Formulation and characterization of Vancomycin loaded Chitosan- Polyvinyl Alcohol Transdermal Patches

Project report submitted in the fulfillment of the requirement for the degree of Bachelor of Technology In

Biotechnology

By

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To

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CERTIFICATE

I hereby declare that the work presented in this report entitled **"Formulation and characterization of Vancomycin loaded Chitosan- Polyvinyl Alcohol Transdermal Patches"** in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the department of Biotechnology and Bioinformatics**,** Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from August 2018 to May 2019 under the supervision of **Dr. Gopal Singh Bisht** (Associate Professor, Dept. of Biotechnology and Bioinformatics).

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Student's Signature)

Rashika Dhar (151827)

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

(Supervisor's Signature)

Dr. Gopal Singh Bisht

Associate Professor

Department of Biotechnology

Dated:

DECLARATION

I hereby declare that the dissertation work that is being presented in this thesis, entitled **"Formulation and characterization of Vancomycin loaded Chitosan- Polyvinyl Alcohol Transdermal Patches"** for the partial fulfillment for the award of degree of Bachelors of Technology in Biotechnology to Jaypee University of Information Technology, Solan, (HP) is an authentic record of my own research work carried out during the academic year 2018-19 under the guidance of **Dr. Gopal Singh Bisht.**

The matter embodied in this thesis is my original work and has not been submitted by me or any other person for the award of any degree in this or any other university.

Place: Solan

Date:

Rashika Dhar

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SNo	Abbreviations	Full form
1.	TP	Transdermal patches
2.	VMC. HCl	Vancomycin hydrochloride
3.	PVA	Polyvinyl alcohol
4.	CH	Chitosan
5.	PVA/CH	Polyvinyl alcohol/ chitosan
6.	Conc.	Concentration
7.	FTIR	Fourier transformation infrared spectroscopy.
8.	TDDS	Transdermal drug delivery systems

ABSTRACT

Administration of drugs through the transdermal route has been considered one of the safest modes of drug administration due to the lesser side effects. In this study, the controlled release "Transdermal patches" of Vancomycin hydrochloride were prepared using chitosan and polyvinyl alcohol polymers. Chitosan was polymerised with polyvinyl alcohol as it is a non-toxic polymer and known for its effective wound healing abilities and by this various Vancomycin hydrochloride loaded patches were prepared in petri plates, giving them a flat cylindrical appearance and were elastic in consistency. The characterization of the gels was carried out by FTIR analysis, calculation of swelling ratio (using different solvents), drug loading and encapsulation studies and drug release calculation. As the antibiotic, Vancomycin hydrochloride shows side effects when administered through the oral or peritoneal route, such as localised irritations, reactions in the gastrointestinal tract and toxicities. Therefore to reduce the toxic effects of Vancomycin hydrochloride, various transdermal patches (F1, F2, F3, F4) were synthesized and characterized. F4 was chosen as best formulation with highest loading capacity, it released 31 % vancomycin hydrochloride in 12 hours. Hence, from this study we can conclude that F4 can be utilized in future as topical antimicrobial formulation.

AIM and OBJECTIVES

- To prepare Vancomycin hydrochloride loaded transdermal patches of chitosan-PVA.
- To characterise the prepared transdermal patches (F1, F2, F3, F4).
- To study the release properties of the best Vancomycin hydrochloride loaded transdermal patch.

INTRODUCTION

Topical formulations are being utilised for range of preparations that are used for dermatological and cosmetic purposes. [1]. Such formulations can be prepared in a variety of types on the basis of consistency which ranges from solid to semisolid to liquid. Delivery of drugs via skin is not only specific but a highly sensitive treatments for localised toxicities and skin related disorders. The transdermal route of drug delivery has gained importance as it puts an end to metabolic reactions, gastrointestinal difficulties and degradation caused by the oral provision of drugs (2,3). Such formulations provide a suitable delivery method for drugs because they are thixotropic, grease proof, easy to spread and remove, moisturizing, non-staining, compatible with several excipients and soluble in water. The drug is released from the formulation and it further penetrated the skin by the percutaneous absorption; to the targeted tissue or cells. The release of the drug from the formulations is directly related to the physicochemical properties of the vector and the drug utilised (4, 5).

Numerous medicated products are applied to the skin that either enhance or restore a fundamental function of the skin or pharmacologically alter an action in the targeted tissues (6). This is because the skin is one of the most convenient route of drug delivery and it also reduces a variety of side effects experienced by patients while undergoing oral and peritoneal administration. Such devices are also known to provide a sustained drug release, cause no pain to the patient and also in case of any difficulties experienced the formulation can be removed with ease. (7).

VMC. HCl is an antibiotic employed in the treatment numerous Gram positive bacterial infections. It is a type of glyco-peptidic antibiotic which proceeds by blocking the synthesis of cell wall (8). It is provided intravenously to treat multiple complications such as severe skin and blood infections, endocarditis, bone and joint diseases, and meningitis resulted via methicillin resistant *Staphylococcus aureus* (MRSA) (9). It is also recommended through the oral route for fatal *Clostridium difficile*colitis. When administered by mouth it is not absorbed very effectively, thus the common repercussions include pain at the site of injection punctures and allergy, occasional hearing difficulties, low blood pressure and in extreme cases there can be bone marrow suppression. This antibiotic can be administered during pregnancy as no case of harm has yet been reported and it doesn't affect during the lactation period as well.(10)

TPs are adhesive, medicated fixtures which are placed on the skin to deliver a specific dose of the drug via the skin into the vascular system. They serve as a medium for sustained and timely drug release into the affected area while over- coming the disadvantages of the other administrative routes. The drug is carefully encapsulated in the porous matrix which is then released through the patch due to the heat released by the body, allowing the release to occur layer wise. The patches consist of a high dose of drug which is retained on the skin and is released timely into the bloodstream via diffusion. Hence, many pharmaceutics are now available in the form of patches due to the clean and painless administration.(11) Drug can enter through skin via three pathways:

a) Through hair follicles.

b) Through sebaceous glands.

c) Through sweat ducts.

Transdermal drug delivery systems are used in a variety of skin disorders and also in the management of angina pectoris, smoking cessation & neurological diseases such as Parkinson's disease (12, 13).

1.1 Types of Transdermal Drug Delivery systems:

1. **Single-layer Drug-in-Adhesive System:**

In this type of patch the adhesive layer of this system contains the drug. The adhesive layer not only serves to adhere the various layers together, along with the entire system to the skin, but it is also responsible for the releasing the drug. The adhesive layer is surrounded by a temporary liner and a backing.

2. **Reservoir System:**

The drug reservoir is placed within a backing layer and a rate controlling membrane. Further the drug releases through micropervious rate controlled membrane as the antibiotic can be in the form of a solution, suspension, gel or dispersed in a rigid polymer matrix in the reservoir compartment.

3. **Matrix System:**

This system is of two types:

a) Drug-in-Adhesive System: For the fabrication of drug reservoir, the drug is first dissolved in an adhesive polymer and then is spread on the medicated polymer adhesive. This is done by solvent casting or by melting the adhesive (while considering hot-melt adhesives) on to an impermeable backing layer.

b) Matrix-Dispersion System:

The drug is dissolved uniformly in a hydrophilic or lipophilic polymer matrix. Then the polymer along with drug is fixed onto an occluding base plate in a division produced from a drug- impermeable backing layer. The adhesive is spread along the circumference to form a strip of adhesive rim (14).

4. Micro-Reservoir System:

This system comprises of 2 systems i.e., matrix and the reservoir system. In which drug is dispersed in an aqueous solution of water-soluble polymer which is further dispersed uniformly in a lipophilic polymer solution. As a result microscopic beads are formed that serve as drug reservoirs. (15).

Every TP has a series of ingredients which are brought together to produce the perfect product. There are various requirements other than the formulation itself, which increase the product efficiency, sustainability, market value and commercial availability. The basic components of TPs are as follows:

5. Polymer matrix/ Drug reservoir:

It is prepared by dispersing the drug in liquid or solid state synthetic polymer base. It should have biocompatibility and chemical compatibility with the drug and other components of the system such as penetration enhancers. Additionally they should provide consistent and effective delivery of a drug throughout the product's intended shelf life and should be of safe status. Polymers used in Transdermal drug delivery systems are classified as

a) Natural Polymers: e.g. cellulose derivatives, gelatin, shellac, waxes, gums, natural rubber and CH etc.

b) Synthetic Elastomers: e.g. polybutadiene, hydrin rubber, silicon rubber, polyisobutylene, acrylonitrile, neoprene, butyl rubber etc.

c) Synthetic Polymers: e.g. poly-vinyl alcohol, polyvinylchloride, polyethylene, polypropylene, poly-acrylate, polyamide, poly-urea, poly- vinyl pyrollidone, polymethyl methacrylate, etc. [16, 17].

- **Liner –** This is the primary packaging material that can protect the patch during application. It is made up of base layer which may be a) Non-occlusive (e.g. paper fabric) b) Occlusive (e.g. polyethylene, polyvinylchloride).

  It is made up of silicon or Teflon. Release liner should be chemically inert & it should be permeable to drug, penetration enhancers & water. (18)

- **Drug** – the gel containing the drug is in direct contact with the liner. Once the liner is removed the drug loaded matrix comes in contact with the skin. These are the following properties of the drugs in use and factors to be considered while preparing TPs:

a) Ideal Properties of Drugs:

Table 1: The ideal properties of a drug

SNo.	Parameters	Properties
1.	Dose	Should be low in weight (less than 20mg/day).
2.	Half- Life	10/ less hours.
3.	Molecular weight	<400 Daltons
4.	Skin permeability coefficient	>0.5* 10^{-3} cm/h
5.	Skin reactivity	Non- irritating, non- sensitizing
6.	Oral bioavailability	Low

b) Factors affecting drugs:

Table 2: The factors affecting the drug

Physicochemical	Pharmacokinetic	Biological
Solubility	Half-life	Skin toxicity

Crystallinity	Volume of distribution	Site of application
Molecular weight	Total body clearance	Allergic reaction
Polarity	Therapeutic plasma conc.	Skin metabolism

- **Adhesive –** it allows all the components to stick together within the patch and also enables the patch to adhere to the skin. E.g.: Poly-acrylates, Poly-isobutylene, silicon based adhesives.
- **Membrane –** found in multi-layer patches promoting controlled release for a second dose.
- **Backing –** It is a supportive material which is impermeable to drugs and also to permeation enhancers. They should chemically compatible with the drug, enhancer, adhesive and other excipients. Ex: Vinyl, Polyethylene and Polyester films (19).
- **Permeation Enhancer –** they serve as promoters for the permeation and enhanced drug delivery into the skin. Ideal Properties of Permeation Enhancers:

  a) They should be non-irritating, non-toxic & non- allergic.
  b) They are not to have any pharmacological activity.
  c) They should be cosmetically acceptable with an appropriate skin feel. [18]

- **Matrix Filler** – it provides bulk to the matrix and also serve as stiffening agents so that the patch stays in place.
- **Other components:** Antioxidants (serving as stabilizers), Preservatives etc.
- **Plasticizers and Solvents:**

a) Solvents: Chloroform, methanol, acetone, isopropanol and dichloromethane.
b) Plasticizers: Dibutylpthalate, triethylcitrate, polyethylene glycol and propylene glycol [19].

1.2 Methods of Preparation of TDDS:

a) "Asymmetric TPX membrane" method.
b) "Circular Teflon mould" method.
c) "Mercury substrate" method.
d) "IPM membranes" method.

e) "EVAC membranes" method.

f) "Proliposomes" method.

g) "Free film" method.

- **"Asymmetric TPX Membrane" Method:** This method was discovered by Berner and John in 1994. According to this, the patches can be prepared by involving heat sealing polyester film along with a backing layer. Drug is dissolved on concave membrane and then covered by TPX [poly (4-methyl-1- pentene)] i.e. the asymmetric membrane. The entire entity is then sealed by an adhesive.

- **"Circular Teflon Mould" Method:** It was discovered by Baker and Heller in 1989. The polymeric solutions used are prepared in varying concs, further divided and subjected to enhancers which are also of different concs. A plasticizer, Di-N-butylphthalate is added and the solution is poured into a circular Teflon moulds. Solvents are left to evaporate and the dried films procured are placed in a desiccator to remove any ageing effects.

- **"Mercury Substrate" Method:** In this procedure drug & plasticizer are submerged in a polymeric solution. It is stirred for a while to produce a uniform dispersion which is then poured into a homogeneous mercury surface and further covered with an inverted funnel. This is done to prevent the solvent from evaporating.

- **"IPM Membranes" Method:** A mixture containing water & polymer (propylene glycol containing Carbomer 940 polymer) is prepared in which the drug is dissolved and stirred in magnetic stirrer. Then the neutralization of this suspension is carried out by the addition of triethanolamine. By using Buffer pH 7.4, the solubility of the drug turns out to be very low, then the formed gel will be amalgamated in the IPM membrane.

- **"EVAC Membranes" Method:** 1% carbopol gel, polyethelene (PE), ethylene vinyl acetate copolymer (EVAC) membrane are all required components. Insoluble drugs are solubilized using propylene glycol and further to this, carbopol resin is dissolved the above solution and neutralized by using sodium hydroxide solution. The gel consisting the drug is fixed on the backing membrane and on this a rate controlling membrane is placed over the gel. The edges are then heat fixed so as to produce a leakage free device.
- **"Proliposomes":** Proliposomes are prepared using carrier method via film deposition technique. For the preparation of proliposomes a round bottom flask is used and in this

mannitol powder is added. These contents are then subjected to Rota-evaporation for 24 hours and then are further lyophilized to obtain a dry powder. Along with this, lecithin and drug ratios are optimized which are then dissolved in a suitable organic solvent mixture and two other aliquots of varying concs, the temperature conditions are maintained at 37°C. After lyophilization of the proliposomes, they are then placed in a desiccator and further passed through a sieve. The collected powder is transferred into a glass bottle and stored at low temperatures until further use.

- **"By using Free Film Method":** Cellulose acetate free film is set up by casting it on mercury surface and along with this 2% w/w polymer arrangement is set up by utilizing chloroform. Plasticizers are to be included at a conc of 40% w/w of polymer weight. At that point 5 ml of polymer solution is poured in a glass ring which is put over the mercury surface in a glass petridish. The rate of dissipation of the dissolvable can be constrained by putting an inverted funnel over the petridish. The film production is noted by watching the mercury surface after complete vanishing of the dissolvable. The dry film will be isolated out and put away between the sheets of wax paper in a desiccator until use. By this procedure we can get ready free films of various thickness can be set up by changing the volume of the polymer solution. [20, 21].

1.3 Advantages of transdermal drug delivery:

- The first pass metabolic activities of the drug are averted.
- Incompatibilities with the gastrointestinal tract are circumvented.
- Self-medication is possible.
- Extension in the duration of action & more predictable.
- Reduced side effects observed.
- The drug plasma concs are regulated.
- Patient compliance is improved as the drug dosage reduces with time.
- Therapeutic value of such drugs gets increased by decreasing problems related to drug such as -lower absorption, GI difficulties, decomposition caused by first pass metabolism in the liver [22, 23].

1.5 Disadvantages:

- Development of allergies on the skin can be observed like- itching, rashes, local edema etc.

- Drugs with molecular size over 600 Daltons have difficulty in permeating the membrane.
- There are different barriers of skin which differ from person to person.
- Drugs with hydrophilic nature is less preferable as compared to drugs with lipophilic nature because of their low permeability [24].

1.6 The Future of Transdermal Drug Delivery Systems

TDDS has been considered ideal for injections as well as orally provided medication, but many drugs cannot permeate the skin membrane effectively due to low permeability of skin barrier. Pharmaceutical companies are now developing new adhesives and substances that magnify molecular absorption as well as permeation.(25) This is being done so as to affect skin permeation and greatly increase the number of drugs which can be provided through this route.. Micro needle technology is more favourable for drug administration via skin as these systems incorporate an arrangement of tiny needle-like structures to create pores in the stratum cornea. This helps in the facilitation of drugs without any cause of pain as they are unable to make contact with the nerve endings. Such systems have been reported to greatly amplify the permeability of macromolecules across skin.(26)

Transdermal technology has also major contributions involving mechanical energy to enhance the drug flux on the skin either by changing the barriers present on the skin or by elevating the energy of the drug molecules. Certain innovations along novel techniques have successfully resulted in various transdermal technologies that are being researched for different drugs. These include “electroporation” (uses pulses of high voltage to cause pores in the skin), “sonophoresis” (uses low frequency ultrasonic waves to break the skin), and “thermal energy” (uses heat to increase the energy of drug molecules which further increases the permeability of the skin). The TPs have been considered an underrated tool for dealing with acute and chronic pain and thus with enhanced delivery and a grand range of medication, it’s expected that the popularity and applicability of this modality to deliver drugs shall increase. The transfer of drugs through the skin holds various advantages such as sustenance and balancing of constant drug level in the blood, reduced side effects and enhancement of bio availability by evasion of hepatic metabolism and increased patient complementarity with respect to drug scheme used for treatment. [27].

Review of Literature

Transdermal drug administration has been considered one of the safest and best route for drug delivery as it does not involve any pain and avoids any sort of metabolic alterations. Even the drugs which cause toxicities while being administered through the other routes i.e., peritoneal, oral, etc., can be easily provided transdermally. The drug used for this study was VMC. HCl which is used for numerous bacterial infections and prominently against Gram positive bacteria. The following antibiotic shows a variety of disadvantages while being administered through the oral or the peritoneal route as it causes difficulties in the gastrointestinal tract, nephrotoxicity and ototoxicity. Other side effects such as nausea, vomiting, rashes and severe body pain. VMC. HCl is majorly used in the treatment for skin infections and methicillin-resistant *Staphylococcus aureus* and the patient suffers from a lot of pain during the treatment. Thus to provide VMC. HCl to the patient without the prevalence of any side effects, the transdermal route was selected. Literature studies were done to comprehend the work done by various scientists on VMC. HCl and TPs.

2.1 LITERATURE REVIEW

K. Vidyalakshmi *et. al.* 2007- PVA-CH blended films were synthesized while varying the conc of PVA in all of the formulations. 1% glacial acetic acid, distilled water, and ethanol were used as solvents for CH, PVA, and curcumin (plasticizers) which were further used to produce a comparative data of the permeation efficiency. As per the results, the swelling behavior and release profiles were best for pure PVA and PVA rich mixtures.(28)

J. Suksaeree *et. al.* 2012- Nicotine infused TPs were prepared using deproteinized natural rubber latex blends and all of the formulations were varied with respect to the backing layer. This was done to determine the moisture vapor transmission rate and lowest oxygen transmission which gives out a higher nicotine release. Components used were DNRL blends, hydroxyl-propyl methyl cellulose and dibutyl-phthalate and they were further characterized using FT-IR spectroscopic, DSC thermograms, X-ray diffraction and SEM techniques. The release and permeation studies were carried out with respect to Higuchi's model and the formulation NCT-BL5 had the lowest oxygen transmission rate and highest vapour transmission.(29)

S. R. Kumar *et. al.* 2012- TPs were developed using hydrophobic ethyl cellulose and di-butyl phthalate and these were prepared to enhance the permeation of colchicine. Three formulations

were prepared using solvent evaporation technique and were subjected to various evaluations such as thickness, weight difference, amount of drug, endurance to folding, percentage moisture content, percentage moisture loss and vapour transmission rate. The drug release and permeation studies were carried out using pig skin and as per the result all three formulations enhanced the permeation of colchicine. (30)

P. Maji *et. al.* 2013- PVA-CH TPs cross linked with maleic anhydride and loaded with alprazolam were prepared using solvent evaporation technique. Four formulations were prepared and the conc of PVA was varied, whereas the other components (drug, CH, etc.) were kept constant. The studies determined that permeation is directly proportion to the amount of PVA used and the formulation with the highest amount of PVA had the highest flux, swelling and permeation. Higuchi's model was used to calculate the regression and kinetics for the release studies of the TPs.(31)

M. Yousuf *et. al.* 2013- formulation of TPs was done using PVA and Eudragit RL- 100, methanol and poly ethylene glycol and the drugs used were salbutamol sulphate and ketotifen fumarate which are antiasthma drugs. Six formulations were prepared using different permeation enhancers such as Tween 20, isopropyl myristate, eucalyptus oil, castor oil and span-20 and various experiments for weight and drug uniformity were carried out. Also the release and permeation studies were carried out using rabbit skin and out of all the formulations, the one using Tween twenty as the enhancer had the highest permeation and drug release activity. Formulations with no enhancers also showed drug release and permeation due to the presence of plasticizer.(32)

L. Charoenchai *et.al.* 2015- *Zingiber cassumunar* was blended with PVA-CH and glycerin to produce patches which were subjected to physicochemical and characterization techniques such as moisture uptake, swelling ratio, erosion, porosity values, FTIR, DSC,
XRD, and SEM**.** The prepared patches were loaded with (*E*)-4-(3′,4′-dimethoxyphenyl) but-3-en-l-ol so as to determine the controlled release on pig skin. Here Zingiber cassumunar is known for its anti-inflammatory activity and (*E*)-4-(3′,4′-dimethoxyphenyl) but-3-en-l-ol is the activation compound. According to the results the blended patches with the active compound were able to depict higher permeation rate and controlled drug release.(33)

2.2 DRUG REVIEW

Generic name: Vancomycin Hydrochloride
Class: Glyco-peptidic antibiotic

Molecular formula: $C_{66}H_{75}Cl_2N_9O_{24}.HCl$

Molecular weight: 1485.723 g/mol

Structure:

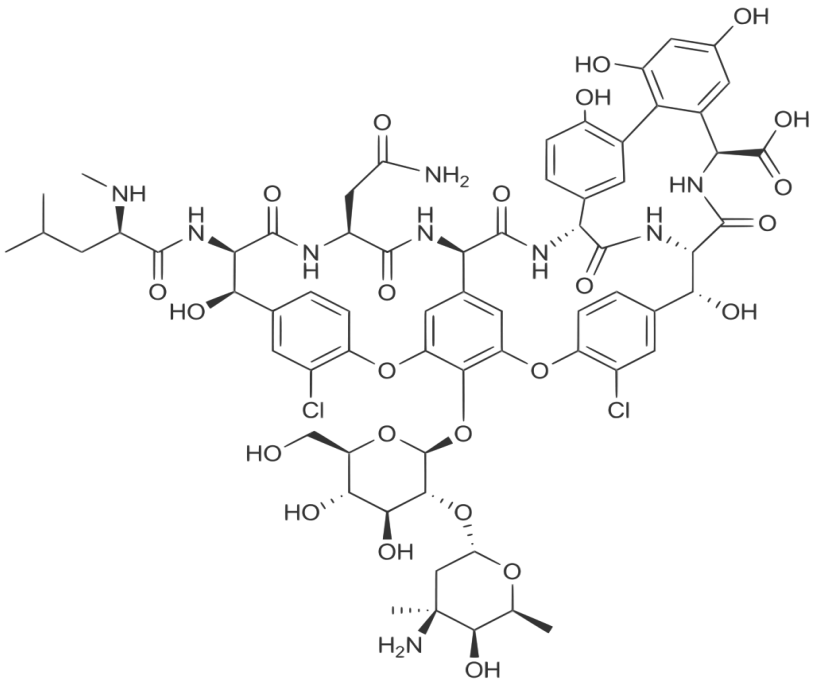


Figure 1: Structure of VMC. HCl

Description: white crystalline powder

Solubility: Water: 100mg/ml, Methanol: 1mg/ml

Pharmacokinetic data:

- Bioavailability: orally negligible
- Metabolism: eliminated without any change
- Elimination: excreted within 4 hours to 11 hours (adults)
- Half -life: 6 d to 10 d (adults vs impaired renal function)
- Excretion: via urine (IV), feces (by mouth)

Uses:

- Treatment of severe infections caused by MRSA

- If the treatment is un-responsive to metronidazole in Pseudomembranous colitis caused by *C. difficile,* VMC. HCl is given orally to the patient.
- Treatment of Gram positive bacterial infections and for persons having allergies to beta-lactam antimicrobials.
- Treatment and prevention of endopthalmitis.(34)

Side effects:

- Local myalgia
- Rashes
- Kidney impairment and nephrotoxicity
- Ototoxicity
- Poor absorption through the oral route. (35)

2.3 EXCIPIENT REVIEW

2.3.1 CHITOSAN

Description: White/grey colored translucent flakes with slight pearl luster which is tasteless, odorless and non- toxic. Procured from the hard exo-skeleton of shellfish, crab, lobster, and shrimp.

Chemical name: (1,4)-2-Amino-2-desoxy- beta-D-glucan

Molecular formula: $C_{56}H_{103}N_9O_{39}$

Molecular weight: 1526.464 g/mol

Empirical formula: $(C_6H_{11}NO_4)_n$

CAS Number: 9012-76-4

Structure:

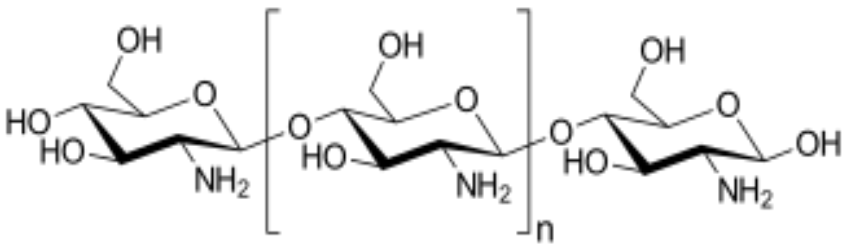


Figure 2: structure of CH

Solubility: 1mg/ml in 1% acetic, adipic, formic, lactic, malic, propionic, or succinic acid.

Uses:

- It's used in hydrogels due to its wound healing properties.
- Used in the filtration process for water processing engineering.
- It removes phosphorous, heavy elements and oils from water.
- Used in shampoos, gels, and creams.
- Also used to treat obesity, high cholesterol and Crohn's disease.
- Used as fillers in tablets so as to improve the dissolution and bitter taste of the tablets.
- Used to reduce inflammation of gums and control cavities.

Side effects:

- Constipation
- Prevents the body from absorbing fat soluble vitamins such as A, D, E, K.
- Sometimes interferes with the working of antivirals.
- Nausea and vomiting.
- Diarrhoea.(36)

2.3.2 POLYVINYL ALCOHOL

Description: colorless, odorless white powder which is a water soluble, synthetic polymer.

Molecular formula: $(C_2H_4O)_x$

Molecular weight: 26,300 to 30,000 g/mol

Structure:

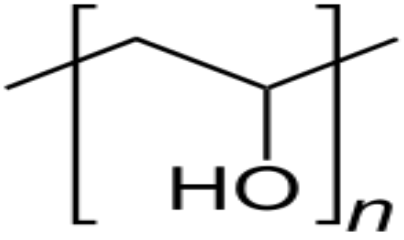


Figure 3: Structure of PVA

Density: 1.19-1.31 g/cm^3

Melting point: 200 °C (392 °F; 473 K)

Refractive index: 1.477 @ 632 nm

CAS Number: 9002-89-5

Lethal dose: 14,700 mg/kg (Mouse)

Uses:

- Used in the coating of medicinal tablets.
- Being water resistant, it's used to coat the safety glasses in vehicles because of the tough films it forms.
- It's non-toxic and hence is used to coat food supplements.
- Used in the strengthening of textile yarns.
- Used in the paper industry to make paper more resilient to oils and greases.
- Prominently used as a component in adhesives, emulsifiers and resins.
- It's also used in eye drops as a lubricant to prevent eye dryness and soreness.
- It doesn't accumulate in the body.(37, 38)

Limitations:

- Minor burning and irritation in the eyes is observed while using the eye drops.
- If allergic, PVA causes rashes, severe dizziness and nausea.
- It's poorly absorbed in the gastrointestinal tract and is eliminated from the body readily.
- In certain cases, increased lacrimation and foreign body experiences are reported. Adverse cases include ocular hyperaemia and eye pruritus.(39)

MATERIALS AND METHODS

3.1 CHEMICALS:

CH, PVA, acetic acid, phosphate buffer (pH 6.8), VMC. HCl HCl and distilled water.

3.2 PREPARATION OF TPS:

The PVA- CH TPs were prepared using the Freeze- Thaw method. According to this method 1% CH was dissolved in 3% Acetic acid and the following solution was mixed in 12% w/v PVA. The process was carried out over a constant heat of 80°C and after mixing the solution was subjected to sonication for 20 minutes. The gel solution was then divided into four tarsons and to three of the tubes, VMC. HCl of varying concs was added. The tubes were then labelled F1 (BLANK), F2 (1:10), F3 (1:1), F4 (1:5), where the ratios represent the varying concs of VMC. HCl. After mixing the antibiotic, the tarsons were sonicated again for 15 minutes and then the gel was poured into Petri plates. The plates were labelled and then stored at -20°C for 18hrs and then were thawed for 30 minutes, this was done for 3 cycles. (40)

3.3 VMC. HCL CALIBRATION CURVE:

The calibration curve was constructed by first preparing a 10ml (1mg/ml) stock of VMC. HCl HCl in Phosphate buffer (pH 6.8). Fresh phosphate buffer was prepared and its pH was set to 6.8. From this stock the following dilutions were prepared: 100µg/ml, 50µg/ml, 40µg/ml, 30µg/ml, 20µg/ml and 10µg/ml**.** To determine the lambda max of VMC. HCl HCl, an absorbance of the stock solution (1mg/ml) was taken in the range of 200- 600 nm. The calibration curve was then plotted by taking the absorbance of the different concs at the lambda max wavelength. (41)

3.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY:

To confirm the compatibility of VMC. HCl with the gel patch, FTIR was used. Also the FTIR spectrum of pure CH, PVA, VMC. HCl HCl and the varying concs of the respective patches. The spectra obtained for pure CH, PVA and VMC. HCl HCl were then compared with the spectra of the patch mixtures.

3.5 EQUILIBRIUM SWELLING RATIO: To determine the swelling, phosphate buffer of pH 6.8 was used. The normal pH of skin is about 5.5 and that of diseased skin is 7.4. The studies were thus carried out at pH 6.8 because it takes into consideration the diseased skin pH as well. Three 7mm discs each were cut out from the different patches and were placed on four watch glasses respectively. These discs were then dried overnight, maintaining a temperature of 50°C and then were weighed after drying. For time intervals of 0, 1, 3, 6, 12 h the discs were swollen and then were weighed on digital weighing balance, till equilibrium was attained. The same method was then repeated using distilled water so as to compare the swelling ratios in accordance to the variable pH of the solvents used.

The ESR was calculated by using the formula."

Equilibrium swelling ratio = (WTtf- WTto)/ WTto

Where WTt (f) determines the weight of the swollen patches at final time or equilibrium and WTt (o) is the weight of the dried patches at tie To.

Percentage equilibrium liquid content of the swollen patches will be calculated by using the following equation:

EWC= (Meq−M0)/ M0 x 100

Where Meq is the mass of the patch at equilibrium while M0 is the mass of dry patch. (42)

3.6 DRUG LOADING and ENTRAPMENT EFFICIENCY:

To carry out the drug loading studies, 50mg of patch of each conc was cut out and was dried overnight at 50°C. The dried patches were then swelled in 50ml phosphate buffer (pH 6.8) for 12hrs. After swelling, the patches along with the buffer were then centrifuged at 13000 rpm for 15 minutes. The solution obtained was filtered and then it was subjected to UV spectroscopy at 281nm (lambda max).

Drug loading was determined by:

% drug loading= (WT of drug in patch)/ (WT of patch) x 100

To deduce the encapsulation efficiency, the dried TPs were then swelled in 3% Acetic acid for 24hrs. After swelling, the gels along with the buffer were then centrifuged. The solution obtained was filtered and then it was subjected to UV spectroscopy at 281nm (lambda max).

Encapsulation efficiency was determined by:

% encapsulation efficiency= (Practical wt of drug in gel)/(theoretical wt of gel) x 100 (43)

3.7 DRUG RELEASE:

The drug release determines the amount of drug which will be released from the formulation onto the skin. Formulations with sustained and controlled release are preferred as they are able to release the drug in small amounts and over a larger period of time. To determine the controlled release, 2g patches were cut out from the different TPs and were incubated respectively in PBS of pH 5.5 at 37°C, 75 rpm. After the time intervals of 0, 1, 2, 3, 4, 5, 6, 12hrs; 2ml of buffer was withdrawn and 2 ml of fresh buffer was added to the incubated gels. The withdrawn samples were filtered and their absorbance was deduced at the lambda max (281 nm). (44)

RESULTS AND DISCUSSION

4.1 Transdermal patches:

TPs were prepared using the Freeze Thaw method and four petri plates of the following concs were made:

F1, F2, F3 and F4

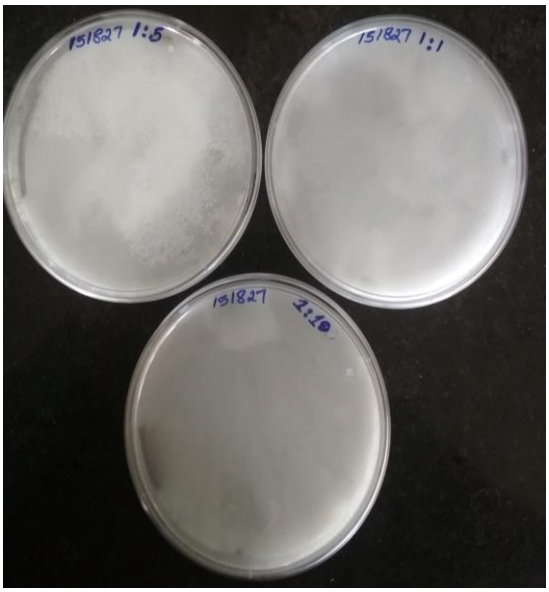


Fig. 4: Frozen patches

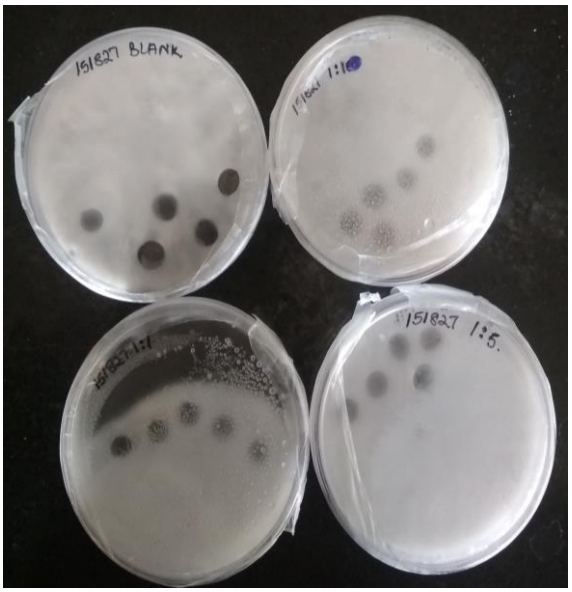
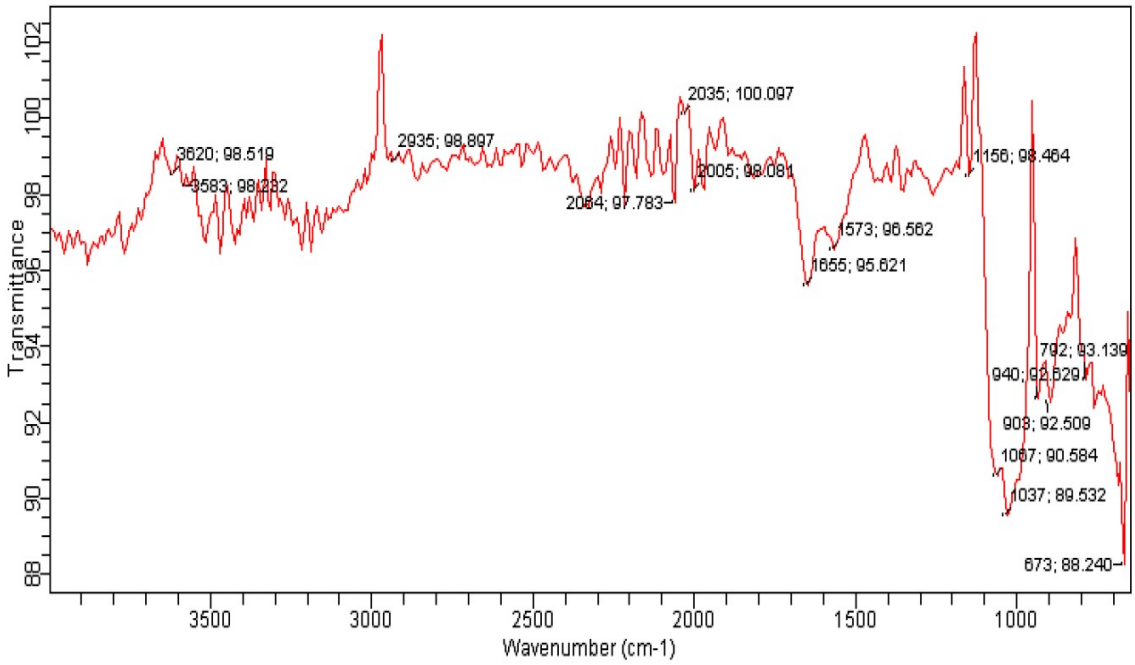


Fig. 5: Patches after disc removal

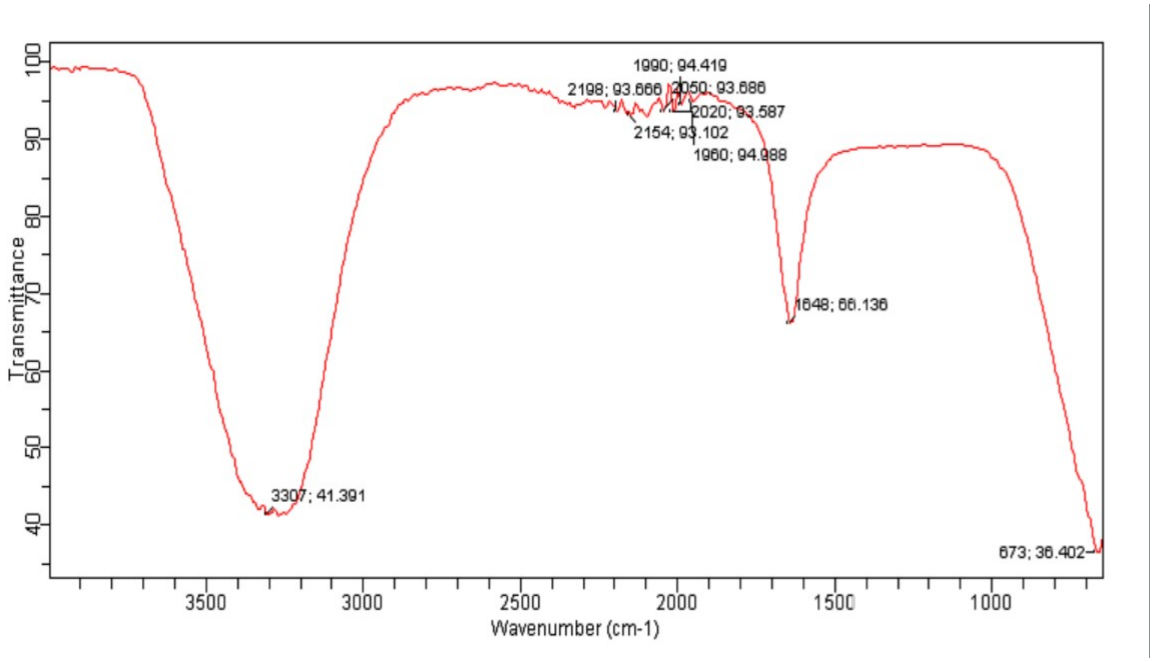
4.2 FTIR:

The infrared spectroscopy gives a relation between the transmittance and the wave number giving out the molecular fingerprint of a particular compound. Through these graphs it has been seen that there are various peaks of different functional groups that have been observed. From the presence of carbonyl and amide bonds to C=C stretching, all of these have been observed in the following graphs. FTIR for this study, depicts the compatibility between the drug used and the polymers and how they will affect the IR spectroscope when combined together as a whole compound.

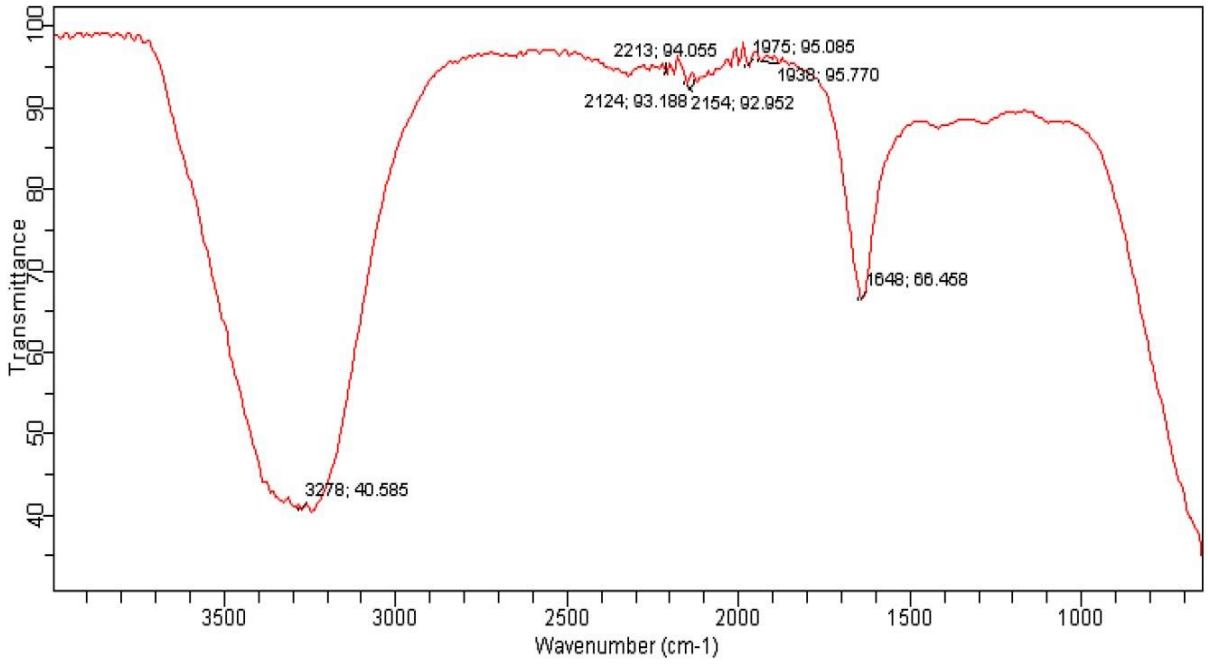
The following results determine the various peaks obtained of transmittance in accordance with the wavenumber of the provided samples. Also by these results it can be deduced that the antibiotic VMC. HCl hydrochloride is compatible with the patch polymers that is CH and PVA. This is because no shift in the peaks of VMC. HCl has been seen in the FTIR results of the antibiotic loaded gels. Thus the following graphs have been obtained for the given samples:



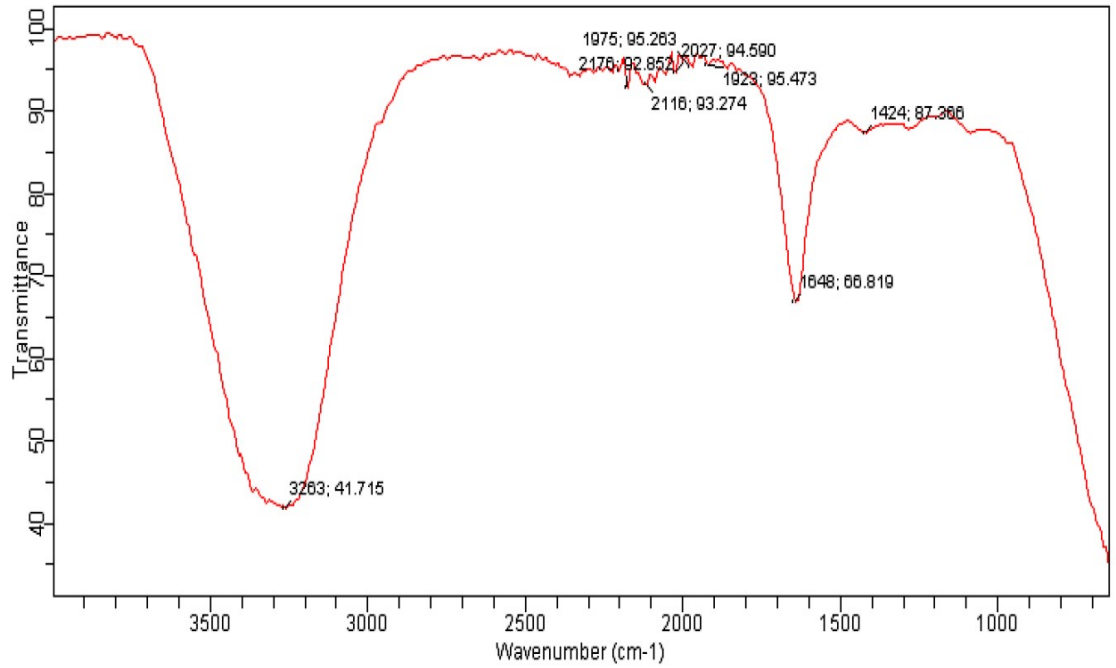
Graph 1: FTIR spectra of CH



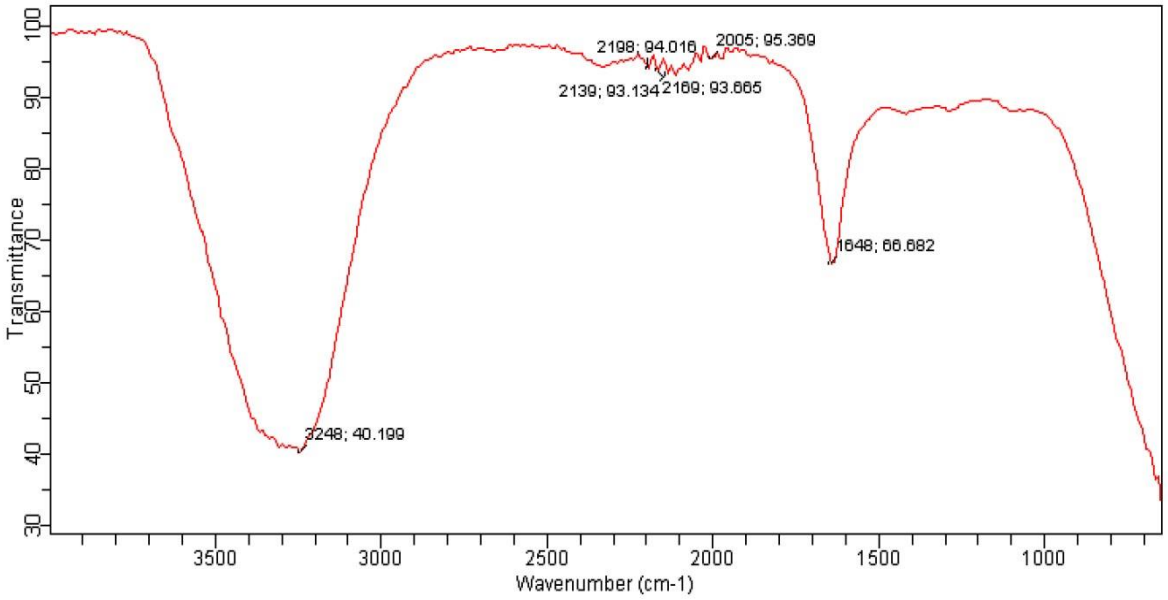
Graph 2: FTIR spectra of VMC. HCl



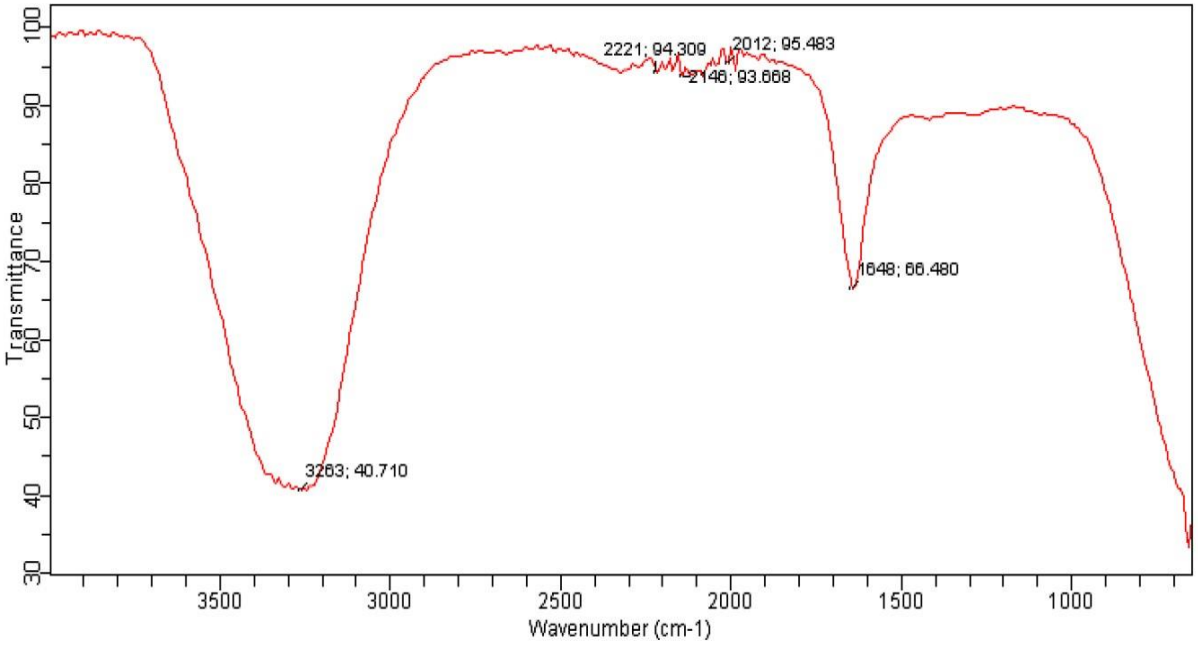
Graph 3: FTIR spectra of **F1**



Graph 4: FTIR spectra of **F2**



Graph 5: FTIR spectra of **F3**



Graph 6: FTIR spectra of **F4**

FTIR was used to note the shifts in the peaks resulted because of the interaction between the blended polymers that are affected by hydrogen bonding or the formation of coordination compounds between the interacting components. Interactions between the proton accepting and donating molecules results in hydrogen bonding whose intensity is dependent on the ionisability of the proton donor and the acceptor and distance between the interacting identities.

The following spectra shows the characteristic stretching, bending and vibrations of the developed PVA/CH patches. The graphs depict the FTIR spectra of CH alone, VMC. HCl alone and F1, F2, F3 and F4 respectively.

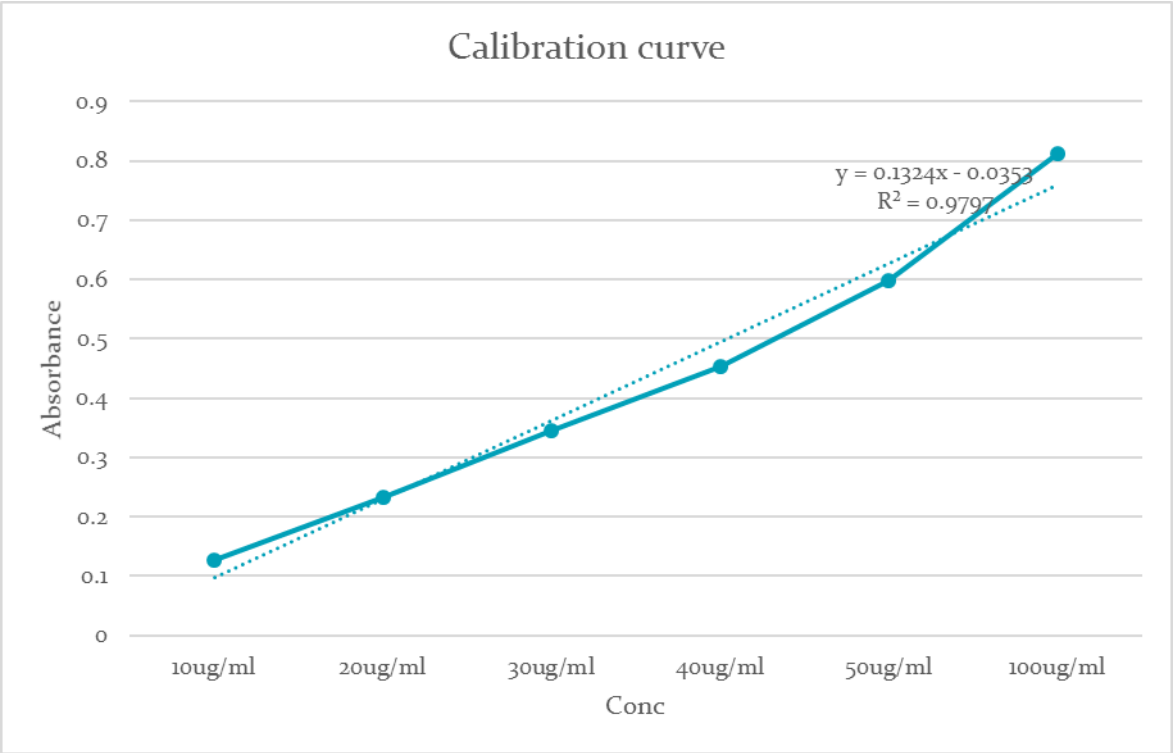
As for the PVA/CH and PVA/CH/VMC patches no major changes or shifts were seen in the peaks. For PVA/CH patches no changes in peaks were seen and the values obtained were maintained. Similarly for the PVA/CH/VMC patches there was no shift in peaks observed thus depicting that the excipient and the drug were compatible.

Table 3: Interpretation of FTIR analysis

S. No	Samples	Peaks observed	Interpretation
1.	CH (alone)	3300-3430 cm^{-1} 890 cm^{-1} 637 cm^{-1}	Represents –OH stretching vibrations and – NH extension vibrations. Stretching and vibrations of aliphatic –CH bonds. Stretching of amide bonds C=O
2.	VMC. HCl (alone)	300 cm^{-1} 918 cm^{-1} 418 cm^{-1} & 1093 cm^{-1}	–OH stretching and vibrations Symmetric –CH2- stretching –C-O groups observed.

4.3 Vancomycin calibration curve:

The calibration curve is prepared so as to determine the conc of an unknown, deduced drug amount, which can be calculated by the equation obtained from the curve. This curve served resourceful during the calculations of the drug loading, release studies and the calculation of the entrapment efficiency. The following curve was prepared by dissolving varied amount of VMC. HCl in distilled water. The concs used were 1mg/ml (to determine the lambda max), 100µg/ml, 50µg/ml, 40µg/ml, 30µg/ml, 20µg/ml and 10µg/ml. The lambda max of VMC. HCl was determined to be 281nm and on the basis of this absorbance the calibration curve was constructed.



Graph 7: VMC. HCl calibration curve

4.4 Equilibrium Swelling Ratio:

As per the given method, from the results it was concluded that the swelling behaviour was governed by the amount of water uptake and the freeze thaw cycles. Along with this, these features also contribute to the morphology of the patches. The gel discs were swollen for 48 hrs till the weight of the gels reached an equilibrium and then the equilibrium swelling ratio was calculated. As per the results, the Equilibrium swelling ratio of **F4** is the highest and thus has shown maximum retention capacity when dispersed in PBS (pH 6.8). Whereas when dispersed in distilled water **F3** has shown the highest retention capacity.

It is a well-known observation that the swelling of TPs is highly associated on the functional groups that are present in it. Also the prepared TPs consist of CH as one its major component and CH consists of different groups that are easily ionisable in acidic and basic media. The presence of such ionisable groups such as amides and hydroxyls allows the TPs to swell easily. The acid dissociation constant (pKa) for CH has been calculated to be 6.5 and to this the various species of amino and carboxyl ions are contributing. The swelling of patches under acidic conditions is predominantly determined by the amino groups present (45). There is a pressure difference observed between the external and internal network of the polymer which is caused by the increased charge density on the polymer. This is observed because, CH is a weak base

with a pKa of 6.5, hence due to protonation and increase in surface charge there are repulsions seen within the NH3+ molecules. This results in the enhancement of the osmotic pressure of the TPs (46).

Figure 6: Swollen patch discs

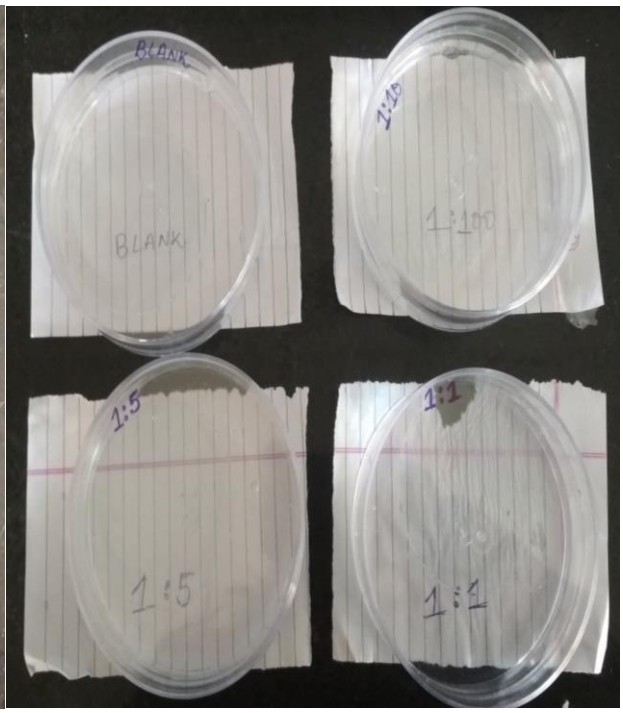
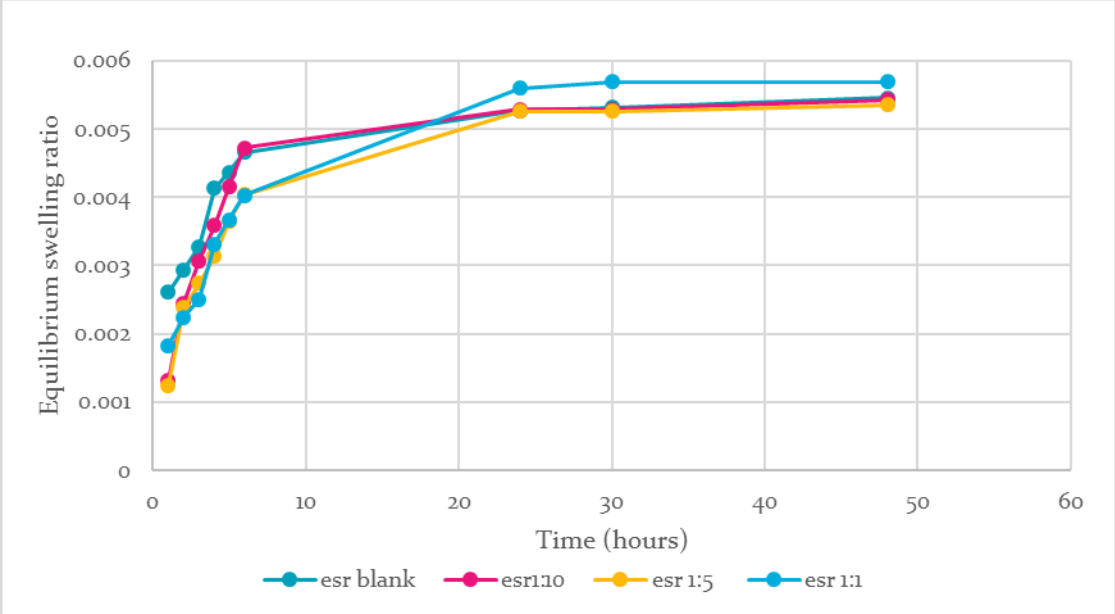
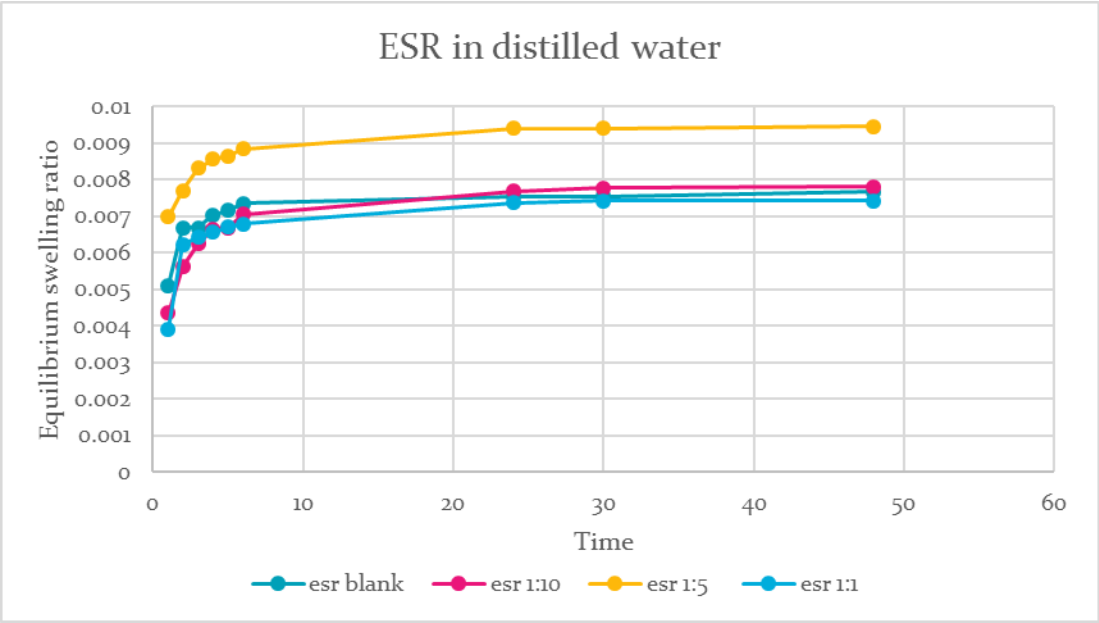


Figure 7: TPs immersed in PBS



Graph 8: Equilibrium swelling curve in PBS for the different concs of the TPs.

The same procedure was then carried out using distilled water and the following results were observed:



Graph 9: Equilibrium swelling curve in distilled water for different concs of the TPs

4.5 Drug Loading and Encapsulation Efficiency:

Discs of equal weight were cut out from the patch and were immersed in PBS (pH 5.5) with constant shaking and at 37°C. After 24 hours these patches were evaluated using a spectrophotometer and the values obtained from there were further placed in the equation of the "VMC. HCl calibration curve". From this the encapsulation efficiency was determined.

From the given formula the following calculations were made, giving out the following results:

% encapsulation efficiency=(Practical wt of drug in gel)/(theoretical wt of gel) x 100

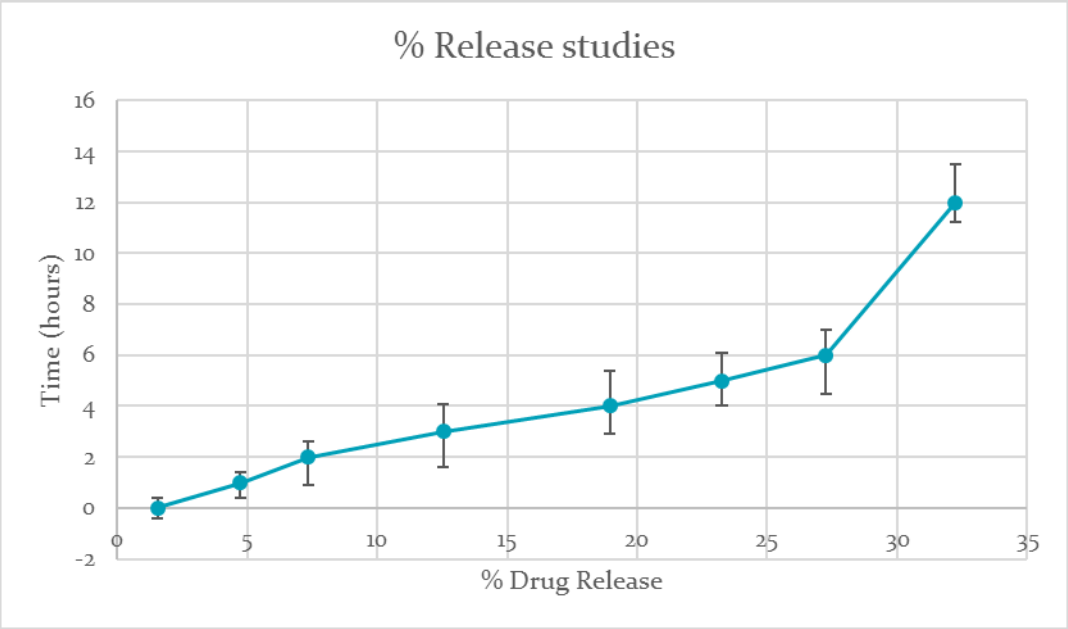
1. F2= 68.7%
2. F3= 72.77%
3. F4= 82.59%

This determines that the **F4** has the highest encapsulation efficiency. This could be because it has the ability to retain higher amounts of drug which could be due to the larger drug content in comparison to the other gels.

4.6 Drug Release Studies:

On the basis of the results of the encapsulation efficiency, the formulation with the highest percentage was selected to carry out the drug release studies. As per the results, **F4** had the highest encapsulation efficiency of 82.59% and hence was subjected to drug release studies. Patches weighing 2g were immersed in PBS (pH 5.5) and the data was collected for 12 hours

so as to determine the controlled release pattern of the used formulation. % release was calculated and the following results were obtained:



Graph 10: Percentage Release of **F4**

The following experiment was carried out in triplicates, thus the graph determines the average results along with the standard deviation. The graph shows an hourly increase in the drug as per the absorbance. The calculations determine that the patches are showing sustained drug release as at the end of 12 hours, 100% release was not obtained. This implies that the patches depict controlled release of the drug and can be used for longer periods of time. Higher release may be contributed by the increased equilibrium swelling ratio of the PVA/CH patches under saline conditions.

CONCLUSION

The following study is being carried out to study the formulation and characterization of Vancomycin hydrochloride loaded transdermal patches. The polyvinyl alcohol/ chitosan based hydrogels as per the results were transparent and elastic in consistency. Though Vancomycin hydrochloride is usually administered intravenously but it has shown profuse complications such as localised toxicities. Vancomycin hydrochloride loaded transdermal patches were prepared to deliver Vancomycin hydrochloride through the topical route as topical drug delivery has various advantages over the other routes. Results of FTIR analysis suggested that the antibiotic is compatible with the excipients as no major shift in peaks of Vancomycin hydrochloride were observed. While the results of Equilibrium swelling ratio suggests a variation in the swelling capacities of the different transdermal patches. As per the graphical depiction of the Equilibrium swelling ratio, the transdermal patch **F4** has the highest values thus depicting that it has the highest swelling capacity out of the four hydrogels prepared. Also the drug loading studies have determined that **F4**, has the highest encapsulation efficiency as per the calculations. The **F4** has shown promising controlled release properties and this formulation can be further developed as topical antimicrobial drug delivery system.

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