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Formulation, Development and Evaluation of transdermal Patches for Anti-Neoplastic Drug Methotrexate

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ABSTRACT

Currently, the most common way to give drugs is by mouth because it's easy. However, there are issues with this method. First, a lot of the drug can be lost in the body's first processing, making it less effective. Second, taking drugs by mouth can lead to ups and downs in the amount of drug in your blood, which can be both expensive and inconvenient.IV infusion is a good way to keep drug levels steady, but it has downsides like needle pain and the need for hospital stays. Later, people found a way to do the same thing without needles by using the skin as the entry point. This is called transdermal administration, and the systems for it are called transdermal patches. They're like stickers that release medicine slowly through your skin, making treatment easier and more convenient. Transdermal drug delivery systems are like smart patches that can send medicine through your skin and into your bloodstream at a steady rate. They keep the right amount of medicine in your body for a long time. This way of taking medicine is better than swallowing pills or getting injections because it's safe, comfortable, and you can stop the treatment just by taking off the patch. Plus, it can help avoid the side effects that some drugs cause when taken in traditional forms. The first three day transdermal patch of scopolamine to treat motion sickness was approved in the United States in 1979. A decade later, nicotine patches became thefirst transdermal blockbuster, raising the profile of transdermal delivery in medicineand for the public in general. The objectives of the proposed study are as to develop low dose maintenance therapyof anticancer drug to reduce the risk of potential side effects, improve the patientcompliance in depressant patients. The

disorders of the central nervous system are creating exciting opportunities for transdermal drug delivery. In 2006, two new products were introduced. The selegiline transdermal system (EMSAM) became the first antidepressant patch approved by the FDA for treating major depressive disorder. The rotigotine patch was authorized for treating restless legs syndrome starting in August 2008. There's also potential for using a transdermal patch with benztrpine for treating Parkinson's disease. The success of the rivastigmine patch, both in terms of effectiveness and patient acceptance, suggests it may represent the future of dementia treatment. (Tanner and Marks, 2008; Hai *et al.*, 2008). Counter from a pharmacy or supermarket (Wiedersbergand Guy, 2014). present study is focused on the developmentof suitable transdermaldrug delivery system(Patches) for sustained delivery of methotrexate.

Keywords: Anticancer drug,Transdermal Patch,Sustained drug delivery,Methotrexate,Side effects

1. INTRODUCTION

Transdermal patches are being developed for various conditions like depression, Alzheimer's, Parkinson's, anxiety, ADHD, heart problems, skin cancer, bone loss after menopause, female sexual issues, and urinary incontinence. Even though only a few drugs use this method right now, the global market for transdermal products is worth around $3 billion. Most of this market is in the USA (56%), followed by Europe (32%), and Japan (7%). Recent reports suggest that the transdermal patch industry is growing at a rate of 12% per year, and more than a billion patches are made every year.(Benson,2005;PrausnitzandLanger,2008).The treatment of psychiatric disorders such as Parkinson's disease, major depression, and attention deficit hyperactivity disorder may rely increasingly on non-oral drug delivery systems like transdermal patches in the future. (Oertel *et al.*, 2007). Diseases and

1.1 ADVANTAGES OF TRANSDERMAL DRUG DELIVERYSYSTEM(TDDS):

The transdermal drug delivery system offers several potential benefits, including:

1. Avoidance of First-Pass Metabolism:This method bypasses the liver's initial processing, increasing the drug's effectiveness.

2. Convenience Over Intravenous Therapy: It eliminates the inconvenience and potential risks associated with intravenous infusion.

3. Large Absorption Surface: The skin provides a relatively large and easily accessible surface area for drug absorption.

4. Termination Flexibility: Drug administration can be stopped quickly by removing the patch in case of toxicity.

5. Gastrointestinal Protection: It avoids drug degradation in the gastrointestinal tract due to pH, enzymatic activity, food interactions, or interactions with other orally administered drugs.

6. Alternative to Oral Administration: It serves as a substitute when oral administration is unsuitable, such as in cases of vomiting and diarrhea.

7. Extended Therapy: It allows for extended therapy, reducing the need for frequent dosing.

8. Reduced Side Effects: By optimizing the blood concentration profile, it can help reduce drug side effects.

9. Enhanced Patient Compliance: Patients find it easier to comply with treatment regimens since it eliminates the need for multiple dosing intervals.

10. Consistency: It minimizes variations in drug absorption between different patients and within the same patient.

1.2 APPROACHES FOR DEVELOPING TRANSDERMALDRUGDELIVERY

1.2.1 MEMBRANE-MODERATEDSYSTEM:

This system consists of a drug reservoir sandwiched between a drug impermeable metallic plastic laminate and a rate-controlling polymeric membrane, e.g., ethylene-vinyl acetate copolymer, which may be micro-porous or non-porous, controlled permeation of drug molecules. In the drug reservoir compartment, the drug solids are homogeneously dispersed in a solid polymer matrix or form a paste-like suspension by viscous liquid medium, e.g., silicone fluid. A thin layer of drug-compatible, hypoallergenic pressure-sensitive adhesive polymer, e.g., silicone adhesive, may be applied to provide intimate contact of the system with the skin surface on the external surface of the polymeric membrane.

1.2.2 ADHESIVEDIFFUSION-CONTROLLEDSYSTEM:

Adhesive diffusion-controlled system is the simplified approach of membrane permeation-controlled system. In this system, the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer and then spreading the medicated adhesive by solvent casting or hot melt onto a flat sheet of drug-impermeable metallic plastic backing to form a thin drug reservoir layer. The thin layers of non-medicated rate-controlling adhesive polymer of a specific permeability and constant thickness are applied on the top of the drug reservoir layer to produce an adhesive diffusion-controlled delivery system.

1.2.3 MATRIXDISPERSIONTYPESYSTEM:

The drug reservoir in matrix dispersion type system is prepared by homogeneouslydispersion of drug particles in a hydrophilic or lipophilic polymer matrix and themedicated polymer is then formulated into a medicated disc with a defined surfacearea and required thickness. This drug reservoir containing polymer disc is then fixedonto an occlusive base plate in a compartment fabricated from a drug impermeablebacking. The adhesive polymer is then spread along the circumference to form a stripof adhesive rim around the medicated disc.

1.2.4 MICRORESERVIORSYSTEM:

This type can be considered as a combination of the both drug reservoir and matrixdispersion-typedrugdeliverysystems. The drug reservoirisprepared by first suspending the solid drug in an aqueous solution of a water soluble polymers and then homogeneously dispersing the drug suspension in a lipophilic polymer by using highshear mechanical technique to form thousands of unleachable microscopic spheres of drug reservoirs. The resultant thermodynamically unstable dispersion is stabilized quickly by immediately polymer crosslinking chain which produces medicated polymer disk with a constant surface area and a desirable thickness.

1. 3 COMPONENTSOFTRANSDERMALSYSTEM:

- Polymers
- Drug
- Permeationenhancers
- Adhesives
- Backingmembrane
- ReleaseLiner

POLYMER:

Polymers are the backbone of a trans dermal drug delivery system as it should keep the drug available on the skin surface with a constant concentration over a long period of time.Systems for transdermal drug delivery are fabricated as multilayered polymeric laminates in which a drug reservoir or a drug

polymer matrix is sandwiched between two polymeric layers, an outer impervious backing layer whichprevents the loss of drug through the backing surface andan inner polymeric layer that functions asan adhesive or rate-controlling membrane.

The polymers are divided into mainlythreegroups:

- **NaturalPolymers**:e.g.cellulosederivatives,chitosan,gelatin,zein,shellac,waxes, gums and naturalrubber etc.
- **Synthetic Elastomers**: e.g. polybutadiene, polyisobutylene, silicon rubber,hydrin rubber, nitrile,acrylonitrile, neoprene, butylrubber etc.

SyntheticPolymers:e.g.polyvinylchloride,polyvinylalcohol,polyacrylate,polyethylene,polyurea,polypropylene,polyamide,polyvinylpyrrolidone,polymethylmethacrylateetc. (SugibayashiandMorimoto, 1994; Kandavilli*et al.*,2002).

DRUG:

The transdermal route is the most attractive option for the drugs with appropriate pharmacology and physical chemistry.For the development of a successful transdermal drug delivery system, the drug should be chosen with great care. Before the development of the transdermal drug delivery system of any drug,various physicochemical properties,pharmacokinetics and pharmacodynamic factors are taken under consideration. It is generally accepted that the best drug candidates for passive transdermal patches must have the following properties:

- Low molecular weight,less than100Daltons,preferably lessthan600
- Melting point should be less than 200 C
- Adequate solubility in oil and water, so the membrane concentration gradient

PERMEATIONENHANCERS:

Skin penetration enhancers are used in many topical and transdermal systems to promote the transport of drugs into and across human skin (Mohammed et al., 2014). Skin permeability is one of the most important criteria relating to transdermal permeation kinetics and disposition. This parameter is not only extensively studied by many researchers but also used as a standard and documented by many health and environmental organizations such as the World Health Organization (WHO), the Organization for Economic Co-operation and Development (OECD), the US Environmental Protection Agency (EPA), the US National Institute for Occupational Safety and Health (NIOSH), and the European Commission (EC) (Chen et al., 2013).The global term percutaneous absorption describes the entry of compounds across the skin. The process is divided into three steps: the entry of a substance into a particular skin layer, the penetration through one layer into another, and finally, resorption, the uptake into the vascular system.

2. MATERIALANDMETHODS

2.1 MATERIAL USED:

Table 1.-List of material used

NameofIngredients	**NameofManufacturer**
Methotrexate	YarrowChemProduct,Mumbai,India
Ethylcellulose	SDFineChem Ltd.,Mumbai, India
Polyvinylpyrrolidone(PVPK-30)	SDFineChem Ltd.,Mumbai, India
Di-n-butylpthalate	SDFineChem Ltd.,Mumbai, India
Dimethylsulphoxide	SDFineChem Ltd.,Mumbai, India
Tween-80	SDFineChem Ltd.,Mumbai, India
Eucalyptusoil	CentralDrugHouse,NewDelhi,India
Oliveoil	CentralDrugHouse,NewDelhi, India
Chloroform	SDFineChem Ltd.,Mumbai, India
n-Octanol	CentralDrugHouse,NewDelhi,India
Methanol	SiscoResearch Laboratory,Mumbai,India
Monobasicpotassiumdihydrogen Orthophosphate	HimediaLaboratories,Mumbai,India
Sodiumhydroxide	CentralDrugHouse,NewDelhi,India

Dialysismembrane	HimediaLaboratories,Mumbai,India
Anestheticether	TKMPharmLtd.Hyderabad,India
Adhesivetape	Johnson &Johnson, India
Faxtin	Ind-SwiftLtd.,Chandigarh,India
Isopropylalcohol	CentralDrugHouse,NewDelhi,India

2.2 METHODS:

2.2.1 PREFORMULATION STUDIES:

Preformulation studies are needed to ensure the development of a stable, therapeutically effective, and safe dosage form. It is a stage of development during which the physical pharmacist characterizes the physicochemical properties of drug substance and its interaction with various formulation components.

2.2.2 IDENTIFICATION OF DRUG: PHYSICAL APPEARANCE

The drug sample was purchased from Yarrow Chem Products, Mumbai, India. The supplied powder of drug sample was a crystalline,white to off white in colour powder of odourless and bitterin taste.

2.2.3 DETERMINATION OF λmax AND PREPARATION OF STANDARD CALIBRATION OF METHOTREXATE:

Principle:

Methotrexate exhibits peak maximum absorbance at 303 nm in 0.1N Hydrochloric acid.

Preparation of standard solution:

100 mg of Methotrexate was exactly weighed in a volumetric flask of 100 ml & solubilized in a small volume of pH 7.4 phosphate buffer. The volume was made up with the 0.1N Hydrochloric acid to get a concentration of 1000 µg/ml (SS-1). From this, SS-2 was prepared containing 100 µg/ml.

Calibration curve:

Primary stock solution:

100 mg of Methotrexate was exactly weighed in a volumetric flask of 100 ml & solubilized in a small volume of pH 7.4 phosphate buffer. The volume was made up with the pH 7.4 phosphate buffer to get a concentration of 1000 µg/ml (SS-1). From this, SS-2 was prepared containing 100 µg/ml.

Secondary stock solution:

5 ml of the primary stock solution was diluted to 100 ml with pH 7.4 phosphate buffer in a 100 ml volumetric flask to obtain a stock solution of 50 µg/ml.

Sample selection:

From the secondary stock solution, aliquots ranging from 1 ml, 2 ml, 3 ml, and 8 ml were pipetted out in a series of 10 ml volumetric flask and diluted to 10 ml with pH 7.4 phosphate buffer to get the concentration of 5 µg/ml, 10 µg/ml, 15 µg/ml, and 40 µg/ml, respectively. Different aliquots were filtered through Whatman No. 1 filter paper and filtrate analyzed at 303 nm by using UV-visible spectrophotometer (Model-1700, Shimadzu, Japan) against pH 7.4 phosphate buffer as a blank. Absorbance was recorded, and a standard curve was plotted with absorbance on the y-axis and concentration on the x-axis for a linear relationship.

2.2.4 DRUG-EXCIPIENTS INTERACTION STUDY:

The Fourier transform infra-red (FTIR) profile of drug alone and physical mixtures of Methotrexate with ethylcellulose (EC) and polyvinyl pyrrolidone K-30 (PVP) wererecorded in order to determine the physicochemical compatibility between drug andpolymers used in the formulation. A pellet of pure drug and physical mixture of drugand polymers (1:1) were prepared by compressing with IR rade potassium bromide in a 100:1 ratio by applying 5.5 metric ton of pressure in hydraulic press. The pelletwas mounted in IR compartment and scanned between wave number 4000-450 cm^{-1}using FTIR spectrophotometer (Model-8400S, Shimadzu,Japan).The obtained FTIR spectra of drug, polymers and physical mixture of Methotrexatewith polymers (EC and PVP) were compared with the references for obtained peak offunctionalgroups(Ammar *et al.,* 2009; B.P., 2009)

2.2.5 FORMULATION OF METHOTREXATE TRANSDERMAL PATCHES:

The matrix-type transdermal patches of Methotrexate were prepared by solvent evaporation technique using different ratios of ethyl cellulose (EC) and polyvinylpyrrolidone K30 (PVP) polymers. The polymers EC and PVP were weighed and mixed in different ratios while keeping the total polymer weight at 1.6 g. They were added to a chloroform solvent using a magnetic stirrer. Dibutyl phthalate, constituting 30% w/w of the polymer, was incorporated as a plasticizer. The drug, making up 20% w/w of the polymer weight, was slowly added to the polymer solution and thoroughly mixed by continuous stirring for 30 minutes to obtain a homogeneous solution.

Five formulations were prepared using the same drug and different polymer ratios without permeation enhancers to determine the optimum combination of drug and polymers. Based on preliminary studies, the optimized polymer ratio of 3:2 (EC:PVP) was mixed with different permeation enhancers such as DMSO, Tween-80, eucalyptus oil, and olive oil. The permeation enhancers were added in three different concentrations, i.e., 2%, 5%, and 10% w/w of the total polymer weight for each. The resulting drug-polymer solution was poured into a petri dish of 64 cm^2. Aluminum foil was uniformly spread on the petri dish on which the drug-polymer solution was poured. The rate of evaporation was controlled by inverting a funnel over the petri dish, and the solvent was allowed to evaporate for 24 hours at room temperature. After 24 hours, the films were collected, and wax paper was applied on the other side of the films as a release liner to complete the formulation (Arora and Mukherjee, 2002; Verma and Chandak, 2009).

2.2.6 PHYSICOCHEMICAL EVALUATION OF METHOTREXATE PATCHES:

2.2.6.1 PHYSICAL APPEARANCE:

All formulated transdermal patches were visually inspected for color, clarity, entrapment of any air bubble, flexibility, and smoothness, which, to a large extent, determines patient acceptability of the patch and also therapeutic efficacy (John et al., 2013).

2.2.6.2 THICKNESS:

The thickness of transdermal patches was measured by using a digital thickness gauge (Muttato Japan). The thickness of rectangular patches (2x2 cm) was determined at four different points, and the average thickness was taken. The same was performed for other patches as well (Patel et al., 2009).

2.2.6.3 WEIGHT VARIATION:

Weight variation study of transdermal patches was performed by individually weighing 10 randomly selected patches of sizes 4.52 cm2 on a digital weighing balance, and the average weight was calculated. The individual weight of patches should not deviate significantly from the average weight (El-Gendy et al., 2009).

2.2.6.4 DRUG CONTENT:

To determine the drug content of transdermal patches, known amounts of Methotrexate patches were cut from casted film and dissolved in chloroform in a 100 ml volumetric flask and placed in a shaking incubator for 4 h. The solution was filtered through a membrane filter (0.45 µm), and 1 ml of the solution was taken and diluted with chloroform to 10 ml. The absorbance of the solution was measured at 303 nm using a UV/visible spectrophotometer (Model-1700, Shimadzu, Japan). Chloroform was used as a blank. The average reading of three patches was taken as the content of the drug in one patch (Limpongsa and Umprayn, 2008).

2.2.6.5 MOISTURE CONTENT:

To determine moisture content of transdermal patches, they were weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24 h. The transdermal patches were weighed repeatedly until they showed a constant weight. The moisture content was calculated by the given formula (Bagyalakshmi et al., 2006; Devi et al, 2003).

$$\%\text{Moisturecontent} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

2.2.6.6 MOISTURE UPTAKE:

Transdermal patches were kept in desiccators at room temperature for 24 h with silica gel and weighed (ws) and transferred to other desiccators to expose to 75% RH using a saturated solution of sodium chloride at 25°C, and patches were reweighed again and again, until a constant weight (wm) was obtained. The moisture uptake capacity was calculated according to the given formula (Amnuaikit et al., 2005).

2.2.6.7 FLATNESS:

Longitudinal strips from the 5 randomly selected transdermal films of each formulation were cut out, one from the center and one from the other side of the patch. The length of each strip was measured, and the variation in length because of the non-uniformity of flatness was measured. 0 % constriction was considered to be 100 % flatness. Flatness was calculated by measuring constriction of strip using given formula (Chandak and Verma, 2008).

2.2.6.8 FOLDING ENDURANCE:

The folding endurance of the patch was expressed as the number of folds (number of times the patch folded at the same place), either to break the preparation or to develop visible cracks. This test was performed to determine the stability of the sample to withstand folding and brittleness. Folding endurance of patches was determined by repeatedly by folding a small strip of patches (approximately 2×2 cm) at the same place until it broke. The number of times patches could be folded at the same place, without breaking, gave the value of folding endurance and it was recorded (Kumar et al., 2013).

2.2.6.9 TENSILE STRENGTH:

The formulated patches were evaluated for its tensile strength to measure their mechanical properties. The tensile strength of the patches was determined by using a self-designed assembly (Department of Pharmacy). Assembly consists of a pan hanged by using a strong thread and the other end of the thread was attached with the center of the patch. The whole assembly was held like a beam balance, and weights were kept on the pan. Weights required to break the patch were noted. Tensile strength was then calculated using the following formula (Bhatia et al., 2012).

Tensile Strength = Break Force/ a . b (1+ ΔL/L)

Where,

a=Width of the patch,

b = Thickness of the patch

L = Length of the patch,

ΔL=Elongation of patch at break point

Break Force=Weight required to break the patch (Kg)

2.2.6.10 pH MEASUREMENT:

The pH of the film-forming solutions was determined using a pH meter which was calibrated before use with buffered solutions at pH 4, 7, and 10 (Abdel et al., 2014).

2.2.7 INVITRO DRUG RELEASE STUDIES OF METHOTREXATE PATCHES:

The dissolution studies were performed using dissolution rate test apparatus (USP-II) for the assessment of the release of the drug from the transdermal patches (3.14 cm2). The commercially available water impermeable adhesive backing membrane was placed over the patch and it was further fixed on a glass slide (2.3x2.3 cm) using cyanoacrylate adhesive. Then the transdermal patch was covered with a dialysis membrane and placed at the bottom of dissolution vessels with the release surface facing upward. The apparatus was equilibrated to 32 ± 0.5°C, and the dissolution medium was 20% methanol in PBS pH 7.4. The paddle speed was kept constant at 50 rpm. The samples were withdrawn at appropriate time intervals up to 24 h and analyzed by a UV spectrophotometer at 303 nm using 20% methanol in PBS pH 7.4 solution as a blank. After each sampling, an equal volume of fresh dissolution fluid was added to the dissolution vessel to maintain a sink condition (Gannu et al., 2007; Shah et al., 1986).

The drug release data of all formulations were fitted to various mathematical models such as zero order as cumulative % of drug released vs. time, first order as log cumulative % of drug remaining vs. time, and Higuchi's model as cumulative % drug released vs. square root of time. To determine the mechanism of drug release from formulations, the data were fitted into Korsmeyer-Peppas equation as log cumulative % of drug released vs. log time (Costa and Lobo, 2001; Prashar et al., 2014) Amounts of drug permeated per sq. cm of patch were calculated and plotted against time. Flux was calculated as the amount of drug permeated per sq. cm per hour. The lag time (Tlag) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa (Amrish and Kumar, 2009; Ubaidulla*etal.*, 2007)

2.2.8 IN-VITRO DRUG PERMEATION STUDIES:

In-vitro drug permeation studies were conducted using a Franz diffusion cell with a receptor compartment capacity of 22 ml. Cellulose acetate, an acetate ester of cellulose, which has been fabricated as semi-permeable membranes for biomedical applications, was utilized for the determination of drug release from the prepared transdermal matrix-type patches. The cellulose acetate membrane (cellulose acetate membrane) was used for the experiment. A cellulose acetate membrane with a pore size of 0.45µ was placed between the donor and receptor compartments of the diffusion cell. The prepared

transdermal film was positioned on the cellulose acetate membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with phosphate buffer at pH 7.4. The entire assembly was fixed on a hot plate magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads. The temperature was maintained at 32±0.5°C, as the normal skin temperature of humans is around 32°C.Samples were withdrawn at various time intervals and analyzed for drug content using a UV Spectrophotometer at 303 nm. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal to maintain sink conditions for in vitro drug release rate of the selected transdermal drug delivery system (TDDS).

2.2.9 STABILITY STUDIES OF METHOTREXATE PATCHES:

Stability studies of formulation FT1 were conducted following ICH (International Council for Harmonisation) guidelines by storing the patches at 40°C and 75% relative humidity (RH) for 3 months. Samples were withdrawn at 30, 60, and 90 days and evaluated for physical appearance and drug contents. The in vitro permeation study was performed after 90 days and compared with the fresh batch (Aggarwal et al., 2011).

3. RESULT AND DISCUSSION:

3.1 PREFORMULATION STUDIES:

A preformulation study was conducted to ensure the authenticity of the Methotrexate sample and to determine various parameters necessary for the formulation development. This included the identification of the drug, determination of its UV absorption maxima, and drug sample identification through FT-IR spectroscopy. Various other studies were performed, and the results were compared with established references.

3.2 IDENTIFICATION:

The Methotrexate drug sample (Batch No. 12820218V) was procured from Yarrow Chem Products in Mumbai, India. The physical characteristics and UV absorption properties of the drug sample were assessed. The drug powder exhibited a crystalline structure and was either white or nearly white in color. It had no discernible odor and a bitter taste.

The identity of the drug sample was further confirmed through UV scanning and FTIR spectroscopy. The drug sample conformed to the expected preliminary identification criteria. UV scanning revealed a λmax (maximum wavelength of UV absorption) of 300nm, closely matching the literature-reported value of 303nm. The IR spectrum was consistent with the characteristic spectrum of pure Methotrexate, confirming the authenticity of the drug sample.

3.3 PREPARATION OF STANDARD CALIBRATION CURVE OF METHOTREXATE:

The standard graph of methotrexate was shown in graph 1, it was observed that the drug obeys beer's law in the concentration range o f2-18µg/ml in phosphate buffer pH 7.4. The linear regression equation generated was used for the calculation of amount of drug release from the implant.The linear regression analysis for standard curve: The linear regression analysis wasdone on absorbance data points. The results are as follows. For standard curve in Phosphate Buffer of pH7.4:

The slope =0.0522

The intercept=0.0368

The correlation coefficient= 0.9979

A straight-line equation (Y= mx + c) was generated to facilitate the calculation of amount of drug. The equation is as follows.

Absorbance=0.0522×Concentration +0.0368

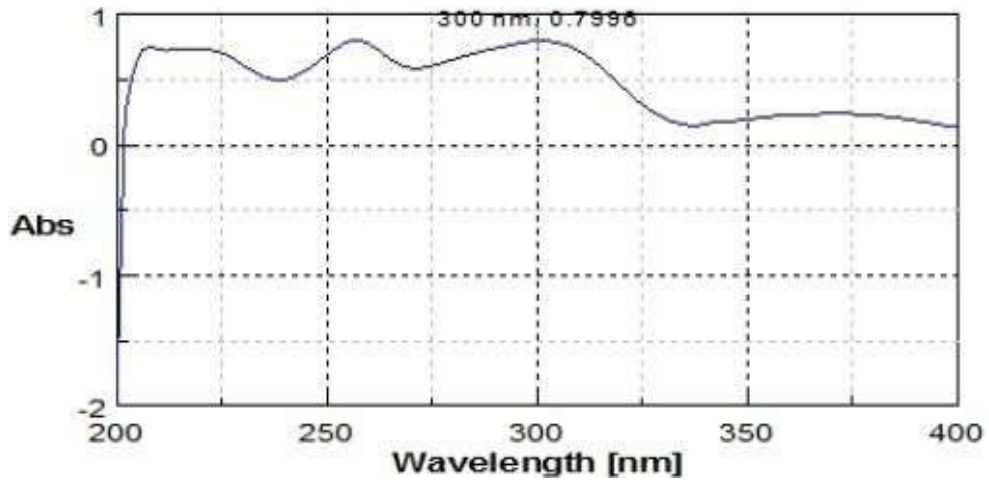


Figure:1-UV Spectra of Methotrexate

Model: V-530, Response: Fast, Measurement range: 200-400 nm, Data pitch: 1 nm,Scanning speed: 1000 nm/min No of cycle: 1, Sample Name: Methotrexate, Operator:BC Doddamani. Peakfound at: 300 nm

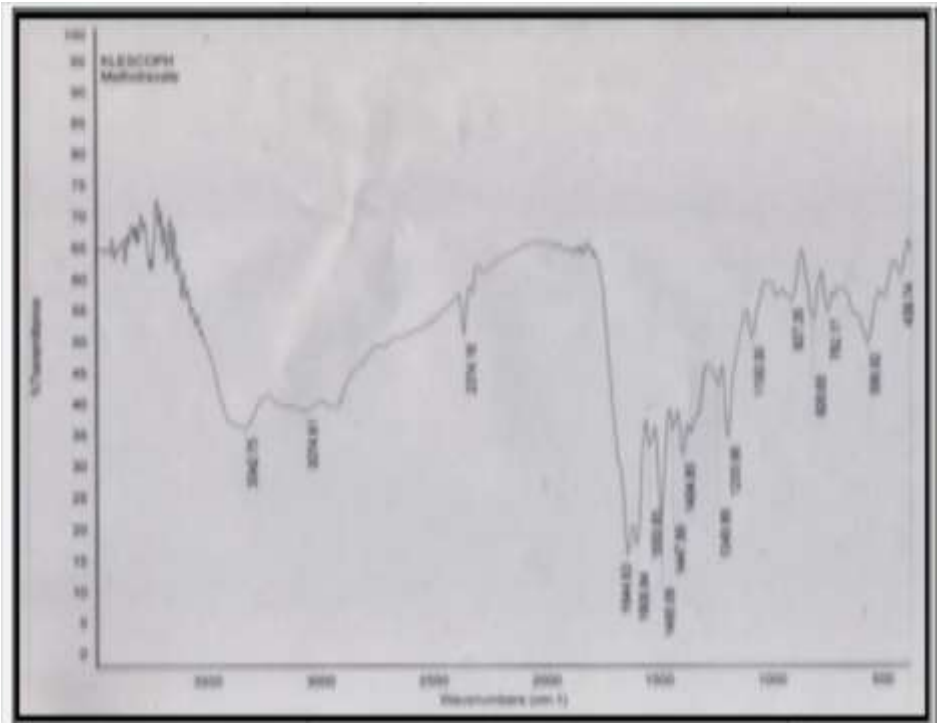


Figure:2- I.R.spectrum of Methotrexate sample

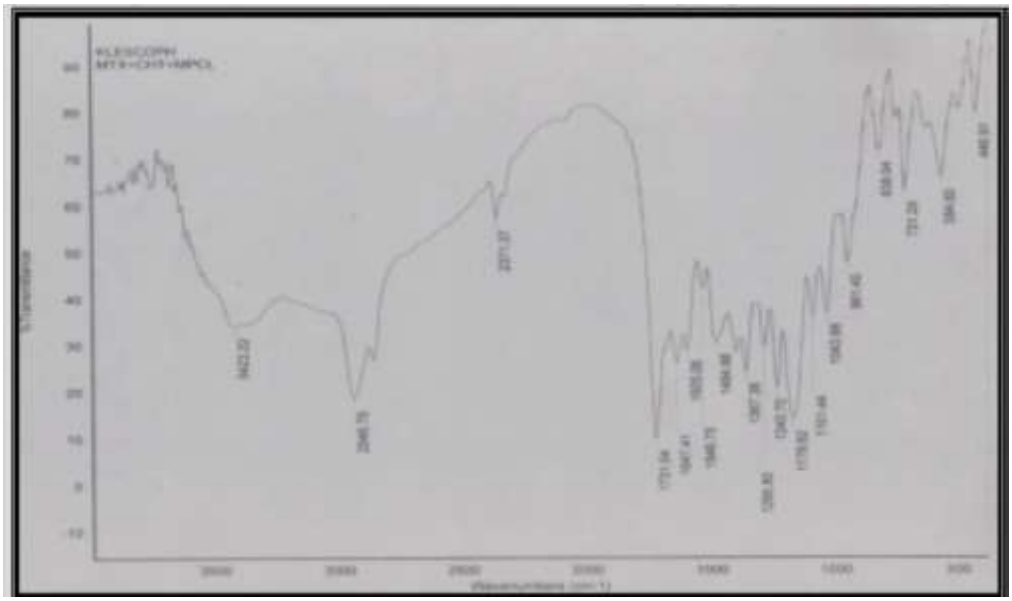


Figure:3-ReferenceI.R.spectrumof Methotrexate-exciepient sample

Table:1-Absorbance of methotrexate in different concentration

Concentration	**Absorbance**
0	**0**
2	0.2001
4	0.3941
6	0.5786
8	0.7998
10	0.9663

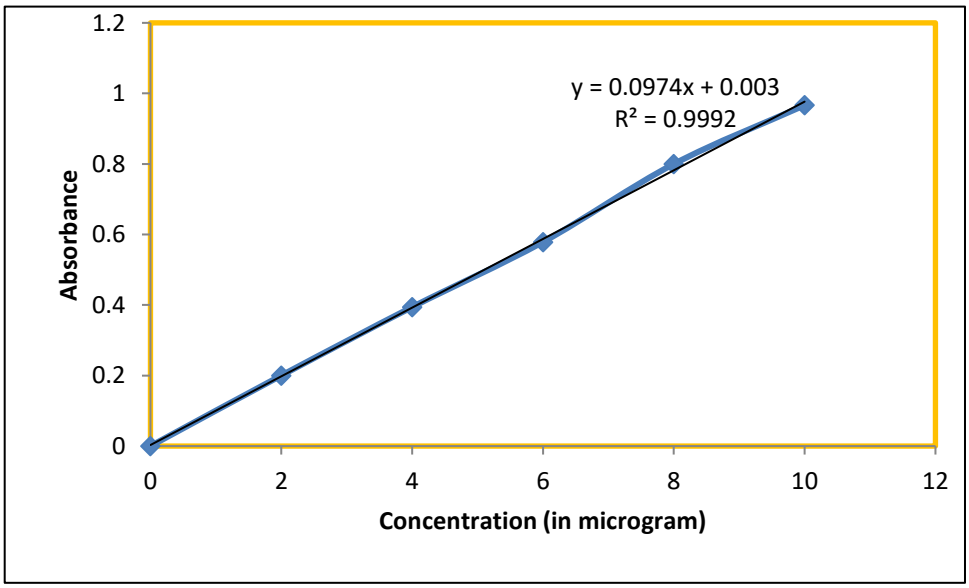


Figure:4 ,Calibration curve of Methotrexate

S. No	Parameter	Observation
1	Physicalappearance	Nature: Crystaline solidColour:Whitetooffwhite Odour:Odourless
2	UVAbsorptionMaxima	303 nm

Table:2-Physical characteristics of Methotrexate

3.4 FORMULATION OF METHOTREXATE TRANSDERMAL PATCHES:

The transdermal patches were formulated using a combination of ethylcellulose (EC) and polyvinylpyrrolidone K-30 (PVP) polymers. EC and PVP are widely utilized polymers in transdermal drug delivery systems due to their compatibility with drugs and their sustained release properties, as reported by Kandavilli et al. in 2012.

In initial experiments, various formulations were prepared, both with and without a plasticizer. It was observed that the transdermal patches made without a plasticizer tended to be brittle. To address this issue, di-n-butyl phthalate was introduced as a plasticizer to enhance the flexibility of the transdermal patches. The studies demonstrated that the addition of di-n-butyl phthalate at a concentration of 30% w/w of the total dry polymer weight resulted in the production of smooth, uniform, and flexible films. Consequently, further formulations were prepared using a plasticizer at a concentration of 30% w/w of the polymer weight in all the patches.Building on the findings from preliminary formulation studies, the optimal polymer ratio was selected for subsequent experiments, which involved the incorporation of various penetration enhancers at different concentrations. The aim of these experiments was to improve the in vitro permeability of the drug molecule through the skin.The fabricated films were valuated for various physiochemical parameters and the composition of formulations is given in Table.3

Table:3-Composition of Methotrexate transdermal patches

S. No.	Formulation Code	Methotrexate(%w/w)	EC: PVP (Ratio)	Permeation Enhancer (%w/w)
1.	F1	20	4.5 : 0.5	-
2.	F2	20	4 : 1	-
3.	F3	20	2 : 1	-
4.	F4	20	3:2	-
5.	F5	20	2:3	-
6.	FD1	20	3:2	DMSO2%
7.	FD2	20	3:2	DMSO5%
8.	FD3	20	3:2	DMSO10%
9.	FT1	20	3:2	Tween-802%
10.	FT2	20	3:2	Tween-805%
11.	FT3	20	3:2	Tween-8010%
12.	FE1	20	3:2	Eucalyptus oil 2%
13.	FE2	20	3:2	Eucalyptus oil 5%
14.	FE3	20	3:2	Eucalyptus oil 10%
15.	FO1	20	3:2	Oliveoil 2%
16.	FO2	20	3:2	Oliveoil 5%
17.	FO3	20	3:2	Oliveoil 10%

All formulations containing dibutylphthalate (30%w/w of polymers weight)as plasticizer and chloroform as a solvent system.

3.5 PHYSIOCHEMICAL EVALUATION OF METHOTREXATEPATCHES:

The prepared transdermal patches underwent a thorough evaluation of their physicochemical characteristics, including physical appearance, thickness, weight uniformity, drug content, moisture content, moisture uptake, flatness, folding endurance, tensile strength, and pH. The results of these evaluations are presented in Table.4.

The formulated patches exhibited the following characteristics:

Physical Appearance: The patches were transparent, smooth, uniform, and flexible, with no air bubbles.

Weight: The weight of the transdermal patches ranged from 164.37 to 172.01 mg, indicating consistency in weight among different batches.

Thickness: Different batches had a thickness ranging from 0.246 to 0.276 mm, with low standard deviation values, ensuring uniformity.

Drug Content: No significant difference in drug content was observed in all formulated patches, with values ranging from 94.12% to 98.23%. This indicates that the method used for patch preparation effectively achieved uniform drug content through homogeneous drug dispersion.

Moisture Content and Uptake: Increased concentration of the hydrophilic polymer (PVP) resulted in higher percentages of moisture content and moisture uptake. This aligns with findings by other researchers. Moisture content ranged from 1.64 ± 0.31 to 6.38 ± 1.04, while moisture uptake ranged from 2.43 ± 0.55 to 9.41 ± 0.75. Higher hydrophilicity led to increased moisture content and uptake. Low moisture content maintains stability and prevents drying, while low moisture uptake protects against microbial contamination and reduces patch bulkiness.

Flatness: Flatness studies confirmed that all patches exhibited 100% flatness, ensuring a smooth surface when applied to the skin.

Tensile Strength: Tensile strength, essential for flexibility and mechanical strength on the skin, ranged from 0.346 to 0.438 kg/mm². Folding endurance, indicating a patch's ability to withstand rupture, fell within the range of 34 to 48. These results suggest that patches from all batches will maintain their integrity even with regular skin folding.

pH: The pH of the film-forming solutions ranged from 5.8 to 6.6, within the ideal pH range for dermatological preparations (pH 4 to 7). This ensures safety and minimizes the risk of skin irritation.

These findings demonstrate that the method employed for patch preparation yielded uniform, stable, and safe transdermal patches suitable for dermatological use.

Table:4-Physiochemical evaluation of Methotrexate transdermal Patches

F. Code	Thickness(mm)	Weight Variation(mg)	Drug Content(%)	Flatness	FoldingEndurance	Tensile Strength(kg/mm2)	pH	F. Code
F1	0.273±0.014	164.87±2.08	96.25±0.42	100	42 ±4.08	0.417±0.02	5.8	F1
F2	0.254±0.017	164.37±1.48	97.26±1.42	100	48 ±6.50	0.438±0.04	5.8	F2
F3	0.266±0.008	167.19±1.88	94.12±0.74	100	44 ±3.43	0.393±0.01	5.8	F3
F4	0.260±0.012	165.20±2.08	96.20±1.11	100	39 ±4.69	0.404±0.03	5.7	F4
F5	0.268±0.011	166.49±1.11	95.03±1.56	100	34 ±3.08	0.357±0.06	5.7	F5
FD1	0.265±0.016	164.40±1.89	96.78±2.14	100	38 ±5.37	0.370±0.07	6.5	FD1
FD2	0.276±0.010	166.72±1.92	94.38±0.92	100	36 ±3.11	0.352±0.03	6.6	FD2
FD3	0.269±0.016	169.61±2.33	96.20±0.61	100	38 ±4.15	0.346±0.05	6.6	FD3
FT1	0.261±0.022	165.20±1.69	97.64±1.04	100	37 ±5.12	0.371±0.02	6.3	FT1
FT2	0.256±0.023	167.57±2.12	95.68±0.62	100	36 ±3.91	0.397±0.04	6.3	FT2
FT3	0.274±0.013	168.97±2.93	95.73±1.80	100	40 ±4.84	0.361±0.02	6.4	FT3
FE1	0.246±0.027	165.40±2.18	98.23±0.78	100	35 ±4.32	0.394±0.03	6.1	FE1
FE2	0.256±0.014	167.60±1.34	95.53±1.21	100	38 ±2.54	0.403±0.04	6.5	FE2
FE3	0.267±0.012	166.76±2.76	97.19±0.96	100	35 ±3.63	0.372±0.03	6.6	FE3
FO1	0.265±0.016	168.56±1.91	94.88±1.13	100	36 ±6.72	0.346±0.02	5.7	FO1
FO2	0.273±0.009	167.95±4.32	94.58±1.34	100	40 ±3.91	0.363±0.04	5.7	FO2
FO3	0.272±0.014	172.01±2.77	96.43±0.69	100	43 ±4.18	0.358±0.05	5.7	FO3

3.5 IN VITRO DRUG RELEASE STUDIES OF METHOTREXATE PATCHES:

Dissolution studies of transdermal patches are of paramount importance to ensure a sustained release pattern. Maintaining a consistent drug concentration on the stratum corneum surface, surpassing the concentration in the plasma, is essential for achieving a constant drug permeation release rate. The modified paddle over disc assembly, employing a dissolution medium of 20% methanol in PBS pH 7.4 at 32 ± 0.5°C, was employed for conducting these dissolution studies. The results of the in vitro dissolution studies for the prepared transdermal patches are presented in Table 5

Cumulative drug release percentages from the control formulations (without enhancer) F1, F2, F3, F4, and F5 were found to be 40.70%, 46.68%, 52.38%, 59.66%, and 50.61%, respectively, within 24 hours. The highest drug release percentage (59.66%) was observed for formulation F4 (EC/PVP, 3:2), which was significantly ($p < 0.05$) greater than the lowest value of 40.70% obtained from formulation F1 (EC/PVP, 4.5:0.5). The order of drug release percentages was as follows: F4 > F3 > F5 > F2 > F1.

It was noted that an increase in the concentration of the hydrophilic polymer PVP led to an increase in the rate of drug release, except for formulation F5 (Mukherjee et al., 2005). The addition of hydrophilic PVP to insoluble ethyl cellulose tends to enhance the release rate constant. This is attributed to the

leaching of the soluble fraction, which results in the formation of pores. Consequently, there is a reduction in the mean diffusion path length for drug molecules into the diffusion medium, an increase in the external film area exposed to the dissolution medium, an increase in internal porosity, and a decrease in tortuosity (Mittal et al., 2009). An initial burst release effect was observed in all formulations. This effect may be due to the higher percentage of PVP in these formulations, and the hydrophilic layer of PVP may require very little "time lag" to establish a concentration profile in the patches, resulting in a burst release in the dissolution studies. Similar findings have also been reported by others (Mutalik and Udupa, 2004).

Formulation F5 exhibited a decrease in the rate of drug release with an increase in the concentration of the hydrophilic polymer. This may be attributed to the earlier finding that a higher concentration of PVP K-30 can reduce the crystalline drug content in the patch, thereby decreasing drug release (Ghosal et al., 2010).

Effect of olive oil:

In case of formulations containing olive oil, the cummulative % of drug release wasalso increased with increase in concentration of olive oil from 2 to 5% (64.50% to73.08% respectively). This may be due to presence of fatty acids in olive oil. But afurther increase in concentration of olive oil to 10%, the % of drug release was found to be decreased (67.93 %) as shown in Table 5. This may be attributed to decrease in solubility of drug in the presence of high concentration of oilin matrix which cannot increase solubility of drug significantly in release media as previously reported for vegetables oils (Rasool*etal.*, 2011).

Table: 5- *In vitro* dissolution profile of Methotrexate from transdermal patches containing EC/PVP in different proportion 4.5:0.5 (F1), 4:1 (F2), 2:1 (F3),3:2(F4) and 2: 3(F5)

Time(h)	**Cumulative %drug release**				
	F1	**F2**	**F3**	**F4**	**F5**
1	0.696 ±0.21	1.81 ± 0.50	2.29 ± 0.46	0.826 ±0.15	3.15 ± 0.21
2	3.81 ± 0.46	3.36 ± 0.69	4.32 ± 0.35	5.68 ± 1.13	6.55 ± 0.40
4	5.23 ± 0.23	7.63 ± 1.73	9.76 ± 1.02	11.52 ±0.63	11.25 ±1.16
6	12.50 ±1.28	13.80 ±1.58	17.52 ±3.05	18.06 ±1.65	19.32 ±2.81
8	20.81 ±2.13	18.49 ±2.08	24.41 ±4.46	28.72 ±3.11	25.51 ±0.64
10	23.89 ±1.77	25.56 ±4.19	33.87 ±1.78	36.55 ±1.57	31.85 ±1.13
12	27.66 ±0.61	32.34 ±1.16	39.67 ±3.10	43.37 ±3.02	37.02 ±2.22
18	35.15 ±2.86	40.76 ±3.53	47.16 ±1.77	52.61 ±2.28	43.96 ±2.39
24	40.70 ±0.49	46.68 ±1.76	52.38 ±0.63	59.66 ±0.86	50.61 ±0.96

3.6 APPLICATION OF KINETIC MODELS TO CHARACTERIZE THE INVITRO DRUG RELEASE FROM METHOTREXATE PATCHES:

In vitro drug release data were analyzed using various kinetic models (refer to Table 6.13) to comprehend the release kinetics. These models included:

1. Zero Order: Cumulative drug released over time.

2. First Order:Logarithm of cumulative drug remaining over time.

3. Higuchi's Model:Cumulative drug released against the square root of time (Prashar et al., 2014).

4. Korsmeyer-Peppas Equation: Logarithm of cumulative drug released plotted against the logarithm of time, with the 'n' exponent indicating the release mechanism.

For matrix systems, an 'n' exponent of 0.5 signifies Fickian diffusion, 0.5 < 'n' < 1.0 suggests non-Fickian diffusion, 'n' = 1.0 corresponds to zero order, and 'n' > 1.0 indicates super case II transport (Dash et al., 2010).

The results revealed that formulations F1, F3, F4, and F5 best followed Higuchi's equation (with the exception of formulation F2) due to the high linearity of the R-squared value. Formulations F2, FD2, FE2, and FO2 displayed an anomalous (pseudo-first order) release pattern. Formulations FT2, FT3, FE1, and FO3 exhibited a pseudo-zero-order release, as indicated by higher coefficients of determination compared to other models. Furthermore, the release pattern of formulations FD1, FD3, FT1, FE3, and FO1 was best fitted by Higuchi's release kinetics.

Table:6- *In vitro* dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) anddifferent proportionofDMSO 2%(FD1), 5%(FD2)and 10%(FD3)

Times(h)	**Cummulative % drug release**		
	FD1	**FD2**	**FD3**
1	1.55 ± 0.27	1.99 ± 0.75	6.67 ± 1.09
2	12.32 ±2.11	6.16 ± 1.46	17.74 ±1.65

4	18.13 ±3.34	11.34 ±1.44	31.46 ±3.40
6	25.86 ±2.32	20.82 ±2.23	42.67 ±3.17
8	30.92 ±2.13	37.65 ±3.56	50.87 ±2.77
10	37.72 ±3.28	44.51 ±3.71	59.35 ±3.72
12	44.17 ±4.30	52.17 ±4.10	65.15 ±3.29
18	51.08 ±2.82	61.88 ±2.77	72.25 ±2.57
24	64.58 ±0.63	70.49 ±1.22	78.29 ±0.76

Table:7- *In vitro* dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and different proportion of tween-80 2% (FT1),5%(FT2)and 10%(FT3)

Times(h)	**Cummulative % drugr elease**		
	FT1	**FT2**	**FT3**
1	2.49 ± 0.32	7.31 ± 1.10	5.76 ± 0.84
2	6.70 ± 1.14	13.82 ±1.35	9.43 ± 1.46
4	14.97 ±1.24	19.39 ±2.08	12.34 ±0.74
6	27.38 ±2.58	25.87 ±2.63	19.47 ±2.11
8	38.94 ±2.63	34.60 ±3.19	23.83 ±1.45
10	53.74 ±4.08	40.61 ±4.44	30.51 ±2.56
12	59.63 ±2.48	48.26 ±3.59	42.16 ±4.05
18	72.61 ±3.75	60.51 ±4.47	54.76 ±4.12
24	88.72 ±0.93	77.32 ±1.27	70.38 ±0.83

Table:8- *In vitro* dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and different proportion of eucalyptus oil 2%(FE1),5%(FE2)and 10%(FE3)

Times(h)	**Cummulative % of drug release**		
	FE1	**FE2**	**FE3**
1	3.65 ± 0.44	1.94 ± 0.21	3.11 ± 0.77
2	9.23 ± 1.11	5.45 ± 1.20	10.40 ±1.14
4	13.29 ±1.61	9.33 ± 0.75	24.10 ±2.02
6	21.41 ±2.37	14.74 ±1.46	31.22 ±1.73
8	25.18 ±2.29	32.58 ±3.25	40.70 ±3.39
10	30.58 ±1.83	39.68 ±4.27	47.10 ±2.28
12	38.40 ±3.25	47.66 ±3.25	53.40 ±1.14
18	52.28 ±4.14	60.28 ±3.19	64.95 ±3.10
24	68.58 ±0.97	76.83 ±0.84	83.52 ±0.81

Table:9- *In vitro* dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and different proportion olive oil 2% (FO1), 5%(FO2)and 10%(FO3)

Time (h)	**Cummulative % of drugrelease**		
	FO1	**FO2**	**FO3**
1	1.72 ± 0.30	0.756 ±0.22	1.31 ± 0.37
2	6.54 ± 0.93	6.51 ± 0.63	3.14 ± 0.25
4	16.18 ±2.25	14.43 ±2.21	8.95 ± 1.32
6	27.29 ±3.09	21.71 ±1.54	14.47 ±2.27
8	32.37 ±2.42	26.22 ±2.31	20.49 ±2.06
10	36.89 ±2.74	35.15 ±3.44	28.92 ±3.24
12	41.17 ±3.17	46.90 ±2.87	40.37 ±3.38
18	49.18 ±2.75	64.47 ±3.20	53.49 ±3.19
24	64.50 ±0.52	73.08 ±1.07	67.93 ±0.89

Figure 5 ***In vitro*** **dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP in different proportion 4.5:0.5 (F1), 4:1 (F2), 2:1(F3), 3:2(F4) and2:3(F5)**

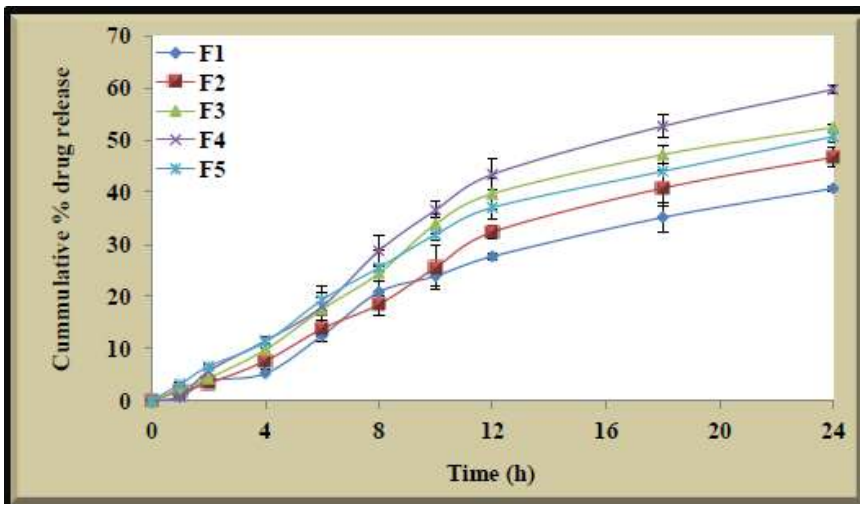


Figure 6 ***In vitro*** **dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and different proportion of DMSO 2% (FD1), 5%(FD2)and10%(FD3)**

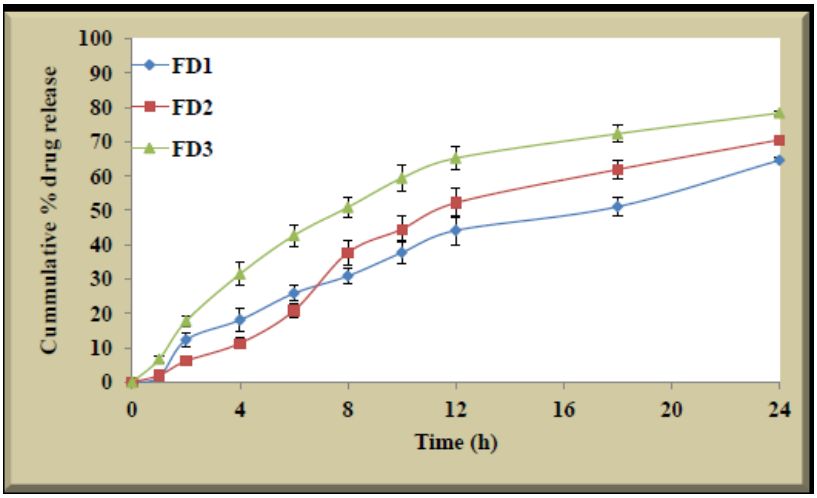


Figure 7 ***In vitro*** **dissolution profile of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and different proportion of tween-80 2% (FT1), 5%(FT2) and10%(FT3)**

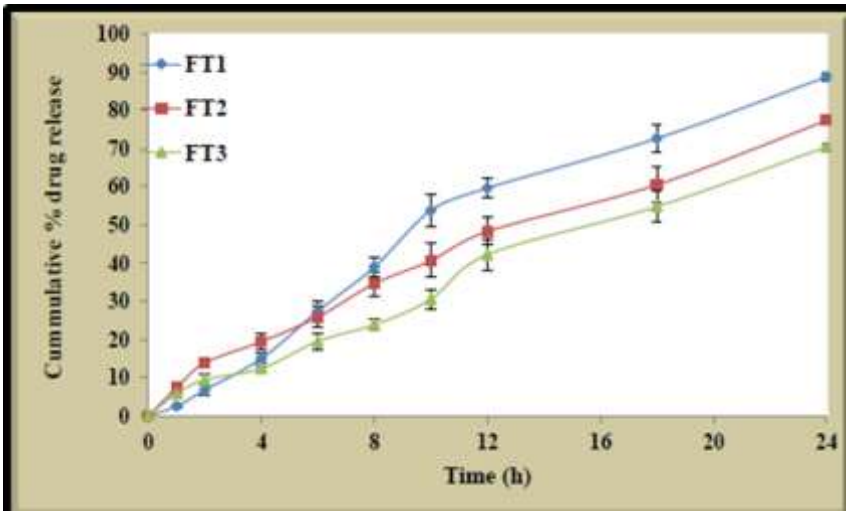
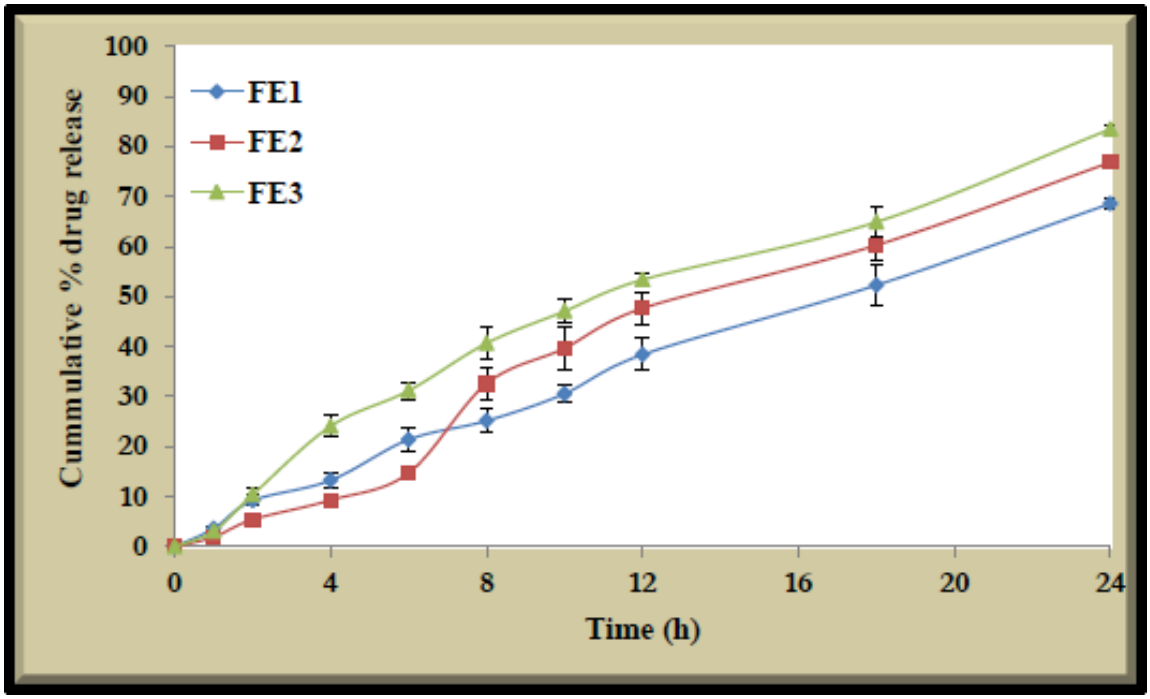


Figure 8 In vitro dissolution profile of Methotrexate from transdermal patches containing EC/PVP (3:2) and different proportions of eucalyptus oil 2% (FE1), 5% (FE2), and 10% (FE3)



3.7 IN-VITRO DRUG PERMEATION STUDIES

In-vitro drug release studies were conducted using a Franz diffusion cell with a receptor compartment capacity of 22 ml. Based on the results of in vitro dissolution studies, the most promising formulations, namely F4, FD3, and FE3, were chosen from the various batches for subsequent in vitro permeation studies across an excised cellophane membrane. Samples were withdrawn at specific time intervals and analyzed for drug content using a UV spectrophotometer set at 303 nm.The effectiveness of permeation enhancers such as DMSO, Tween-80, and eucalyptus oil was assessed by comparing the in vitro permeation and steady-state flux of Methotrexate from transdermal patches with and without the enhancer (control patch). The in vitro permeation profiles are presented in Table 11. DMSO exhibited a cumulative drug permeation of 637.78 ± 31.63 µg/cm2 in 24 hours, with a flux of 27.39 ± 1.76 µg/cm2/h and a lag time (Tlag) of 1.65 ± 0.82 hours. This represented a 2.73-fold enhancement, underscoring DMSO's effectiveness as a penetration enhancer.Transdermal patches containing Tween-80 as the permeation enhancer demonstrated the highest cumulative drug permeation, measuring 1020.29 ± 40.88 µg/cm2 in 24 hours, with a flux of 43.91 ± 1.29 µg/cm2/h and an enhancement of 4.36 times. The lag time was found to be 0.72 ± 0.58 hours. It's important to note that Tween-80 is a nonionic surfactant known for its efficacy as a permeation enhancer. The transdermal patches containing tween-80 as permeation enhancer showed highest cumulative amount of drug permeated 1020.29±40.88µg/cm 2 in24 h with flux 43.91±1.29µg/cm2/h and enhancement of 4.36times and T lag was 0.72±0.58h.Tween-80 is anionic surfactant

Table:10- Release kinetic of Methotrexate transdermal patches

F. Code	**Zero order**		**First order**		**Higuchi Model**		**Korsmeyer Peppas Model**
	r2	K0	r2	K1	r2	Kh	(n)
F1	0.938	1.820	0.965	0.010	0.975	11.07	1.260
F2	0.960	2.096	0.980	0.012	0.976	12.61	1.096
F3	0.923	2.349	0.958	0.014	0.970	14.37	1.065
F4	0.935	2.669	0.976	0.017	0.980	16.31	1.289
F5	0.935	2.131	0.970	0.013	0.984	13.05	0.907
FD1	0.945	2.539	0.985	0.018	0.991	15.52	1.028
FD2	0.917	3.165	0.975	0.023	0.967	19.39	1.160
FD3	0.844	3.277	0.955	0.027	0.958	18.92	0.756
FT1	0.950	3.876	0.979	0.039	0.982	23.52	1.156
FT2	0.986	2.985	0.982	0.025	0.985	17.80	0.728
FT3	0.990	2.877	0.976	0.021	0.952	16.84	0.801
FE1	0.995	2.766	0.981	0.020	0.971	16.30	0.885
FE2	0.966	3.401	0.982	0.027	0.964	20.28	1.191

FE3	0.956	3.316	0.975	0.030	0.996	20.19	0.981
FO1	0.939	2.581	0.979	0.018	0.990	15.82	1.089
FO2	0.975	3.263	0.988	0.025	0.979	19.47	1.332
FO3	0.987	3.035	0.984	0.021	0.960	17.86	1.128

Several studies have reported that essential oils, such as eucalyptus oil, can significantly enhance the permeability of both lipophilic and hydrophilic drugs (Fox et al., 2011). This enhancement is likely attributed to their interaction with the stratum corneum, leading to increased penetration of lipophilic drugs through an enhanced partition coefficient and hydrophilic drugs through improved diffusion coefficients. Eucalyptus oil has been found to enhance skin permeation by disrupting intracellular lipids within the stratum corneum membrane, as observed by Rajan and Vasudevan in 2012.

The results of Methotrexate permeation, which is a lipophilic drug, support the above observations. The formulation FE3, containing 10% eucalyptus oil, demonstrated a cumulative drug permeation of 806.60 ± 60.25 µg/cm2 over 24 hours, with a flux of 36.70 ± 2.63 µg/cm2/h, representing a 3.64-fold enhancement. The lag time (Tlag) was measured at 0.95 ± 0.36 hours.

Among the penetration enhancers evaluated, the following order of effectiveness was determined: tween-80 > eucalyptus oil > DMSO > olive oil. Transdermal patches FT1 exhibited a significantly higher ($P < 0.05$) cumulative drug permeation of 1020.29 µg/cm2 in 24 hours, with a flux of 43.91 µg/cm2/h, compared to the control formulation F4, which showed a permeation of 217.19 µg/cm2 in 24 hours, with a flux of 10.06 µg/cm2/h. While not as effective as tween-80, eucalyptus oil also displayed notable enhancing effects.

Table:11- *In vitro* permeation studies of Methotrexate from transdermal patchescontaining EC/PVP (3:2) and without enhancer (F4), DMSO 10% (FD3),tween-802%(FT1),eucalyptusoil10%(FE3),oliveoil 5%(FO2)

Time(h)	**Cummulative amount of drug permeated (µg/cm2)**				
	F4	**FD3**	**FT1**	**FE3**	**FO2**
1.	1.22 ± 0.38	3.42 ± 1.67	13.59 ±2.58	6.41 ± 2.13	1.57 ± 0.48
2.	4.47 ± 1.17	14.22 ±3.47	57.43 ±6.97	32.34 ±3.72	3.46 ± 0.83
3.	13.09 ±2.90	40.20 ±6.08	105.21 ±8.77	87.85 ±12.43	13.60 ±2.70
4.	18.96 ±2.35	73.53 ±9.10	147.77 ±16.06	110.32 ±23.61	42.73 ±5.26
5.	27.49 ±4.59	109.02 ±8.68	289.20 ±36.40	143.87 ±22.35	68.09 ±9.29
6.	48.22 ±3.78	141.14 ±20.56	331.58 ±22.47	180.30 ±17.86	121.58 ±31.12
8.	69.26 ±7.90	167.06 ±22.57	426.39 ±32.22	257.31 ±36.20	150.71 ±27.90
10.	93.11 ±13.53	232.83 ±35.26	492.86 ±46.76	314.89 ±30.39	193.91 ±15.04
12.	120.13 ±8.10	295.92 ±29.01	543.80 ±29.69	484.24 ±58.34	251.57 ±44.28

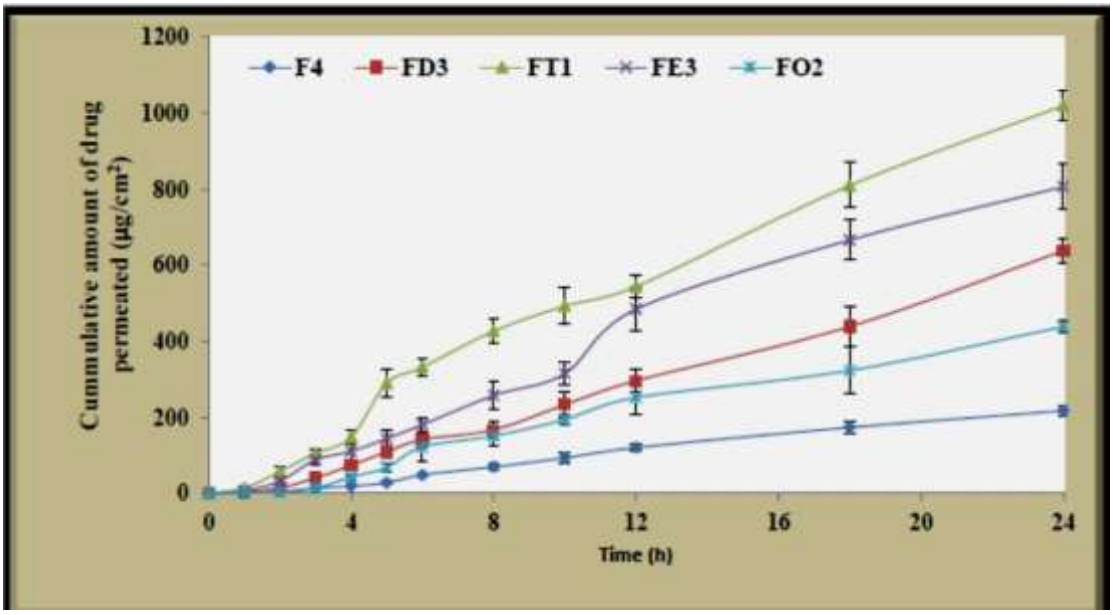


Figure:9-*Invitro* permeation studies of Methotrexate from transdermal patches containing EC/PVP (3:2) and without enhancer (F4), DMSO 10%(FD3),tween-802%(FT1),eucalyptus oil10%(FE3),oliveoil5%(FO2)

3.8 STABILITY STUDIES OF METHOTREXATE PATCH (FT1):

The stability assessment of the optimized formulation (FT1) was conducted in strict adherence to ICH guidelines, involving storage at 40°C and 75% relative humidity over a duration of three months. The findings indicated the absence of any alterations in the physical appearance of the patch over the entire 90-day period. Moreover, the drug content remained remarkably consistent, with recorded values of 97.11%, 96.91%, and 96.84% at the 30, 60, and 90-day intervals, respectively. Crucially, no statistically significant changes ($p > 0.05$) were noted throughout the three-month study. As a result, based on these compelling results, it can be confidently asserted that the optimized Methotrexate transdermal patch (FT1) exhibited outstandingstability.

The highest cumulative percentage of drug release, i.e., 59.66%, was observed in 24 hours from formulation F4 (EC/PVP, 3:2). Consequently, formulation F4 was chosen for the incorporation of permeation enhancers in three different concentrations: 2%, 5%, and 10%, to enhance permeation.

Effect of Dimethyl Sulfoxide (DMSO):

For the formulations containing DMSO as a permeation enhancer, the release rate was found to be directly proportional to the concentration of DMSO. Specifically, drug release percentages were 64.58%, 70.49%, and 78.29% for 2%, 5%, and 10%, respectively, within 24 hours in the transdermal patches. DMSO, being relatively polar in nature with a small and compact structure, likely contributes to the higher release rate (Budhathoki and Thapa, 2005). The results of in vitro drug release studies are presented in Table 10

Effect of Tween-80:

In the case of batch FT, i.e., the formulation containing tween-80 as a permeation enhancer, the highest percentage of drug release (88.72% in 24 hours) was observed with 2% of tween-80 (FT1). This may be attributed to the solubilization effect of tween-80. However, with a further increase in the concentration of tween-80 from 5% (FT2) to 10% (FT3), a decrease in the percentage of drug release was observed from FT2 and FT3, measuring 77.32% and 70.38%, respectively. Tween-80 contributes to achieving the critical micelle concentration (CMC). Concentrations of surfactants above CMC may form drug micelles, which could be challenging to diffuse out from the patch. The higher the amount of surfactant above CMC, the greater the micelles of drug formed, potentially retarding the drug release rate further (Soh et al., 2012; Samy et al., 2013). The in vitro drug release profiles of patches containing tween-80 as a permeation enhancer are outlined in Table 11

Effect of Eucalyptus Oil:

Transdermal patches containing 2% (FE1), 5% (FE2), and 10% (FE3) of eucalyptus oil exhibited 68.58%, 76.83%, and 83.52% drug release in 24 hours, respectively. It was observed that as the concentration of eucalyptus oil increased, the cumulative amount of drug release also increased, as depicted in Table 12 . This may be attributed to the presence of terpene constituents in eucalyptus oil. Eucalyptus oil containing cineol as a principal terpene is likely to increase drug release, probably due to the enhanced saturation solubility of the drug in the matrix (Heard et al., 2006).

4. CONCLUSION

The objective of the present study was to create a transdermal matrix patch for Methotrexate and evaluate its suitability for transdermal administration.Skin's inherent low permeability often presents a barrier to drug delivery, limiting the effectiveness of many medications. To address this issue, the study investigated the impact of permeation enhancers, including DMSO, Tween-80, eucalyptus oil, and olive oil, on the permeation of Methotrexate from the matrix patch through the skin. The formulated patches were then subjected to various tests, including in vitro drug release, in vitro permeation studies, and stability studies.The results of the Methotrexate transdermal matrix patch research highlighted that the most promising formulation was FT1. This particular formulation contained EC: PVP in a ratio of 3:2, Methotrexate at 20%, dibutyl phthalate at 30%, and 2% Tween-80, all expressed as percentages of the total weight. FT1 demonstrated the ability to deliver Methotrexate over a 24-hour period at a flux rate of 43.91 µg/cm2/h across rat skin.In summary, the optimized transdermal matrix patch for Methotrexate, utilizing polymers such as EC and PVP with Tween-80 as a permeation enhancer, showcased its capability to provide sustained release. This was achieved through excellent drug release and permeation characteristics, ultimately impacting the efficacy of Methotrexate as an antidepressant. The development of this Methotrexate formulation is expected to enhance patient compliance, create a more effective dosage regimen, and offer maintenance therapy for patients dealing with cancer. The promising results showed the feasibility of delivering Methotrexate through transdermal matrix patch.

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