicrobial Resistance*, 6 Editas Medicine *International Advisor Corresponding Author. *International Advisor*, *76*(45), 45–65. https://doi.org/10.1111/j.1875-9114.2016.01868.x

Madawi, E. A., Al Jayoush, A. R., Rawas-Qalaji, M., Thu, H. E., Khan, S., Sohail, M., Mahmood, A., & Hussain, Z. (2023). Polymeric Nanoparticles as Tunable Nanocarriers for Targeted Delivery of Drugs to Skin Tissues for Treatment of Topical Skin Diseases. *Pharmaceutics 2023, Vol. 15, Page 657*, *15*(2), 657. https://doi.org/10.3390/PHARMACEUTICS15020657

McDonald, E. M., Ram, F. S. F., Patel, D. V., & McGhee, C. N. J. (2014). Topical antibiotics for the management of bacterial keratitis: An evidence-based review of high quality randomised controlled trials. *British Journal of Ophthalmology*, *98*(11), 1470–1477. https://doi.org/10.1136/bjophthalmol-2013-304660

Meyerhoff, A., Albrecht, R., Meyer, J. M., Dionne, P., Higgins, K., & Murphy, D. (2004). US Food and Drug Administration approval of ciprofloxacin hydrochloride for management of postexposure inhalational anthrax. *Clinical Infectious Diseases*, *39*(3), 303–308. https://doi.org/10.1086/421491

Mohamed, M. A., Jaafar, J., Ismail, A. F., Othman, M. H. D., & Rahman, M. A. (2017). Fourier Transform Infrared (FTIR) Spectroscopy. In *Membrane Characterization*. Elsevier B.V. https://doi.org/10.1016/B978-0-444-63776-5.00001-2

Mohan Bhagyaraj, S., Oluwafemi, O. S., & Oluwafemi, O. S. (2018). Nanotechnology: The Science of the Invisible. In *Synthesis of Inorganic Nanomaterials: Advances and Key*

Technologies. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-101975-7.00001-4

Moolakkadath, T., Aqil, M., Ahad, A., Imam, S. S., Iqbal, B., Sultana, Y., Mujeeb, M., & Iqbal, Z. (2018). Development of transethosomes formulation for dermal fisetin delivery: Box–Behnken design, optimization, in vitro skin penetration, vesicles–skin interaction and dermatokinetic studies. *Artificial Cells, Nanomedicine and Biotechnology*, *46*(sup2), 755–765. https://doi.org/10.1080/21691401.2018.1469025

Moore, N. F. E., Batten, T. J., Hutton, C. E. J., White, W. J., & Smith, C. D. (2021). The management of the shoulder skin microbiome (Cutibacterium acnes) in the context of shoulder surgery: a review of the current literature. *Shoulder and Elbow*, *13*(6), 592–599. https://doi.org/10.1177/1758573220945226

Moris, V., Lam, M., Amoureux, L., Magallon, A., Guilloteau, A., Maldiney, T., Zwetyenga, N., Falentin-Daudre, C., & Neuwirth, C. (2022). What is the best technic to dislodge Staphylococcus epidermidis biofilm on medical implants? *BMC Microbiology*, *22*(1), 1–14. https://doi.org/10.1186/s12866-022-02606-x

Muhammad, W., Khan, M. A., Nazir, M., Siddiquah, A., Mushtaq, S., Hashmi, S. S., & Abbasi, B. H. (2019). Papaver somniferum L. mediated novel bioinspired lead oxide (PbO) and iron oxide (Fe2O3) nanoparticles: In-vitro biological applications, biocompatibility and their potential towards HepG2 cell line. *Materials Science and Engineering C*, *103*(February), 109740. https://doi.org/10.1016/j.msec.2019.109740

Mulat, M., Khan, F., & Pandita, A. (2020). Chemical Composition and Antibacterial, Anti-biofilm and Anti-virulence Activities of Plant Extracts Against Human Pathogenic Bacteria. *The Natural Products Journal*, *12*(1), 54–68. https://doi.org/10.2174/2210315510999200527142244

Mustarichie, R., Sulistyaningsih, S., & Runadi, D. (2020). Antibacterial Activity Test of Extracts and Fractions of Cassava Leaves (Manihot esculenta Crantz) against Clinical Isolates of Staphylococcus epidermidis and Propionibacterium acnes Causing Acne. *International Journal of Microbiology*, *2020*. https://doi.org/10.1155/2020/1975904

Nayak, D., Tawale, R. M., Aranjani, J. M., & Tippavajhala, V. K. (2020). Formulation, Optimization and Evaluation of Novel Ultra-deformable Vesicular Drug Delivery System for an Anti-fungal Drug. *AAPS PharmSciTech*, *21*(5), 1–10. https://doi.org/10.1208/s12249-020-01681-5

Nayak, D., & Tippavajhala, V. K. (2021). A comprehensive review on preparation, evaluation and applications of deformable liposomes. *Iranian Journal of Pharmaceutical Research*, *20*(1), 186–205. https://doi.org/10.22037/ijpr.2020.112878.13997

Otlewska, A., Baran, W., & Batycka-Baran, A. (2020). Adverse events related to topical drug treatments for acne vulgaris. *Expert Opinion on Drug Safety*, *19*(4), 513–521. https://doi.org/10.1080/14740338.2020.1757646

Otto, M. (2009). Staphylococcus epidermidis - The "accidental" pathogen. *Nature Reviews Microbiology*, *7*(8), 555–567. https://doi.org/10.1038/nrmicro2182

Paiva-Santos, A. C., Silva, A. L., Guerra, C., Peixoto, D., Pereira-Silva, M., Zeinali, M., Mascarenhas-Melo, F., Castro, R., & Veiga, F. (2021). Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations. *Pharmaceutical Research*, *38*(6), 947–970. https://doi.org/10.1007/S11095-021-03053-5/FIGURES/5

Qiu, L., Wang, C., Lan, M., Guo, Q., Du, X., Zhou, S., Cui, P., Hong, T., Jiang, P., Wang, J., & Xia, J. (2021). Antibacterial Photodynamic Gold Nanoparticles for Skin Infection. *ACS Applied Bio Materials*, *4*(4), 3124–3132. https://doi.org/10.1021/acsabm.0c01505

Rahmdel, S., & Götz, F. (2021). The Multitasking Surface Protein of Staphylococcus epidermidis: Accumulation-Associated Protein (Aap). *MBio*, *12*(5), 1–4. https://doi.org/10.1128/mBio.01989-21

Rodríguez-Luna, A., Talero, E., Ávila-Román, J., Romero, A. M. F., Rabasco, A. M., Motilva, V., & González-Rodríguez, M. L. (2021). Preparation and In Vivo Evaluation of Rosmarinic Acid-Loaded Transethosomes After Percutaneous Application on a Psoriasis Animal Model. *AAPS PharmSciTech*, *22*(3), 1–18. https://doi.org/10.1208/s12249-021-01966-3

Rose, G., Crocco, P., Agrawal, R., Hu, A., & Bollag, W. B. (2023). The Skin and Inflamm-Aging. *Biology 2023, Vol. 12, Page 1396*, *12*(11), 1396. https://doi.org/10.3390/BIOLOGY12111396

Ruchiatan, K., Rizqandaru, T., Satjamanggala, P. R., Tache, N., Cahyadi, A. I., Rezano, A., Gunawan, H., Sutedja, E. K., Dwiyana, R. F., Hidayah, R. M. N., Achdiat, P. A., Sutedja, E., Suwarsa, O., & Hindritiani, R. (2023). Characteristics of Biofilm-Forming Ability and Antibiotic Resistance of Cutibacterium acnes and Staphylococcus epidermidis from Acne Vulgaris Patients. *Clinical, Cosmetic and Investigational Dermatology*, *16*, 2457–2465.

https://doi.org/10.2147/CCID.S422486

Sabitha, M., Sanoj Rejinold, N., Nair, A., Lakshmanan, V. K., Nair, S. V., & Jayakumar, R. (2013). Development and evaluation of 5-fluorouracil loaded chitin nanogels for treatment of skin cancer. *Carbohydrate Polymers*, *91*(1), 48–57. https://doi.org/10.1016/j.carbpol.2012.07.060

Saeb, A. T. M., Alshammari, A. S., Al-Brahim, H., & Al-Rubeaan, K. A. (2014). Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. *Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/704708

Saeed, U., Insaf, R. A., Piracha, Z. Z., Tariq, M. N., Sohail, A., Abbasi, U. A., Fida Rana, M. S., Gilani, S. S., Noor, S., Noor, E., Waheed, Y., Wahid, M., Najmi, M. H., & Fazal, I. (2023). Crisis averted: a world united against the menace of multiple drug-resistant superbugs - pioneering anti-AMR vaccines, RNA interference, nanomedicine, CRISPR-based antimicrobials, bacteriophage therapies, and clinical artificial intelligence strategies to safeguard global antimicrobial arsenal. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/FMICB.2023.1270018

Sakai, K. (2019). Atomic Force Microscope (AFM). *Measurement Techniques and Practices of Colloid and Interface Phenomena*, *56*(9), 51–57. https://doi.org/10.1007/978-981-13-5931-6_8

Sala, M., Diab, R., Elaissari, A., & Fessi, H. (2018). Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. *International Journal of Pharmaceutics*, *535*(1–2), 1–17. https://doi.org/10.1016/j.ijpharm.2017.10.046

Schaming, D., & Remita, H. (2015). Nanotechnology: from the ancient time to nowadays. *Foundations of Chemistry*, *17*(3), 187–205. https://doi.org/10.1007/s10698-015-9235-y

Sharma, P. C., Jain, A., Jain, S., Pahwa, R., & Yar, M. S. (2010). Ciprofloxacin: Review on developments in synthetic, analytical, and medicinal aspects. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *25*(4), 577–589. https://doi.org/10.3109/14756360903373350

Shi, J., Votruba, A. R., Farokhzad, O. C., & Langer, R. (2010). Nanotechnology in drug delivery and tissue engineering: From discovery to applications. *Nano Letters*, *10*(9), 3223–3230. https://doi.org/10.1021/nl102184c

Shi, Z., Zhou, Y., Fan, T., Lin, Y., Zhang, H., & Mei, L. (2020). Inorganic nano-carriers based smart drug delivery systems for tumor therapy. *Smart Materials in Medicine*, *1*(March), 32–47. https://doi.org/10.1016/j.smaim.2020.05.002

Singh, R., Ray, P., Das, A., & Sharma, M. (2010). Penetration of antibiotics through Staphylococcus aureus and Staphylococcus epidermidis biofilms. *Journal of Antimicrobial Chemotherapy*, *65*(9), 1955–1958. https://doi.org/10.1093/jac/dkq257

Song, C. K., Balakrishnan, P., Shim, C. K., Chung, S. J., Chong, S., & Kim, D. D. (2012). A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and in vitro/in vivo evaluation. *Colloids and Surfaces B: Biointerfaces*, *92*, 299–304. https://doi.org/10.1016/j.colsurfb.2011.12.004

Song, H., Wen, J., Li, H., Meng, Y., Zhang, Y., Zhang, N., & Zheng, W. (2019). Enhanced transdermal permeability and drug deposition of rheumatoid arthritis via sinomenine hydrochloride-loaded antioxidant surface transethosome. *International Journal of Nanomedicine*, *14*, 3177–3188. https://doi.org/10.2147/IJN.S188842

Subramani, K., Elhissi, A., Subbiah, U., & Ahmed, W. (2019). Introduction to nanotechnology. In *Nanobiomaterials in Clinical Dentistry*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-815886-9.00001-2

Tan, S. Y., Khan, R. A., Khalid, K. E., Chong, C. W., & Bakhtiar, A. (2022). Correlation between antibiotic consumption and the occurrence of multidrug-resistant organisms in a Malaysian tertiary hospital: a 3-year observational study. *Scientific Reports*, *12*(1), 1–9. https://doi.org/10.1038/s41598-022-07142-2

Tiwari, A. K., Mishra, A., Pandey, G., Gupta, M. K., & Pandey, P. C. (2022). Nanotechnology: A Potential Weapon to Fight against COVID-19. *Particle and Particle Systems Characterization*, *39*(1), 1–26. https://doi.org/10.1002/ppsc.202100159

Unemo, M., Lahra, M. M., Cole, M., Galarza, P., Ndowa, F., Martin, I., Dillon, J. A. R., Ramon-Pardo, P., Bolan, G., & Wi, T. (2019). World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): Review of new data and evidence to inform international collaborative actions and research efforts. *Sexual Health*, *16*(5), 412–425. https://doi.org/10.1071/SH19023

Velmurugan, P., Lee, S. M., Cho, M., Park, J. H., Seo, S. K., Myung, H., Bang, K. S., & Oh, B. T. (2014). Antibacterial activity of silver nanoparticle-coated fabric and leather against odor

and skin infection causing bacteria. *Applied Microbiology and Biotechnology*, *98*(19), 8179–8189. https://doi.org/10.1007/S00253-014-5945-7/FIGURES/6

Walduck, A., Sangwan, P., Vo, Q. A., Ratcliffe, J., White, J., Muir, B. W., & Tran, N. (2020). Treatment of Staphylococcus aureus skin infection in vivo using rifampicin loaded lipid nanoparticles. *RSC Advances*, *10*(55), 33608–33619. https://doi.org/10.1039/D0RA06120D

Wang, Yanhan, Kuo, S., Shu, M., Yu, J., Huang, S., Dai, A., Two, A., Gallo, R. L., & Huang, C. M. (2014). Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of Propionibacterium acnes: Implications of probiotics in acne vulgaris. *Applied Microbiology and Biotechnology*, *98*(1), 411–424. https://doi.org/10.1007/s00253-013-5394-8

Wang, Yanhong, Wang, R., Qi, X., Li, W., Guan, Q., Wang, R., Li, X., Li, Y., Yang, Z., & Feng, Y. (2021). Novel transethosomes for the delivery of brucine and strychnine: Formulation optimization, characterization and in vitro evaluation in hepatoma cells. *Journal of Drug Delivery Science and Technology*, *64*, 102425. https://doi.org/10.1016/j.jddst.2021.102425

Wu, X. W., Ji, H. Z., & Wang, F. Y. (2015). Meta-analysis of ciprofloxacin in treatment of crohn's disease. *Biomedical Reports*, *3*(1), 70–74. https://doi.org/10.3892/br.2014.368

Zhang, G. F., Liu, X., Zhang, S., Pan, B., & Liu, M. L. (2018). Ciprofloxacin derivatives and their antibacterial activities. *European Journal of Medicinal Chemistry*, *146*, 599–612. https://doi.org/10.1016/j.ejmech.2018.01.078

Zhu, Y., Yu, X., & Cheng, G. (2023). Human skin bacterial microbiota homeostasis: A delicate balance between health and disease. *MLife*, *2*(2), 107–120. https://doi.org/10.1002/mlf2.12064