WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

"DEVELOPMENT AND OPTIMIZATION OF TRANSDERMAL HYDROGEL SYSTEM LOADED WITH TETRACYCLINE HYDROCHLORIDE AGAINST BROAD SPECTRUM ANTIGENS"

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ABSTRACT :

The rising worldwide threat of antimicrobial resistance calls for novel drug delivery strategies. This study seeks to develop and optimize a transdermal hydrogel formulation for targeted and sustained delivery of tetracycline hydrochloride (TCH), a broad-spectrum antibiotic. Transdermal delivery has advantages over conventional means, including reduced systemic side effects, improved patient compliance, and targeted therapy for surface infections. The research aims at developing biocompatible and stable hydrogels using blends of natural and synthetic polymers (chitosan, Carbopol etc.). Primary objectives include systematically incorporating tetracycline hydrochloride in these matrices as well as fully characterizing the physicochemical properties of the loaded system, such as pH, viscosity, spreadability, as well as drug content. Experimental design strategies will be used to optimize the best polymer and tetracycline hydrochloride concentrations with optimal drug release kinetics and permeation properties. Ex vivo skin permeation experiments, using Franz diffusion cells with excised animal skin, will quantify tetracycline hydrochloride flux and overall permeation as a function of time.

Notably, the efficacy of the maximized transdermal hydrogel as an antimicrobial drug will be comprehensively measured in vitro against a variety of broad-spectrum bacterial antigens including common Gram-positive and Gram-negative strains. This will involve measurement of minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and zone of inhibition. Biocompatibility will be determined by cytotoxicity tests and preliminary skin irritation tests. The objective of this study is to formulate a robust, efficacious, and patient-friendly transdermal tetracycline hydrochloride delivery system. Successful formulation would enhance the therapeutic outcomes for skin infections by providing targeted and sustained drug action, and potentially minimizing systemic side effects, thereby significantly contributing to antimicrobial stewardship efforts. The findings will form a basis for future preclinical and clinical evaluations.

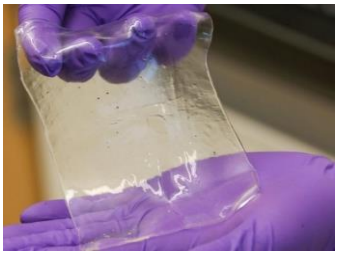
KEYWODS: Transdermal drug delivery, Hydrogel, Tetracycline hydrochloride (TCH), Broad-spectrum antigens, Drug release kinetics, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Biocompatibility, Dermatological infections.

INTRODUCTION

1.1 Hydrogel:

A hydrogel contains a three-dimensional hydrophilic polymer network since chemical or physical crosslinking occur within the chains for this it hold an acceptable level of water or biological fluids without dissolving.

Fig1: Hydrogel (https://images.app.goo.gl/FmVVch1PxqjJhawq8)



Different definitions of hydrogels have been put forward by different authors throughout the years.A polymeric material that swells to contain a large quantity of water in its form but is not able to dissolve in water (1). Hydrogels are biocompatible, soft, and elastic and therefore have widespread applications in medical, pharmaceutical, agricultural, and industrial purposes. 1Hydrogels can be prepared from almost any water-soluble polymer, regardless of its physical properties in the bulk state and chemical structure. Hydrogels can also be formed in a range of physical forms, such as slabs, microparticles, nanoparticles, coatings, and films. (2) The presence of chemical or physical crosslinking sites in the network maintains the three-dimensional character of hydrogels when in a swollen state. In hydrogels that have undergone chemical crosslinking, the polymer chains are connected by covalent bonds, which are aided by crosslinking agents (3).

1.1.1 Classification of hydrogel: According to their origin, structure, method of preparation, crosslinking process, charge, responsiveness, physical shape, and degradation, hydrogels are classified into a very diverse range of varieties. (4)

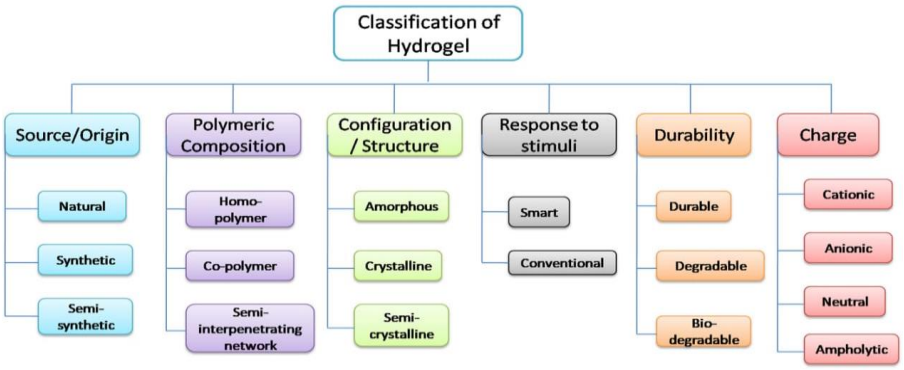


Fig2: Classification of hydrogel(https://www.mdpi.com/2310-2861/8/4/205)

On the basis of source: Fundamentally, hydrogels can be divided into two categories depending on where they originate like natural and synthetic hydrogel..PEG, polyvinyl alcohol (PVA), polyethylene oxide (PEO), poly(methacrylic acid), poly(acrylamide) (PAM), poly(N-isopropylacrylamide) (PIPAM), (4)

On the basis of cross linking: Hydrophilic polymer chains are crosslinked using numerous various processes to create hydrogel polymer networks. The agents, in most cases detrimental chemicals requiring their removal 1from the gels prior to use, can be harmful to the integrity of materials to be trapped, such as proteins and cells. (5)

1.1.2 Preparation of hydrogel:

A collection of polymers with water soluble ability is known as hydrogels. Chemical methods focus on polymerization through 1the creation of covalent bonds, whereas physical methods rely upon molecular assembly that is cross-linked by hydrogen and/or ionic bonds between the biopolymers (6).

a. Bulk polymerization:Crosslinking agent in small quantities is incorporated in the majority of hydrogel preparations. The polymerization process is initiated by radiation, ultraviolet rays, or chemical catalysts. The choice of initiator depends on the combination 1of monomers and solvents. There are several ways of manufacturing the polymerized hydrogel, 2e.g., 1 rods, particles, films and membranes, and emulsions (7).

b. Free radical polymerization: Acrylates, vinyl lactams, and amides are the main monomers employed in this process to produce hydrogels. There are a variety of thermal, ultraviolet, visible, and redox initiators that can be used to generate radicals 1in the initiation step. The radicals react with the monomers to transform them into active states (8).

c. Grafting to a support: The delicate architecture of hydrogels produced through bulk polymerization requires enhancing the mechanical properties of the hydrogel to graft the hydrogel onto a more robust support. To form a chain of monomers covalently attached to the support, free radicals are initially formed onto a more robust support surface, and afterward monomers are instantaneously polymerized onto it (9).

d. Polymerization by irradiation: Initiators like ionizing high energy radiation, e.g., electron beams and gamma rays, have been used to form hydrogels of unsaturated molecules Finally, a cross-linked structure is formed when the macro-radicals on different chains recombine to form covalent bonds. Irradiation polymerization is achieved with the aid of poly (vinyl alcohol), poly (ethylene glycol), and poly (acrylic acid). The process produces hydrogels that are rather pure and initiator-free (10,11,12).

1.2 Wound healing:

The etiology of cutaneous wounds is a failure in the integritiy of the skin. Healing is influenced by systemic mediators, local wound parameters, the type of injuries and the underlying disease. (13). The logical process of wound healing is a succession of overlapping yet successive stages of increasing complexity. But it may last as long as five or seven days after the crash. The third phase, proliferation and repair, tends to happen a week to three weeks after injury (14).

1.2.1 Classification:

From the management point of view, diagnosis, choice of therapy, healing time required, and assessment of risk, wound classification is important and stop infections that may occur during the healing process. As of now, this area does not have a universal and standard classification. On the basis of beneficial characteristics, these groups have been suggested to classify a minimal amount of wounds (15).

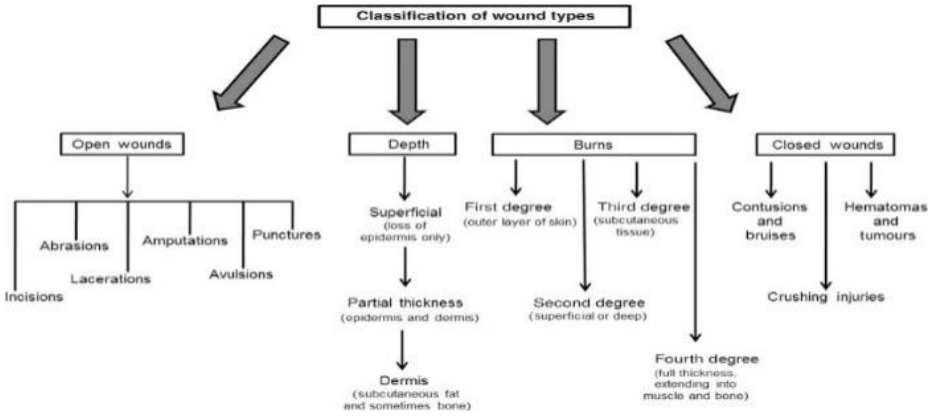


Fig3: Classification of wound healing(https://doi.org/10.1002/jps.24068)

1.2.2 Phases of wound healing:

An acute wound heals in a normal progression of events. This sequence of events is in a precisely controlled mechanism which can be replicated from wound to wound(16). While stages of the healing process for wounds are concurrent, they are expounded in a linear process in the sake of simplicity. Five stages of the healing process for wounds are-

1.Hemostasis (Immediate):

A cascade of serine protease reactions engineered to stop blood loss, the first events after injury are engineered to establish hemostasis in the first minutes to hours of trauma. In addition to hemostasis, platelet activation also leads to 1the 1release of growth factors like platelet-derived growth factor (PDGF) and immunological mediators that activate the immune system and commence the inflammatory process of wound healing (17).If bleeding into the open wound site due to tissue injury, then the extrinsic clotting cascade is set in motion, with such mediators as serotonin being released causing regional vasoconstriction and the beginning of the phase of haemostasis (18,19,20).

2.Inflammation (0–3 days):

Within the first 72 hours following tissue injury, the inflammatory period and early hemostasis are quite alike to one another(21). To prevent unnecessary injury to tissues and the elimination of pathogens and foreign debris, this step is largely typified by a multifaceted pattern of chemical signals that ultimately make Infiltration of neutrophils and monocytes into the wound bed(22).Local signals play a secondary role to promote the attraction of the cells responsible for inflammation to the wound site(23). Damaged host cells are necrotic in an open injury and spill out substances that serve as danger signals, such as extracellular matrix material, DNA, RNA, and uric acid. Collectively, these products have been referred to as damage-associated molecular patterns (DAMPs)(24,25,26).

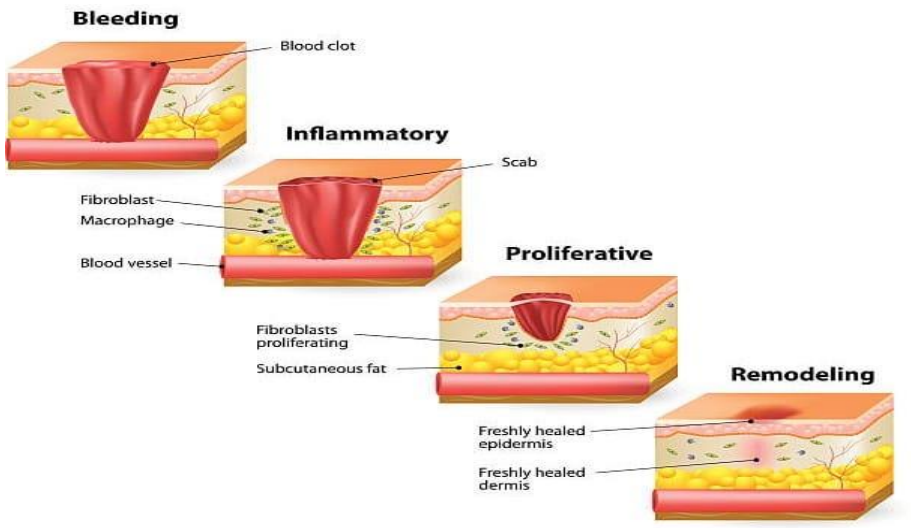
3.Proliferation: (3-10 days):

This stage occurs 14 days following the injury and starts 4 days following the injury. The body discharges a variety of cells like those which are in charge of proliferation and migration as inflammation subsides. Keratinocytes on the wound edge's lowest stratum migrate and differentiate into epithelial stem cells from nearby hair follicles or sweat glands to be employed in reepithelialisation (27,28,29).

4.Remodeling and Maturation (weeks to months):

Remodeling or maturation represents the concluding stage of wound healing. Healing can take years or sometimes just months, and it is fairly consistent across all types of wounds (30).Scar tissue replaces granular tissue after two weeks of injury(31).

Fig4: Phases of wound healing (https://images.app.goo.gl/qdy9tCH7HtASdVt76)



1.3 Tetracycline Hydrochloride:

Tetracycline hydrochloride is an antibiotic effective against gram-positive and gram-negative bacteria. The parent compound chlortetracycline was synthesized in 1947 from Streptomyces aureofaciens. The current research endeavors to bridge this knowledge gap by gathering information regarding the various degradation processes, mechanisms of both biodegradable and non-biodegradable processes (32,33,34). Antibiotics represent complex molecules characterized by excellent antimicrobial activity. Since the serendipidous discovery of penicillin in 1928, many antibiotics have subsequently been constructed. Nevertheless, the effectiveness and risk of allergic or toxic reactions of antibiotics that belong to the same structural class are comparable (35,36). Beyond their application as drugs, antibiotics have also been effectively applied in animal rearing for improvements in feed efficiency and growth promotion (37,38).

1.3.1 Mechanism of Action:

By binding itself to the 30S ribosomal subunit and preventing aminoacyl-tRNA from binding to the mRNA-ribosome complex, tetracycline hydrochloride prevents bacteria from protein synthesis. This action stops bacterial growth, making it a bacteriostatic antibiotic. (39,40,41).

1.3.2 Structure:

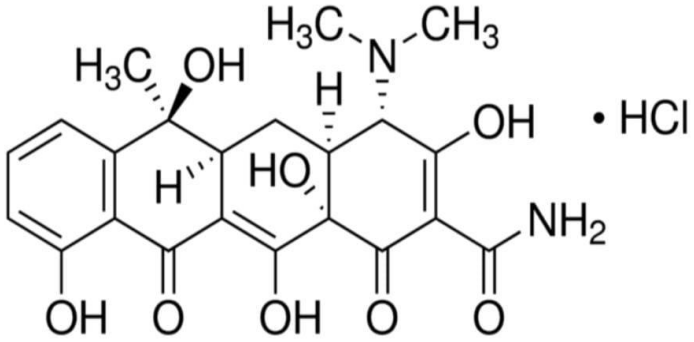


Fig5: Structure of tetracycline hydrochloride(https://images.app.goo.gl/9pLACmGu5ZyRXuvf9)

1.4 Gram Positive Bacteria:

Gram-positive bacteria are those that react positive to the Gram stain, a test which has been employed for many years to quickly classify bacteria into two large groups according to the nature of cell wall they possess Rates of major Gram-positive pathogens' antibiotic resistance are still increasing alarmingly in different regions of the world. (42). Methicillin-resistant Staphylococcus strains occur in medical institutions in most countries at considerably high frequencies (43). The annual increase in penicillin-resistant pneumococci is also alarming (44)

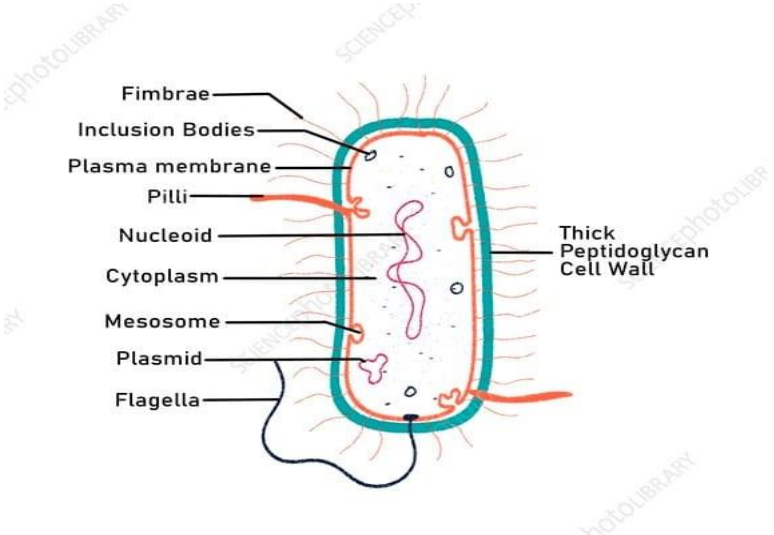


Fig6: Gram Positive Bacteria(https://images.app.goo.gl/rKhWiwLYUHNJCzek9)

1.4.1 Example:

1.Staphylococcus

2.Enterococcus (45)

1.5 Gram Negative Bacteria: The crystal violet dye used in the Gram staining method of bacterial differentiation is not retained by gram-negative bacteria, which is different from gram-positive bacteria. Because they threaten both morbidity and mortality and put patients at the ICU, or intensive care unit at critical risk, these bacteria are of significant clinical importance in hospitals (46).

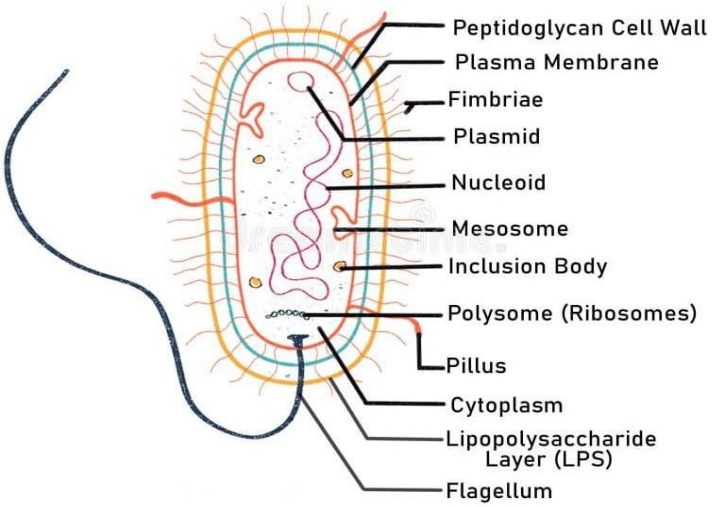


Fig7: Gram Negative Bacteria(https://images.app.goo.gl/dQo9VWoWfkUyTdwR8)

1.5.1 Example:

1.Pseudomonas,

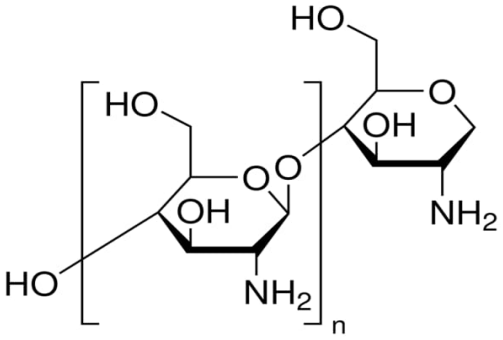
2.Klebsiella(47)

1.6 Chitosan:

Chitosan existed as early as 1859., when Rouged spoke about the deacetylated species of the parent nature polymer chitin (48). The naturally occurring source of chitosan is the exoskeleton of insects, crustaceans, and fungi, which has been found to be biodegradable and biocompatible (49). Chitosan polymers are semi-synthetic amino polysaccharides with multiple uses in the biomedical and other industries, and novel architectures, multidimensional properties, and very complex activity (50).

1.6.1 Structure of Chitosan:

Fig8: Structure of Chitosan(https://images.app.goo.gl/VLzaMW53pypDer4b6)



1.6.2 Application of chitosan:

a. Tissue engineering:

Tissue engineering techniques sometimes demand three-dimensional (3D) scaffolds for inducing initial cell adhesion and further tissue growth. Chitosan is structurally similar to glucosamine glycans (GAGs), which are found in the extracellular matrix of many human tissues. It has thus been extensively utilized in tissue engineering because of its ability to allow for cell attachment and retention of differentiation properties (51).

b. Cosmetics: Antifungal properties exist in chitin and chitosan, and they can be employed to form fine solvents for cosmetic use by using organic acids. Chitosan is the sole cationic gum to naturally thicken when an acid is added. They are used in creams, lotions, and permanent waving lotions. There have also been reports on the use of some chemicals as nail lacquers (52).

PLAN OF WORK

1.Preformulation study of chitosan, gelatine and tetracycline hydrochloride.
2.Preparation of chitosan film.
3.Preparation of gelatine solution.
4.Preparation of chitosan-gelatine hydrogel.
5.Casting of chitosan-gelatine hydrogel.
6.Addition of tetracycline hydrochloride.
7.Antimicrobial efficacy of prepared film.

WORK DONE (METHODOLOGY)

3.1. Materials:

SL.NO	CHEMICAL NAME	COMPANY NAME
1	Chitosan	Loba Chemie Pvt. Ltd
2	Gelatine	Nice Chemicals (P)Ltd.
3	Tetracycline Hydrochloride	Nice Chemicals (P)Ltd.
4	Beef Extract	Loba Chemie Pvt .Ltd
5	Sodium Chloride	Nice Chemicals (P)Ltd.
6	Agar agar Bacteriological	Nice Chemicals (P)Ltd.
7	Peptone Powder	Loba Chemie Pvt. Ltd.

Table 1: List of Chemicals

3.2. Preformulation Study of chitosan, Gelatine. Tetracycline hydrochloride:

3.2.1. Preformulation Study of chitosan:

a. Solubility:

Chitosan, a natural polymer of chitin, is predominantly present in crustacean exoskeletons. Both the degree of deacetylation and pH have a significant impact on its solubility. Because its amino groups are protonated, chitosan becomes soluble in diluted acidic solutions like acetic acid, but it remains insoluble in water and the majority of organic solvents. Chitosan receives a positive charge as a result, which enables it to dissolve and create a transparent, viscous solution. Chitosan usually dissolves easily at pH values lower than 6.5, but at neutral or alkaline pH, it precipitates out. Because of its crystalline structure and the existence of strong intermolecular hydrogen bonding, it is typically insoluble in neutral and alkaline aqueous solutions. The percentage of deacetylated units in the polymer, or the degree of deacetylation (DDA), has a significant impact on solubility; greater DDA values (usually above 70%) make more amino groups available for protonation, which improves solubility.

b. Melting point:

As chitosan is a high-molecular-weight biopolymer and degrades prior to the occurrence of a melting stage, it does not have a true melting point. At high temperatures, chitosan begins to thermally degrade instead of dissolving like small organic molecules. Subject to the level of deacetylation, molecular weight, and moisture content, this degradation typically occurs between 250°C and 300°C. The evaporation of bound water makes chitosan show an initial endothermic peak at around 100–150°C when analyzed through techniques like thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). It is followed by an exothermic peak associated with breakdown.

3.2.2. Preformulation Study of gelatine:

a. Solubility:

The solubility of gelatine is not usually calculated through a constant formula—it is rather experimentally determined depending on various factors such as temperature, pH, concentration, and type of gelatine used (Type A or Type B). To find gelatine solubility, a definite weight of gelatine is added gradually to a certain volume of solvent normally water at a regulated temperature (usually around 40–60°C), and stirred until totally dissolved. This process is reiterated at different temperatures to plot the solubility curve. Solubility may be affected by pH (with solubility at a maximum at or around

neutral pH) and by the presence of other salts or solutes. In analytical chemistry, gelatine solubility may be quoted in grams per 100 mL of solvent per degree at a particular temperature.

b. Melting point:

The melting point of gelatine is not worked out by an equation but experimentally measured as it is influenced by a number of factors like gelatine concentration, bloom strength (a parameter of gel hardness), molecular weight, and conditions like temperature and pH. Gelatine lacks a clear-cut melting point like pure crystalline substances but has a range of melting, commonly 30°C to 35°C, slightly below the temperature of the human body.A gelatine gel is prepared first by dissolving a specified amount of gelatine in water and then leaving it to set.This transition temperature is assigned as the melting point.

3.2.3. Preformulation Study of tetracycline hydrochloride:

a. Solubility:

A major step in preformulation studies of tetracycline hydrochloride is the solubility test, which determines the way the drug dissolves in various solvents and pH levels. The test helps to determine the best conditions for formulation design. To find the qualitative and quantitative solubility of tetracycline hydrochloride, it is dissolved in several solvents, such as water, ethanol, methanol, acetone, and chloroform. Because of water solubility, which is very high, particularly at acidic pH (pH 2–6), To determine its solubility, a definite excess quantity of tetracycline hydrochloride is added to a given volume of solvent—often water or an aqueous buffered solution—at a particular temperature, frequently 25°C or 37°C.

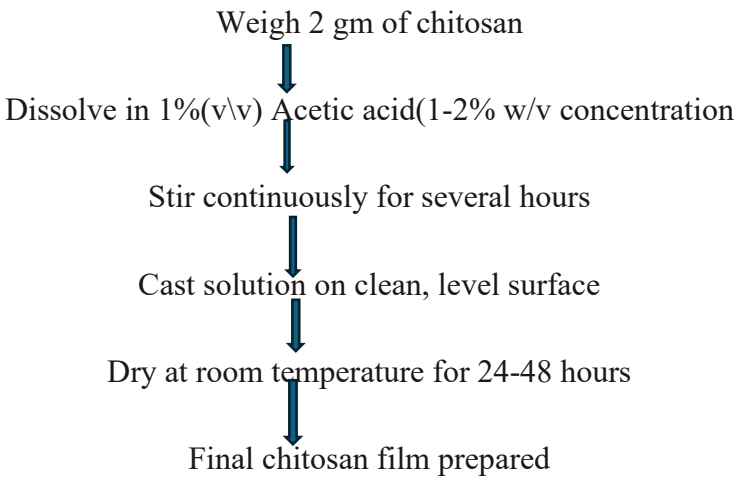
b. Melting point:

The melting point of tetracycline hydrochloride is not usually determined by a mathematical equation but experimentally measured by thermal analysis methods. This is due to the fact that tetracycline hydrochloride, as with many complex organic molecules, tends to have a certain melting behavior caused by its crystal form, hydrogen bonding, and purity, which are not easily determinable theoretically.

Experimental Determination: The difference between the two temperatures is known as the melting point range, which is typically between 220°C and 230°C in pure tetracycline hydrochloride.

3.3. Preparation of chitosan film:

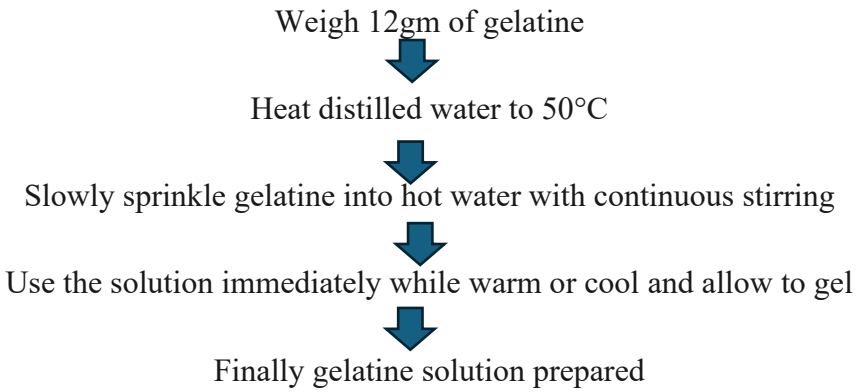
Chitosan film preparation includes dissolving chitosan powder in a weak acidic solution, typically acetic acid, to get a clear, uniform polymer solution. Chitosan is dissolved at concentrations of 1-2% (w/v) in 1% (v/v) acetic acid under steady stirring for several hours for complete solubilization.Add 2ml acetic acid, dissolved in water and mixed with chitosan solution.After dissolving, the solution tends to be filtered to eliminate undissolved particles or impurities. The solution prepared is then poured onto a clean leveled surface like a glass or Teflon plate and left to dry under room temperature or in a controlled setting (e.g., an oven at 40–50°C). Following thorough drying, which could take anywhere from 24–48 hours based on conditions, the developed chitosan film is gently removed. Add 2 gm chitosan in 90 ml water. The thickness, tensile strength, and other characteristics of the film can be controlled by varying the concentration of chitosan, plasticizer, or drying process.



Flow chart 1: Preparation of chitosan film (Self-copyright)

3.4. Preparation of gelatine solution:

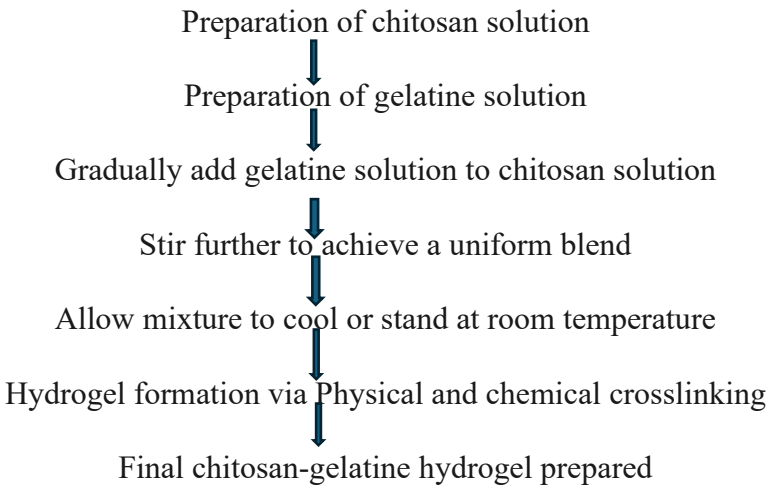
To dissolve the gelatine, 40 milliliters of cold distilled water were mixed with 50°C. Since gelatine soaks up and swells in water but not in cold water, it can be dissolved more easily by soaking in a small amount of cold water for a few minutes before being heated.12 gm gelatine dissolve in 40 ml water. After the gelatine is completely dissolved, the mixture is warmed by gentle heating with stirring until the gelatine dissolves completely and appears to be a clear or pale yellowish solution.



Flow chart 2: Preparation of gelatine solution (Self-copyright)

3.5. Preparation of chitosan-gelatine hydrogel:

Preparation of chitosan–gelatine hydrogel entails mixing both biopolymers under defined conditions to produce a stable, biocompatible gel for biomedical, pharmaceutical, or tissue engineering applications. The process begins by dissolving chitosan in a little bit acidic solution, typically 1% (v/v) acetic acid, between concentrations of 1% to 2% (w/v), and agitating the mixture for a couple of hours until it is dissolved thoroughly. Chitosan and gelatine are blended 2:1 ratio. Independently, gelatine is dissolved in hot distilled water (approximately 40–50°C) at an equivalent concentration, also under constant stirring. After both solutions are completely dissolved and homogeneous, the gelatine solution is added to the chitosan solution slowly under gentle agitation in order to maintain uniform mixture and prevent precipitation. The formed mixture is then stirred further to obtain an evenly blended mixture. The blended solution is subsequently cooled or left at room temperature to form a hydrogel network due to physical and/or chemical crosslinking. Casting of a chitosan–gelatine hydrogel includes pouring a prepared hydrogel solution into a mould or onto a planar surface to create a film or scaffold having a specified shape and thickness.



Flow chart 3: Preparation of chitosan-gelatine hydrogel (Self-copyright)

3.6. Casting of chitosan-gelatine hydrogel:

Once the homogeneous hydrogel solution is prepared by dissolving gelatine in hot water and chitosan in diluted acetic acid, and then mixing both solutions at a high rate of stirring, the viscous solution is poured into clean, levelled casting moulds like Petri dishes, glass plates, or silicone trays immediately. The amount poured is accurately measured to provide equal thickness. Where crosslinking agents such as glutaraldehyde are employed, casting is frequently preceded and followed by curing in order to facilitate correct network development and structural stabilization. After complete drying or gelling, the cast chitosan–gelatine hydrogel is gently stripped from the mould. The obtained material can be additionally washed, neutralized, or sterilized according to the targeted use in biomedical, pharmaceutical, or packaging applications.

Fig 9: Casting of chitosan gelatine hydrogel (Self-copyright)

3.7. Addition of tetracycline hydrochloride:

To create the chitosan–gelatine hydrogel, chitosan is dissolved in a solution of weak acetic acid and gelatine in a 2:1 ratio, which is then heated in boiling water.The two solutions are subsequently mixed under gentle stirring to form an even slurry.A crosslinking agent like glutaraldehyde can be added in order to increase the mechanical and structural properties of the hydrogel. The combination is left to cool or be at room temperature, which leads to the formation of a soft, elastic, and biocompatible hydrogel network. Then casting the chitosan-gelatine hydrogel. Following the casting of chitosan-gelatine hydrogel, 0.16 gm of tetracycline hydrochloride into this chitosan-gelatine hydrogel.

FORMULATION NO	CHITOSAN (gm)	GELATIN (gm)	TETRACYCLINE HYDROCHLORIDE (gm)
Formulation 01	4	6	0.16
Formulation 02	1	1	0.16
Formulation 03	2	1	0.16

Table 2: Formulation table of chitosan, gelatine, tetracycline

In this study formulation 1 and formulation 2 was not appropriate as desire so result of formulation 3 was considered as standard.

3.8. Antimicrobial efficacy of prepared film:

One widely employed technique for determining bacteria's susceptibility to antibiotics is the disk diffusion technique, also known as the Kirby-Bauer test. 2. The principle behind this method is that an antibiotic is diffused out of a paper disc into an agar plate that has been uniformly contaminated with a test bacterium. Larger zones typically indicate greater sensitivity, while smaller or absent zones suggest resistance. Standardized interpretation charts, such as those from CLSI or EUCAST, are used to classify bacteria as sensitive (S), intermediate (I), or resistant (R) based on the measured zone diameters. This method is simple, cost-effective, and suitable for routine use in clinical and research laboratories. However, it provides only qualitative results and is most effective with non-fastidious, rapidly growing aerobic bacteria on Mueller-Hinton agar.

3.8.1. Microorganism species used:

E. Coli (ATCC 8739), B. subtilis (ATCC 6633), Candida albicans (ATCC 10238), and Staphylococcus aureus (ATCC 6538) were the bacterial strains (ATCC, CDL, Kolkata) used. Escherichia coli, Bacillus subtilis, Candida albicans, and Staphylococcus aureus are popular test micro-organisms used for antimicrobial testing based on their varied properties and clinical significance. They are suitable for testing the broad-spectrum antimicrobial activity of drugs because they belong to various types of pathogens, including fungi (C. albicans), Gram-positive bacteria (B. subtilis and S. aureus), and Gram-negative bacteria (E. coli).E. coli, B. subtilis, C. albicans, and S. aureus for anti-microbial screening of hydrogel would yield an extensive evaluation of its possible anti-microbial activity. This would enable testing of the plant extract's efficacy against a variety of clinically important pathogens, including both bacterial and fungal pathogens. Yet it should be noted that the anti-microbial effect can differ based on the compounds in the tetracycline hydrochloride and their modes of action against various micro-organisms.

3.8.2. Preparation of nutrient agar media:

a. Nutrient Agar: A great agar medium for verifying purity prior to biochemical or serological testing is nutritional agar, which has nutrients that are appropriate for the sub culturing of a broad variety of bacteria.Also, the addition of agar solidifies nutrient agar, making it suitable for microorganism growth.

b. Preparation nutrient agar: In 1 liter of distilled water, dissolve 28 grams of nutrient agar powder. Stir thoroughly to ensure complete mixing. Autoclave at 121°C for 15 minutes to sterilize.

TYPICAL FORMULA	NUTRIENT AGAR (GM/LITRE)
Beef Extract	5gm
Distilled water	500ml
Peptone	5gm
Sodium Chloride	3gm
Agar	12.5gm

Table 3: Composition of Nutrient Agar

3.8.3. Inoculation of bacterial suspension:

• The nutritional agar media are cooled to 45° C after autoclaving. They are then poured onto the sterile petri plates to create a deep layer (6 mm) and not disturbed for two hours. Petri plates which are sterile are taken and labelled 1 and 2.

•After 2 hours when the media solidifies, from test tube 0.5 ml of bacterial suspension is taken and inoculated in petri plate, in an aseptic condition (laminar air-flow chamber).

•In the same way, petri plates 1 and 2 are inoculated with the suspension from the test tubes 1 and 2.

3.8.4. Preparation of Test and Standard Solutions:

For obtaining the concentrations of 5 mg/mL, hydrogel was dissolved in distilled water and diluted later with sterile distilled water. Similarly, standard chloramphenicol was prepared at the same concentrations (5mg/mL) using sterile distilled water. All solutions were freshly prepared before each experiment.

3.8.5. Agar Well Diffusion Method:

The agar well diffusion technique was employed to assess the antibacterial activities of the hydrogel and the commercial chloramphenicol. Two sterile Petri plates were prepared for each of the microbial strains tested, one for hydrogel (test group) and one for the standard drug (chloramphenicol).Sterile nutritional agar was utilized for bacterial cultures, while Sabouraud dextrose agar (SDA) was employed for Candida albicans.About 20 mL of melted agar medium was filled in every 90 mm Petri dish aseptically and let to set. After setting, the agar surfaces were seeded by swabbing them uniformly with 100 µL of the calibrated microbial inoculum that had been diluted to achieve the 0.5 McFarland turbidity standard. After the diffusion time, the plates with the bacterial strains were incubated for 24 hours at 37 °C, whereas the plates with the Candida albicans were incubated for 48 hours at 28 °C.

3.8.6. Zone of Inhibition:

Post-incubation, the diameter of the zone of inhibition surrounding each well was measured in millimeters (mm) using a transparent ruler or Vernier caliper. All tests were performed in triplicate to ensure reproducibility, and the mean values were recorded.

RESULTS

4.1. Preformulation Study of chitosan, Gelatine. tetracycline hydrochloride:

4.1.1. Solubility chart of chitosan:

SOLVENT	SOLUBILITY	REMARKS
Water	Insoluble	Chitosan does not dissolve in water unless the pH is acidic

Dilute Acetic Acid(1%v/v)	Soluble	Most commonly used solvent for chitosan, gives clear solution
Dilute Hydrochloric Acid	Soluble	Effective for dissolving chitosan
Dilute Lactic Acid	Soluble	Biocompatible acid
Sodium Hydroxide	Insoluble	Chitosan precipitates out in alkaline conditions
Methanol, Ethanol, Acetone	Insoluble	Chitosan is not soluble in organic solvents
Acetic Acid	Soluble	May enhance solubility

Table 4: Solubility chart of chitosan

4.1.2. Melting point of chitosan:

The standard melting point of chitosan is – 102.5°C

NUMBER OF ATTEMPTS	MELTING POINT
1st	100°C
2nd	101°C
3rd	102°C

Table 5: Melting point of chitosan

Average:

(100+101+102/3) = 101.6°C

4.1.3. Solubility of gelatine:

SOLVENT	SOLUBILITY	REMARKS
Cold Water	Insoluble	Gelatine swells and absorb water but does not dissolve
Hot water	Soluble	Dissolves completely with stirring to form a clear viscous solution
Boiling Water	Fully Soluble	Rapidly dissolve
Acetic Acid	Soluble	May enhance the solubility

Table 6: Solubility of gelatine

4.1.4. Melting point of gelatine:

The standard melting point of chitosan is 22-40°C

NUMBER OF ATTEMPTS	MELTING POINT
1st	20°C
2nd	30°C

3rd	20°C

Table 7: Melting point of gelatine

Average:

(20+30+20/3) = 23.3°C

4.1.5. Solubility of tetracycline hydrochloride:

SOLVENT	SOLUBILITY	REMARKS
Water	Highly Soluble	Approx. 50 mg/ml at room temperature forms a yellow solution
Hot water	Very Soluble	Solubility increases with temperature
Ethanol	Slightly Soluble	Limited solubility
Methanol	Slightly Soluble	Slightly more soluble than ethanol
Dilute Acid	Soluble	Stable and soluble in acidic medium

Table 8: Solubility of tetracycline hydrochloride

4.1.6. Melting point of tetracycline hydrochloride:

The standard melting point of tetracycline hydrochloride is 215-217°C.

NUMBER OF ATTEMPTS	MELTING POINT
1st	180°C
2nd	185°C
3rd	180°C
4th	185°C
5th	177°C
6th	190°C
7th	183°C

Table 9: Melting point of tetracycline hydrochloride

Average:

(180+185+180+185+177+190+183/7) = 182.85°C

4.2. Preparation of chitosan film:

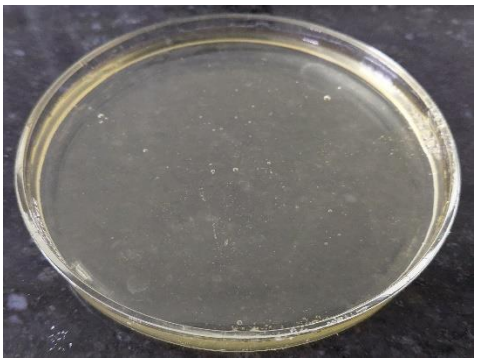


Fig10: Preparation of chitosan film (Self copyright)

4.3. Preparation of gelatine solution:

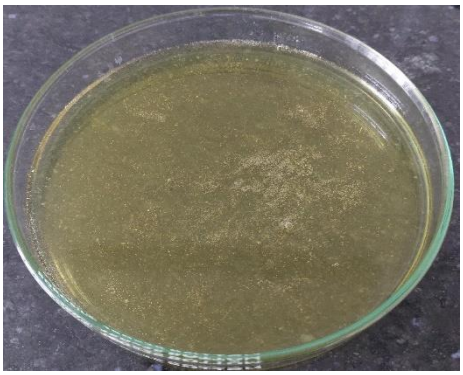


Fig11: Preparation of gelatine solution (Self copyright)

4.4. Preparation of chitosan-gelatine hydrogel:

Fig12: Mixing of chitosan-gelatine hydrogel (Self-copyright)

Fig13: Preparation of chitosan-gelatine hydrogel (Self–copyright)

4.5. Casting of chitosan-gelatine hydrogel:

Fig14: Casting of chitosan-gelatine hydrogel (Self-copyright) **Fig15: Storage of hydrogel (Self-copyright)**

4.6. Antimicrobial efficacy of prepared film:

4.6.1. Comparative Antimicrobial Evaluation Of hydrogel and chloramphenicol using agar well:

We can use the bacterial strains (ATCC, CDL, Kolkata) were E. coli (ATCC 8739) denoted as "A", B. Subtilis (ATCC 6633) denoted as "B", Candida albicans (ATCC 10238) denoted as "C", and Staphylococcus aureus (ATCC 6538) denoted as "D" to perform our evaluation of antimicrobial activity in hydrogel.

4.6.2. Preparation of Standard solution, Test solution and Agar media:

The Standard can be made with chloramphenicol with different concentration of 5mg/mL and the test solution of hydrogel is also made as same concentration of standard solution. Then takes two different petri plates with nutrient agar solution. Pour the plate with agar solution and leave it for a while to freeze in room temperature to content agar media. After get the agar media we can boring four different area on it to give the bacterial and standard solution. Then we made the bacterial solution with Nacl solution in aseptic chamber. Then we pour filter paper in the previously boring area and denoted "A", "B", "C", "D", to identify the bacterial solution and then give 9drops (20µl each drop) of bacterial solution with micro pipette in every petri plates. Then give 9drops (20µl each drop) of standard solution with micro pipette in three petri plates parallelly. And 9drops (20µl each drop) of test solution with micro pipette in three petri plates parallelly. Therefore, it locked in BOD chamber for 5-7 days. After 5-7 days we can see the microbial growth of standard and test solution plates to known about the anti-microbial activity.

There the standard solution plates:

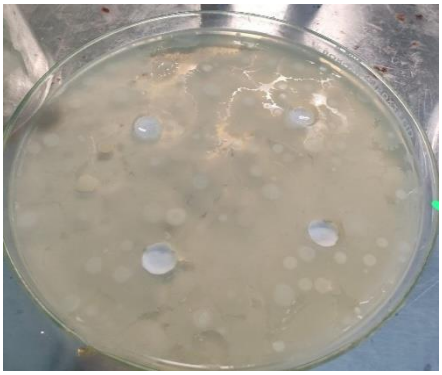


Fig16: Standard solution plate (Self-copyright)

From the above pictures we see that the microbes can growth perfectly in the solution of chloramphenicol.

There the test solution plates:

Fig17: Test solution plate (Self-copyright)

From the above pictures we see that the microbes cannot growth perfectly in hydrogel.
Hence, we proved the anti-microbial activity is present in hydrogel.

4.6.3. Zone of inhibition:

SL NO	MICROORGANISM SPECIES	CONCENTRATION (mg/mL)	HYDROGEL TETRACYCLINE HYDROCHLORIDE (mm)	CHLORAMPHENICOl (mm)
1	Escherichia coli	5	2.3	2.3
2	Bacillus subtilis	5	1.5	2.4
3	Staphylococcus aureus	5	3.5	2.0
4	Candida albicans	5	4.0	2.2

Table 10: Antimicrobial activity of hydrogel containing tetra cyclin hydrochloride and Chloramphenicol against Pathogenic microorganism

DISCUSSION

The creation of a transdermal hydrogel system with a loading of tetracycline hydrochloride (TCH) for broad spectrum antibacterial activity capable of transforming the therapy of a multitude of infections is the creation of a transdermal hydrogel system with a loading of tetracycline hydrochloride (TCH) for broad spectrum antibacterial activity. Numerous of the drawbacks of conventional oral and cutaneous TCH formulations are avoided by this method. For example, traditional topical therapy is usually sloppy and of temporary and short-distance action, whereas oral TCH causes stomach distress and irregular absorption. In contrast, transdermal delivery circumvents the digestive system and can decrease systemic side effects through prolonged release of the drug via the skin. Tch, a very potent antibiotic that has the ability to combat diverse bacteria, can be delivered more efficiently to the affected site by this method, and it is therefore a very good option for the treatment of many skin and soft tissue infections. The main focus of this investigation is to carefully choose hydrogel materials that have the potential to encapsulate and deliver tch as well as maintain stability and biocompatibility. There is a necessity to optimize others such as tch content, type and quantity of permeation enhancers, and pH of gel to improve the ability of the drug to penetrate the skin. There is also the need for rigorous testing to ensure that the gel is effective against various diseases and non-toxic in humans, including phases of drug release in the laboratory conditions and performance and safety in animal models. Ultimately, an effective transdermal tch hydrogel may decrease side effects, improve patient compliance with treatment, and offer a more targeted and effective means of preventing infections that are prevalent.

CONCLUSION

The work in the development and optimization of a tetracycline hydrochloride transdermal hydrogel system is a significant milestone in the delivery of antimicrobial drugs and provides a potent substitute for traditional topical and oral therapy. In comparison with conventional topical formulations, the system can improve activity and retention by exploiting the exclusive characteristics of hydrogels to deliver TCH in a targeted and sustained manner, avoiding common problems like gastrointestinal side effects and unpredictable bioavailability related to oral administration.
Optimization of the desired kinetics of drug release, skin permeation, and patient acceptability requires delicate adjustment of key parameters such as polymer type and amount, TCH loading, pH, and careful use of permeation enhancers. To determine the physicochemical stability of the system, pattern of drug release, in vitro and in vivo broad-spectrum antimicrobial activity against a variety of infections, and most critical.
Finally, an intelligently engineered transdermal TCH hydrogel system has tremendous potential to enhance patient outcomes in the treatment of many soft tissue and skin infections. With the increasing issue of antibiotic resistance, its potential to enable convenient, localized, and sustained broad-spectrum antimicrobial therapy to revolutionize treatment regimens, reduce systemic exposure and correlate adverse effects, and make it easier to use antibiotics judiciously is real.

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