‘FORMULATION AND EVALUATION OF HERBAL TRANSDERMAL PATCHES IN TREATMENT OF WOUND HEALING”

Miss. SAILI PRAKASHSINGH RAJPUT [Author]:

Student of Bachelor of Pharmacy Final year,
Shreeyash Institute of Pharmaceutical Education and Research.
Aurangabad Maharashtra.

Mr. RUPESH BHAGWAN KHALSE [Co-Author]:

Assistant Professor at Shreeyash Institute of Pharmaceutical Education and Research.
Aurangabad Maharashtra

Mr. GANESH.G. TAPADIYA [Co-Author]:

Principal at Shreeyash Institute at Pharmaceutical Education and Research.
Aurangabad Maharashtra

Mr. PRAKASHSINGH.G. RAJPUT [Co-Author]:

(M.A B.Ed.) Assistant Professor at Godavari Public School
Aurangabad Maharashtra

ABSTRACT:
Wound is the term which means the damage or tearing of cells and its anatomy and cell function. Wound are classified as surgical, traumatic, diabetic, venous, arterial wound and etc. The wound healing is a process which involves coagulation, Ephilization, granulation, and remodelling of tissue. The proposed study was done and performed to evaluate the wound healing capacity of the herbs like ocimum sanctum (tulsi) and aloe Vera when formulated in form of transdermal patches. In this study Natural wound healing was enhanced by the various phytochemicals present in tulsi and aloe Vera. The present includes the drug delivery through transdermal patches for treating, curing, preventing various skin allergy, infection or wound healing. The main aim of this study was to formulate the herbal transdermal patches in which tulsi plant extract is loaded in aloe vera patches which help to treat the skin infection like rashes, redness, and in wound healing. Herbal formulation is still the mainstay about 75-80 % of world’s population in various country for health care because it has fewer side effects. And they also have better compatibility as compare to synthetic drugs. Herbal formulation consists of the extract of herbs, plants and its part like root system and shoot system which are rich in various phytochemicals which helps to treat various injuries, disease or infection. In various study it has been seen and observed that the plants like tulsi and aloe have the wound healing activities. Various Research Study and Surveys States that there are Topical and Transdermal Medicated Formulation for Dealing with Treatment of Skin Infections but this Study States the Transdermal Drug Delivery System has wide range of Advantages over Topical Formulation. This TDDS has wide scope in future so it involves various New Approaches like Iontophoresis, Photomechanical waves etc.

KEYWORDS: - Transdermal, Wound Healing, Herbal, Transdermal Patches Aloe Barbadense, Ocimum Sanctum

INTRODUCTION TO NOVEL DRUG DELIVERY SYSTEM:

Drug delivery essentially involved in development of controlled or site-specific delivery of drug. Controlled drug delivery improves the bioavailability of drug and prevents premature degradation, enhance the drug uptake thus maintains drug concentration. Every drug molecule needs a delivery system to carry drug to site of the action upon administration to the patients. Delivery of drug can be achieved using developed dosage forms like tablet, capsule, creams, ointments, liquids, aerosols etc. To reduce the fluctuation of drug level, minimize side effects while therapeutic outcome of drug is improved. The ultimate goal of Pharmacotherapeutics is to maximize the therapeutic efficacy of drug while minimizing the related and associated adverse effects. Historically Drug delivery system were developed primarily for conventional routes of administration such as oral and intravenous. However there has been explosion in research on delivery option like Transdermal, Nasal, and Ocular, Pulmonary etc. This system employees a variety of rate controlling mechanism including matrix diffusion, membrane diffusion, Biodegradation and Osmosis.

There are 2 main objectives of drug delivery system and they are as follows: -

1. **Drug Targeting: -** This means to deliver a drug to desired location in body.
2. **Controlled Release: -** This states to deliver a drug at desired rate over a desired length of time.

The Drug Delivery System usually cover up Sustained, Controlled, Targeted, Smart intelligent novel therapeutic programmed etc. delivery system for drugs. The Drug Delivery may be developed encompassing 3 components i.e., Drug Input Function, The Pharmacokinetics response and Pharmacodynamics response.
It is Important to understand and evaluate different terms used under broad category of NDDS and they are as follows-

1. **Localized Drug Delivery Device: -** This delivers drug through spatial control of drug release directly to the target site.
2. **Modified Release Drug Product: -** This term is used to describe the products that alter things or release rate of drug.
3. **Extended-Release Dosage Form: -** When absorption of drug over extended or prolonged period of time or at constant release rate. When Absorption of drug is greater than its elimination then that release is extended drug release.
4. **Sustained Release: -** This includes drug delivery that achieves and ensure slow release of drug over extended or prolonged period of time or at constant release rate. Here Absorption is Equal to Elimination.
5. **Controlled Release: -** Any drug delivery system from which drug is delivered at predetermined rate for prolonged period of time.
6. **Delayed Release Dosage Form: -** A dosage form that release a discrete fraction of drug at a time although 1 portion may release immediately after administration. For Example, Enteric coated dosage form.
7. **Targeted Release of Drug: -** A dosage form that release drug at or near physiological or target site. Target release may be immediate or extended.
8. **Repeated Action Dosage Form: -** This is a type of modified dosage form that is designed to release 1 dose or drug initially followed by 2nd dose of drug latter time.
9. **Prolonged Action Dosage Form: -** They release the drug slowly and provide continuous supply of drug over an extended period of time.

HISTORY AND INTRODUCTION TO TRANSDERMAL DRUG DELIVERY SYSTEM:
Routine use of transdermal drug delivery system became common practice in later 3rd of 20th century when there was more development in drug delivery systems. (Pastore, March 2015)Topical remedies are used since the origin of man. A millennium and half later **GALEN** a Greek physician introduced the compounding of herbal drugs and other excipients in dosage form. He is widely considered to be **"FATHER OF PHARMACY"**. And his work and practices are called as **"GALEN PHARMACY".** The concept that certain drugs cross the skin appears to have been applied by **Ibn Sina** (AD 980-1037) a Persian physician best known as **AVICENNA** within western world.

In **THE CANNON OF MEDICINE**, he proposed that topical drugs have 2 spirits or state i.e., soft and hard. He suggested that when topical products are applied to skin then soft part penetrates in the skin whereas the hard part does not. He also proposed that dermally applied drug not only have local effect but also affects tissue immediately as well as have systemic action. Other forerunners of modern transdermal medication include Mercurial ointments which were used for treatment of syphilis in late 15th century.

In 1904, **Schwenkenbecker** generalized that skin was relatively permeable to lipid soluble substance but not to water and electrolytes. However, the ointments were needed to be applied several times a day which concerns remain about exact amount of drug being applied each time.

Dale Wruster and his student **Sherman Kramer** stated that the absorption can be enhanced or modified by varying the diffusion area of cell by changing the level of skin hydration.

Scopolamine (Hyoscine) patch for treatment of motion sickness was the first transdermal patch to reach the market. Then after scopolamine Nitro-glycerine patches for Angina pectoris were evolved. Before marketing the transdermal scopolamine patches the nitro-glycerine ointments was the only transdermal product of nitro-glycerine. (Pastore, Transdermal patches:history,development and pharmacolgy , March 2015)

Then after this many patches came in market such as Fentanyl patches for pain treatment, Clonidine in Hypertension treatment, Oestradiol patches for female hormone replacement therapy and many more.

The **Zero order kinetics** (constant rate of drug release) of transdermal drug delivery has one of the Cornerstone in Future Development of Transdermal System.

Transdermal system is also used to produce clinical effects as local anaesthesia and anti-inflammatory activities deep in the skin. Transdermal drug delivery system is a system having more scope now a days because it's a type of novel drug delivery system. T.D.D.S helps to enhance the benefits and drug safety. TDDS also have many advantages over other routes of drug delivery. (Ayalasomayajula, May 2021) Transdermal drug delivery system can bypass the first pass metabolism and can also be used for immediate release of drug which also gives high bioavailability and steady plasma drug concentration. Transdermal drug delivery system is a developing drug delivery system which is now more explored in last one to two decades. In TDDS the drug is transported to the epidermal and dermal tissues present in the skin for local and systemic therapeutic action.

Transdermal drug delivery system maintains the drug concentration within the therapeutic window for prolonged time period to ensure that the minimum effective concentration or exceed the maximum effective concentration. Transdermal drug delivery system

has competed with oral route as most successful innovative research area in drug delivery. Transdermal drug delivery system includes different formulations like ointments, patches and gels etc. In recent years many Novels transdermal formulations are like example Liposomes, Nanoparticles, Micro needles etc. (Cheng, 30 october 2020)

SKIN STRUCTURE AND ANATOMY:

The very complex structure of skin and its properties helps into efficient outermost defence line against external and environmental factors and also helps to maintain the Homeostasis of human body. This role is mainly played by stratum corneum which is the outermost layer of epidermis. (Boer, 2008)

The Thickness of outer layer of epidermis, size of corneocytes and composition of superficial lipid impact the properties of skin which contributes to various courses of dermatological diseases. The anatomical area with thick epidermis is more resistant to the external factors. The area with relatively this skinny layer such as face are characterized by high chances to damaging factors but also quick and very fast regenerating ability. The Condition of epidermis Barrier depend on the amount of Sebum, Hydration, Loss of water, etc.

The absorption of substance from Skin surface depends on Degree of size of corneocytes of layer and is inversely proportional to size of cells. Skin is the visible organ that interacts with environment. Skin also has role in synthesis of Vit-D by converting 7-Dehydrocholesterol through 2 hydroxyl group in body to activate Vit-D.
Various Layers of skin are discussed below-

EPIDERMIS:

It is a keratinized stratified squamous epithelial layer which is derived from ectoderm and forms outermost layer of skin. In the skin with heavy hairy area their epidermis is thin and if skin area is non hairy then there the epidermis is thick. Epidermis has 2 types of cells i.e., Keratinocytes and Melanocytes. The Keratinocytes are the Major cells. The keratin containing cells are arranged in interlocking structure. Epidermis is composed of various layers like stratum corneum, stratum lucidium, stratum granulosum, stratum spinosum.

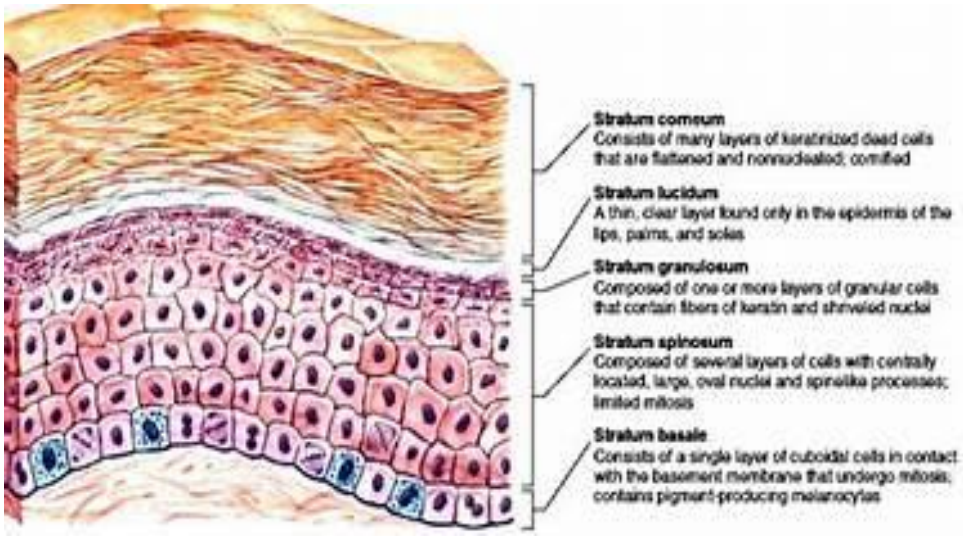


Figure 1:-Structure of Epidermis

1. **STRATUM CORNEUM**: -
   It is 15-20 um in size. (Lizelle T. Fox, Dec 2011)It is the outermost layer of epidermis is stratum corneum which has several layers of keratinized flat dead cell which continuously sheds off the skin and they have direct contact with the environment. This layer is anucleate and also lack cytoplasmic organs. The stratum corneum layer prevents both penetration of substance from environment and insensible loss of body water from surface to the environment. As it is outermost it contains blocks of cytoplasmic protein embedded in lipid.

2. **STRATUM LUCIDIUM**: -
   It is present in specific area of body where skin is thick and lack hairs. This contains the layer of keratinized compact dense cells.

3. **STRATUM GRANULOSUM**: -
   This layer is a layer below stratum lucidium. This contains or has 3-5 layers of flat cells and also contain irregular shaped, non-membrane bound, keratohyalin granules. This granule has structured protein profilaggrin which are involved in keratinization and barrier function of skin.

4. . **STRATUM SPINOSUM: -**
   This has several layers of irregular polyhedral shaped cells and in uppermost layer contain small granules or membrane coating granules.

5. **STRATUM BASALE**: -
   It is also called as Stratum Germinativum which is made of a single layer consisting of columnar or cuboidal in shape.

DERMIS:

Dermis is composed of network of collagen and elastin fibres embedded in mucopolysaccharides matrix which contains blood vessels, lymphatic nerve endings, etc. Dermis is a mesodermal in origin which support to epidermis. It contains the network of dense irregular connective tissue and extend from basement membrane to hypodermis or subcutaneous tissue. Matrix of connective tissue has collagen, elastic and reticular fibres embedded in substance of mucopolysaccharides. Dermis is approx. 3-5 mm thick layer.

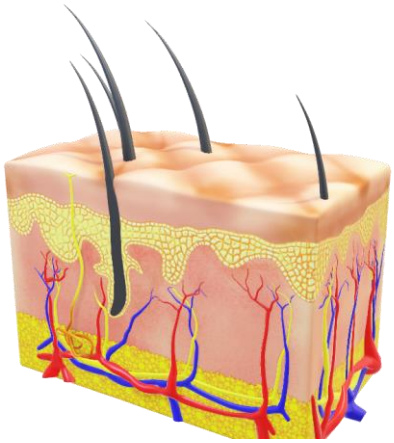


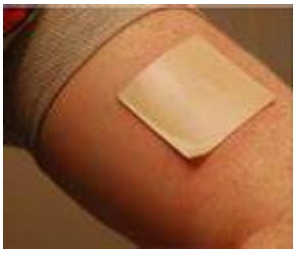
Figure 2:- 3Dmodel of Skin

DRUG PERMEATION THROUGH SKIN:

The drug is absorbed through follicular epithelium and sebaceous Gland. Then when the steady state is achieved then diffusion through intact Stratum corneum occurs. Drug penetrates in skin via two routes and that are

1. **Trans epidermal route: -**If drug penetrates through this route, then drug penetrate through 2 route like Trans cellular and Intercellular.
2. **Trans follicular: -** Here drug is transported via sweat gland and hair follicles. This route has high permeability but it is of minor importance.

TRANSDERMAL PATCHES:

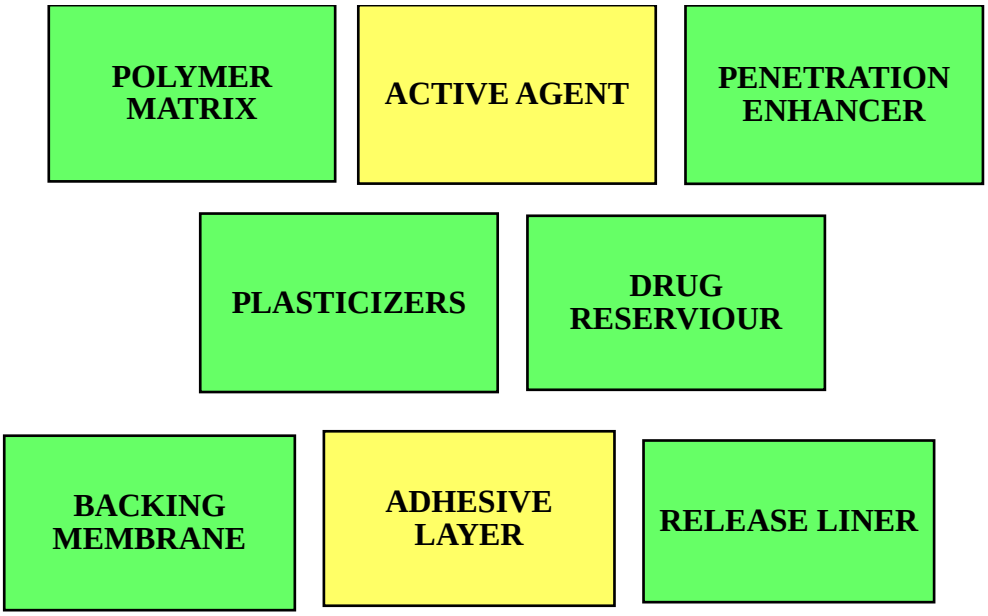


Transdermal patches are a mediated adhesive patch which have coating of drug and is then placed on skin to deliver the drug in the blood stream through the skin. The delivery technology like TDDS helps to enhance the convenience for patients and also increases their effectiveness and protection of drug. Transdermal patches are formulated mainly to deliver drug through skin which diffuse through various skin layer and reach systemic circulation i.e., blood.

TDDS patches are defined as self-contained dosage form which when applied to the skin and deliver the drug through the skin and drug reach the systemic circulation at the controlled rate for prolonged period.

Transdermal patches can avoid or bypass the first pass metabolism which can't be bypassed by oral route. It is easy to stop the drug uptake in blood can be stopped easily by removing patches from the skin. Several synthetic drugs are prepared by transdermal patches for example Nicotine patches, Lidocaine patches, Ketoprofen patches, Diclofenac patches and many more. In the mechanism of transdermal patches skin act as a partition membrane to create barrier that control release and absorption of drug.

VARIOUS COMPONENTS OF TRANSDERMAL PATCHES:



1. POLYMER MATRIX:

This mainly helps to release the drug from transdermal patches depend on or controlled by the polymer. As we increase the concentration of polymer then it forms a very dense matrix which results in slow-release rate of drug. Polymer forms the backbone of transdermal drug delivery system. The drug diffusion across the polymer matrix and release rate of drug depends on the concentration and various physiochemical properties of drug as well as polymer.

- **IDEAL PROPERTIES OF POLYMER MATRIX ARE-**
  1. It should be inert and should not react with drug.
  2. It must not get decomposed in presence of drug and excipients.
  3. It should not interfere in stability of drug.
  4. It should be easily available.
  5. It should be inexpensive.
  6. It must not lead to any type of Antagonistic effect.
  7. It should not result in any type of Hypersensitivity reaction.
- **EXAMPLES OF POLYMER MATRIX: -** Gelatine, Hydroxy propyl methyl cellulose, PVA (polyvinyl Alcohol), PVC (polyvinyl chloride), Starch, PVP, Polyethylene etc.

2. ACTIVE INGRIDIENT:

Drug reservoir is most important component of transdermal patches. It should be selected with very much intense care. Drug that ionizes rapidly are not the suitable agents for formulating transdermal patches because ionized drug molecule has poor skin permeation and penetration.

- **IDEAL PRPERTIES FOE ACTIVE INGRIEDIENT ARE: -**
  1. It should be non-irritant to human skin.
  2. It should have short biological half-life.
  3. It should be potent to impart the required pharmacological action.
  4. It should not show any type of hypersensitivity reaction when administered.
  5. It should be non-toxic in nature.
  6. Drug should have the affinity towards lipophilic and hydrophilic phases.
  7.

3. PENETRATION ENHANCERS: -

This are the substances which enhance the skin permeability by enhancing properties of skin to drug. Polar, Non-polar and polar/nonpolar are 3 pathways for drug penetration through skin. Penetration is enhanced by altering one of these pathways. Polar pathway can be altered by causing protein conformational changes. Non-polar pathway can be altered by altering the rigidity of the lipids.

- **IDEAL PROPERTIES OF PENETRATION ENHANCERS ARE**

1. It should not damage the layer of skin permanently.
2. It should be pharmacologically inert.
3. It should be non-toxic.
4. It should be non-allergic.
5. It should be action specific.
6. It should be non-irritant.

a. SURFACTANTS: - These are added when drug used shows hydrophilic character. They enhance polar pathway transport of the drug. Cationic surfactant is not used. They are considered to be most irritating to skin. Example of Non-ionic surfactant is Pluronic F127. An example of Anionic surfactant is SLS (sodium lauryl sulphate)

b. SOLVENTS: - The example of solvents used are Ethanol, Methanol, Glycerol, Propylene glycol.

4. PLASTISIZERS: -

They are used to reduce or minimize the brittleness of polymer film. They provide or give Flexibility and elasticity to the polymeric film. If Plasticizer are used in high concentration, then they make the film sticky and damp.

- **IDEAL PROPERTIES OF PLASTICIZER ARE: -**

1. It should be easy to handle.
2. It should be non-reactive and non-irritant.
3. It should be pharmacologically inert.
4. It should not affect the stability of drug.
5. It should be cost effective.
6. It should be easily and readily available.

- **EXAMPLES OF PLASTICIZERS ARE**- Glycerol, Propylene glycol, Dibutyl Phthalate, Polyethylene glycol.

5. DRUG RESERVIOUR COMPONENT:

This is a component that contains one polymer or the combination of polymers in various different concentrations and ratios.

6. BACKING LAMINATES: -

This helps to give and provide support. They should prevent the release of drug surface which is not in contact with skin. It should be compatible with drug and excipients. While selection the flexibility, strength, elasticity should be considered. This imparts appearance, flexibility, occlusions to transdermal drug delivery system. While selecting backing laminates the excipients compatibility should be considered. The most suitable backing laminates is the one with high flexibility.

- **EXAMPLE OF BACKING LAMINATES ARE: -** Metallic Plastic Laminates, Polyurethane, Aluminium foil.

7. ADHESIVE LAYER: -

This layer adheres the transdermal device on surface of skin at proper site and position.

- **IDEAL PROPERTIES OF ADHESIVE LAYERS ARE: -**

1. It should have ability to stick with minimum pressure.
2. It should not interfere with release rate of drug.
3. It should not affect solubility of drug.
4. It should be non-irritant to the skin.

8. RELEASE LINERS: -

These are the protective layer which are removed before the application of transdermal patches on skin. They are helpful to prevent drug loss during storage and transportation condition.

- **EXAMPLES OF RELEASE LINERS ARE: -** Teflon, Silicon, Polyester etc.

VARIOUS TYPES OF TRANSDERMAL PATCHES: - Following are various types of transdermal patches- (Hardainiyan, June 2014)

1. **SINGLE LAYER PATCHES**
2. **MULTI LAYER PATCHES**
3. **RESERVOIR**
4. **MATRIX**
5. **VAPOUR PATCH**

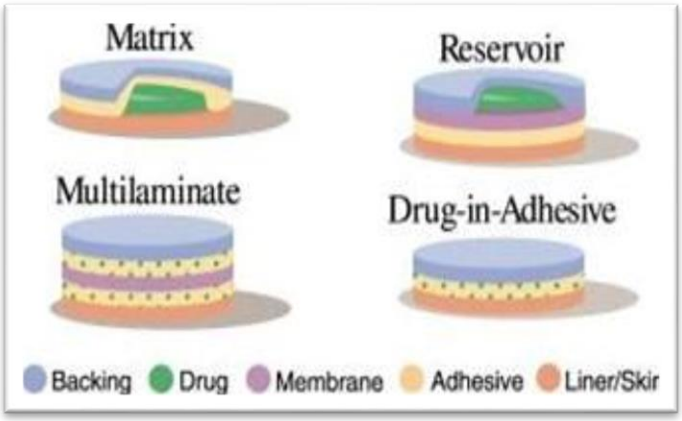


Figure 3:- Types of Transdermal Patches

1. SINGLE LAYER PATCHES: -

Here the adhesive layer not only serves to adhere the various layers together along entire system to skin but also helps and is responsible for drug release from the patch. This adhesive is also surrounded by the backing laminate and release liner. This type of patches is characterized by inclusion of drug directly in the skin contacting adhesive placed onto the epidermis.

2. MULTI LAYER PATCHES: -

These patches are similar to single layer patches. It consists more than one layer. One of the layers is for immediate release of the drug and other one is for control release of the drug from temporary liner layer and a permanent backing layer. In this patch the drug release depends on the membrane permeability and diffusion capacity of drug molecule.

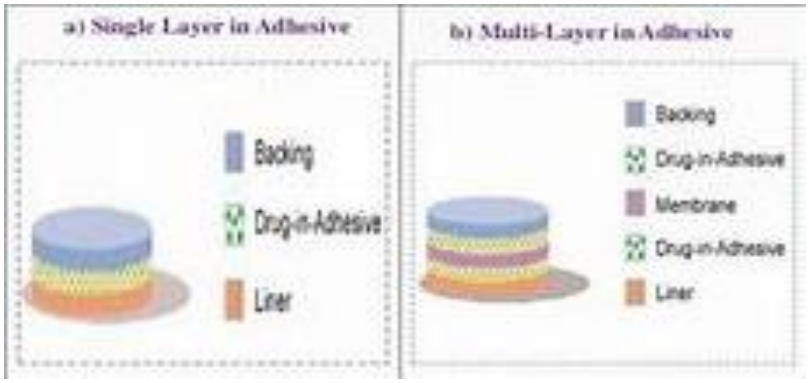


Figure 4:- Multilayer Patches

3. RESREVOIR TYPE PATCH: -

In this patch there is a separated drug layer. The drug layer is a liquid compartment containing a drug solution or suspensions. Here the drug compartment is totally encapsulated in shallow compartment moulded with metallic plastic laminates which have rate controlling membrane made with polymer. This patches also has backing membrane present.

Figure 5:- Reservoir Type Patches

4. MATRIX TYPE PATCH: -

The Matrix system has a drug layer of semisolid matrix which contains drug in solution or suspension form. This is also called as monolithic device.

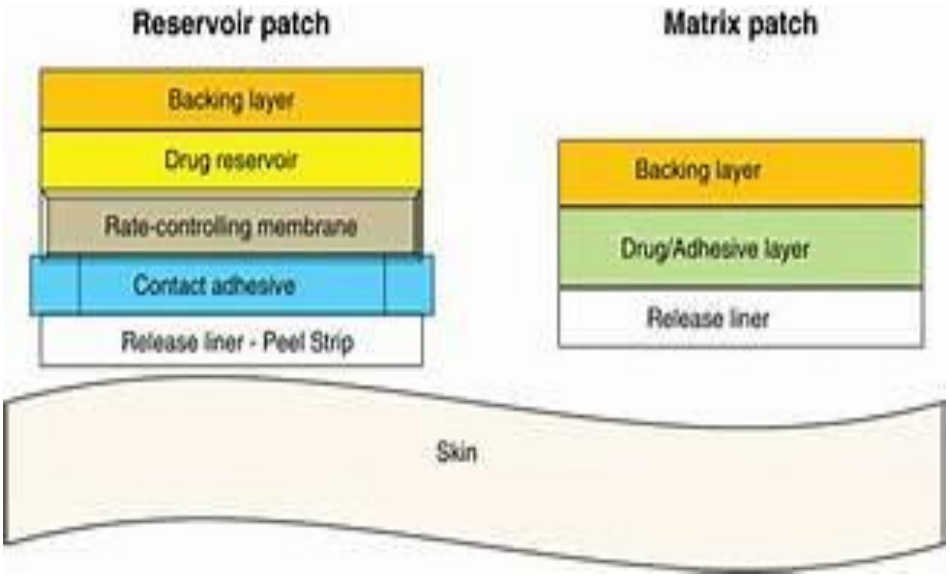


Figure 6:-Matrix Type Patches

5. VAPOUR PATCHES: -

In this patch the adhesive layer carries out two roles one to adhere the various layers and other one to release the vapours. This vapour patches release the essential oils for up to time period of 6 hours. This vapour patches are mainly and widely used for decongestion. Other vapour patches are formulated to enhance the quality of sleep and as an aid for smoking cessation.

FACTORS AFFECTING THE PERMEATION OF TRANSDERMAL PATCHES: -

There are 3 types of main types of factors which affects transdermal permeation and they are as follows-

- **A.** Physiochemical properties of penetrant
- **B.** Physiochemical properties of delivery system
- **C.** Physiological and pathological Skin conditions

A. PHYSIOLOGICAL PROPERTIES OF PENETRANT: -

1. **PARTITION COEFFICIENT: -**
   The partition coefficient value of 1 or more is ideal for the transdermal drug delivery.
2. **PH CONDITION: -**
   The rate of absorption of acidic or basic drug are affected by PH whereas unchanged form of drug exhibits better penetrating capacity. Moderate PH is a suitable for transdermal patches. High or low PH can cause destruction of skin or can cause damage to the skin.

3. **COMPOSITION OF DRUG DELIVERY SYSTEM: -**
   This includes concentration of various component such as drug, polymer, plasticizer, thickness of membrane etc. The transdermal permeability across mammalian skin in passive diffusion process that depends on concentration of penetrant molecule on skin surface.

B. PHYSIOLOGICAL PROPERTIES OF DRUG DELIVERY SYSTEM: -

1. **AFFINITY OF VEHICLE FOR DRUG MOLECULE: -**
   Solubility in carrier determine the release rate of drug. Mechanism of drug release depends on whether the drug is dissolved or suspended in delivery system.
2. **COMPOSITION OF DRUG DELIVERY SYSTEM: -**
   This affects the rate of drug release and also permeability of subcutaneous layer by hydration.
3. **ENHANCEMENT OF TRANSDERMAL PERMEATION: -**
   The subcutaneous layer is dead in nature. Permeation enhancer can cause physiological changes in subcutaneous layer and increases drug penetration through the skin.

C. PHYSIOLOGICAL AND PATHOLOGICAL SKIN CONDITION: -

1. **SKIN AGE: -** The permeability of foetal and infant skin is more than that of mature adult skin. Therefore, percutaneous absorption of topical steroid is rapid in children's as compare to adults.
2. **LIPID FLIM: -** Formation of thin lipid film of skin occurs by excretion of sebaceous gland like sebum.

3. **SKIN HYDRATION: -** Transdermal permeation can be increased by hydration of subcutaneous layer.
4. **SKIN TEMPERATURE: -** Increased skin temperature enhance the skin permeation rate. It tends to increase the vasodilation of blood vessels in contact with skin. This increases the percutaneous absorption.
5. **SPECIES DIFFERENCE: -** Skin of different mammalian species shows various anatomical differences like thickness of subcutaneous, hair follicles etc.

FACTORS AFFECTING THE RATE OF DRUG RELEASE: -

Following are the various factors the rate of drug release from the transdermal patches and they are as follows-

1. Pore size of rate controlling membrane
2. Molecular weight of drug
3. Molecular size of drug
4. Solubility of the drug.
5. Thickness of the membrane

NEW APPROCHES IN TRANSDERMAL DRUG DELIVERY SYSTEM: Following are the various new approaches related to transdermal drug delivery system. These new approaches are emerging and are having a great scope of innovation and development. (Hardainiyan, June 2014)

1. **IONTOPHORESIS: -**
   Iontophoresis is defined as facilitation of drug permeation across skin by applying electrode. Here the charged electrodes are connected to drug reservoir and current is applied. So, in the Presence of electric current the permeability of stratum corneum is increased which help to enhance the drug release.

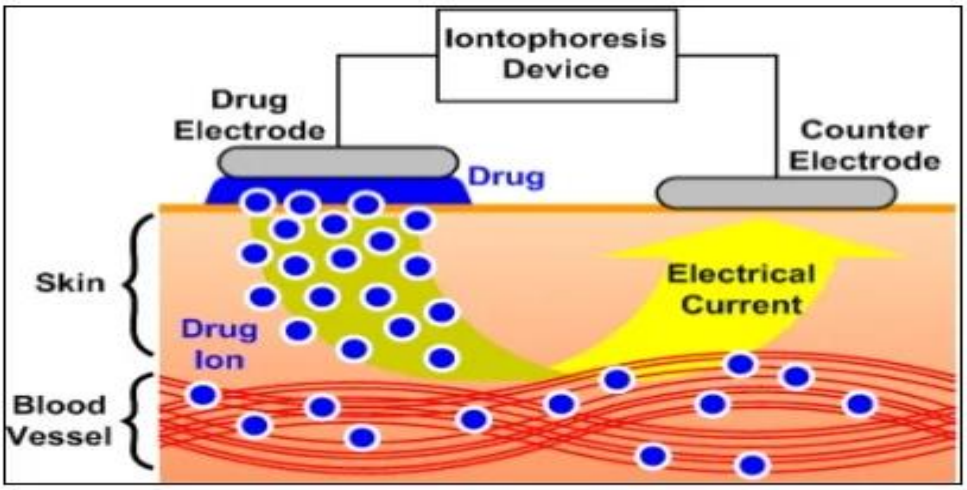


Figure 7:-Iontophoresis

2. **ELECTROPORATION**: -

There is formation of small pores with the help of electric pulses in stratum corneum through which drug is transported.

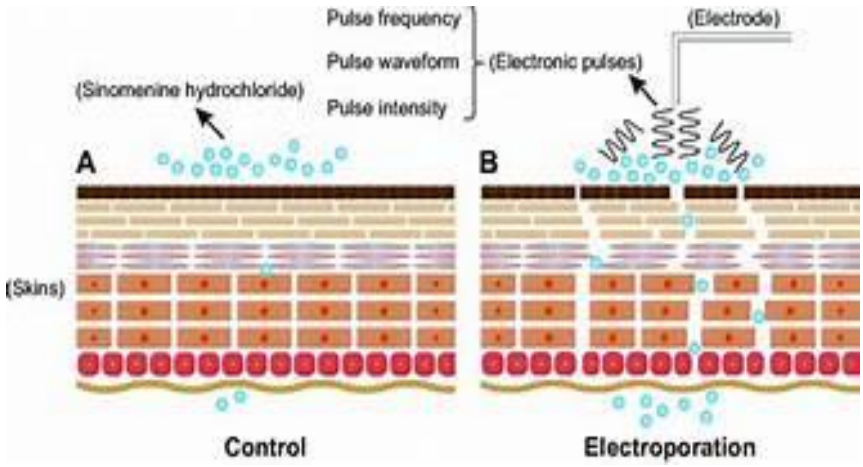


Figure 8:- Electrophoresis

3. PHOTOMECHANICAL WAVE: -

This wave makes stratum corneum permeable to drug by developing transient channel.

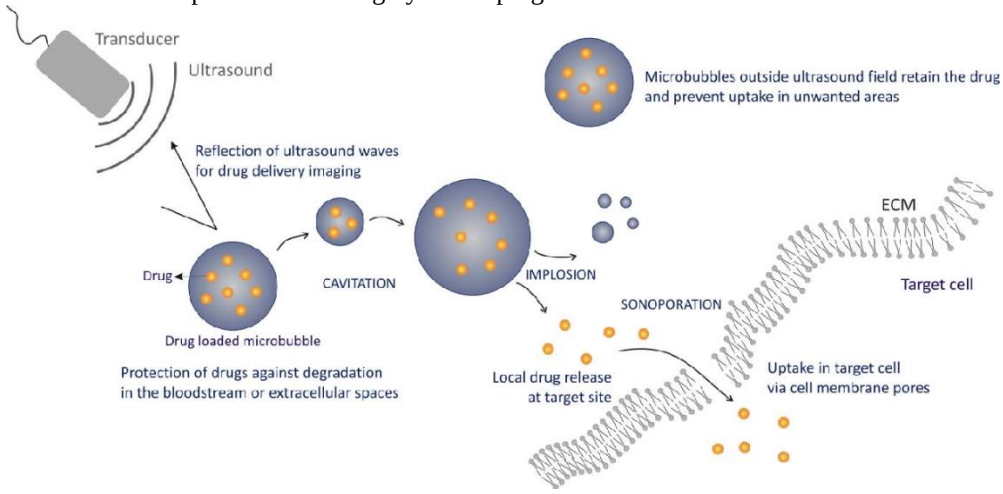


Figure 9:- Photomechanical Wave

4. MEDICATED TATTOOS: -

This are also called as Med-Tats. Med-Tats contains active drug substance. This is beneficial and used for drug administration in children's which do not take or believe in traditional dosage form. Here there is no predetermined duration of therapy. So, manufacturer gives colour chart which is then compared with patients tattoo to analyse when tattoo is to be removed.

5. MICRONEEDLE: -

This was seen or observed first in 1976. Micro needle which are 50-100mm long are used. They are penetrated from reservoir to stratum corneum for the drug delivery.

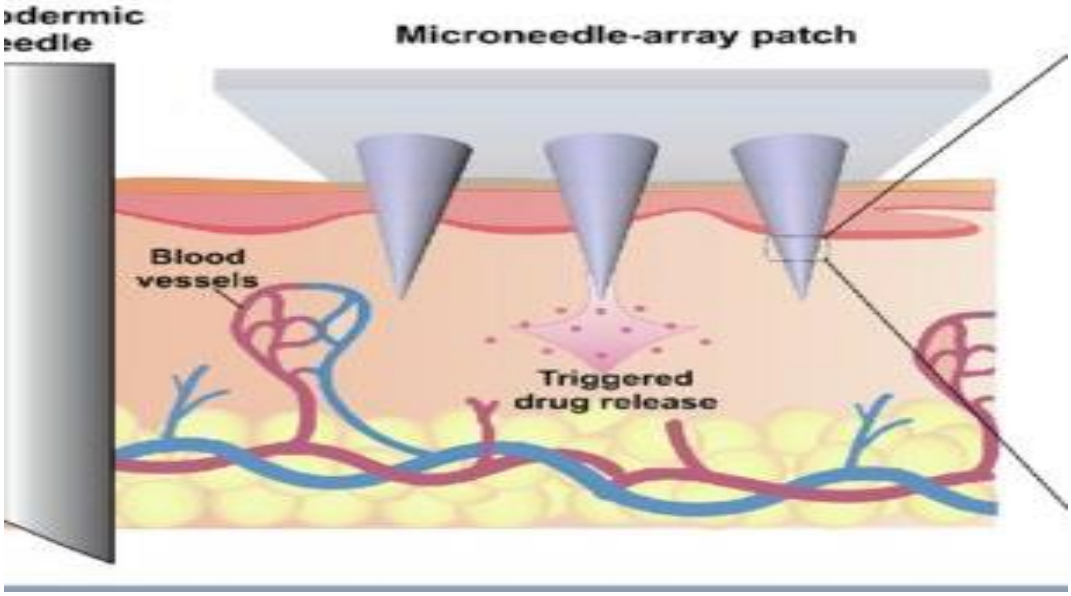


Figure 10:- Microneedle

6. SKIN ABRASSION: -

Here there is removal and destruction of upper layer of skin to enhance the permeation of medicament. Such technique is used in treatment of acne, scars, skin blemishes etc.

7. LASER RADIATION: -

In this there is a direct use and exposure of laser to skin which results in ablation of stratum corneum without any damage caused to epidermis this is beneficial for lipophilic or hydrophilic drugs delivery.

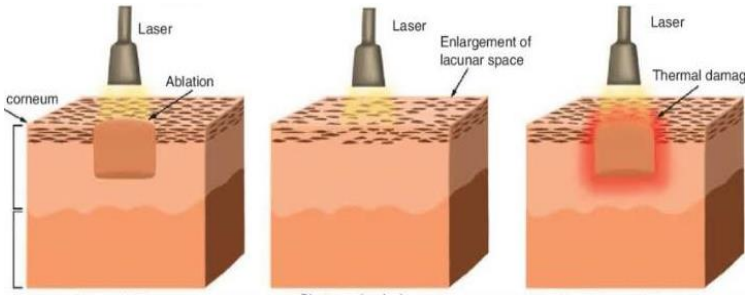


Figure 11:- Laser Radiation

8. **ULTRASOUND: -**
Here there is use of low frequency ultrasound for average 15 seconds to enhance the permeation of skin.

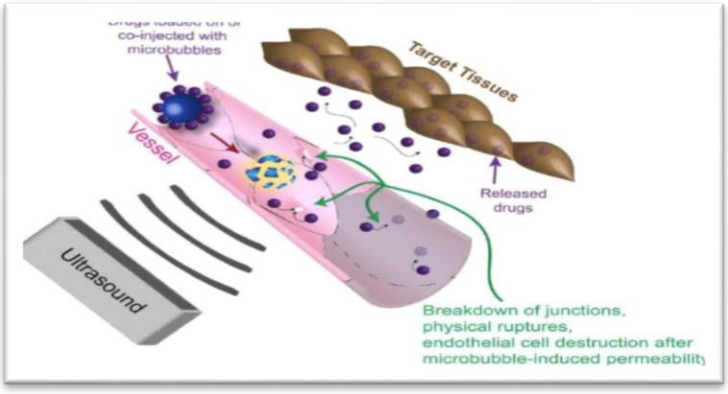


Figure 12:- Ultrasound Method

GENERAL METHODS FOR FORMULATION OF TRANSDERMAL PATCHES: -

1. Circular Teflon mould method.
2. Mercury substrate method
3. By using EVAC membrane method
4. Aluminium Backed Adhesive film method.
5. By using free film method.

GENERAL PROCEDURE FOR APPLYING ANY TYPE OF TRANSDERMAL PATCHES: -

Following are the steps which should be followed for applying the transdermal patches and they are as follows-

1. Initially wash your hands and area where patch will be applied.
2. Make the area clean and sterile.
3. Hold the patch so that the plastic backing is facing to side applicator.
4. Peel off one side of the patches backing.
5. Apply exposed half of patch to the skin in the spot you have chosen.
6. Now then press sticky side of patch against the skin and smooth it down.

ADVANTAGES OF TRANSDERMAL PATCHES AND TRANSDERMSL DRUG DELIVERY SYSTEM: -

1. It helps to maintain the steady infusion of drug for prolonged period of time.
2. It is an alternate dosage form for the patient who can't tolerate oral dosage forms.
3. It helps to increase the therapeutic value of drug.
4. Herbal Transdermal patches helps as an aid in smoking, relive stress, detoxification etc.
5. It acts as a best and convenient alternative for conventional dosage form.
6. It helps to increase the patient compliance.
7. It bypasses the first pass metabolism.
8. It helps to minimize fluctuations in physiological and pharmacological response.
9. It helps to decrease side effects due to reduced plasma concentration.
10. It is helpful in administrating drugs having short biological half-life and drug with low therapeutic index.
11. It helps to administer the drug with low melting point.
12. It helps in Easy termination of drug therapy at any time.
13. The gastrointestinal drug absorption difficulties covered by gastrointestinal ph., enzymatic activity, and drug interaction with food, drinks and other orally administered drug can be avoided.
14. Drug given orally can be given as transdermal patches if the patient is vomiting or having diarrhoea.
15. It is non-invasive and avoid the inconvenience caused by parenteral route.

16. It provides extended therapy with single application.
17. It Help to reduce inter and intra patient variability.
18. Drug level can be maintained in systemic circulation within therapeutic window.

DISADVANTAGES OF TRANSDERMAL PATCHES AND TRANSDERMAL DRUG DELIVERY SYSTEM: -

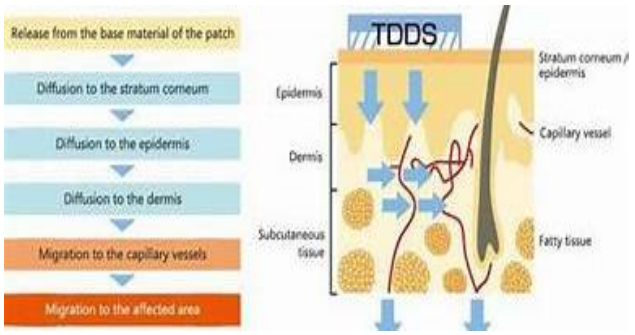
1. Drug with high molecular weight is difficult to penetrate stratum corneum.
2. In this delivery system the drug dose is a limitation factor.
3. If drug which metabolize in liver are given through transdermal route, then results into low bioavailability.
4. The drug causing irritation locally or systemically are not suitable for transdermal drug delivery system.
5. Only potent drugs can be incorporated into transdermal patches due to natural limit of drug entry through skin.

WHAT ARE THE GENERAL DIFFERENCES BETWEEN TOPICAL AND TRANSDERMAL FORMULATION? (Wilbur)

	TOPICAL FORMULATION		***TRANSDERMAL FORMULATION***
1.	Topical medication works on surface of skin and do not reach the blood stream.	**1.**	Transdermal medication penetrates the skin and enters the blood and also distribute through whole bloodstream.
2.	For Example: - Hydrocortisone ointment for skin rashes	**2.**	For Example: - Nitro-glycerine patches to treat chest pain
3.	Topical formulations are cream, ointments, lotion, sprays, foam, powder etc.	**3.**	Transdermal formulations are Patches, Nano gels, and Pastes.
4.	These formulations can't avoid first pass metabolism	**4.**	Transdermal formulation bypass and avoids first pass metabolism.
5.	There are some chances for occurrence of local skin irritation and rashes.	**5.**	Can Help to minimize adverse drug reactions due to low drug concentration.
6.	Drug when given through topical route Just show superficial action	**6.**	Here drug penetrates deep inside the layers of tissues.
7.	Topical medication works on the surface of the skin and do not reach the Bloodstream	**7.**	Patches do not enhance ability of drug molecule but increase drug absorption by prolonged application of patches.

GENERAL PROCEDURE OF TRANSDERMAL DRUG PERMEATION:

1. Sorption of drug through Stratum Corneum
2. Uptake of drug by Capillary Network.
3. Activation of Pharmacological Response.
4. Release of Medicament from vehicle.
5. Penetration through Skin Barriers.
6. Penetration of drug through viable epidermis.



IDEAL PROPERTIES OF DRUG FOR TRANSDERMAL DRUG DELIVERY SYSTEM

PROPERTIES	REQUIRED CRITERIA
HALF LIFE (HOURS)	10 or less
MOLECULAR WEIGHT	< 500 Dalton
LOG P	Between 1-3
Ph	5-9
Aqueous Solubility	>1 mg/day
Dose Deliverable	<10 mg/day
Melting Point	<200 degree Celsius
Oral Bioavailability	Should be Low
Therapeutic Index	Should be Low

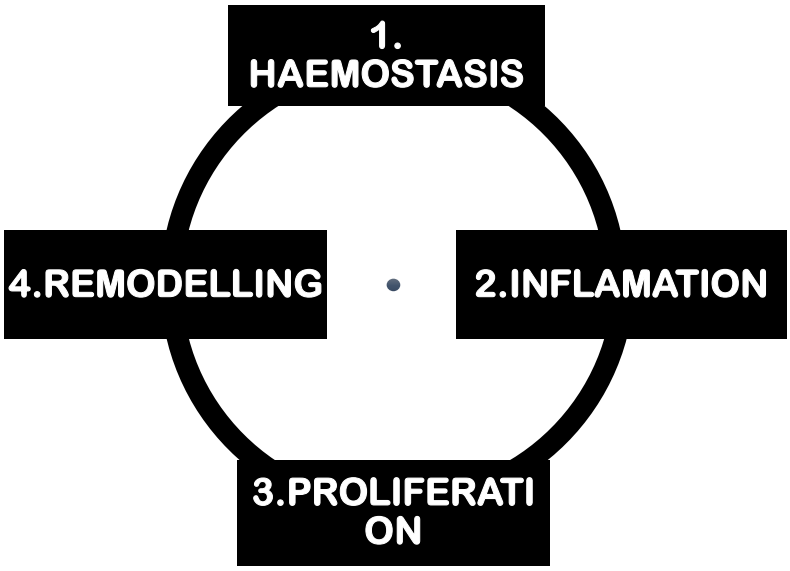
INTRODUCTION TO WOUND HEALING: -

Mainly, Wound is defined as the lesions on skin or rupture of skin surface which is caused by various physical or thermal trauma. (Hashemi, 2015)Skin wounds are typically of two types acute and chronic. Acute wounds are traumatic or surgical wounds that usually heal over time according to normal healing process. Acute skin wounds vary from superficial scratches to deep wounds with loss of tissue, damage to blood vessel. If the wound is large or injury is intense then there is intensive response from a body to wound. Acute wound healing is a complex process that is regulated by different types of cells and growth factors. (Nuutila, March 2014)

During the healing process cells such as inflammatory cells, platelets, endothelial cells, fibroblasts, and keratinocytes undergo changes in their gene expression. Since skin serve as a protective barrier against physical and chemical threats, exposure to radiation or thermal stress, and pathogen entry, a wound radially compromises the functionality of the barrier. The wound site is under

pressure and thus superficial skin wound is practically never sterile. The paramount cellular signalling events and activities in healing of wound are controlled by different type of growth factors like EGF (Epidermal growth factor), TGF (Transforming growth factor), FGF (Fibroblastic growth factors), and IGF (Insulin like growth factor). There are lots of factors which affects the time and Quality of wound healing and some factors are like blood flow in that area, wound size, microbial attack, age of patient, and a nutrition of patient etc. Skin wounds and wound healing are major concerns for public health sector. Complex and lengthy treatments cause as increasing burden on healthcare system.

STEPS OF WOUND HEALING: -There are Four main phases of wound healing and they are given as follows-



1. **Haemostasis: -** Haemostasis is process of the wound healing closed by clotting. Haemostasis starts when blood leaks out of the body. The first step of haemostasis is when blood vessels constrict to restrict the blood flow. Next, platelets stick together in order to seal the break in the wall of blood vessels. The haemostasis stage of wound healing happens very quickly. This establishes the fibrin provisional wound matrix and platelets provide initial release of cytokines and growth factor in the wound.

2. **Inflammation: -** Inflammation controls both bleeding and prevents infection. The fluid engorgement allows healing and repairs cells to move to site of wound. During this phase damaged cells, pathogens and bacteria are removed from wound area. This is mediated by neutrophils and macrophages which remove bacteria and denatured matrix components that retard healing and are the second source of growth factors and cytokines. Prolonged inflammation retards healing due to excessive level of protease and reactive oxygen. That destroy essential factors.

3. **Proliferation: -** Proliferative phase of wound healing is when the wound is rebuilt with new tissues made up of collagen and matrix. A new network of blood vessels must be constructed. Fibroblasts supported by new capillaries, proliferate and synthesize disorganized ECM. Basal epithelial cells proliferate and migrate over the granulation tissue to close wound surface.

4. **Remodelling: -** Also called as maturation phase. In this phase collagen is remodelled from type 3 to type1 and wound fully closes. During maturation phase collagen is aligned along tension lines and water is reabsorbed so that collagen fibres can lie and cross link. Generally remodelling begins about 21 days after injury and can continue for a year fibroblasts and capillary densities decreases and initial scar tissue is removed and replaced By EMC that is more similar to normal skin. Cellular functions during wound healing re regulated by key cytokines, chemokines and growth factors.

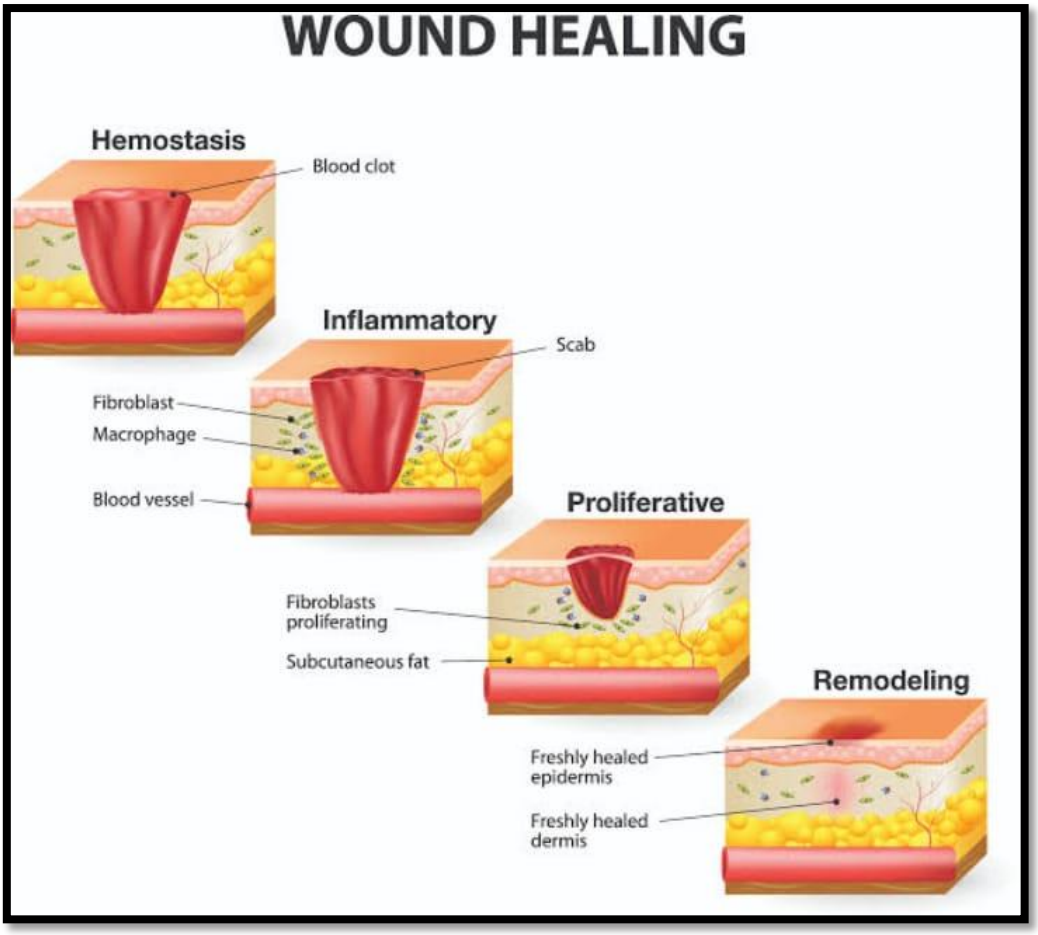


Figure 13:- Steps of Wound Healing

VARIOUS SKIN INFECTION: -

Skin and soft tissue infections involve microbial invasions of skin and underlying soft tissue. They have variable presentation, aetiologies and severities. Approximately 7% to 10% of hospitalized patients are affected by skin infections. The diagnosis of skin and tissue infections is difficult because they may generate other clinical syndromes. The selection of anti-microbial therapy is predicated on knowledge of the potential pathogens, the instrument of entry, disease severity and clinical complications.

Skin infections are caused by various pathological agents and following are some examples-

- **Bacteria** cause Cellulitis, impetigo, and staphylococcal infections.
- **Virus** cause shingles, warts, and herpes simplex
- **Fungi** cause athletes foot and yeast infections.
- **Parasite** cause body lice, head lice, and scabies.

Q. WHO IS AT A HIGHER RISK FOR VARIOUS KIND OF SKIN INFECTIONS?

An individual with following criteria's is more prone to skin infections-

1. Have poor blood circulations.
2. Have diabetes.
3. Have immune system disease like HIV/AIDS.
4. Have a weakened immune system because of chemotherapy or other medicine that suppress immune response.
5. Person who are malnourished.
6.

VARIOUS SKIN INFECTIONS: -

Following is the various skin infection commonly seen in wide range of populations

1. **ECZEMA: -** Atopic dermatitis commonly referred as eczema, causes the skin to become red, itchy, dry, and inflamed. In this skin becomes scaly and cause red bumps. Eczema can appear on face, neck, wrists, elbows, hands, ankles and feet.
2. **COLD SORES: -** Cold sore is a group of tiny painful blisters caused by herpes simplex virus. Cold sores are embarrassing and can be quite painful. This can be treated with creams and reduce its intensity.
3. **DRY SKIN: -** Dry itchy skin is uncomfortable and can make look older than you are. A dermatologist can treat dry skin by reducing its discomfort.
4. **PSORIASIS: -** It is a skin disease that cause red itchy scaly patches most commonly seen on knees elbows trunk and scalp. It is a chronic disease with no permanent cure.

5. **VITILIGO: -** It is a skin disease that causes loss of skin colour in patches. Here the discoloured areas get larger with time. The main cause of this is Lack of pigment called Melanin in the skin. The melanin is produced by Melanocytes.
6. **CONTACT DERMATITIS: -** It is a red, itchy rashes caused by direct contact with a substance or an allergic reaction to it. Example of Causative chemical is soaps, bleach, dyes, and solvents. There are to forms of contact dermatitis i.e., irritant and allergic.
7. **ROSACEA: -**This is a chronic swelling of face with redness associated with pimples. It is most commonly seen in women over age 30.
8. **MELASMA: -** Melasma cause grey brown patches to appear on skin on the face. This is commonly seen in pregnant women.
9. **WARTS: -**It is a skin infection caused by Human papillomavirus. There is no cure for these warts. These warts are small grainy skin growths that occur often on hands and fingers.
10. **ACTINIC KERATOSIS: -**It is a rough, scaly patch on skin that develops from years of sun exposure. Keratosis can be cured if one avoids all sun for few years.
11. **ACNE: -** Acne is caused by blocked hair follicles and oil glands of skin and also triggered by hormonal changes in individuals. The term acne not only means pimples but also blackheads, cyst, and nodules.
12. **SHINGLES (Herpes Zoster): -** This virus cause and results into red blistered rash and appear anywhere in the body.
13. **SUNBURNS: -** This occurs when there is too much exposure to ultraviolet light from sun. In this skin turns red, painful, and hot to touch.
14. **ATHLETES FOOT: -** It leads to extreme itching, redness, and cracked skin on feet and toes. It takes a week to clear the infections. It can be prevented by keeping your feet clean.

TULSI AND ITS ROLE IN WOUND HEALING: -

Figure 14:- Tulsi

Tulsi is an erect much branched shrub which is 30-60 cm tall with simple opposite green or purple leaves. In traditional system of medicine different parts of ocimum sanctum have been recommended for treatment of bronchitis malaria diarrhoea, dysentery, skin disease arthritis eye disease insect bites etc. Tulsi is known as holy basil because it has pure energy. Tulsi is the aromatic perineal plant that's native to Indian subcontinent. Tulsi has Ayurveda qualities like warming, clarifying, supporting lung health, support flow of life cycle, helps in circulation etc. Cultivation of tulsi plant have both spiritual and practical significance that connects the creative powers of nature.

KINGDOM	Plantae
CLADE	Tracheophytes
CLADE	Angiosperms
CLADE	Eudicots
CLADE	Asterids
ORDER	Lamiales
FAMILY	Lamiaceae
GENUS	*Ocimum*
SPECIES	O. *Sanctum* L.

Tulsi is adaptogen which helps in stress prevention, mental clarity, prevent exhaustion. The ocimum sanctum has also been suggested to have anti-fertility, anti-cancer, analgesic, anti-spasmodic actions. Ocimum sanctum also known as Ocimum tenuiflorum is used for 1000 years in Ayurveda for its diverse healing properties.

Tulsi is Queen of herbs the legendary "Incomparable one" of India. It is one of holiest and a most cherished of many healings and health-giving herbs of orient. Tulsi extract are used in Ayurveda remedies for common cold, headache, stomach disease, inflammation, heart disease or in malaria etc.

Tulsi is native throughout the world and has widespread as a cultivated plant. Tulsi contains vitamin C & A and also contains some minerals like calcium, zinc and iron. The chemical composition of tulsi is highly complex due to presence of many nutrients. The stem and leaves contain variety of constituents like saponin, flavonoids, Tannins and Volatile oils. The volatile oil from leaves contains Eugenol. Eugenol (1-hydroxy-2-methoxy-4-allybenzene) the active constituent present in ocimum sanctum is responsible for therapeutic potential. The ethanolic extract of ocimum sanctum tends to show decrease in blood glucose level and show anti-Diabetic action. The hydro alcoholic extract of ocimum sanctum helps to prevent chronic resistant stress. The ocimum sanctum may be useful in management of abnormal healing such as Keloids & Hypertrophic scars. The extract increased wound healing breaking strength wound epithelializes fast and wound contraction was increased. The oil of ocimum sanctum shows good anti-bacterial activities against staphylococcus aureus, Bacillus pumices and Pseudomonas aeruginosa. (Yamani, 2016).Eugenol shows 97% cycloxgenase-1 inhibitory action.

Tulsi also shows anti-oxidant property which is helpful in wound healing. Leaves of ocimum sanctum have anti-inflammatory, analgesic action. Flavonoids present in ocimum sanctum shows Antilipoperoxidant action. The free radical scavenging action of plant helps in healing of wounds. Free radical scavenging activity is a major mechanism by which ocimum sanctum protect against cellular damage. Ocimum sanctum may act on various level in immune mechanism.There are mounting evidences that tulsi can address physical, chemical, metabolic and psychological stress through unique combination of pharmacological action. Tulsi also been shown to counter metabolic stress through normalization of blood glucose level, Blood pressure, and lipid level.
Various actions of ocimum sanctum are discussed below: -

1. **Reduce the stress and anxiety: -** All Parts of basil acts as adaptogen. Adaptogen is a natural substance that helps your body for adapting the stress and promote mental balance. The concept of adaptogen has a holistic approach.
2. **Stimulate and vitalize your body: -** Holy basil is rich in antioxidant and help your body to detox.
3. **Protect against infection and treat wounds: -** Extract of tulsi has anti-bacterial, anti-viral, anti-fungal, anti-inflammatory and analgesic action.
4. **Helps to reduce blood sugar level: -**In animal and human studies have shown that holy basil helps to prevent symptoms of diabetic like high cholesterol, insulin resistance, hypertension etc.
5. **Protects your stomach: -** Tulsi can counteract the effect of stress induced ulcer. It naturally increases defence mechanism by reducing acid level in stomach, increases mucus secretion. And extend the life of mucus cells.

Leaves of ocimum sanctum contains volatile oils which is composed of Limonene, borneol, caryophyllene, phenolic compounds, flavonoids, aromatic compounds. Various Oils like eugenol, citronellol, and linalool has enzyme inhibitory action and flavonoids and antioxidants present strengthen the immune system.

ALOE VERA AND ITS ROLE IN WOUND HEALING: -

KINGDOM	Plantae
CLADE	Tracheophytes
CLADE	Angiosperms
CLADE	Monocots
ORDER	Asparagales
FAMILY	Asphodelaceae
GENUS	*Aloe*
SPECIES	A. vera

Figure 15:- Aloe Vera

Aloe vera is an herbaceous and perineal plant that belongs to Liliaceae family. (Hekmatpou, Jan 2019)Aloe Vera is a medicinal plant traditionally used since 1500 BC in worldwide in many countries and areas. Aloe vera is an indigenous plant from tropical

Madagascar, Saudi Arabia and Iran belongs to Liliaceae family. The mucilage tissue is present at centre of leaves which is also called as aloe gel which is widely used in cosmetics. Aloe vera is widely known for its many different activities like Anti-tumour, Anti-inflammatory, Anti-diabetic, Anti-viral, Anti-septic, Anti-bacterial, skin protection, and wound healing. Various aloe vera studies has shown the enhancing and positive results of aloe vera to treat wounds such as psoriasis, mouth sores, diabetes, burn wounds etc. Due to anti-bacterial and anti-fungal action of aloe vera it tends to reduce dandruff of hair scalp. Aloe vera is effective in inhibiting inflammatory reaction by inhibition of IL-6 and IL-8, it reduces the adhesion of WBC and also decrease TNF alpha levels. (Shedoeva, 2019)Aloe vera increases amount of collagen in wound and also tends to change composition of collagen. It also increases the cross linking then hence it promotes wound healing. Various scientific studies have shown that aloe vera gel tends to increase flexibility and also reduce the skin frangibility.

The Mucopolysaccharides along with amino acids and in present in aloe tends to skin integrity, moisture retention, and also helps to prevent skin ulcers. Topical administration of aloe vera enhance healing process of dermal injuries. Aloe vera is most suitable in case of wound dressing, Aloe vera is also referred for traditional remedy for burns. The past history states that aloe vera has been effective in chronic wounds such as ulcers, diabetic ulcers, or wounds caused by accident, psoriasis and genital herpes.The mucilage od aloe consist some glycoproteins which prevents pain and stimulate skin growth and healing of external as well as internal wounds. Aloe vera has a significant stimulatory effect on cell proliferation and migration. Aloe vera shows healing process due to presence of Glucomannan compound which is polysaccharide like mannose. This Glucomannan stimulates action of proliferation in the cells. Aloe vera also contains vitamin E and C and also some Amino acids which also have key role in enhancing the speed of wound healing where Vit. C increases the collagen production and Vit. E act as a strong Antioxidant.

MATERIALS AND METHODS:

FORMULATION OF HERBAL TRANSDERMAL PATCHES: -

Requirements and Materials

Collection of Plant Sample: -

The Plant Sample was Being Collected from Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad Maharashtra.

Figure 16:-Department of Botany, BAMU

Figure 17:-Tulsi Plant, BAM

Figure 18:- Aloe Vera, BAMU

Instruments: - Electrical Stirrer, Magnetic stirrer, Desiccator, Ph. Meter, Vernier calliper etc.

Figure 19:-Electric Stirrer

Figure 20:- Desiccator

Figure 21:- Ph Meter

Figure 22:-Weighing Balance

Apparatus: - Beakers, Measuring cylinder, Glass rod, Petri Plate

Figure 23:- Apparatus

Chemicals: - PEG-400, Propylene Glycol, HPMC, Chloroform, Methanol, Tulsi extract, Aloe vera Extract.

Figure 24:- Chemicals

CONTENT: -

Sr No.	NAME OF INGRIDIENTS	QUANTITY REQUIRED
1.	Aloe Vera Extract	5 ml
2.	Tulsi Extract	5ml
3.	Polyethylene Glycol-400	2.5 ml
4.	Propylene Glycol	2.5 ml
5.	HPMC	5 gm
6.	Chloroform	8 ml
7.	Methanol	4 ml

PROCEDURE FOR PREPARATION OF HERBALPLANT EXTRACT: -

1. Tulsi Extract: - Initially the Leaves of Ocimum Sanctum were collected. Then the leaves were dried under sunlight. Then the leaves were Grinder into fine powder using mortar pestle. Then to that fine powder the Organic solvent i.e., ethanol was added and was macerated for 48 hours. Then That Extract was filtered and concentrated.

2. Aloe Vera Extract: - The Scaly Leaves of Aloe vera were collected from the plant. Then those fleshy leaves were thoroughly washed using clean water to remove yellow latex. Then the covering was removed and the Gel like Part was collected separately. (Puttarak, January 2015)

PROCEDURE FOR FORMULATION OF TRANSDERMAL PATCHES: -

1. Initially weigh the required ingredients for the formulation.
2. Then add 8 ml chloroform and 4 ml methanol in the beaker and mix them properly using the electric stirrer or Magnetic stirrer.

3. Then add 2.5 ml Propylene Glycol and 2.5 ml Polyethylene Glycol and again stir it continuously using electric or magnetic stirrer.
4. Each medicinal plant extract i.e., 5 ml Tulsi extract and 5 ml Aloe vera extract were added with constant stirring for 10-15 minutes.
5. Now then add HPMC with constant stirring using electric stirrer. But add 1 gm. HPMC at the Time Interval of 1 minute.

Figure 25:- Mixing using Electric Stirrer

6. After time period of 10-15 minutes when the formulation becomes viscous then was added to the glass petri plates which were coated using the Aluminium Foil.
7. Then the petri plate was placed in the Hot Air Oven at 50 degrees for certain time period for the Evaporation of solvent.

Figure 26:- Petri Plate Coated with Aluminium Foil

Figure 27:-Content in petri plate

EVALUATION TEST OF PREPARED TRANSDERMAL PATCHES: -

The various evaluation test was being performed on laboratory level for the evaluation of formulated transdermal patches. Following are the Test performed: -

1. Organoleptic Characteristics
2. Surface Ph Determination
3. Phytochemical Screening of Tulsi
4. Phytochemical Screening of Aloe vera
5. Measurement Of Thickness of patches
6. UV Spectroscopy
7. % Moisture content

1. **ORGANOLEPTIC CHARECTERISTICS: -**
   In this the organoleptic properties were studied like colour, odour, appearance, and etc.
   A. **Colour: -** The Colour of patches was evaluated to be whitish cream.
   B. **Texture: -** The texture of the formulated patches was evaluated to be smooth and uniform.
   C. **Appearance: -** The Appearance of the Formulated patches was Turbid.
   D. **Odour: -** The Odour of the Formulated Patches was evaluated to be Herbal plants like.
2. **SURFACE PH DETERMINATION: -**
   In this evaluation test the Ph of the surface of transdermal patches was evaluated using Ph Meter.
3. **PHYTOCHEMICAL SCREENING OF TULSI: -**
   In Phytochemical screening of Tulsi extract the chemical test named as Mayer's test, Ferric Chloride test, Killer Kilani test, Benedict's test, Ninhydrin test were performed.
4. **PHYTOCHEMICAL SCREENING OF ALOE VERA: -**
   In Phytochemical screening of Aloe vera Extract the Chemical tests like Ferric Chloride test, Mayer's test, Steroid test, Lieberman's test, Ninhydrin test were Performed.
5. **MEASUREMENT OF THICKNESS OF PATCHES: -**
   The Thickness of Formulated Transdermal Patches was Evaluated Vernier Calliper.

Figure 28:-Vernier Calliper

6. UV SPECTRSOCOPY: -

The Formulated Transdermal Were Exposed to the UV Spectroscopy to Study the Absorbance and **209nm** Wavelength. For performing the UV Spectroscopy, the sample was been send to MIT-CARS Aurangabad for evaluation.

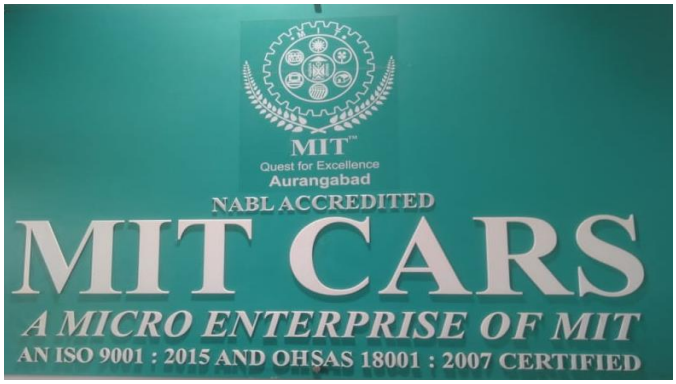


Figure 29:-MIT-CARS

7. PERCENTAGE MOISTURE CONTENT: -

The % Moisture content was studied using Desiccator. Initially the individual patches were weighed and then kept in the desiccator containing activated silica at the Room temperature for time period of 24 hours. Then afterwards the Patches were reweighed.

%Moisture Content= [Initial Wt.-Final Wt.]/Initial Wt. *100

Figure 30:-%Moisture content Result

RESULTS OF EVALUATION TEST: -

1. Results of Organoleptic Tests: -

SR.NO	CHARECTERISTIC	OBSERVATION
1.	COLOUR	Whitish cream
2.	TEXTURE	Smooth and Uniform
3.	APPEREANCE	Turbid
4.	ODOUR	Herbal Extract

2. Result of Ph determination: - The Ph of Formulated Patches Was found to be in Range of **5-9.**

Figure 31:-Ph Determination

3. Results for phytochemical screening of Tulsi and Aloe vera: -

Sr. No.	Name of Chemical Test	Procedure	Result for Tulsi	Result for Aloe vera
1.	**Mayer's Test**	Take 5 mg of extract and then add 1% of Hcl and then gently heat the test tube.	+	+
2.	**Ferric Chloride Test**	Take 5mg of extract and add 1ml of water and 0.5ml ammonia solution and also add conc.H2SO4.	+	+
3.	**Lieberman Test**	Take 5mg of extract and then add 2ml of chloroform and 2ml acetic acid. Then cool the test tube in ice and then add 1ml H2SO4.	+	+
4.	**Killer Kilani test**	Take 5mg of extract and add 1ml glacial acetic acid. Then add 2%Fecl2 +1ml H2SO4.	+	+
5.	**Steroid Test**	Take 5mg of extract and add 1ml Chloroform and then add 1 drop of H2SO4.	-	+
6.	**Ninhydrin test**	Take the extract and add 2ml 0.2% of Ninhydrin solution and boil test tube for 2 minutes.	-	-
7.	**NaOH Test**	Take 5mg of extract and add 1ml of 10% NaOH and when yellow colour occurs then add 1ml Hcl.	+	---
8.	**Benedict's Test**	Take 5mg of extract and add benedict's reagent and boil them.	+	---

[+ =Positive Test; **-** =Negative Test**]**

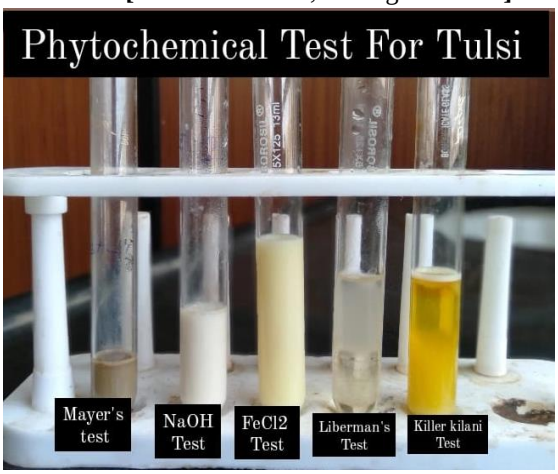


Figure 32:-Test result for Tulsi

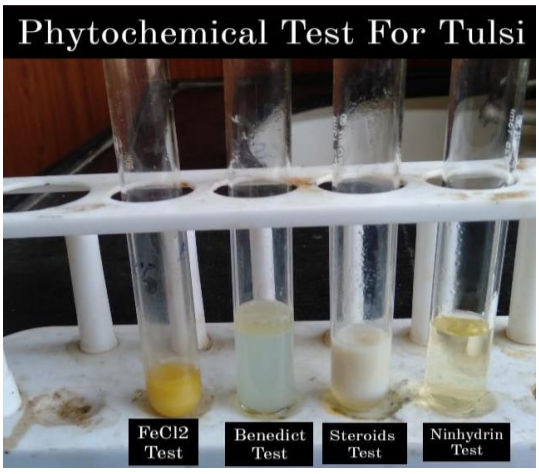


Figure 33:-Test result for Tulsi 2

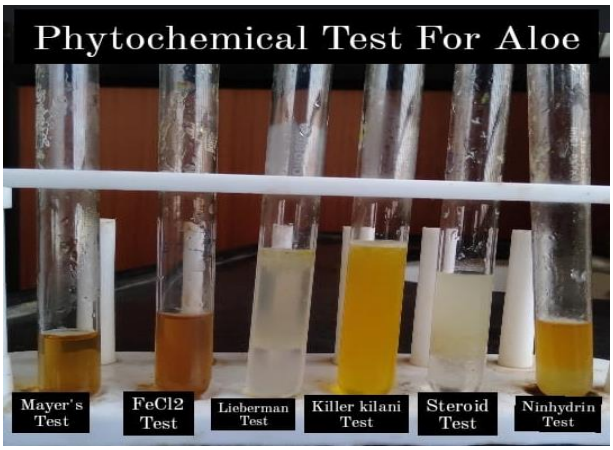


Figure 34:-Test result for Aloe Vera

4. Result of Thickness Measurement: - The Thickness of Formulated transdermal patches were evaluated to be **0.22mm** using vernier Calliper. This Thickness was evaluated by measuring the average Thickness from three Sites of the patches.

5. Result For %Moisture Content: -

Here,

Initial weight=0.6gm and Final weight=0.3gm so By Using the formula of %MC

%Moisture content= [0.6-0.3]/0.6*100=50%

Hence, the %Moisture Content was evaluated to be **50%**

6. Result of UV Spectroscopy: -

MAHARASHTRA INSTITUTE OF TECHNOLOGY,
CENTER FOR ANALYTICAL RESEARCH AND STUDIES (MIT-CARS)
Recognized By Central Pollution Control Board, New Delhi, Recognized By (State Level Laboratory Grade) Department of Agriculture, Government of Maharashtra, Recognized By Scientific and Industrial Research Organizations (SIROs)
MIT-CARS, MIT Campus, Aurangabad (MH)-431010.India.
Website: https://cars.mit.asia, Email: cars@mit.asia,0240-2375330

Page 1 of 1

Test Report No.: 9736 **Date: 24.06.2022**

Issued To :
Saili Prakashsingh Rajput
Shreyash College of Pharmacy Aurangabad
Kind Attention : Mr. Saili Prakashsingh Rajput
Contact No. : 9637338525
Mail ID: sailirajput9290@gmail.com
Sample Collected By: Saili Rajput

Customer Sample ID Details:

Sample Inward No. MIT-CARS/2021/9736
Inward Date 17.06.2022
Quot. Reference
Reference Date
Dispatch Mode: Mail/ By hand

Analysis Start	18.06..2022
Analysis End	23.06.2022
Sample Category	Food
Group	Herbal Powder
Sub-Group	

Test Report

S. N.	Test Parameter	Wavelength Use	Test Method	Requirement as per	Test Result(Abs)
1	Herbal Powder	209nm	Spectrophotometer	---	**2.259**

Dr. Deepak T. Bornare
Deputy Director

NOTE: ● Please see Barcode "Original Test Report" to confirm the authenticity of this report.● Results shall be referred to tested sample(s) and applicable to tested parameters only. ● Test report shall not be reproduced except in full without prior written approval of MIT-CARS. ● Liability of MIT-CARS is limited to invoiced amount only. ● Non-perishable and perishable sample(s) shall be disposed off after 30 days and 15 days respectively from the date of issue of Test Report, unless specified otherwise. ● 'mg/l' is equivalent to 'ppm'. ● 'µg/l' is equivalent to 'ppb'. ● BDL- Below detection limit. ● ND- Not Detected ●DL- DL Indicates detection limit of instrument /method and shall be considered as 'absent'. ●CFU/g- Colony forming Unit per gram.
REMARKS: As requested by the client, sample was tested for above parameters only.
----------**END OF REPORT**----------

Figure 35:-Result of UV Spectroscopy

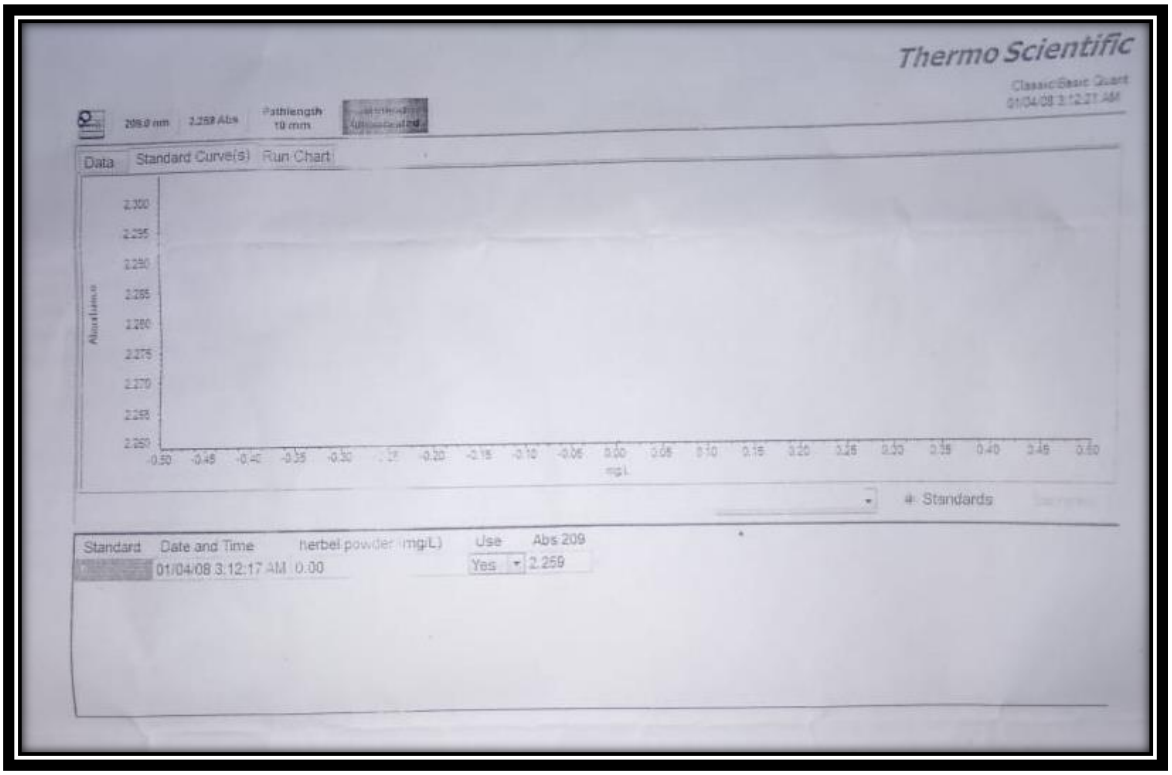


Figure 36:-Graph of UV Spectroscopy

7. Result Of Formulated Transdermal Patches: -

Figure 37:-Formulated Herbal Transdermal Patches

8. Label Of Formulated Transdermal Patches: -

Figure 38:-Label for Formulated Patches

CONCLUSION: -

The Transdermal patches with the incorporation of herbal extract of Ocimum Sanctum and Aloe barbadense were formulated. As Earlier it was discussed that now in the emerging world there is more demand to the Herbal formulation. In the latest research studies, it has come to see that there is a wide scope for Implementation of Novel Drug Delivery System. As we all are known to some overpowering benefits of Novel drug delivery system over the traditional drug delivery system. As it is in concern with the drug delivery through the skin the Transdermal Drug delivery system has an effective benefit over the topical method of drug delivery. The advancement in drug delivery system is allowing wide range of drugs to be administered through transdermal drug delivery system. As discussed earlier there are lots of differences between topical and transdermal system which leads the selection of drug product very crucial and complex.

The Herbal transdermal Patches including aloe and tulsi were aimed to heal the wound or any type of injury or the skin infection like eczema etc. The Evaluation studies states that the patches have the optimum thickness and is within the suitable range of Ph. In the various research it's seen that transdermal drug delivery system has great scope in future for developing drug delivery system in NDDS. Transdermal drug delivery system is widely accepted now-a-days because it causes the drug penetration through skin layers and reach systemic circulation without causing any damage to skin or rupturing it. TDDS also benefits for controlled release of drug for prolonged period of time. More research and innovation will bring the wide acceptance in the use of various other transdermal drug delivery system like iontophoresis, Ultrasound technology, Med Tat etc.

REFERENCE: -

1. Transdermal Patches: History, Development and Pharmacology. Michael N Pastore, Yogeshwar N Kalia. PMID:25560046
2. Transdermal Drug Delivery Enhancement by Compound of Natural Origin Lizelle. Fox PMCID: PMC6264261
3. Herbal Transdermal patches made from optimized polyvinyl alcohol blended film: Herbal Extraction process, Film Properties and in Vitro study.
4. Transdermal Delivery System of Natural Products Applied to Skin Therapy and care Yin-Chen Ching, PMID:33143260
5. An Insight into Delivery of Drug through the skin: Transdermal Drug Delivery System May2021.Research Journal of Topical and Cosmetics Sciences.
6. Structural and Biophysical Characteristics of Human skin in Maintaining Proper epidermal barrier functions. Magdalena Boel, Ewa Duchnik PMID:26985171
7. Bacterial Skin and Soft Tissue Infection in Adults: A Review of Their epidemiology, Pathogenesis, Diagnosis, Treatment and Site of care Article ID 846453
8. Preparation Of Centella Extract Loaded Aloe Vera Transdermal Patches for Wound Healing Purpose January 2015. Advanced Materials Research 1060:54-47
9. A Review on the recent Innovations in Transdermal drug delivery for Herbal Therapy Swati Hardainiyan.

10. Human Wound Healing Research: Issues and perspectives for Studies Using Wide-Scale Analytical Platforms Kristo Nutila, Shintara Katayama PMID:24761360
11. Wound Healing and the use of Medicinal Plants Aleksandra Shedoeva, David Leavesley.
12. Soma Shankar Shetty, Sarawati Udupa and Laxminarayan Udupa Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of *Ocimum sanctum Linn* in Rats [PMC2249741]
13. Tulsi- *Osmium sanctum*: An Herb for all reasons DOI:10.4103/0975-9476.146554. PMID:25624701
14. Antimicrobial Activity of Tulsi Essential Oil and Their Major Constituents against Three Species of Bacteria.
15. The clinical Efficacy and Safety of Tulsi in Humans: A Systematic Review of the Literature Negar Jamshidi and Marc.M. Cohen PMID:28400848
16. The effect of aloe vera on wound healing in cell proliferation, migration and viability Eric Tiplicki PMID:30256753
17. The review on Properties of Aloe vera in healing of Cutaneous Wounds. Seyyed abbas Hashmi, Seyyed Abdollah Madani.
18. The effect of Aloe vera Clinical Trials on Prevention and Healing of Skin Wound: A Systematic Review Davood HekmatPou PMID:30666070
19. The difference between Topical and Transdermal Medication. White Paper by Robert L Wilber, PharmD Executive director, Medical Affairs, and Gensco Pharma.