List of abbreviations:

Short form	Full form
CAM	Complementary and alternative medicines
RA	Rheumatoid arthritis
HPMC 15 cps	Hydroxy propyl methyl cellulose
PVA	Poly vinyl alcohol
PVP	Poly vinyl pyrolidone
EC	Ethyl cellulose
PG	Propylene glycol
PEG 400	Poly ethylene glycol
w/w	Weight by weight
BA	Boswellic acids
PDA	Photo diode arrar
HPLC	High pressure liquid chromatography
µg	Micro gram
HPTLC	high performance thin layer chromatography
FTIR	Fourier transfer infrared spectroscopy
ATR	Attenuated total reflectance
KBr	Potassium bromide
H_2SO_4	Sulphuric acid
mg	Milligram
ng	nano gram
µl	micro litre
TTS	Transdermal therapeutic system
TDDS	Transdermal drug delivery system
MDTS	Metered dose transdermal system
TDS	Transdermal delivery system
LDL	Low density lipoproteins
HDL	High density lipoproteins
VLDL	Very low density lipoproteins
BSA	Bovine serum albumin
CD	Crohn's disease
UC	Ulcerative colitis
GS	Guggulsterones
TNBS	Tri nitro benzene sulfonic acids
TLC	Thin layer chromatography
ME	methanolic extract
EAF	ethyl acetate fraction
%	Percentage

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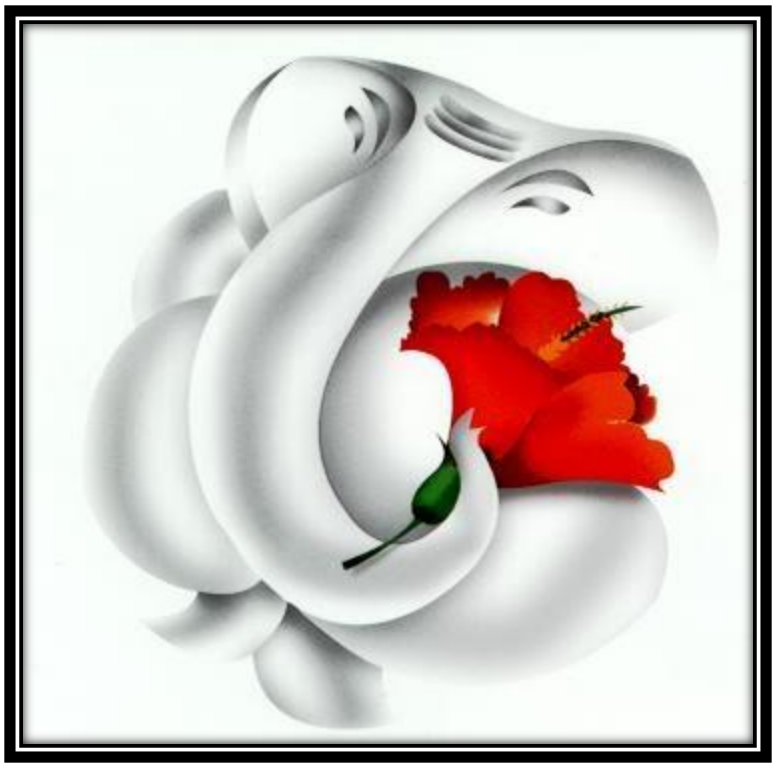
Date: ***DHRUV N. RAVAL***

Place: Institute of Pharmacy,

Nirma University,

Ahmedabad.



DEDICATED TO MY FAMILY, TEACHERS AND FRIENDS

"FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES OF BOSWELLIC ACIDS AND GUGGULSTERONES FOR THE TREATMENT OF RHEUMATOID ARTHRITIS"

A THESIS SUBMITTED TO

NIRMA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

MASTER OF PHARMACY

IN

PHYTOPHARMACEUTICALS AND NATURAL PRODUCTS

BY

DHRUV N. RAVAL
(09MPH506), B. PHARM.

UNDER THE GUIDANCE OF

PROF. (DR.) VIMAL KUMAR

DEPARTMENT OF PHYTOPHARMACEUTICALS AND NATURAL PRODUCTS
INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
AHMEDABAD-382481
GUJARAT, INDIA

APRIL 2011

CERTIFICATE

This is to certify that ***Mr. Dhruv N. Raval*** *has prepared his thesis entitled "Formulation and Evaluation of Transdermal Patches of Boswellic acids and Guggulsterones for the Treatment of Rheumatoid Arthritis", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under my guidance. He has carried out the work at the Department of Phytopharmaceuticals and Natural Products, Institute of Pharmacy, Nirma University.*

Guide:

Dr. Vimal Kumar
M. Pharm., Ph.D.
Professor and Head,
Department of
Phytopharmaceuticals and
Natural Products,
Institute of Pharmacy,
Nirma University

Forwarded Through:

Dr. Manjunath Ghate
M. Pharm., Ph.D.
I/c Director
Institute of Pharmacy,
Nirma University

Date : 29th April, 2011

DECLARATION

I declare that the thesis "Formulation and Evaluation of Transdermal Patches of Boswellic acids and Guggulsterones for the Treatment of Rheumatoid Arthritis" has been prepared by me under the guidance of Dr. Vimal Kumar, Professor and Head, Department of Phytopharmaceuticals and Natural Products, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Mr. Dhruv N. Raval (09MPH506)
Department of Phytopharmaceuticals and Natural Products
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 29th April, 2011

TABLE OF CONTENTS

1. ABSTRACT:

Traditional herbal medicines have great potential, but due to poor understanding of the formulation aspect and bioavailability of the herbal medicines, these medicines are consider into the complementary and alternative medicines (CAM). Various alternative therapies have been used to treat the diseases like diabetes, asthama, arthritis etc.

Rheumatic diseases have affected mankind since ages and are one of the commonest inflammatory conditions in developing countries like India and China. Rheumatoid arthritis (RA) forms a major prototype of rheumatic diseases and it is a common cause of disability.

Boswellic acids and guggulsterones both are very well pharmacologically proved phytoconstituents for the treatment of rheumatoid arthritis. Boswellic acids a natural mixture isolated from oleo gum resin of *Boswellia serrata* comprised of four major pentacyclic triterpene acids: β-boswellic acid (the most abundant), 3-acteyl-β-boswellic acid, 11-keto-β-boswellic acid, and 3-acetyl-11-keto-β-boswellic acid, is reported to be effective as anti-inflammatory, immunomodulatory, antitumor, anti-asthmatic and in chronic colitis disorders. It inhibits pro-inflammatory mediators in the body, specifically leukotrienes via inhibition of 5-lipoxygenase, the key enzyme of leukotriene synthesis, is the scientifically proved mechanism for its anti-inflammatory/anti-arthritic activity.

Guggulsterone [4,17(20)-pregnadiene-3,16-dione] is a plant derived steroid isolated from the gum resin of the *Commiphora mukul* tree, termed guggulipid, extensively used in the Ayurvedic medicine to treat conditions associated with inflammation such as hyperlipidemia, obesity, and arthritis.

In this present study we have isolated total boswellic acids and guggulsterones from *Boswellia serrata* and *Commiphora mukul* respectively, which are very well pharmacologically proved for the treatment of rheumatoid arthritis. Identification of those isolated phytoconstituents was also done by thin layer chromatography.

Initial optimization and evaluations were conducted for the transdermal films and formulation of transdermal films were done by polymer hydroxyl propyl methyl cellulose (HPMC) 15cps (2.5 %), plasticizer polyethelene glycol 400 (PEG400) (30 %) and solvent methanol. Physical evaluations (e.g. tensile strength, thickness, folding endurance and % elongation) were also carried out for these formulations.

For the isolated fraction of the total boswellic acids and guggulsterones the transdermal films were formulated with optimized polymer and plasticizer concentrations and different batches of films were evaluated for their physical parameters.

For estimation of the total boswellic acids from the formulations, we have developed in-house colorimetric method. Calibration curve of total boswellic acids was taken in chloroform using Liebermann-Burchard reagent (cold acetic anhydride + Conc. Sulphuric acid) and linearity was observed between 10µg/ml to 500µg/ml with R square value of 0.998. For *in-vitro* release studies the transdermal systems were administered to the Franz diffusion cells. Diffusion of boswellic acids from the formulations through the membrane (dialysis bag) was also studied using diffusion cells. The results were found satisfactory. After 10 hrs % drug release were found 46.57%, 77.83% and 79.27% of the batches B1, B2 and B3 respectively. For *ex-vivo* studies, we have used rat skin and human cadaver skin. The % drug release after 10 hrs in *ex-vivo* studies in rat skin was found to be 48.36% and 75.97 % in human cadaver skin. Boswellic acids transdermal films were further carried out for the *in-vivo* drug release using male wistar rats. The Cmax was found 125.667 µg/ml in 3 hrs.

Transdermal film formulations of pharmacologically active phytoconstituents like boswellic acids and guggulsterones could be useful to cure arthritis and inflammation effectively in human.

2. INTRODUCTION:

Rheumatic diseases have affected mankind since ages and is a major prototype disease causing disability (Raut et al., 1991). Rheumatoid arthritis (RA) is a chronic inflammatory disorder and is believed to result from an immune reaction (Zvaifler, 1973). RA is both an extravascular immune complex disease and a disorder of cell-mediated immunity leads to chronic inflammation, granuloma formation and joint destruction. (Rang et al., 1999).

The present investigation was aimed to formulate anti-inflammatory transdermal patch by incorporating herbal phytoconstituents like total boswellic acids and guggulsterones. The incorporation of boswellic acids (*Boswellia serrata* Rox.) and guggulsterones (*Commiphora mukul*) in the topical formulations can be useful to the arthritic patients (Singh et al., 2008). Boswellic acids (BA), a mixture comprised of four major pentacyclic triterpene acids: beta-boswellic acid, 3-acteyl beta boswellic acid, 11-keto-beta-boswellic acid and 3-acetyl-11-keto-beta-boswellic acid, isolated from the oleo gum resin of *Boswellia serrata* is reported to be effective as anti-inflammatory (Singh et al., 1996), anti-tumor (Huang et al., 2000), anti-asthmatic (Gupta et al., 1998) and in chronic colitis (Gupta et al., 2001). Its anti-inflammatory activity has been attributed to the inhibition of 5-lipoxygenase in a selective, enzymelinked nonredox and noncompetitive manner (Ammon et al., 1991) (Safyhi et al., 1992). Boswellic acids, which is already proved as a leukotriene inhibitor and is in clinical use as an anti-arthritic agent has shown similar results with topical application as with systemic administration in both acute and chronic models of inflammation (Singh et al., 2008). Guggulsterones is a steroidal phytoconstituent isolated from the gum resin of the plant *Commiphora mukul* and variously used in ayurvedic formulations for hyperlipermia and obesity (Burris et al., 2005). Guggulsterones exerts potent anti-inflammatory effects by suppressing the activation of the transcription factor NF-kappa B in response to different pro-inflammatory mediators including TNFa and IL-1b. (Shishodia & Aggerwal, 2004), (Lee et al., 2008). Both the phytoconstituents Boswellic acids and Guggulsterones were selected on the basis of their anti-arthritic and anti-inflammatory activity.

Transdermal drug delivery system (TDDS) was selected as it has various advantages over the other dosage forms. Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin. Transdermal drug delivery system can deliver certain medication to systemic circulation in a more controlled, convenient and effective way than is possible with conventional dosage form. The transdermal patch dosage form is user-friendly, convenient, painless, and offers multi-day dosing, it generally leads to improved patient compliance (Audet et al., 2001). Consequently, the transdermal therapeutic system is of particular clinical significance for the prevention and long-term treatment of diseases like arthritis.

The aim of the present study was to investigate isolated phytoconstituents transport from a transdermal patch system. For boswellic acids the diffusion as well as the *in-vivo* study has done to check the delivery of the drug from the formulations. We have tried to develop such a correlation with the great potential of traditional system of medicines with the modern novel drug delivery system (NDDS).

2.1 Introduction to Disease (Rheumatoid Arthritis):

Rheumatic diseases have affected mankind since ages and is a major prototype disease causing disability (Rang et al., 1999). Rheumatoid arthritis (RA) is a both extra vascular immune complex disease and disorder of cell-mediated immunity leading to chronic inflammation, granuloma formation and joint destruction. The etiopathogenesis of RA involves diverse and complex factors such as genetic background, rheumatic factor (circulating antibodies), immune complexes, compliment activation, lymphocytes, arachidonic acid metabolites, free oxygen radicals etc. (Nuki et al., 1999) . Rheumatoid arthritis is a long-term disease that leads to inflammation of the joints and surrounding tissues. It can also affect other organs.

2.1.1 Causes, Incidence and Risk Factors

The cause of RA is unknown. It is considered an autoimmune disease. The body's immune system normally fights off foreign substances, like viruses. But in an autoimmune disease, the immune system confuses healthy tissue for foreign substances. As a result, the body attacks itself. RA can occur at any age and especially women are affected more often than men. RA usually affects joints on both sides of the body equally. Wrists, fingers, knees, feet, and ankles are the most commonly affected. The course and the severity of the illness can vary considerably. Infection, genes, and hormones may contribute to the disease.

2.1.2 Symptoms

The disease often begins slowly, with symptoms that are seen in many other illnesses: fatigue, loss of appetite, low fever, swollen glands, weakness, eventually, joint pain appears. Morning stiffness, which lasts more than 1 hour, is common. Joints can even become warm, tender, and stiff when not used for as little as an hour. Joint pain is often felt on both sides of the body. The fingers (but not the fingertips), wrists, elbows, shoulders, hips, knees, ankles, toes, jaw, and neck may be affected. The joints are often swollen and feel warm and boggy (or spongy) to the touch. Over time, joints lose their range of motion and may become deformed.

Other symptoms include, chest pain when taking a breath (pleurisy), eye burning, itching, and discharge, nodules under the skin (usually a sign of more severe disease), numbness, tingling, or burning in the hands and feet, joint destruction may occur within 1 - 2 years after the disease appears.

Currently, synthetic drugs are used in the management of arthritis. The conventional drug treatments of RA consist of analgesic, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) and cortico-steroids. These agents act at various sites in schema of pathogenic mechanism.

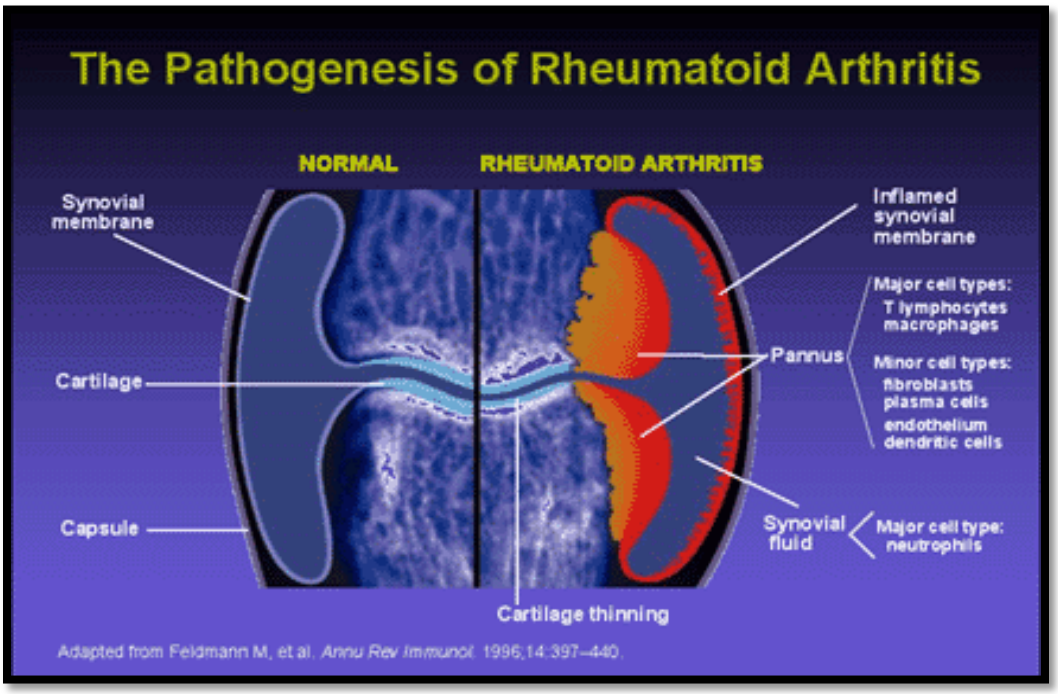


FIG 2.1 PATHOGENESIS OF RHEUMATOID ARTHRITIS

2.2 Transdermal Drug Delivery System (TDDS):

Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin. Transdermal drug delivery system (TDDS) can deliver certain medication to systemic circulation in a more convenient and effective way than is possible with conventional dosage form. TDDS can minimize first-pass metabolism associated with gastro-intestinal administration of drugs. The TDDS can maintain constant drug level in blood. A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication through the skin to treat systemic conditions. Since the early 1980s, this dosage form of transdermal therapeutic system (TTS) has been available commercially. Such a system offers a variety of significant clinical benefits over other systems, such as tablets and injections. For example, it provides controlled release of the drug, and produces a steady blood-level profile, leading to reduced systemic side effects and, sometimes, improved efficacy over other dosage forms (Ranade, 1991). In addition, the transdermal patch dosage form is user-friendly, convenient, painless, and offers multi-day dosing, it generally leads to improved patient compliance (Audet et al., 2001). Consequently, the transdermal therapeutic system is of particular clinical significance for the prevention and long-term treatment of diseases like arthritis.

2.2.1 Types of Transdermal Patches

2.2.1.1 Reservoir/Membrane Transdermal Patches

There are two traditional designs for transdermal patches. With the membrane, or reservoir, system, a membrane that lies between the drug and the skin controls the rate of release from a reservoir. This patch design can provide a true zero-order release pattern to achieve a constant serum drug level.

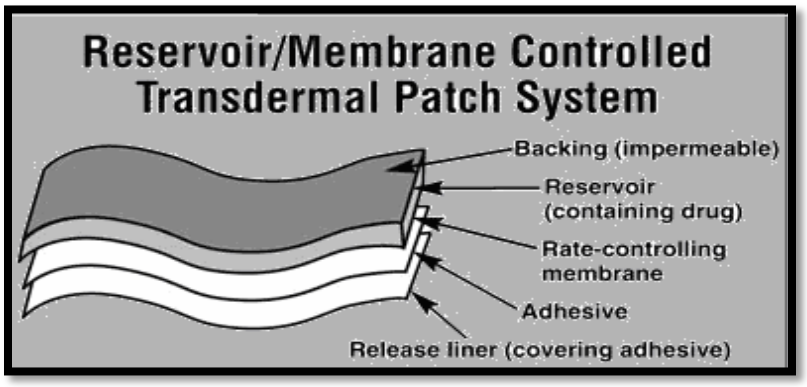


FIG 2.2 RESERVOIR TRANSDERMAL PATCHES

2.2.1.2 Matrix Transdermal Patches

The second type of traditional transdermal patch is the matrix system. The active drug in this type of patch is contained in a polymer matrix. The drug is released at a rate governed by the components in the matrix. In a matrix patch, the drug, adhesive, and polymer matrix are combined. Matrix patches are not designed to provide true zero-order release because as the drug closest to the skin is released, the drug deeper within the patch must travel a longer distance to reach the skin. The longer diffusional path slows the rate of absorption from the patch over time. For most well-designed matrix patches, however, the decrease in release rate is so slight that it does not significantly affect the rate of drug absorption. The first transdermal patches incorporated the reservoir technology, and this type of patch maintains a reasonable share of the market. However, most transdermal patches reaching the market today use the matrix technology. Matrix

patches may be smaller and thinner than their reservoir predecessors due to advances in design. Therefore, these new patches have the benefit of improved patient acceptability (Prochazka, 2000) (Hadgraft, 2001) (Chein, 2005).

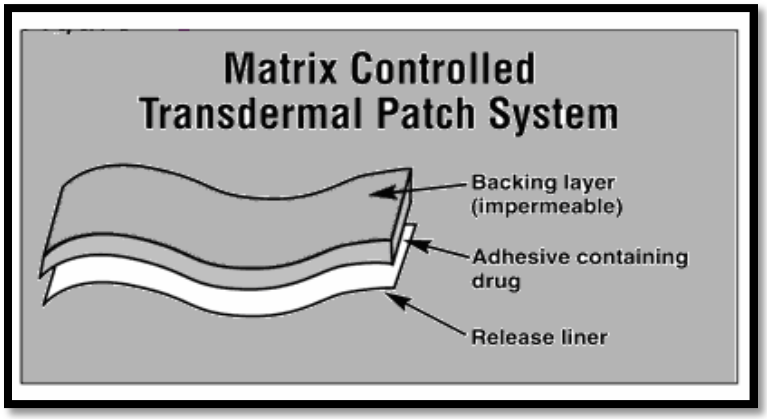


FIG 2.3 MATRIX TRANSDERMAL PATCHES

Transdermal patches are innovative drug delivery systems intended for skin application in view of achieving a systemic effect. Among the different types of systems, the drug-in-adhesive products, in which the drug is included in the adhesive layer contacting the skin, are very commonly used, being thin, conformable and comfortable. More and more efficient systems are introduced into the market, with the advantage of reducing the size of the patch to the size of a stamp (DOT-Matrix_, Novogyne Pharmaceuticals). Transdermal patches are generally occlusive, i.e., they do not allow water to be released from the skin surface, and this is often the reason for skin irritation (Zhai & Maibach, 2002). On the other hand, occlusion generally increases drug transport because it augments the water content of the stratum corneum, although the effect is not the same for different permeants (Treffel et al., 1995). An alternative to water permeable patches (MInghetti et al., 1997) is the use of non-occlusive gels or, more recently, of quick-drying sprays. Metered-dose transdermal systems (MDTS, Acrux Inc., Melbourne, Australia) are constituted of a solution of the drug in volatile and non-volatile solvents. Upon atomization, the volatile solvents evaporate quickly, leaving a concentrated drug solution in the non-volatile component that is taken up into the stratum corneum and

forms a reservoir from which the drug can be released. Transdermal drug delivery systems offer many advantages over conventional dosage forms, which include controlled delivery, improved patient compliance and reduced side effects (Barry, 2004). Transdermal pharmaceutical products--whether ointments, matrix formulations, or reservoir systems--provide the considerable advantages of a noninvasive parenteral route for drug therapy. Rate-controlled transdermal dosage forms can provide, in addition, precise regulation of drug concentrations in plasma and thus a high degree of safety and selectivity of action for some drugs. Another corollary of rate-controlled drug delivery is its capability of lengthening dosage intervals to an unprecedented degree.

Transdermal dosage forms provide a safer and more convenient method of parenteral therapy than, for example, intravenous infusions; yet they retain many advantages associated with drug therapy via the parenteral route. These stem mainly from avoidance of the gastrointestinal tract variables (acidity, motility, enzymatic activity, food intake, and transit time) that frequently make absorption from the gut unpredictable. A first pass through the liver prior to reaching the systemic circulation is also avoided, minimizing drug degradation by that organ. Thus, transdermal systemic drug input can be more reliable than oral dosing, for some drugs, and smaller daily doses of drug may be efficacious. The reliability of transdermal drug administration is dependent on elimination of any variables that can make permeation of drugs through skin itself unpredictable.

2.2.2 Advantages of Transdermal Drug Delivery

Transdermal drug delivery offers several important advantages over more traditional dosage forms. The steady permeation of drug across the skin allows for more consistent serum drug levels, often a goal of therapy. Intravenous infusion also achieves consistent plasma levels, but it is more invasive than transdermal drug delivery. The lack of peaks in plasma concentration can reduce the risk of side effects. Thus, drugs that require relatively consistent plasma levels are very good candidates for transdermal drug delivery. In addition, if toxicity were to develop from a drug administered transdermally, the effects could be limited by removing the patch. Another advantage is convenience,

especially notable in patches that require only once a week. Such a simple dosing regimen can aid in patient adherence to drug therapy. Transdermal drug delivery can be used as an alternative route of administration to accommodate patients who cannot tolerate oral dosage forms. It is of great advantage in patients who are nauseated or unconscious. Drugs that cause gastrointestinal upset can be good candidates for transdermal delivery because this method avoids direct effects on the stomach and intestine. Drugs that are degraded by the enzymes and acids in the gastrointestinal system may also be good targets. First pass metabolism, an additional limitation to oral drug delivery, can be avoided with transdermal administration.

2.2.3 The Course of Transdermal Development

Only in the last decade or so has any major effort been made to utilize the limited permeability of skin for systemic drug administration. Prior to that, drugs were applied topically for localized effects only; any other actions constituted unwanted side effects. Systemic drug administration by the transdermal route first became a part of modern therapeutics in the form of drug-releasing ointments or creams. Preparations of nitroglycerin, etofenamate, and 17fl-estradiol, for example, were found efficacious to varying degrees. Systemic drug absorption from ointments or creams was unpredictable, however, because patients inevitably did not apply the ointments to the skin in a reproducible manner. Variations in area of ointment applications could lead to an undershoot or overshoot of drug input to the circulation. Differing thicknesses of application unpredictably affected duration of drug input, which in most cases were only a few hours. Additional inconveniences were the messiness of applications and the need to cover them to prevent evaporation of drug and stained clothing. Nevertheless, ointment formulations demonstrated that systemic therapy via the transdermal route could be efficacious. For this route to become more practical and reliable, it was necessary that dosage forms provide predictable drug input, with simplified application procedures and regimens. In the 1970s attention was initially paid to the development of such dosage forms. Transdermal dosage forms of defined surface area are now available that deliver drug to the surface of intact skin for periods up to 1 week after application. These dosage

forms provide a preprogrammed rate and duration of drug delivery; the rate is such that the dosage form and not the barrier properties of skin predominantly control or limit systemic drug input. Thus, many of the nuisance features and unreliability of ointment applications for systemic therapy are avoided.

Interest in transdermal drug delivery then fell away until the late 1960s and early 1970s, when many advances were made with transdermal drug delivery devices. In 1981, the first transdermal patch, Transderm-Scop was developed by Alza1 (Mountain View, CA, USA), quickly followed by Transderm-Nitro. During the 1980s, several other transdermal delivery systems (TDSs) were developed, including Nitro-Disc (Searle, Peapack, NJ, USA) and Catapress-TTS. Many other patches were introduced during the 1990s, including Estraderm (CIBA, East Hanover, NJ, USA), Duragesic (Janssen, Titusville, NJ, USA), Testoderm (Alza), Deponit (Wyeth–Ayerst, New York, NY, USA), Nitrocine (Schwartz Pharma, Manheim, Germany), Minitran (3M, Minneapolis,MN, USA) and Nicotine patches. The interest in these devices can be attributed to the many advantages of TDSs2–4. They avoid variables that affect the gastrointestinal absorption of the medication, such as pH, enzymatic activity and drug–food interactions. There are no first-pass effects (drug inactivation by digestive and liver enzymes). Multiday therapy is possible with a single application. They allow the use of low therapeutic index drugs and improve patient compliance. Drug effects can be terminated rapidly. The activity of drugs with short half-lives can be extended.

Most transdermal testing is performed using hairless mouse skin. However, other models are sometimes used including rat, guinea pig, rabbit and shed snake skin, artificial composite membranes, and, more recently, living skin equivalent (Ayman et al., 2000). Although there are many similar features between these models and human cadaver skin, no model has yet been tested that fully mimics the results obtained with human cadaver skin (Panchagnula, 1997).

TABLE 2.1: IDEAL PROPERTIES OF A TRANSDERMAL DRUG DELIVERY SYSTEM	
Properties	Comments
Shelf life	Up to 2 years
Patch size	< 40 cm2
Dose frequency	Once a daily to once a week
Aesthetic appeal	Clear, tan or white color
Packaging	Easy removal of release liner and minimum number of steps required to apply
Skin reaction	Non irritating and nonsensitizing
Release	Consistent pharmacokinetic and pharmacodynamic profiles over time

2.2.4 Physical Evaluations of Transdermal Patches

2.2.4.1 Thickness

Patch thickness should be measured using digital micrometer screw gauge (Mitutoyo, Japan) at three different places and the mean value was calculated.

2.2.4.2 Folding endurance

Folding endurance of patches should be determined by repeatedly folding a small strip of film (2 cm x 2 cm) at the same place till it broke. The number of time the film could be folded at the same place without breaking was the folding endurance value (Tanwar et al., 2007).

2.2.4.3 Tensile strength

The tensile strength should be determined by using a modified pulley system. Weight was gradually increased so as to increase the pulling force till the patch broke. The force required to break the film was consider as a tensile strength and it was calculated as kg/cm^2 (Peh & Wong, 1999).

2.2.4.4 Weight variation

Weight variation should be studied by individually weighing 10 randomly selected patches. Such determination should be performed for each formulation.

2.2.4.5 Drug content

A 5 cm film should be cut into small pieces; the drug content should be determined by suitable validated analytical method.

2.2.4.6 Flatness

Three longitudinal strips should be cut out from each film: one from the center, one from the left side, and one from the right side. The length of each strip should be measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, ith 0% constriction equivalent to 100% flatness (Mukherjee et al., 2005) (Arora & Mukherjee, 2002).

2.2.4.7 In vitro diffusion

The use of TDDS to control drug permeation across the skin has seen extensive research in the past two decades. The permeation rate of the drug across the skin has been measured using several different kinds of *in vitro* skin permeation apparatus. A typical apparatus has three main components (Schaefer, 1996). The first is the donor compartment, where the drug is applied uniformly. From the donor compartment, the drug passes through a permeation barrier or membrane (i.e. skin), which is the second compartment, and into the receptor solution, which is the third compartment. Properties of the receptor solution, such as temperature and buffer composition, can have a significant effect on drug permeation through the skin (Roy, 1994). Typically, physiological saline or a phosphate-buffered solution maintained at 37ºC is used (Setoh, 1995). This will keep the skin surface at approximately 32ºC, which simulates the temperature of the human skin. Generally, antibiotics and preservatives are added to the receptor solution to prevent microbial growth, enzymatic degradation, and to stabilize the skin. Drug permeation across the skin is evaluated using different *in vitro* models. These

include horizontal-type skin permeation system, Franz diffusion cell and the flow-through diffusion cell (Keleb et al., 2010).

2.2.4.7.1 *Franz diffusion cell*

The cell is composed of two compartments: donor and receptor. The receptor compartment has a volume of 5–12 ml and an effective surface area of 1.0–5.0 cm2.The diffusion buffer is continuously stirred at 600 rpm by a magnetic bar. The temperature in the bulk of the solution is maintained by circulating thermostated water through a water jacket that surrounds the receptor compartment (Stinecipher, 1997).

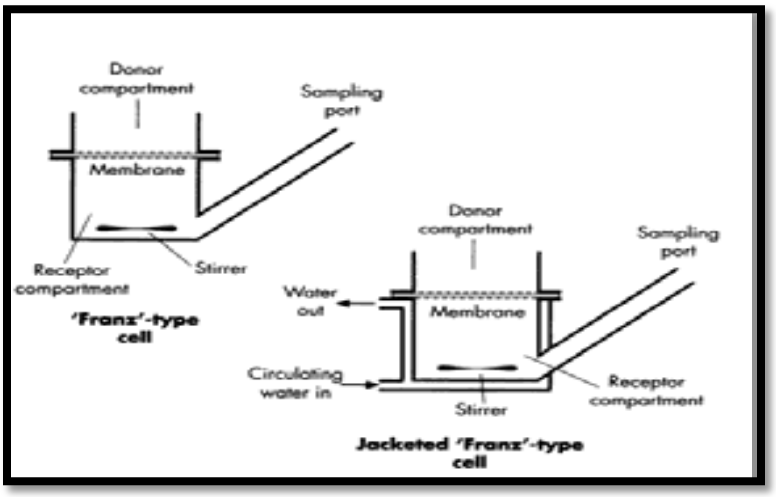


FIG 2.4 FRANZ DIFFUSION CELL

2.3 Introduction to Phytoconstituents and their plants:

2.3.1 Boswellic acids (*Boswellia serrata* Roxb.)

Classification:

Kingdom	**:**	Plantae
Division	**:**	Angiospermae
Class	**:**	Dicotyledoneae
Order	**:**	Geraniales
Family	**:**	Burseraceae
Genus	**:**	*Boswellia*
Species	**:**	*serrata* Roxb

FIG 2.5 BOSWELLIA SERRATA ROXB.

Vernacular names:

English	**:**	Indian Olibanum or Indian frankincense
Hindi	**:**	Kundur, Salai
Bengali	**:**	Kundur, Salai
Gujarati	**:**	Dhup, Gugali
Kannada	**:**	Chitta, Guguladhuph
Malayalam	**:**	Parangi, Saambraan
Tamil	**:**	Parangi, Saambraani
Telugu	**:**	Phirangi, Saambraani
Sanskrit	**:**	Ashvamutri, Kundara, Shallkai

FIG 2.6 GUM OF SALAI GUGGUL

Part used: Gum

Botanical description: It is a deciduous medium sized tree, with ash coloured bark, peeling off in thin flakes, shoots are young and leaves pubescent. Leaves are long, opposite, sessile variable in shape ovate or lanceolate, obtuse flowers in auxiliary racemes, shorter than leaves. Calyx is pubescent outside. Petals are long and ovate. Drupe is tringonous (Prakashanand, 1992).

Geographical distribution: The tree is common at the foot of the Western Himalayas, in Rajasthan, Gujarat, Maharashtra, Madhya Pradesh, Bihar, Orissa, Andhra Pradesh and further south in the peninsular. In many places, the tree forms almost pure forests yielding an abundant supply of timber. Large forests of this tree occur in the khandesh and Nagpur-Wardha divisions in Maharashtra and Khandwa-Nimar division in Madhya Pradesh and Adilabad in Andhra Pradesh.

Traditional uses: The gum-resin is sweet, bitter, astringent, anti-pyretic, anti-dysenteric, expectorant, diaphoretic, diuretic, stomachic, emmenagogue. It is useful in fevers, diaphoresis, convulsions, dysentery, urethrorrhea, orchiopathy, bronchitis, asthma, cough, stomatitis, syphilitic diseases, chronic laryngitis, jaundice and arthritis (Indian Medicinal Plants, A Compendium of 500 species, 1994).

2.3.2 Guggulsterones (*Commiphora mukul*)

Classification:

Kingdom	**:**	Plantae
Division	**:**	Angiospermae
Class	**:**	Eudicots
Order	**:**	Sapinadales
Family	**:**	Burseraceae
Genus	**:**	*Commiphora*
Species	**:**	*mukul*

FIG 2.7 COMMIPHORA MUKUL

Vernacular names:

English	**:**	Indian Bdellium
Hindi	**:**	Guggul
Sanskrit	**:**	Guggulu, Kaushika, Devadhupa, Palankasha
Ayurvedic	**:**	Guggul, Guggal, Guggulu

FIG 2.8 GUM OF GUGGUL

Part used: Gum Resin

Botanical description: *Commiphora mukul* ranges from a woody shrub to a small tree, with spirally ascending branches. Leaves 1-3 foliate, rhomboid to avate in shape, irregularly toothed edges. Flowers are small, from brown to pink in color and are unisexual. Its fruit is red and oval in shape. The tree grows in rocky and rough terrain in warm and semiarid areas of India. It is found on the slopes of hills and foothills. The ash coloured bark comes off in rough flakes exposing the underbark which also peels off in thin papery rolls. The oleoresin from *Commiphora mukul* has been mentioned in the

ancient Indian texts Atharvaveda, and in the early medical texts of Charaka, Sushuta, the Samhitas and Nighantus which are over a thousand years old. Textbooks of Ayurvedic Medicine distinguish between fresh and old varieties of Guggul. (Raghunathan & Mittra, 1982).

Geographical distribution: The tree commonly found in the arid, rocky tracts of Rajasthan, Gujarat and Karnatka in India. A healthy tree yields 250-500 grams of resin in one season, and Guggul plants typically begin yielding resin after five years.

Traditional uses: An ancient Ayurvedic remedy proven to lower cholesterol (lowers bad LDL and raised good HDL cholesterol). Suggested as a protection against heart attacks and strokes. Ayurvedic literature is full of praises for Guggul and its divine actions, from healing bone fractures and inflammations to treating cardiovascular disease, obesity and lipid disorders. Gum Guggul has Carminative, Antispasmodic, Diaphoretic, Antisuppurative, Emmenagogue and Aphrodisiac qualities. In Tibetan medicine, the plant is used for skin diseases, Anemia, Edema, Salivation and heaviness of stomach. Guggul is used for Ulcers, Tonsillitis, Sore throat, hay Fever, Nasal catarrh, Laryngitis and Bronchitis. Gum from the Guggul plant is used in the treatment of Rheumatism, Neurological disorders, Obesity, Syphilis, Urinary disorders and thyroid conditions. Guggul has also been proven helpful for regulating cholesterol levels. The plants lipid lowering properties have been noted among practitioners of Ayurvedic medicine, and modern scientific research is validating these observations. Guggul works to balance conditions of both low and high cholesterol whether brought on by diet, lack of exercise, chronic stress, or genetic predilection. Gum Guggul does not create any of the harmful side effects associated with drugs commonly used for cholesterol disorders (Rastogi & Mehrotra, 1994).

3. LITERATURE REVEIW:

3.1 Literature Review on Boswellic Acids and Guggulsterones:

Singh et al., 2008 showed that Boswellic acids: A leukotriene inhibitor also effective through topical application in inflammatory disorders. Boswellic acids (BA), a natural mixture isolated from oleo gum resin of *Boswellia serrata* comprised of four major pentacyclic triterpene acids: b-boswellic acid (the most abundant), 3-acteyl-b-boswellic acid, 11 keto-b-boswellic acid, and 3-acetyl-11-keto-b-boswellic acid, is reported to be effective as anti inflammatory, immunomodulatory, antitumor, anti-asthmatic and in chron's disease. It inhibits pro inflammatory mediators in the body, specifically leukotrienes via inhibition of 5-lipoxygenase, the key enzyme of leukotriene synthesis, is the scientifically proved mechanism for its anti-inflammatory/anti arthritic activity. All previous work on BA for its biological activity has been done through the systemic application but no pre-clinical data reported for its anti-inflammatory activity by topical application.

Gupta et al., 1986 reported anti-arthritic activity of boswellic acids in bovine serum albumin (bsa)-induced Arthritis. The effect of boswellic acids on bovine serum albumin (BSA)-induced arthritis in rabbits was studied. Oral administration of boswellie acids (25, 50 and 100 mg/kg/day) significantly reduced the population of leucocytes in a BSA-injected knee and changed the electrophoretic pattern of the synovial fluid proteins. The local injection of boswellic acids (5, 10 and 20 mg) into the knee 15 min prior to BSA challenge also significantly reduced the infiltration of leucocytes into the knee joint, reduced the infiltration of leucocytes into the pleural cavity and inhibited the migration of PMN *in vitro*. The leucocyte-inhibitory activity of boswellic acids was not due to its cytotoxic effect. Sharma et al., 1989 report that the boswellic acids do not show any detergent or surfactant properties.

Atal, et al., 1980 tested anti-inflammatory and anti-arthritic activities against carrageenan-induced paw edema adjuvant arthritis in rats defatted alcoholic extract of salai guggul treatment caused inhibition of the carrageenan induced rat hind paw oedema by 39 - 75% and 65 - 73%, administered orally (p.o.) in dose ranges of 50-200 mg per kg^{-1} and intraperitoneal (i.p.) in dose range of 50 -100 mg per kg^{-1} respectively, compared to 47% inhibition seen with phenylbutazone (50 mg/kg^{-1} p.o.). The anti-inflammatory effect was equally well marked in adrenalectomized rats. In the anti-arthritic study on the mycobacterial adjuvant-induced poly-arthritis in rats, salai guggal showed 34% and 49% inhibition of paw swelling with 50 and 100 mg per kg^{-1} (p.o.) doses respectively as compared to controls. Phenylbutazone in doses of 50 & 100 mg per kg^{-1} (p.o) showed 26% and 60% inhibition respectively. The P values in the above finding were less than 0.01-0.001

Singh et al., 1984 reported the anti-inflammatory activity of mixture of Boswellic acid (Composed of 5 acids with a Boswellic acid as the major component) in the dose range of 25-200 mg per kg. In acute carragenin tests, it showed 27-46% inhibition of paw oedema in rats and mice. In chronic test of formaldehyde arthritis it exhibited 45- 67% anti-arthritic activity in a similar dose range. The fraction was effective in both adjuvant arthritis (35-59%) as well as established arthritis (54 84%). It also showed antipyretic effect, with no ulcerogenic effect and well tolerated in as high a dose as 2 gm/kg p.o. mice.

Atal et al., 1984 showed that salai guggal (500-200 mg/kg p.o.) caused dose related (39-72%) inhibition in carrageenin and dextran induced hind paw oedema in rats. The effect was equally well marked in adrenalectomized rats. In a dose of 100 mg/kg p.o. in mice it reduced oedema induced by carrageenin, formaldehyde, dextran, serotonin and histamine by 42.85, 49.42, 40.42, 29.26 and 20.83% respectively. In a dose range of 50-200 mg/kg p.o. reduced the foot swelling by 34.89% to 58.38% in mycobacterium induced polyarthritis in rats while phenylbutazone in 50 & 100 mg/kg p.o. doses showed 26.17 and 59.73% inhibition respectively.

Boswellic acids in a dose range of 50-200 mg/kg on oral administration produces significant dose related anti-inflammatory activity (25.71-64.28%) in carrageenin, histamine and dextran induced oedema tests in rats and mice (Pachnanda, 1981). The activity was found to be similar in adrenaectomised rats. In formaldehyde arthritis its activity was found to be 52.27 to 81.88% (P-value < 0.05 to < 0.01). Boswellic acids displayed marked anti-arthritic effect in both developing and established adjuvant arthritis (41.32 to 79.82%) with P- value < 0.001. Marked anti-arthritic activity (41.94-68.02%) with Boswellic acids was also reported in Sodium monourate-induced gouty arthritis in dogs in a dose range of 100-300 mg/kg. Boswellic acids inhibited the arthritis, increased values of total leucocyte counts, serum transaminase levels, E.S.R. in these test models.

Sharma, 1984 conducted clinical trials of 175 patients for boswellic acids. The patients were suffering with musculoskeletal rheumatism inducing rheumatoid arthritis and ankylosing spondylitis of moderate to severe type 1-6 years duration in the age group of 10 to 50 years of either sex and has undergone treatment at various centres with all the available antirheumatic drugs. 122 patients out of 175 patients who were either bed ridden incapacitated from doing normal work and suffered from morning stiffness, showed abatement of symptoms in 2-4 weeks of initiation of treatment. 17 of these 122 patients when put on placebo treatment showed recurrence of symptoms within 10 days. Of the rest 53 cases, 35 showed good results and the other 18 had no appreciable improvement within a week after starting treatment. None of these patients complained of any undesirable side effects.

Four weeks toxicity study of Boswellic acids in rats in doses of 500 and 100 mg/kg orally and histology revealed no significant change in haematological and biochemical parameters and histology of vital organs (Atal, 1982). Acute oral and i.p. LD50 was greater than 2 g/kg. Chronic toxicity studies were conducted in 16 normal healthy monkeys divided in four groups. Each group comprised of two male and two female monkeys. Their body weight was recorded before and at monthly intervals after drug administration. Haematological and biochemical estimations were done prior to drug

administration and at monthly intervals after drug treatment. Defatted alcoholic extract of salai guggul was administered orally in three dose levels to three groups i.e., low dose of 2 X ED50, medium dose of 5 X ED50 and high dose of 10 X ED50 for six months and one group served as a control. All the animals were maintained under uniform husbandry conditions throughout the experiment. Biochemical haematological, histopathological, and other observations revealed no toxicity.

Gum contains different sugars like D-galactose, D-arabinose, D-xylose and Dmannose. It also contains volatile oil and uronic acids (Pardhy & Bhattacharya, 1978). A range of triterpene acids has been isolated viz. b Boswellic acids, Acetyl-b-boswellic acid, keto-b-boswellic acid and acetylketo-b-boswellic acid, which are responsible for its medicinal value (Graham, 1969).

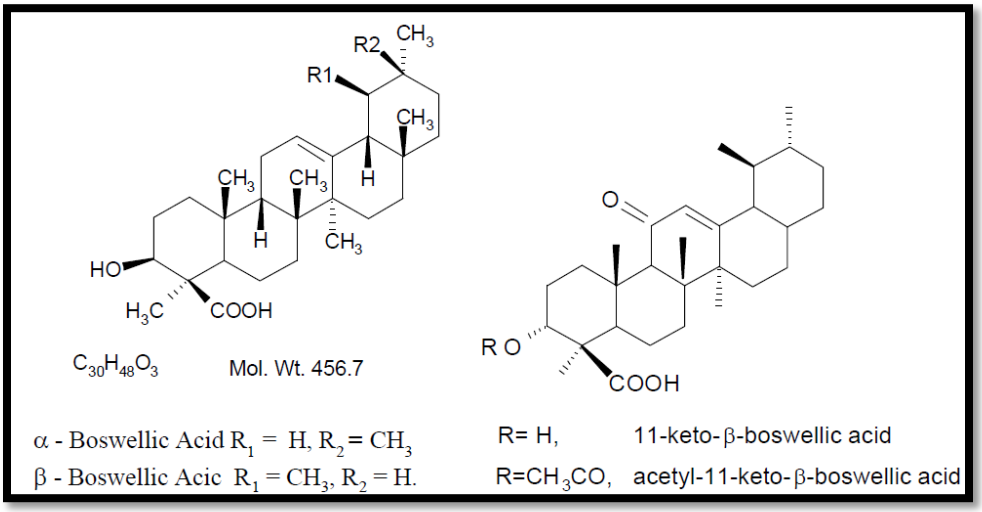


FIG 3.1 BOSWELLIC ACIDS

Mencarelli et al., 2009 reported that the plant sterol guggulsterone attenuates inflammation and immune dysfunction in murine models of inflammatory bowel disease. Inflammatory bowel diseases (IBD) are chronic inflammatory and relapsing diseases of the gut that may manifest as either Crohn's disease (CD) or ulcerative colitis (UC). CD

and UC are immunologically different diseases characterized by exacerbated Th1 and Th2 response. T-cell resistance against apoptosis contributes to inappropriate T-cell accumulation and the perpetuation of chronic mucosal inflammation. In this present study author investigated the effect exerted by guggulsterone (GS) a plant derived steroid isolated from the gum resin of the *Commiphora mukul* tree, in two models of intestinal inflammation induced in mice by trinitro-benzene sulfonic acid (TNBS) and oxazolone. Author provided evidence that E-GS protects mice against development of sign and symptoms of colon inflammation. EGS effectively attenuated the severity of wasting disease and the fecal score and colon inflammation as assessed by measuring the macroscopic- and microscopic-damage scores. Administration Z-GS failed to ameliorate colon inflammation in TNBS-induced colitis and had a partial effect in oxazolone-induced colitis. In vitro, mechanistic studies carried out using CD4+ cells isolated from the intestinal lamina propria demonstrate that GS effectively regulates the function of effector T cells by modulating cell signaling activation pathway caused by CD3/CD28. The net biological effects resulting from exposure to GS includes attenuation of generation of interleukin-2 and -4 and interferon-g as well as T cell proliferation. In conclusion, GS is an anti-inflammatory compound with the capacity to prevent and ameliorate T-cell-induced colitis. These data ground the use of GS, a natural cholesterol lowering agent, in the treatment of chronic inflammatory diseases.

Agrawal et al., 2004 reported HPTLC method for guggulsterone. I) Quantitative determination of E- and Z guggulsterone in herbal extract and pharmaceutical dosage form. A sensitive, selective, precise and robust high-performance thin-layer chromatographic method of analysis of E and Z stereoisomers of guggulsterone (the hypolipidemic agent in the gum-resin exudates of *Commiphora mukul*) both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene–acetone (9:1, v/v). Densitometric analysis of guggulsterone was carried out in the absorbance mode at 250 nm. This system was found to give compact spots for E- and Z-guggulsterone (*R*f value of 0.38 ± 0.02 and 0.46 ± 0.02, respectively) following double development of chromatoplates with the same mobile

phase. The linear regression analysis data for the calibration plots for E- and Z-guggulsterone showed good linear relationship with r^2 = 0.9977 ± 0.054 and 0.9975 ± 0.068, respectively, in the concentration range of 100–6000 ng/spot. The mean value of slope and intercept were 0.11 ± 0.006 and 0.11 ± 0.005, 14.26 ± 0.56 and 10.92 ± 0.76, respectively, for E- and Z-guggulsterone. The method was validated for precision, robustness and recovery. The limit of detection and quantitation were 12, 10 and 24, 20 ng/spot, respectively, for E- and Z-guggulsterone. Statistical analysis proves that the method is repeatable and selective for the estimation of the said drug. Since the proposed mobile phase effectively resolves the E- and Z-isomers of guggulsterone, this HPTLC method can be applied for identification and quantitation of these isomers in herbal extracts and pharmaceutical dosage form.

Srivastav et al., 1991 reported that the ethyl acetate extract of *Commiphora mukul* significantly protect the albino rats against the development of experimental atherosclerosis. The drug not only prevented deteriorating changes in serum cholesterol, triglycerides and plasma fibrinogen level but also favorably increase plasma fibrinolytic activity. The oleoresin fraction of guggul possesses significant anti arthritic and anti inflammatory activities. The minimum effective dose being 12.5 mg/ 100 gm body wt. (Shanthakumari et al., 1964). The crude aqueous extract of the oleo gum resin was found to suppress acute rat-paw edema induced by carrageenin. Gum guggul also had a suppressive action against the granuloma pouch test. In adjuvant arthritis, the extract suppressed the secondary lesions very effectively without having any significant action on the primary phase. The side effects such as gastric ulceration, loss of weight and mortality were negligible in the animals treated with the extract as compared to those treated with Betamethasone (Satyavati et al., 1969).

Clinical trial with purified Guggul (*Commiphora mukul*) has been carried out in 35 patients of rheumatoid arthritis in order to assess its antirheumatic activity, dose requirement, resistance development, side effects, and effects on hematology (ESR).

From the results obtained it has been indicated that Guggul acts as a digestive and analgesic agent without the toxic or side effects (Vyas & Shukla, 1987).

Verma & Bordia, 1988 were carried out clinical trials on twenty patients of Hyperlipidemia. The patients were administered 4.5 g. of purified gum Guggul in two divided doses daily for 16 weeks. Serum cholesterol and serum triglyceride levels decreased at the end of the 4th to 8th weeks. HDL cholesterol showed a gradual increase while VLDL and LDL cholesterol showed significant decrease at all time points. This shows the potent anti hyperlipidemic activity of the gum guggul.

The oleoresin contains 0.37% essential oil containing mainly Myrecene, Dimyrecene, and Polymyrecene. Alcohol extraction gives a soluble resin and an insoluble carbohydrate gum. Solvent extraction, hydrolysis and column chromatography over silica gel of Guggul resin identifies a number of compounds - a diterpene hydrocarbon, a diterpene alcohol, Z-Guggulsteron, E-Guggulsterone, Guggulsterol-I, Guggulsterol-II and Guggul sterol-III. Cholesterol, sesamin and camphorene are also found. The anti-inflammatory and Hypolipidemic fractions have been isolated. Guggulsterono VI and Z-Guggulsterone isolated from gum resin along with 20a-hydroxy-4-preg nen-3-one, 20b hydroxy-4-pregnen-3-one, 16b-hydroxy 4,17 (20oz) - pregnadien3-one (Bajaj & Dev, 1981). Ketone fraction that is extracted from the resin contains the most potent cholesterol lowering components. This is composed of C21 or C27 steroids, with the major components being Z- and E-guggulsterone. Guggul contains resin, volatile oils, and gum. The extract isolates ketonic steroid compounds known as guggulsterones. These compounds have been shown to provide the lipid-lowering and anti inflammatory actions noted for guggul (Bajaj & Dev, 1982).

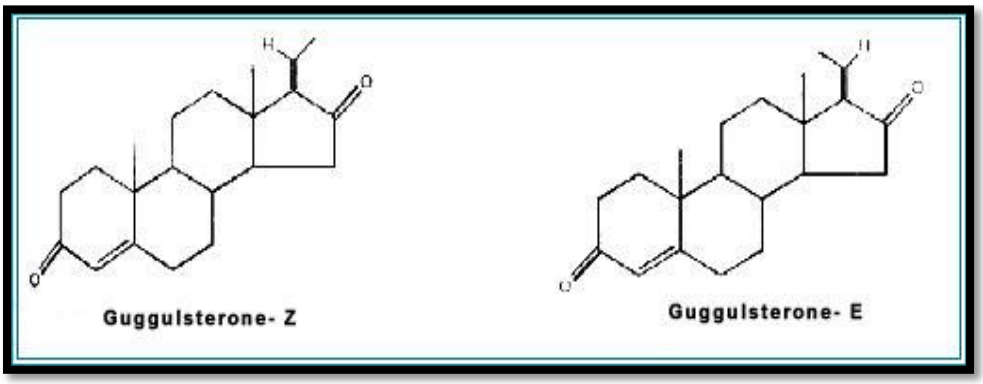


FIG 3.2 GUGGULSTERONES E AND Z

3.2 Literature Review on Polymer and Formulation:

Suruse at al., 2009 carried out study on formulation and development of sustained release anti inflammatory transdermal pad using herbal extracts. The investigation was aimed to formulate anti-inflammatory transdermal pad by incorporating herbal extracts. The incorporation of herbal drugs such as boswellic acid (*Boswellia serrata* Rox.), shivlingi extract (*Bryonia laciniosa* Linn.), guggul extract (*Commiphora mukul* Hook.) and isolated compounds from raladhupa namely CS1 and CS2 (*Canarium strictum* Rox.) were envisaged. The drugs were selected on the basis of their synergistic action in suppressing inflammation. The anti-inflammatory transdermal pads were evaluated for their physical properties like thickness of film, moisture absorption and diffusion studies across shaved rat skin. The qualitative drug release of each constituents of formulation on TLC plate indicates drug release occurred at a constant rate. The skin irritation study on albino rabbit skin showed that the formulation does not produce any irritation. From the results it has been showed that transdermal pads can be effective for the inflammation.

Verma et al., 2000 developed transdermal drug dosage formulation for the Anti-rheumatic ayurvedic medicinal plants. The investigation was aimed to formulate transdermal films incorporating herbal drug components. The allopathic system of

medicine includes two conventional lines of treatment for rheumatoid arthritis, which come along with certain side effects. Hence, turning to safe, effective and time-tested ayurvedic herbal drug formulation would be a preferable option. With this view transdermal films incorporating herbal drug components such as boswellic acid (*Boswellia serrata) a*nd curcumin (*Curcuma longa)* was envisaged. The drugs were selected on the basis that they produce synergistic action in suppressing inflammation and are proved time tested and safe drug. The polymeric films were evaluated for their physical properties like percentage flatness, thickness uniformity, and drug content and diffusion studies across hairless mouse skin. The average per cent release of curcumin was found to be 50.70% and 69.84% for boswellic acid (peak 1) and 64.84% for boswellic acid (peak 2) in the hydroalcoholic diffusion medium at the end of 9 hrs. The graphs obtained for the average per cent release through transdermal film indicate drug release occurred at a constant rate. The skin irritation study done on albino rabbit skin showed that the formulation does not produce irritation to the skin. Overall, it is observed that the well-known ayurvedic drugs have been found to be effective through modern pharmaceutical formulation techniques.

One practicable attitude to minimize the device associated adverse skin reactions of transdermal therapeutic systems is to employee highly biocompatible polymers for their fabrication (Thakarodi & Rao, 1995). Polymer should provide consistent, effective delivery of a drug throughout the product's intended shelf life or delivery period and have generallyrecognized- as-saf status (Davis & Illum, 1998).

Polymers are the backbone of a transdermal drug delivery system. Systems for transdermal 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 that prevents the loss of drug through the backing surface and an inner polymeric layer that functions as an adhesive and/or rate-controlling membrane (Kandavilli et al., 2002).

Ethyl cellulose (EC) and polyvinylpyrrolidone (PVP) matrix films with 30% dibutyl phthalate as a plasticizer have been fabricated to deliver diltiazem hydrochloride and indomethacin. The addition of hydrophilic components such as PVP to an insoluble film former such as ethyl cellulose tends to enhance its release-rate constants. This outcome can be attributed to the leaching of the soluble component, which leads to the formation of pores and thus a decrease in the mean diffusion path length of drug molecules to release into the dissolution medium. The result is higher dissolution rates. Substances such as PVP act as antinucleating agents that retard the crystallization of a drug. Thus they play a significant role in improving the solubility of a drug in the matrix by sustaining the drug in an amorphous form so that it undergoes rapid solubilization by penetration of the dissolution medium (Ramarao & Diwan, 1998).

Hydroxypropyl methylcellulose (HPMC). A hydrophilic swellable polymer widely used in oral controlled drug delivery, also has been explored as a matrix former in the design of patches of propranolol hydrochloride. HPMC has been shown to yield clear films because of the adequate solubility of the drug in the polymer. Matrices of HPMC without rate-controlling membranes exhibited a burst effect during dissolution testing because the polymer was hydrated easily and swelled, leading to the fast release of the drug (Guyot & Fawaz, 2000).

Formulation and *in-vitro* characterization of monolithic matrix transdermal systems using HPMC/Eudragit S100 polymer blends. Monolithic matrix transdermal systems containing tramadol HCl were prepared using various ratios of the polymer blends of hydroxy propyl methyl cellulose (HPMC) and Eudragit S 100 (ES) with triethyl citrate as a plasticizer. A 3^2 full factorial design was employed. The concentration of HPMC and ES were used as independent variables, while percentage drug release was selected as dependent variable. Physical evaluation was performed such as moisture content, moisture uptake, tensile strength, flatness and folding endurance. *In vitro* diffusion studies were performed using cellulose acetate membrane (pore size 0.45 μ) in a Franz's diffusion cell. The concentration of diffused drug was measured using UV-visible spectrophotometer (Jasco V-530) at λ max 272 nm. The experimental results shows that

the transdermal drug delivery system (TDDS) containing ES in higher proportion gives sustained the release of drug (Garala et al., 2009).

A comparison of test methods for determining *in vitro* drug release from transdermal delivery dosage forms. Three test methods for determining *in vitro* drug release rate from transdermal delivery dosage forms were tested for equivalency of results, ease of implementation and precision. The 'paddle-over-disk' (POD) method is under consideration by the USP as a standarized method for release-rate testing of all transdermal delivery dosage forms. The 'reciprocating disk' (RD) and 'diffusion cell' (DC) methods are both commonly employed throughout the pharmaceutical industry. The three methods were demonstrated to be equivalent in terms of release rate profile (curve shape) and total drug released over the lifetime of the dosage form tested (Transderm-Scop). The precision for the RD method as measured by the mean relative standard deviation over all time points was 4.6%; the precision of the POD method was 5.4% and that for the DC method was 6.7%. Steady-state flux values derived from the POD and RD methods were equivalent (~4 µg cm $^{-2}$ h^{-1}) but were ~25% greater than the steady-state flux value derived from the DC method (--3 µg cm $^{-2}$ h^{-l}). All three methods gave results which were within the specifications of the manufacturer (CIBA-GEIGY). The POD method was the easiest to use on a routine basis, required the least amount of specialized equipment and most resembled the current test methodology for dissolution testing of other dosage forms such as tablets or capsules (Mazzo et al., 1986).

4. AIM AND OBJECTIVE:

Traditional herbal medicines have great potential. Many traditional medicines are used for the treatment of many vital diseases. One of the major draw back of traditional system is poor understanding of the formulation aspects. In this present study we have tried to incorporate the traditional medicines in to the novel drug delivery system to check the *in-vitro*, *ex-vivo* and *in-vivo* release of the formulation. Transdermal drug delivery system is the novel approach of the drug delivery system.

Our major aim and objective of the present study was

- To isolate the phytoconstituents (boswellic acids and guggulsterones) from the *Boswellia serrata* (Salai guggul) and *Commiphora mukul* (Guggul) respectively. These phytoconstituents are very well reported as an anti arthritic and anti inflammatory agents. Hence both the phytoconstituents had been selected for the formulation of the transdermal films.
- Optimization of the effective and stable transdermal films.
- To formulate and evaluate topical formulations (transdermal film) using isolated boswellic acids & guggulsterones.
- To compare those formulations by their diffusion profiles.
- To check the diffusion of the drug from the formulations by *in-vitro* and *ex-vivo* studies.
- To check the *in-vivo* drug release of the phytoconstituent in rats.

5. MATERIALS AND METHODS:

5.1 Materials And Equipments

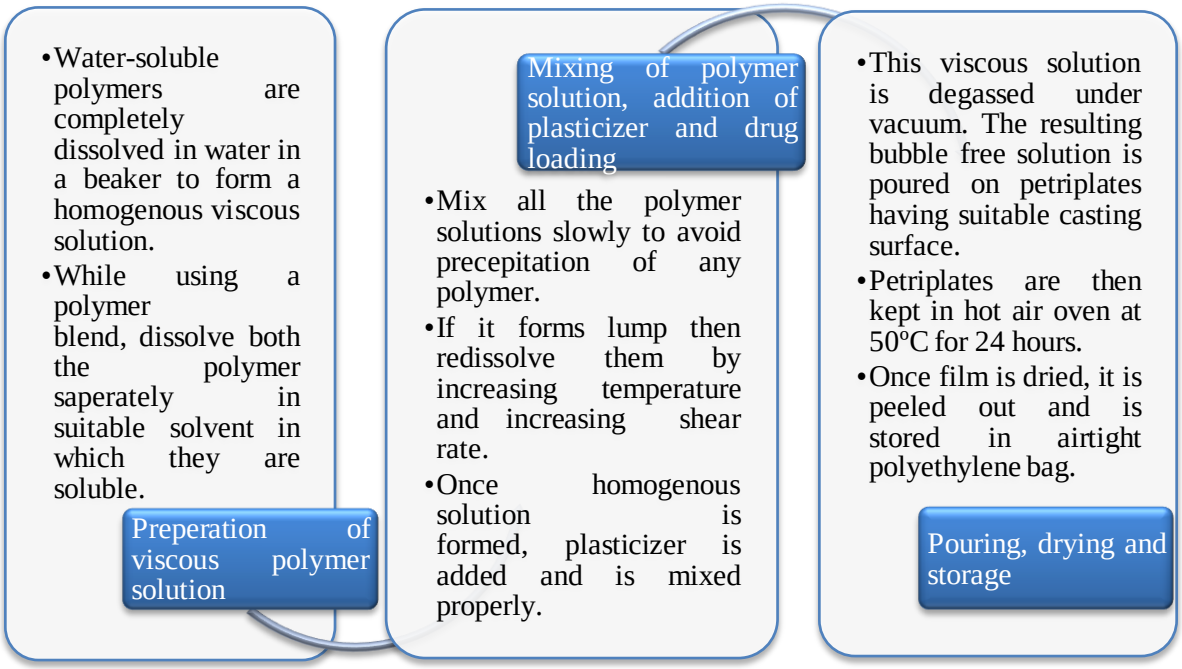
TABLE 5.1 MATERIALS AND EQUIPMENTS		
Materials		**Company name**
Crude drugs	Gum of *Boswellia serreta and* Gum resin of *Commiphora mukul*	L.V.G., Ahmedabad.
Polymer	HPMC (15 cps)	Colorcon Asia Pvt. Ltd
	HPMC (50 cps)	S.D.Fine-Chem Ltd
	Polyvinyl alcohol (PVA)	HIMEDIA Laboratory Pvt. Ltd
	Ethyl cellulose	S.D.Fine-Chem Ltd
Plasticizer	Polyethylene glycol (PEG 400)	HIMEDIA Laboratory Pvt. Ltd
	Propylene glycol	HIMEDIA Laboratory Pvt. Ltd
	Glycerol	CDH Pvt. Ltd
Solvent	Ethanol (70%)	CDH Pvt. Ltd
	Methanol	CDH Pvt. Ltd
	Chloroform	CDH Pvt. Ltd

TABLE 5.2 LIST OF EQUIPMENTS	
Equipments	**Manufacturing**
Digital Tensiometer,	EIE Instruments, Ahmedabad
Micrometer screw gauge	Durga Instruments, Baroda
Franz diffusion cell	Jencons Ltd
Magnetic stirrer with hot plate	EIE Instrument Pvt Ltd
Sonicator Bath	Trans-o-Sonic D-Compact
Digital pH meter	Elico LI 612 and ANALAB
FTIR spectrophotometer	Jasco Corporation Ltd
UV/Visible spectrophotometer	UV 2450 Shimadzu scientific instrument, Japan
Electronic Balance	BL-220H, Shimadzu corporation

5.2 Formulation of Transdermal Patches:

5.2.1 Method of Preparation of Transdermal Patches

Solvent casting method



5.2.2 Evaluation Parameters of Transdermal Patches

TABLE 5.3: EVALUATION PARAMETERS OF TRANSDERMAL PATCHES

Sr. No.	Evaluation parameters
1	Physical appearance
2	Thickness
3	Folding Endurance
4	Tensile Strength
5	Percentage elongation at break
6	Content Uniformity
7	Drug Release Study

1) Physical appearance:

The films were observed visually for their physical appearance such as colour and transparency.

2) Thickness

Thickness of the patches was measured using micrometer at different places. The average film thickness and standard deviation were computed.

3) Folding endurance

Folding endurance of the film was determined by repeatedly folding one patch at the same place till it broke or folded manually, which was considered satisfactory to reveal good film properties. The number of times of film could be folded at the same place without breaking gave the value of the folding endurance. This test was done for three films.

4) Tensile Strength and percentage elongation at break

The mechanical properties of films were evaluated using a Digital Tensiometer, (Jainson). Film strip in 2 cm X 6 cm of dimension and free from air bubbles or physical imperfections, was held between two clamps positioned at a distance of 5.5cm. During measurement, the film was pulled by top clamp at a rate of 20 mm/minutes. The force and elongation were measured when the films broke. Measurements were run three times for each film. The tensile strength and elongation at break were calculated as below:

$$\text{Tensile strength } (N/cm^2) = \frac{\text{Force at break (kg)}}{\text{Cross sectional area of sample } (cm^2)} \times 9.8$$

$$\text{Elongation at break } (\%) = \frac{\text{Increase in length at breaking point (cm)}}{\text{Original length (cm)}} \times 100\%$$

5) Content Uniformity

Drug content uniformity was determined by colorimetry for boswellic acids transdermal patches and by HPTLC for guggulsterones transdermal patches. For boswellic acids transdermal patches the patch (1 cm × 1 cm) was dissolved in 10 ml of chloroform and filtering with Whatman filter paper (0.45 µm). The stock solution was diluted with chloroform and the Liebremann-Burchard reagent was added to the final diluted solution, the solution was kept on waterbath for 30 min at 50º C to develop the colour and the intensity of the colour was analyzed at 458 nm using a UV spectrophotometer. For guggulsterones transdermal patches the content uniformity was determined by HPTLC method. The patch of 1 cm x 1 cm was dissolved in 10 ml of chloroform and filtering with Whatman filter paper (0.45 µm). From the stock solution the 10 µl of solution was kept on precoated silica tlc plate and was analysed by HPTLC method. The experiments were performed in triplicate, and average values were reported.

$$\text{Content Uniformity} = \frac{\text{Actual amount of drug in film}}{\text{Theoretical amount of drug present in film}} \times 100$$

6) Drug Release study

Transdermal patch measuring preferred area was subjected to *in vitro* diffusion testing using Franz diffusion cell. Suitably prepared membrane for *in vitro* diffusion was clamped between the donor and receptor compartments and the patch was placed over the skin. The receptor compartment contained phosphate buffer (pH 7.4) at 37^{o} C ± 1^{o}C. The medium was magnetically stirred and the amount of drugs diffusing into the receptor compartment across the membrane were determined by withdrawing 3 ml samples over the duration of experiment and an equivalent amount of diffusion medium was added to the receptor compartment to maintain a constant volume (Bhalla & Bhate, 1994). The 3 ml of samples were again extracted with the chloroform and the chloroform portion was further taken for the estimation. Drug content was determined using respective standard calibration curve. The cumulative amount of drug permeating through the membrane was then calculated and average per cent release and flux values were determined.

5.2.3 Optimization of Formulation

Drug-free films containing different proportions of polymers Ethyl cellulose (EC), Hydroxy propyl methyl cellulose (HPMC 15 cps), Hydroxy propyl methyl cellulose (HPMC 50 cps), Poly vinyl alcohol but constant proportion of plasticizer PEG 400 were prepared. Prepared films were taken for the physical evaluations. After physical evaluations films having polymer HPMC 15 cps were selected and optimization of plasticizer were done by taking different proportions of glycerine, propylene glycol (PG) and polyethylene glycol 400 (PEG 400). From the physical evaluations the polymer and plasticizer were selected with solvent which gave the transparent and stable film.

5.2.4 Formulation and Characterization of Transdermal Patches with Optimized Concentration of Excipients

From the optimization of the concentration of polymer and plasticizer was decided. The solvent was also selected. Further transdermal films of isolated phytoconstituents were made with optimized concentrations of excipients and characterizations were also done of those batches.

Gum of salai guggul (*Boswellia serrata* Roxb.) and gum of guggul (*Commiphora mukul*) were procured from L.V.G., Ahmedabad and authenticated by Dr. B.L. Punjani, senior botanist, S.M. Panchal science college, Talod.

5.3 Boswellic acids Transdermal Patches:

5.3.1 Isolation of Total Boswellic acids

The fraction containing BAs was prepared by extracting *B. serrata* gum resin (100 g) successively with methanol in a percolator and evaporated under reduced pressure on a thin film evaporator at 40 ºC to obtain a thick brown residue. The total extract was stirred

with 3 % potassium hydroxide (KOH) till it form the uniform emulsion. The emulsion was extracted with the successive qts of chloroform. Combined chloroform layer was then treated with the dilute acid to form precipitates. The precipitates were dried in a vacuum oven at temperature below 50 ºC to yield 30 g creamish powder of Bas. This mixture was used for further studies.

5.3.2 Identification of Isolated Boswellic acids

5.3.2.1 Comparative thin layer chromatographic profile of methanolic extract of *B.serrata* and isolated total Boswellic acids

Test samples of methanolic extract and isolated total boswellic acids were prepared in methanol.

Stationary phase	:	Silica gel G
Mobile phase	:	Hexane : Ethyl acetate (7 : 3)
Derivatization	:	10% H_2SO_4 solution

5.3.2.2 FTIR Spectra of isolated fraction of total Boswellic acids

A IR Prestige-21 FTIR (Shimadzu, Japan) spectrometer equipped with attenuated total reflectance (ATR) accessory was used for analysis of isolated fraction of total boswellic acids and their physical mixture (in 1:1ratio) was carried out using solid state as potassium bromide (KBr) pellets at moderate scanning speed between 4000-600 cm^{-1}. The powder sample was dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. FTIR spectroscopy is one of the most powerful methods for material characterization. However, the sensitivity of this analytical tool is often very limited especially for materials with weak infrared absorption or when spectral bands of the targeted trace material overlap with the spectral bands of major components. Fortunately, for heterogeneous samples, there is an opportunity to improve the sensitivity of detection by using an imaging approach. FTIR Spectra of isolated fraction of total

Boswellic acids was measured and it was compared with the standard FTIR spectra of Boswellic acids.

5.3.3 Estimation of Total Boswellic acids

In the present work, total boswellic acids was estimated by UV/Visible spectrophotometer, UV 2450 Shimadzu Scientific by colorimetric method. Liebermann-Burchard reagent(Cold acetic anhydride & conc. H_2SO_4) was used to develop the colour. The reagent can be used for a qualitative and quantitative estimation of the steroids. (Schoenheimer & Sperry, 1934). Series of known concentrations of total boswellic acids were prepared in chloroform and Liebermann-Burchard reagent was added to develop the colour and the spectra of those solutions were taken. From the spectra the λmax was selected. A solution of total boswellic acids was prepared in chloroform. The solution was sonicated for 10 min, after adding the reagent UV/VIS Spectra and standard curve was taken using UV/Visible spectrophotometer, UV 2450 Shimadzu Scientific.

5.3.3.1 Selection of wavelength maxima (λmax)

Wave length maxima for the calibration curve was selected by making the series of known concentrations of the boswellic acids in gradually increasing range and the UV spectra was taken. According to Beer's law absorbance increases with the increase in concentration of the drug. The wavelength which gave gradual increase in absorbance by gradually increase in concentration was selected.

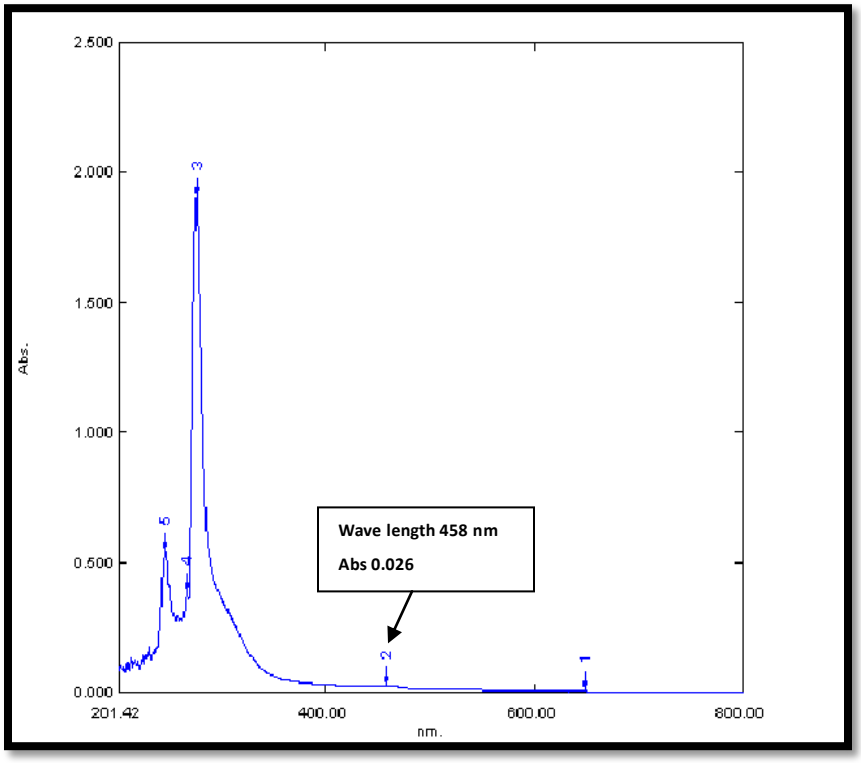


FIG 5.1 BOSWELLIC ACIDS 10µG/ML IN CHLOROFORM 800-200 NM

TABLE 5.4 BOSWELLIC ACIDS 10 ppm 800-200 nm

Wavelength (nm)	Abs.
648.00	0.004
458.00	**0.026**
276.00	1.905
266.50	0.383
245.50	0.539
416.50	0.023
268.50	0.360
259.50	0.275
211.50	0.083

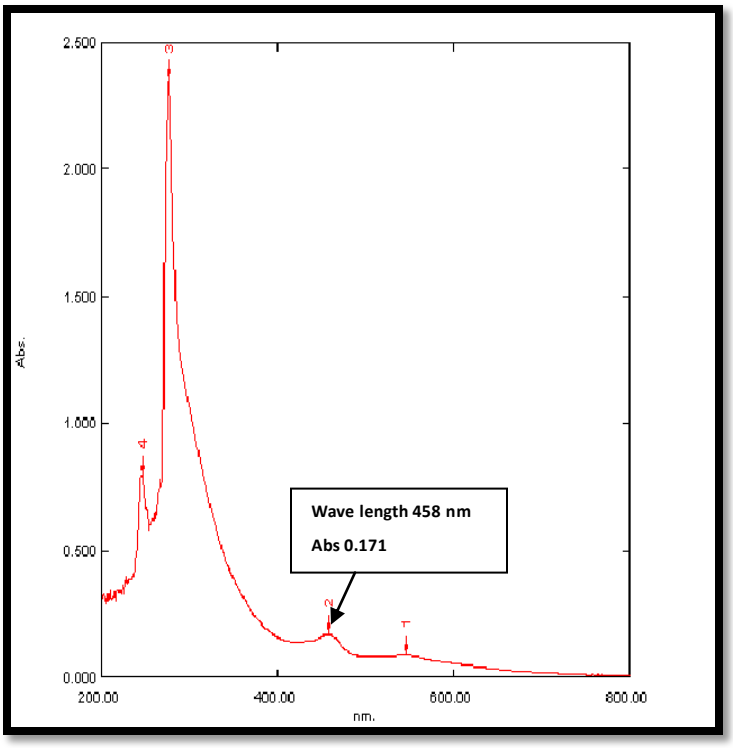


FIG 5.2 BOSWELLIC ACIDS 100µG/ML IN CHLOROFORM 800-200 nm

TABLE 5.5 BOSWELLIC ACIDS 100 ppm 800-200 nm	
Wavelength (nm)	**Abs**
545.50	0.089
458.00	**0.171**
276.00	2.353
246.50	0.800
754.00	0.008
512.00	0.082
420.50	0.138
254.00	0.582

By observing the UV spectra of the boswellic acids different concentrations the wavelength 458 nm was selected.

5.3.3.2 Preparation of calibration curve in chloroform

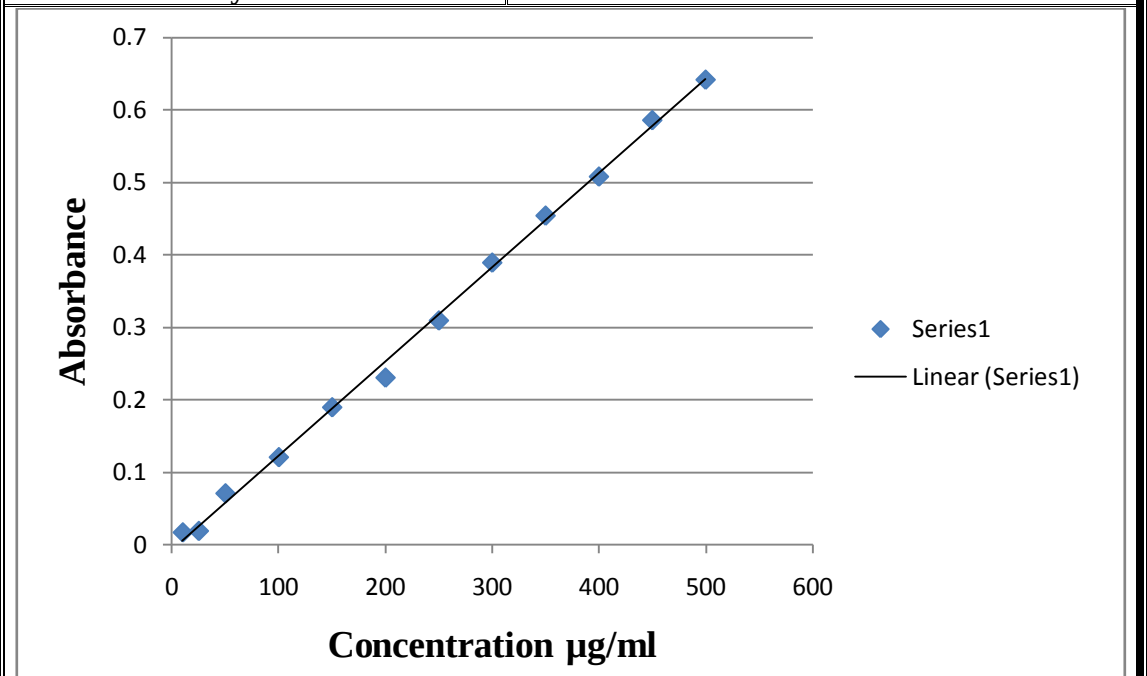
Total boswellic acids (100mg) was accurately weighed and transferred into the 100 ml volumetric flask. It was dissolved in small amounts of chloroform and volume was made up to the mark and sonicated for 10 min. This was standard stock solution with concentration of 1000 µg/ml. From the standard stock solution a series of dilutions were made to get 10 to 500 µg/ml using the same medium. To each solution Liebermann-Burchard reagent (Cold 2 ml acetic anhydride & 0.2 ml conc. H_2SO_4) was added to develop the colour (Schoenheimer & Sperry, 1934). These series of solutions were allowed to keep on waterbath for 30 min. at 45-50°C. Greenish brown colour was developed and the intensity of the colour was measured at 458 nm against chloroform as a blank using UV/Visible spectrophotometer. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated.

TABLE 5.6: CALIBRATION CURVE OF BOSWELLIC ACIDS IN CHLOROFORM BY LIEBERMANN-BURCHARD COLOUR REACTION AT WAVE LENGTH (λ) 458 nm.

Conc.(µg/ml)	Absorbance			Average absorbance ± S.D
	A1	A2	A3	
10	0.015	0.016	0.016	0.016 ± 0.0104
25	0.016	0.020	0.018	0.018 ± 0.0065
50	0.068	0.068	0.074	0.07 ± 0.0113
100	0.127	0.117	0.116	0.12 ± 0.0107
150	0.205	0.181	0.181	0.189 ± 0.0066
200	0.250	0.217	0.223	0.230 ± 0.0107
250	0.300	0.298	0.329	0.309 ± 0.0175
300	0.432	0.368	0.367	0.389 ± 0.0045
350	0.447	0.440	0.475	0.454 ± 0.0067
400	0.489	0.507	0.528	0.508 ± 0.0102
450	0.612	0.566	0.580	0.586 ± 0.0111
500	0.640	0.654	0.632	0.642 ± 0.0267

FIGURE 5.3 CALIBRATION CURVE OF BOSWELLIC ACIDS IN CHLOROFORM BY LIEBREMANN-BURCHARD METHOD

y = 0.001x - 0.007	**R^2 = 0.998**



Equation of line	
Concentration = (Absorbance + Intercept)/slope Concentration = (Absorbance + 0.007)/0.001	
R^2	0.998
Y-axis intercept	-0.007
Slope	0.001

5.3.4 Formulation of Boswellic acids Transdermal patches

In the optimization of batches, polymers with good film forming properties were selected for further optimization by addition of drug. With optimized concentration of polymer (HPMC 15cps) and Plasticizer PEG 400 at concentration of 30 %w/w of polymer the boswellic acids patches were formulated according to procedure mention in the section 5.2.1. Each formulation was evaluated for the following parameters like thickness, tensile strength, % elongation, folding endurance, content uniformity, *in vitro* drug release and *ex vivo* permeation.

5.3.5 Evaluation of Boswellic acids Transdermal patches

Formulated transdermal patches of boswellic acids were further taken for the physical evaluations. The evaluations were done as described in section 5.2.2. Formulated batch were again kept for the diffusion study to check the release profile.

5.3.6 Drug Release Study

5.3.6.1 Preparation of pH 7.4 Isotonic Phosphate Buffer :

Place 50 ml of 0.2 M Potassium dihydrogen phosphate in 200 ml volumetric flask and 39.1 ml of 0.2 M NaOH solution was added and then add distilled water was added to adjust the volume to obtain pH 7.4. The pH of the prepared solution was checked with the Digital pH meter (Elico LI 612).

5.3.6.2 *In-Vitro* Release Study

A cell fabricated on the lines of the Franz (Franz, 1975) diffusion cell with a diffusional area of 6.74 cm^2 was used. Boswellic acids transdermal film measuring respective area was subjected to *in-vitro* diffusion testing using Franz diffusion cell. The dialysis membrane (Himedia Mol.Wt 12000) was kept in intimate contact with the release surface

of the TDDS (kept in the donor cell). The receiver phase was 30 ml Isotonic Phosphate Buffer of pH 7.4 stirred at 100 rpm on a magnetic stirrer. The whole assembly was kept on an water bath at 37±0.5 ℃. 3 ml samples were taken at appropriate time intervals up to 10 hours. The volume was replenished with an equal quantity of pre-warmed receiver solution. The 3 ml samples of phosphate buffer were further extracted with 6 ml of chloroform and the amount of drug permeated was determined by method described in section 5.3.3. The cumulative amount of drug permeating through the membrane was calculated and average per cent release and flux values were determined.

5.3.6.3 *Ex-Vivo* Release Study (Rat Skin)

A cell fabricated on the lines of the Franz (Franz, 1975) diffusion cell with a diffusional area of 6.74 cm2 was used. Boswellic acids transdermal film measuring respective area was subjected to *ex-vivo* diffusion testing using Franz diffusion cell. Male Wistar rats weighing 180-220g, were used in this study. The skin was removed from the abdominal portion of rat after killing the animal. The hair and fat were removed from the skin. The stratum corneum side of the skin was kept in intimate contact with the release surface of the TDDS (kept in the donor cell). The receiver phase was 30 ml Isotonic Phosphate Buffer of pH 7.4 stirred at 100 rpm on a magnetic stirrer. The whole assembly was kept on an water bath at 37±0.5 ℃. 3 ml samples were removed at appropriate time intervals up to 10 hours. The volume was replenished with an equal quantity of pre-warmed receiver solution. Those samples were further extracted with 6 ml chloroform and the amount of drug permeated was determined by method described in section 5.3.3. The cumulative amount of drug permeating through the skin was then calculated and average per cent release and flux values were determined.

5.3.6.4 *Ex-Vivo* Release Study (Human Cadaver Skin)

The permeation kinetic throughout the human cadaver skin was evaluated using Franz diffusion cells. Human cadaver skin was procured from the V.S. Hospital, Paldi, Ahmedabad. After sampling, all specimens were immediately placed in ice box and transferred to the laboratory within. Excesses of hair and fats were removed from the skin and the skin specimens were cutted and mounted in the Franz diffusion cells. In the donor compartment was then placed patch (boswellic acid patch of measured area) in the acceptor compartment was placed isotonic phosphate buffer pH 7.4 (30 ml). The acceptor solution was stirred by means of a magnetic stirrer 100 rpm or shaker bath. At regular time intervals, samples (3 ml) were withdrawn from the acceptor compartment. To avoid saturation phenomena and maintain the ‘‘sink” conditions, the sample volume taken out was replaced by fresh isotonic phosphate buffer pH 7.4. The samples were again extracted with chloroform (6 ml) and the amount of drug permeated was determined by the respective appropriate method as described in section 5.3.3. Each experiment was carried out for 10 hours. The cumulative amount of drug permeating through the skin was then calculated and average per cent release was determined.

5.3.7 *In-Vivo Drug Release* In Rats

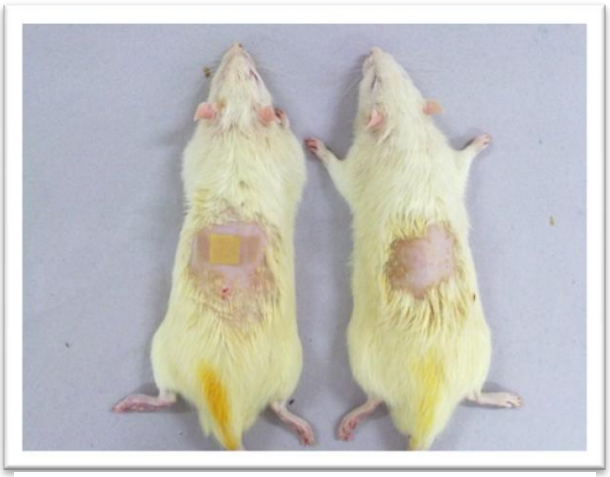


FIGURE 5.4 IN-VIVO DRUG RELEASE IN RATS

The *in-vivo* release rate of the formulations was determined on the male wistar rats weighing 250-300g after the approval of the institutional animal ethical committee (IPS/PCOG/MPH10/010). The rats were randomly divided into two groups. Each group was containing 3 animals. All experiments were carried out on the same day. Group I was applied with the transdermal formulation of the boswellic acids with the same dose with same respective area and group II was being treated as normal (without any application). One day prior to the application of the patch, on group I animals the back of each rat was carefully shaven and the skins were cleaned by wiping with water and alcohol (70 %) containing cotton. Before application of the patch the shaved skin were again cleaned with the alcohol (70 %). The applied patch was protected with the adhesive bandage. 1 ml blood was collected after the 1 hr time interval from the each animal. Every blood sample was heperinized to avoid clotting. After the blood collection the plasma was separated. Heparinized plasma (0.4 ml) was collected and and it further extracted with the chloroform (1 ml) to remove excesses of fats. The chloroform portion was further taken for the analysis and the amount of drug was determined by the respective method as described in section 5.3.3. The experiments was carried out for 8 hrs.

5.4 Guggulsterones Transdermal patches:

5.4.1 Isolation of Guggulsterones

The fraction containing guggulsterones was prepared by extracting *C.mukul* gum resin (100 g) successively with ethyl acetate in a percolator for 6-8 hrs. The solvent were filtered and collected. Ethyl acetate extract of guggul was taken round bottom flask and 0.5 N alcoholic potassium hydroxide (KOH) was added and kept reflux for 90 min on waterbath. Luke warm water was added. It was further extracted while liquid is warm with 3 successive qty of petroleum ether. The petroleum ether fractions were combined and washed with water. Petroleum ether fractions were evaporated till the dryness. The residue were dried, collected and weighed.

5.4.2 Identification of Isolated Guggulsterones

5.4.2.1 Comparative TLC of ethyl acetate extract of gum guggul and isolated guggulsterone

Test samples of ethyl acetate extract and isolated guggulsterone were prepared in methanol.

Stationary phase	:	Silica gel G
Mobile phase	:	Touene : Ethyl acetate (97 : 7)
Detection	:	In UV at 365 nm
Derivatization	:	Vanillin-Sulphuric acid reagent

5.4.3 Formulation of Guggulsterones Transdermal Patches

In the optimization of batches, polymers with good film forming properties were selected for further optimization by addition of drug. With optimized concentration of polymer (HPMC 15cps) and Plasticizer PEG 400 at concentration of 30 %w/w of polymer the boswellic acids patches were formulated according to procedure mention in the section 5.2.1. Each formulation was evaluated for the following parameters like thickness, tensile strength, % elongation, folding endurance, content uniformity, *in vitro* drug release and *ex vivo* permeation.

5.4.4 Evaluation of Guggulsterones Transdermal Patches

Formulated transdermal patches of guggulsterones were further taken for the physical evaluations. The evaluations were done as described in section 5.2.2. Formulated batch were again kept for the diffusion study to check the release profile of the formulations.

6. RESULTS:

6.1 Optimization of Formulation

6.1.1 Optimization of Polymer with same Plasticizer PEG 400

6.1.2 Optimization of Plasticizer with optimized concentration of HPMC (15 Cps)

6.2 Boswellic Acids Transdermal patches

6.2.1 Identification of Boswellic acids

6.2.1.1 Comparative Thin Layer Chromatography of methanolic extract of gum of of *B.serrata* and isolated boswellic acids

- 4 spots were observed in boswellic acid methanolic extract and 3 spots were observed in isolated boswellic acids.
- Rf value of standard 11-α keto boswellic acid is 0.27.

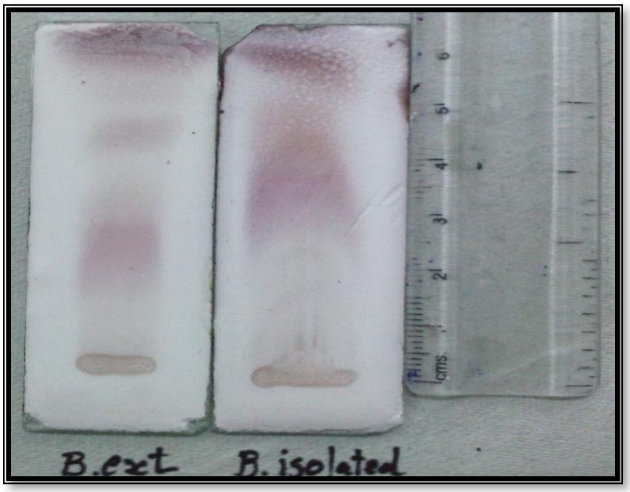


FIGURE 6.1 THIN LAYER CHROMATOGRAPHY OF ISOLATED FRACTION OF TOTAL BOSWELLIC ACIDS

6.2.1.2 FTIR Spectra of Isolated Fraction of Total Boswellic acids

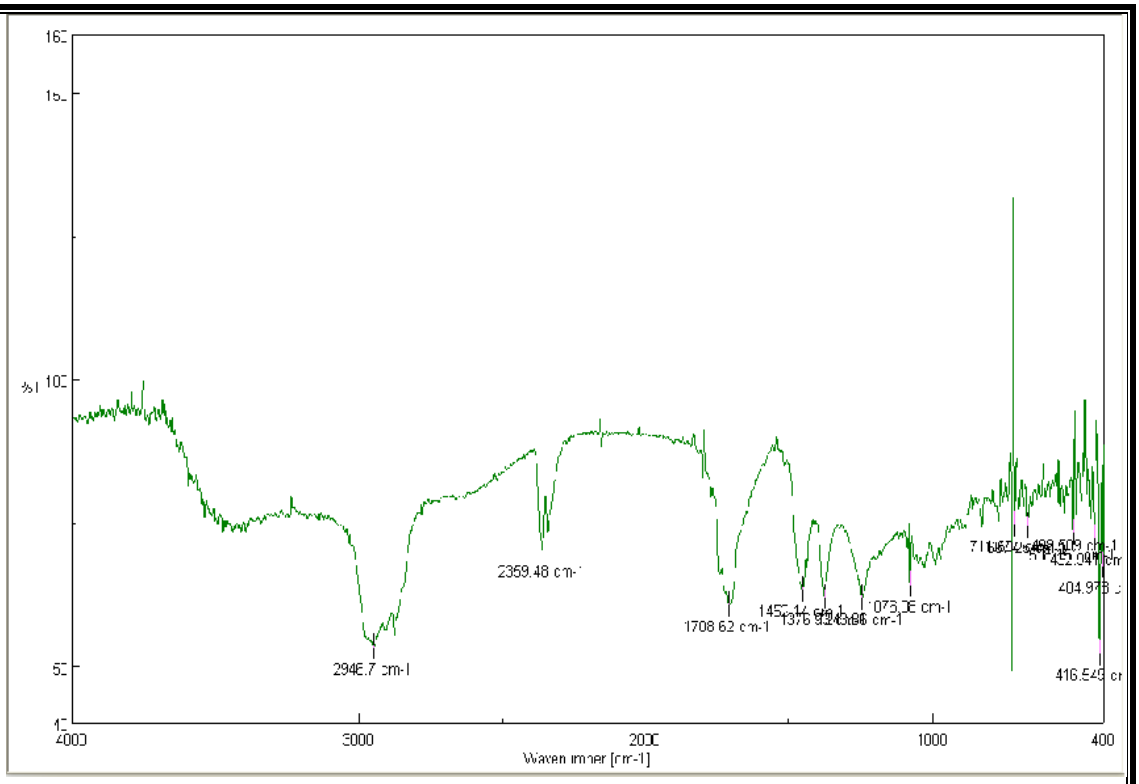


FIG 6.2 FTIR SPECTRA OF TOTAL BOSWELLIC ACIDS

TABLE 6.5:OBSERVED FREQUENCIES IN FTIR SPECTRA OF ISOLATED FRACTION OF TOTAL BOSWELLIC ACIDS

6.2.2 Formulation of Boswellic acids Transdermal Patches

Polymer used: HPMC 15Cps (2.5 %)

Plasticizer used: Polyethylene glycol (PEG 400) (30 % w/w of polymer)

Solvent used: Methanol

Active ingredient: Total Boswellic acids with different concentrations

Method: Solvent casting method

Diameter of petridish: 7.2 cm

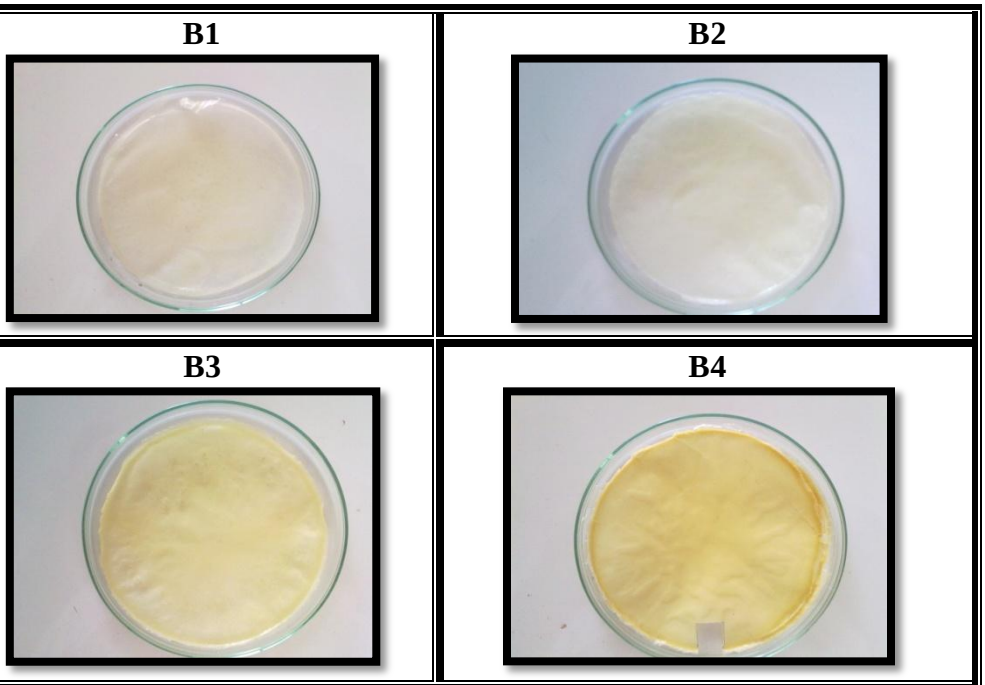


FIG. 6.3 FORMULATED DIFFERENT BATCHES OF BOSWELLIC ACIDS TRANSDERMAL PATCHES

6.2.3 Evaluations of Boswellic acids patches

6.2.4 Drug Release Study of Boswellic acids

6.2.4.1 *in-vitro* Release Study

FIGURE 6.4 IN-VITRO DRUG RELEASE PROFILE OF BATCH 1

FIGURE 6.5 IN-VITRO DRUG RELEASE PROFILE OF BATCH 2

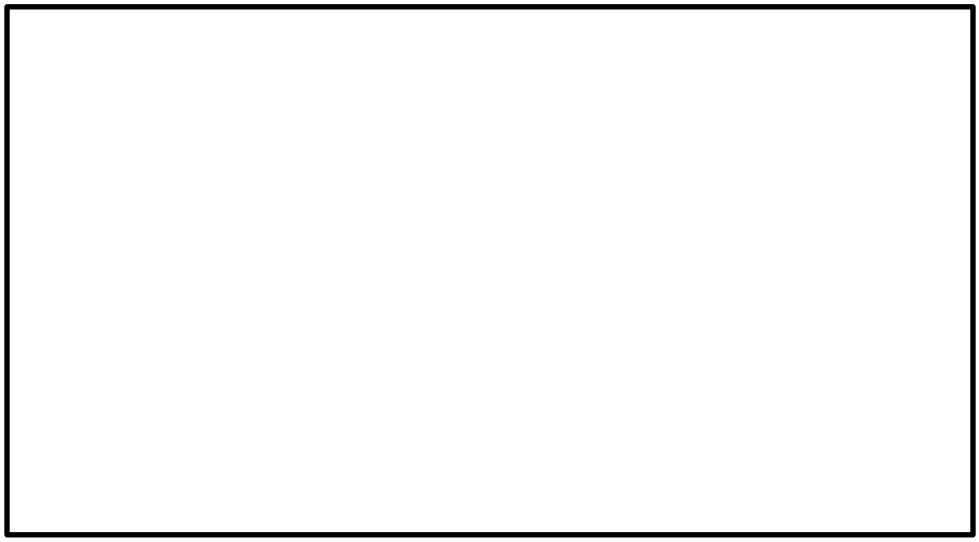


FIGURE 6.6 IN-VITRO DRUG RELEASE PROFILE OF BATCH 3

6.2.4.4 Comparision of Percentage Drug Permeation (*in-vitro*)

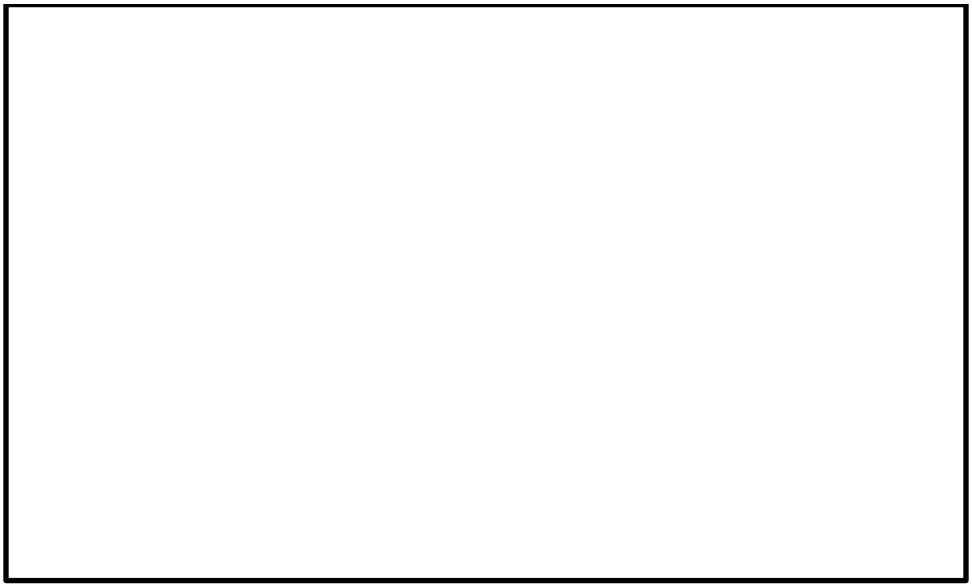


FIGURE 6.7 COMPARISION OF % DRUG PERMEATION OF BATCHES B1, B2 & B3

6.2.4.2 *ex-vivo* Release Study using Rat Skin

FIGURE 6.8 EX-VIVO DRUG RELEASE PROFILE

6.2.4.3 *ex-vivo* Release Study Using Human Cadaver Skin

FIGURE 6.9 EX-VIVO DRUG RELEASE PROFILE USING HUMAN CADAVER SKIN

6.2.4.5 Comparision of Percentage Drug Permeation (*ex-vivo*)

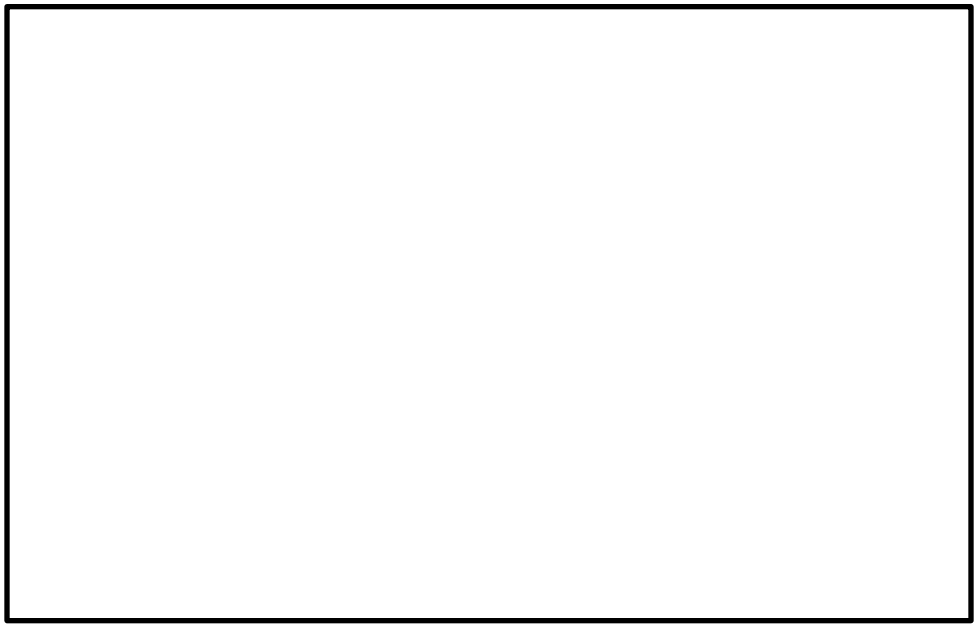


FIGURE 6.10 COMPARISION OF % DRUG PERMEATION OF EX-VIVO BATCHES

6.2.4.6 Comparision of Percentage Drug Permeation *In-Vitro* and *Ex-Vivo*

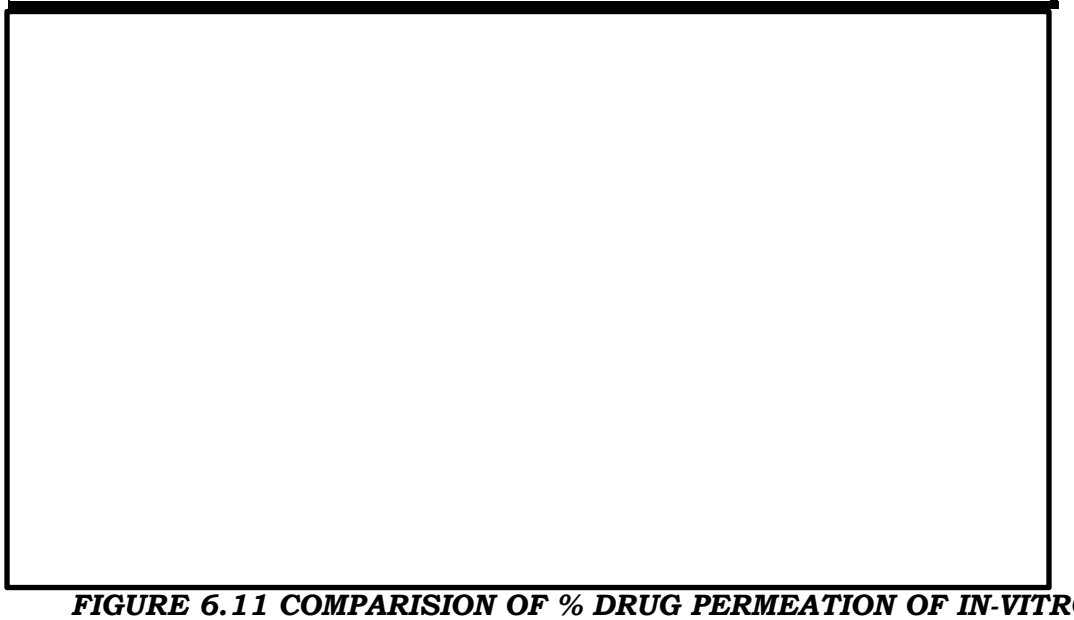


FIGURE 6.11 COMPARISION OF % DRUG PERMEATION OF IN-VITRO AND EX-VIVO BATCHES

6.2.4.6 *in-vivo* Drug Release

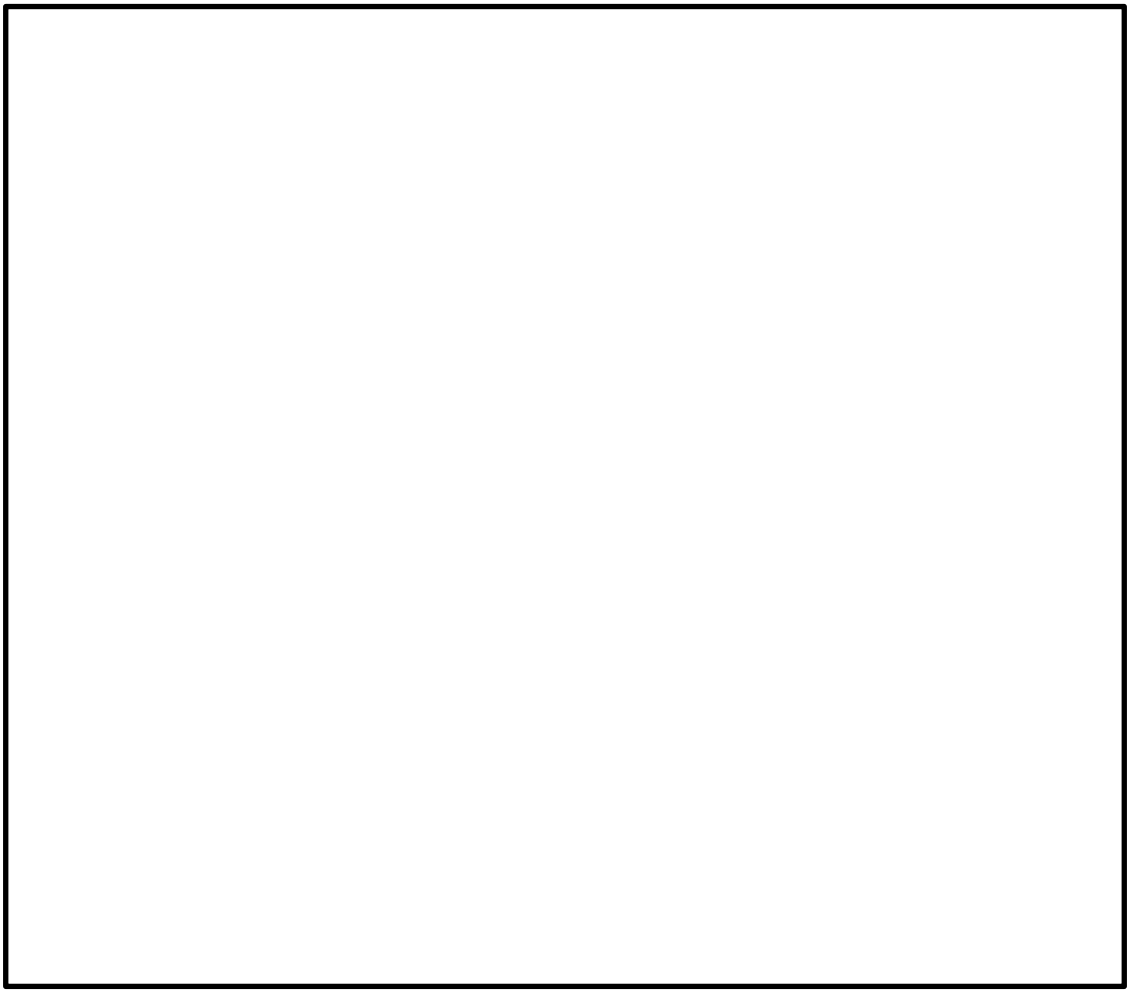


FIGURE 6.12 AREA UNDER CURVE OF THE BOSWELLIC ACIDS IN RATS

Concentration maxima Cmax = 127.667 µg/ml

Time at peak plasma concentration Tmax = 3 hr.

6.3 Guggulsterones Transdermal patches

6.3.1 Identification of Guggulsterones

6.3.1.1 Comparative TLC of ethyl acetate extract of gum guggul and isolated guggulsterones

- 4 spots were observed in ethyl acetate extract and 4 spots were observed in isolated guggulsterones.
- Standard guggulsterone mixture having E and Z guggulsterone give Rf at 0.38-0.46 by two compaque spots.

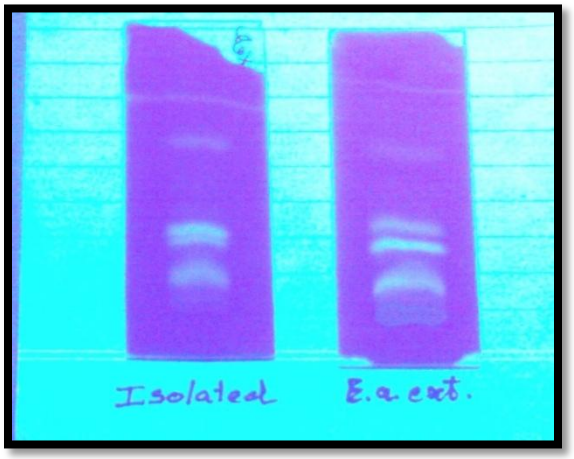


FIGURE 6.13 THIN LAYER CHROMATOGRAPHY OF ISOLATED GUGGULSTERONES

6.3.2 Formulation of Guggulsterones Transdermal Patches

Polymer used: HPMC 15Cps (2.5 %)

Plasticizer used: Polyethylene glycol (PEG 400) (30 % w/w of polymer)

Solvent used: Methanol

Active ingredient: Isolated Guggulsterones with different concentrations

Method: Solvent casting method

Diameter of petridish: 7.2 cm

6.3.3 Evalation of Guggulsterones Transdermal patches

7. DISCUSSIONS:

Transdermal drug administration generally refers to topical application of agents to healthy intact skin either for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin. Transdermal drug delivery system (TDDS) can deliver certain medication to systemic circulation in a more convenient and effective way than conventional dosage form. The TDDS can minimize first-pass metabolism associated with gastro-intestinal administration of drugs and it can maintain constant drug level in blood (Ranade, 1991). As a result of above reasons a new formulation of herbal constituents was undertaken for present study.

Boswellic acids are leukotriene inhibitor and are also effective through topical application in inflammatory disorders (Singh et al., 2008). The anti-inflammatory and anti-arthritic activity of the boswellic acids have been well documented, thus it was selected as one of the component for the formulation.

Initial formulations for the development of various films, involved the use of different polymers i.e. Ethyl cellulose (4.5 %), Poly vinyl alcohol (2.5 %) with plasticizer polyethelene glycol 400, but the resulting films were not transparent and could not peeled out properly. Again films were formed with polymer, Hydroxy propyl methyl cellulose 15 cps (2.5 %) and plasticizer, PEG 400 with chloroform and methanol. These films were found to be transparent and possess good peeling property. (Table 6.2)

While carrying out the optimization of plasticizer, preliminary trials revealed that the polymer (HPMC 15 cps) films can be formed using different concentrations of the plasticizer (PEG 400), using glass surface and methanol as a solvent. The results showed that as the plasticizer increases tensile strength and percent elongation increases. This increase in percent elongation may be due to bond formation between the polymer and

plasticizer. The films formed using plasticizer PEG 400 (30 % w/w of polymer) with polymer HPMC 2.5 % showed good plasticity, good elasticity and best tensile strength. Hence polymer HPMC (2.5 %), plasticizer PEG 400 (30 % w/w of polymer) and solvent methanol were selected for further studies.

Boswellic acids were used as one of the active constituents for the transdermal film formulations; the isolated fraction of *B.serrata* was identified by thin layer chromatography (TLC) and FTIR spectra. Comparative TLC profile of isolated fraction and methanolic extract of the gum of *B.serrata* has been shown in figure 6.1,6.2 and in table 6.4,6.5. The TLC profile and FTIR spectra showed the presence of boswellic acids in the isolated fraction.

The formulated films of the isolated fraction of the total boswellic acids were evaluated using physical evaluation parameters such are tensile strength, % elongation, thickness, folding endurance and drug content. All the batches of HPMC 15 cps (2.5 %), PEG 400 (30 % w/w of polymer) with the active phytoconstituents formed uniform film and all the films were peeled out uniformly. It was also found that batch B1 showed more stickiness than other batches (B2, B3 and B4). The results also revealed that increase in amount of active constituents, decreases % elongation and tensile strength, which may be due to bond formation between polymer and active constituents. All the batches of transdermal films showed folding endurance more than 100 times. All the transdermal patches were smooth in appearance, having uniform thickness; proper weight and drug content. Batches B1- B4 were found to be ideal for drug loading.

The comparative diffusion studies using dialysis membrane for different formulated batches B1, B2 and B3 were determined (fig 6.7). The results showed gradual increase in the drug diffusion, which may be due to film swelling. The drug release from the film was initially affected by the swelling of the film. The cumulative amount of total boswellic acids permeated through dialysis membrane has shown in Table 6.14. The batch B3 showed higher permeability in comparison to batch B1 and B2. The *in-vitro* release study performed on different batches showed that more than 70 % drug release

after the duration of 10 hrs. The release was in sustained manner. Further, it can be seen by comparing the release profile of all the three batches that with the increase in drug: polymer ratio there was increase in drug release. All three batches were found to be suitable for the transdermal sustained delivery.

Comparative diffusion studies of the formulated batches on rat topical skin and human cadaver skin were carried out and it was seen that there was a gradual increase in drug release with time (fig 6.10). The cumulative amount of release on rat topical skin and human cadaver skin has been given in table 6.14. The amount of drug permeated through human cadaver skin was to found be 70 % higher than the amount permeated through rat skin. Results also showed 50 % drug release had been achieved through human cadaver skin in 7 hrs as compared to rat skin in 10 hrs.

Comparative *in-vitro* and *ex-vivo* diffusion studies for formulated batches were determined (fig 6.11, table 6.20). The *in-vitro* and *ex-vivo* result comparison indicates that the *ex-vivo* release from the human skin was very much similar to the *in-vitro* releases.

In-vivo drug release was performed on the male wistar rats weighing 200-240 gm and the results of the *in-vivo* release are shown in table 6.21. As amount of cholesterol interfere with the concentration of the boswellic acids in to the blood, the normal group of animals without treatment were taken. The C_{max} (peak plasma concentration) was found 125.667 µg/ml in 3 hr.

The plant sterol guggulsterone attenuates inflammation (Mencarelli et al., 2009). The anti-inflammatory activity of the guggulsterones have also been well documented, thus it was selected as another component for the formulation.

The TLC was carried out for the identification of the isolated fraction of gugulsterones and compared with the ethyl acetate extract of the gum guggul (fig 6.13), (table 6.22).

The R_f values suggested that the isolated mixture consist of the mixture of the guggulsterone E and guggulsterone Z.

Using the optimized concentration of the polymer and plasticizers the films containing guggulsterones were formulated and evaluated. Evaluation parameters of the formulated films of the isolated fraction of the guggulsterones were carried out and the results obtained were outlined in table 6.24. All the batches of HPMC 15 cps (2.5 %), PEG 400 were uniformly formed and all the films were peeled out uniformly. All films were having smooth surface and stickiness. Results also revealed that the amount of active phytoconstituents are showing good plasticizing property. As increase in amount of active constituents, % elongation and tensile strength also increases. All the batches showed folding endurance more than 100 times. The patches were found to have uniform thickness and proper weight. Our present study may be useful for patient suffering from rheumatoid arthritis.

8. CONCLUSION:

In this present work transdermal patches of boswellic acids and guggulsterones were formulated. The *in-vitro*, *ex-vivo* and *in-vivo* drug release pattern were determined of the transdermal patches of the boswellic acids. By observing the release profiles of the *in-vitro* batches, we may conclude that % drug release increases with the increase in the drug : polymer ratio.

By comparing the *ex-vivo* release pattern of the same drug : polymer ratio batches we found that the % drug release through human cadaver skin was much higher than the % drug release through the rat skin. Therefore, we may conclude that the controlled release due to the pachyderm layer present in the rat skin the release is controlled. So the rat skin may be the rate limiting step for the drug release. The *in-vivo* release in rats Cmax was achieved in 3 hrs.

As our target population was human subjects. The % drug release in *ex-vivo* diffusion through human cadaver skin was found more than 70 % after 10 hrs. So, we may conclude that the same release pattern would be expected in human subjects.

Further work are needed to assess the pharmacodynamic of the transdermal patch of boswellic acids and refine it in order to attain suitable clinical levels in patients suffering from Rheumatoid arthritis.

Further work are also needed to establish the diffusion profile of the transdermal patches of the guggulsterones. The effectiveness of transdermal batches would be evaluated pharmacologically by suitable animal model.

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