Encapsulation of Lauric Acid inside the Polymers and use against Sweat Bacteria

Wajeeha Shah

(Registration no: 0000362826)

Department of Biomedical Engineering & Sciences

School of Mechanical and Manufacture Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

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Wajeeha Shah

(Registration no: 0000362826)

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Supervisor: Dr. Nosheen Fatima Rana

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Signature: 

Name (Supervisor): Nosheen Fatima Rana

Date: 11 - Mar - 2024

Signature (HOD): 

Date: 11 - Mar - 2024

Signature (DEAN): 

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National University of Sciences & Technology (NUST)

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We hereby recommend that the dissertation prepared under our supervision by: Wajeeha Shah (00000362826)
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Examination Committee Members

1. Name: Muhammad Asim Waris Signature:

2. Name: Aneeqa Noor Signature:

Supervisor: Nosheen Fatima Rana Signature:

Date: 11 - Mar - 2024

11 - Mar - 2024

Head of Department Date

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This is to certify that the research work presented in this thesis, entitled "**Encapsulation of Lauric Acid inside the Polymers and use against Sweat Bacteria''** was conducted by Mr./Ms **Wajeeha shah** under the supervision of **Doctor Nosheen Fatima Rana.** No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the School of Mechanical and Manufacture Engineering in partial fulfilment of the requirements for the degree of Master of Science in Field of Biomedical sciences Department of **SMME** National University of Sciences and Technology, Islamabad.

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Supervisor Name: Dr Nosheen Fatima Rana Signature:

Name of HOD: Dr Asim Waris Signature:

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I certify that this research work titled **''Encapsulation of Lauric Acid inside the Polymers and use against Sweat Bacteria''** is my work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledge referred.

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Dedicated to my exceptional parents and friends and my supervisor whose Tremendous support and cooperation led me to this Wonderful Accomplishment.

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ABSTRACT

In society, the problem of human odor has caused serious problems. The development of Disagreeable odor on the skin of contemporary humans is the consequence of the commensal microbiota converting naturally released, odourless precursor molecules into Volatile odorants. Human odour is a major problem in society which originates from commensal bacteria that converts odourless compounds into volatile odorants on the skin that cause bad smell. The majority of the axillary bacteria consist of gram-positive bacteria such as *Corynebacterium*, *Propionibacterium* and *Staphylococcus*. There is a need for a deodorant/antiperspirant composition that successfully avoids body odor without irritating skin or having unfavourable effects, considering the shortcomings of current solutions. Use of essential oils and maintaining good hygiene play vital role in maintaining the microbiota development. The existing deodorants are problematic because they contain aluminium and other toxins that can affect the skin so a natural alternative that avoids irritants like aluminium and toxins is recommended. This research paper emphasized the synthesis of deodorants containing the naturally occurring fatty acids like Lauric acid, which is well known for its antibacterial properties. Polymers e.g. Chitosan, Polyvinyl alcohol and Gelatin is use in this formulation. Lecithin and Ethanol are used to dissolve lauric acid in the gel. Remarkably, neither aluminium nor parabens are present in the deodorant because they are connected to cancer and hormone imbalance.

Keywords: Lauric acid, chitosan, deodorants, *Corynebacterium*, aluminium, lecithin, unfavourable, odorants.

CHAPTER 1: INTRODUCTION

1.1 Lauric acid

Lauric Acid is plentiful in nature and comes from primary sources like VCO(virgin coconut oil) and palm kernel oil(Ubgogu, Onyeagba, & Chigbu, 2006). It is famous as a beneficial saturated fatty acid (Jadhav et al., 2013).

With the molecular formula C12H24O2, the chemical structure is made up of 12 carbon (C), 24 hydrogen (H), and 2 oxygen (O) atoms. Lauric Acid is a white solid that has a molecular weight of 200.32 g mol–1 and a melting point of 43–44°C. Furthermore, it is a fatty acid that can be made via the Colgate-Emery steam process, the base-catalyzed hydrolysis of pure fatty acid methyl ester, and the hydrolysis reaction of vegetable oils utilizing an inorganic-base catalyst or a lipase enzyme.

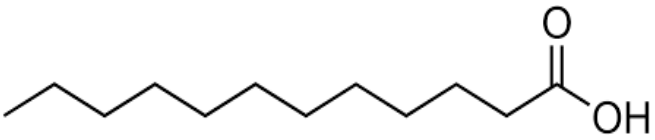


Figure 1.1: Chemical structure of Lauric acid

According to chemical structure, the presence of the -OH group and the oxygen atom in the carbonyl group appears to be responsible for the hydrophilic qualities of lauric acid. Pathogenic microorganisms' cell walls' polar portion can create hydrogen bonds with both functional groups. Lauryl groups, on the other hand, contribute to its lipophilic qualities. They may create Van der Waals interactions with the non-polar regions of microorganisms' cell walls. Lauric Acid's surfactant qualities improve its interaction with cell walls to prevent and even eradicate pathogenic organisms. Furthermore, a wide range of antibacterial properties of lauric Acid against bacteria, fungi, and viruses have been established(Fischer et al., 2012).

1.2 Source of Lauric acid

About half of the fatty acid content of coconut milk, coconut oil, laurel oil, and palm kernel oil (which should not be confused with palm oil) is comprise of lauric Acid, which is a component of triglycerides. If not, it is not very common. Moreover, goat's milk (3.1%), cow's milk (2.9%), and human breast milk (6.2% of total fat) all include it(Beare-Rogers, Dieffenbacher, & Holm, 2001).

Plant-based sources of LA include a variety of vegetable fats, including those derived from coconut and palm kernel oils. While the amount of LA present in food is safe for health, there is inadequate data to determine whether it has therapeutic value. LA can be present in human breast milk, cow's milk, and goat's milk, among other animal resources. However, the process of extracting resources from plants is far more efficient than that of extracting resources from animals(Aghedo, Owolabi, & Ogbeide, 2021).

Figure 1.2: Physical appearance of Lauric acid

1.3 Health benefits of Lauric Acid

Medium-chain triglycerides, or MCFA, have a friendly safety profile and are used to treat a wide range of conditions, including the maintenance of cholesterol levels (Shilling et al., 2013) weight loss (Tsuji et al., 2001), immunomodulatory effects (Nurul-Iman, Kamisah, Jaarin, & Qodriyah, 2013), cardiovascular uses (Zakaria et al., 2011)and more recently, hepatic toxicity (Wang, Wu, Simonavicius, Tian, & Ling, 2006) in Alzheimer's disease (Augustin et al., 2018).Several pharmacological characteristics of LA have been demonstrated, such as anti-diabetic (D. L. Sheela et al., 2017)hypotensive in spontaneously hypertensive rats (Júnior et al., 2021), and neuronal cell protection through the promotion of ketone body synthesis (Nonaka et al., 2016).

It is implied that MCFAs have a brief half-life in the body and are unlikely to cause obesity by direct storage in adipocytes since they are less effectively stored than other fatty acids and are

extremely vulnerable to oxidative metabolism once consumed (Bach, Ingenbleek, & Frey, 1996).

It has been suggested that individuals trying to control their weight should use MCTs as an oil source (Papamandjaris, MacDougall, & Jones, 1998).

By encouraging signalling through specific toll-like receptors—TLR2 heterodimers and TLR4 homodimers—LA can activate macrophages (Huang et al., 2012).

Several studies on lauric acid's anticancer effects in vitro have been reported. Lauric Acid is one of the saturated medium-chain fatty acids that has been demonstrated to be an extremely effective cancer therapy. Utilizing human colorectal cancer cell lines (HCT-15, HCT-116, and CACO2), the effects of several medium-chain fatty acid classes (capric, caprylic, caproic, and lauric acids) were examined. Cell growth was shown to be dose-dependently inhibited by lauric acid (D. Sheela, Narayanankutty, Nazeem, Raghavamenon, & Muthangaparambil, 2019).

For children with refractory epilepsy, the high-fat, low-carb ketogenic diet is frequently utilized as a therapy. The application of the diet seems promising in the treatment of epilepsy, according to a Cochrane evaluation of the classic ketogenic diet (Levy & Cooper, 2003). Decanoic acid inhibits the expression of the AMPA receptor by binding to it. In hippocampal slices, it also suppresses excitatory neurotransmission. The anti-convulsant action of medium-chain fatty acids is promote by this mechanism (Chang et al., 2016).

The influence of ketones on brain metabolism underscores the promise of the ketogenic diet to cure the underlying metabolic abnormalities that underlie Alzheimer's disease. Medium-chain fatty acids influence the absorption and metabolism of medium-chain triglycerides (MCTs) into ketone bodies. Typically, glucose serves as the brain's primary energy substrate. The liver produces the ketones, β-hydroxybutyrate (β-HB) and acetoacetate (AcAc), when there is a fast or restriction on carbohydrates in the diet.

Ketones can supply up to 80% of the brain's energy needs during extended periods of fasting. The impact of ketones on brain metabolism underscores the promise of the ketogenic diet to cure the underlying metabolic abnormalities that underlie Alzheimer's disease. Medium-chain fatty acids improve the absorption and metabolism of medium-chain triglycerides (MCTs) into ketone bodies. Typically, glucose serves as the brain's primary energy substrate. The liver produces the ketones, β-hydroxybutyrate (β-HB) and acetoacetate (AcAc), when there is a fast or restriction on carbohydrates in the diet. Ketones can supply up to 80% of the brain's energy

needs during extended periods of fasting (Han, Ramprasath, & Zou, 2020). Moreover, research suggests that MCFA, may be able to lessen neuronal hyperactivity, a common feature of AD, and enhance mitochondrial function (Reger et al., 2004). Nowadays, it is believed that ketones may be used as a therapeutic approach to treat a variety of illnesses, including diabetes, cancer, heart disease, and neurodegeneration (Henderson et al., 2009).

MCT and its component MCFA were extracted from milk and skin lipids. They have been explore as a potential antibiotic substitute for animals because they directly inhibit a range of pathogenic gram-positive and gram-negative bacteria, including Clostridium perfringens, Listeria monocytogenes, and Campylobacter jejuni, at high concentrations (Fischer et al., 2012).

1.4 Polymers

Any natural or synthetic material designed to collaborate with biological processes to guide medical therapy is referred to as a biomaterial(Williams, 2009). Biomaterials have to be biocompatible, which means they have to work with the right kind of host responsive.

Polymers play a significant role in many commercial products and technologies, including industrial parts, household goods, medical components, packaging, and other items. Polymers' ability to be blended in a variety of formulations to produce products with a wide range of physical and chemical qualities is their fundamental benefit(Fakirov, 2017). Hence, the physicochemical properties of the polymer can be modified to suit a particular application. These properties are contingent upon the type of polymer (linear, grafted, and cross-linked), its molecular weight, and polydispersity, as well as the nature and chemical structure of the monomer (aliphatic, aromatic, polar, nonpolar, or containing specific functional groups) and its configuration (blocks, branched, or random) in the case of a copolymer. Additionally, blends including various suitable polymers can be created to produce a range of end qualities(Pillay et al., 2013).

Three broad categories of polymers exist: semi-synthetic, biopolymers, and synthetic polymers. The unique qualities of synthetic polymers include their comparatively easy large-scale synthesis and their ability to be modified to acquire precise and specified features(Englert et al., 2018).

Poly (lactic acid) (PLA), Poly (caprolactone) (PCL), Poly (ethylene glycol) (PEG), Poly (vinyl alcohol) (PVA), Poly (acrylic acid) (PAA), and Poly (2-hydroxyethyl methacrylate) (PHEMA)

are a few examples of polymers that are commonly employed in analytical applications in medicine. These polymers have several main advantages, most relating to their high mechanical, biological, and pH stability, which allows them to be used for extended periods in a variety of biological media at varying temperatures and pH levels without significantly changing their efficiency(Cardoso et al., 2020).

The most prevalent natural Polymers, also known as biopolymers(Deb, Kokaz, Abed, Paradkar, & Tekade, 2019), include proteins, polyesters, and polysaccharides that come from both plant and animal worlds (Aravamudhan, Ramos, Nada, & Kumbar, 2014). These materials exhibit superior biocompatibility and reduced cytotoxicity when compared to Synthetic Polymers (Mkedder et al., 2013). Biopolymers like Proteins, Nucleic acids, and Polysaccharides are utilized in a wide range of analytical procedures; chitosan is the most often used, followed by cellulose, alginate, pectin, chitin, starch, and lignin(Beata Łabowska, Michalak, & Detyna, 2019). Naturally occurring Polymers were significantly more widely employed in biological applications than Synthetic Polymers, which were initially used in the 1960s(Kaur, Mehta, & Gupta, 2018). Often used, naturally occurring polymers fall into three categories: Proteins (silk, collagen, soy, fibrin gels), Polysaccharides (chitin/chitosan, alginate, and derivatives of hyaluronic acid), and Polynucleotides (DNA and RNA) (ALEXANDER et al., 1996).

1.5 Polymeric hydrogel

Cross-linked networks known as hydrogels can absorb vital amounts of solvent without disintegrating. Depending on the type of solvent used in the gel, these substances can be categorized as hydrogel or sol-gel. Sol gels contain organic solvents, but hydrogels contain hydrophilic functional groups linked to the polymer backbone (Okay, 2010).

Hydrogels are networks of hydrophilic polymers that are insoluble in water and have a hydrophilic quality (Abdeen, 2011). Polymeric hydrogels are widely used in many domains, including sophisticated technology, agriculture, health, industry, pharmacology (Buchholz & Peppas, 1994), and environmental protection (Abdeen, 2011).

Hydrogels are a type of material possessing special physical qualities that allow them to be used in many different biomedical applications (Hoffman, 2012). Hydrogels have so found extended application as scaffolds in the fields of tissue engineering (Lee & Mooney, 2001),restorative medicine (OZ, Khademhosseini, & Peppas, 2009), diagnostics (Buenger, Topuz, & Groll, 2012), biomolecules separation membranes (Kim & Park, 1998), or drug

encapsulation, transportation, and release matrixes(Graham & McNeill, 1984). Hydrogels' primary attributes, including their high water content, high porosity, high deformability, and moldability, as well as their adaptability—most of which can be simply adjusted by varying the degree of crosslinking—represent significant benefits that support their use in biomedicine(Saha, Saarai, Roy, Kitano, & Saha, 2011). Particularly, chitosan-based hydrogels play a significant role in this class of soft materials due to their high biocompatibility and consequent applicability, which is assist by both the high water content and the inherent qualities of chitosan, which include stability, susceptibility to enzymatic degradation, nontoxicity, and so forth (Ahmadi, Oveisi, Samani, & Amoozgar, 2015). Furthermore, from a compositional and mechanical standpoint, the hydrogels based on polysaccharides, such as chitosan, provide the benefits of being comparable to the extracellular matrix outside of cells (El-Sherbiny & Yacoub, 2013).

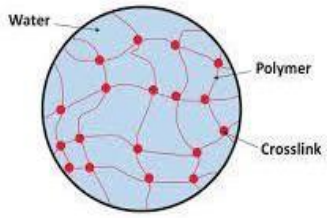


Figure 1.3: **Polymeric hydrogel**

1.6 Skin-friendly deodorants

Sweating in the body helps us to regulate body temperature. Although it is very important for the body because it helps us to maintain our body temperature but high level of sweating sometimes causes the person to feel uneasy in public places due to bad smell and stains in clothes. Various bacteria are present in human skin that produce various types of chemicals that give a bad smell. Defendants are products that are applied to the skin to kill bacteria that produce sweat and are sometimes used to suppress the odor produced by bacteria.

Triclosan is the most important antibacterial agent that is used in various personal care products such as deodorants, shampoo, and toothpaste. It can kill the bacteria but there are some safety concerns about safety and their environmental impact. Another important element that is used in deodorants to kill bacteria is Aluminium, but aluminium is not skin skin-friendly product because it can be seen that aluminium is related to Skin Cancer, particularly Breast Cancer.

Breast cancer is common in most of those people who use Aluminium in skin care products but still, there is insufficient data available on it. So we need most of those skin care products including deodorants that are Aluminium free and environment-friendly. There is a lot of demand to develop deodorants that control sweat and eliminate unpleasant smells without affecting human health (Arora et al., 2022).

CHAPTER 2: LITERATURE REVIEW

2.1 Polymers in the Cosmetics industry

Cosmetics and personal care products are any material or mixture of materials that comes into contact with the external surfaces of the human body, such as the lips, nails, skin, external genital organs, or the teeth and mucous membranes of the oral cavity, with the intention of cleaning, enhancing scent, altering appearance, preserving, or regulating body odours (Savary, Grisel, & Picard, 2016).

Based on these standards, any material meant to be an ingredient in a cosmetic product is included in the category of cosmetics and personal care products, as are Creams, Emulsions, lotions, Gels, and Oils created with the intention of Cleaning, Moisturising, and makeup application for the skin and hair. Natural and edible Polymers are utilized as Conditioners, hydrating agents, water-soluble binders, thickeners, rheological modifiers, sensory compounds, active substances, texturing agents, and conditioners in personal care products and cosmetics (Bais, Trevisan, Lapasin, Partal, & Gallegos, 2005).

Rheology modifiers, such as Polymers, are used in cosmetics and personal hygiene products. Here, the polymers are introduced to make the formulation more viscous and, frequently, gel. Sometimes natural polymers such as polysaccharides, starch, xanthan gum, guar gum, carrageenan, alginates, pectin's, gelatin, agar, etc. are used to improve the viscosity of water-based systems (Laba, 1993).

2.2 Polymers in Biomedical and Pharmaceuticals

In the field of biomedicine, edible polymers find extensive use in wound dressing, protein release, tissue engineering, regenerative medicine, medication delivery, and biomedical devices, among other areas. Emulsions stabilized with edible particles can be used as a vehicle for the delivery of active ingredients including medications, biological cues, antioxidants, etc. The creation of a thick coating of ingestible particles around the droplets helps to regulate the release of the drug within the inner polymer layer. In rare circumstances, the loaded medication may additionally interact with the core layer to provide a prolonged release profile (Frelichowska et al., 2009).

The rapid advancement of drug delivery Nano devices can be ascribed to the latest developments in nanofabrication techniques and nanotechnology. Owing to their smaller size in comparison to traditional delivery methods, these Nano devices provide focused delivery, which reduces adverse effects and extends the duration of release. Nowadays, a range of drug delivery Nano devices, including Liposomes, Nanoparticles, Dendrimers, Micelles, and Nanorods, have been created using Biodegradable Polymers. (Felice, Prabhakaran, Rodríguez, & Ramakrishna, 2014).

The investigation of Edible Polymers in film form for medication delivery is the main focus of ongoing research. This covers their usage in the oral delivery of drugs that need to be absorbed quickly, like those for allergic reactions, sleep issues, and nervous system problems. Furthermore, topical distribution of pharmacological agents, such as analgesics and antibacterial drugs, is being investigated for edible polymers in film form, specifically for uses in wound healing (Bala, Pawar, Khanna, & Arora, 2013), When medications are delivered through the vagina, a film is physically applied and reacts with the vaginal fluid to form a hydrogel. Soluble films are used in gastro-retentive drug administration to carry water-soluble and weakly soluble compounds that can be broken down by the gastrointestinal tract's pH or enzyme production. (Tiwari, Umashankar, & Damodharan, 2018).

The use of edible polymer hydrogels as extracellular matrix in Tissue Engineering and regenerative medicine has been extensively studied. A hydrogel based on Chitosan was developed by Gao et al to carry mesenchyme stem cells (MSCs) for the treatment of kidney injury. (Gao et al., 2012).

Hydrogel materials made of edible polymers have been studied extensively as Wound dressings outside of Tissue Engineering. Their outstanding mechanical and barrier qualities, little cytotoxicity, biocompatibility, and simplicity of disposal account for this. Alginate-based wound dressings are widely recognized in academic literature and from a business perspective, particularly for haemorrhaging wounds. Because of its capacity to create a gel, it can absorb liquids in a less sticky way, remove dressings with minimal stress, and lessen the associated pain. Additionally, it creates a humid atmosphere that promotes quick granulation and re-epithelialization (Chakravarthy, 2019).

2.3 Polymeric hydrogels

Polymeric networks that can absorb a lot of water yet remain insoluble due to chemical or physical cross-linking are known as hydrogels(Buhus, Peptu, Popa, & Desbrieres, 2009).

Three-dimensional network systems are hydrogels. Polymeric chain cross-linking leads to the formation of the system. Physical contacts, covalent bonds, hydrogen bonds, and van der Wall interactions can all result in cross-linking(Vashist & Sharif, 2013).

Because of their intelligence, hydrogels can react to changes in their environment, including changes in pH, temperature, ionic strength, electrostatic field, and enzyme presence(Kalshetti, Rajendra, Dixit, & Parekh, 2012).

Hydrophilic functional groups that are affixed to hydrogels cause them to be able to absorb water (Ahmed, 2013). They mimic normal tissues because they absorb 90% of the water(Kalshetti et al., 2012).

It is possible to create hydrogels using synthetic or natural polymers. Among the natural polymers are Alginate, Pectin, Chondroitin sulphate, and Dextran, While Poly(vinyl alcohol) is one type of synthetic polymer, Poly (ethylene oxide), Poly (hydroxyethyl methacrylate), and Poly (nisopropylacrylamide) (Crispim et al., 2012).

PHGs are substances that have network structure and viscoelastic characteristics brought about by the solvent and the cross-linker, respectively. PHG compounds are increasingly present in everyday products like soap, shampoo, toothpaste, hair gel, and contact lenses, depending on their intended use(Mailra, 2005). PHGs are made up of three-dimensional networks that are created when polymer chains are cross-linked. These polymers' cross-linking is caused by covalent bonds, hydrogen bonds, van der Waals forces, or physical entanglements(Laftah, Hashim, & Ibrahim, 2011).

PHG materials are categorized according to various viewpoints. They are divided into five categories based on their morphology: particle, powder, spherical, fiber, membrane, and emulsion. PHG can also be separated into natural macromolecules, semi-synthesized polymers, and synthesized polymers from the perspective of material resources. They are categorized as radiation cross-linking, graft polymerization, cross-linking polymerization, and network production of water-soluble polymer from the perspective of the preparation process. Additionally, PHG can be categorized based on cross-linking type, environment response, and original constitution(B. Singh, Sharma, & Gupta, 2008).

2.4 Applications of hydrogel

Hydrogels have become increasingly significant for various uses because of their unique properties, which include softness, compatibility, softness, flexibility and high water content. One of the main qualities of hydrogels is their similarity to biological tissues, which may open up new possibilities for them in the food and pharmaceutical industries. Hydrogels are now often utilised in the manufacturing of food additives(X. Chen et al., 1995), Tissue engineering, Agriculture(Saxena, 2010),Biomedical applications(Seliktar, 2012),Pharmaceuticals(Plunkett & Moore, 2004), and Hygiene products (Singh et al. 2010) and Biosensors(Peppas & Van Blarcom, 2016).

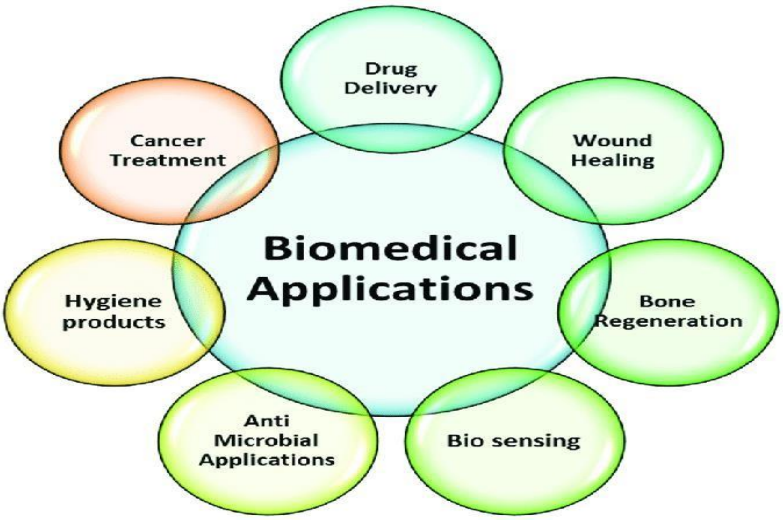


Figure 2.1: Biomedical application of hydrogel

2.5 PHGs for transdermal drug delivery

Compared to other medication administration techniques, the transdermal drug delivery system is superior in many ways, including a) first-pass metabolism through the liver; b) easy dosing; and c) patient-controlled start and stop of therapy. There are numerous transdermal dose forms available, including gels, Ointments, Creams, and more(Jahan, Ferdaus, Shaheen, Sultan, & Mazid, 2011).

Gels are a type of material that resembles jelly and can be solid, hard, or tough. Dilute cross-linked systems without flow in steady state conditions are referred to as gels. Additional classifications for gels include hydrogels, xerogels, and organogels(Buhus et al., 2009).

When used topically or trans dermally, Hydrogels offer numerous benefits, such as avoiding hepatic metabolism, which boosts medication efficacy and bioavailability(Zaman et al., 2015)

These days, Hydrogels are more popular because of their regulated and prolonged drug release. The medications can be released at appropriate and precise locations. The adoption of a transdermal medication delivery method allows for consistent drug release. Unlike other dosage forms like patches and ointments, hydrogels can be removed with ease because of their swelling and resemblance to live tissues. Drugs can be administered topically or systemically using transdermal drug administration. For example, transdermal hydrogels containing glucocorticoids and budesonide are made(Trookman, Rizer, Ford, & Trancik, 2007).

Hydrogels can have macroporous, microporous, or Nano porous network structures. Drug release occurs by the diffusion mechanism, and macroporous size ranges from 0.1 to 1 micron meter. Small pore sizes of 100–1000 angstroms characterize microporous materials, which release drugs through molecular diffusion and convection. However, drug release from Nanoporous materials with a mesh size of 10-100 angstroms occurs solely by diffusion mechanisms(Zaman et al., 2015).

2.6 Advantages of Polymeric hydrogels

There are some advantages of polymeric hydrogels which are given under

Posse's extreme elasticity is comparable to those of genuine tissues, They can be injected because they are biocompatible and biodegradable, They might release the medication in response to PH or temperature changes, They are applied locally, so first-pass metabolism is passed(R. Singh, Goel, Sharma, & Agarwal, 2021).

2.7 Axillary odor

Axillary odor, also referred to as "body Odor," is the characteristically foul-smelling smell of adults. It is the most noticeable note among the smells coming from different parts of the body. The best possible apocrine sweat is the cause of axillary odor; when it is visible on the skin, it

is odorless and sterile. When Apocrine Sweat and resident microbes mix, a strong stench is produced (Shelley, HURLEY, & Nichols, 1953).

Regarding the kinds of Bacteria that can release the compounds in odiferous, there is disagreement. Initially, it was believed that a variety of both gram-negative and gram-positive microorganisms may cause the distinctive odor. However, Shehadeh and Kligman showed that this ability was exclusive to Gram-Positive Bacteria (Shehadeh & Kligman, 1963)

It is unknown if the axillary microflora of people with varying degrees of body odor differs in terms of quantity or quality.

2.8 Axillary microbiology

A significant and ongoing population of microorganisms lives in the axilla (underarm) and is fed by secretions from the Sebaceous, Apocrine, and Eccrine glands. This resident microbiota is primarily composed of Gram-Positive Bacteria belonging to the genera *Staphylococcus, Micrococcus, Corynebacterium*, and *Propionibacterium*, according to conventional culture-based microbiological research. The axilla (underarm) is home to a sizable and persistent population of microorganisms that are fed by secretions from the Eccrine, Apocrine, and Sebaceous glands. Conventional culture-based microbiological research indicates that the majority of the Gram-Positive Bacteria in this resident microbiota are members of the genera *Corynebacterium, Micrococcus, Staphylococcus and Propionibacterium*. (Leyden, McGinley, Hölzle, Labows, & Kligman, 1981). Malodor production on the axillary skin and other body places has been linked to the biotransformation of odourless natural secretions into volatile, odorous compounds by the surrounding microbial populations since the 1950s. (Shelley et al., 1953).The general assumption is that *Corynebacterium* is the primary cause of underarm odour; however, evidence supporting this notion was found in a 2003 study conducted by Taylor et al. that examined the relationship between microbial counts and malodor intensities. Although the bacteria that were treated to this were initially discovered through culture on a selective medium, this work used 16S rRNA gene sequencing as a genetic technique to further analyse the *Corynebacterium* population of axillary skin. (Taylor et al., 2003). The individual species found by genetic and biological research are now identified by pyro sequencing and culture-independent met genomics methods. Thus, data generation has significantly improved both breadth and depth.

2.9 Axillary Cosmetics

People in our society are very conscious of their hygiene and are strongly opposed to offensive body odor. Cosmetics for the underarms are essential for controlling sweating and the development of bad odor. These goods help people live better lives and have more self-confidence in social situations. Since deodorants and antiperspirants are used by around 90% of the population in the US, the industry generates revenues of over $1 billion annually. To combat bad odor and leave the skin smelling nice, Deodorants are applied to the Axillae and feet, among other parts of the body. A subset of deodorants called Antiperspirants function by obstructing sweat glands to reduce sweating and improve odor control. Antiperspirants are approved for use by the US Food and Drug Administration as "over-the-counter drugs" following stringent testing. Conversely, Deodorants are seen as cosmetics that help cover up body odor and give the skin a more agreeable scent. Contrary to popular belief, Antiperspirants are not always used as deodorants. As per European legislation, Deodorants and Antiperspirants are classified as Cosmetics (Arora et al., 2022).

In the axillary region, *Propionibacterium*, *micrococcus*, *Corynebacterium*, and *Staphylococcus* are among the microorganisms that live there. These microbes release several enzymes that are meant to turn sweat that doesn't smell bad into foul-smelling sweat. A strong axillary malodor is linked to the presence of aerobic lipophilic *Corynebacterium* spp. (from the Actinobacteria phylum), while other axillary species and *Staphylococcus* spp. (from the Firmicutes phylum) produce less odor. In addition, scientists have discovered that human sweat contains short volatile branched-chain fatty acids, sulfonyl alkanols, and derivatives of steroids. It is also clear that *Corynebacterium* spp. is involved in all three phases (Y. T. Chen, Shih, Chen, & Chen, 2015).

2.10 Antibacterial Deodorants

Deodorants and Antiperspirants contain antimicrobials to reduce the number of Bacteria on the skin. Benzalkonium chloride, Triclosan, metal salts, and Propylene glycol are often used as antimicrobial agents in these products because they have Antifungal and Antibacterial qualities. Furthermore, several flavourings, like Geraniol, Eugenol, and Cinnamon aldehyde, have antibacterial qualities. Although the number of germs is reduced by these compounds, there hasn't been any evidence of total eradication of microbial colonies as of yet. Some people claim that using these deodorants results in a noticeable decrease in pleasant axillary odor. The effectiveness of the substances is being investigated further to determine whether they affect

microbiota in a specific way or in a more general way. This study looks into the effects of stopping and starting deodorant use again. Evaluating whether such consumption can affect the dynamics, diversity, and structure of the autochthonous microbial community is the main objective of this study (Ermenlieva, Georgieva, & Milev, 2020).

2.11 Lauric Acid:

About half of the fatty acid content of laurel oil, Coconut oil, Palm kernel oil (which should not be confused with palm oil) and Coconut milk is composed of Lauric Acid, which is a component of Triglycerides. If not, it is not very common. Moreover, human brest milk(6.2% of total fats), cow milk(2.9%) goat's milk (3.1%) and all include it(Beare-Rogers et al., 2001).

The 12-carbon atom chain that makes up the saturated fatty acid LA is also referred to as Duodecylic acid, Laurostearic acid, n-dodecanoic acid, 1-undecanecarboxylic acid, Dodecoic acid. It is a white, powdered substance that is insoluble in water and has a slight bay oil smell(Aghedo et al., 2021).An acid-base neutralisation reaction takes place when a particular type of carboxylic acid releases hydrogen ions and a base is available to absorb them; the reaction results in the production of salt and water as by-products. The manufacture of soaps and cosmetics is the primary application for this neutralization technique. Soap is produced when sodium laurate, or LA, is neutralized with Sodium Hydroxide(Anneken et al., 2000).

Many vegetable fats, such as those made from coconut and palm kernel oils, are plant-based sources of LA. Although the amount of LA found in food is safe for consumption, not enough information is available to assess its potential therapeutic benefits. Among other animal products, goat’s milk, human breast milk, and cow's milk can all contain LA. However, the process of extracting resources from plants is far more effective than that of extracting resources from animals(Aghedo et al., 2021).

By turning a larger percentage of LA into Monolaurin, also known as Glyceryl Laurate, the body absorbs LA. Eventually, this monolaurin is converted into HDA cholesterol, or 2-hydroxydecynoic acid, which helps the body fight against bacterial infections. Monolaurin is commonly found in deodorants.(May, 2012).

LA is a sort of MCT component that is present in human breast milk and helps to enhance the body's metabolism. It is a component of triglycerides. An MCT component appears colourless, transparent, low viscosity "water-like" liquid oil, and odorless components at room temperature(Takeuchi, Sekine, Kojima, & Aoyama, 2008).

About half of the lauric acid and 6-7 percent of the capric acid are found in coconut oil and some coconut products. Often, people wonder if consuming lauric acid can result in a notable amount of endogenous creation of Monolaurin. The amount of Lauric Acid that is genuinely converted to monolaurin in the human body does not seem to be well-documented. However, there is proof that some are created. The primary antiviral and antibacterial agent in human breast milk is lauric acid. The antiviral and antibacterial activity of Lauric Acid (C12) is higher than that of other medium-chain triglycerides such as Myristic acid (C14), Caprylic acid (C8), and Capric acid (C10). When it comes to killing viruses and bacteria, monolaurin is many times more biologically active than Lauric Acid(Kabara, Vrable, & Lie Ken Jie, 1977).

Lauric Acid is safe to handle, affordable, has a long shelf life and nontoxic, similar to many other fatty acids. It is mostly utilized in the manufacturing of soaps and cosmetics. For these uses, Sodium Hydroxide and lauric acid react to produce sodium laurate, a soap. The most widely used process for making sodium laurate involves saponifying various oils, notably coconut oil. These starting materials yield sodium laurate and various soap combinations(Zaminpira & Niknamian, 2013).

2.12 Antibacterial Properties of Lauric Acid

Because Lauric Acid can combat a variety of bacterial infections, using it may help avoid skin conditions. For instance, coconut oil has been used for many years to treat bacterial and fungal skin problems. Although this LA makes up around 50% of the contents in coconut oil, there isn't much research on using pure LA directly to treat skin conditions. According to a recent study by Teruaki Nakatsujiet al. (2009), Propionibacterium acnes, which has been identified from the most prevalent skin condition affecting humans, acne vulgaris, which affects up to 80% of people in their lifetime, is inhibited from growing by LA.(Nakatsuji et al., 2009). Consequently, the pharmaceutical sectors may require more LA due to its exceptional antibacterial qualities. Moreover, monolaurin, the monoglyceride derivative of LA, shows much stronger antibacterial properties against many pathogenic Gram-Positive Bacteria, lipid-coated RNA and DNA viruses, and a range of Pathogenic Protozoa (Paul May, 2012). Furthermore, Coconut Oil has been claimed by James and Rahman (2005) to be used in engine lubrication, transformer oil, and skin moisturizing. There have also been reports that Coconut Oil's acids can be employed as herbicides(James & Rahman, 2005).

CHAPTER 3: METHODOLOGY

All applied reagents and chemicals were of analytical grades. Chitosan (low molecular weight) was obtained from Sigma Aldrich Company. Some other chemicals were Polyvinyl alcohol (PVA, MW =72KDa), Edible bovine gelatin (Gel), Glacial acetic acid, distilled water, ethanol, and lecithin obtained from Sigma Aldrich Company. Saturated fatty acid (Lauric acid) obtained from Sigma Aldrich.

3.1 Methodology

3.1.1 Preparation of Polymeric hydrogel

We combined Chitosan, Edible bovine gelatin, and Polyvinyl alcohol to create the necessary Polymeric hydrogel. Polyvinyl alcohol and Edible bovine gelatin are dissolved in distilled water while chitosan is mostly dissolved in glacial acetic acid. We take 1% of Glacial acetic acid. For the preparation of 1% of glacial acetic acid, we take 1 ml of glacial acetic acid and dissolve it in 99 ml of distilled water to make a total of 100 ml of acetic acid .First, we take one gram of Polyvinyl alcohol and let it dissolve in twenty millilitres of distilled water for a full day at room temperature while stirring. Then we prepared Edible bovine gelatin. For that, we took 1 gram of edible bovine gelatin and dissolved it in 10ml of distilled water under stirring for 2 hours. In order to prepare chitosan, one gram of Chitosan is dissolved in ten millilitres of glacial acetic acid and stirred for twenty-four hours at room temperature. After preparation of Polymeric solutions of different polymers, we take 5 ml of Polyvinyl alcohol solution, 10 ml of Chitosan solution, and 10 of Edible bovine gelatin and mix all these concentrations to make a solution 25ml solutions in 1:2:2 concentration. We mix all these solutions under stirring for 2 hours at room temperature.

3.1.2 Incorporation of LA inside the gel

After the formation of the desired hydrogel then we add our active ingredient (lauric acid) inside the hydrogel. Lauric acid is not directly dissolved in the Polymeric hydrogel, it can be dissolved in ethanol solution but it cannot directly dissolve in ethanol so we use lecithin for the dissolution of Lauric Acid inside the ethanol. We take 50mg of Lecithin and dissolve it in 10 ml of Ethanol. After the dissolution of Lecithin, we add 150mg of Lauric Acid inside this

ethanol lecithin solution under stirring for 2 h to dissolve Lauric Acid. When lauric acid is fully dissolved we stop stirring. We separate 5 ml of polymeric solution from 25 ml solution and we add 2 ml of lauric acid solution inside 5 ml of polymer solution under stirring for 3 hours at 37 °C to make our desired polymeric hydrogel.

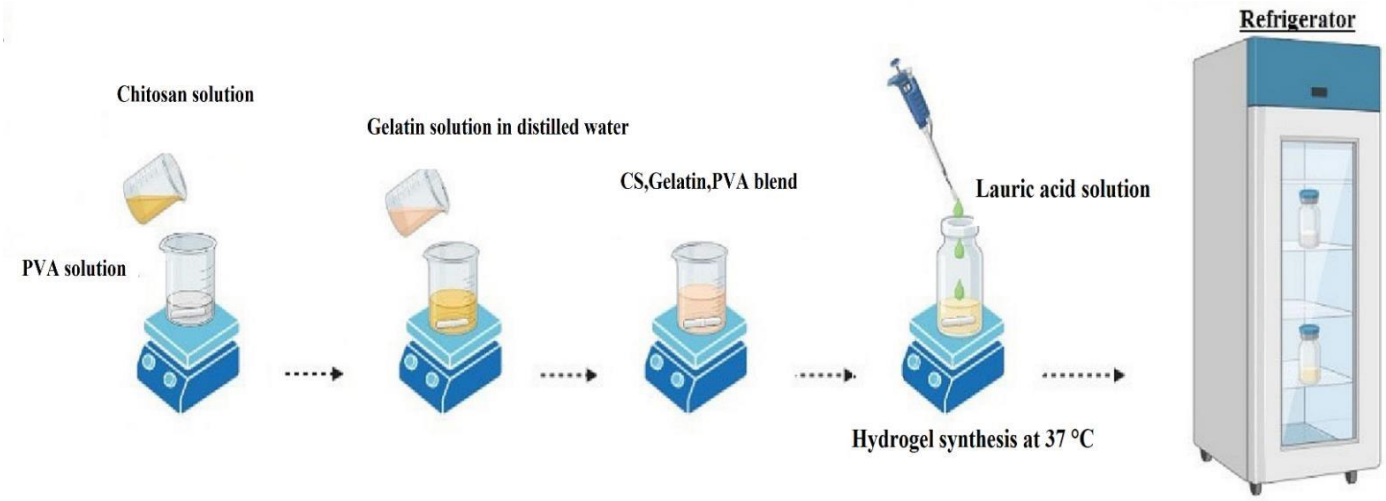


Figure 3.1: Synthesis of lauric acid loaded Polymeric hydrogel

3.2 Characterization of polymeric hydrogel, PH

3.2.1 Ultraviolet-Visible (UV-Visible) Spectroscopy Analysis

UV-visible spectroscopy is the most common technique used in industrial as well as medical fields. When the sample is traversed by a beam of light, its absorption capacity is gauged by a reflecting light beam. The light beam is divided into two halves, one pass through the reference cuvette (containing only the solvent) and the only pass through the sample cuvette. The outcomes are scrutinized and computed at a specific wavelength within the anticipated range. The resulting spectrum shows the absorbance registered at a particular wavelength and is graphically represented, depicting absorbance against the wavelength. The absorbance peak at a specific wavelength known as Lambda Max is a key parameter. The UV-visible spectroscopy works on the principle of Beer-Lambert Law expressed as A=EcL or E=A/cL where A represents the absorbance denotes the concentration of the sample is the length of light that passes through the cuvette in centimeters and E stands for molar absorptivity. This process allows the measure of electronic transition in molecules. The absorption of the sample is

directly proportional to the molar concentration, making the molecular absorptivity value a valuable metric for comparing different compounds.

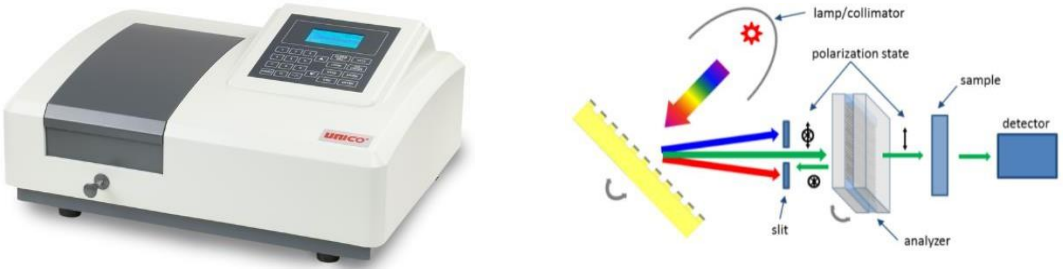


Figure 3.2: UV spectrophotometer and its working principle

3.2.2 Fourier transform infrared spectroscopy analysis (FTIR)

Fourier transform infrared spectroscopy analysis is referred to as FTIR. It is a technique used to identify and quantify the material based on absorption of infrared light. The interaction of molecules with infrared light is measured using FTIR spectroscopy, which yields important details about the sample's chemical makeup. The spectrum was obtained ranging from 500-4000 cm by using the attenuated reflectance technique.FTIR is one of the techniques that is used to detect the functional group in a compound. Photoconductivity, absorption, emission, or Raman scattering of gas, liquid, or solid molecules can be detected by FTIR. It is used for the surface analysis and characterization of polymers and nanoparticles. It is used for surface analysis and characterization of polymers.

The surface of polymers contains different absorbents which cause peaks in the FTIR spectrum in comparison with samples having no absorbents. It easily detects changes in the surface. It has a complicated mechanical and electrical system that detects changes on the surface of samples. It helps to analyze a wide range of materials such as paste, bulks, fiber, films, or powder. Both qualitative and quantitative analysis can be done by determining the size of the peaks. The frequency of absorption is made based on vibrionic coupling, shape of the surface, and atomic mass.

3.2.3 Viscosity testing

A fluid's viscosity describes its internal resistance. Although viscosity testing is frequently used to assess fluids in engineering systems, it becomes essential when studying hydrogels to investigate their rheological characteristics. The rheological examination is important since it informs choices about how to process or store the fluid later on.

3.2.4 Scanning Electron Microscopy

One tool that is frequently used to image materials at the Nano scale is scanning electron microscopy. An electron beam is used in scanning electron microscopy. The sample's surface and the picture are scanned using the electron beam. The beam of electrons interacts with the atoms and molecules of the sample which gives rise to various signals and thus provides information about the composition, morphology, and topography of the sample. The raster scan pattern is used by a beam of electrons to scan. Detected signals along with the position of the beam of electrons give the combined effects and a highly refined image is produced. More than 1nm resolution is checked with scanning electron microscopy. The conventional SEM uses high vacuum for imaging whereas if we have environmental SEM we use low vacuum and wet conditions.

Imaging can also be done at high temperatures but that is done by specified instruments. The device checks the secondary electrons depending on the topography of the sample to be checked. The image can be created when the secondary electron is detected by the detector, the sample is scanned and the topographic images of the specimen are created.

In this imaging technique, it is important to note whether the sample can withstand the high-energy electron beam and the vacuum conditions or not. So the sample is prepared according to the situation. The sample should be small enough so that it can be adjusted and fixed on the specimen stage. A stub is used to mount the sample and conductive adhesive is used. Some scanning electron microscopes have the ability they be rotated so the sample is viewed by 360° rotation.

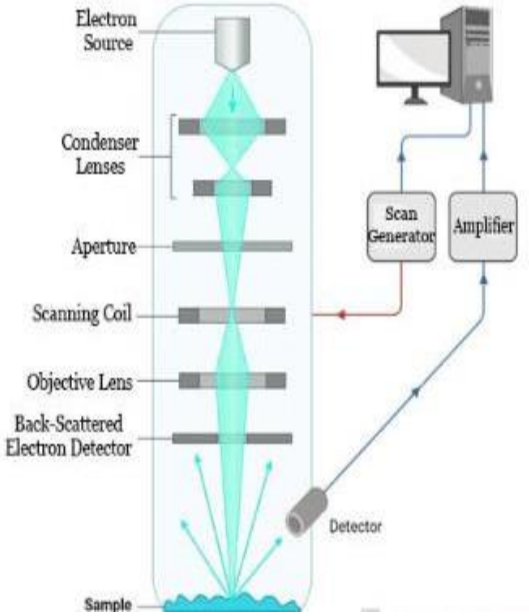


Figure 3.3: SEM and its working principle

3.2.5 Zeta

The ZP, or electro-kinetic potential, is the potential at the slipping/shear plane of a colloid particle moving in an electric field(Kaszuba, Corbett, Watson, & Jones, 2010).Zeta potential gets its name from the Greek letter ν, which was initial y used to describe it in mathematical calculations(Vidal-Iglesias, Solla-Gul ón, Rodes, Herrero, & Aldaz, 2012).The electro-kinetic, also known as the zeta-potential, is an aspect of the electrical properties of solid/liquid and liquid-gaseous interfaces and is a crucial part of the electrical double layer(Clogston & Patri, 2011).Zeta potential is known to be dependent only on the pH of the medium being studied, as well as the charge density of the particles and the kind, amount, and nature of ions and counterions in solution (López-León, Carvalho, Seijo, Ortega-Vinuesa, & Bastos-González, 2005).

3.2.6 Antibacterial activity

3.2.6.1 Sample collection

A total of fifty volunteers were selected aged between 20-50 years. These included twenty-five male and twenty-five female subjects. All the volunteers were in good health. They refrained

from taking any medication including Antibiotics, one week before sample collection. They were also asked not to use any kind of deodorant or antiperspirants 6 hours before sample collection.

To study the axillary microflora Sweat samples were collected using sterile cotton swabs. The swabs were rubbed for 30s over a 2cm diameter skin area. The swabs were then pooled in sterile phosphate buffer saline (PBS) and stored at 4 °C for not more than 24h.

3.2.6.2 Non-selective growth

The samples were spread in Tryptic Soy Agar (TSA) for non-selective growth.TSA plates were prepared by taking 40g of Tryptic Soy Agar media and mixing it in 1L of distilled water. Autoclave the media at 121°C at 15psi for 15min.The plates were prepared by pouring 20 ml of sterile media aseptically.50 µl of the samples were spread using pasture pipettes on TSA plates. The plates were incubated at 37 °C for 24 to 48 hours.

3.2.6.3 Antibacterial activity

To study the antibacterial activity, TSA was used as it allows the growth of axillary microflora. The antibacterial activity was carried out using the agar well diffusion method. The TSA plates were prepared as mentioned above and wells were created using pipette tips. Bacterial inoculum was prepared as mentioned above and 50 µl of inoculum was spread on TSA plates using pasture pipettes. Then 50 µl of test samples were loaded into the wells and the plates were incubated for 24-48h at 37°C. Observe the zones after incubation.

3.2.7 Drug Release Assay

Up to 48 hours were spent monitoring drug release through Polymeric hydrogel in conjunction with the administration of a predetermined amount of Phosphate Buffer Saline (PBS). A drug release assay was performed to see how much quantity of drug was released from Polymeric hydrogel over time. In order to create the buffer solution for the drug release test, initially one buffer tablet was dissolved in 100 millilitres of distilled water. Then we add 20 ml of that buffer into a small beaker. We took a 1cm dialysis membrane and added 2 ml of our drug-loaded polymeric hydrogel to that dialysis membrane. Then we dip this dialysis membrane with polymeric hydrogel in a beaker containing buffer solution and put that beaker in a water bath for shaking. We adjusted the water bath's temperature to 37°C and its shaking speed to 100

rpm. The beaker starts shaking. Now we took 90 µl of buffer after 1,2,4,6,8,24 hours and checked their absorbance to see the result of drug release over time.

3.2.8 Hemolytic assay

Haemolys**is** is a mechanism in which destruction of Red Blood Cells occurs. Red blood cells burst and Haemoglobin is released in the bloodstream. There are many reasons for Haemolysis like drug toxicity, any disease in blood, or any other reason. We perform the Haemolytic test in our research to check the haemolytic activity of polymeric hydrogel containing Lauric Acid.

For that purpose, we perform a haemolytic test on the Red Blood Cells of a healthy person.10 ml of blood were taken from a healthy person. To separate the red blood cells and plasma, 1 milliliter of blood was placed in an Eppendorf tube and centrifuged for 10 minutes at 3000 rpm. Supernatants were discarded and pallets containing RBCs were removed. The Red Blood Cell pellet was washed three times with PBS buffer to remove the plasma properly.

The sample was prepared from a stock solution of Polymeric hydrogel and PBS was used as a solvent. We prepared different concentrations of drugs like 20 µg/ml, 30 µg/ml, and 40 µg/ml. Positive and negative controls were also used. Triton X-100 was utilised as the positive control and PBS as the negative control. Blood suspensions were prepared by dissolving 1 ml of blood in 4 ml PBS to make 5 ml blood suspensions. We take 5 eppendrof and take 1 ml of blood suspension in these 5 Eppendorf. In the first eppendrof, we take 1 ml PBS as negative control and in 2nd eppendrof, we take 1 ml Triton X-100 as a positive control. In the remaining 3 eppendrof we add different concentrations of drugs as above mentioned in remaining three eppendrofs..We put these 5 eppendrof in an incubator for 1 hour at t 37ºC. After the incubation, we take this eppendrof from the incubator and centrifuge all these at 3000 rpm for 10 minutes. The supernatant was removed and pallets were discarded. We take supernatant from all 5 eppendrof and put it in 96 well plates to check their absorbance at 545 nm. 100% absorbance is displayed by the positive control, while 0% absorbance is displayed by the negative control. The following equation was used to get the hemolysis %

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

3.2.9 Determination of the Inhibitory Activity of Polymeric hydrogel against S.epidermidis and other Sweat bacteria

A microliter plate spectroscopic experiment was used to assess the Biofilm activity of Sweat Bacteria. It is the most common technique for determining the compound Antimicrobial and Anti-biofilm properties. Briefly, 5 millilitres of TSB were used to generate a fresh culture of *S.epidermidis* and the other two Sweat Bacteria, which were then incubated at 37°C for the entire night. Following incubation, 100µl of each diluted culture of *S.epidermidis* and the other two Sweat Bacteria strains was put into each well of 96 well plates. Following that, the culture was 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 well and dispose of the content through aspiration. After adding 250µl of 0.1% w/v Crystal Violet Stain, the wells were allowed to stand at room temperature for 15 minutes while the lid was closed. Wells were incubated and washed with distilled water and then allowed to dry. Then each well was filled with 300µl of 95% ethanol, and the lid was closed for 15 minutes. After carefully mixing the contents with a gentle pipette, 150µl of Crystal Violet and Ethanol solution was transferred to fresh 96 well plates, and the Multiskyscan Sky Microplate Spectrophotometer was used to detect the optical density at 630nm. Four wells were used for the studies, with one well acting as a media control (blank). The following formula was used to determine the percentage of biofilm inhibitory activity.

Where C is the absorbance of the control (biofilm, no treatment), B is the absorbance of the blank (just TSB), and T is the absorbance of the test (biofilm and treatment), the formula is [(C – B) – (T – B) (C – B)*100%].Ethanol, 96%, and distilled water were employed as positive controls in this experiment.

CHAPTER 4: RESULTS

This study includes the isolation of sweat bacteria which is responsible for malodor and the antibacterial effect of LA-loaded polymeric hydrogel-based deodorant formulation on that bacteria.

4.1 Synthesis of Polymeric hydrogel

The polymeric hydrogel produced by blending and stirring of three different polymers was milky white with a semi-solid consistency. The developed Polymeric Hydrogel was a semi-solid milky gel that was free of any fragrance. The produced Polymeric Hydrogel was semi-solid at room temperature and was very feasible to be used for incorporation into the gel.

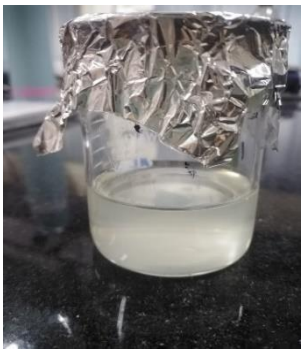


Figure 4.1: Synthesized Polymeric hydrogel

4.2 Synthesis of Lauric Acid loaded Polymeric hydrogel

The Deodorant gel prepared was clear and thick. It gave a yellow appearance due to the use of a small quantity of Lecithin. The gel was semi-solid, yellowish, and had a viscous appearance. The consistency is good enough to resist skin fraction. The resulting product was free of Aluminum and other toxic chemicals that can cause any skin or health problem. The clear consistency makes it reasonable to be used for commercial-scale products.

Figure 4.2: LA-loaded Polymeric hydrogel

4.3 Characterization of Polymeric hydrogel

4.3.1 Ultraviolet-Visible Spectroscopy

Ultraviolet-visible spectroscopy analysis of lauric acid encapsulated in polymeric hydrogel shows peaks between 200 to 300 nm due to presence of lecithin and lauric acid because these two show peaks between 200-300 nm, while blank polymeric hydrogel do not show any peak.

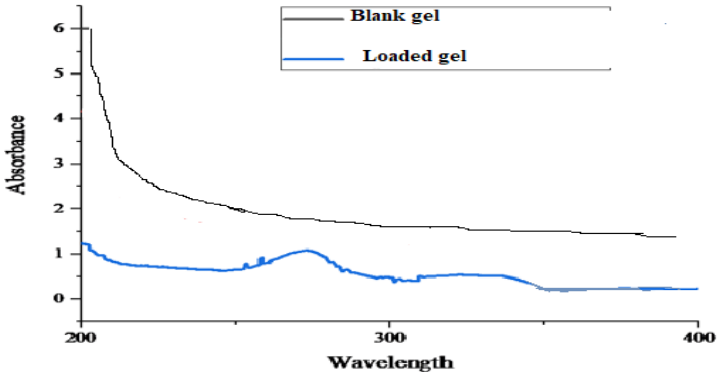


Figure 4.3: UV spectroscopy of hydrogel

4.3.2 Analysis using Fourier transform infrared spectroscopy (FTIR)

FTIR peaks of blank polymeric hydrogel show peaks at 1000(C=O), It show peak at 1623 due to (C=C), peak at 2900 is due to OH stretch. Lauric Acid loaded Polymeric hydrogel show peaks at 1641 and 2922 due to presence of lecithin. Loaded gel show peaks at 2870(CH3) and

3285(OH) due to presence of Lauric Acid. The Lecithin spectrum indicated peaks at 1641/cm (NC=C amide carbonyl) and 2992(CH2) stretch, 3299 (OH) stretch. In ethanol it shows peaks at 3299 due to the OH stretch, it shows peaks at 1051 due to the (C-O ester group) stretch, it shows peaks at 881 due to the (C-H) stretch. In PVA peak at 3300 is due to (OH) group, peak at 2900 is due to (C-H) from alkyl group, peak at 1700 is due to (C=O).In chitosan peak at 3300 is due to (NH2 amino group), peak at 200 is due to C-H stretch, peak at 1636 is due to (C-O carbonyl group), peak at 1300 is due to (C-N), peak at 1000 is due to (C-O) stretch.

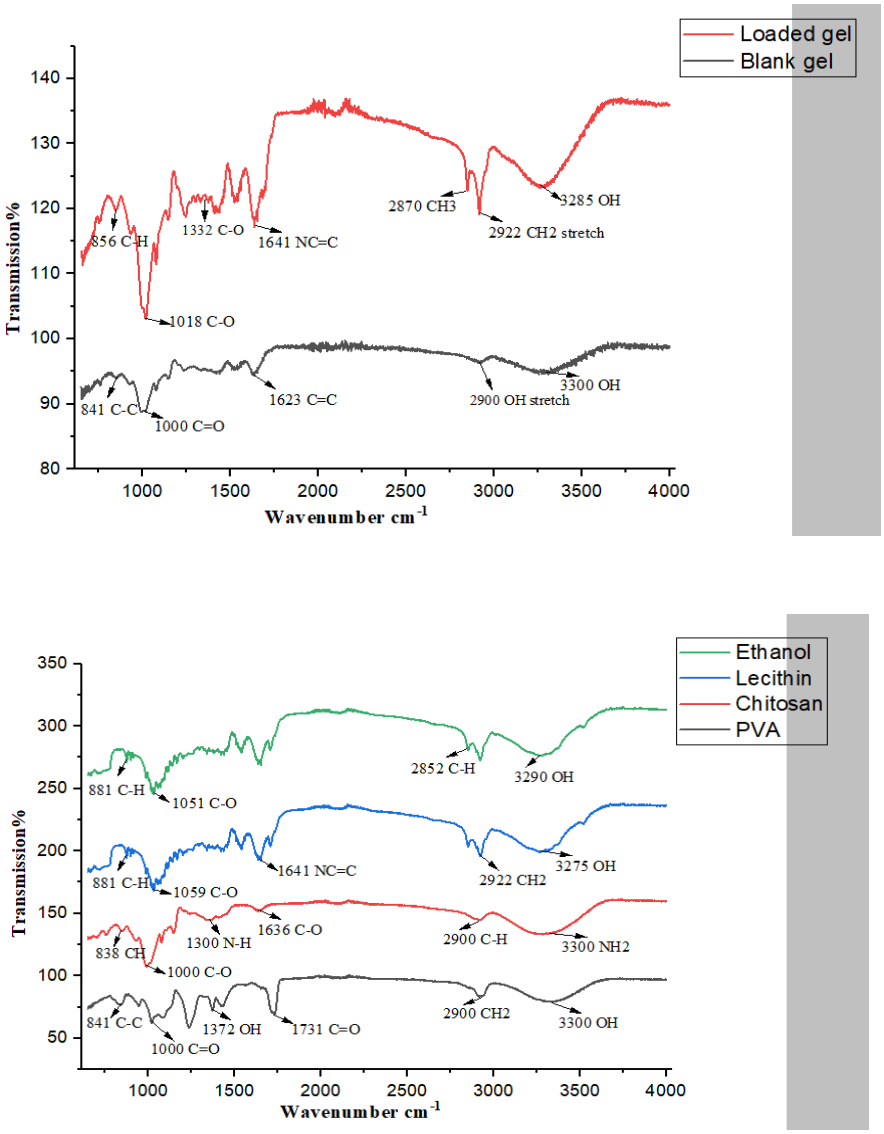


Figure 4.4: FTIR spectrum of hydrogel and its components

4.3.3 Viscosity

The incorporation of Lauric Acid inside the Polymeric hydrogel tends to stabilize the hydrogel. The viscosity of Blank Polymeric hydrogel and loaded Polymeric hydrogel with Lauric Acid are given below

Table 1: Viscosity of Polymeric hydrogel

	Viscosity(cp)	**Acceleration(±cp)**	**Temperature(°C)**
Unloaded gel	160	6.40	37
Loaded gel	276	6.40	37

4.3.4 Size of Particles

Utilising Scanning Electron Microscopy, the particle size is ascertained. Scanning Electron Microscopy is used to provide information about the size, shape, and morphology of the sample. Scanning Electron Microscopy revealed that Polymeric hydrogel without Lauric Acid are homogeneous and smooth surfaces which showed that gel components were mixed well with each other. The addition of Lauric Acid inside hydrogel revealed the hollow cavities inside the gel and these hollow cavities revealed the presence of lauric acid inside the gel. A Scanning Electron Microscope revealed that the Lauric acid-loaded polymeric hydrogel was spherical in size and about the size of 60nm, 66nm, and 90nm.

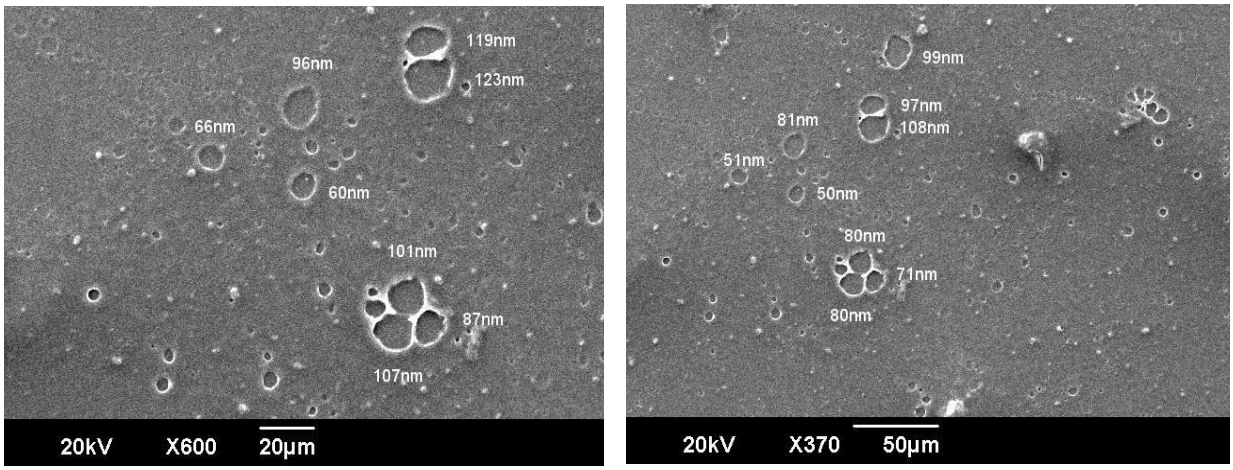


Figure 4.5: SEM image of LA loaded Polymeric hydrogel

4.3.5 Zeta

Greater positive readings indicated a stable Zeta Potential, indicating the stability of NPs(Talib, Ahmed, Khan, Khan, & ur Rehman, 2021).Zeta potential of lauric acid loaded Polymeric hydrogel is 14.96 mv.

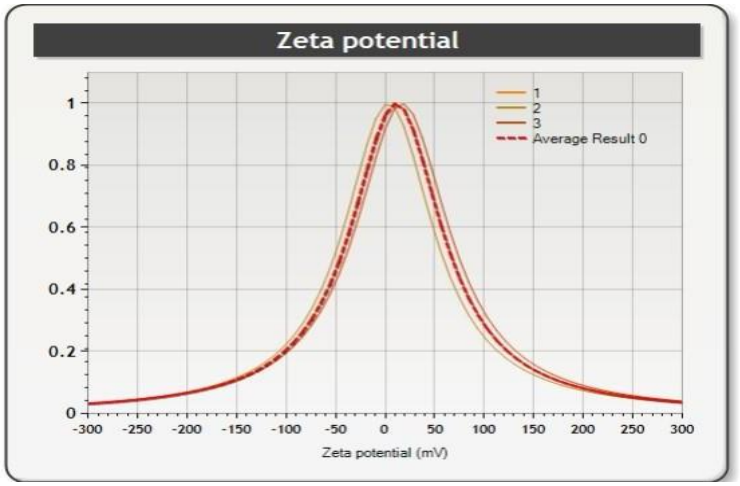


Figure 4.6: Zeta Potential value of loaded hydrogel

4.3.6 Antibacterial testing

4.3.6.1 Non-selected growth

The non-selective growth was carried out on TSA plates. The colonies were picked and put in Tryptic Soy Broth in autoclaved test tubes and placed that test tubes in an incubator for 24 hours.

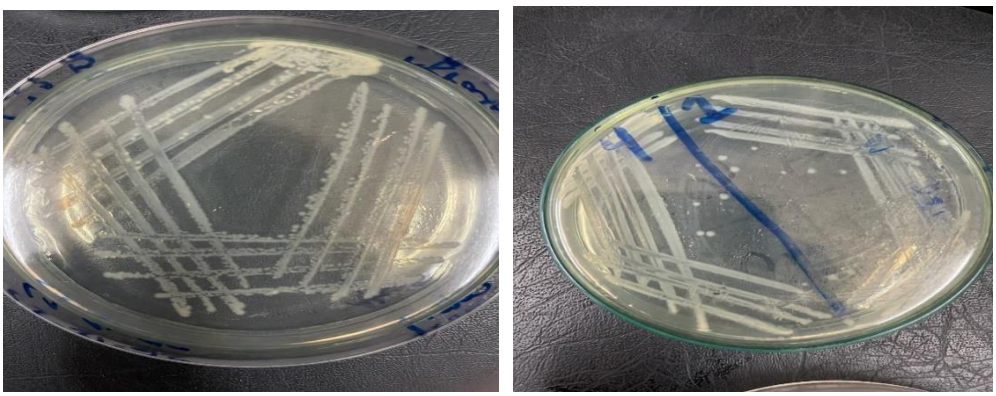


Figure 4.7: Non-selective growth of human sweat bacteria

4.3.6.2 Agar well diffusion assay

Agar well diffusion assay was used to check the antibacterial activity of any sample. We took 50µl of preculture bacteria from each test tube and spared that bacteria on the agar plates to show the bacterial growth. The wells were created on the TSA plates and after the incubation of plates loaded with the test samples, the zones were observed. We had three sweat bacteria so three plates were used in this method. Samples include blank gel, loaded gel with Lauric Acid, and Pure Lauric Acid solution in Lecithin ethanol. Three plates contain three different Sweat Bacteria. Each bacterial culture on a different plate. Three wells were created in three plates. We put 50 µl blank gel, loaded gel with Lauric Acid, and Pure Lauric Acid in ethanol lecithin solution in each plate. After 24 hours we measure the Zones in three plates. The first plate with *Corynebacterium* has three zones 10mm for blank gel, 15 mm for loaded gel, and 24 mm for pure lauric acid solution. Plate 2 has *Staphylococcus aureus* consisting of three zones e.g. 15mm for blank gel, 20mm for loaded gel, and 30 mm for pure lauric acid solution. Plate three has *Staphylococcus epidermis* which has three zones e.g. 25 mm for blank gel, 30mm for loaded gel, and 40mm for pure drug solution. So after measuring different zones, we plot a graph on graph pad prism and ANOVA is applied on that graph.

***Table 2*:** Zone of inhibition of blank gel, loaded gel and drug solution against *S. epidermidis*

Blank gel	25 mm
Loaded gel	30 mm
Drug solution	40 mm

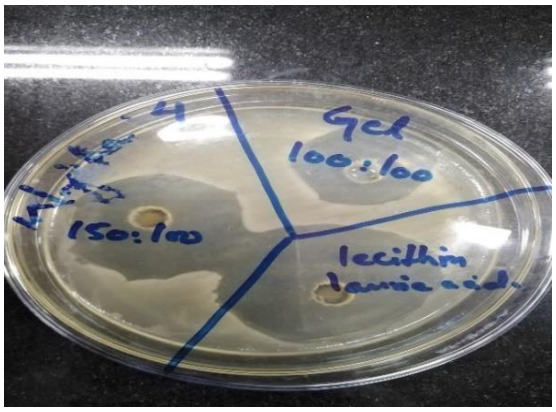


Figure 4.8: Zone of inhibition for *S.epidermidis*

Table 3: Zone of inhibition of blank gel, loaded gel and drug solution against *S.aurius*

Blank gel	15mm
Loaded gel	20mm
Drug solution	30mm

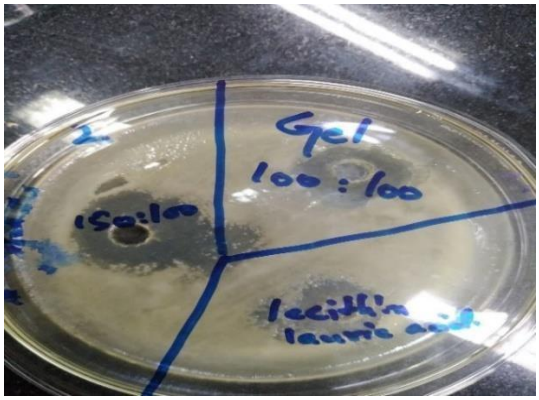


Figure 4.9: Zone of inhibition for *S.aurius*

Table 4: Zone of Inhibition of blank gel, loaded gel and drug solution against *Corynebacterium*

Blank gel	10mm
Loaded gel	15mm
Drug solution	24mm

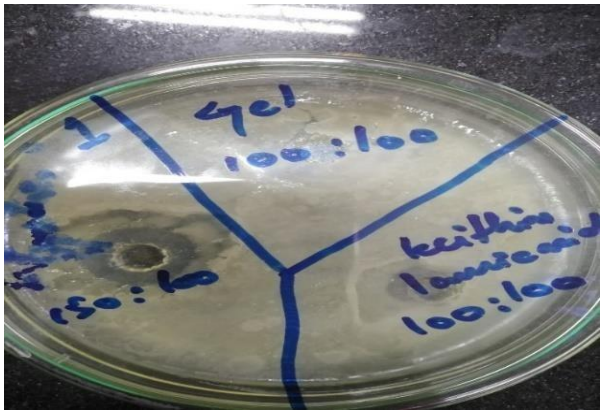


Figure 4.10: Zone of inhibition against *Corynebacterium*

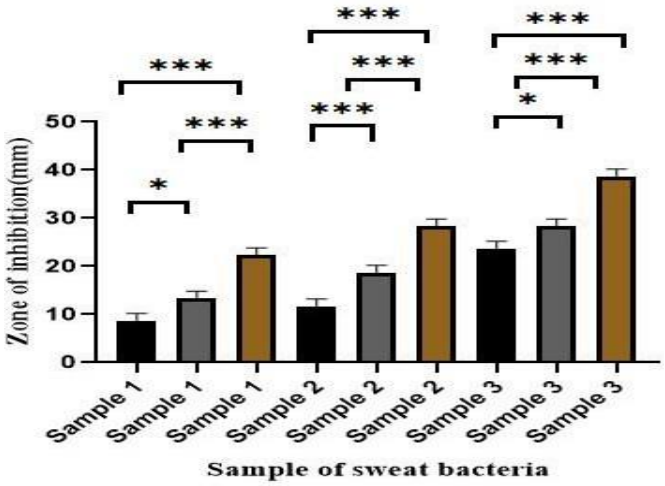
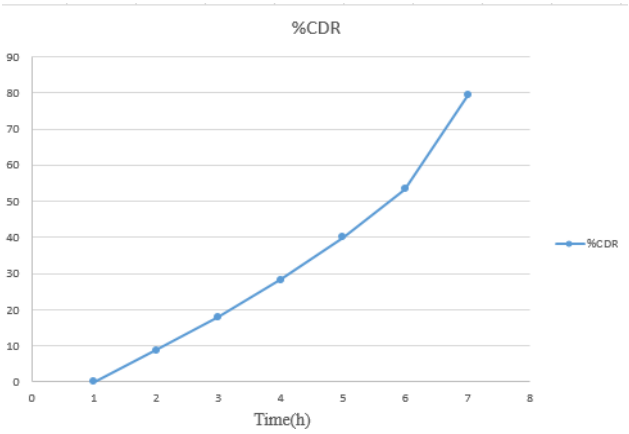
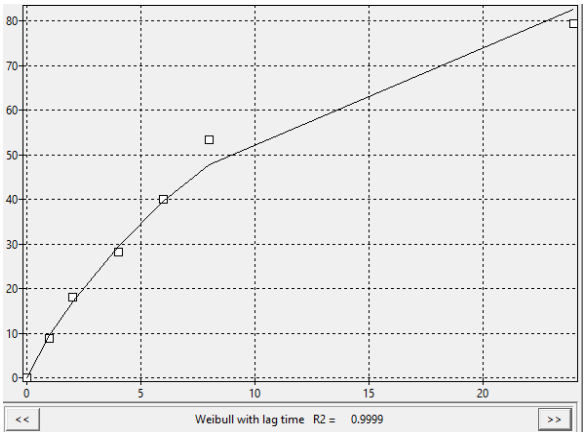


Figure 4.11: Graph showing antibacterial activity of lauric acid loaded Polymeric hydrogel against sweat Bacteria

4.3.7 Drug release efficiency

The cumulative drug release measures up to 24 hours by Lauric acid-loaded Polymeric hydrogel is 40%, 53%, and 79%. The slow and steady drug release depicted by the KinetDS software curve fits the Weibull model for drug release. The drug is released by diffusion following this model.





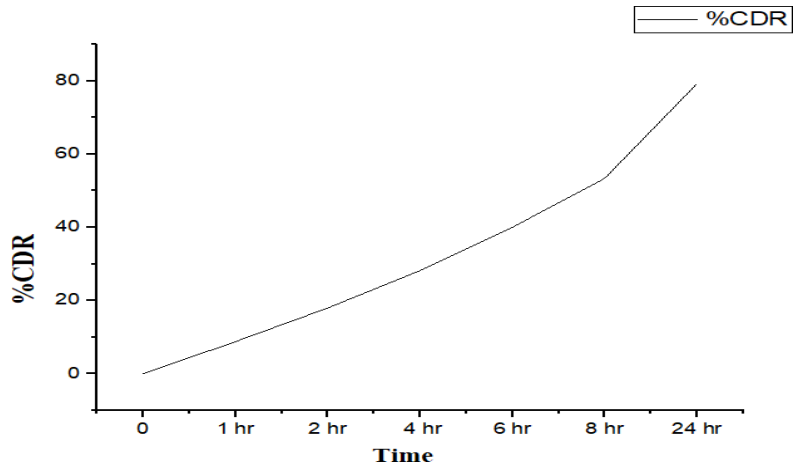


Figure 4.12: Graph showing drug release analysis of Lauric Acid-loaded Polymeric hydrogel

4.3.8 Hemolytic assay

A Hemolysis test is performed to check the Hemolytic activity of Polymeric hydrogel containing Lauric Acid. Different concentrations of Polymeric hydrogel with or without Lauric Acid were taken like 20 µg/ml, 30 µg/ml and 40 µg/ml and their Haemolytic activity was checked. It was indicated that blank gel without lauric acid showed very less Hemolysis and gel with Lauric Acid showed little more Hemolysis as compared to pure gel. An increase in Hemolysis activity is indicated by the drug's concentration rising and maximum hemolysis is shown when we take 40 µg/ml of the drug which shows the percentage of haemolysis is 4%. Hemolysis behavior increases with an increase in the concentration of the drug but overall Hemolysis results showed that the percentage of Hemolysis is less and this drug and the polymeric hydrogel is nonhemolytic. Graph is plotted on graph pad prism and ANOVA is applied. ANOVA is applied because there are more than two groups and if there are more than two groups then we applied ANOVA. Significant difference between means of two groups are indicated by Asterisks and here are three asterisks which indicates that P value is less than 0.05. If P value is less than 0.05 it means that value is significant and is indicated by 1 asterisks .Here three Asterisks indicates that P value is less than 0.001.

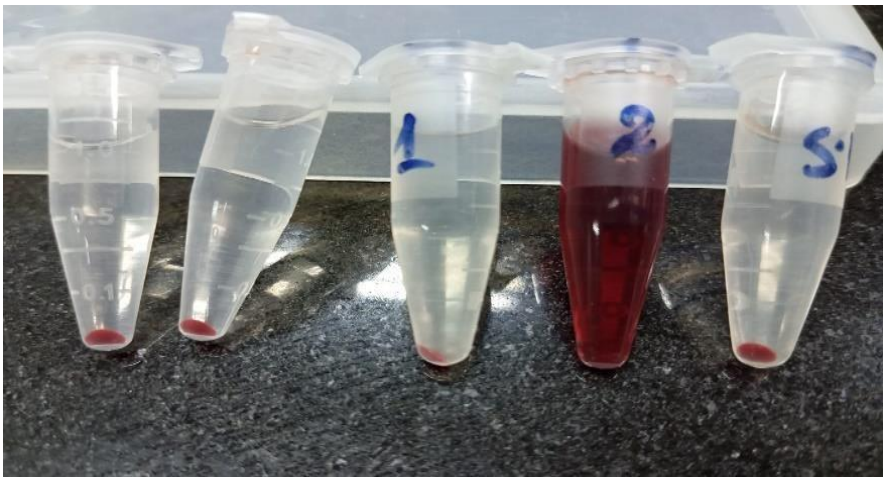


Figure 4.13: Haemolysis of the hydrogel at different concentrations

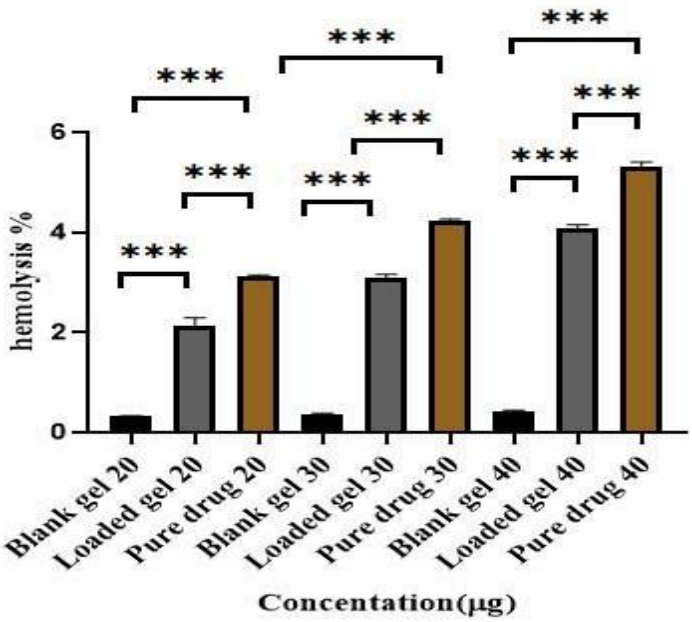


Figure 4.13: Graph showing hemolysis of blank gel, loaded gel and Pure drug solution at different concentrations

4.3.9 Evaluation of the Test Compound's Inhibitory Activity against Sweat Bacterial Biofilms

The following formula was used to determine the percentage of inhibitory activity of Biofilm: [(C – B) – (T – B) (C – B) * 100], where C represents absorbance of control (biofilm without treatment), B represents absorbance of blank (just TSB), and T represents absorbance of the test (biofilm and treatment).Concentration of drug were taken in microgram (µg). Lauric Acid-loaded Polymeric hydrogel shows maximum Biofilm Inhibition as compared to pure drug solution.

Biofilm inhibitory activity of different concentrations of Blank gel, loaded gel, and pure drug against *S.epidermidis*. Graph indicates three asterisks and two asterisks which indicates that P value is less than 0.05 and P value is significant. If P value is less than 0.05 it means that P value is significant and indicated by 1 asterick.If P value is equal to 0.01 it is indicated by 2 asterick.If P value is less than 0.01 it is indicated by three asterisks and it indicates that P value is significant and there is huge difference between means of three groups which reject the null hypothesis.

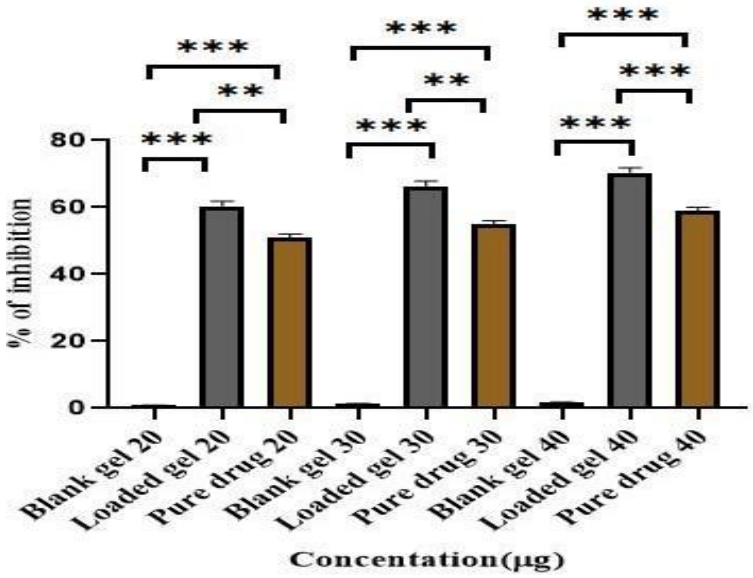


Figure 4.14: Graph showing biofilm inhibition of blank gel, loaded hydrogel and Pure Drug solution against *S.epidermidis*

Biofilm inhibitory activity of different concentrations of Blank gel, loaded gel, and Pure Drug against *S.aurius*. Graph indicates three asterisks and two asterisks which indicates that P value is less than 0.05 and P value is significant. If P value is less than 0.05 it means that P value is significant and indicated by one asterick.If P value is equal to 0.01 it is indicated by 2 asterick.If P value is less than 0.01 it is indicated by three asterisks and it indicates that P value is significant and there is huge difference between means of three groups which reject the null hypothesis.

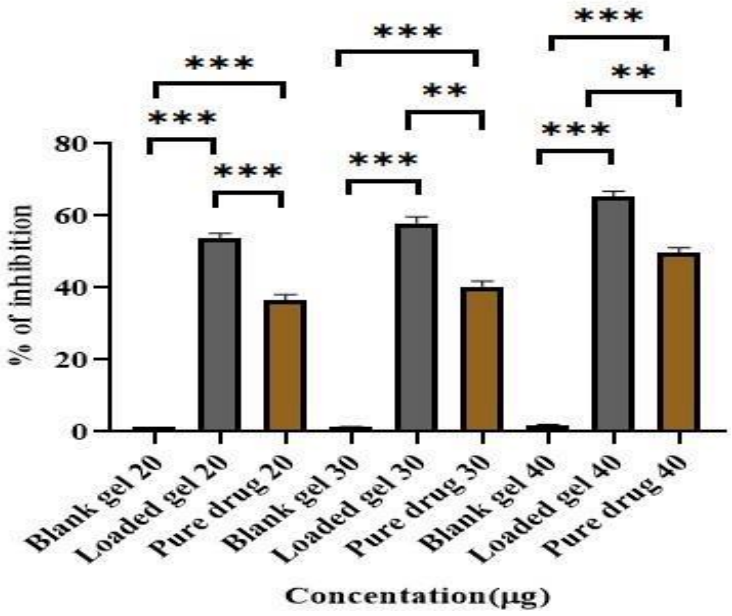


Figure 4.15: Graph showing biofilm inhibition of blank gel, loaded hydrogel and Pure Drug solution against *S.aurius*

Biofilm inhibitory activity of different concentrations of blank gel, loaded gel, and pure drug against *Corynebacterium*. Graph indicates three asterisks and two asterisks which indicates that P value is less than 0.05 and P value is significant. If P value is less than 0.05 it means that P value is significant and indicated by 1 asterick.If P value is equal to 0.01 it is indicated by 2 asterick.If P value is less than 0.01 it is indicated by three asterisks and it indicates that P value is significant and there is huge difference between means of three groups which reject the null hypothesis. If P value is less than 0.001 it is indicated by four asterisks which indicates that P

value is significant and it rejects null hypothesis which says that there is no significant difference between mean of three or more group.

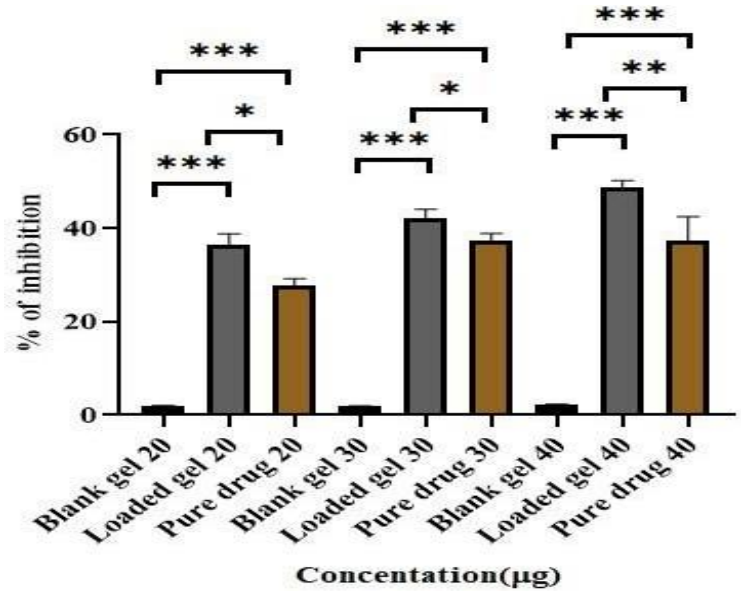


Figure 4.16: Graph showing biofilm inhibition of blank gel, loaded hydrogel and Pure Drug solution against *Corynebacterium*

All these graphs shows the biofilm inhibitory activity of Blank gel, loaded gel and Lauric acid loaded Polymeric hydrogel against three Sweat bacteria because sweat bacteria has ability to form biofilm on skin surface and study shows that Lauric Acid has ability to inhibit the biofilm growth on skin surface.

CHAPTER 5: DISCUSSION

In this study, we evaluated a Lauric Acid's antibacterial qualities by adding it to particular delivery systems made to transfer lipid contents to the intended location while maintaining their original features. Many bacteria classified according to their effects on health and disease are present in human skin. The development of Pathogenic and Odor-causing germs must be specifically inhibited to foster a healthy symbiotic interaction between Humans and Microorganisms. The Lauric Acid family of saturated fatty acids, which includes Antibacterial, Antifungal, Anti-inflammatory, and Antioxidant capabilities that promote general well-being, attractiveness, and health support, has attracted increasing attention from the cosmetic sector. Lauric acid, which was formerly mainly connected to the fragrance industry, is now being used in a wider range of continuously changing applications. They are used as natural preservatives in hair and skin care formulations. Although Lauric Acid has played a significant role in the cosmetic industry's transition to more environmentally friendly and sustainable products, safety concerns about their use in finished goods highlight the importance of exercising caution when determining dose. Consequently, even while Lauric Acid unquestionably includes bioactive molecules that are fundamental to the Beauty industry, their application circumstances need to be carefully considered to ensure safe and effective use.

However, there are benefits to using Lauric Acid in cosmetics and personal care products that go beyond their ability to improve appearance and serve as preservatives. It makes a major contribution to improving the commercial products' brand image. Therefore, it is essential to do additional studies to improve our comprehension of these chemicals' biological roles and investigate any potential toxicological ramifications. This endeavor creates new opportunities for the development of Lauric Acid-based Cosmetics. Thus, more systematic studies assessing the true effectiveness of Lauric Acid in finished formulations are desperately needed, especially in light of the Cosmetic industry's trend towards substituting ecologically friendly bioactive ingredients for conventional actives. Notably, the body absorbs chemicals from Deodorants and Antiperspirants through the olfactory system and the armpits. This suggests that the ingredients in the Deodorants we use regularly are absorbed into our bloodstream, filling our systems with more chemicals and pollutants with each application. Making the switch to natural deodorant is a big step towards establishing a natural skincare regimen that fits with a clean lifestyle. Natural deodorant is better for the environment and our skin; it works just as well as traditional roll-ons and sprays. Since the underarm area has a high concentration of

odor-producing glands, Lauric acid is a great natural alternative to deodorant and antiperspirant. The primary component of coconut oil with antibacterial qualities is lauric acid. Saturated fatty acids like lauric acid are part of the medium-chain fatty acid group. The body transforms lauric acid into monolaurin, which has been demonstrated to possess antiviral, antifungal, and antibacterial qualities. Because of these characteristics, Lauric Acid and its derivative monolaurin are beneficial against a wide range of pathogens, including germs like *Streptococcus pneumonia* and *Staphylococcus aureus* and many other bacteria such as sweat bacteria.

The aim of the Lauric acid introduction into polymeric hydrogels was to optimize the synergistic effects of the fatty acids, particularly by utilizing their antibacterial, anti-inflammatory, moisturizing, and calming characteristics. These Polymers, which take the shape of hydrogels, have exceptional adhesive qualities once they come into contact with the skin's surface. Beyond their advantages for skincare, Polymeric hydrogels show promise in the delivery of active ingredients and the treatment of dermatological diseases. Hydrogel carrier systems are ideal options for the development of topical formulations due to their properties. Testing was done on the formulation, which included the Lauric acid blend loaded in the polymeric hydrogel, against the major sweat-odor-causing bacteria, *Corynebacterium* and *Staphylococcus*, which are major players in the human skin microbiota. It was also taken into consideration to investigate *Propionibacterium acnes*, a member of the *Propionibacterium* genus that generates propionic acid and is linked to offensive odor in elderly bedridden patients. Using conventional culture-based microbiological research, the resident microbiota—which is mainly composed of Gram-positive bacteria such as *Staphylococcus*, *Micrococcus*, *Corynebacterium*, and *Propionibacterium*—was successfully isolated.

The formulation and its constituent parts were tested against every isolated bacterial strain in an agar well diffusion experiment to determine the formed product's Antibacterial activity. The outcomes showed that odor-causing bacteria were resistant to the antibacterial qualities of the gel-containing polymers with loaded Lauric acid. This formulation's suitability for eco-friendly skincare has been demonstrated, and it has shown no adverse effects. Its potential for usage on a commercial scale is encouraging, providing the public with a toxin-free substitute for traditional products made of Aluminium and other toxic compound.

CHAPTER 6: CONCLUSION

Three different polymers e.g. Chitosan, Polyvinyl alcohol, and Edible bovine gelatin were used to make the Polymeric hydrogel. These three polymers were dissolved in different solvents chitosan was dissolved in glacial acetic acid and Polyvinyl alcohol and Edible bovine gelatin was dissolved in distilled water. Different characterization techniques like UV-Vis spectroscopy analysis, zeta, FTIR, Scanning electron microscopy, and Viscosity have shown that this process results in effective formulation. The final deodorant gel had a neutral smell, Consistency, and Viscosity that was skin and environment-friendly. The deodorant formulation is free of Aluminium and other toxins. Drug release efficiency was used to see how much drug was released over time. Other different techniques like hemolytic assay were used to see whether this gel caused hemolysis of blood or not when coming in contact with skin. Biofilm assay was used to see the antimicrobial and anti-biofilm activity of polymeric hydrogel. The antibacterial activity of the gel was seen by the Agar well diffusion method in which preculture was done and after that activity of the sample was checked against three sweat bacteria by measuring the Zone of inhibition. The Zones produced by blank gel were smaller as compared to loaded gel and pure lauric acid solution. The formulation may be altered even more to accommodate other alterations that will result in an even higher level of antibacterial activity.

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